Adjuncts to Pre-Hospital Resuscitation Strategies for Haemorrhagic Shock and Blast Injury: Supplemental Oxygen and Recombinant Activated Factor VII

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Abstract

Explosion is responsible for almost 80% of Coalition injuries in today's conflicts. Haemorrhage is the leading cause of death and blast lung injury is evident in 11% of Coalition casualties surviving to reach the (UK) Field Hospital. Military prehospital evacuation times can be prolonged and the combined insults of haemorrhage and blast injury present a 'double hit' to oxygen delivery. Resuscitation strategies must be capable of preserving life from such trauma for several hours. Alongside fluid therapy, adjuncts to resuscitation might improve battlefield survival.

This randomized controlled animal trial assessed two adjuncts: supplemental inspired oxygen and recombinant activated Factor VII (rFVIIa). Neither adjunct is currently available in the far-forward military echelon, but with modern technology, both are potentially deployable.

18 terminally anaesthetized swine were exposed to blast, controlled haemorrhage and grade IV liver laceration (uncontrolled haemorrhage). Animals were allocated randomly into three treatment groups. All animals were resuscitated with normal saline to a hypotensive systolic target (80mmHg), which continued until the 8hr end point. Thirty minutes after the onset of resuscitation each group received one of the following: single (180mcg/kg) dose of rFVIIa; supplemental oxygen (min FiO_2 0.3 to maintain SaO_2>95%) or the control group (breathed air throughout and received saline placebo 0.18ml/kg).

5/6 control animals died within 4 hours. Supplemental oxygen improved survival (4/6 survival to 8h endpoint, P=0.014). Single dose rFVIIa did not prolong survival

compared to control (2/6 survived, p=0.65). Oxygen arrested physiological decline while control and rFVIIa animals continued to decline until death.

Supplemental oxygen is a useful adjunct to fluid resuscitation in the context of haemorrhage and blast injury. Delivery of oxygen support capability to forward echelon units is recommended. By contrast, a single intravenous (pre-hospital) dose of rFVIIa was not an effective treatment for blast lung based on our model of complex battlefield injury.

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ABE	Actual base excess		KPa	KiloPascal
ABG	Arterial Blood Gas		LWI	Lung Weight Index
AIS	Abbreviated Injury Score		MAP	Mean Arterial Pressure
ARDS	Acute Respiratory Distress Syndrome		MERT	Medical Emergency Response Team
ATLS	Advanced Trauma Life Support®		MOF	Multi Organ Failure
BATLS	Battlefield Advanced Trauma Life Support		ms	Millisecond
BP	Blood pressure		Mg	Milligram
bpm	Beats per minute		NH	Novel Hybrid fluid resuscitation strategy
BV	Blood volume		NO	Nitrous Oxide
CaO2	Arterial oxygen content		O2	Oxygen
CI	Confidence Interval		OER	Oxygen extraction ratio
CNS	Central Nervous System		PaCO2	Arterial CO2 tension
CO	Cardiac output		PaO2	Arterial oxygen tension
CO2	Carbon Dioxide		PBI	Primary blast injury
CXR	Chest Radiograph		PVC	Polyvinylchloride
DAH	Diffuse Alveolar Haemorrhage		RTS	Revised Trauma Score
DO2	Oxygen Delivery		RBC	Red Blood Cell
DSTL	Defence Science and Technology Laboratories		ROTEM	Rotational Thromboelastometry
EFP	Explosively Formed Projectile		PRBC	Packed Red Blood Cells
EBW	Enhanced blast weapons		PCR	Polymerase Chain Reaction
FAE	Fuel air explosive		rFVIIa	Recombinant activated Factor VII
HR	Heart rate		SBP	Systolic blood pressure
ICU	Intensive Care Unit		se	Standard error
ISS	Injury Severity Score		SV	Stroke volume
IV	Intravenous		SVR	Systemic vascular resistance
IED	Improvised Explosive Device		TNT	Trinitrotoluene
Kg	Kilogram		TEG	Thromboelastography

1 Introduction

Trauma accounts for 10% of all deaths worldwide (1;2). In western countries, it is the leading killer before 40 years of age (NCEPOD Report 2009) and the cost of trauma to the United Kingdom lies at well over £1.2 billion per annum (Department of Health 1998).

Military operations in the Middle East have increased awareness of military trauma. In Afghanistan (OP HERRICK) since 2001, over 360 UK Military or MOD civilians have died as a result of hostile action. Since 2006, 1871 UK military or MOD civilian personnel have been wounded in action. Of these, 267 have been listed as 'Very Seriously Ill' and a further 275, 'Seriously Ill'(4).

Trauma represents a significant burden on both humanitarian and financial levels. Measures that ameliorate the consequences will lessen the impact of this disease.

1.1 Classification by mechanism

Trauma is typically classified into either blunt or penetrating mechanisms. Blunt mechanisms cause the majority of trauma injuries in the developed urban environment (5), although modern US data shows an increasing burden from penetrating injury (6). Protagonists of conflict deliberately employ ballistic weapons and high explosive fragmenting munitions to incapacitate their enemies, so military trauma is predominantly penetrating.

In addition to the typical division of traumatic injury into blunt and penetrating mechanisms, blast injury represents a third injury mechanism of particular relevance

to the military. Exploding munitions and improvised explosive devices (IED) are increasingly responsible for both military and civilian trauma casualties.

1.2 Civilian and Military Trauma

Urban trauma has demonstrated a tri-modal distribution of deaths (7) . Approximately half die within minutes from catastrophic wounding (Immediate deaths): there is little one can do for these victims; instead the priority lies in injury prevention. A second peak is seen over the ensuing few hours. This peak is termed 'early deaths' and is the area most relevant to military trauma care and to this thesis. The third peak, 'late deaths', occurs days to weeks after injury, usually as a result of sepsis or multiple organ failure. As trauma systems mature, this third cohort represents a declining proportion, now accounting for only 7% of all deaths (6).

Military data has not reproduced Trunkey's tri-modal distribution. Instead a large proportion of fatalities die instantly (6). Most subsequent deaths occur in the 'early' phase and late deaths are uncommon. The greatest potential for improvement remains in preventing the 'early deaths'.

It is understood that evacuation to surgical facilities must be achieved swiftly to maximise survivability after major trauma. National and regional frameworks are capable of covering the population in a timely manner (8). 95% of all 'Category A' calls should be reached by a fully equipped ambulance within 19 minutes. Ambulances are manned by highly trained paramedics. Patients normally reach hospital within 1 hour of wounding. Time delay to surgery following penetrating

trauma at The Royal London Hospital has a mean time of 86 min and all were in surgery within two hours from wounding (9).

On military operations, logistic constraints and the tactical situation potentially impede evacuation. The UK Armed Forces deploy medical assets in a manner which enables rapid recognition, assessment and management of high priority casualties, swift evacuation to a surgical facility and, thereafter, onwards repatriation to the UK. Individual soldiers and embedded medics instigate medical aid in the immediate post-injury period. If there is considerable distance between the 'front line' and the surgical facility, the Regimental Aid Post (RAP) provides an interim echelon in the evacuation chain. High priority casualties should reach here within the hour. The RAP is manned by at least one doctor. At this point casualties receive the battlefield-modified version of advanced trauma life support (10). A recent paper by Tai and military surgical colleagues, recommends that all battlefield casualties should reach a surgical facility within two hours from wounding (11). This facility may be designed to perform only damage control procedures, but in current conflicts is usually a dedicated Field Hospital.

Despite good planning, delayed evacuation on military operations is sometimes unavoidable. US data from Operation Iraqi Freedom demonstrated evacuation times of up to 20 hours (12). Data from the US mission in Afghanistan specifically mentioned delay to initial surgical care (5 hours) as a contributing factor for casualty mortality (13). Short evacuation times, although achievable for the majority of casualties, can neither be guaranteed in the military context; nor after a major civilian incident.

1.3 The Problem of Haemorrhage

Haemorrhage is the principal cause of battlefield death; responsible for 44% of pre-hospital fatalities after wounding (14). In urban trauma, haemorrhage is the second commonest cause of pre-hospital death, but remains the leading cause of early hospital deaths (15). Addressing haemorrhage effectively will improve survival.

The body mounts a physiological response to haemorrhage in an effort to maintain perfusion of vital organs and minimise bleeding. If despite this compensation, organ perfusion begins to suffer, the casualty enters 'haemorrhagic shock'. This progressive phenomenon is the result of increasingly compromised oxygen delivery to the tissues: the level of 'shock' depends on the rate and duration of haemorrhage, and the time delay before restoration of normal perfusion. Metabolic acidosis develops as a result of insufficient oxygen delivery and can be considered a marker of oxygen debt. Coagulopathy, common after traumatic haemorrhage, is an independent predictor of poor outcome (16). It develops as a result of several contributing events after injury, and recent work suggests that, in the early phase after injury, hypoperfusion plays a key role in coagulopathy, irrespective of acidosis or dilution (17). Coagulopathy and acidosis reduce survival from trauma (18;19), but also increase morbidity in surviving patients (20;21). With hypoperfusion driving acidosis and coagulopathy, the degree and duration of the haemorrhagic shock state is critical to overall outcome.

The most obvious intervention is to stop the bleeding. Extremity haemorrhage should be controllable with simple first aid interventions, but Bellamy's data from the Vietnam war showed that a third of battlefield exsanguinations occurred from

extremity injury (22). Troops carry a range of products to help control extremity haemorrhage. Novel haemostatic dressings may gain temporary control in some cases of junctional injury (23;24), but incompressible haemorrhage requires surgical control.

It is clear that haemorrhage must be stopped as quickly as possible and that this normally requires surgical intervention in severely wounded casualties. In the pre-surgical interval, prompt restoration of tissue oxygen delivery, without precipitating rebleeding, should reduce mortality and morbidity.

1.4 The Problem of Blast

'Blast injuries' describe the sequellae of exposure to an explosive detonation. They represent an increasing problem in both military and civilian environments (25). US data from Iraq and Afghanistan indicated that blast injuries are present in between 31% and 55% of combat casualties (26-29). Another US paper illustrated that 78% of wounds are sustained as a result of exploding munitions (29).

There is a growing threat posed by enhanced blast weapons (EBW) (30). EBW specifically exploit the capabilities of a blast wave to incapacitate or kill enemy over a larger target area than conventional weapons. The blast wave can penetrate traditional defences and the prolonged over-pressure can destroy buildings (30). Thermobaric weapons incorporate both enhanced blast and thermal output and they have been used in conflicts before (30). If EBW are deployed, we should expect large numbers of casualties with primary blast, burns, translational and crush injuries (31).

As protagonists of terror deploy increasingly lethal explosive devices our perceived vulnerability to terror attacks increases (25). Terrorist attacks have produced several civilian casualties with primary blast injury, particularly where explosions have occurred in confined spaces, such as buildings and vehicles (32).

Exposure to high explosive can injure through several means. The wave might cause few external signs of injury, yet may still be fatal. It particularly affects the lungs causing profound hypoxia (33). Exposure to the blast wave has been shown to modify the response to haemorrhage (35). Other features of explosive munition detonation make it likely that, aside from sustaining lung injury, a blast victim will also sustain both penetrating and blunt trauma (34). These injuries will result in severe haemorrhage.

We know that injury and blood loss produce significant physiological sequellae; and that the principal cause of deterioration after haemorrhage is inadequate delivery of oxygen to the tissues. If thoracic exposure to a blast wave is added, oxygenation of what blood remains is reduced. The blast-injured bleeding casualty therefore sustains a ‘double hit’ to his capacity to deliver oxygen to tissues and will deteriorate more swiftly.

1.5 Current and Potential Pre-hospital Interventions

We cannot rely on prompt access to surgery, so we must develop pre-hospital strategies to maintain life and delay physiological demise after trauma.

The physiological goal of pre-hospital resuscitation is to maximise tissue oxygen delivery. By augmenting the depleted intravascular volume, fluid therapy bolsters cardiac output and maintains tissue oxygenation. The type of fluid and what targets should guide therapy remain subjects of debate, with Bickell's study even questioning whether fluid should be given at all in the first hour or so after injury (36). Work previously undertaken at Dstl Porton Down has illustrated the survival and physiological penalties of prolonged hypotensive resuscitation after blast and haemorrhage (37;38). The problem with the hypotensive concept is its inability to support perfusion enough to prevent physiological decay. A subsequent study assessed a 'novel hybrid' fluid resuscitation protocol (39): this addressed the concerns of inadequate tissue perfusion, while also avoiding rebleeding from sites of injury; a potential risk of more aggressive fluid administration (40).

Fluid therapy aside, it would be advantageous to improve oxygen delivery to the tissues after trauma, without increasing the hydrostatic pressure exerted on a blood clot. Two such adjuncts have been identified: supplemental oxygen and recombinant activated Factor VII.

Although pre-hospital supplemental oxygen is standard in civilian emergency medicine, it is not a part of far forward UK military medical doctrine. The principal limitation is logistic: regular resupply is difficult to achieve and forward medical assets must remain 'light' to respond to the battle picture. However, modern technologies increase the feasibility of deploying remote oxygen support.

Supplemental oxygen should increase the oxygen content of arterial blood (CaO2) and improve tissue oxygen delivery, independent of fluid resuscitation. This should be particularly effective in hypoxic blast-injured casualties.

Recombinant Activated Factor VII (rFVIIa) is a haemostatic agent. It has been shown to increase the blood pressure at which re-bleeding occurs after swine aortotomy (41). Rebleeding is a concern during fluid resuscitation. In blast lung, by enabling strong clots at sites of haemorrhage, rFVIIa may reduce the volume of intra-alveolar haemorrhage and so preserve blood oxygenation. rFVIIa has been shown to reduce the incidence of Adult Respiratory Distress Syndrome (ARDS) and Multiple Organ Failure (MOF) in severely injured trauma patients (42). ARDS and MOF are significant contributors to 'late deaths' from trauma, so any 'anti-inflammatory' effect could improve outcomes. rFVIIa has demonstrated Level IV efficacy in diffuse alveolar haemorrhage, a pathology similar to blast lung (see section3.3.6). An Israeli clinician, with experience in managing blast-injured casualties, has reported anecdotally that rFVIIa might reduce lung injury after blast (43). A heat stable preparation of the drug is now available and FDA approved; improving its potential for use as a pre-hospital drug in austere environments (44).

Both haemorrhage and blast injury produce significant clinical problems. There is a requirement to develop effective strategies to manage casualties injured by these means. This thesis will explore current understanding of the problems of haemorrhage and blast injury, both as discrete and combined insults, and assess two potential interventions that might attenuate the sequellae of these events.

2 Basic Science of Haemorrhage and Blast Injury

After traumatic injury, casualties are subject to a range of stimuli capable of producing physiological responses. The term, 'Shock', describes inadequate oxygen delivery to the tissues: it is common after trauma. Oxygen delivery (DO_2) is the total amount of oxygen delivered to tissues per unit of time. It is a product of blood flow through the circulation in a minute (cardiac output) and the amount of oxygen within that blood (oxygen content) (Equation 1). The degree and duration of the oxygen delivery shortfall will correlate with survival and morbidity. The goal of trauma resuscitation is to restore normal oxygen delivery as soon as possible. During this Basic Sciences Chapter, the physiological effects of haemorrhage and blast injury, their interactions, and the potential benefits of therapy, will be related to their impact on this oxygen delivery equation.

(DO_2) (ml/min) = cardiac output (CO) x arterial oxygen content (C_aO_2)

Equation 1 - Oxygen delivery equation

CO = Stroke Volume (SV) (ml) x Heart Rate (HR) (bpm)

C_aO_2 = [Haemoglobin (Hb)] (g/dl) x Oxygen Saturation (SaO_2) (%) x 1.34* x 10 / 100[1]

* Hüffner Constant – Oxygen carrying capacity 100% saturated Haemoglobin (ml O2/g Hb)

Example:

CO = [75 (SV) x 70 (HR)] = 5.25 l/min

CaO2 = [14 [Hb] x 98 (SaO2) x 1.34 10/100] = 183.85

DO2 = 5.25 x 183.85 = 965 ml/min

[1] *The amount of unbound O_2 dissolved in plasma at 1 atmosphere is clinically negligible and not therefore included in this representation of the DO_2 equation*

2.1 Haemorrhage

The primary purpose of the circulatory system is to distribute oxygen and nutrients to the organs and tissues. Under normal circumstances (patient with normal levels of haemoglobin breathing air at atmospheric pressures) most of the oxygen (approximately 99%) is carried in blood bound to haemoglobin, which is found inside erythrocytes. The remainder of the oxygen is simply dissolved in plasma (3.2.1). One of the functions of the circulatory system is to transport the erythrocytes to the tissues via the vasculature, driven by pumping of the heart. Damage to the vasculature or the pump can rapidly impair the transfer of oxygenated blood. Loss of blood from injured vessels is termed haemorrhage.

Significant blood loss produces shock, with reduced circulating volume and haemoglobin levels leading to impaired oxygen delivery – a critical factor of trauma outcome.

2.1.1 Shock

The term ‘Shock’ is defined as pathologically inadequate tissue oxygen delivery (relative to the tissue’s needs). Tissue oxygen delivery is dependent upon both the oxygen content of arterial blood and the flow of blood into tissues (Equation 1). Therefore one impairment that can lead to shock is poor tissue perfusion. This can be the result of several processes including: haemorrhage; myocardial failure and spinal cord injury. Haemorrhage is the leading cause of death on the battlefield and the most common cause of shock in trauma (45).

The sequellae of shock occur in a dose-dependent fashion: the longer and more severe the shock state, so the more severe the physiological penalties and the

higher the mortality. An understanding of key features of the response to haemorrhage and the pathophysiology of shock allows one to identify potential targets for therapy.

2.1.2 Biphasic response to simple haemorrhage

In controlled laboratory environments, where isolated haemorrhage can be imposed upon test subjects, there is a predictable sequence of physiological responses to ongoing haemorrhage. Of course, in the world of trauma medicine, isolated haemorrhage is uncommon. Factors such as tissue injury, pain and blast injury all have significant effects on the response to 'simple' haemorrhage and will therefore be discussed after the basic response to blood loss.

Phase one is characterized by maintenance of arterial blood pressure, tachycardia and increased vascular resistance. Phase Two occurs when blood pressure, heart rate and vascular resistance fall.

2.1.2.1 Phase One Events

Phase one occurs because the baroreceptor reflex attempts to maintain the arterial blood pressure despite a haemorrhage-induced fall in cardiac output. To describe the events chronologically, this phase is initiated by reduced venous return to the heart following blood loss from the circulation. Reduced venous return results in decreased filling of the heart during diastole. Starling's law of the heart (46) dictates that decreased end diastolic volume will result in a reduced cardiac stroke volume. Reduced stroke volume in turn reduces cardiac output:

(Cardiac Output (CO) = Stroke Volume (SV) x Heart Rate (HR))

Equation 2 - Cardiac Output Equation

Reduced CO could result in a fall in blood pressure, as blood pressure is determined by the product of cardiac output and systemic vascular resistance.

Mean Arterial Blood Pressure = Systemic Vascular Resistance (SVR) x CO

Equation 3 - Mean Arterial Pressure

However, the baroreceptor reflex is able to maintain blood pressure despite a fall in CO. This is achieved because baroreceptors are responsive to the rate of change of blood pressure as well as mean pressure (at normal or sub-normal pressures) (47) Reduced SV results in a reduced arterial pulse pressure, which in turn leads to unloading of baroreceptor afferent discharge (through detection of rate of change of pressure mentioned above). The baroreceptor response to this unloading involves initial withdrawal of Vagal tone which results in initiation of tachycardia (48). Very soon thereafter (a few seconds later), there is an increase in sympathetic efferent activity to both the heart and vasculature (49).

In contrast to the effects of vagal stimulation of the heart, which are only chronotropic, the sympathetic efferents innervate both the pacemaker cells to influence heart rate and the ventricular myocardium to increase the force of contraction (50).

As a consequence of this sympatho-activation there is an increased tachycardia and a positive inotropic effect which, in part, offsets the fall in SV which has resulted from the Starling mechanism described earlier.

The end result of these mechanisms on the heart is that the fall in CO (initiated by haemorrhage) has now been limited (but not reversed). The final part of the baroreceptor reflex to maintain blood pressure relies on effects of sympatho-activation on the vasculature. Sympathetic-driven vasoconstriction increases vascular resistance and therefore helps maintain blood pressure (Equation 3 - Mean Arterial Pressure). The effects of this sympathetically-mediated vasoconstriction on different vascular beds is not however uniform. Some beds experience intense vasoconstriction, while others undergo little or no contraction. Blood flow to critical organs, such as the brain, is therefore maintained at the expense of reduced flow into other organs, such as the gut; kidneys and skeletal muscle, which are more tolerant of transient ischaemia (51;52).

This compensatory baroreceptor-mediated response to haemorrhage cannot however maintain blood pressure indefinitely. Despite the aforementioned mechanisms, loss of approximately 30% of blood volume will result in a cardiac output of about 50-60% of baseline (53). At this stage a second phase, 'Phase Two' takes over (54).

2.1.2.2 Phase Two Events

With ongoing haemorrhage, a reversible depressor phase occurs; characterised by a profound bradycardia and a significant drop in systemic vascular resistance, which in turn results in a profound drop in blood pressure (54;55) . This drop is so severe that most patients will faint at this stage (48).

At the end of Phase one, the heart is beating very forcefully around very under-filled chambers and there is marked deformation of the myocardial wall. This deformation

is thought to activate a group of mechanoreceptors in the ventricular wall, which relay in unmyelinated Vagal C-fibres (53). Stimulation of these receptors is known to produce a bradycardia (due to increased vagal efferent activity) and a decreased vascular resistance (due to sympatho-inhibition) (56;57).

While counter-intuitive for survival advantage at first glance, this bradycardia has two potentially beneficial effects. First, the bradycardia increases diastolic filling time, which results in a small increase in stroke volume. Second, the same increase in diastolic time allows better perfusion of the heart musculature and may therefore be cardio-protective. Indeed, attempts to block the bradycardia of Phase Two with atropine have lead to increased mortality in a clinical study (58). Furthermore, the reduction in systemic vascular resistance that occurs due to the sympatho-inhibition described above, reduces afterload on the heart and therefore reduces cardiac work at a time when coronary perfusion is in danger of being impaired. This reflex was actually described by Hunter in 1794 as an increase in skeletal muscle perfusion noted in individuals as they approached the point of fainting during venesection (59).

This concept of a ventricular cardiac inhibitory reflex being the sole cause of the Phase Two response to haemorrhage is not uniformly accepted however, since Scherrer and colleagues demonstrated the depressor effect in a cardiac transplant subject with presumably no ventricular cardiac innervation (60).

2.1.3 Auto-resuscitation during Haemorrhage

Haemorrhage results in loss of whole blood. Therefore, immediately after haemorrhage, the haematocrit (HCT) and haemoglobin (Hb) concentration remain unchanged. A little later however, fluid shifts expand and dilute the intravascular

compartment and reduce both HCT and Hb. As we have seen in the previous paragraphs, the Phase One response to haemorrhage involves significant vasoconstriction. Most of this constriction occurs in arterioles which precede capillary beds. The constriction upstream reduces capillary pressure within these vascular beds. Filtration and fluid shifts in capillary beds are governed by Starling's Forces (61). Decreased capillary pressure (Pc) results in reduced filtration (↓Jv) of blood and an increase in intravascular fluid volume as blood exits the capillary bed. This process increases blood volume and dilutes the blood. Experimental plasma volume measurements in sheep resulted in 57% restoration of lost volume at 3 hours following 15% controlled haemorrhage and 42% restoration following 45% controlled haemorrhage (62).

$$Jv = Kf([Pc - Pi] - \sigma[\pi_c - \pi_i])$$

Jv = Net fluid movement across (capillary) membrane
Kf = Filtration coefficient (water permeability of capillary)
Pc= Capillary hydrostatic pressure
Pi = Interstitial hydrostatic pressure
σ = Reflection coefficient (1= zero permeability to water)
π_c = Capillary oncotic pressure
π_i = Interstitial oncotic pressure

Equation 4 - Starling's Forces

Of course, a mainstay of medical treatment following haemorrhage is intravascular fluid resuscitation, which will rapidly both expand and dilute the blood.

2.1.4 Coagulopathy and Haemorrhage

With ongoing haemorrhage driving shock it is vital to arrest bleeding as swiftly as possible. While extremity haemorrhage can usually be controlled by direct means, incompressible haemorrhage is often impossible to stop in the pre-hospital environment. Cessation of bleeding relies on surgical control and a functioning coagulation system. However, surgery may not be available for many hours following injury and trauma is frequently associated with deranged coagulation.

Coagulopathy requiring transfusion after injury has been defined as an increase in Prothrombin (PT) and Activated partial Thromboplastin times (APTT) to greater than 1.5 times normal(63). Coagulopathy has been demonstrated in 24% of 1088 severely injured urban trauma patients (Injury Severity Score > 15) at a median time of 73 minutes following injury. 75% of these patients had suffered blunt trauma (16). Presence of coagulopathy at this early stage increased mortality 4-fold and was present only in those with increased base deficit, but independent of the amount or type of pre-hospital fluid therapy. Another survey of the German Trauma Registry (64) demonstrated similar findings from a population of 8724 severely injured (96% blunt trauma) patients. 34% demonstrated coagulopathy on admission to hospital. A third retrospective survey of civilian trauma data found an incidence on admission of 28% for abnormal PT and 8% for abnormal APTT in a cohort of 7,638 trauma patients with median ISS 9 (19). All of these studies underline the early generation of coagulopathy after injury. Its importance is clear as mortality and morbidity are both increased in the presence of early coagulopathy (16;19;65)

2.1.4.1 Proposed Mechanisms of Coagulopathy Following Trauma

Coagulopathy in trauma is multifactorial. Researchers and clinicians have attempted both to address its consequences and establish the mechanisms involved in its development following injury. The problem has been approached from several angles, focussing on: dilution; consumption of clotting factors; changes in clotting factors' environment and more complex mechanisms, which people now believe are highly relevant in the acute phase following injury. These include anticoagulation and fibrinolysis due to tissue hypoperfusion. Cosgriff and his colleagues reviewed a series of 58 massive transfusion patients with a mean ISS of 30.6 (66). 47% of 58

patients developed life-threatening coagulopathy. They identified four risk factors for coagulopathy development: pH<7.1; Temp<34°C; ISS>25 and SBP<70mmHg. Absence of any of these risk factors yielded a 1% chance of life-threatening coagulopathy development. 1 of 4 led to a 10-40% incidence of coagulopathy development and 4 of 4 risk factors produced a 98% incidence of coagulopathy. Coagulopathy is not however confined only to these variables. Some important aspects of coagulopathy are discussed below.

Dilutional Coagulopathy

Haemorrhage results in loss of whole blood. As mentioned previously (2.1), intravascular fluid becomes diluted following major haemorrhage as fluid from the interstitium shifts to the blood. The auto-resuscitated fluid now in the vessels has low protein and cellular content and therefore dilutes the blood.

The problem of dilution is exacerbated by large-volume fluid therapy, which is given to support flow of blood to the tissues in shock. Most resuscitation fluids (Crystalloids and colloid plasma substitutes), contain few or no clotting elements and packed red blood cells also contain little plasma. Aside from oxygen transport capacity, the haematocrit is important for haemostasis. Turitto demonstrated a five-fold increase in platelet adhesion as haematocrit rose from 10% to 40% (67). This effect of haematocrit is thought to be due to its influence on the transport of platelets to site of endothelial damage for adhesion.

Hiippala assessed clotting factor levels in patients undergoing major surgery, whose haemorrhage volume was replaced with packed red cells and colloid (68). Replacement of 1.4 times blood volume caused fibrinogen levels to fall below critical

values for haemostasis (1g/L). Replacement of 2-2.3 times' blood volume caused platelet count to fall below $50x10^3/mm^3$ and the activity of Prothrombin, factor V and factor VII to fall below their critical levels for haemostasis. During haemodilution, fibrinogen levels fall sooner than other coagulation factors. Large animal models of injury and blood loss have been used to assess the efficacy of fibrinogen therapy in reversing dilutional coagulopathy. Fries demonstrated that a single dose (250 $mg.kg^{-1}$) of fibrinogen could normalise clot propagation following 65% haemodilution with a colloid fluid, used to replace haemorrhage volume caused by a Grade III liver laceration (69). In a subsequent study (70) he administered fibrinogen in combination with a prothrombin complex concentrate (Beriplex) in a placebo controlled trial. Animals again underwent 65% replacement haemodilution with hydroxyethyl starch and then suffered the liver injury. Combination therapy significantly improved survival (100% vs. 20% survival) and blood loss (240ml vs.1800ml). TEG and PT both improved significantly in the treatment arm. Histology and autopsy found no sign of thromboembolic complications of this combined therapy.

Another approach has been to administer antifibrinolytic agents to reduce blood loss and compensate for dilutional compromise in fibrinogen action. The CRASH 2 randomised controlled trial (71) recently reported efficacy of Tranexamic Acid (TXA) in acute traumatic injury. 20,211 trauma patients with, or at risk of severe bleeding, were entered within 8hr from injury. Patients were enrolled in 274 hospitals and 40 countries. 10,096 patients received 1g TXA over 10 minutes followed by an infusion of another 1g over 8hr. All cause mortality within 4 weeks was significantly reduced in the TXA group vs. placebo (14.5% vs. 16%, RR 0.91(0.85-0.97)). Death from haemorrhage was also reduced (4.9% vs. 5.7%, RR 0.85 (0.76-0.96). TXA therapy

has recently been recommended for bleeding trauma patients in a Cochrane review (72).

Modern massive transfusion strategies, including those now employed by the UK and US militaries; recognise the importance of early factor replacement. Aggressive protocols recommend plasma administration at ratios of 1:1 with packed red cells. A retrospective study of 246 combat casualty patients, (median ISS=18), who underwent massive transfusion was performed, based on Joint Theatre Trauma Registry data (73). It remarkably improved mortality rates with high FFP:PRBC ratios (65% with 1:8 ratio vs. 19% with 1:1.14 ratio ($p<0.001$). Despite some difficulties with the equivalence of groups in terms of injury severity, the mortality rates remained significantly different as ratios increased from 1:8 (FFP:PRBC) to 1:1.14; even when thoracic and neuro-trauma patients had been excluded from the cohorts. While retrospective, this data nevertheless supports expert consensus that high ratio FFP and factor replacement protocols should be used in the management of haemorrhaging trauma patients. The UK military massive transfusion protocol is illustrated below (Figure 1).

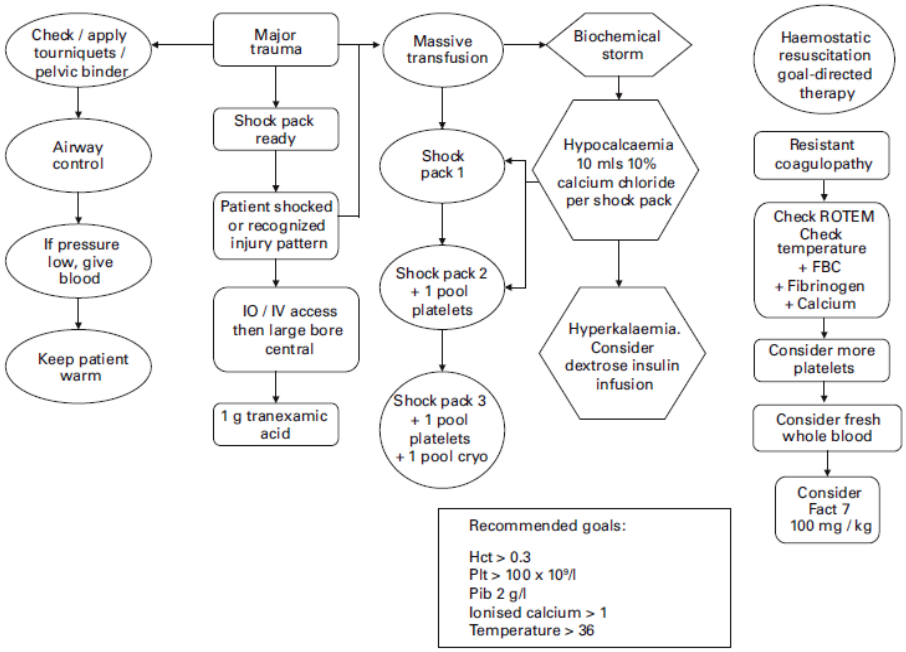


Figure 1 UK Military Massive Transfusion Protocol. Taken from Joint Doctrine Publication: Clinical Guidelines for Operations: management of massive haemorrhage on operations. Section 8, Policy 2, 2009:21-3 (JDP 4-03.1).

Consumption of Clotting Factors

Explosive injury can generate enormous tissue damage (74). Tissue injury initiates coagulation as endothelial damage exposes sub-endothelial collagen and tissue factor (TF). These bind Von Willebrand factor, platelets and activated FVII (75). The FVIIa/TF complex activates factors X (and co-factor V) to initiate the process of thrombin generation. TF also activates factor IX which then mediates the propagation phase of coagulation on the surface of activated platelets (76). Widespread activation of coagulation, at numerous sites of endothelial damage, results in consumption of circulating coagulation factors and platelets (77).

As well as inducing thrombin generation, tissue injury and ischaemia promotes fibrinolysis as endothelial damage releases tissue plasminogen activator (tPA) (78). The presence of thrombin can also act as an anticoagulant. Thrombomodulin binds thrombin both to impair its capacity for fibrinogen cleavage and to facilitate binding

with Protein C (79). Activated Protein C inactivates cofactors Va and VIIIa therefore limiting the progression of clotting (80). As widespread activation is controlled by this mechanism, inactivated cofactors build up in the local environment. Clearance of these inactivated factors is impaired and these then interfere with active factor complex assembly (81). In a situation of limited tissue injury, a balance is achieved between controlling bleeding at the site of injury and limiting widespread inappropriate activation of the coagulation system. With extensive tissue insult, however, this localisation may be lost (82), so developing a state of both excessive clot formation and hyperfibrinolysis. The net result is consumption of available coagulation factors. Despite initiating both coagulation and fibrinolysis, tissue injury alone may not be enough to generate clinical coagulopathy (83). More likely, it contributes to the problems generated by acidosis, hypothermia, dilution and the acute coagulopathy of trauma shock.

Clotting Factor Environment

Trauma and hypovolaemic shock effect major changes on the intravascular and interstitial environment. Among these changes, acidosis and hypothermia have particular relevance to coagulopathy. Cosgriff highlighted the contribution of acidosis and hypothermia to development of life-threatening coagulopathy (66). Of the four risk factors for identified in the study, acidosis was the most important and hypothermia the second most influential.

Laboratory studies by Meng et al have illustrated dynamic changes in activities of factor VIIa and the VIIa/TF complex as acidosis develops (84). At a pH of 7, activity of FVIIa falls by 90% and the activity of the VIIa/TF complex by 60%.

Martini and colleagues induced acidosis in anaesthetized swine via hydrochloric acid infusion (85). Animals were then subjected to a standardized liver laceration. Acidosis increased splenic bleeding time by 47% compared to controls. Fibrinogen concentration fell by 18%. Thrombin generation in acidotic animals was over 50% reduced compared to controls.

It is thought that excess hydrogen ion concentrations interfere with interactions between coagulation proteins and complexes and the negatively charged phospholipid activated platelet surface (86).

Hypothermia has been reported on admission to hospital in two thirds of seriously injured patients (87). Severe hypothermia in the operating theatre in patients undergoing trauma laparotomy (temp 33.8 +/- 0.5°C) resulted in a 2.4 fold increase in blood loss, compared with an injury severity-matched group, whose temperature was 36.1 +/- 0.7°C (88).

Laboratory studies have assessed the effects of hypothermia on coagulation factor, platelet and TF/VIIa complex activity (84;89). Watts studied 112 adult trauma patients with ISS>9 fewer than 2 hours post-injury (89). 64% were hypothermic and 6% were severely hypothermic with temperatures below 34°C. 34°C was found on multivariate analysis of TEG, PT and APTT to be the critical point, below which there was significant decrease in enzyme activity and platelet function. Fibrinolysis was not however affected.

Cosgriff's study, mentioned earlier in this section, demonstrated that core temperature below 34ºC increased the odds of developing life-threatening coagulopathy by nearly nine-fold (66).

In response to current understanding of the dangers of hypothermia, modern trauma systems seek to prevent hypothermia at all stages of evacuation and resuscitation and it is now a rare occurrence for a military trauma patient to experience severe hypothermia (90).

Acute Coagulopathy of Trauma Shock and Protein C Pathway

Shock has recently been described as the prime driver of coagulopathy early after injury, with severity and duration of hypoperfusion influencing the impact (91). A base-deficit >6mM suggests significant hypoperfusion and was associated with coagulopathy in 25% of admitted patients at The Royal London Trauma Centre (16). Mortality was increased 4-fold in those patients with coagulopathy on arrival. Coagulopathy in the acute stage (approximately 30 minutes post injury) was independent of injury severity score, and only occurred with hypoperfusion. There were also no changes in fibrinogen levels in any patients. This suggests that hypoperfusion is a key driver of early coagulopathy and that dilution and factor consumption are not responsible at this stage (91). The mechanisms of this early (arrival at trauma centre) coagulopathy have been attributed to activation of Thrombomodulin-Protein C pathway and fibrinolysis. Hypoperfusion after injury increases Thrombomodulin, which impairs thrombin's ability to generate fibrin and instead prompts activation of protein C. Activation of Protein C results in prolongation of PTT and PT clotting times and increased fibrinolysis with raised tPA levels (91). Increased thrombomodulin levels and reduced (un-activated) Protein C

levels were associated with increased mortality, blood transfusion requirements and organ injury rates (91). Later on in a patient's progress from time of injury, the effect of widespread activation of Protein C might be consumption of (unactivated) stores. This could lead to a depletion of Protein C pathway activity and the relative hypercoagulability that is seen hours or days after injury (91).

Blast Induced Hypercoagulability

Previous experimental work at DSTL has demonstrated a hypercoagulative state that begins 'ultra early' after primary blast wave exposure in anaesthetized swine, and which is visible on Thromboelastography (TEG) analysis (93). The first samples to demonstrate the hypercoagulability were drawn almost immediately following blast wave exposure. The hypercoagulability persisted over the next hour, during which the animals sustained 30% haemorrhage and received hypotensive normal saline resuscitation. The animals later became hypocoagulable. Brohi's paper, which demonstrated 'early' hypocoagulability, looked at early clotting dynamics in seriously injured civilian trauma patients, 75% of whom had suffered blunt injury and none of whom had sustained primary blast injury (16). The DSTL work looks at 'ultra early' (minutes from blast until 1 hour post injury) effects of trauma on clotting and demonstrates hypercoagulability after blast injury. The mechanism of this ultra-early hypercoagulability is unclear, but it is possible that blast injury exposes enormous quantities of tissue factor to circulating blood, causing un-localised activation of the coagulation process.

Inflammation and coagulopathy

It is increasingly clear that coagulation and inflammatory pathways overlap (92). Coagulation factors induce inflammation via protease receptors and complement

activation. Also, monocytes can express TF and adhere to activated platelets to promote coagulation.

Initially, haemorrhaging trauma patients tend to be hypocoagulable (16), but later, through inflammatory pathways and perhaps depletion of anticoagulant stores (91), they become hypercoagulable with increased risk of thrombotic events. A detailed examination of inflammation following haemorrhage and coagulopathy is beyond the scope of this thesis. However, some important markers of inflammation are discussed later (2.1.5) in order to provide context for results of inflammatory markers used in this study.

The diagram below (Figure 2) integrates the concepts discussed in this section and affords a modern perspective on trauma coagulopathy.

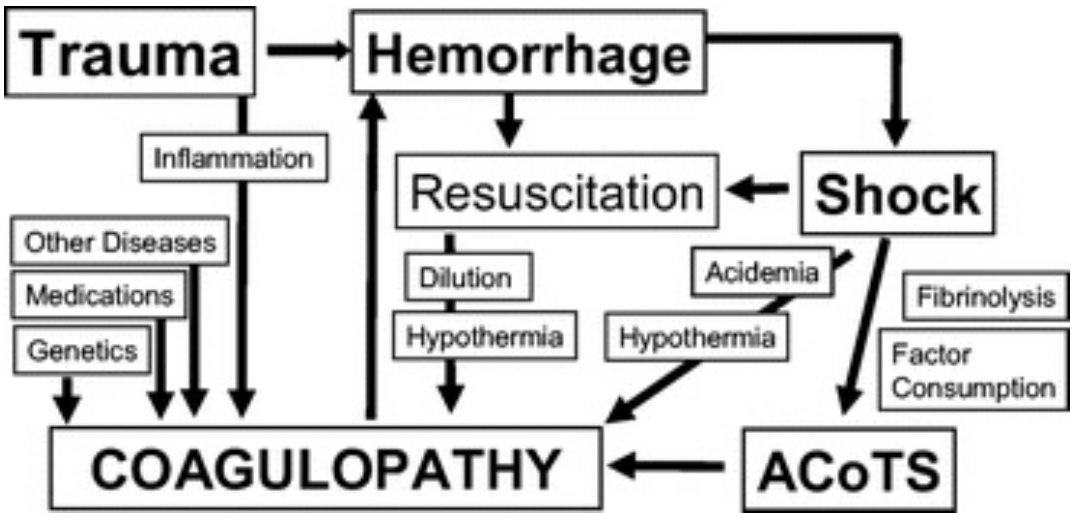


Figure 2 Hess JR et al. Mechanisms of Coagulopathy following Trauma. Reproduced from (94)

2.1.5 Markers of Inflammation following haemorrhage

The majority of deaths from trauma occur either at the scene, or within the first 24hrs of injury, but a later phase of deaths (>48hr to weeks after injury) also occurs in those who have survived the first two days following trauma (7). Systemic

inflammation is considered a key driver of conditions such as ARDS, sepsis and Multi Organ Failure (MOF): common causes of these late trauma deaths (5). The inflammatory response to injury tends to peak around days 3 to 5 and begins to subside by days 7-10 (95). An individual's progress towards either recovery or demise is influenced by: injury severity; duration of hypoperfusion; pre-injury physiological reserve; delay until adequate treatment and the presence of subsequent insults (95). Figure 3 illustrates the concept of initial shock followed by hyper-inflammation and the possibility of further organ failure over the ensuing days to weeks. What causes some patients to recover without complications, while others (similarly injured) succumb to later complications is not yet clear.

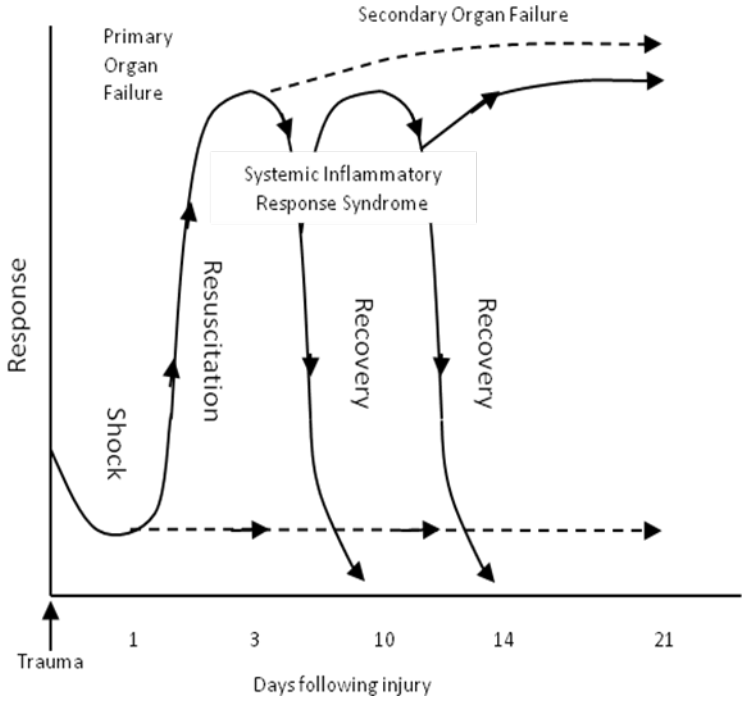


Figure 3 Patterns of Inflammatory Response following initial survival from injury. Modified from (95)

Recent work has questioned the established notion that anti-inflammatory processes sequentially follow initial hyper-inflammation predisposing to second hits and that

these hits induce further gene expression changes. Xiao and colleagues measured genome-wide expression in collected leukocytes within 12hr out to 28 days in 167 severe blunt trauma patients (mean ISS 31) who were shocked or acidotic and required blood product transfusion (96). Gene expression was compared with 37 healthy controls and analysed within the trauma group based on complicated or uncomplicated recovery. Their data illustrated rapid alteration of over 80% of the genome's expression, which was maintained up to 28 days: the authors refer to the term, 'genomic storm'. Rather than a discreet inflammatory phase, followed by anti-inflammatory expression, there was simultaneous expression of pro and anti-inflammatory genes. Further, there was no evidence of a second hit in terms of gene expression profiles. Complicated recovery resulted in longer alteration of gene expression, but neither new gene recruitment, nor dropout compared to those with uncomplicated recoveries. Strategies that manipulate the balance between inflammatory and anti-inflammatory pathways have the potential to reduce complication rates after injury.

While it is important to understand the importance of inflammation after haemorrhage and trauma, a complete review of inflammation is beyond the scope of this thesis. However, certain molecules, from among the whole range of mediators involved in inflammation, have been used as markers of inflammation in both experimental and clinical practice; some have been shown to correlate with organ failure and death. The inflammatory markers used in our study merit discussion in this section.

2.1.5.1 Inflammatory Cytokines

Cytokines are small protein molecules, released by many types of cell, that influence interactions with and behaviour of other cells. During inflammation, both pro- and

anti-inflammatory cytokines are produced. Tumour Necrosis Factor, Interleukin-1 and Interleukin-6 have been implicated in sepsis pathways and the inflammatory response (97).

Interleukins are a family of cytokines and many have been described.

Interleukin-1

IL-1 cytokines has several effects in inflammation. IL-1 receptors exist in both membrane bound and soluble forms. IL-1 is present in the epidermis of normal human skin and is vital for the maintenance of effective skin barrier against microorganisms (98). Many other immune cells can also be stimulated to produce IL-1 including: macrophages; mast cells; fibroblasts and endothelial cells.

IL-1 induces the release of other factors, including Prostaglandin E2, collagenase and nitric oxide and increases expression of adhesion molecules on endothelial cells; a function it shares with TNF (TNF can also increase neutrophil adhesion molecule expression). Alongside TNF, it has been implicated in the development of septic shock (99). TNF and IL-1 both rise following ischaemia/reperfusion injury in rat models (100). The same experiments demonstrated that a rise in these cytokines was associated with Interstitial oedema formation (101).

Interleukin-6

IL-6 functions as both a pro-inflammatory and anti-inflammatory cytokine. It is principally secreted by T-cells and macrophages, but can also be produced by exercising muscle, osteoblasts (102) and vessel wall muscle cells. It is one of the most important acute phase mediators. It can cross the blood-brain-barrier (BBB) and acts on the hypothalamus to alter the baseline temperature threshold (103).

This produces pyrexia. It also stimulates catabolism of protein and mobilisation of lipid reserves, both of which processes generate heat. As such, IL-6 is a strong driver of fever.

IL-6 levels are raised within minutes after trauma and their levels correlate with injury severity and mortality as early as six hours after injury (104). As such, IL-6 can be used as both a marker of injury extent and a prognostic tool (105).

Tumour Necrosis Factor (TNF)

Although TNFα is an important mediator of inflammation in sepsis, and can be elevated in some species after trauma, it does not appear to play a major role in porcine models of trauma (106), and for the sake of brevity will not be considered further in this thesis.

2.1.5.2 Adhesion molecules

As endothelial cells line blood vessels, effects of their damage correlate closely with organ dysfunction (107). In normal conditions the endothelium displays anticoagulant and anti-platelet surface properties. Following injury, the endothelium plays an important role in the orchestration of the inflammatory response. Activated neutrophils in the tissues are critical protagonists of the tissue damage and organ dysfunction (108;109). In order for neutrophils to migrate from the intravascular compartment to the tissues, they must interact with, and then cross, the endothelium. Cell adhesion molecules facilitate this process and they are derived from three families: the Immunoglobulin super-family; the Integrins and the Selectins (110;111). Three steps, adhesion (rolling), tethering and diapedesis have been characterised in this process of migration of neutrophils from the blood to the tissues.

Haemorrhagic shock and subsequent resuscitation represents a 'whole body' ischaemia-reperfusion injury (112). Prolonged cellular hypoxia and subsequent reperfusion produces endothelial cell swelling, loss of cytoskeletal organisation and altered membrane dynamics (113). This occurs particularly in the post-capillary venules. This damage results in: leukocyte adhesion; trans-endothelial migration; platelet and leukocyte aggregation and oxidative molecule formation. If localised this contributes to the classic features on inflammation (pain, swelling, heat, redness and loss of function). Widespread endothelial damage can lead to multiple organ dysfunction syndromes and death. Adhesion of neutrophils is a key step in the inflammatory response following ischaemia-reperfusion injury (108). Markers of activation in this pathway to inflammation after injury are relevant to studies investigating different resuscitation strategies for haemorrhage and blast injury.

Selectins

Three Selectins have been described: L-Selectin; E-Selectin and P-Selectin. All 3 are lectin-domain glycoproteins (111). Selectins are responsible for the first step in the process of neutrophil migration: 'rolling'. Rolling slows neutrophil transport along the endothelium and allows firm attachment, which is required before transmigration can occur (114). Leukocytes begin to roll along venule walls within minutes after tissue reperfusion (115) and Selectin levels remain high for at least 2 hours (116). Both in vitro and in vivo studies have examined the contributions of Selectins to leukocyte accumulation and migration (117) and they are implicated in remote tissue injury following ischaemia-reperfusion (114).

L-Selectin is expressed on the surface of most circulating neutrophils, eosinophils and monocytes (118) and shed from their surface following activation by cytokines

(119). L-Selectin mediates rolling attachment of neutrophils to cytokine-stimulated endothelium, even in the absence of neutrophil activation (111;120). Vedder demonstrated improved survival following haemorrhagic shock by treating rabbits with anti-L-Selectin antibodies (108). Schlag and colleagues employed anti-L-Selectin antibodies in primates following haemorrhagic shock and found improved mortality, haemodynamics, organ damage and reduced fluid requirements in the L-Selectin-blocked group (121).

P-Selectin is stored in basal conditions within Weibel-Palade bodies of the endothelial cytoplasm and within α granules of platelets (109;122). Within 5 minutes it is exported to the cell surface, following activation by Thrombin; Histamine and cytokines (109;111;122) It is therefore the first adhesion molecule to be expressed on the endothelial cell after trauma (109). After 3-4hr its expression can also be induced by TNF and lipopolysaccharide (122). P-Selectin mediates rolling of neutrophils, as well as facilitating platelet adhesion to activated neutrophils and monocytes (111). The importance of P-Selectin in leukocyte-endothelial adherence following haemorrhage and reperfusion has been demonstrated in several experimental studies. Scalia employed a mouse model of ischaemia-reperfusion, where P-Selectin deficient mice and those given monoclonal antibodies to P-Selectin, were compared to wild type controls (115). MAP was significantly higher following 45min ischaemia in P-Selectin-blocked subjects. Leukocyte rolling increased threefold from baseline very soon (few minutes) after reperfusion in subjects with intact P-Selectin, but remained at baseline levels in those groups with P-Selectin deficiency or monoclonal antibody. The same study also demonstrated significant reductions in neutrophils within liver, lung and intestinal tissues following

reperfusion in P-Selectin deficient subjects (115). Another small animal model demonstrated survival increase following severe haemorrhage and resuscitation, from 30% to 70% at 3 days in the P-Selectin-blocked group (109). Liver injury parameters were also decreased in the P-Selectin –blocked group. A clinical study has demonstrated the prognostic significance of raised soluble P-Selectin levels in acute lung injury (123).

E-Selectin requires synthesis within endothelial cells and therefore takes approximately 4 hours to peak following cytokine activation of endothelium (124). Like the other Selectins, E-Selectin's function is to facilitate neutrophil rolling (111).

Ramos-Kelly and colleagues used a Sialyl Lewis antagonist molecule, known to block L-Selectin, P- and E-Selectin, in an uncontrolled haemorrhagic shock model in rats (125). Treatment resulted in survival advantage at 3 days of 80% compared to 20% in untreated controls. There was also a significant reduction in liver enzymes, myeloperoxidase and histological evidence of hepatic parenchymal destruction.

Cellular Adhesion Molecules (ICAM, VCAM and PECAM)

Once the (Selectin-mediated) process of rolling along the endothelial wall has occurred, the next phase in the interactions between leukocytes and the endothelium involves firm adherence of leukocytes to the endothelium.

Leukocytes express 'binding proteins' on their surface, which enable them to adhere to activated endothelium. Neutrophils express two surface monomers: CD11 and CD18. The pair forms a complex, CD11/CD18. This complex is the ligand for ICAM-1. Both are required for leukocyte adherence to endothelial cells and subsequent

migration into tissues, so the CD11/CD18 – ICAM-1 interaction is pivotal in neutrophil mediated inflammation within tissues (108;126).

ICAM-1

ICAM-1 is a cell-surface molecule. It can be expressed on several cell surfaces, including damaged or activated endothelial cells and alveolar pneumocytes (127). ICAM-1 expression is potentiated by the pro-inflammatory cytokines(128), Tumour Necrosis Factor (TNF)-α and Interleukin (IL)-1(129) and by activated platelets (130). In addition to facilitating binding of leukocytes via the CD11/CD18 ligand, ICAM-1 expression reduces the barrier function of the endothelium and thus further enables leukocyte migration into the tissues (131). Soluble ICAM-1 can be detected in blood using PCA techniques and can therefore be used as a marker of systemic inflammation and endothelial damage. Circulating levels of ICAM-1 increase within 4 hours of haemorrhagic shock and remain raised at 24hr (132). Following haemorrhagic shock in rats, ICAM-1 was expressed in gastrointestinal smooth muscle and associated with neutrophil invasion and muscle dysfunction. Sun et al demonstrated almost immediate up-regulation of both ICAM and vascular cell adhesion molecule (VCAM) in a rat model of haemorrhage and fluid resuscitation (133). Both ICAM-1 and VCAM were elevated in the lung and spleen and associated with tissue injury. In the lung, ICAM-1 can be expressed on type-1 pneumocytes and capillary endothelium. Expression is rapidly up-regulated in Goodpasture's syndrome, a pathology which results in diffuse alveolar haemorrhage (134). ICAM-1 expression was associated with early influx of activated neutrophils (1.5hr) and later accumulation of macrophages (6hr).

ICAM-1 expression has been examined in clinical studies of trauma patients and in sepsis. A correlation exists both between the degree of trauma and ICAM-1 expression, and between initial levels of ICAM-1 and subsequent development of MOF(135). ICAM-1 levels increase following haemorrhagic shock and predict mortality at day 0 in sepsis (136).

VCAM-1

A second member of the Immunoglobulin family is VCAM-1, which is also expressed on activated endothelium. Like ICAM-1, VCAM-1 mediates the adhesion of leukocytes through interaction with Very Late Antigen-4 (an Integrin expressed on activated leukocyte surface) (111). Up-regulation of VCAM-1 is stimulated by cytokines such as TNF and IL-1. Sun's rat model of haemorrhage and resuscitation, demonstrated an almost immediate up-regulation of VCAM in spleen and lung tissue (133). As for ICAM, raised VCAM levels were associated with tissue injury. Another clinical study assessed admission adhesion molecule levels after both trauma and sepsis (137). While sepsis produced higher levels of adhesion molecules than did trauma, for both pathologies, mortality was associated with raised VCAM-1 levels.

Platelet Endothelial Cell Adhesion Molecule (PECAM-1)

PECAM-1 is the third member of the Immunoglobulin super-family involved in leukocyte-endothelial interactions. In vessels, it is highly concentrated around the junctions between endothelial cells and is a key player in the final step in leukocyte migration into the tissues (138). It is also expressed on the surface of platelets and leukocytes. After Selectins, Integrins, and their ligands, have achieved 'rolling' and 'adhesion', leukocytes need to cross into the tissues. PECAM-1 blockade with

monoclonal antibody reduces trans-endothelial migration by 70 to 90% (138). Blockade of PECAM-1 also reduces neutrophil accumulation and suggests therefore a role also in neutrophil recruitment (139). Animal studies of ischaemia-reperfusion in the myocardium have demonstrated reduced infarct size in PECAM-1-blocked subjects (139).

2.1.5.3 Summary of Inflammatory Markers

We focussed on two Integrins in our study, ICAM and VCAM. These have been shown to rise rapidly in injured ling tissue before being shed into the circulation for systemic analysis using PCR techniques. Their expression correlates with mortality in a clinical trauma study. Analysis will highlight any significant differences between groups and should therefore reflect the impact of the different adjuncts to resuscitation on post-injury inflammation.

2.2 Blast Injury

As mentioned previously (1.4), blast injuries represent an increasing threat to civilian and military populations.

2.2.1 Physics of Blast

Explosives can generate an explosion from within their own substance. They may be termed ‘high’ or ‘low’ explosives: low explosives (black gunpowder) burn, rather than detonate. High explosives, such as Tri-Nitro-Toluene (TNT), detonate very rapidly with blast wave velocities reaching 7000m/s in air. Their ability to release enormous amounts of energy over a very short time is integral to explosives’ enormous power as weapons.

On detonation, chemical bonds begin to decay in an exothermic chain, and temperature rises exponentially. While contained within the charge, this hugely increases pressure, forming a supersonic detonation wave which propagates through the explosive. A detonation wave describes a shock, supported by a trailing exothermic reaction. This wave travels through the highly combustible medium (e.g. high explosive) and is driven by the chemical energy released from the trailing exothermic process. As the detonation wave reaches the limits of the explosive it generates a **'shock wave'** in the surrounding medium (normally air), by compressing a rim of air around the sphere of explosive products. The pressure rise from the resultant shock wave lasts only a matter of milliseconds, too short a time for significant translational effects to be exerted on victims, but nevertheless capable of causing significant injury. In enclosed environments (buildings or vehicles), reflection of the shock wave results in amplification of overpressure and formation of complex blast waves, increasing the chance and severity of shock wave injuries (32;140).

The heated cloud of gaseous explosive products initially expands supersonically and remains attached to the shock wave rim of compressed air. However, as gases expand, they cool and slow down, so the sphere's velocity falls to subsonic speeds. At this point the shock wave detaches to continue its journey through the atmosphere (Figure 4). Therefore, close to the explosion, the blast wave comprises a high pressure shock wave and adjoined heated gas products. Further away, gaseous products are absent.

Figure 4 Separation of Shock Wave (black arrows) and Expanding Gaseous Sphere

The Friedlander waveform (Figure 5) offers a clear illustration of pressure changes after detonation of an explosive in a free field environment. Pressure changes are recorded at a given point over time. There is a virtually instantaneous rise in pressure; the maximum amplitude is termed **'peak overpressure'**. As the wave passes the given point, pressure rapidly decreases, falling beyond atmospheric pressure because of initial overexpansion, before recovering to baseline.

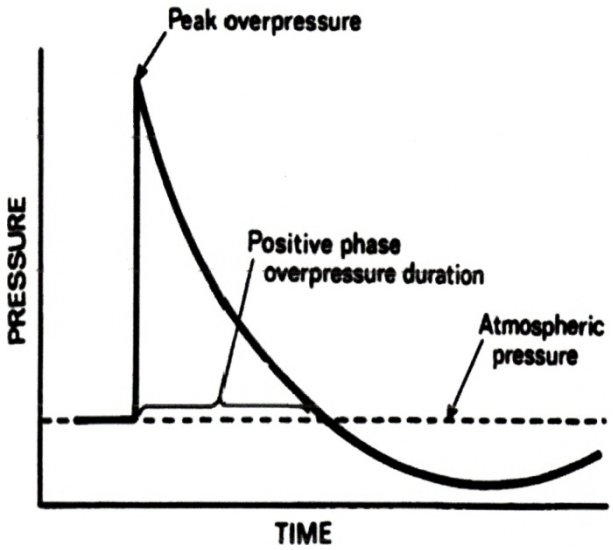


Figure 5 Friedlander's 'Ideal Blast Wave'

The peak overpressure of a shock wave declines rapidly as it propagates through the atmosphere in an inverse cube relation (distance x 2 → amplitude / 8) (Figure 6).

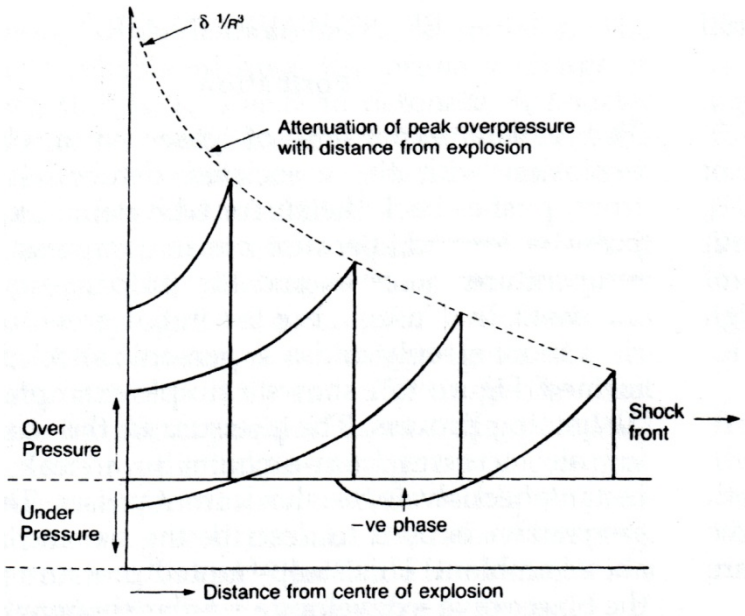


Figure 6 Attenuation of Peak Overpressure with Distance

As well as increasing air pressure, expanding gases accelerate the surrounding medium (air or water). This mass movement of air is called **'the dynamic overpressure'** or **'blast wind'**. This dynamic overpressure, a separate entity from the cloud of gaseous products, lasts long enough to produce significant translation of objects in its path.

Together, the shock wave and the dynamic overpressure are termed, **'blast wave'**. Aside from these elements, one must remember that the casings of explosive munitions, (often preformed metallic fragments), become energised by the explosion. If these collide with people, penetrating injury will result. Furthermore, exposure to the heated gases of an explosion can wound through burning and inhalational injury.

2.2.2 Classification of Blast Injuries

Zuckerman classified blast injuries into four groups (Table 1) (34). A fifth group, recently proposed by Kluger, involves a hyper-inflammatory response to blast, seen in victims of a high explosive Penta-Erythrittol -TNT suicide bombing in Israel (141).

Type of Blast Injury	Mechanism of injury
Primary	Interaction of shock wave with body
	Ear, lung, abdominal hollow viscera, (brain)
Secondary	Energised bomb fragments and debris, or environmental debris accelerated by blast wind
	Penetrating trauma
Tertiary	Translational Effects - body thrown by blast wind
	Blunt trauma from impact, or crush from building collapse
Quaternary	Other miscellaneous effects
	Psychological; burn; radiation and inhalational injury
(Quintinary)	*Hyper-inflammatory response to PE-TNT explosion*

Table 1 Zuckerman Classification of Blast Injuries (34)

Primary blast injuries occur when a shock wave interacts with the body. As the shock wave encounters materials of differing densities it is coupled into the body. Once within the body, as the wave encounters materials of differing acoustic impedance, energy is deposited. This causes injury predominantly at gas-tissue interfaces such as the lung and bowel. The brain too is increasingly believed to be affected by primary blast injury; although the mechanism is likely to be different here as there is no gas-tissue interface in the brain (142;143). Solid organs are relatively protected from blast (Mayo and Kluger 2006) and the skin is also resistant; indeed casualties with serious blast injuries may exhibit few or no visible external signs of injury (144;145).

Secondary blast injury results from fragments and other missiles, energised by the explosion, colliding with the body. Explosive munitions may have metallic casings, or house pre-formed fragments in order to maximise the fragment burden. These fragments and missiles will cause predominantly penetrating injury and, following conventional munition detonation in open areas, will be responsible for the majority of injuries from blast (30).

Tertiary blast injury occurs when the body is accelerated by the dynamic overpressure of the blast wave. Victims can be thrown against objects and limbs can be amputated or stripped by the force. In the case of lower limb amputation from blast, it is believed that the shock wave fractures the tibia and the blast wind then strips the soft tissues to complete the amputation (146).

Quaternary injuries include a miscellaneous group of mechanisms, such as burns; inhalational injury; crush and asphyxiation.

The overall trauma burden from each type of blast injury depends on the quantity and nature of the explosive; the design of the explosive device; the standoff of casualties from the blast and the environment in which detonation occurs. It is therefore difficult to predict. Table 2 gives some idea of the scale of gross damage and injury one might expect with various overpressures, assuming a 4 millisecond duration (147).

Overpressure (KPa)	Probable effect
7	Damage to standard buildings and windows break
14	Slight risk of perforation of tympanic membrane
100	50% chance of tympanic membrane rupture
275	Reinforced structures suffer significant damage
480	50% chance of marked pulmonary damage
900	50% mortality risk

Table 2 Likely effects given 4ms exposure to blast overpressure - adapted from (147)

In 1968, Bowen produced curves to estimate threshold for lung injury and lethality based on amplitude and duration of overpressure and position of a 70kg man with respect to free field blast wave propagation (148). Later, Cooper and colleagues at Dstl produced injury modelling in 16-60Kg swine with 2ms overpressure (149) and Yang and colleagues exposed sheep (15-42Kg) to blast overpressures of up to 5ms duration (150). The lethality and lung injury thresholds for these experiments are presented together in Courtney's recent article relating to traumatic brain injury (151).

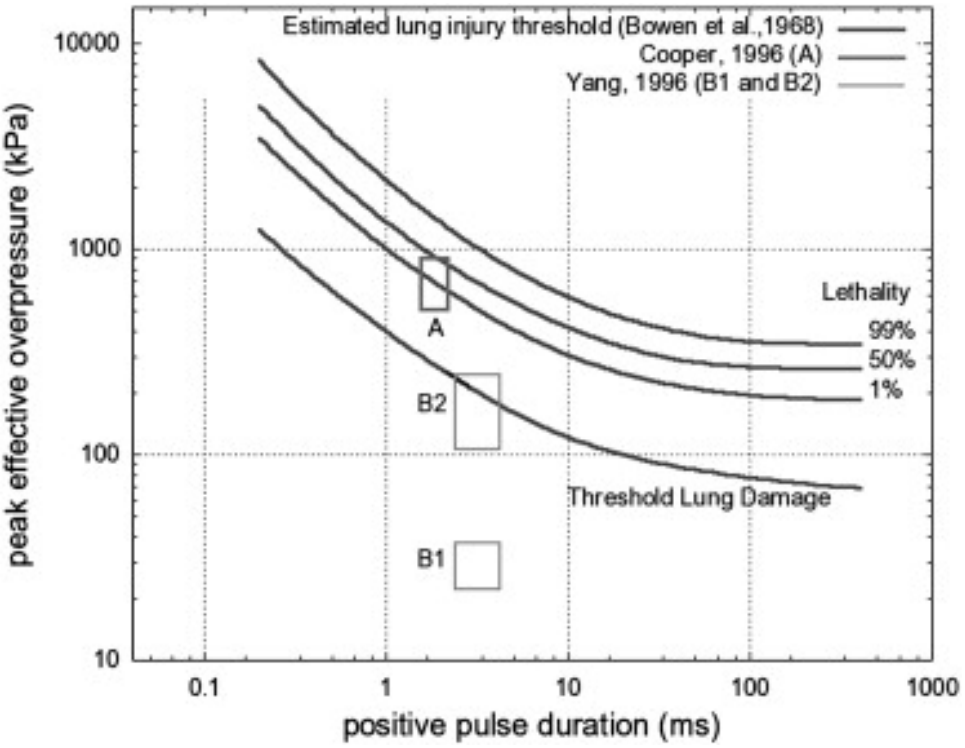


Figure 7 Lethality and Lung damage thresholds from experimental data. Bowen curves, Coopers's 2ms swine overpressure data to produce respiratory tract injury (A) and Yang's lung injury threshold (B1) and overpressure required to produce severe lung injury (B2) given 5ms overpressure in sheep. (Reprinted from (151) with permission)

There is clearly significant variation in observed lung injury threshold, even in laboratory conditions. Furthermore, other authors in small animal models have demonstrated antioxidant depletion in the lungs after single low overpressure blast (62KPa). This depletion occurs in the absence of macroscopic lung tissue damage and is accompanied by inflammation that progresses beyond 24h (152). It is therefore probable that we underestimate the true incidence of microscopic blast lung and potential vulnerability of lung tissue to secondary damage.

As mentioned previously, fragments (secondary blast injury), cause the most injuries in an open environment. The amplitude of the shock wave declines very sharply with increasing stand off from the explosion. For an unprotected individual in an open environment, secondary blast injuries are therefore likely to be more common in

survivors than primary blast injuries. Taking the example of a commonly used indirect fire munition, the 81mm mortar, these rules can be related to a clinically relevant prediction of damage. The 81 mm mortar typically contains about 1Kg of TNT. If set to ground detonation, an overpressure peak of 1300KPa will occur at a stand off of one metre. By two metres, however, this peak pressure has declined to 280KPa. The overpressure would last for 2ms. At 2 metres, the pressure and duration are below the threshold for lung damage (149). It is therefore unlikely that an 81mm mortar round will result in primary blast injury; instead it will injure through penetrating injury (secondary blast effects) from energised metallic casing fragments.

The likely burden of blast injury may, however, change. Improvements in combat body armour will increase survivability for a given fragment burden, potentially affording survival within the zone of danger for primary blast injury. Also, the deployment of enhanced blast weapons (153) is likely to increase. These carry little fragment burden, but possess the capability to produce prolonged blast overpressures over a wide radius. Civilian terrorist attacks have already exploited the influence of creating explosions in confined spaces, where reflection amplification of the shock wave increases the chance and severity of primary blast injury (140;154;155).

2.2.3 Primary Blast Injury

Blast lung injury is the most important factor with regards mortality after exposure to a blast wave (149). The term blast lung describes direct damage to the organ produced by the shock wave of an explosion: hypoxia can result from pulmonary contusions, pneumothoraces; haemothoraces; air emboli and oedema, but contusion

and pulmonary oedema are most common (34;156). Blood enters the alveoli, reducing their capacity to exchange gas. The degree of injury can range from occasional petechiae to widespread and contiguous haemorrhage of the entire lung. The amplitude and duration of the blast overpressure correlate with the extent of lung injury (157).

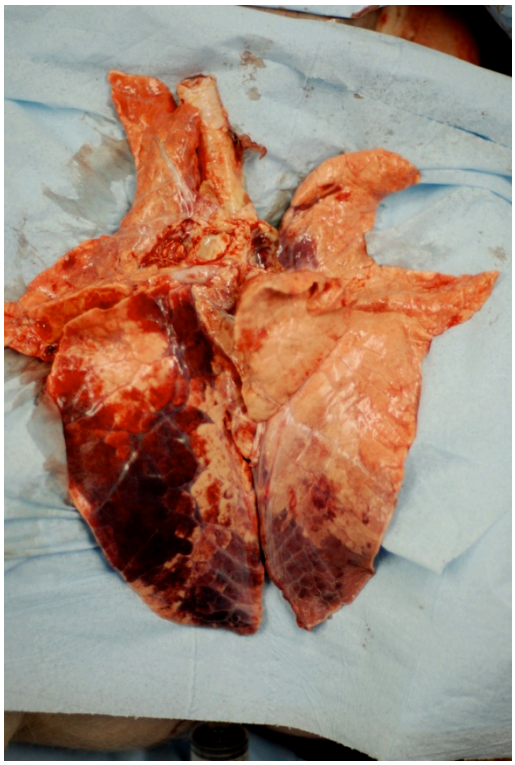


Figure 8 Pulmonary Contusion in Porcine right lung

Table 3 summarises the progress of blast lung over time. While contusions can affect both lungs, they are often confined to the lung nearer the explosion. A physiological redirection of blood to better ventilated (uninjured) regions occurs. Lung oedema follows alveolar haemorrhage and an acute inflammatory response occurs. Interstitial oedema reduces the compliance of a contused lung, increasing the work of breathing and leading to hypoxia (158). In more severe cases this can later develop into the Acute Respiratory Distress Syndrome (ARDS). Although the extent of the lung injury can propagate up to days after exposure (159), the worst of

the respiratory compromise will normally occur within the first 72 hours (158). This is a condition which can, at first, appear relatively benign, but later develop into profound respiratory failure. This not only presents a triage challenge to clinicians, but also directs research into the pathophysiology and early treatment of blast lung.

Time	Event	Clinical Features	CXR
0 (h)	Shock Wave Alveolar capillary rupture Interstitial and alveolar haemorrhage Redirection of blood flow Reduced Lung Compliance	Hypoxia *Tachypnoea* *Haemoptysis/Cough* *Pneumothorax* *Haemothorax* *Retinal air emboli*	Contusion *Pneumothorax* *Haemothorax* *Emphysema* *Rib Fracture*
3	Free Hb & blood Free radical reactions / Oxidative Stress Pro Inflammatory Response / Chemotaxis Increasing Oedema	Hypoxic resp. failure High airway pressures	Contusion Oedema
12-72	Leukocyte Accumulation Disrupted tissue architecture Increased oxidative stress Increased Inflammation Increasing Oedema	ARDS SIRS MOF	Diffuse pulmonary infiltrates
5 -10 days	Resolution	Improved gas exchange Improved compliance	Resolution of lung contusions

Table 3 Natural History of Blast Lung

2.2.4 Pathology of Blast Lung

Irwin outlines three mechanisms of lung damage that have been described due to the blast wave of an explosion: ‘spalling’; ‘implosion’ and ‘inertial damage’ (160).

Spalling occurs at tissue/air interfaces. Cavitation and turbulence ‘throws’ the denser media (lung tissue) into the less dense media (air).

Implosion occurs as the shock wave passes through gas containing organs. There is a rapid compression and decompression of the gas; instantaneous expansion of this gas causes small 'secondary explosions'.

Inertial damage describes shearing forces that occur because the pressure wave accelerates tissues with differing densities.

These effects can produce: alveolar capillary rupture; extravasation of blood and fluid into the alveoli and interstitium; pneumothorax; air embolism and even massive pulmonary haemorrhage.

Electron microscopy of blast wave-exposed rat lungs demonstrated capillary rupture; intra-alveolar haemorrhage; tearing of the inter-alveolar septa; changes to the epithelium and type II pneumocytes (156). In lung less exposed (on the far side from the focus of the blast) there were oedematous changes within the interstitium and alveoli, capillary endothelial swelling and alveolar rupture.

Aside from impairing diffusion directly, the resultant free haemoglobin within alveoli following blast wave exposure has been shown to augment the inflammatory response to blast lung through increased oxidative stress. This occurs rapidly (within a few hours) and can progress for 24h or more. The evidence for this process is discussed later (3.2.6).

2.2.5 Cardio-Respiratory Physiological Consequences of Blast

In addition to the direct effects on lung tissue, significant physiological responses occur. Hooker described a *'condition of shock which was unrelated to obvious*

trauma since no external wounds were visible' in soldiers exposed to the *'concussion of large shells'* during the First World War (144). As for the biphasic response to simple haemorrhage, the cardio-respiratory response is largely mediated by the autonomic nervous system, but other mediators such as Nitric Oxide (NO) contribute.

During the Second World War, Barrow described bradycardia and hypotension in 200 soldiers after air blast exposure (161). More recently, Irwin noted hypotension in survivors of the Oklahoma bombing in 1995, despite apparent absence of external injury (160).

Most work on the physiology of primary blast injury employs animal experiments. Hooker pioneered this work in the 1920s, when he studied muzzle blasts in dogs and noted changes to the pattern of respiration and blood pressure (144).

While blast lung is the most life-threatening problem after exposure to a blast wave, a rapid-onset triad of cardio-respiratory reflexes also occurs; characterised by **apnoea** (162-164), **bradycardia** (160;161;164;165) and **hypotension** (161;162;165). These reflexes may be immediately fatal (asystole), or contribute to the mortality from accompanying injuries victims of an explosion are likely to have suffered.

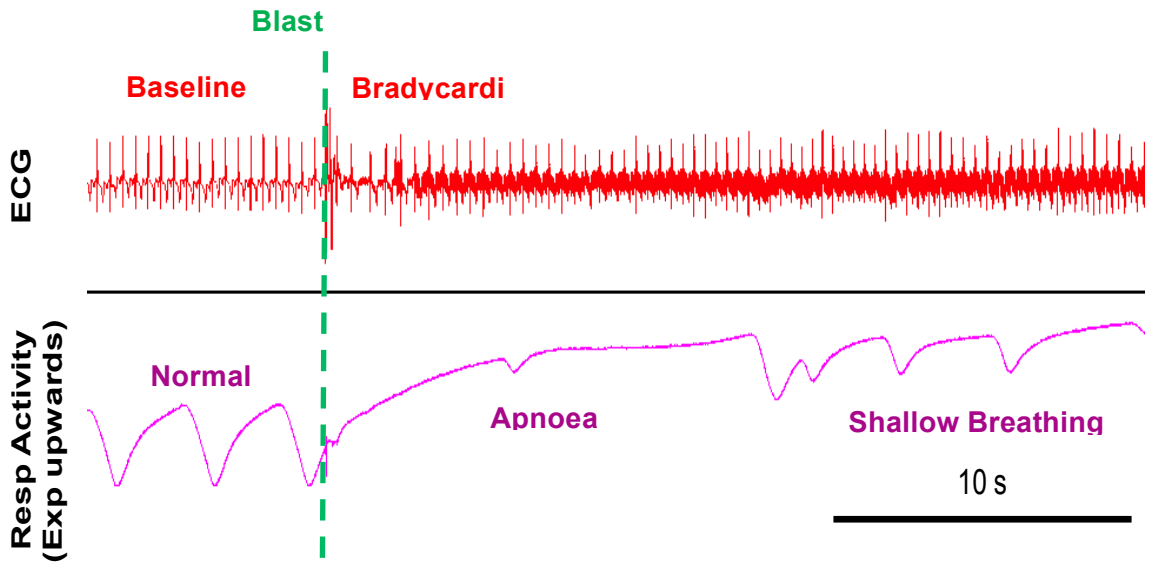


Figure 9 Blast pad traces of ECG and respiratory pattern from a typical animal. Green dotted line represents moment of blast exposure. Respiratory pattern detected via thermocouple device attached to endotracheal tube. Increased temperature of exhaled breath produces upwards deflection in trace pattern. 10s scale line drawn to illustrate duration of apnoea and bradycardia.

Vagal afferent and efferent activity has been implicated in these reflexes (160;162). Ohnishi exposed rats to primary thoracic blast, or sham blast and recorded breathing, heart rate and blood pressure immediately after exposure (166). Animals exposed to blast demonstrated almost immediate apnoea, bradycardia and hypotension. If animals received pre-blast vagotomy, the apnoea and bradycardia were removed, but hypotension only partially ameliorated. Atropine pre-treatment attenuated the bradycardia response, but had no effect on the apnoea or hypotension. These results suggest the Vagus nerve is responsible for the bradycardia and apnoea; and for part of the hypotensive response. The authors note that this reflex triad is similar to that produced by stimulation of pulmonary afferent C-fibres (J receptors) which travel within the Vagus nerve (167;168). Ohnishi explains the partial response of the hypotension to vagotomy by postulating an impairment of the baroreceptor reflex by blast; which would normally produce a sympathetically-mediated increase in vascular tone and a tachycardia (166).

2.2.6 Interactions between Injury Mechanisms

2.2.6.1 Blast and Haemorrhage

Sawdon linked knowledge of haemorrhage physiology to the reflex responses associated with a primary blast injury in a study employing anaesthetized rats. Groups received a blast, or sham blast thoracic insult. They were then subjected to haemorrhage (169). Blast exposure reduced the compensatory tachycardic response to haemorrhage and enhanced the bradycardic hypotensive phase.

The implications of this effect can be related to oxygen delivery. The PaO_2 of blast-exposed subjects is reduced for a prolonged period, despite swift return to normal respiratory patterns within 5 min (166). Hypoxia persists, with or without hypercarbia, because gas transport across the alveolar tissue-blood barrier is impaired in blast lung. At the same time, the haemorrhagic sequellae of blast injury (secondary and tertiary blast injury) reduce cardiac output and so compromise the flow component of the oxygen delivery equation.

In the clinical setting this means that casualties exposed to the combined insults of blast wave exposure and major haemorrhage, have sustained a ‘double hit’ to their capacity to deliver oxygen to their tissues.

2.2.6.2 Tissue Injury and Haemorrhage

Simple haemorrhage, uncomplicated by pain or tissue damage, is uncommon in trauma. Certainly, trauma victims suffer a degree of tissue damage and the effect of an afferent nociceptive storm could also influence the response patterns to haemorrhage.

Pain and tissue injury can produce a variety of stimuli that alter one's response to haemorrhage. Little and colleagues showed that musculoskeletal injury produces a reflex tachycardia and hypertension (170) and his rat model of haemorrhage and tissue injury (54), showed that hind limb ischaemia reduced the depressor response to haemorrhage, delaying hypotension and limiting the bradycardia. Opioid receptors have also been examined for their impact on response to haemorrhage. Ohnishi's rat haemorrhage model compared groups pre-treated with morphine (into a cerebral ventricle) against controls and those given Naloxone (an opioid receptor antagonist). Rats treated with morphine demonstrated a delayed onset of hypotension and no bradycardic response. Those animals given Naloxone responded in the same fashion as the control groups (171). A subsequent study by the same group employed intravenous morphine with similar findings. They also administered morphine at a late stage in the haemorrhage, by which time bradycardia and hypotension had become established. Again, morphine removed the bradycardia and limited the hypotension (172).

While it may be thought that this would improve tissue perfusion and reduce the degree of 'shock' suffered by the trauma victim, this reflex has been shown by Overman and colleagues to decrease survival in dogs after haemorrhage (173). Perhaps the bradycardic response is protective? With low venous return and a very high heart rate, stroke volume will be very small and myocardial perfusion poor. If the heart rate were to reduce, this would allow more time for ventricular filling and myocardial perfusion.

2.2.6.3 Haemorrhage, Blast and Musculoskeletal Injury

Military trauma has the potential to inflict all three types of insult simultaneously. All three insults can modify the potential requirements for resuscitation and the response to therapy. The net result of these responses is not known. By incorporating all three insults, this study will produce a relevant model of severe, but not immediately overwhelming, military wounding, against which background adjuncts to fluid resuscitation can be assessed.

3 Current and Potential Interventions

3.1 Intravascular Fluid Therapy

The study employs only a simple, widely available crystalloid (0.9% Saline) for resuscitation. A detailed discussion surrounding fluid therapy is not therefore relevant. However, a brief summary will add context to the problem we are trying to address.

In 1977 Baker performed an epidemiological autopsy study into trauma deaths in San Francisco (5). This highlighted both the significant burden of trauma and the need for improved trauma care. In reviewing Baker's data, Trunkey described a tri-modal distribution of death after trauma: 'immediate deaths' - within 2hrs of injury (≈45% of deaths); 'early hospital deaths' - within 4hrs of injury (≈ 34%) and 'late' - days to weeks after injury (≈20%) (7). This produced 'windows of opportunity' for clinicians to prevent trauma deaths after injury and, from this, the concept of 'The Golden Hour' arose. Since the early 1980s, a systems approach for delivering trauma care has been implemented in several States within the US and they have been shown to improve outcome after trauma (174-177). While modern data has questioned whether this pattern is still relevant, the concept is still widely taught in ATLS courses worldwide (6;178). A mainstay of the prehospital intervention has been fluid resuscitation. This aims to augment the intravascular volume which has become depleted as a result of traumatic haemorrhage, thus preserving preload and maintaining cardiac output. Of course, the oxygen content of the blood will reduce as haemoglobin-rich blood is replaced with fluid, but the net effect is still to increase DO_2 to tissues.

Fluid therapy strategies have changed over time as our understanding of resuscitation and trauma physiology improves and as we see the potential side effects of accepted doctrine (179). To this day clinicians, both civilian and military, debate the ideal fluid type and administration regimes for post-trauma resuscitation. While most accept that whole blood is an ideal resuscitation fluid, practically this is not available to most pre-hospital casualties.

3.1.1 Blood Pressure Resuscitation Target

As with the type of fluid used, there is ongoing debate regarding the ideal blood pressure target that should be employed, to guide the rate and volume of fluid to be given. There are two well established concepts for prehospital fluid resuscitation: the Advanced Trauma Life Support system (178), which advocates a normotensive resuscitation target and the permissive hypotensive target, preferred by the National Institute for Health and Clinical Excellence (NICE). Each has advantages and potential limitations.

3.1.1.1 Normotensive Resuscitation

The normotensive target dictates that baseline blood pressure, or Mean Arterial Pressure (MAP) be restored as swiftly as possible. It is an aggressive concept and includes an initial one-to-two litre bolus of fluid for the clinically shocked adult trauma patient. Advanced Trauma Life Support (ATLS) is a course designed to provide a safe and logical framework for doctors to employ when managing casualties. It has now been taught to over 1 million doctors worldwide and is a compulsory qualification for completion of surgical training in the UK. Brief note only is made of the concept of delaying aggressive fluid resuscitation until surgical haemorrhage control has been achieved (178). By rapidly restoring the intravascular volume, the preload will increase and this will increase cardiac output. The remaining

haemoglobin (and its bound oxygen) will therefore be delivered more rapidly to the tissues. There are concerns, though, that this rapid volume expansion will place a large hydrostatic pressure on any clots that have formed at sites of vessel damage; the risk is bursting of the clot and rebleeding. In the prehospital environment and in the presence of incompressible haemorrhage, rebleeding could precipitate exsanguination.

3.1.1.2 Hypotensive Resuscitation

The permissive hypotensive approach accepts temporary hypoperfusion in order to allow a strong clot to form at sites of vessel injury. The aim is to support perfusion of critical organs just enough to allow temporary survival, while not disrupting any clots that have managed to form. Landmark evidence supporting this concept is Bickell's prospective clinical trial in an urban major trauma centre of immediate versus delayed fluid resuscitation for shocked (SBP<90mmHg) penetrating adult (>16yrs) torso trauma (36). Patients were randomised to receive either no fluid therapy from point of wounding until they entered the operating room, or they received standard prehospital fluid therapy. Randomisation was based on even vs. odd days of the month. Fluid therapy comprised continuous high flow infusion of Ringer's Lactate with transfusion of red cells as indicated. Other than fluid therapy, treatment pre-surgery was identical.

The study enrolled 598 adult trauma patients. 309 received prehospital fluid therapy, 289 received no pre-surgical fluid therapy. Demographics were similar between groups, except the interval to surgery was marginally longer in the delayed group (134±101 vs. 114±105 min). Overall survival was improved in the delayed fluid therapy group: 70% vs. 62%. There was a trend towards greater surgical

haemorrhage in the immediate treatment group. Immediate treatment resulted in longer hospital stay. There was a trend towards more complications in the immediate fluid therapy group.

This study shaped modern prehospital trauma doctrine and it's findings were influential when NICE developed their prehospital guidelines (180). Hypotensive-target fluid therapy was also, until recently, recommended by the Battlefield Advanced Trauma Life Support (BATLS) doctrine (10).

There are important differences between those patients enrolled in Bickell's study and the modern combat trauma patient. First, the delay to first treatment of any kind is extremely short (8±6min) with interval to arrival in the trauma centre averaging 44 and 52 min. Surgery interval was around the two-hour mark. While in mature theatres of operation, evacuation times to surgical care might match this; military evacuation is unpredictable and, as mentioned before, can be very prolonged. If we are to maintain a casualty in a pre-surgical environment for several hours, will this hypoperfusion cause irreversible metabolic dysfunction? Furthermore, no casualty in Bickell's series had been exposed to a primary blast injury (although Bickell describes a 'shotgun-blast' mechanism in 3 and 6%, this does not equate to the shock wave of an explosion interacting with the body. Add the problem of blast injury, with its impaired oxygenation and myocardial depression, to prolonged reduction in oxygen delivery from delayed fluid therapy or hypotensive strategies and there will be potentially severe consequences. Third, despite a mean ISS of 26±14, admission arterial gases demonstrated mean pH 7.29. While this is clearly acidotic, the degree of acidosis is not reflective of the seriously injured casualties arriving in

military surgical facilities after injury from explosive munitions. Perhaps the casualties in Bickell's study are less severely injured and therefore more tolerant of a delay in restoring oxygen delivery?

Balancing the risk of 'blowing off nascent clots' against the penalties of prolonged hypotension forms a fulcrum for the debate regarding fluid therapy strategies in trauma. From the military perspective, we must be prepared to maintain casualties for prolonged periods without surgical haemorrhage control and cannot ignore the problem of blast injury that is present in a significant number of our injured.

3.1.1.3 Work at the Defence Science and Technology Laboratories Porton Down

The Defence Science and Technology Laboratories (DSTL) at Porton Down have been investigating the implications of these strategies in the context of prolonged resuscitation after blast injury and haemorrhage.

3.1.1.4 Blast and Haemorrhage Programme

Previous work at DSTL has examined the efficacy of prolonged permissive hypotensive resuscitation (37;181) to address two areas of concern for military casualties, namely extended evacuation timelines and concomitant blast injury. Terminally anaesthetized swine were subjected to blast loading and 30% controlled haemorrhage. These animals were compared to a similar group who received sham (no) blast, but the same haemorrhagic insult. Fluid therapy (normal saline) was targeted to either the hypotensive systolic target of 80mmHg (control group) or to a normotensive target (systolic arterial pressure of 110 mmHg, the original ATLS strategy). The purpose of this study was to compare the physiological problems

associated with prolonged hypotensive resuscitation with those associated with the aggressive fluid administration necessary to attain a normotensive target.

In the presence of a combined blast and haemorrhage insult, prolonged hypotensive resuscitation was not compatible with life (Figure 10). Survival was initially good over the first hour of resuscitation, but thereafter fell sharply resulting in approximately 50% mortality after two hours of resuscitation and 100% mortality after 4 hours of resuscitation.

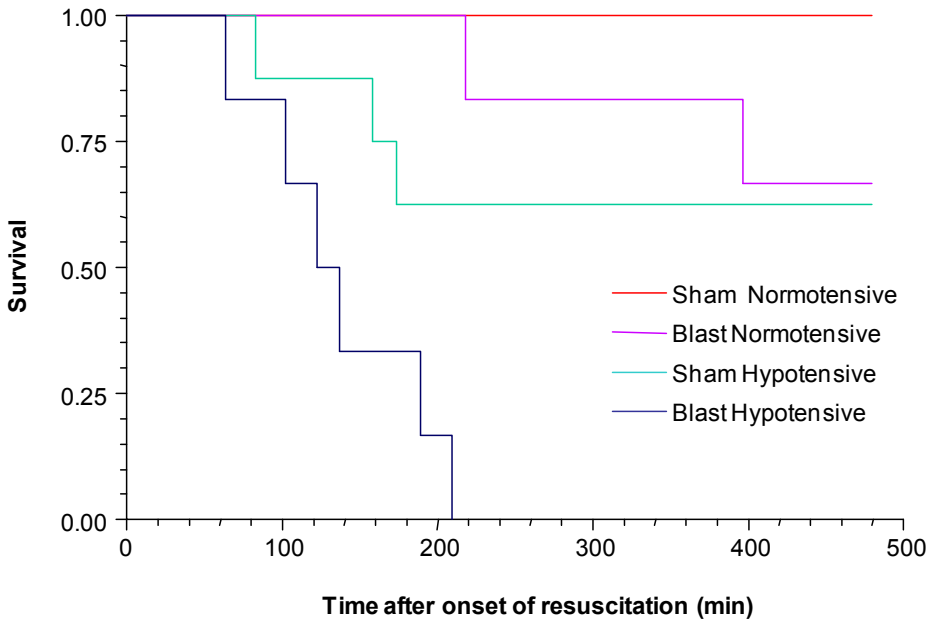


Figure 10 Kaplan Meier survival plot for four groups of animals. Two groups received blast exposure; sham animals received no blast. Two groups were resuscitated (normal saline for all groups) to a normotensive target of 110mmHg; the other two were resuscitated to a hypotensive target of 80mmHg.

Although survival was good with hypotensive resuscitation after haemorrhage in the absence of blast, significant physiological penalties were seen.

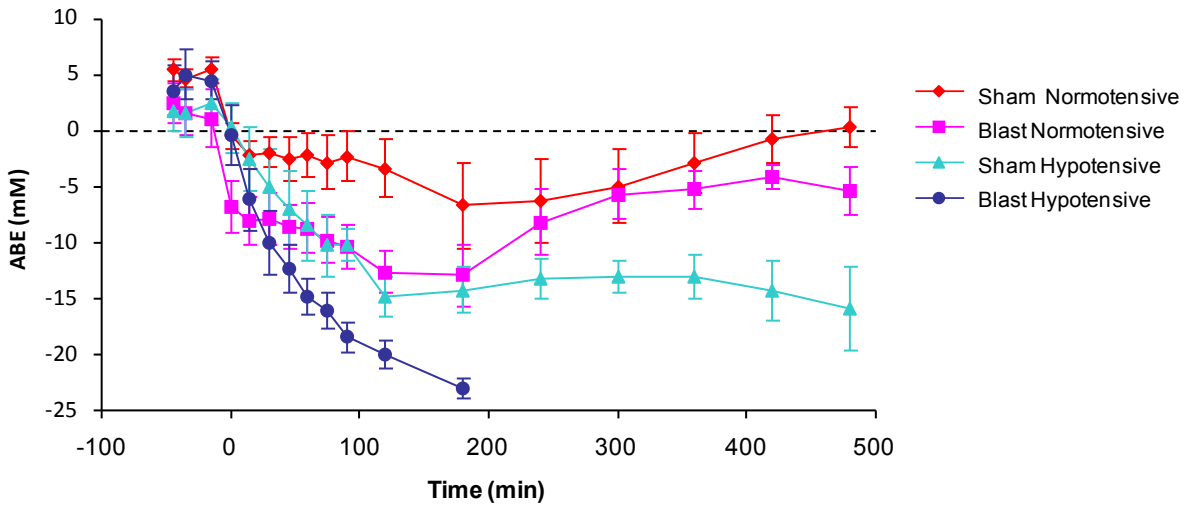


Figure 11 Actual Base Excess plots for four groups of animals. See legend to Figure 10 for explanation of groups.

The underlying problem with prolonged hypotensive resuscitation was found to be inadequate tissue oxygen delivery, due to the low blood flow state during hypotension. Tissue oxygen delivery became grossly inadequate when there was concomitant blast injury, which reduced arterial oxygen content and therefore compounded the problem of low tissue blood flow (37;181).

The study did not however allow for the potential re-bleeding that is a feared consequence of normotensive resuscitation. It was therefore necessary to develop strategies that could improve the delivery of oxygen to tissues, without increasing the risk of rebleeding. Implicit in this objective was the need to alter the animal model to allow for rebleeding.

3.1.1.5 Novel Hybrid Programme

The next project involved development of a capacity for rebleeding while continuing the theme of blast/sham blast exposure and controlled haemorrhage.

A liver snare was developed that could be implanted at time of preparatory surgery and later deployed to produce a Grade IV liver injury (39) (Figure 22). This is both an arterial and venous injury with the capacity to re-bleed at any stage after initial clot formation.

A novel fluid resuscitation strategy was designed (Novel hybrid – NH), which assimilated the best of both the hypotensive and normotensive resuscitation philosophies. For the first hour of fluid therapy, the resuscitation target was the hypotensive 80mmHg. Thereafter, however, the target increased to the normotensive 110mmHg. Normal Saline was used throughout. The first hour's low pressure target allowed nascent clot formation without exerting undue hydrostatic pressure across sites of vessel injury. By one hour, clot integrity has reached 80% of maximum (182) and it was felt that improving flow (and hence oxygen delivery) was now more important than protecting the clot.

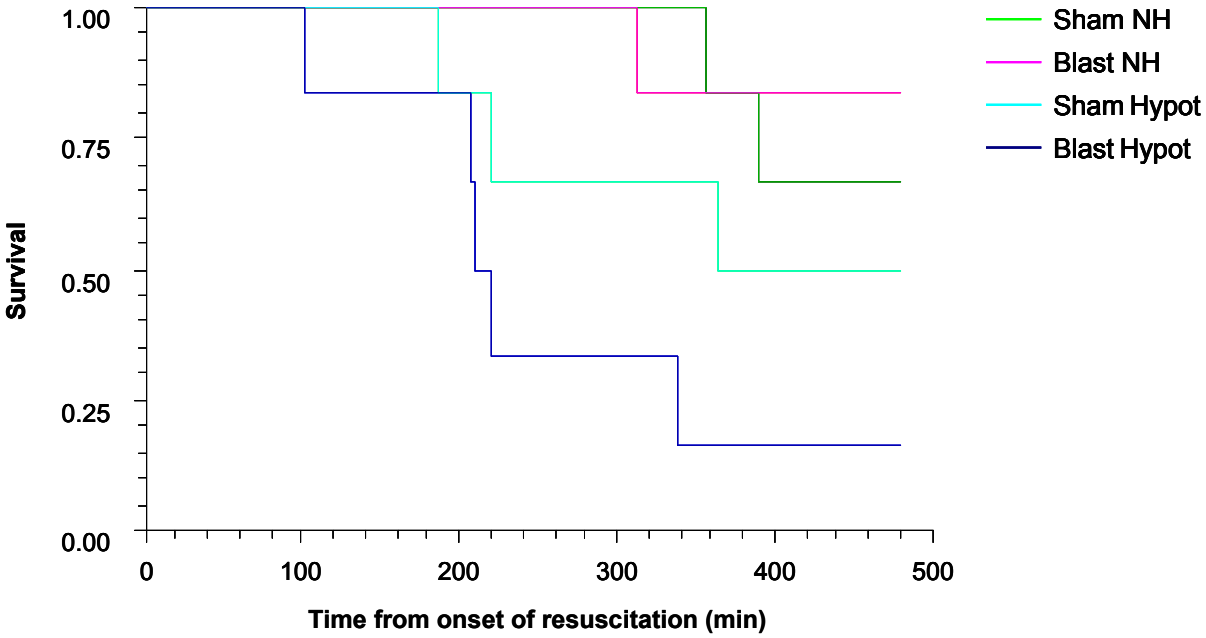


Figure 12 Novel Hybrid study survival plot for four groups of animals. Blast groups received primary blast injury, the 'sham' group received blast wave exposure. All animals received total 35% blood volume controlled haemorrhage and grade IV liver injury. NH animals received hypotensive fluid therapy for the first hour and

then normotensive fluid resuscitation (SBP 110mmHg) for the remainder of the experiment. Hypotensive animals received fluid therapy to the hypotensive (SBP 80mmHg) target throughout.

The novel hybrid strategy proved effective and safe (Figure 12). Animals survived to the end of the experiments and demonstrated physiological recovery – evidence that oxygen delivery had been restored enough to repay an acquired oxygen debt (Figure 13). Importantly there was no evidence of increased rebleeding compared to hypotensive controls (39).

Figure 13 Base Deficit and Oxygen extraction ratio plots for the same four groups of animals. First dotted line represent onset of fluid resuscitation. Second dotted line represents T60, when NH animals began to receive normotensive fluid therapy.

While NH proved effective and no increase in rebleeding was identified in the study, there are other approaches to augmentation of oxygen delivery, which exert no increased pressure across blood clots: two such adjuncts were selected for this study.

3.2 Supplemental Inspired Oxygen

Fluid resuscitation attempts to address the impaired flow component (cardiac output) of the oxygen delivery equation after haemorrhage (Equation 1**)**

As mentioned before, the NH strategy is effective in the prolonged prehospital setting after haemorrhage, and in the presence of primary blast injury, where lung injury produces hypoxia (39). It is recommended as the default prehospital fluid resuscitation strategy for UK military casualties (10). Although NH did not cause increased bleeding in the animal model, there may be clinical situations where the nature of the injuries suggests an unacceptably high risk of re-bleeding were arterial pressure to be elevated to normal levels. A potential alternative strategy in this

circumstance would be to increase arterial oxygen content. The simplest means of achieving this would be to increase inspired oxygen, although this would only have a large effect on oxygen content if pre-treatment arterial oxygen saturation was significantly depressed while breathing air.

In the civilian prehospital arena, oxygen therapy is ubiquitous. However, oxygen support capability is not routinely available in the far-forward military environment.

How does military trauma differ from civilian trauma and where is the role for supplemental oxygen? A recent epidemiological study has supported the widely held consensus that civilian trauma is predominantly blunt in nature (183). A recent prospective study into blunt thoracic injury reported a 6.3% incidence of significant chest injury in 2 urban trauma centres (184). 31 of 492 consecutive patients had significant thoracic injury (including any one or more of: aortic injury, two or more rib fractures; sternal fracture; pulmonary contusion; haemothorax or pneumothorax). Chest pain and hypoxia on admission to the Emergency Department were the two best diagnostic indicators.

In contrast to the predominance of blunt injury in civilian trauma, Schreiber's study confirmed that military trauma is predominantly penetrating, with 80% of combat-injured casualties suffering penetrating injury (183). In addition to penetrating (missile or fragment) injury, the effects of primary blast must also be considered. While it is difficult to estimate the true incidence of blast lung, we know that 78% of combat casualties between 2005 and 2006 in Afghanistan and Iraq have been injured in an explosive event (185). A recent UK paper has demonstrated an 11%

incidence of blast lung in casualties surviving to reach the field hospital in Afghanistan (186). However, another case series from Iraq, reported a lower incidence of primary blast injury (3.7%) in UK casualties (187). The Joint Theater Trauma Registry (JTTR) – the database of the US Department of Defence – has yielded a 4.6% incidence of blast lung in soldiers wounded between 2005 and 2006 (188). So how worried should we be about primary blast? It is important to note that, for both Ramasamay's series and the US JTTR report, Iraqi insurgents were deploying a very specific form of IED, the explosively formed projectile (EFP). EFPs contain a relatively small, shaped explosive charge and function by creating a supersonic molten slug of copper which is designed to defeat vehicle armour. The blast overpressure transmitted into the vehicle is much smaller than the overpressure adjacent to the vehicle. Champion's paper cites test data addressing this concept (188). A bare 17Kg C-4 explosive charge was detonated 3m from a vehicle. Pressure readings were gathered both within and outside the vehicle. The peak overpressure within the vehicle was 28 times lower than the peak pressure adjacent (outside) to the vehicle. Inside the vehicle, a peak pressure recorded of approximately 6PSI would be expected to produce tympanic membrane rupture, but lies well below the threshold for blast lung injury. Those outside the vehicle would in contrast suffer significant or fatal blast exposure. Injury patterns from EFPs followed a bimodal distribution: those hit by the main slug sustained catastrophic, normally unsurvivable, injuries; while those adjacent to the slug's path sustained minor fragmentation injuries from spalling within the vehicle (personal experience from Op TELIC 10).

We know that, following open-air blast, blast overpressure declines by the inverse cube law with distance from the explosion (e.g. a doubling of the distance reduces peak overpressure to one eighth) (2.2.1). In previous conflicts, those close enough to a blast epicentre to suffer primary blast injury have normally succumbed to the devastating fragment load at that range (189). A recent review of over 1000 large animal (<30ms) blast injury experiments supports this concept (190). The authors concluded that blast lung would not occur in an open-air environment when the subject was over 20m from the epicentre of a 100Kg TNT explosion. 20m however, would lie within the lethal fragment zone of a real-world explosive device detonation. The lethal fragment zone of a 155mm (100Kg) shell is 24m and significant injury from fragments remains a risk out to well beyond 100m (188). Another laboratory study demonstrated that 17-24 PSI overpressures would be required to produce blast lung. Again this would equate to a distance from the explosion of 10-20m (191).

In Afghanistan, the current operation in which UK forces are deployed in large numbers, several conventional high explosive munitions are being piled together to form IEDs with a huge total quantity of explosive charge. The geography and military taskings within the country mandates a high proportion of dismounted operations; rendering troops more vulnerable to primary blast effects. Recent UK data confirms that casualties with significant primary blast lung are reaching the Field Hospital (186).

It seems therefore that the Ramasamay and Champion's figures may not represent the true risk in the current Afghanistan conflict. EFPs do not feature as the IED design of choice; the quantities of explosive charge used by insurgents in

Afghanistan are larger and dismounted operations are more common in Afghanistan than Iraq; removing the protective effect of the vehicle hull in reducing the overpressure of blast. Other potential reasons for the increased incidence of primary blast injury being reported from Helmand Province are the improvements in issued body armour; prehospital haemorrhage control and resuscitation and shortened evacuation times to hospital. All these potentially increase the survivability of exposure to significant fragment burden and potentially enable survival from fragmentation within the zone of primary blast effect of an explosive detonation.

If a potential benefit from supplying oxygen to far forward, highly mobile military medical assets is acknowledged, then the delivery of such a capability would incur a logistic expense. Many severely wounded military casualties will not derive significant benefit from an increase in inspired oxygen concentration: without thoracic trauma, their arterial oxygen saturation (SaO_2) will be over 95%. The potential increase in DO_2 from supplementing oxygen is therefore very limited and one must not ignore the issue that high concentrations of oxygen might produce unwanted sequellae (3.2.6.1).

A complete military prehospital resuscitation strategy must look ahead to future threats. Enhanced blast weapons have been discussed previously (1.4), as have the effects of closed-space explosions on blast wave amplitude and duration. These weapons could significantly increase the incidence of severe primary blast injury in otherwise survivable casualties. Hypoxia must not therefore be ignored.

3.2.1 Oxygen Transport

The lungs comprise conducting airways and a respiratory zone. The conducting airways do not play a part in gas exchange. The respiratory zone contains alveoli, the functional units of gas exchange. Each alveolus has a diameter of approximately 200 micrometers (μm) and the total gas exchange surface area is approximately 70m^2. The interface layer between the alveolus and capillary is, in normal circumstances, extremely thin, optimizing diffusion. The internal surface of the alveolus is thinly coated with surfactant, which reduces surface tension and the tendency towards atelectasis.

3.2.2 Gas Exchange in the Lung

Diffusion of gases is governed by Fick's law (Equation 5), which states that the diffusion rate of a gas through a tissue is proportional to tissue area and the difference in partial pressure of gas on either side of the tissue, and inversely proportional to the thickness of the tissue through which diffusion must occur.

$$Vgas \propto \frac{A}{T} \times D \times (P1 - P2)$$

Equation 5 Fick's Law of Diffusion. Vgas = Volume of gas per unit time which diffuses across a membrane. A = Surface Area for Diffusion. D = Diffusion Constant or permeability coefficient of the gas: D α Solubility/√Molecular Weight. P_1-P_2 = Difference in partial pressure of gas on either side of tissue. T = Thickness of tissue sheet across which diffusion must occur.

The healthy lung, with huge surface area and extremely thin diffusion distance, is optimised to allow efficient transfer of gases: in normal conditions, a single erythrocyte will complete its journey through the gas exchange zone of a lung capillary in approximately 0.75s, but PO2 in blood will equilibrate with alveolar partial pressure by 1/3 of its journey through the capillary (Figure 14). There is a large functional reserve for diffusion. The amount of oxygen taken up by the blood therefore depends on the flow and exchange is described as 'perfusion limited'.

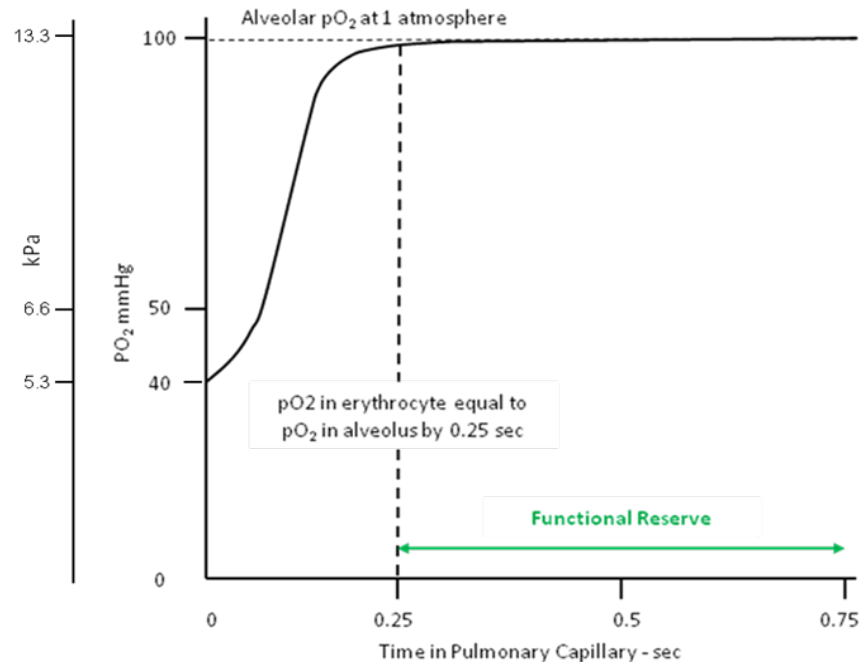


Figure 14 Oxygen diffusion in capillary

During exertion, flow increases and an erythrocyte may spend less time in the capillary, but there is normally still sufficient time for equilibration to occur. However, any pathology that increases the thickness of this barrier will slow diffusion. If a difference between alveolar PO2 and end-capillary PO2 exists, oxygen exchange will have become 'diffusion limited'. Blast lung is characterized by interstitial haemorrhage and oedema, both of which significantly increase the diffusion distance. At the same time, haemorrhage and hypotensive resuscitation reduce the flow. Given the common finding of hypoxia, it is likely that diffusion limitation exists in the context of blast lung.

3.2.3 Haemoglobin and Gas Transport

Oxygen can be carried in the blood either dissolved in solution, or bound to haemoglobin. The amount of dissolved oxygen is proportional to the tension of oxygen in the plasma, which is in turn influenced by the alveolar oxygen tension. For

every mmHg O2 (0.13KPa) in alveolar gas, the blood will contain 0.003ml O2/100ml blood. At sea level, the alveolar gas oxygen tension (PAO2) is 13.3KPa. This equates to a dissolved content of 3ml O2/L of blood: assuming a cardiac output of 5L/min and dissolved oxygen will deliver only 15ml O2/min to the tissues. The metabolic demand for oxygen far exceeds this value. A more potent vehicle for oxygen carriage is therefore required; this is delivered by haemoglobin; the principal oxygen carrying protein.

The oxygen dissociation curve demonstrates the saturation of Hb with O2 for a given oxygen tension (Figure 15). There is rapid binding of oxygen up to a PO_2 of approximately 6.6kPa; thereafter the curve flattens. The broad plateau means that a moderate drop in alveolar pO2 (down to 8.7kPa for example) will have little effect on either the Hb saturation or the oxygen concentration in blood. The steep lower part of the curve facilitates unloading of oxygen into the tissues where lower oxygen tension exists.

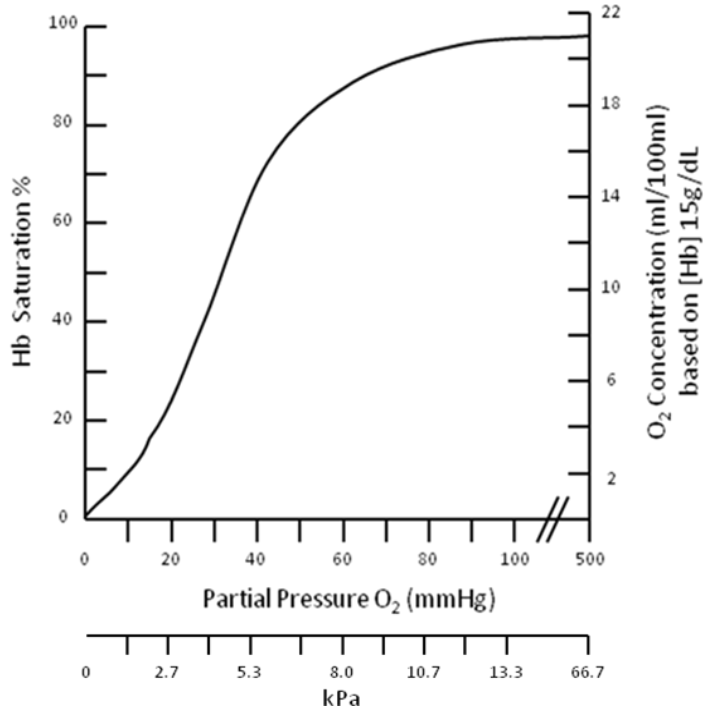


Figure 15 Oxygen dissociation curve

The normal P50 of Hb is 26.6mmHg. The curve can be shifted by changes in the erythrocytes' environment. An increase in [H+], PCO_2 or temperature will shift the curve to the right. This was discovered by Bohr through laboratory experiments on fresh dog blood and his curves are illustrated below (192) (Figure 16).

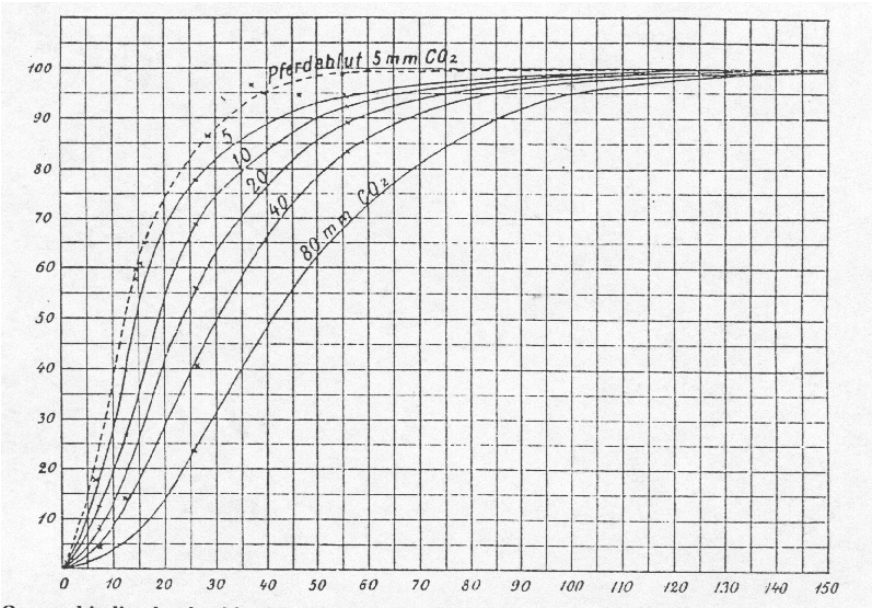


Figure 16 Bohr's dissociation curves at various CO2 levels in dog blood

2,3-disphosphoglycerate (2,3-DPG) a product of erythrocyte metabolism, also pushes the curve to the right (193). Levels are increased in states of chronic hypoxia, such as altitude and chronic lung disease (3.2.3.2). A right shift in the oxygen dissociation curve facilitates unloading of oxygen in the peripheral tissues.

The oxygen dissociation curve; the red cells' environment (temperature; and PCO2); Hb concentration ([Hb]) and the saturation of the Hb allow us to predict the arterial oxygen content of blood. This can be illustrated in equation form:

$$CaO2 = (SaO2 \times [Hb] \times 1.34)$$

Equation 6 Arterial Oxygen Content

CaO_2 is one of the key variables in the oxygen delivery equation. Haemoglobin concentration and $PaCO_2$ can rapidly be assessed by blood gas analysis. The saturation of the Hb can be estimated by using a pulse-oximeter. The absorption spectra of oxy-haemoglobin and deoxy-haemoglobin differ. This gives them bright red and blue/purple colours respectively and allows pulse oximetry to detect the percentage of oxy-haemoglobin saturation in arterial blood.

3.2.3.1 Oxygen Carrying Capacity

One gram of Haemoglobin that is 100% saturated with oxygen can carry 1.39ml O2 (However, a figure of 1.34ml O2/gram haemoglobin is generally used, as a fraction of Hb is in met-haemoglobin form and carries no oxygen). This figure is termed the 'Hüffner constant'. An average male might have a [Hb] of 15g/dL. This person would be able to carry a maximum amount of 20.1ml oxygen bound to haemoglobin per 100ml blood. Assuming a resting cardiac output at sea level of 5L/min; O2

delivery to the tissues would equal 1005ml O2/min (20.1 x 50 (bound to Hb) + 0.3 x 50 (dissolved)).

Clearly changes in haemoglobin concentration will influence maximum oxygen carrying capacity. An anaemic Hb of 10g/dL will have a maximum bound O2 capacity of 13.4 O2/100ml blood, reducing oxygen delivery at sea level (CO of 5L/min) to 670ml O2/min.

3.2.3.2 How Altitude affects the alveolar pO2

Altitude	pO2 air (mmHg)		pO2 alveolus (mmHg)		Likely SaO2 %
	mmHg	KPa	mmHg	KPa	
Sea level (0m)	160	21.3	100	13.3	98-100
1000m	141	18.8	85	11.3	97
2000m	128	17.1	70	9.3	93
3000m	117	15.6	58	7.7	86

Table 4 Partial Pressures of Oxygen at Altitude

The declining alveolar pO2 at altitude reduces the concentration gradient for diffusion and slows equilibration of capillary and alveolar partial pressures. Furthermore there is a right shift in the O2 dissociation curve at altitude secondary to a rise in 2,3-DPG within a week of acclimatization at high altitude (194). While this assists unloading of oxygen to the periphery, it slows O2 diffusion into the pulmonary capillary. Much of Afghanistan lies above 2000m (Figure 17). Injured soldiers will be hypoxic so oxygen would be useful even in the absence of blast lung.

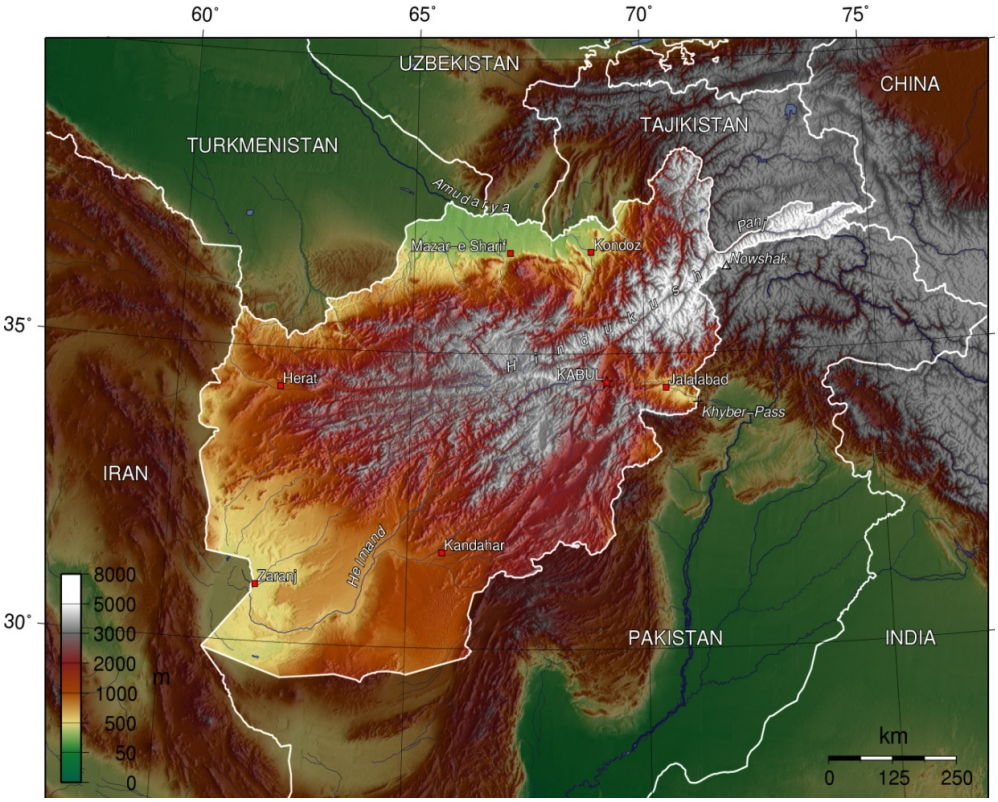


Figure 17 Topographical Map of Afghanistan

3.2.3.3 Tissue Oxygen Delivery

Oxygen delivery (DO2) is the total amount of oxygen delivered to tissues per unit of time. It is a product of blood flow through the circulation in a minute (cardiac output) and the amount of oxygen carried within that blood (oxygen content). The equation is repeated here for clarity.

(DO_2) (ml/min) = cardiac output (CO) x arterial oxygen content (C_aO2)

Equation 7 Oxygen delivery equation. CO = Stroke Volume (SV) (ml) x Heart Rate (HR) (bpm). C_aO2 = [Haemoglobin (Hb)] (g/dl) x Oxygen Saturation (SaO2) (%) x 1.34 x 10 / 100^2.

[2] *The amount of unbound O2 dissolved in plasma at 1 atmosphere is clinically negligible and not therefore included in this representation of the DO2 equation*

In summary, the delivery of adequate quantities of oxygen to tissues is critical for maintaining cellular aerobic respiration. Oxygen delivery is affected by blood flow and oxygen content. Oxygen content in turn is influenced by [Hb]; the state of shift on the dissociation curve of Hb and the oxygen saturation of the Hb.

3.2.4 Gas Exchange in the Periphery

As an erythrocyte travels through a peripheral capillary, it must be able to offload its oxygen and take up CO2. Again, this process occurs via diffusion. In tissues, the diffusion distances are greater (50 microns in muscle) than the 0.3 microns seen in the lung. As oxygen diffuses from the capillary, oxygen tension decreases. Nevertheless, with adequate perfusion of oxygenated blood, there is still adequate supply to all the tissue (see Figure 18 for example of muscle tissue bridge). As oxygen delivery declines after trauma, a tissue bridges between adjacent capillaries begins to receive inadequate oxygen. This forces cells within the zone of hypoxia to metabolise anaerobically and begins the process of ‘shock’. Prolonged cellular ischaemia leads to loss of integrity of mitochondrial membranes with resultant mitochondrial dysfunction and eventually apoptosis. The dysfunction that results will depend on the tissue type affected: in the kidneys for example, hypoperfusion results in acute tubular necrosis – a recent epidemiological study of renal failure found that ischaemia was the second most common cause of tubular necrosis (195).

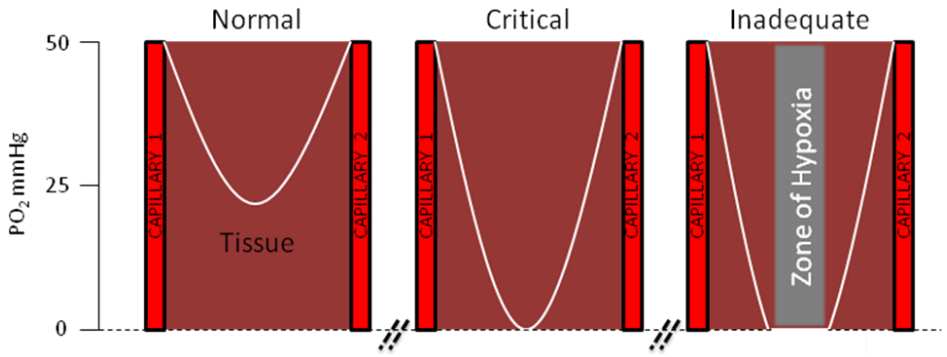


Figure 18 Simplified schematic of decreasing oxygen tension in tissue between capillaries

3.2.5 Oxygen Consumption

Cellular metabolism requires a constant supply of energy. Cells cannot, however, simply use glucose as a fuel for work. They must convert the potential energy from digested nutrients into a usable energy storage molecule or 'currency'. Adenosine Tri-Phosphate can be thought of as our principal energy currency and 90% of our absorbed oxygen is used as the terminal electron acceptor for oxidative phosphorylation of ADP to ATP. ATP can be produced via either aerobic or anaerobic pathways. To maintain normal conditions, aerobic pathways must form the principal source of energy creation and this is why oxygen delivery to the tissues' mitochondria is so critical.

Glycolysis is a common metabolic pathway for both aerobic and anaerobic metabolism. It occurs within the cytoplasm of cells and is independent of the need for oxygen. Two Pyruvate molecules form the end product of glycolysis. In aerobic respiration, the action then moves to the mitochondria where the Kreb's cycle and electron transport generate large quantities of ATP (theoretical maximum of 34 ATP per molecule of glucose).

In shock states, respiration can still occur, but the Kreb's cycle and electron transport cannot take place. Instead glucose's energy is extracted by lactate fermentation. Pyruvate is converted into lactate and ATP. This process generates only 2 molecules of ATP per glucose molecule. Prolonged depletion of oxygen causes lactic acid accumulation and the extracellular fluid becomes acidic.

This thesis focuses on oxygen delivery shortfall as a key driver of early of mortality and morbidity after blast and haemorrhage injury, it is important that the oxygen that delivered to the tissues be gainfully employed - the tissues must be capable of undertaking aerobic metabolism.. Oxygen consumption data are presented in this thesis to confirm whether or not increased DO2 correspondingly increases VO2. Addressing the disordered mitochondrial function following haemorrhagic shock represents another focus for trauma research (196;197)

3.2.6 Hypoxia after Blast

The pathophysiology of blast lung has been described earlier (2.2.4). Hypoxia can result from several sequellae of blast. Pulmonary contusion (diffuse haemorrhage) and oedema are most common (34). Also discussed earlier is the cardio-respiratory response to blast (2.2.5). The apnoea and subsequent shallow breathing exacerbates the hypoxia associated with the physical sequellae of blast lung.

The pathophysiological progress of blast lung over time is summarised in Table 5 below.

Time	Event	Clinical Features	CXR
0 (h)	Shock Wave	Hypoxia	Contusion
	Alveolar capillary rupture Interstitial and alveolar haemorrhage	*Tachypnoea* *Haemoptysis/Cough*	*Pneumothorax* *Haemothorax*

	Physiological Shunt Reduced Lung Compliance	*Pneumothorax* *Haemothorax* *Air emboli*	*Emphysema* *Rib Fracture*
3	Free Hb & blood Free radical reactions / Oxidative Stress Pro Inflammatory Response / Chemotaxis Increasing Oedema	Hypoxic resp. failure High airway pressures	Contusion Oedema
12-72	Leukocyte Accumulation Disrupted tissue architecture Increased oxidative stress Increased Inflammation Increasing Oedema	ARDS SIRS MOF	Diffuse pulmonary infiltrates
5 -10 days	Resolution	Improved gas exchange Improved compliance	Resolution of lung contusions

Table 5 Natural History of Blast Lung

Capillary rupture; intra-alveolar haemorrhage; tearing of the inter-alveolar septa and changes to the epithelium and type II pneumocytes have been demonstrated in blast-exposed rat lungs (156). The result is a diffuse haemorrhage picture with interstitial and intra-alveolar bleeding. The increased diffusion distance for oxygen exchange results in hypoxia.

In addition to the localised gas exchange effects of intra-alveolar haemorrhage and oedema, free Hb within alveoli have been shown to recruit activated polymorphonuclear neutrophils. These neutrophils, along with the extravasated blood, generate reactive oxygen and nitrogen species that, in turn, result in secondary oxidative damage to the lung tissues following intra-alveolar haemorrhage (198). Gorbunov and colleagues, using rat models and shock tube overpressure, expanded on this work to present a temporal cascade of inflammatory alterations in

the injured lung following exposure. They implicated iron dependant pathways of oxidative damage (199)

Shock Wave	Influx of blood into alveoli Cardio-pulmonary reflex events Reactive Oxygen and Nitrogen species formation
1-6h	Leukocyte sequestration, migration and degranulation. Phagocytosis of free RBCs Endothelial damage Pulmonary oedema Myeloperoxidase accumulation Accumulation of stress proteins: Copper, Zinc Superoxide Dismutase (SOD) and Haem-Oxygenase Type 1 (HO-1)
12-24h	Extensive phagocytosis and lysis of RBC leading to solid phase Hb Hb oxidation and decomposition Protein nitration Parenchymal expression of stress Proteins SOD and HO-1
36-56h	Phagocytosis of solid phase Hb Free Fe deposition in parenchymal and phagocytotic cells Type I and II pneumocyte damage Alveolar exudates Clumping of surfactant released from lamellar bodies

Table 6 Temporal Sequence of Haemorrhagic alterations in alveoli following Blast Overpressure - modified from (199)

Within only 3 hours, a significant influx of leukocytes occurred within zones of intrapulmonary haemorrhage. Levels of Myeloperoxidase (MPO), which are abundant in inflammatory leukocytes, continued increasing beyond 24h – an indication of developing inflammation.

Another paper from the same group examined changes to antioxidant levels; peroxidation and architecture after single and repeated low-impulse overpressure (200). Vitamin C and E levels dropped significantly within 1hr after a low-level single exposure to 62KPa shockwave. Lipid peroxidation increased immediately. At the same time, there were few macroscopic changes noted in the lungs, with <10% of the lung surface recognisably altered. Microscopic analysis demonstrated multifocal alveolar haemorrhage with infiltration of RBC and alveolar wall rupture – this progressed out to 24hr endpoint. This paper supports the previous work and

discusses the link between the secondary inflammatory processes and the clinical picture similar to ARDS that can occur many hours after exposure to a blast overpressure insult (164). While the extent of the lung injury can propagate up to days after exposure (159), the worst of the respiratory compromise will normally occur within the first 72 hours (158). It also illustrates that blast lung, at a cellular level, may occur at exposures far below those traditionally thought of as thresholds for lung damage.

While very local pathological and inflammatory effects are occurring in the lung tissue shortly after blast exposure, a more systemic response is also taking place. In a mouse model of isolated thoracic blast exposure, circulating TNFα and Interleukin-6 (IL-6) were significantly elevated (and to a level seen in Systemic Inflammatory Response Syndrome following chest trauma) after only 3 hours (201). Clinical studies have demonstrated a rise in both circulating TNFα and IL-6 very soon (at scene of injury and after 30 minutes) after thoracic trauma and their levels correlate with injury severity from major trauma (202) and with prognosis in ARDS (203).

Alongside the pathological features of blast, reduced compliance is important. Compliance describes the volume change in the lung per unit of pressure change. Reduced compliance requires the chest wall to work harder to deliver gas to and from the respiratory zone. This increased work of breathing increases oxygen consumption and, in severe cases, compromises gas exchange(158).

To demonstrate how supplemental inspired oxygen should attenuate the hypoxia of blast, Fick's law is again used. Blast lung increases the diffusion distance for oxygen

exchange (red arrow) and the development of 'diffusion limitation' at the blood-gas barrier.

$$Vgas \quad \alpha \quad \frac{A}{T} \quad . \quad D \quad . \quad (P_1 - P_2)$$

Vgas = Volume of gas per unit time which diffuses across a membrane
A. = Surface Area for Diffusion
D. = Diffusion Constant of the gas: $D \propto \frac{\text{Solubility}}{\sqrt{\text{Molecular Weight}}}$
P_1-P_2 = Difference in partial pressure of gas on either side of tissue
T = Thickness of tissue sheet across which diffusion must occur

Supplemental inspired oxygen can reduce the impact of increased 'T' by increasing the difference between alveolar and blood oxygen tension on either side of the tissue barrier (blue arrow). Because carbon dioxide is so soluble, the Diffusion Constant 'D' is far greater than that of O2; allowing adequate exchange despite significant increases in 'T'.

3.2.6.1 Potential Side Effects of Supplemental Oxygen Therapy

A small percentage of reactions in normal conditions during mitochondrial oxidative phosphorylation generate superoxide anions. These anions are produced when mono-electron-reduction of oxygen occurs during the electron transport process. The mitochondria house large numbers of anti-oxidants that 'mop up' these free radicals and prevent organelle damage: these include manganese superoxide dismutase; glutathione peroxidise and Vitamins C and E. As we have seen from Elsayed's work, antioxidants are depleted very soon after blast exposure (152). Lipid peroxidation and other markers of free radical reactions also appear. These factors contribute to the pathophysiology of blast lung and potentially render the lungs vulnerable to further oxidative stress. Adding high flow, high concentration oxygen to inhaled gas could provoke further free radical damage.

Following experimental lung insult with lipopolysaccharide, even moderate concentrations of supplemental oxygen (FiO2=0.6) have been shown to produce hyperoxia associated lung injury (204). Markers of macrophage activation, alveolar neutrophil counts; signs of histological injury and epithelial barrier insufficiency were raised. These indicate a relationship between supplemental oxygen and secondary lung injury when support is offered to a damaged lung. Our experimental model was resuscitated out to 8hrs post blast. Sampling protocols included adhesion molecules implicated in ALI/ARDS after major trauma (ICAM and VCAM). Lung weight indices were calculated at the end of each experiment, and histology performed to assess damage that could be correlated to oxygen therapy.

3.2.7 Considerations for Experimental Method - Supplemental Inspired Oxygen

Mechanical ventilators are not available to far-forward medical echelons. We must therefore address the problem of oxygen supplementation during spontaneous ventilation. Our model must reflect a practically deliverable protocol.

While face mask, nasal speculums or regulators could be used to deliver the supplemental oxygen during spontaneous breathing, we felt a mask would provide the most relevant comparison to methods to oxygen support likely to be used in the field. A standard human face mask would not however fit a porcine snout. A nose cone was therefore adapted to include a port for oxygen tubing. This was placed loosely around the animal's snout. The concentration of oxygen being presented to the airway by this method was measured using an oxygen analyser. A sensor attached to the endotracheal tube also measured end tidal CO2, which was used to confirm that no rebreathing was taking place (Figure 29).

While the ATLS approach of 100% oxygen (from the cylinder) flowing at a minimum 11L min^{-1} (178) might seem ideal for maximising oxygen levels in the alveoli, this does not consider the remote pre-hospital environment: portable, cylinder-based systems would last only a short time at such a rate. Furthermore, there is the concern regarding the side effects of oxygen therapy. We therefore chose a relatively modest minimum FiO2 of 0.3 as we felt this would be enough to ameliorate hypoxia, while conserving supply and minimising side effects. We used a clinically relevant endpoint of 95% SaO2 as the target for oxygen therapy.

3.3 Recombinant Activated Factor VII

As we have seen in the previous chapter, blast lung injury is characterised by diffuse intra-alveolar haemorrhage and a prolonged oedematous inflammatory response. The inflammatory response that is in part initiated by the presence of blood in lung parenchyma, develops over the first few hours after blast exposure, and can be prolonged. Both intra-alveolar haemorrhage and pulmonary oedema compromise gas exchange and produce hypoxia. An agent that could reduce both diffuse haemorrhage and inflammation might improve outcome after blast injury. Since both problems have, to some degree, pulmonary haemorrhage as a common starting point, then an agent targeting the bleeding has the capacity to attenuate lung compromise significantly if given sufficiently early after blast exposure.

Recombinant activated Factor VII (rFVIIa) was developed for, and is licensed for use at times of bleeding or major surgery in patients with Haemophilia A and B and inhibitors (antibodies) to coagulation factors VIII and IX (205). Its first clinical use

was reported by Hedner in 1983 (206). The drug has since been used off licence in the management of a wide variety of complex clinical scenarios, in the absence of pre-existing coagulopathy (207). It's first successful use in a trauma patient was reported by Kenet in 1999 (208) and several case reports now exist. There has been one paired randomised controlled trial assessing its use in major haemorrhage after civilian trauma (42) (3.3.4). There have been no prospective trials assessing its use in blast lung injury. There are however several small case series and reports that document rFVIIa use in diffuse pulmonary haemorrhage from other causes.

This chapter will discuss the mechanism of action of rFVIIa, the evidence for its efficacy in diffuse pulmonary haemorrhage and consider a possible anti-inflammatory effect. These will be related to a potential impact on blast lung.

3.3.1 Mechanism of rFVIIa Action

rFVIIa is produced by Novo Nordisk A/S, Bagsvaerd, Denmark and marketed as Novoseven®. It is a copy of the activated form of the plasma-derived glycoprotein clotting factor, factor VII. The manufacturing process involves expression in a baby hamster kidney cell line; a chromatography-based purification process and includes a step that results in auto-activation. The result is a protein qualitatively identical to plasma-derived FVIIa (205). Novoseven® is currently licensed in the US for bleeding haemophilia patients with inhibitors to factors VIII and IX. In the European Union, it is also licensed for use in acquired Haemophilia, FVII deficiency and Glanzmann Thrombasthenia refractory to platelet transfusion (209). The use of rFVIIa in trauma remains an unlicensed indication, but it is prescribed off-licence on a named-Consultant basis to treat non-surgical major haemorrhage after trauma

3.3.2 FVIIa's Role in Clotting

In 1964, Davie described the "Waterfall Sequence for Intrinsic Blood Clotting" (210). This cascade involved two initiator pathways: the "extrinsic" and "intrinsic" systems, which resulted in serial activation of clotting factor co-enzymes present in the blood. Both initial pathways resulted in activation of factor X, which then led to activation of the 'common stem'; converting pro-thrombin to thrombin; the thrombin in turn promoting fibrin polymerisation. This coagulation factor-centred model conveniently explained the Prothrombin Time (PT) and Activated Partial Thromboplastin Times (APTT), which had been designed to monitor therapeutic Warfarin levels, but did not account for cells or platelets and their contribution to the haemostatic process. Within this cascade model, FVII and Tissue Factor formed the extrinsic pathway.

More recently, Hoffman proposed a "Cell-Based Model of Coagulation" (211), which has since gained acceptance as an improved hypothesis for *in vivo* coagulation. This model emphasises the roles of specific cell surfaces in controlling the coagulation process. Three overlapping phases of coagulation are described: Initiation; Amplification and Propagation.

3.3.2.1 Initiation

Initiation takes place on cells bearing Tissue Factor (TF). TF is a trans-membrane protein, expressed on surface of sub-endothelial vessel wall cells when activated by growth factors and immune mediators (212). Vessel injury exposes TF to FVII within circulating blood and binds it to form an active complex. This in turn activates factors IX and X on the TF-bearing cell (211). Factor Xa, in conjunction with its cofactor Va, produces a small amount of thrombin (IIa) on the TF-bearing cell. This thrombin is responsible for the Amplification process (Figure 19).

3.3.2.2 Amplification

Amplification involves the activation of co-factors V and VIII and factor XI. IIa also activates platelets as they adhere and accumulate activated cofactors on their surface. It occurs as the “action moves from the TF-bearing cell to the platelet surface” (211).

3.3.2.3 Propagation

In the Propagation phase of haemostasis, IXa (activated in the initiation phase) forms a complex with activated factor VIII (VIIIa). This complex on the activated platelet surface produces large quantities of Xa. Platelet surface-derived Xa is responsible for the “thrombin burst” required to make a stable and firm fibrin clot.

As with the Waterfall model, plasma protease inhibitors such as Antithrombin III and Tissue-Factor Pathway Inhibitor (TFPI), localise the coagulation response by “mopping up” wandering activated cofactors.

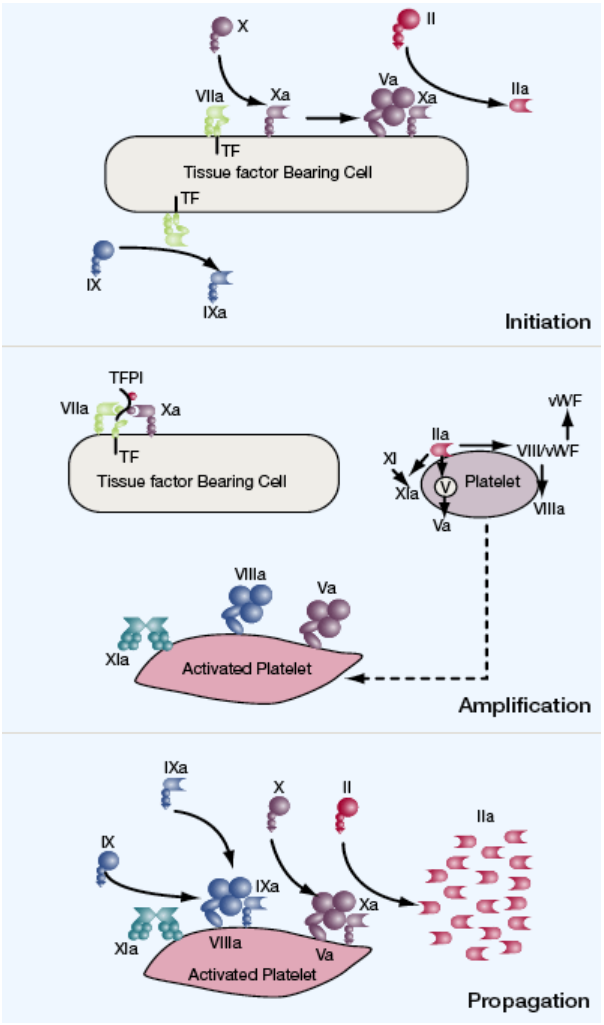


Figure 19 Cell based model of haemostasis illustration - taken from www.medi-cell.co.uk

As pharmaceutical rFVIIa has an identical action to the human activated factor, so it binds to exposed TF at the site of vascular injury (213). The resulting TF-rFVIIa complex catalyses the activation of factors IX and X on the TF-bearing cell surface. In this way, rFVIIa initiates the haemostatic pathway.

Further to this, however, Allen describes a mechanism where, at pharmacologic levels, rFVIIa binds directly to IIa-activated platelet membranes, resulting in factor X cleavage (214). Although this process is a less potent producer of Xa than the first route, it nevertheless contributes to significant IIa generation on the platelet surface.

Xa on the platelet surface is necessary for the formation of the Prothrombinase assembly (215-217).

3.3.3 Localised Effect at Sites of Bleeding

So, through both of these mechanisms, rFVIIa promotes thrombin generation where TF is exposed to circulating blood. TF exposure normally occurs only at sites of vascular injury, so rFVIIa is an intuitive drug to employ in the management of traumatic haemorrhage. However, some patients will express TF elsewhere; for example those with unstable atherosclerotic plaques, or gram negative sepsis (212). As such there is a potential risk of complications, such as myocardial infarction and stroke and there are case reports of adverse thrombotic events associated with rFVIIa administration (218).

3.3.4 Evidence for rFVIIa in Major Bleeding

Clinical evidence supporting efficacy of rFVIIa in this role began with promising anecdotal reports and progressed through to large case series (219). Eventually NovoNordisk sponsored a prospective randomised controlled trial, performed by Boffard and colleagues (42). He lead two parallel, multi-centre, prospective, randomised, placebo-controlled, double-blind trials of rFVIIa use in trauma (42). Inclusion criteria were: patient had received six or more units RBC before administration of rFVIIa; fewer than 4hrs from injury and Glasgow Coma Score >8/15. Blunt and penetrating groups were separately randomised. An initial dose (200mcg/Kg) was given after the eighth unit of RBC, followed by further (100mcg/Kg) doses at 1 and 3 hrs if required. End points were mortality, transfusion requirements and organ failure. 143 blunt injuries and 137 cases of penetrating trauma were included into the treatment arms. There was no significant difference in mortality. Blunt trauma patients who received rFVIIa required significantly fewer Red Blood

Cells (RBC) and blood product units (after excluding those who died within 48hr) with a mean reduction of 2.6 units and a 56% reduction in the number of patients requiring >20 units of RBCs. The penetrating injury group failed to reach significance for blood product requirement.

The amount of blood transfused is an independent risk factor for mortality and development of infection and ARDS (220-222). When blunt and penetrating cases were pooled, there was a significantly reduced incidence of MOF and ARDS in the rFVIIa-treated patients. There was a trend towards reduced time spent on a ventilator and fewer Intensive Care Unit (ICU) days.

Several animal studies have demonstrated haemorrhage reduction after rFVIIa administration in major haemorrhage models (223-228). Survival improvement has been shown in only two of these studies (223;224)

In 2006 Vincent attempted, with a European panel of experts, “to develop consensus guidelines for use of rFVIIa in massive haemorrhage” (229). He relied heavily on Boffard’s paper, as it was the only published randomised controlled trial at the time. Lists of key recommendations were tabled:

1. For rFVIIa to be indicated, conventional means of haemorrhage control must have failed.
2. In blunt trauma, it can be recommended as an adjunct therapy to surgery and blood product administration.
3. It is not recommended for use in penetrating trauma.

4. Monitoring of effect should be based on visual inspection and the need for ongoing transfusions.

In the military Role 3 setting, rFVIIa is being used, often in multiple doses, as part of an aggressive surgical and blood product resuscitation approach for the most severely injured bleeding casualties, most of whom have been injured by explosion. Anecdotal reports suggest potential benefit. Despite Vincent's attempt to provide guidelines, there is no consensus on the efficacy of and indications for rFVIIa use in major bleeding from combat trauma.

3.3.5 Proposed Mechanism of Action in Diffuse Alveolar Haemorrhage

Diffuse alveolar haemorrhage can occur with a range of pathologies, including leukaemia; malignancy; fungal infections and auto-immune diseases. It causes dyspnoea, haemoptysis, hypoxia and anaemia and can be fatal. Over 50% of patients require assisted positive pressure ventilation (230). As described earlier, blast lung injury typically results in diffuse pulmonary haemorrhage.

Alveolar macrophages, activated by acute lung injury, produce high levels of Tissue Factor Pathway Inhibitor (TFPI) (20 times normal) (231). TFPI inhibits the action of the FVIIa/TF complex. This inhibition renders the injured lung more vulnerable to ongoing diffuse haemorrhage. By exploiting the (TF-Independent) direct factor X cleavage effect of rFVIIa when bound to thrombin on a platelet surface, the inhibitory effect of the high levels of TFPI might be overcome.

The actions of intrinsic FVIIa and pharmacological rFVIIa in alveolar haemorrhage and the impact of TFPI are illustrated in the diagram below:

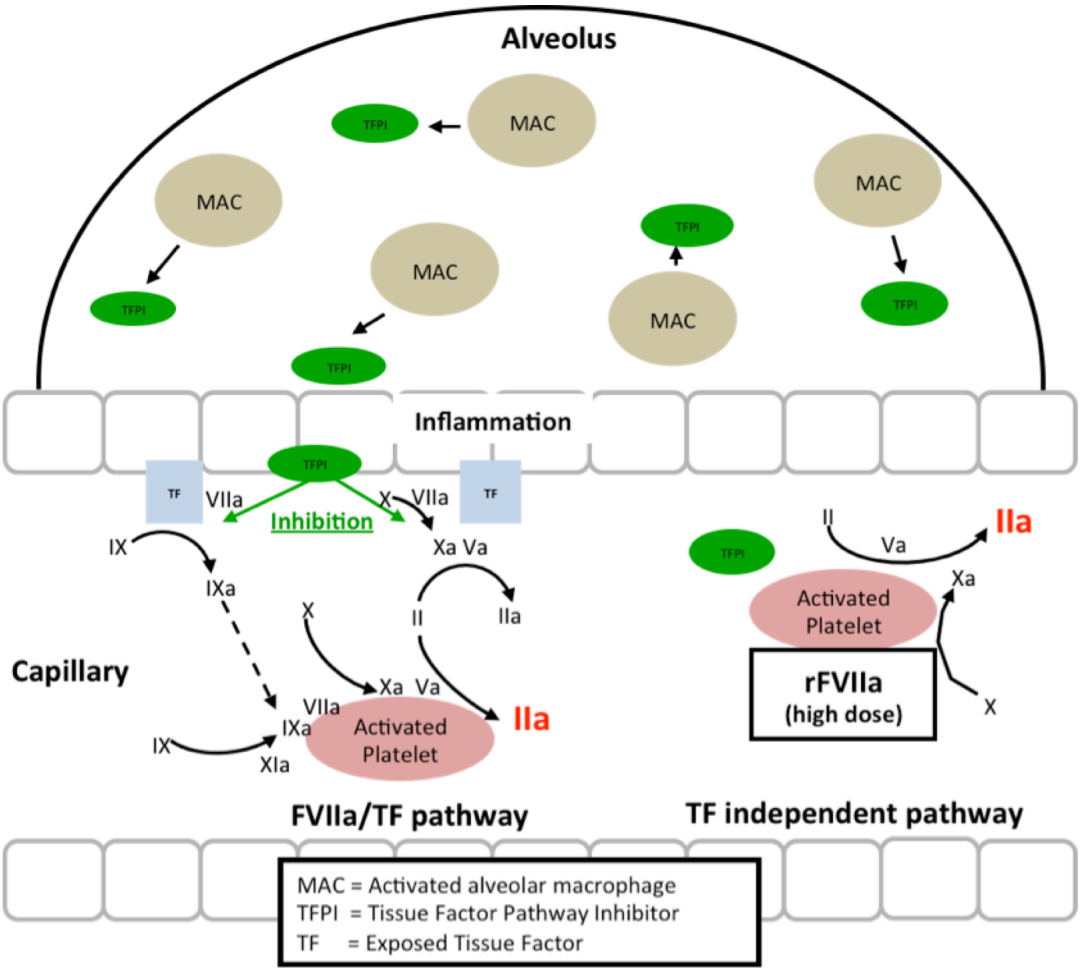


Figure 20 FVIIa action and TFPI in alveolar haemorrhage. Modified from (232)

Although no work exists to prove elevated levels of TFPI following blast lung, inflammatory changes in the lungs and increased systemic inflammatory markers have been shown to occur following blast exposure (233). It is therefore probable that activated macrophages produce raised TFPI levels, which then play a part in the pathology of diffuse haemorrhage following blast lung injury. In this case, therapeutic levels of rFVIIa could ameliorate haemorrhage through the TF-independent pathway.

3.3.6 Evidence for rFVIIa use in Diffuse Alveolar Haemorrhage

No clinical studies have investigated the use of rFVIIa to attenuate the diffuse bleeding associated with blast lung injury. Several reports however, describe rFVIIa use in the treatment of pulmonary haemorrhage. The majority are case reports or small series and relate to diffuse alveolar haemorrhage. Although positive reporting bias must not be overlooked, these reports do demonstrate the potential efficacy of rFVIIa in arresting pulmonary haemorrhage. They are summarised below (Table 7).

Authors	Report Type	No. Patients	Condition(s)	Findings
Small vessel / diffuse bleeding				
O'Connor et al (234)	Case report	1 Adult	Blunt trauma Diffuse bilateral haemorrhage	"Near instantaneous cessation of bleeding" after single dose 100mcg/kg rFVIIa. No adverse effect reported.
Tien et al (235)	Case report	1 Adult	GSW chest Lung contusion and haemoptysis with no definitive source in non-coagulopathic patient.	Control of haemoptysis, no adverse events.
Kamphuisen et al (236)	Case report	1 Adult	Blunt trauma Lung contusion and massive pulmonary haemorrhage	First 60mcg/kg dose of rFVIIa reduced bleeding from 200ml/hr to 30ml/hr via drain. Second dose at 12h stopped bleeding. No adverse effects. Full pulmonary recovery.
Betensley et al (237)	Case report	1 Adult	DAH secondary to microscopic polyangiitis	Bleeding resolved, patient survived, no thromboembolic complications noted
Estella et al (238)	Case series	2 Adults	INTRAPULMONARY ADMINISTRATION DAH in one patient with leukaemia and another with HIV during post-myocardial infarction-thrombolysis	Bleeding resolved, patients survived, no thromboembolic complications noted

Authors	Report Type	No. Patients	Condition(s)	Findings
Henke et al (239)	Case series	3 Adults	DAH in 2 small-vessel vasculitis–associated diseases and bone marrow transplantation	Oxygenation improved within minutes of rFVIIa administration, patients survived, no adverse events
Hicks et al (240)	Case report	1 Adult	DAH in leukaemic patient after bone marrow transplant	Improvement in lung function after multiple doses of rFVIIa, deterioration on discontinuation followed by resolution after further doses. No adverse effects
Pastores et al (241)	Case report	1 Adult	DAH after allogeneic haematopoietic stem-cell transplantation	The haemoptysis rapidly subsided after two doses of rFVIIa, and no further doses of rFVIIa were administered. No adverse effects due to rFVIIa were observed.
Shenoy et al (242)	Case report	1 Adult	DAH after stem cell transplantation	Control of bleeding after two doses of rFVIIa. No adverse effects reported.
Wheater et al (243)	Case report	1 Adult	Pulmonary haemorrhage associated with metastatic chorio-carcinoma	Control of bleeding after single dose of rFVIIa. No adverse effects reported.
Yildrim et al (244)	Case report	1 Adult	DAH in a patient with pulmonary–renal syndrome	Bleeding controlled by three doses of rFVIIa. Subsequent episodes also controlled by rFVIIa and patient survived to discharge. (Patient later died from a further episode of pulmonary bleeding)
Courtney et al (245)	Case report	1 Adult	Bleeding from lung parenchyma and distal bronchi after cardiopulmonary bypass	5 min after rFVIIa administration blood loss slowed substantially from the ETT and ventilation slowly improved. No thrombotic complications.
Heslet et al (246)	Case series	6 Adults	INTRAPULMONARY ADMINSTRATION DAH from a variety of medical conditions: leukaemia (3); stem cell transplant; Neurosarcoidosis; Wegener's granulomatosis & HIV.	"An excellent response, defined as complete and sustained haemostasis after a single dose of rFVIIa, was seen in 3 patients. A good response (sustained haemostasis was achieved by a repeated rFVIIa administration) was seen in the remaining 3 patients." No adverse effects reported.

Authors	Report Type	No. Patients	Condition(s)	Findings
Large vessel / focal bleeding				
Lau et al (247)	Case series	4 Adults	Massive haemoptysis associated with cystic fibrosis	Control of haemoptysis, no adverse events.
Macdonald et al (248)	Case report	1 Adult	Massive haemoptysis due to necrotizing community-acquired pneumonia.	Control of haemoptysis, no adverse events.
Samarzija et al (249)	Case report	1 Adult	Massive haemoptysis associated with chronic necrotising aspergillosis.	Control of haemoptysis. No thromboembolic or other adverse events seen.
White et al (250)	Case report	1 Adult	Pulmonary haemorrhage secondary to aspergillus infection leukaemic patient with acquired FVII deficiency	Control of haemoptysis after two doses of rFVIIa, no adverse events.
Felten et al (251)	Case series	4 Adults	Surgical bleeding from pulmonary artery during lung transplant	A single dose, of rFVIIa ranging from 68 to 105 μg/kg, was successful and allowed chest closure with no need for surgical re-exploration. 3/4 patients subsequently died but none of the adverse events could directly be linked to rFVIIa administration.
Paediatric				
Brady et al (252)	Case series	9 Children (2 relevant)	Diverse but included 2 patients with pulmonary haemorrhage	Bleeding resolved, no complications relating to rFVIIa found.
Poralla et al (253)	Case series	3 Children	Massive pulmonary haemorrhage in preterm neonates	Bleeding controlled within minutes of administration of a single dose of rFVIIa. No children showed any clinical, laboratory or ultrasound derived adverse events; especially no signs of thrombotic or embolic events in the following clinical course.

Authors	Report Type	No. Patients	Condition(s)	Findings
Yilmaz et al (254)	Retrospective review	13 Children	Diverse Included 8 patients with pulmonary haemorrhage	Bleeding was stopped completely in 10 patients at least for 24 h and decreased in 3 patients 30/45 min after rFVIIa administration. *Two patients had thrombotic complications attributed to rFVIIa administration.* No other complication was observed in the other patients.
Olomu et al (255)	Case series	2 Children	Pulmonary haemorrhage associated with very low birth weight	Bleeding resolved. No complication associated with rFVIIa reported.

Table 7 Literature Concerning rFVIIa administration in Pulmonary Haemorrhage

Four reports concern paediatric patients. While the clotting characteristics of children differ from adults, (longer clotting times; reduced clotting factor levels, less thrombin generation (256)), these reports nevertheless add to the data on pulmonary haemorrhage and rFVIIa use.

Two of the reports describe intra-pulmonary administration, rather than IV delivery of rFVIIa. Heslet's six patients all responded to therapy, even after one of them had failed to respond to an IV dose (257). Estella decided on intra-pulmonary therapy as both patients had just received thrombolytic therapy for myocardial infarction, so systemic rFVIIa administration (and its potential for TF binding remote from the target site) was avoided (238).

3.3.7 Timing and route of rFVIIa administration

The timing of rFVIIa administration could affect the impact of the agent in arresting both major and diffuse alveolar haemorrhage. While in the early stages of diffuse alveolar haemorrhage, bleeding control is of key importance to preserve gas

exchange and limit inflammatory sequellae, later on following injury; fibrin deposition and permanent scarring might be exacerbated by rFVIIa administration. Fibrin deposition has been demonstrated in ARDS (258) and attributed to over-expression of TF and FVIIa (259). Permanent fibrosis has also been linked to transient intra-pulmonary fibrin deposition (260). So great was the interest surrounding this phase of ARDS that an international trial was set up to investigate use of a FVII inhibitor during established ARDS (24-48hr following injury). The study was terminated early due to excess mortality in one of the treatment cohorts (261). However, unlike recovery from ARDS from other causes, it is also unclear whether transient fibrin deposition is even a real problem following blast lung. The limited data suggests recovery of normal lung examination; radiograph and lung function testing at one year following blast-induced ARDS (262).

Most authors reporting successful use of rFVIIa in diffuse pulmonary haemorrhage have employed intravenous administration of the drug. Intrapulmonary (inhalational) administration has been reported by only two authors (238;257). Military blast injury is a combined trauma producing significant external haemorrhage as well as blast lung. As a first step in investigating the efficacy of rFVIIa for improving survival in a blast and haemorrhage model, it seems logical to employ an intravenous administration route and a single-dose strategy that might be easily translated to a field protocol. The present study therefore seeks to establish whether early use of rFVIIa can improve mortality following a combined blast and haemorrhage injury. Therefore a single intravenous dose of human rFVIIa was used, in conjunction with permissive hypotensive fluid resuscitation. A dose of 180μg/kg was selected as this had previously been shown to increase survival time in an animal model with

incompressible major vessel injury (263). The drug was administered at 30 minutes after the onset of fluid resuscitation.

3.4 Study Aims

The overarching aims of this thesis are to use a clinically relevant animal model of battlefield injury to provide clear answers to the following questions:

1. Does supplemental inspired oxygen improve survival and preserve physiology after a combined primary blast and haemorrhage injury?
2. Can a single intravenous dose rFVIIa improve survival and preserve physiology after a combined primary blast and haemorrhage injury?

The following chapters will discuss the experimental model (Chapters 4 and 5) and present the results of the interventions (Oxygen, Chapter 6 and rFVIIa, Chapter 7).

4 Method Development

A clinically relevant model of severe battlefield injury was used in terminally anaesthetised pigs to evaluate the efficacy of supplementary oxygen and rFVIIa as adjuncts to resuscitation. The model was designed to represent a severely injured military casualty, wounded by an explosive munition. Exposure of the animal to a blast wave detonation produced a primary blast injury. Severe haemorrhage represented the bleeding sequellae of injury from energised munition fragments. The potential for uncontrolled haemorrhage was included in order to demonstrate any rebleeding.

The study formed part of an ongoing programme of work at DSTL Porton Down. A preceding study had employed an animal model that incorporated blast and both controlled and uncontrolled haemorrhage (39). The injury model in this study was similar. Modifications and their rationale are described in the following sections.

4.1.1 Explosive Charge

Uncased 2.2Kg Octol high explosive charges were used for all blast exposures in this study. This particular explosive was chosen because of the high degree of reproducibility in its output. Previous experience with this explosive on the same breed of pig led to the stand-off (distance between animal and explosive charge) being set at 2.10m from the centre of the charge to the body wall at the level of the 8^{th} rib. Physiological changes and post mortem analysis confirmed this was reliably producing a severe, but not immediately overwhelming blast insult.

4.1.2 Haemorrhage volumes and liver section

The original model incorporated an initial haemorrhage of 30% total estimated blood volume followed by a second controlled haemorrhage of 5% blood volume to simulate a re-bleeding episode during resuscitation. This re-bleeding episode was not required in our study and was therefore removed from the experimental protocol. The model also incorporated a Grade IV liver injury, induced at the end of the controlled haemorrhage. This led to an incompressible haemorrhage, which was found to be consistent between groups and provided tissue injury and a capacity for re-bleeding. Although this study was not expected to produce rebleeding, the liver section was included because of its tissue injury. Tissue injury is normally present on a large scale in victims of explosive injury, so this liver section was felt to increase the validity of the model.

4.1.3 Oxygen therapy target of SaO2 95%

A clinically relevant target was chosen to guide oxygen support. Rather than sticking dogmatically to a given FiO2, oxygen therapy was titrated to a target SaO2 of 95%. Pulse oximetry is widely available in the field and medics of all levels are familiar with its application. Both in this study, and in the field, oxygen concentration and/or flow can easily be titrated to achieve and maintain the target saturation.

4.1.4 180mcg/Kg dose and intravenous administration of rFVIIa

There is no consensus regarding the minimal effective dose of rFVIIa in trauma, nor in DAH. Barletta's review of rFVIIa use in trauma revealed that most trials had used doses between 60-120mcg/kg (264). 180mcg/kg therefore seems to be a relatively high dose in human terms. The rationale for selecting this high dose was the fact that ***human*** rFVIIa was used in pigs. Although the pig is responsive to human rFVIIa, the sensitivity is reduced compared to humans. The resolution to this insensitivity is to

increase the dose, and a dose of 180 mcg/kg in the pig has been adopted by several investigators of swine injury models as ‘equivalent’ in terms of effect to a human clinical dose (224-227;265). Further, a previous study at DSTL Porton Down demonstrated improved survival in a swine model of severe incompressible haemorrhage in the absence of blast injury (223;266). It is noteworthy that Boffard’s randomised controlled clinical trials also adopted a high initial dose (200mcg/kg) of rFVIIa, aiming to achieve a plasma concentration of 40ng/L; an amount approximately three times greater than would be achievable via cryoprecipitate and FFP transfusion. No safety concerns were raised from Boffard’s study.

That the drug was administered intravenously in this study is also in line with previous work and the most common form of use in clinical practice for diffuse alveolar haemorrhage.

4.1.5 Shared control group

Both oxygen and rFVIIa groups required a control (untreated) group. The corresponding control for the supplementary oxygen group is to breathe air, while the control for the rFVIIa group is to receive placebo. Since both treatment strategies are superimposed on combined blast injury and haemorrhagic shock, followed by hypotensive resuscitation, a single group given air to breathe throughout and treated with 0.18 ml/kg body weight 0.9% saline 30 min after the onset of resuscitation served as a control for both oxygen and rFVIIa treatments. The volume of placebo amounted to 9 ml for a 50 kg pig (0.38% estimated blood volume at the end of haemorrhage) and is therefore negligible as a resuscitation volume. For these reasons, the control group was shared, resulting in fewer animals sacrificed

(consistent with the requirements of the Animals Scientific Procedures Act 1986). This also reduced the financial cost.

4.1.6 Power Calculation

A power calculation, based on an increase from 0.1 to 0.8 in the proportion surviving to 8 hours from the onset of resuscitation in the previous Novel Hybrid study indicated that 7 animals would be required in each of the hypotensive and oxygen treatment groups (Power 0.8, Alpha 0.05, Chi Squared test).

There was insufficient clinical and experimental evidence regarding rFVIIa's effect in blast lung and haemorrhage to guide group size requirement for the primary outcome measure. A group of the same size was chosen and an interim analysis planned at n=6 for each group. This would determine whether a clear (statistical) conclusion had been attained and, if not, how many additional animals would be required to provide an unequivocal conclusion.

5 Methods

5.1 Licensing and Animal Husbandry

Prior to commencing the study, the programme of work underwent Local Ethical Review (DSTL Porton Down) and gained a United Kingdom Home Office Project Licence (PPL 30/2022). The entire study was carried out in accordance with the Animals (Scientific Procedures) Act 1986.

Immature female Large White pigs were sourced from a commercial supplier, whose animals are classed as Specific Pathogen Free. Animals were delivered by trailer at least 7 days in advance of the trials, attended to twice daily and fed and watered *ad libitum* (16% protein home-milled coarse mix). The animals were weighed three times per week and on the afternoon before a trial. Mean trial-day weights were 51.8 ± 2.2 [48-56] Kg (mean±SD [range]). Feeding was discontinued 18 hours before planned induction of anaesthesia and the animals restricted to water only.

5.2 Anaesthesia

Animals underwent terminal anaesthesia, i.e. anaesthesia was induced and maintained throughout the study, followed by humane killing at the end with an overdose of anaesthetic without recovery of consciousness.

5.2.1 Induction and Intubation

Sedation was achieved with 5ml of Midazolam hydrochloride (approx. 0.1mg/kg) via intramuscular injection. Once sedated, anaesthesia was induced by inhalation of Isoflurane 5%, driven by a 1:1 ratio of Oxygen (O2) and Nitrous Oxide (NO) via a nose cone. Endotracheal intubation with a size 8.0 silicone oral/nasal tube (Vygon

514.80, France) followed induction. Aspiration, chest wall movement and end-tidal Carbon Dioxide (CO2) monitoring (Propaq 106 EL, Dräger Medical) confirmed correct tube position. An Oesophageal Doppler Probe (CardioQ™, Deltex Medical) was placed. The endotracheal tube was then secured with strong adhesive tape and the mouth loosely packed with gauze.

5.2.2 Maintenance of Anaesthesia in Operating Theatre

Once intubated, the animals were moved to the operating theatre, where anaesthesia continued with Isoflurane (1-2%) and a mixture of O2 and NO (ratio 1:2) (Blease Frontline Plus 690). The animals were then ventilated (Blease Frontline Manley MP3 Anaesthetic Ventilator) for the duration of the surgical procedure. Monitoring comprised a Propaq 106 EL (Dräger Medical) monitor for continuous 3-lead ECG, End Tidal CO_2 and rectal temperature measurements. Once central venous access was secured at surgery, inhalational anaesthesia was replaced by an intravenous (IV) infusion of Alfaxalone (Alfaxan®, Vétoquinol), at a starting rate of 1ml/Kg/hr (IVAC Medical Systems™ P2000 syringe driver). The anaesthetic infusion rate was adjusted to maintain surgical anaesthesia. Total Intravenous anaesthesia continued for the remainder of the experiment. At the end of surgery mechanical ventilation was discontinued and the animals breathed air spontaneously.

5.2.3 Maintenance of Anaesthesia in Blast Arena

For obvious safety reasons, there is a short period surrounding the time of explosion when nobody can be at the animal's side. To ensure reliable anaesthesia during this phase, two separate anaesthetic lines were connected to independent syringe drivers, protected from the explosion within padded metal containers placed behind large concrete blocks. One of these was connected by remote cord to the range

bunker as a back up system. On returning to the animal after the blast exposure, the infusion status was checked immediately after airway and breathing.

5.2.4 Maintenance of Anaesthesia in the Range Bunker

The IV infusion of Alfaxan® continued, titrated to depth of anaesthesia. The animal was only ventilated (Evita 2, Dräger Medical) if its Arterial Blood Gas (ABG) showed a PaO2 < 7 KPa, or a PaCO2 >6 KPa. Mechanical ventilation was discontinued if the animal showed signs of spontaneous breathing, (to determine if the animal had regained the capacity for adequate ventilation and gas exchange).

5.2.5 Temperature Control

Warming pads, a silver-foil blanket, a wool blanket and a portable electric heater were used to maintain the animal's normal rectal temperature of 38.7-39.8°C (267). If the temperature rose above 39°C, the animal was fanned and cooling pads applied. Resuscitation fluids were warmed in an incubator bath set to 45°C (some cooling occurred in the infusion lines, so the fluid entering the animal was below 40°C). Core body temperature was measured by a rectal temperature probe, connected to the Propaq 106 EL monitor (Dräger Medical).

5.3 Surgical Preparation

After intubation, surgical fields were shaved and prepared with Pivodine Iodine (Betadine®, Purdue Pharma) antiseptic. The animal was placed supine, covered with a large sterile surgical drape, cut to expose the neck; abdomen and left groin.

5.3.1 Vascular Access in the Neck

Both carotid sheaths were exposed via a midline neck incision. The left internal carotid artery and internal jugular vein were cannulated with Portex 8 French lines,

(Hythe, UK), flushed and sutured in position (Figure 21). A pulmonary artery catheter (744MF75 Swann-Ganz, Edwards Life Sciences Ltd, Newbury, UK) was floated through an introducer sheath (Desivalve Catheter Introducer, Vygon, Cirencester UK) in the right internal jugular vein. Pulmonary arterial placement was confirmed by pressure wave monitoring (Propaq 106 EL, Dräger Medical AG &Co. Lübeck, Germany). The neck incision was closed with a silk blanket suture.

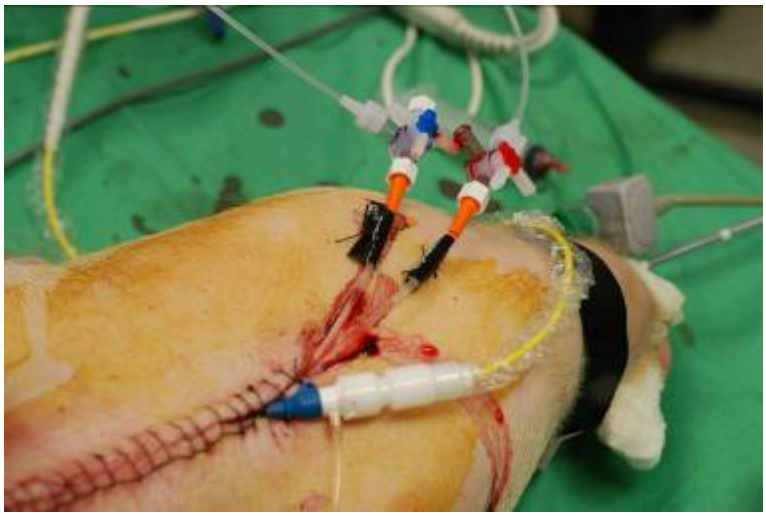


Figure 21 Vascular Access in Neck

5.3.2 Splenectomy

Pigs' spleens are invested with smooth muscle and the organ acts as a reservoir for a significant amount of blood (268). This smooth muscle contracts during the sympathetic response to haemorrhage; squeezing blood into the circulation. In order to replicate more closely the human response to haemorrhage, the spleen was therefore removed in all animals. After mobilisation, the short gastric vessels were ligated and divided. With the main pedicle still patent, 1ml 1:1000 Epinephrine was dripped onto the surface and massaged onto the spleen. This contracted the smooth muscle, emptying the organ of most of its stored blood. The splenic pedicle was then

double-ligated and divided. The spleen was removed and weighed to estimate retained blood volume: this guided crystalloid fluid replacement at a ratio of 3:1.

5.3.3 Liver Snare

Dstl Porton Down has developed a liver snare which creates a grade IV liver injury in the middle lobe of the swine liver (39). Figure 22 illustrates the technique. The two pull-cords are exteriorised and pulled at a later stage in the experiment to create a mixed arterial and venous injury, with the potential to re-bleed (uncontrolled haemorrhage).

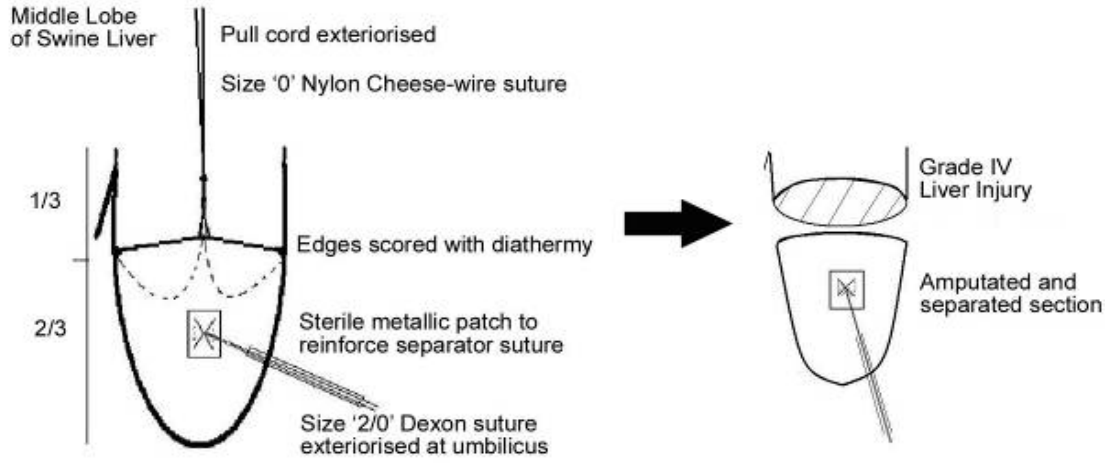


Figure 22 Liver Section schematic

5.3.4 Open Suprapubic Cystostomy

Two 2/0 Polyglactin 910 (Vicryl™, Ethicon, UK) purse-string sutures were placed on the anterior wall of the bladder. A cystostomy was made with diathermy in the centre of the purse-string and a 14G Foley Catheter placed into the bladder (Bard Ltd, Crawley, UK). The balloon was filled with 10ml sterile water and the sutures drawn up and tied. The bladder was emptied.

5.3.5 Abdominal Closure

The abdominal incision was closed en-mass with continuous size ‘0’ Nylon double-stranded sutures (PDS™, Ethicon, UK). The two liver snare pull-cords and the Foley catheter were exteriorised without snagging. The wound was dressed with thick black adhesive tape.

5.3.6 Femoral Vessel Cannulation

Approached through a left-sided groin incision (Figure 23), the femoral vessels were distally ligated and cannulated with trimmed 8Fr Dog catheters (Arnolds®, Smiths Medical International Ltd. Hythe, UK). Three-way taps (Vyclic-Color, Vygon, Ecouen, France) were placed and the lines secured. The incision was closed with a silk blanket stitch.

In the pig, the profunda femoris artery, gluteal vessels, circumflex femoral arteries and their collaterals supply the musculature of the hind limb above the knee. The profunda vessel separates proximally (Figure 24). The superficial femoral artery, which we ligate, has few branches above the knee. Below the knee in the swine, there is little muscle and, while some tissue ischaemia will have resulted from ligation, frank ischaemia was not seen in any animal even at the 8hr endpoint of the study. Any distal ischemia will have been similar for all animals and worse for those living longest.

Figure 23 Femoral Vessel Cannulation

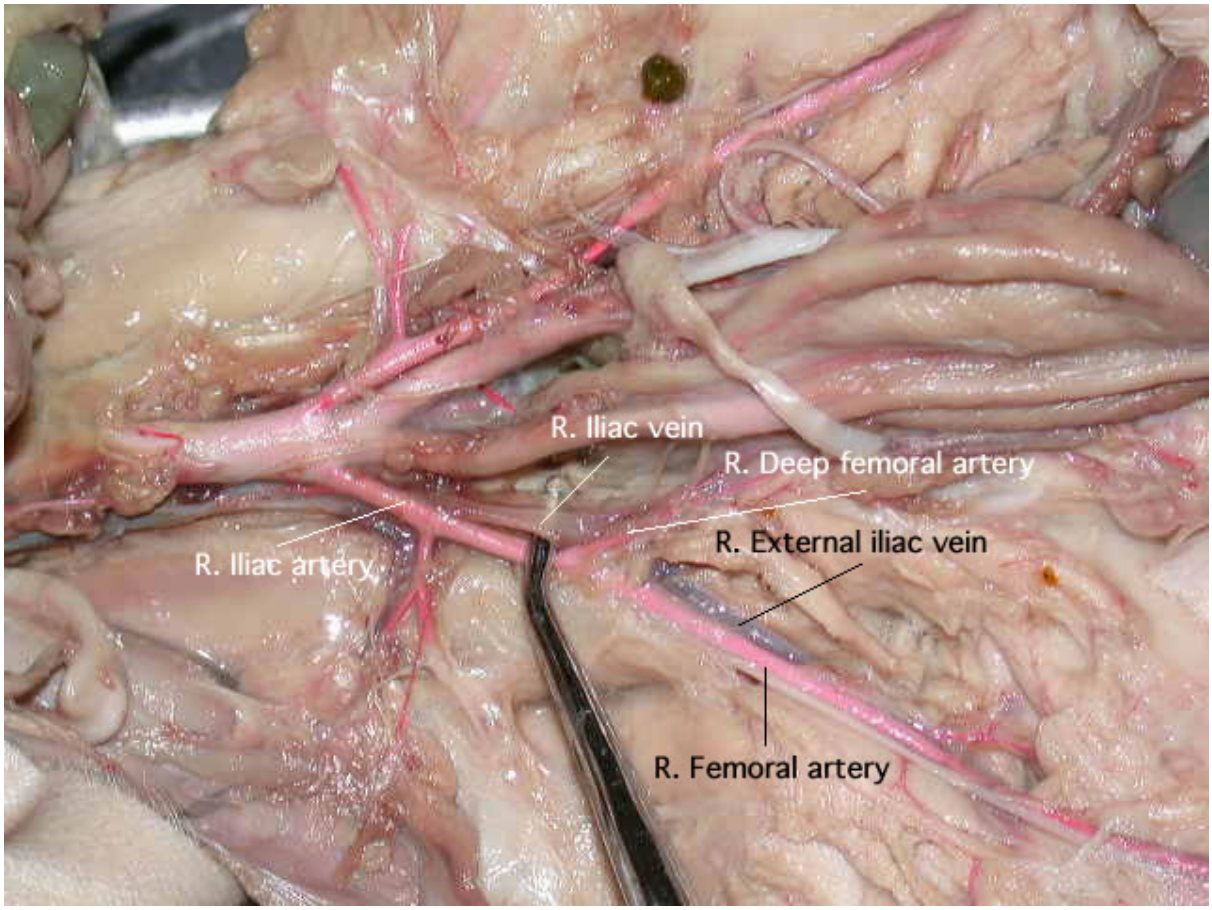


Figure 24 - anatomic swine specimen. Note proximal division of common femoral artery into deep and superficial branches. (Photograph taken from www.anselm.edu)

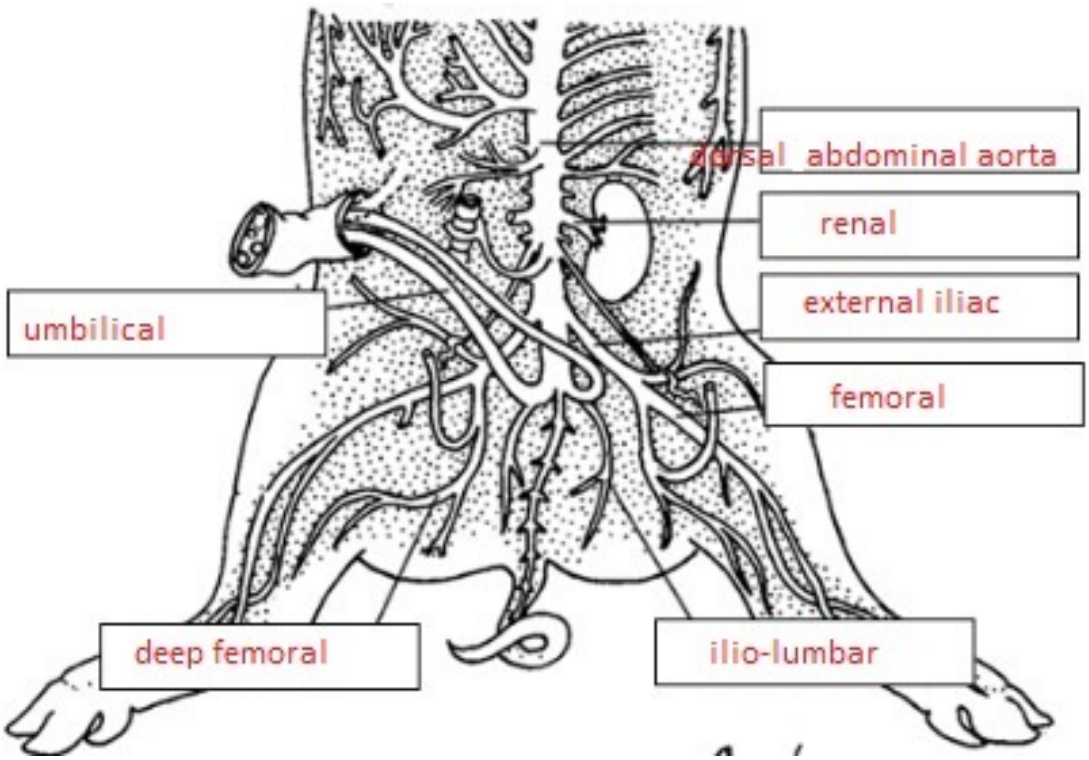


Figure 25 schematic of swine hind limb vasculature (taken from www.biologycorner.com)

5.4 Physiological Measurements and Sampling

Several parameters were measured continually; other measurements and samples were taken at intervals. Table 8 provides an overview of the monitoring methods. Table 9 illustrates the blood sampling protocol:

Time	Device	Uses	Manufacturer
Monitoring	Propaq 106 EL Monitoring System	End Tidal CO2, Respiratory Rate Continuous ECG Core Temp (°C)	Dräger Medical AG &Co. Lübeck (Germany)
	DP12 Oesophageal Doppler Probe CardioQ™ Monitor	Stroke Volume, Haemodynamics	Deltex Medical Ltd. Chichester (UK)
Central Lines and Catheter	Left Internal Jugular 8Fr Dog Catheter	Intravenous Anaesthetic	Smiths Medical Instruments, Hythe (UK)
	Left Carotid Artery 8Fr Dog Catheter	Arterial Pressure Arterial Blood Gas	
	Right Internal Jugular Desivalve Catheter	Cardiac Output (Vigilance™ Monitor) Central Venous	Vygon, Ecouen (France) &

	Introducer & 744MF75 Swann-Ganz Catheter	Pressure Pulmonary Artery (PA) Pressure PA Wedge Pressure Temperature Mixed Venous Blood Gas	Edwards Life Sciences (UK) & SensoNor 840, SensoNor a.s., (Norway)
	Left Femoral Artery 8Fr Dog Catheter	Controlled Haemorrhage Haematology Sampling	Smiths Medical Instruments, Hythe (UK)
	Left Femoral Vein 8Fr Dog Catheter	Resuscitation Fluid Infusion	
	Foley Catheter (18Ch)	Urine output Urine biochemistry Microalbuminuria	Bard, Ltd, Crawley (UK)
(Blast Arena)	3-lead ECG End Tidal CO2	Remote monitoring during blast exposure Laptop Computer	Maclab 8/s, & Chart v4.2.3, ADInstruments, UK

Table 8 Monitoring devices and parameters

Time	Arterial Blood Gas	Mixed Venous Blood Gas	Full Blood Count	TT, PT, APTT, Fibrinogen	U&E	C-RP	IL-6	Calcium	ROTEM TEG	ICAM VCAM
Line (Carotid)	■		■	■	■	■	■	■	■	■
Line (Jugular)	■	■	■	■					■	
Post Surgery	■	■	■	■					■	■
Baseline 1	■	■		■					■	
Baseline 2			■	■	■	■	■	■		
Baseline 3	■	■								
Post Blast	■			■					■	
Pre Haemorrhage	■	■	■	■	■	■	■	■	■	
Post Haemorrhage	■	■		■	■		■	■		
T0	■	■	■	■		■	■	■		■
T15	■	■		■			■	■		
T30	■	■	■	■	■	■	■	■	■	
T45	■									
T60	■	■	■	■		■	■	■	■	■
T90	■	■			■					
T120 (2hr)	■	■	■	■		■	■	■	■	■
T150	■	■			■					
T180 (3hr)	■	■	■	■		■	■	■	■	
T210	■	■			■					
T240 (4hr)	■	■	■	■		■	■	■	■	■
T270	■	■			■					
T300 (5hr)	■	■	■	■		■	■	■	■	
T330	■	■			■					
T360 (6hr)	■	■	■	■		■	■	■	■	■
T390	■	■			■					
T420 (7hr)	■	■	■	■		■	■	■	■	
T450	■	■			■					
T480 (8hr)	■	■	■	■		■	■	■	■	■
(Preservative)	Heparin	Heparin	EDTA	Sodium Citrate (SC)	Lithium Heparin	Lithium Heparin	SC	Lithium Heparin	SC	Serum

Table 9 blood sampling protocol

Dots indicate collection of sample	
TT	Thrombin Time
PT	Prothrombin Time
APTT	Adjusted Partial Thromboplastin Time
U&E	Urea and Electrolyte Panel
ROTEM	Thromboelastometry
TEG	Thromboelastography
IL-6	Interleukin 6

5.4.1 Cardiovascular Measurements

Arterial blood pressure was recorded via the carotid artery cannula. Pulmonary arterial and central venous pressures were recorded via the flow-directed balloon-tipped flotation catheter, which was also used to determine cardiac output as a 6 minute rolling average (Vigilance™ Volumetrics CEDV, Edwards Lifesciences™, USA). Additional assessments of cardiac stroke volume (stroke distance) and haemodynamics were made using trans-oesophageal echo (Deltex Medical Ltd. Chichester (UK)). Physiological pressure measurements were made using strain gauge manometers (SensoNor 840, SensoNor a.s., Norway): zero pressure for all transducers was set at heart level. Core body temperature was measured by rectal probe (Propaq 106 EL) and maintained at approximately 38°C using external heating/cooling and blankets as appropriate.

All cardiovascular variables were recorded using a computerized data acquisition system (Maclab 8/s, ADInstruments, UK) and associated software (Chart v4.2.3, ADInstruments, UK) for subsequent analysis.

5.4.2 Blood Gas Analysis

Blood gas analysis was performed alongside cardiovascular measurements, but an extra set was taken immediately after blast exposure. Arterial and venous blood samples were taken anaerobically into heparinised syringes from the carotid and pulmonary artery catheters respectively for blood gas, base excess and lactate analysis (Gem Premier 3000 Blood Gas Analyzer, Instrumentation Laboratories, Warrington, UK).

5.4.3 Biochemistry, Haematology and Urinalysis

Table 9 above illustrates the blood sampling protocol. Urine output was recorded hourly by evacuation of the bladder. Urine samples were taken each hour for biochemistry and detection of microalbuminuria.

For assessment of coagulation and inflammatory responses, arterial blood samples were collected into citrated vacutainers (9NC 0.105M Vacutainer 367691, Beckton Dickinson, UK), centrifuged at 1500 x g for 10 min and the plasma separated and stored at -80°C. Prothrombin time (PT) was determined using the ACL Elite (Beckman Coulter, UK) by turbidimetry.

5.5 Experimental Protocol

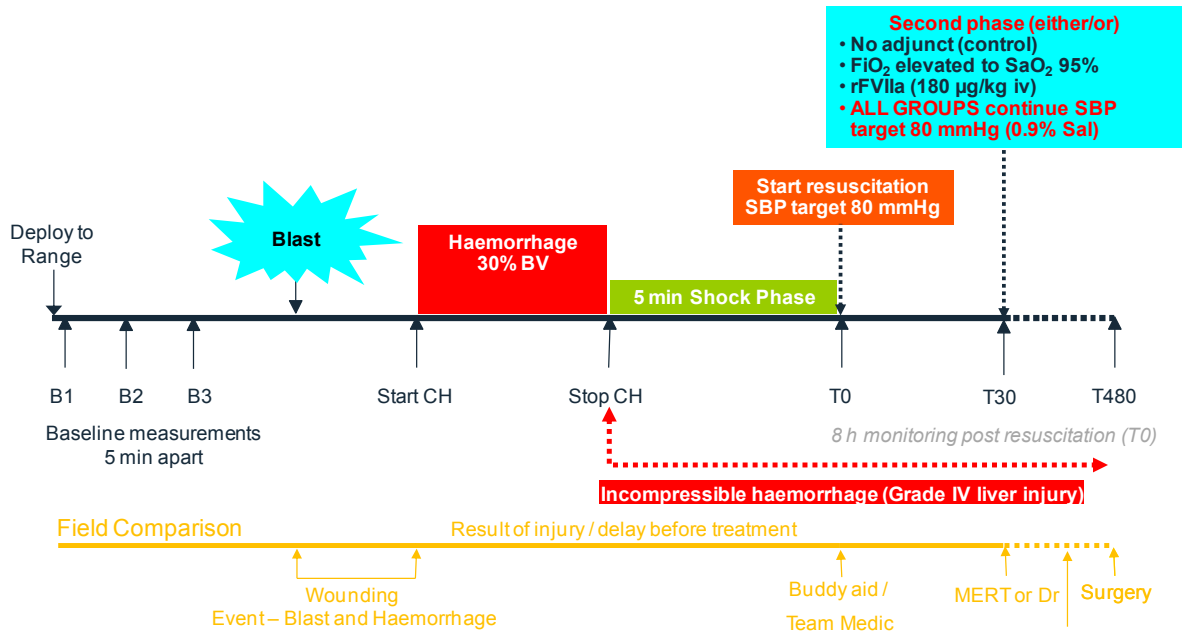


Figure 26 Experimental Protocol

5.5.1 Blast Arena and Exposure

A large van was used to transport the instrumented animal for the 15-minute journey from the operating theatre to the range. The animal was accompanied and

monitored throughout. Once at the range, three baseline measurements and blood samples were taken at five minute intervals; the first one hour after the end of surgery. The animal was then mounted supine onto the blast rig, with the right thorax nearest the charge, and wrapped in a Kevlar® blanket to protect from any fragment injury. ECG and respirometry were relayed to a laptop computer in a bunker.

Figure 27 Blast arena prior to explosive insult. Cardboard mounting tube for placement of uncased charge (see Figure 28). Sliding rig on right side with animal wrapped in Kevlar to protect from fragment debris (monitoring and anaesthesia lines out of view). Stand-off 2.1m from charge to animal body wall.

A stand-off of 2.10m was measured from the centre of the charge to the body wall at the level of the 8th rib. This protocol was determined based on previous work performed by the Trauma and Biophysics Team at DSTL Porton Down, who have a broad experience in exposing large animal models to blast injury (269).

The same batch of 2.2kg uncased cylindrical high explosive Octol charges was used for all experiments in the series.

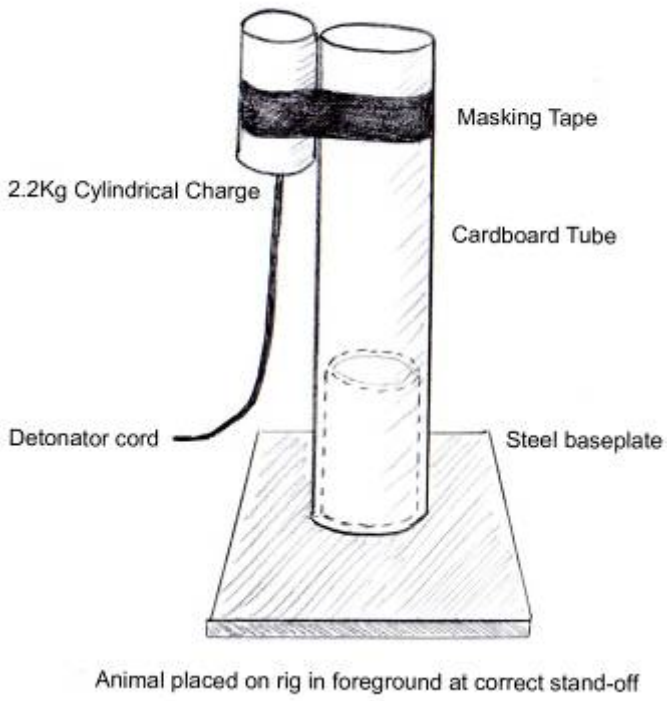


Figure 28 Charge set up. See photograph of blast pad (Figure 27) for scale and position of charge in relation to animal on rig.

After detonation of the charge and a safety check of the area, the surgical team quickly returned to the animal, always arriving within 120 seconds from charge detonation. Airway patency, breathing and anaesthetic delivery were confirmed. Concurrently, blood samples were taken from the femoral arterial line. The animal was recovered to the range bunker for the rest of the experiment.

5.5.2 Controlled Haemorrhage

15 minutes after the blast, 30% of the animal's blood volume, calculated from body weight (Equation 8), was pumped (MasterFlex® LIS® Computerised Drive Pump, Cole-Palmer Instrument Co. Il, USA) from the femoral arterial line, through silicone tubing (MasterFlex® Precision Tubing, Cole-Palmer), and collected into 3 blood donation bags (CPDA-1, Baxter Healthcare Corp. Il, USA) placed on scales

(Sartorius LP2201, accurate to 0.1g). The bleed occurred over 5.3 minutes, with a progressively decreasing rate of bleeding, designed to reflect the reducing flow over time from a real arterial injury (270). By monitoring the weight of removed blood, the accuracy of exsanguination volume was confirmed. The calculations below dictate the quantity and rates of haemorrhage adjusted to body weight:

Total Blood Volume (B_0) (ml/kg) = $161.4751(Wt^{-0.2197})$ (271)

Equation 8 Blood Volume by Swine Body Weight.

Arterial Rate of Exsanguination (V) = $B_0(1-e^{(-0.04t)})$ (270)

Equation 9 Haemorrhage Rates in Arterial Exsanguination (V= the total blood loss (ml/Kg) and t= the percent time until death)

5.5.3 Uncontrolled Haemorrhage

The two pull-cords of the previously described liver snare were pulled immediately after completion of the controlled haemorrhage. The 'cheese-wire' was pulled first to achieve amputation, followed by the 'separator' cord to ensure complete avulsion of the amputated segment. The resulting grade IV liver injury (272) produces both arterial and venous incompressible haemorrhage with the potential for rebleeding.

5.5.4 Shock phase and onset of fluid resuscitation

Following completion of the haemorrhage, there was a 5 minute 'lock-out' period, designed to reflect a realistic minimum time interval before resuscitation could commence in a military environment. Time '0' began at the end of the lock-out phase. Warmed 0.9% Normal Saline (Aquapharm®, Animalcare Limited, York, UK) was infused into the femoral venous line, controlled by a Masterflex® computerised

pump, at a rate of 3ml/kg per minute. Fluid was given continuously until the target systolic blood pressure (SBP) was attained: further aliquots were given to maintain the target SBP whenever required for the duration of the experiment.

5.5.5 Permissive Hypotension

The target SBP for resuscitation was 80mmHg; representing the hypotensive strategy advocated by the National Institute for Health and Clinical Excellence (NICE) for prehospital fluid resuscitation (273).

5.5.6 Randomisation and Death before Treatment group separation

Animals were allocated randomly at the outset of the study to the treatment groups using a random number table. The study was not designed to be 'an intention to treat' analysis, but rather an investigation of physiological effects of resuscitation. Despite the injury being standardised as far as possible (e.g. reproducible explosive charges, fixed distance between charge and animal, placement of liver snares to anatomical landmarks) a few animals succumbed e.g. due to ventricular fibrillation after combined blast and haemorrhage or catastrophic blood loss during the uncontrolled haemorrhage (verified at post-mortem examination) before instigation of treatment. As such, the few animals that did not reach the point of treatment initiation were excluded, as they contributed no meaningful data to these physiological responses. Therefore, although the intended group for each individual animal was pre-determined according to a random table, if a death occurred prior to commencement of treatment then the animal was excluded and the following animal allocated to the treatment paradigm.

5.5.7 Supplemental Oxygen Group

For the oxygen group, oxygen support was initiated at T30. The oxygen (minimum FiO2 = 0.3) was delivered via an anaesthetic machine and the use of a nose cone overlapping the snout of the intubated animal (Figure 29). The FiO2 was titrated to maintain a SaO_2 of 95%. SaO2 was recorded by blood gas analysis. The 'Blast Air' control group received no oxygen therapy, but did receive a very small saline bolus of approximately 9ml (0.18ml/kg), as this group also served as the control group for the rFVIIa study limb.

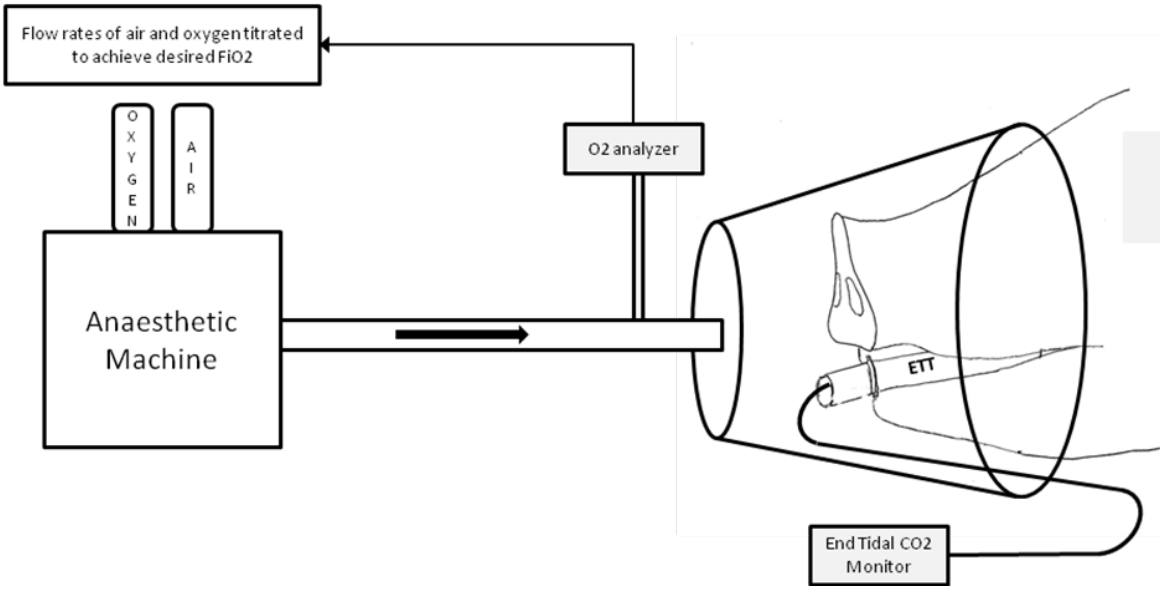


Figure 29 Nose Cone set up for oxygen supplementation at desired FiO2. End tidal CO2 monitoring ensured animal was not 'rebreathing'.

5.5.8 Recombinant Activated Factor VII Group

Recombinant activated Factor VII (Novo Seven™) was purchased from NovoNordisk, Denmark and refrigerated at 4ºC. After preparation, (in line with the manufacturer's instructions), it was administered intravenously as a single dose at T30, adjusted for body weight. The dose was 180mcg/Kg. This dose had previously increased survival time in an animal model of severe incompressible haemorrhage (223).

5.5.9 Control Group

The test groups shared a control group, termed ‘Blast Air’ or ‘Placebo’. As placebo for the rFVIIa study, animals received 0.18ml/Kg of normal saline at T30 (9ml for 50Kg animal). This is a clinically negligible volume of fluid and does not compromise the use of the animals as controls for the oxygen study.

5.5.10 Experimental End Point

The primary end point of the experiments was survival, up to a maximum of 8 hours (T_{480}) after the onset of resuscitation. Death was defined as a pulse pressure of zero. If the animal died prior to T480**,** a Phenobarbitone overdose was administered IV to confirm euthanasia (Euthatal, Merial Animal Health Ltd). If the animal survived to T480, the same overdose was administered to euthanize the animal.

5.6 Post-Mortem Examination

Immediate post mortem examination was performed. Table 10 illustrates data sets and tissue sampling protocols.

Abdominal sutures were cut and the abdomen carefully opened. Free Intra-abdominal blood was collected with suction and pre-weighed gauze packs. The volume of intra-abdominal fluid as an indicator of uncontrolled haemorrhage was calculated. The liver section was examined for completeness of amputation and a note was made of the quality of the clot on the raw surface of the middle lobe. The intestines were examined to identify evidence of blast bowel injury (Figure 31). Sections of non-contused lung, heart, small bowel, kidney and liver were removed (Table 10). Tissue samples for light microscopy were fixed in neutral buffered

formalin (VWR International Ltd. Lutterworth, UK) and prepared in a standard fashion (5.5.8).

The thoracic cavity was opened and the lungs and heart removed. The lungs were examined for blast injury (Figure 30) and a percentage score for blast damage was given to each lobe. Froth or clots in the trachea were noted. The lungs were stripped of mediastinal attachments and weighed to allow calculation of the lung weight index (lung weight/body weight). The heart was inspected for contusions.

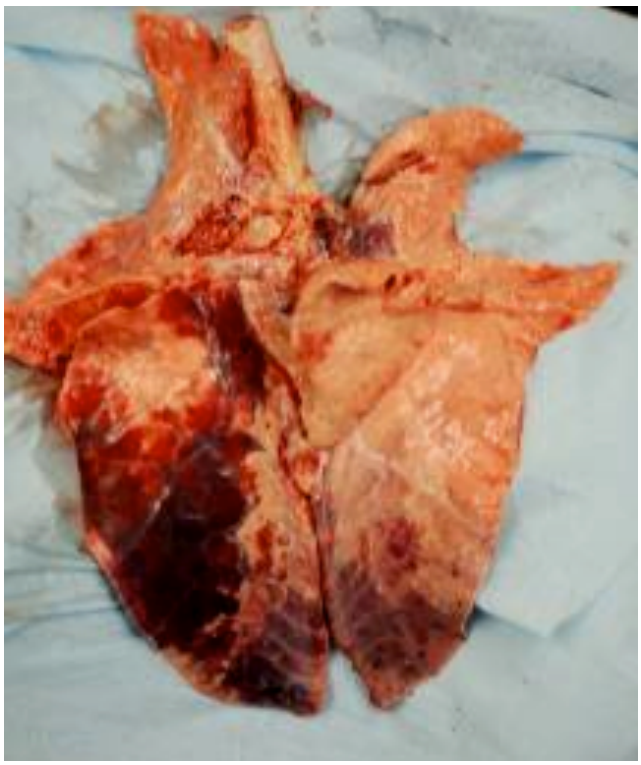


Figure 30 Post Mortem Blast Lung

Figure 31 Post mortem blast bowel

Animal No:	Date:	Wt:	O2 / FVIIa
ABDOMEN		**Uncontrolled Haemorrhage Estimation**	
Volume by Suction			**ml**
***Weight* of Blood in Packs**			**gm**
***Volume* of Blood in Packs (1ml = 1.036 gm)**			**ml**
Total Volume of Intraperitoneal Blood			**ml**
LUNG		**Weight**	**gm**
LIVER section		**Weight**	**gm**

PM Appearance				
Chest Wall				
Liver				
Kidneys				
Heart				
Small Bowel				
Large Bowel				
Lung	Trachea			
	Right	Upper	% *(contused)*	
		Lower	%	
	Left	Upper	%	
		Lower	%	

Samples	**Yes**	**No**	
Liver			*3 samples, away from middle lobectomy: RNA later*
Lung			*Not from contused area: RNA later*
Lung wet/dry			*Not from contused area, 1 sample each lobe (six). Glass pot*
Small Bowel			*Formulin*
Kidney			*Formulin*
Left Ventricle			*Formulin*

Table 10 Post Mortem datasheet

Samples were also taken from the lung and liver for quantitative PCR detection of adhesion molecules, ICAM and VCAM. These were preserved in 'RNA later' (Sigma, UK); an RNA stabilisation solution for tissue sections and refrigerated at 4ºC before analysis. The pathologists examining specimens were blinded to the animals' treatment status.

In order to determine the safety of systemic rFVIIa administration, lung, liver, kidney, small intestine and left ventricular samples were immersed in 10% neutral buffered formalin (NBF) for a minimum of 48hrs. Samples were sectioned to a thickness of 3mm, rinsed in 20% alcohol and processed overnight in a Tissue-Tek Infiltration Processor (VIP). The processed samples were embedded in wax, cut into 5 micron sections, mounted on glass slides and duplicate sections were stained with haematoxylin and Eosin and Martius Scarlet Blue. The slides were examined for evidence of microthrombi or microemboli by Histopathologists blinded to the treatment groups.

5.7 Statistics

All data are presented as mean ± s.e. mean unless indicated otherwise. Survival times in the oxygen, rFVIIa and control groups were compared using a Kaplan-Meier analysis (Mantel-Cox log rank test) using SPSS v10. Data from animals still alive after 8 hours were treated as right-censored. Cardiovascular, blood gas and chemistry data were compared using two-way analysis of variance (ANOVA) with repeated measures over time. Single time-point analyses were made using a Student's t test. Where data was found to be non-normal a non-parametric

equivalent was used as indicated in the text. In all cases a significance level of $P \leq 0.05$ (two tailed) was used.

6 Effects of Supplemental Inspired Oxygen

6.1 Introduction

This study has investigated two adjuncts to fluid resuscitation in an animal model of prolonged resuscitation after haemorrhage and blast injury. Both adjuncts seek to improve oxygen delivery, without increasing the hydrostatic pressure exerted across nascent blood clots.

The relevance of our blast and haemorrhage model to military clinical reality is brought into focus by the fact that the commonest mechanism of injury in current (Afghanistan) and recent (Iraq) conflicts has been the improvised explosive device. In Afghanistan, this has produced an 11% incidence of primary blast injury seen in casualties surviving to reach the field hospital in Afghanistan (186) and a 48% incidence in those sustaining thoracic trauma from blast (JTTR Review of 1678 seriously injured Casualties from Iraq and Afghanistan between Jan 2003 and Oct 2009). Although evacuation times are short in mature theatres, entry operations and immature combat zones tend to incur long evacuation timelines of several hours' duration. The need to prolong life and preserve physiology in critically injured battlefield casualties, without relying on the ability to achieve rapid surgical haemorrhage control, provides the context for this work.

Fluid therapy has been shown to improve survival after blast and haemorrhage, but the efficacy of supplementing oxygen, while adhering to a permissive hypotensive resuscitation fluid target has yet to be determined. The hypoxia that results from blast injury impairs oxygen delivery (the key factor in shock) and haemorrhage reduces both oxygen carrying capacity and flow to the tissues. Supplemental

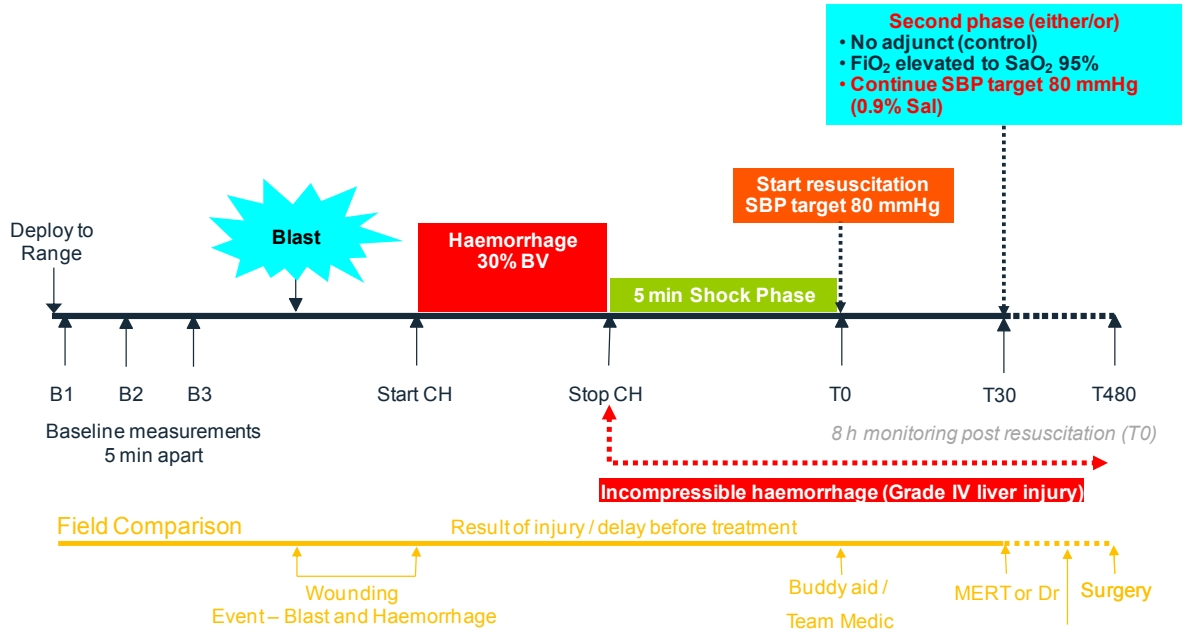
oxygen has the potential to improve oxygen delivery in victims of blast and haemorrhage by increasing the arterial oxygen content. It should exert no increased hydrostatic pressure across a nascent blood clot and will therefore be acceptable to clinicians who hold concerns regarding re-bleeding at sites of vascular injury. Modern oxygen support technologies have made feasible the possibility of supplying oxygen to far forward and remote units.

6.2 Aims

The aim of this part of the study is to provide robust data to answer the question whether oxygen has the potential to preserve life and physiology in critically injured (blast and haemorrhage) prehospital casualties. If proven to be effective, the data will support investment into, and adoption of, a sustainable; remote oxygen support capability.

6.3 Methods

12 terminally anaesthetised swine were exposed to a standardised injury model of primary blast insult, 30% (controlled haemorrhage) blood volume haemorrhage and grade IV liver laceration (via deployment of surgically implanted liver snare to create uncontrolled haemorrhage (5.3.3). All were resuscitated with warmed 0.9% saline to a hypotensive target blood pressure of 80mmHg and the study was run to 8hrs following onset of fluid therapy (5.5.5 and Figure 26). Randomisation separated animals into two groups at 30 minutes: control (‘blast air’) or oxygen supplementation. Oxygen supplementation involved titrated delivery of oxygen via nose cone at a minimum FiO2 0.3 to maintain SaO2 of 95% (5.5.7). Various data were collected throughout the experiments.



6.4 Results

Three animals died before T30 and separation of treatment strategies[3]. These were excluded from the study and are not presented, nor included in statistical analysis. The table below illustrates pre-blast baseline data for the complete oxygen and control groups.

[3] Two animals were destined to be given supplementary oxygen after 30 min of resuscitation, one died of ventricular fibrillation at the end of the controlled haemorrhage and the other failed to respond to fluid resuscitation and died after 28 minutes of resuscitation.

One animal, destined for the control group, failed to respond to fluid resuscitation and died after 10 minutes of resuscitation.

	Blast O2 Mean ± SEM	Blast Air Mean ± SEM	O2 vs. Air (t-test)
n	6	6	
Wt (kg)	51.7 ± 0.8	50.8 ± 1.2	0.563
PaO2 (kPa)	9.1 ± 0.2	8.9 ± 0.3	0.688
PaCO2 (kPa)	6.5 ± 0.1	6.6 ± 0.2	0.86
Art pH	7.43 ± 0.01	7.42 ± 0.02	0.405
ABE (mM)	7.0 ± 0.7	6.1 ± 1.0	0.477
OER	0.27 ± 0.02	0.3 ± 0.02	0.295
Hct (%)	38.7 ± 1.1	37.8 ± 1.1	0.562
T Hb (g/dl)	12.7 ± 0.4	11.6 ± 0.4	0.102
CaO2 (ml/dl)	16.2 ± 0.5	14.2 ± 0.5	0.031*
Art K+ (mM)	3.8 ± 0.1	3.9 ± 0.1	0.305
T (oC)	38.1 ± 0.1	38.7 ± 0.3	0.095

Table 11 Table of baseline data for oxygen support group and air control groups. P-values given for two tailed t-test between groups. * Although there was a statistically significant difference in CaO2 between groups at baseline the difference was small and not likely to be of clinical significance; values in both groups are within normal limits for the anaesthetised pig.

6.4.1 Efficacy of Oxygen Supplementation Method

Blast exposure resulted in a rapid reduction in arterial partial pressure and saturation of oxygen from baseline values to a nadir of 58.1 ± 7.8% and 50.9 ± 6.7% respectively for control and oxygen supplementation groups (Figure 32)[4]. Subsequently, the animals were all given a haemorrhage of 30% blood volume and, by the end of the 'shock phase', arterial oxygen saturation had spontaneously recovered to 71.7 ± 4.4% and 79.9 ± 3.1% respectively for control and oxygen groups. By thirty minutes after the onset of permissive hypotensive fluid resuscitation, arterial oxygen saturation was 68.5 ± 3.4% and 71.1 ± 6.1% respectively. Both groups were therefore profoundly hypoxic and there were no significant differences in arterial oxygen saturations between groups to this point. Thereafter the oxygen group received supplementary oxygen to a target SaO2 of

[4] Note: at this stage of the protocol, both groups are breathing air since oxygen therapy has not yet been initiated.

95%. Administration of oxygen by nose cone led to a significant increase in PaO2 and arterial oxygen saturation. The target SaO2 of 95% was quickly attained and sustained for the remainder of the experiment. By contrast, arterial oxygen saturation failed to improve and remained significantly lower in the control group, which continued to breathe air. There were parallel changes in arterial oxygen tension (Figure 32)

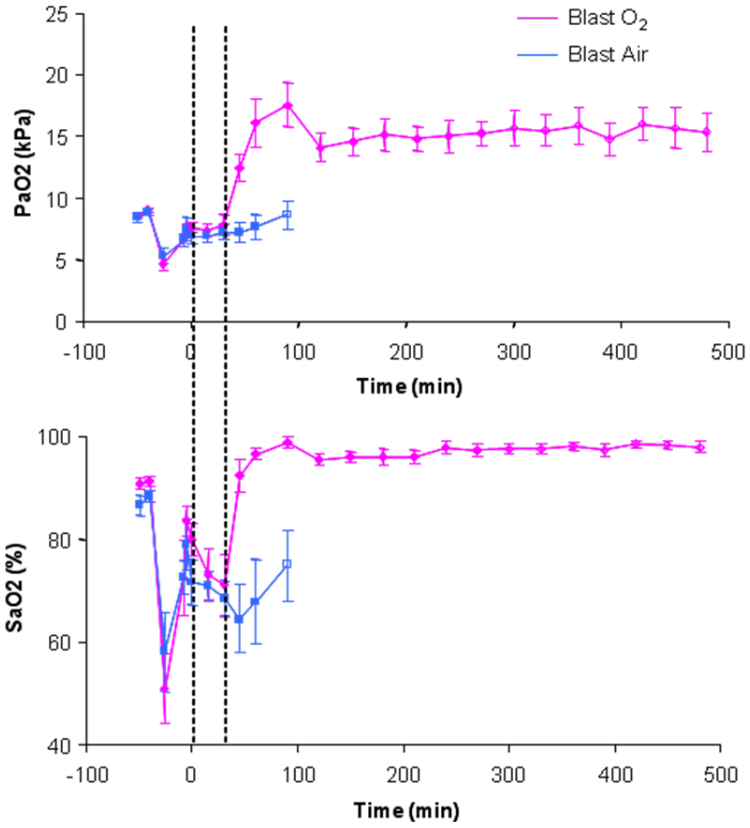


Figure 32 Arterial oxygen tension (PaO2) and saturation (SaO2) in two groups of animals subjected to blast injury, haemorrhage and hypotensive resuscitation. Time '0' means onset of resuscitation and is represented by the first dotted line. The second line is T30 and represents the first point where treatment separates by group.

Figure 33 below illustrates the arterial oxygen content and haemoglobin levels of both groups. By T30, the combined insults and 30 minutes' hypotensive fluid therapy had produced a decline in Hb from baseline (12.7 ±0.4 and 11.6 ±0.4 g/dl) to 9.4 ± 0.5 and 8.8 ± 0.5. By T60, Hb was 9.0 ± 0.5 and 7.5 ± 0.6g/dl respectively (P=0.093). As recorded above, severe hypoxia had also developed at T30.

Accordingly the CaO2 reduced significantly in both groups from baseline (16.2 ± 0.5 and 14.2 ± 0.5 ml/dl) to 10.6 ± 0.6 and 8.3 ± 0.3. By T60 however, 30 minutes after the onset of oxygen supplementation, the CaO2 had diverged significantly between groups: CaO2 in the oxygen group was 12.9 ± 0.3 compared to 7.2 ± 1.1 in the air-breathing group (P=0.006). The CaO2 in the oxygen group remained higher than that in air control group at all times.

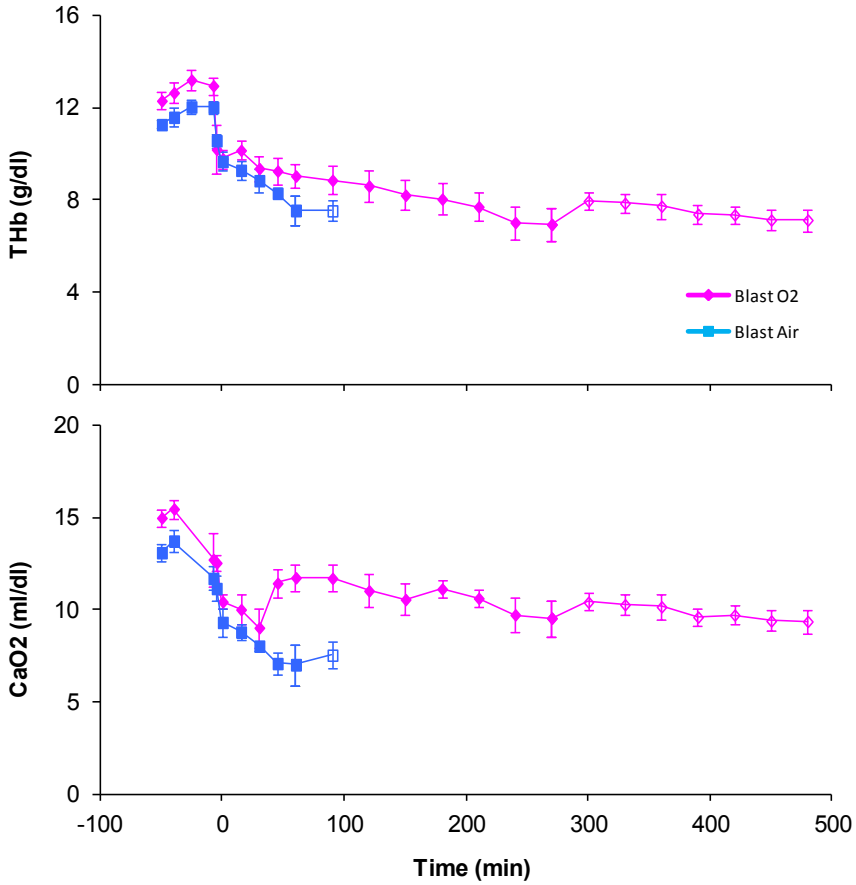


Figure 33 Total haemoglobin concentration and arterial oxygen content for both groups.

6.4.2 Survival

The primary endpoint of the study was survival. Supplemental oxygen significantly increased survival time from 195 [84-306] min (mean [95% CI]) in the control group

to 414 [340-489] in the group given supplementary oxygen (P=0.014 Mantel Cox log rank) (Figure 34).

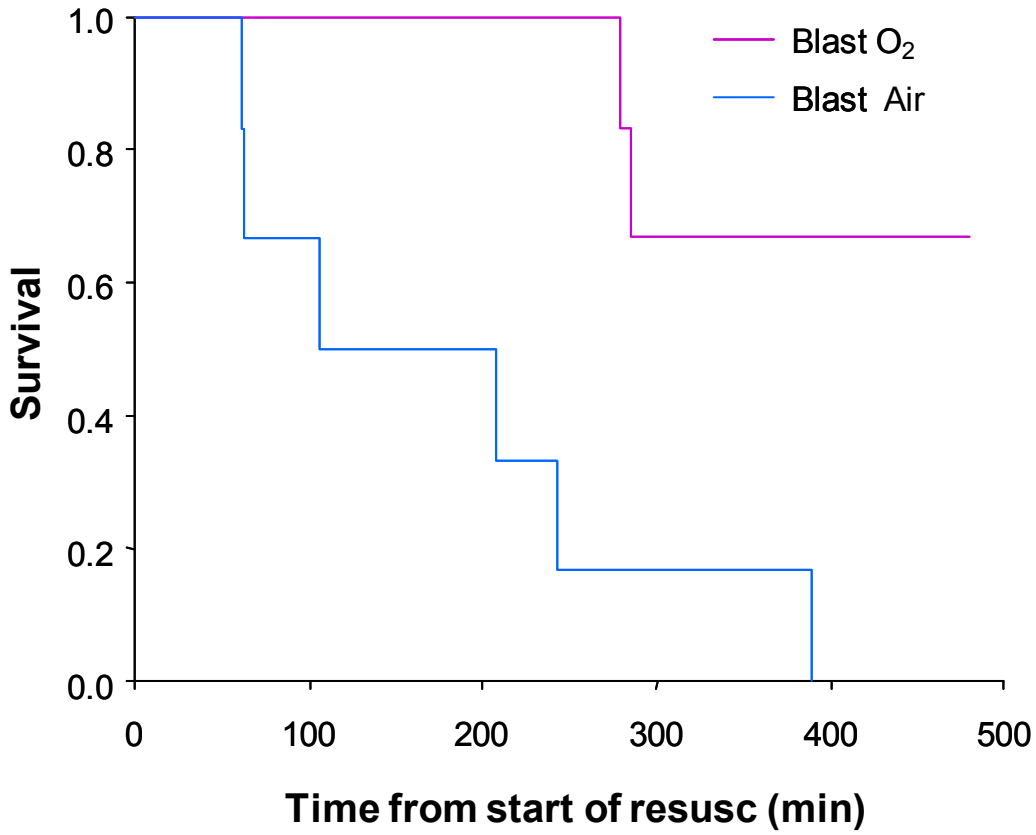


Figure 34 Kaplan-Meier Survival plot for two groups of animals subjected to blast injury, haemorrhage and hypotensive resuscitation. The Blast O_2 group was given supplemental oxygen to elevate the SaO_2 to ≥95% and was begun after 30min hypotensive resuscitation. Blast Air animals simply breathed room air (FiO_2 0.21) throughout.

6.4.3 Physiology

As well as improving survival, supplementary oxygen had an effect on the development of metabolic acidosis. The combined insults of blast injury and haemorrhagic shock led to a profound, statistically significant, fall in arterial base excess in all animals (Figure 35) (P=0.0151).

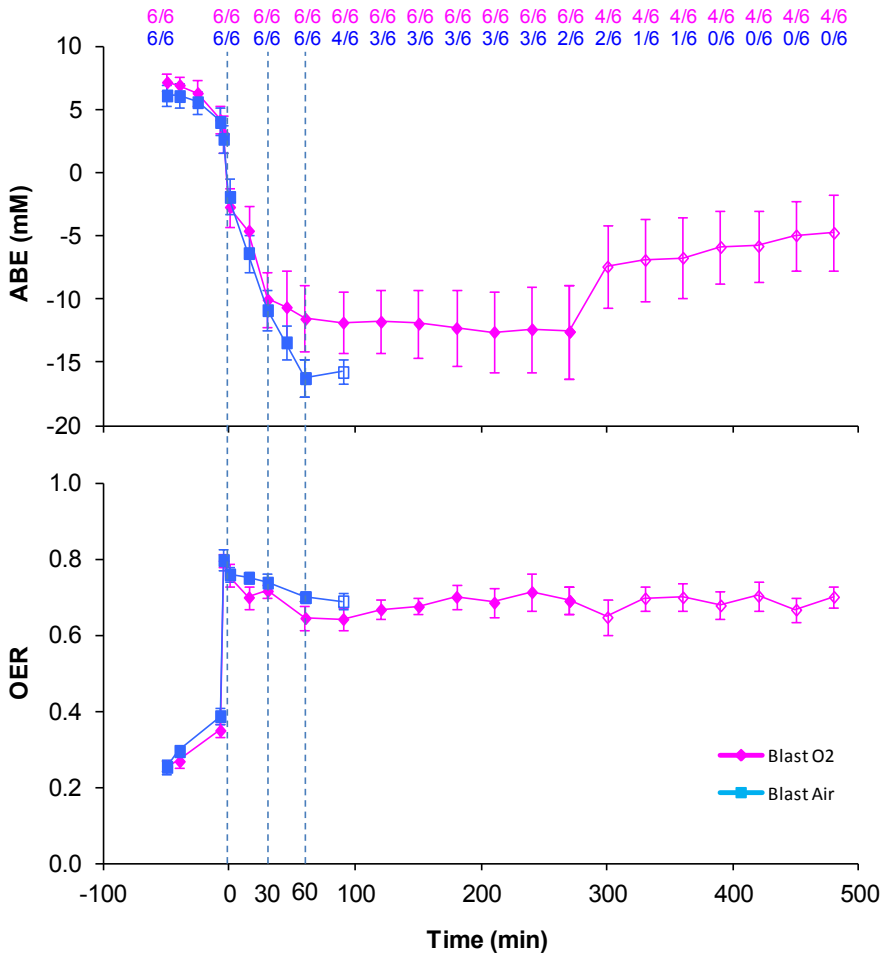


Figure 35 Arterial base excess (ABE) and Oxygen Extraction Ratio (OER) in two groups of animals subjected to blast injury, haemorrhage and hypotensive resuscitation. Time indicates time from onset of resuscitation which was initiated at the first dotted line. Second dotted line (30 min after onset of resuscitation) represents the divergence of treatment groups with supplemental inspired oxygen being introduced into the Blast O2 group while the Blast Air group continue to breathe air and the third dotted line indicates 30 min after the divergence of the treatment groups. First two values represent Baseline, followed by Post Blast, Pre and Post Haemorrhage and then onset of resuscitation. Open symbols indicate 66% of animals surviving. No data plotted when proportion surviving fell to 50% or below. Numbers across top of figure indicate number of surviving animals/original number for each group (colour coded as indicated). Mean values ± SEM.

By 30 minutes after the onset of hypotensive fluid resuscitation, the ABE had declined to -10.6 ± 1.6mM and -10.0 ± 2.2mM for control and oxygen groups respectively. At this stage there was no significant difference between groups (P=0.2412). 30 minutes later, ABE had declined further, to -16.2 ±1.5mM in the control group breathing air. However, in the oxygen group the decline was arrested

and, at the same time point, the ABE was -11.5 ±2.6mM. The difference between treatments (air vs. oxygen) was statistically significant (P=0.0137). Beyond this time point there was rapid loss of animals in the control group (Figure 35). By contrast, in the oxygen group, base excess remained constant without recovery or deterioration until 270 min from the onset of resuscitation. Thereafter there appears to be an increase in base excess, however this improvement is more apparent than real since this is due to the death of the two most acidotic animals (Figure 36).

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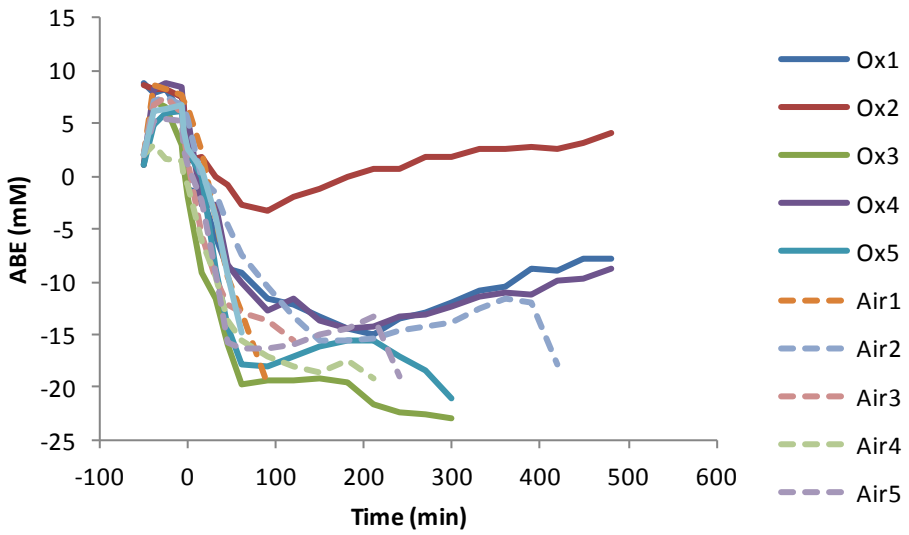

Figure 36 Individual ABE data for oxygen and blast air control animals. Oxygen group in solid lines.

It is clear from Figure 36 that one animal (Ox2) did not become as acidotic as the others in the group, even before the administration of oxygen. There is no identifiable reason for this difference as it was treated identically to the others throughout, and therefore the difference is likely to represent normal the normal biological variation that sometimes results clinically in unexpected survivors. Since this animal was an outlier a 'what if' examination of the data was made to determine whether the same overall outcome would be seen in the absence of this animal. The

base excess portion of Figure 35 has been redrawn using mean data derived after excluding animal Ox2, and the result shown in Figure 37.

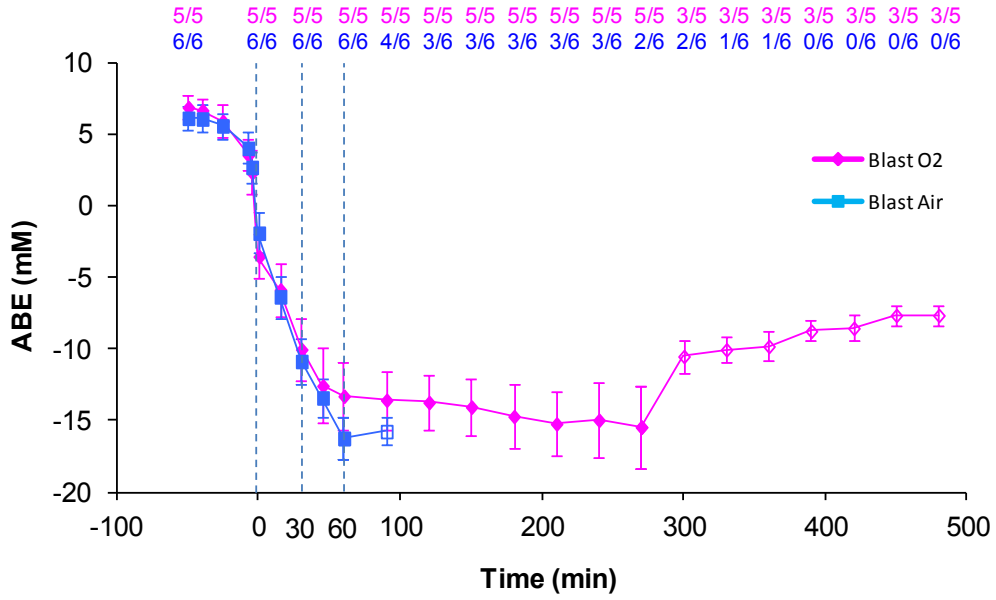

Figure 37 Arterial base excess (ABE) in two groups of animals subjected to blast injury, haemorrhage and hypotensive resuscitation. The data for the group that breathed air throughout (Blast Air) is identical to that shown in Figure 35, while the data for the group given supplemental oxygen 30 min after the onset of resuscitation (Blast O_2) represents mean values from the group after exclusion of an outlier (Ox2). See legend to Figure 35 and text for further explanation.

Statistical re-evaluation, with Ox2 excluded, revealed no significant difference in ABE between the air and supplemental oxygen groups from the onset of resuscitation to 60 min after the start of resuscitation (P=0.4756). Given the small number of animals in the study, and the dangers associated with excluding individuals without a clear *a priori* rationale, it is impossible to be certain whether supplemental oxygen has or has not resulted in an early, significant, improvement in base excess. However, regardless of this, it is clear that supplemental oxygen has had an overall beneficial effect, even if Ox2 is excluded, since the outcome regarding the primary variable, survival, is unaffected by exclusion of Ox2 (a significant difference in survival time still exists between air and supplemental oxygen groups even after exclusion of Ox2, P=0.034).

Returning to the full dataset, blast exposure led to a small increase in OER from baseline values: 0.30±0.02 and 0.27±0.02, to 0.39±0.02 and 0.35±0.02 in the control and oxygen groups. Haemorrhage however led to a large increase in OER in both groups, to the physiological maximum 0.80±0.03% and 0.80±0.02% respectively. At T30, there was no difference in OER between groups 0.74±0.02% and 0.72±0.02% (P=0.470). By T60 minutes there was a trend toward a slightly reduced OER in the oxygen group: 0.65±0.03% vs. 0.70±0.01 in the control group, (P=0.170).

Consistent with the changes in OER, there were also alterations in systemic oxygen delivery and consumption. Oxygen delivery (DO2) and consumption (VO2) were both similar between groups before the initiation of oxygen therapy (30 min after the onset of resuscitation). Thereafter in the oxygen group, there was a trend towards increased oxygen delivery and importantly a corresponding increase in oxygen consumption, although neither achieved statistical significance (P=0.093 and P=0.181 respectively for group differences in DO2 and VO2) (Figure 38), possibly because of the small group sizes.

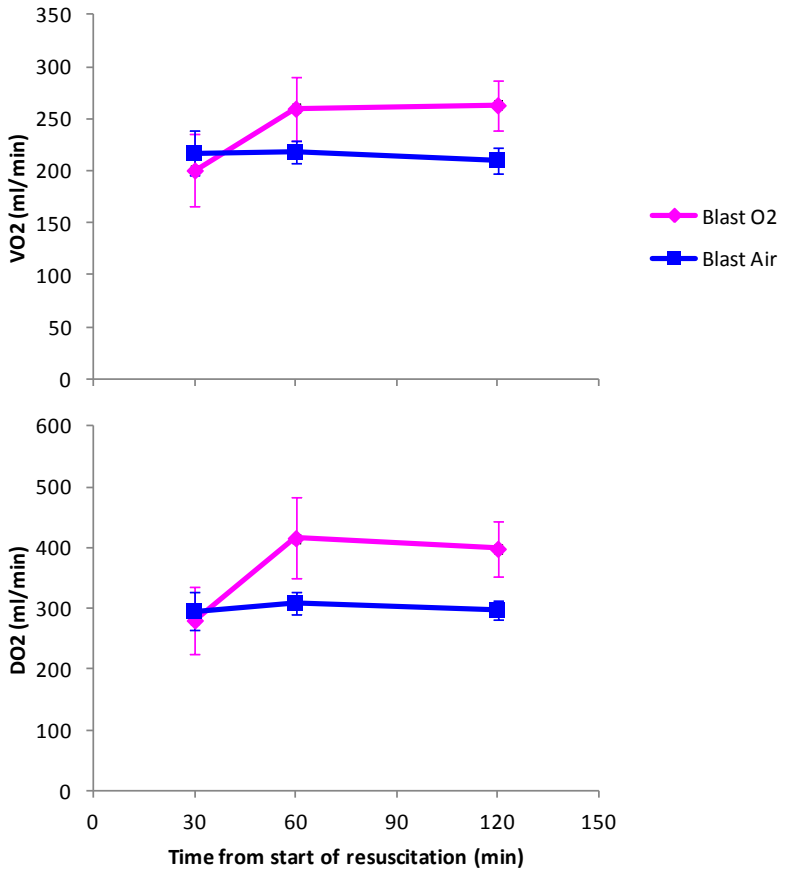

Figure 38 Oxygen delivery (DO2) and consumption (VO2) immediately before initiation of oxygen therapy 30 min after the onset of resuscitation and again at 60 and 120 minutes after the onset of resuscitation. Mean ± SEM.

This trend could be consistent with oxygen consumption being supply-dependent before the initiation of oxygen therapy, and a consequent improvement in delivery allowing an increase in consumption in at least some of the animals given oxygen therapy. Perhaps a more informative way of evaluating this possibility is to examine the relationship between DO2 and VO2 in individual animals (Figure 39).

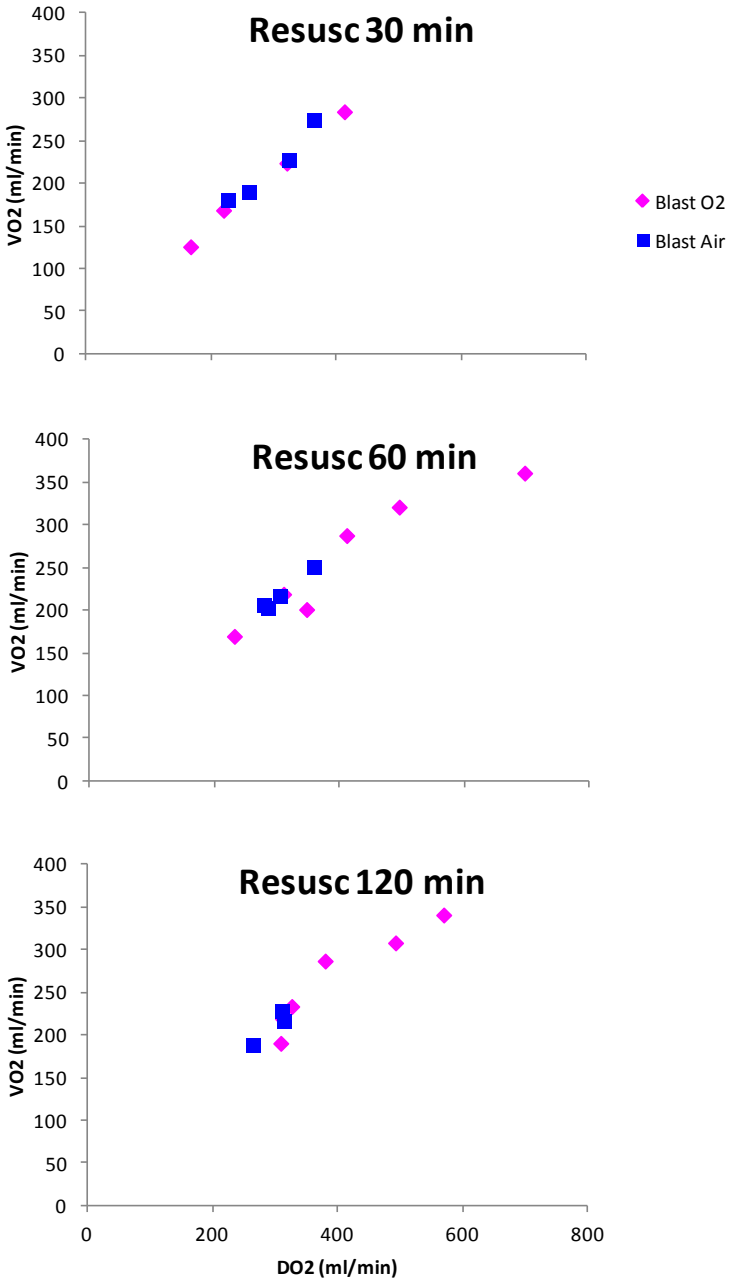

Figure 39 Relationship between oxygen consumption (VO_2) and delivery (DO_2) in individual animals immediately before initiation of oxygen therapy (30 min after the onset of resuscitation, top panel) and again 60 and 120 min after the onset of resuscitation (middle and bottom panels respectively). Note that these are not DO_2/VO_2 curves for individual animals but rather single points on the DO_2/VO_2 relationship for each animal at each timepoint.

Although full DO2/VO2 curves for each individual animal would be required to determine whether they are on the supply-dependent or supply-independent portion of the relationship, the data shown here, coupled with both the ABE and OER data,

suggests that the animals are predominantly on the supply-dependent part of the relationship before the administration of oxygen. By 60 min after the onset of resuscitation (30 min after initiation of oxygen therapy) a number of animals in the oxygen group have increased DO_2 and seem also to have been able to elevate VO_2, suggesting an improvement in their oxygen transport status compared to the air-breathing controls. This pattern persists 120 min after the onset of resuscitation.

CO2 and arterial pH levels were measured in both groups (Figure 40). There was a significant rise in PaCO2 and a corresponding drop in arterial pH following blast injury. Haemorrhage did not further increase PaCO2, but pH dropped significantly. There was no difference in PaCO2 between groups, but levels remained below baseline in all animals.

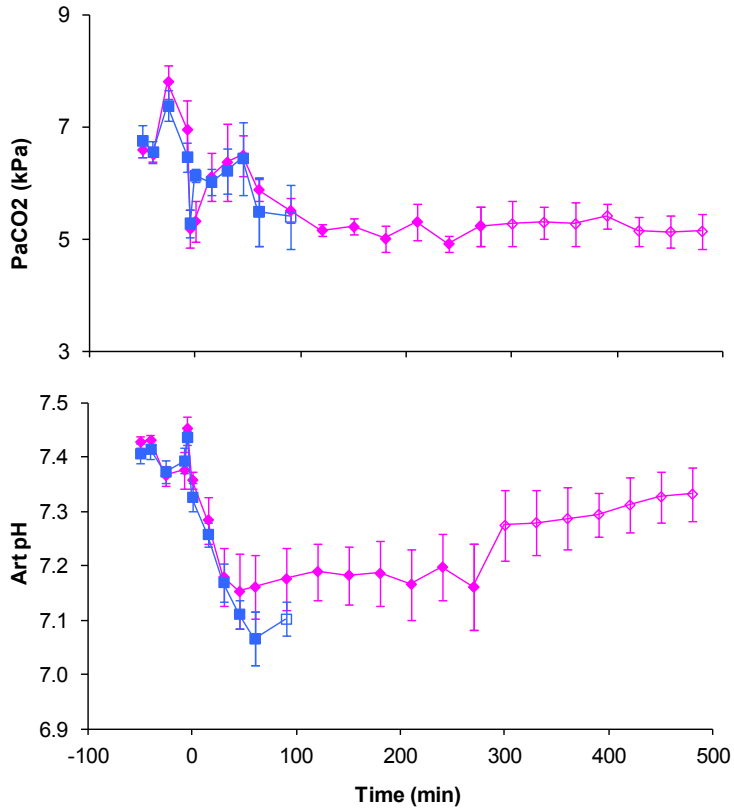

Figure 40 Arterial partial pressure of CO2 and arterial pH for both groups. The apparent recovery of pH in the oxygen group, as mentioned for ABE, is due to the demise of the two sickest animals, rather than a true recovery.

6.4.4 Coagulation

As discussed earlier, coagulopathy in trauma is a significant problem. This institution has already described an ultra-early hypercoagulability after blast injury and haemorrhage (274). Brohi and colleagues at The Royal London Hospital have revealed an early hypocoagulopathy early after trauma in civilian patients and coined a term 'acute coagulopathy of trauma shock' (17). Tissue hypoperfusion has been promoted as a key driver of this coagulopathy. This study assesses interventions designed to improve tissue perfusion, one of which is a recombinant coagulation factor, so it is relevant to establish whether the improved tissue oxygen delivery (demonstrated above) is accompanied by an improvement in clotting parameters. Thromboelastography (TEG) samples were taken at standard intervals during this

study (Figure 41). However, due to a high degree of variability, particularly in the air group, there was no significant difference between the air and supplementary oxygen group. Overall a trend of worsening clotting dynamics (increased K time) was seen during the early phase of resuscitation in the air group when compared to those given supplementary oxygen.

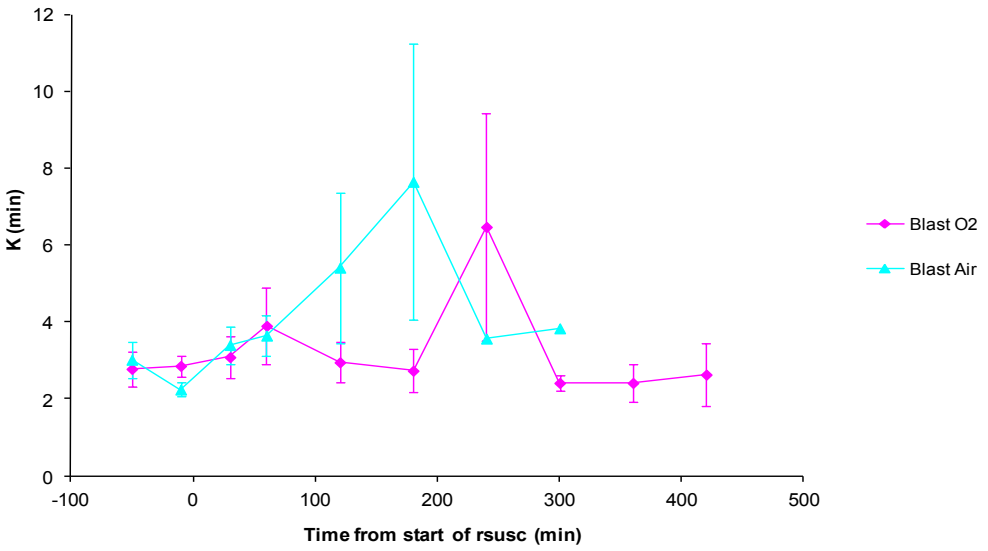

Figure 41 K Time (Mean +/- se of mean) for blast oxygen and blast air control groups.

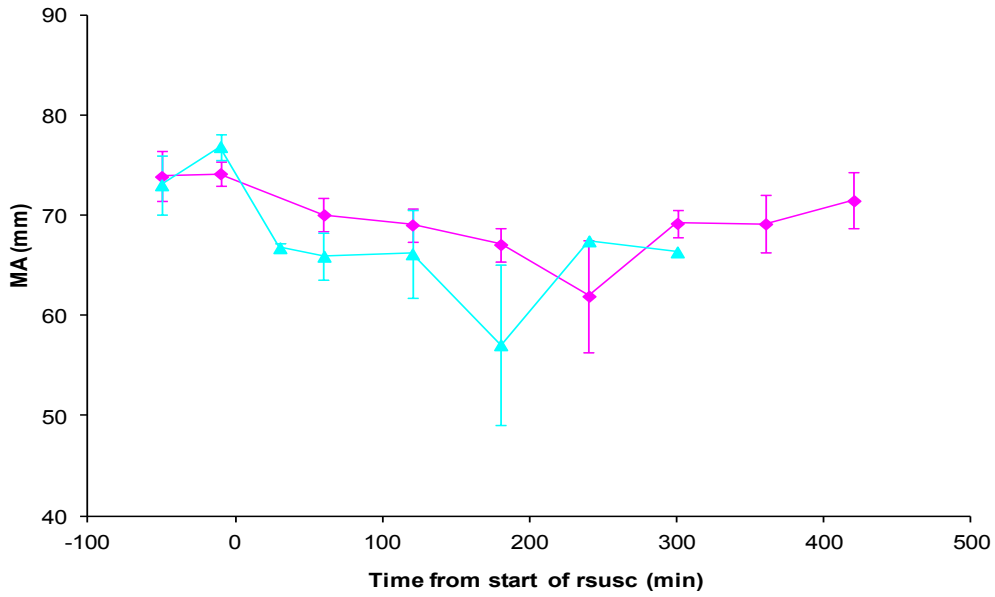

Figure 42 Maximum Amplitude (Mean +/- se of mean) for blast oxygen and blast air control groups.

MA (representing clot strength) was found to fall significantly between baseline and 120 min after the onset of resuscitation (P=0.0254; statistical analysis was not continued beyond this point due to the loss of animals from the air-breathing group). As with K time, there were no significant difference in MA between treatment groups.

6.4.5 Lung Inflammatory Changes

The relative expression of the endothelial adhesion molecules, ICAM and VCAM from lung tissue are shown in (Figure 43). Overall there was less expression of both molecules in the oxygen group. Although this did not achieve statistical significance for VCAM (P=0.126), it barely failed to achieve significance for ICAM (P=0.052, Pfaffl method (275)).

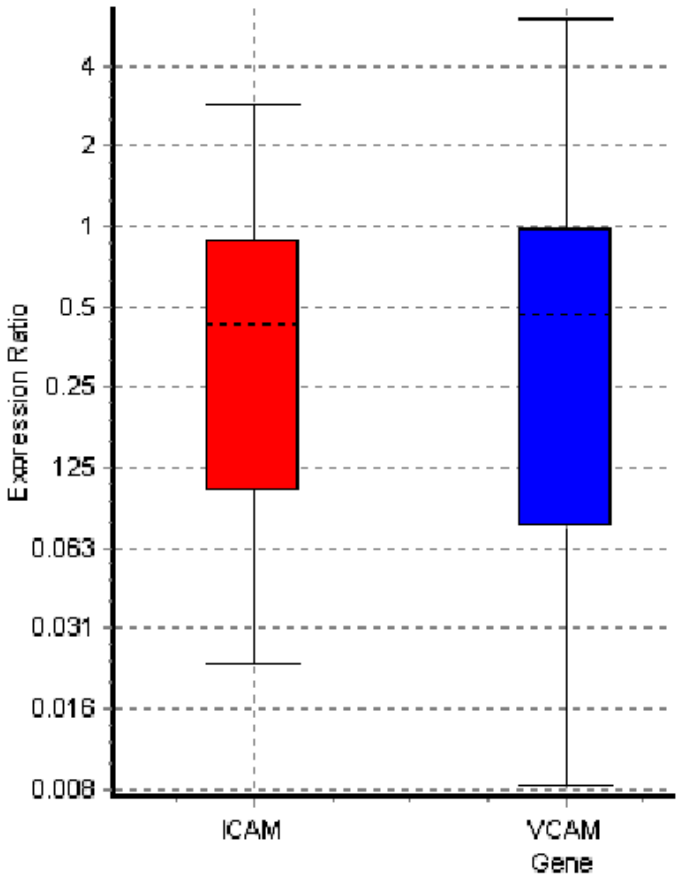

Figure 43 Relative expression in oxygen group as a proportion of expression in the air control group. A value below 1 suggests less expression in the oxygen group

Lung tissue was harvested at post-mortem for all animals. No difference was seen between groups on gross morphological examination. Both groups exhibited clinically significant pulmonary contusions (Figure 44 and Figure 45) although this was not quantified. The lungs were however weighed immediately post-mortem and the lung weight index calculated for each animal. There was no significant difference in lung weight indices between groups: 13.2±0.7 and 12.9±0.5 g/kg respectively in the air and oxygen groups, (P=0.72), suggesting that the degree of lung water content was similar between groups. However, this latter observation must be caveated with the finding that survival time; and hence the time available to develop pulmonary oedema; was significantly different between groups.

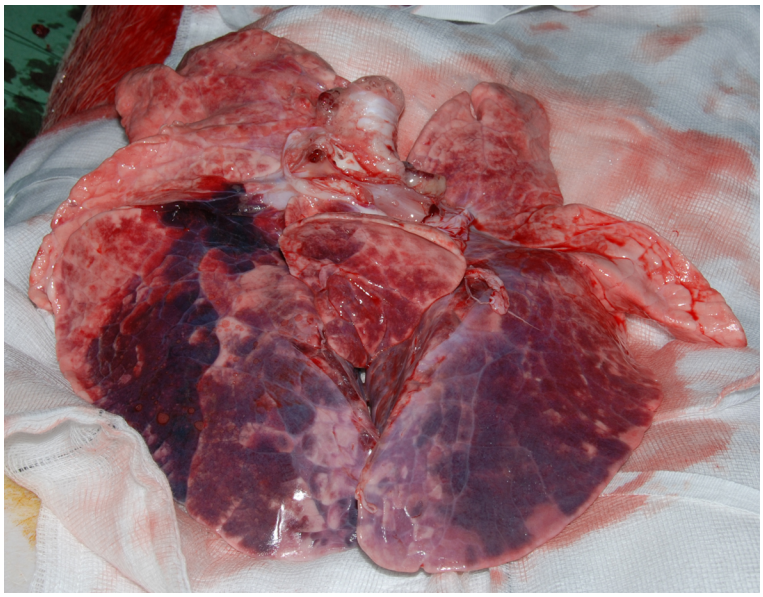

Figure 44 Post mortem photograph of blast lung injury (right side nearer explosion)

Figure 45 Sectioning of same right lung demonstrates extent of contusions

6.5 Discussion of Oxygen Supplementation Results

This study has shown that supplemental inspired oxygen significantly increases survival in a large animal model of combined blast and haemorrhage injury. In addition, oxygen therapy halted the physiological deterioration (increasing base deficit), but did not deliver recovery of physiology to normal. There was no evidence of an increase in lung damage or early inflammation in animals given supplementary oxygen. Furthermore, there was no evidence of increased haemorrhage associated with supplemental oxygen therapy.

6.5.1 Injury model

The details of blast exposure are discussed in the methods section (5.5.1). Significant blast injury was confirmed in all animals by the reflex bradycardia, apnoea and hypotension that immediately followed the exposure and by noticeable changes in PaCO2; OER; SaO2 and pH following blast exposure. Mean SaO2 at T30 was 69%. Post-mortem analysis demonstrated significant lung contusions in all animals. At the same time, the blast loading was not immediately overwhelming and was compatible with survival of the animals.

Liver snare deployment successfully amputated the desired section of the middle lobe of the liver – this was confirmed at post mortem (Figure 46). This created a Grade IV liver injury – a mixed arterial and venous insult with the potential for rebleeding at any subsequent stage of the study.

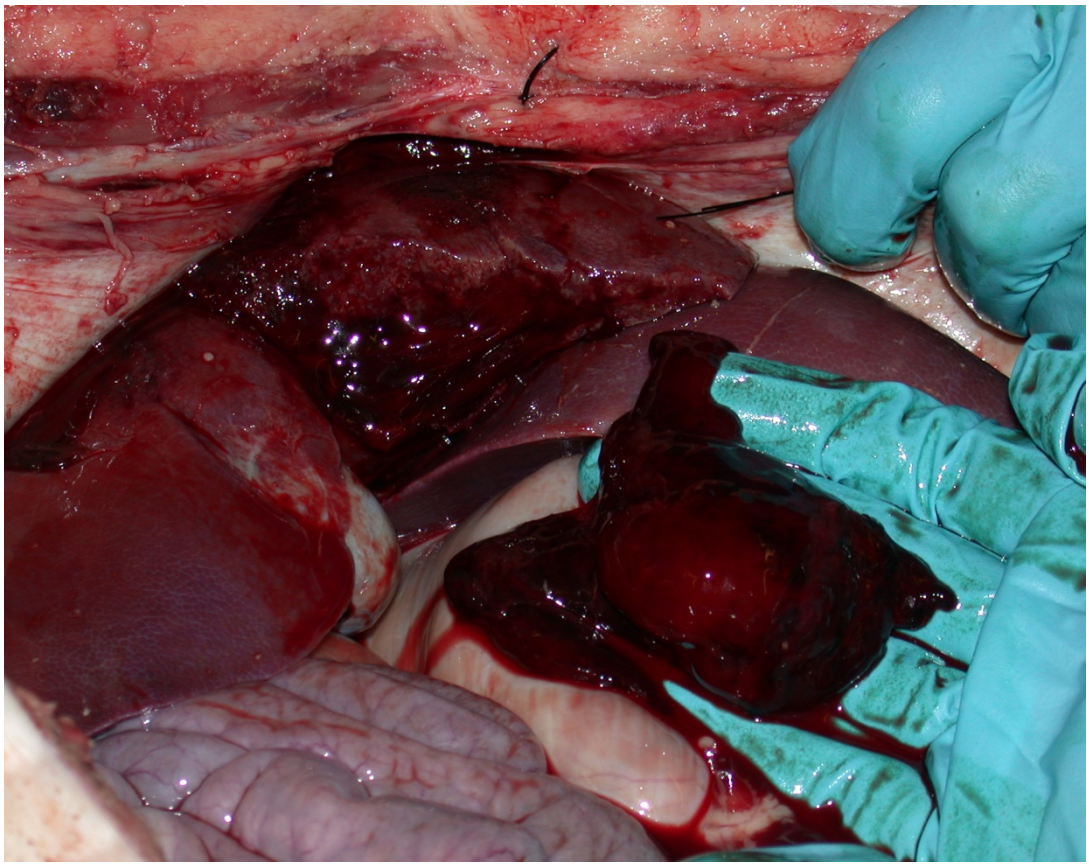

Figure 46 Post mortem photograph of removal of adherent clot (in hand) from raw amputation site on middle lobe of liver. Note complete amputation.

The controlled 30% blood volume haemorrhage was successfully completed via pump-controlled exsanguination from the femoral arterial line (5.5.2). Haemorrhage produced significant changes in OER; ABE and pH. By T30, (35 minutes after completion of controlled haemorrhage and liver snare deployment and after 30 minutes' hypotensive fluid therapy), all animals were in profound metabolic acidosis.

The two injury mechanisms combined produced a significant physiological insult in the animals, but this was not so severe that all animals died regardless of treatment modality. The injury model, coupled with prolonged hypotensive resuscitation therefore presents a significant challenge to potential resuscitation adjuncts that is relevant to battlefield casualty care.

6.5.2 Effects of supplementary oxygen on survival

The combined insults of blast lung and haemorrhage invoke a 'double hit' on oxygen delivery: hypoxia from blast lung reduces arterial oxygen content while haemorrhage primarily reduces flow (although later, arterial oxygen content is also reduced through reduced haemoglobin concentration). Standard trauma fluid resuscitation strategies attempt to restore the flow component of DO2, but when restricted to hypotensive resuscitation, this is very limited and, alone, incompatible with prolonged survival in the presence of blast injury. In the blast-injured casualty, because of the hypoxia, there is scope to improve the arterial oxygen content and, hence, an additional 'angle' one can employ to improve DO2. The DO2 equation is repeated below for clarity and discussed in more detail earlier (Equation 1).

(DO_2) (ml/min) = cardiac output (SV x HR) x arterial oxygen content ([Hb] x SaO2 x 1.34)

Administration of oxygen improves survival by increasing arterial oxygen content and, hence, tissue oxygen delivery. This is borne out by oxygen's effect on physiology. By T30, all animals were in profound metabolic acidosis, with ABE between -10 and -11. Thereafter, in animals given oxygen, this metabolic acidosis remained at similar levels, but deteriorated no further. By contrast, control group animals suffered inexorable physiological decline until death. To our knowledge this is the first study that has systematically tested the efficacy of oxygen supplementation alongside hypotensive fluid therapy following combined blast injury and haemorrhage.

6.5.3 Physiology

6.5.3.1 Oxygen effect

At first glance, it may seem a foregone conclusion that oxygen therapy would improve survival in this study scenario, but there are two important reasons why this might not have been the case in this study. First, the profound shock, coupled with prolonged hypotensive resuscitation, might have been so severe that, even despite increasing CaO2, animals would all have succumbed. Second, the lung injury from the blast insult might have obstructed gas exchange to such an extent, that despite increasing FiO2 to maximum, CaO2 would not increase enough to influence outcome.

By T30 (30 min after onset of hypotensive fluid therapy) ABE averaged between -10 and -11mM and OER was at an almost maximal figure of 72-74%. Despite this, supplemental oxygen was able to increase CaO2 to such an extent that life could be maintained and further development of acidosis arrested.

The effects of blast lung have been described earlier (2.2.4). Diffuse alveolar haemorrhage is a key very early pathological consequence and it significantly impairs gas exchange. Areas of macroscopic contusion seen at post-mortem represent underlying diffuse haemorrhage. When injured segments of lung cannot exchange gas, but perfusion to these segments continues, a shunt occurs. In the lungs, this describes a mismatch between ventilation and perfusion. If large enough, a shunt leads to significant percentages of cardiac output passing through the lungs without experiencing gas exchange. A shunt, at least in part, contributes to the hypoxia associated with blast wave exposure. However, oxygen therapy was rapidly

able to improve saturations from 69% to >95%. This highlights the ability of improved FiO2 to improve overall gas exchange, despite the presence of a shunt, in severely blast-injured lungs. In addition, the additional oxygen may have improved oxygenation in areas of marginal gas exchange, thereby effectively reducing shunt fraction. In the military setting, a casualty who has sustained 'total' blast lung would probably die at the scene. Potentially salvageable casualties should however benefit from oxygen support.

6.5.3.2 Base Deficit and Oxygen Extraction

At T30, there was no difference in ABE or OER between groups: all animals had significant metabolic acidosis and maximal OER. From this point, control animals continued to deteriorate until all succumbed. In contrast, oxygen therapy in 4/6 animals halted the deterioration in ABE that occurred in control animals, but did not restore ABE to physiological levels (Figure 36). This lack of physiological recovery demonstrates that inadequate blood flow was the limiting factor. Improving the CaO2 alone does not improve DO2 enough to allow repayment of the 'oxygen debt' already acquired.

OER data support this notion. At T30 both groups had similar, almost maximal OER (0.72 ± 0.02 and 0.74 ± 0.02). At T60 there was only a trend towards slight reduction in OER in the oxygen group (0.65±0.03% vs. 0.70±0.01 in the control group, P= 0.170). If oxygen therapy were able to restore DO2 to physiological levels, one would expect to see a larger drop in OER. In essence, DO2 is improved by oxygen therapy from 'grossly inadequate', to 'barely adequate' levels.

6.5.3.3 Carbon Dioxide

CO_2 levels were seen to drop a little in both control and oxygen supplementation groups. There was no significant difference between groups. This fall in CO_2 is probably due to the effects of hypoxia and acidosis on central and peripheral chemoreceptors. Chemoreceptors influence ventilation in response to alterations in plasma $[H^+]$; PaO_2 and $PaCO_2$.

Peripheral chemoreceptors lie within the carotid and aortic bodies. They primarily respond to decreased PaO_2, but also to increased $[H^+]$. Efferent activity from these receptors results in increased ventilatory drive. All animals were rendered hypoxic and acidotic by the injury model. The combination of both will therefore have produced significant efferent activity from these peripheral chemoreceptors and, hence, an increase in respiratory minute volume. Increased respiratory minute volume will reduce $PaCO_2$. The acidosis remained throughout the experiment, so peripheral chemoreceptors will have continued drive additional ventilation throughout. In the oxygen group, hypoxia was rapidly reversed, removing one potent stimulator of peripheral chemoreceptors. Acidosis also stabilized, rather than continuing to deteriorate, further reducing the stimulus on peripheral chemoreceptors in comparison to control animals. One might therefore have expected a relative rise in the CO_2 levels in the oxygen group compared with controls as the peripheral chemoreceptors would be relatively unloaded (respiratory minute volume was not measured in this study). There were however, no significant differences in $PaCO_2$, perhaps because control animals did not live long enough to demonstrate an effect. In future, measuring respiratory minute volume may add clarity to these concepts.

Central chemoreceptors in the medulla are relatively insensitive to PaO2 fluctuations and cannot respond directly to plasma [H+] as H+ cannot cross the blood-brain-barrier (BBB). Instead, they are acutely sensitive to changes in PaCO2. CO2 diffuses easily across the BBB, so levels in CSF closely match those in plasma. In CSF, CO2 forms carbonic acid, which then dissociates into H^+ and $HCO3^-$. The H^+ stimulates the chemoreceptor which, via Intercostal and Phrenic nerves, increases rate and volume of breathing. Blast exposure produced a reflex period of apnoea, which was followed by rapid, shallow breathing. This led to a significant rise in PaCO2. The increased CO2 will have triggered the central chemoreceptors to increase the volume and rate of breathing and contributed to the reduction in PaCO2 that followed the initial rise. Once CO2 had reduced below baseline levels, the central chemoreceptors would have ceased to contribute.

6.5.3.4 Flow limitation and oxygen carrying capacity

Another way to improve oxygen delivery is to address the flow component. When using crystalloid solutions such as normal saline, one must bear in mind that dilution of the intravascular fluid will occur and this will, in turn, reduce CaO2 by reducing haemoglobin levels and hence oxygen carrying capacity. For a benefit to be seen, this reduction in CaO2 would need to be outweighed by the benefit of improved flow. A previous DSTL study developed the novel hybrid strategy (hypotensive target with normal saline fluid therapy for first hour, followed by normotensive fluid resuscitation), which improved DO2 to such a degree that ABE recovered to normal levels (39). There was no evidence of re-bleeding. These results suggest that optimising the flow component overcomes the dilution effect and is more potent than manipulation of CaO2 (by simple increase of arterial oxygen saturation) on a background of hypotensive fluid therapy. However, if novel hybrid fluid strategies

AND supplemental oxygen were employed in tandem, it is logical to predict that the consequences of hypoperfusion would more rapidly be reversed and physiology therefore quickly optimised in the pre-hospital environment.

So, improved oxygen content and flow are both able to improve outcome. Together, their effects should complement each other. Another approach would be to employ a fluid which did not cause haemodilution. Such a fluid could have a dramatic effect on oxygen delivery and, therefore, physiology. This might be achieved by infusing a fluid that can carry oxygen. Such a fluid could increase both oxygen carrying capacity and restore intravascular volume: it would therefore address both the CaO2 and the flow components of oxygen delivery.

A logical first step might be to consider giving packed red blood cells (PRBC) as the primary resuscitation fluid. This approach is however limited by a maximum haematocrit that can be tolerated, before hyperviscosity impairs microcirculation – this is thought to lie around 70% (276). While limited blood products (2 units PRBC; 2 units FFP) are now available on the Medical Emergency Response Team (MERT) airframe, blood is not routinely available for use at the scene of wounding. Storage, resupply and issue of blood products is complex and remains beyond the scope of far forward units,

So what other products might be used to improve oxygen carrying capacity? Much research effort has gone into development and evaluation of synthetic haemoglobin oxygen carrying (HBOC) solutions for use in trauma and high-risk surgery and four products have been assessed in clinical trials since 1990. These solutions contain

polymerized and purified human/bovine/ recombinant haemoglobin and attempt to reproduce blood's haemoglobin concentration, affinity for oxygen and osmotic pressure. They are an attractive pre-hospital option and, could dramatically reduce blood product use in the acute stages of trauma resuscitation. Unlike blood products, they do not induce an inflammatory response (277) and are easily stored. Treatment would increase oxygen carrying capacity, independent of the need for oxygen support, thereby potentially removing the need for oxygen at all, or reducing the volume of oxygen required to achieve target saturations. This could reduce the logistic demands on the delivery of oxygen or blood products to remote environments and pacify concerns surrounding the risks of oxygen therapy in blast-injured lungs.

Despite early promise in clinical trials of HBOC use in trauma (278), products tested in clinical trials have shown toxicity and vasoactivity which have hampered their development (279). The tendency of HBOCs to induce vasoconstriction has been attributed to nitric oxide scavenging by free haemoglobin (280) and oversupply of oxygen along the vasculature to deplete endothelial NO (279). Increased mortality and ARDS / MOF rates (attributed to NO vasoactivity) have terminated two clinical trials and elderly patients seem particularly vulnerable (281).

No HBOC product is currently approved for use in trauma patients. However, one recently developed HBOC, maleimide-polyethylene glycol haemoglobin (MP4OX or Hemospan) (Sangart Inc, San Diego, CA) has been developed to possess low Hb concentration, high affinity for oxygen and have high colloid osmotic pressure. These properties aim to maintain intravascular volume, thus minimizing tissue NO

scavenging and to target oxygen unloading to areas of hypoxia (282). These designed differences should theoretically improve the safety and efficacy of MP4OX. It has shown preclinical promise in a large animal model of uncontrolled haemorrhage where survival from aortotomy-induced haemorrhage was significantly improved compared to crystalloid controls (283). A recent RCT in orthopaedic hip surgery with spinal anaesthesia, showed reduced hypotensive episodes following MP4OX, but did demonstrate a transient increase in liver transaminase enzymes. There was however no significant difference in major adverse events. MP40X has recently completed a Phase IIa international RCT in trauma haemorrhage-induced lactic acidosis . Full results are yet to be published, but presented headline data (51 patients) revealed improved lactate clearance and a trend towards shorter hospital stay and improved 28 day survival (284). There was also an acceptable safety profile. MP40X is currently undergoing a Phase IIb clinical trial (TRA-205: http://clinicaltrials.gov/ct/show/ NCT01262196). This will recruit 360 patients with similar inclusion criteria to the TRA-204 trial.

So far therefore, MP40X is promising and may escape the vasoactivity that has limited other products. Ongoing trials will shortly clarify its role and safety in trauma haemorrhage. However, as a pre-hospital resuscitation adjunct, MP40X might be assessed for military value in a similar injury model to that used in this study.

6.5.4 Coagulation

Coagulopathy in trauma has been discussed earlier (2.1.4) and is a significant problem in military trauma. This institution has already described an ultra-early hypercoagulability after blast injury and haemorrhage(285). Brohi and others have revealed an early hypocoagulopathy early after trauma in civilian patients and coined

a term ‘acute coagulopathy of trauma shock’ (91). Tissue hypoperfusion has been promoted as a key driver of this coagulopathy. This study assesses an intervention designed to improve arterial oxygen content and hence oxygen delivery. This should improve shock and it is therefore relevant to establish whether the improved tissue oxygen delivery (demonstrated above) is accompanied by an improvement in clotting parameters. The current study was not designed to test this hypothesis, and no significant difference was seen between air and supplementary oxygen groups, although overall there was a trend for early improvement in coagulation dynamics in the group given oxygen compared to those given air throughout. This is clearly an area of significant interest and future studies should be designed specifically to examine the relative impact of enhanced arterial oxygen content versus improved blood flow on acute trauma coagulopathy.

6.5.5 Oxidative Stress and Lung Inflammation

As discussed earlier, (3.2.6.1) lung injury increases free radical formation and depletes stored levels of antioxidants in the lung (152). This renders damaged lungs vulnerable to further oxidative stress. Aggarwal demonstrated further oxidative lung damage with FiO2 0.6 oxygen therapy in lipopolysaccharide-damaged lungs (204). It was therefore important to determine the safety of oxygen support in this study.

Two adhesion molecules were measured, VCAM and ICAM: both are described earlier (2.1.5.2). ICAM-1 is expressed on the surface of damaged capillary endothelial cells and alveolar pneumocytes. ICAM-1 expression is critical in enabling the migration of neutrophils into the tissues. It is rapidly up-regulated in Goodpasture’s syndrome – a condition which produces diffuse alveolar haemorrhage

- and correlates with very early lung inflammation (neutrophils within 1.5hrs and macrophages within 6hrs) (134).

VCAM is another endothelial cell adhesion molecule involved in inflammation. It is almost immediately up-regulated in lung tissue in experimental models of haemorrhage and resuscitation (133). Admission levels of VCAM and ICAM after trauma and sepsis correlate with mortality (137).

VCAM and ICAM levels were lower in the oxygen group. ICAM in the oxygen supplementation group reduced almost to a statistically significant level. The study was not powered to detect a difference in adhesion molecules, so it is possible that lack of significance here represents a type II error. Certainly, there was no increased expression in the oxygen group.

Lung weight indices were recorded at post-mortem to establish lung water content; a marker of pulmonary oedema and therefore inflammation. There was no difference in lung weight indices between oxygen and air control groups. It is important to note, however, that the oxygen group animals survived significantly longer than air controls. This allowed more time for inflammatory oedema to develop.

At worst, one can therefore say that oxygen supplementation has had no deleterious effect on lung inflammation, but it may actually have reduced inflammation relative to air-breathing controls. The concerns regarding oxygen therapy in blast damaged lungs are not upheld and should not preclude the adoption of oxygen support capability in the prehospital environment.

6.5.6 Logistic considerations of oxygen support

Animals in the oxygen support group initially required an average FiO2 of 0.56 to achieve recovery of SaO2 to 95%. Thereafter, the FiO2 required to maintain SaO2 95% reduced and, by 90 minutes into oxygen support, mean FiO2 was a modest 0.36.

In the past, the logistics of delivering oxygen capability to remote military units were prohibitive and the indication unjustified for the vast majority of casualties. The incidence of blast lung is now reported at 11% in casualties surviving to the field hospital in Afghanistan. This study demonstrates survival advantage from oxygen supplementation in a prehospital scenario. Investment into modern oxygen support technologies is therefore justified if this can make the field use of oxygen a practical proposition for a prolonged evacuation. Two approaches to oxygen support are described below.

A 4L cylinder filled at 200bar can store 800L O2. At the ATLS-recommended minimum flow rate for trauma (11L min^{-1}) (178), a 4L cylinder at 200bar would last only 1.2 h. Advanced regulator valves can now deliver a controlled dose of oxygen at the leading edge of the patient's inspiratory cycle. This capability reduces hugely the wastage of gas and significantly prolongs the working time of an oxygen cylinder. An industry example is the Summit Oxygen products (www.summitoxygen.com). This company designs and manufactures oxygen support solutions for mountaineering applications and offers robust, integrated, battery-free systems, capable of delivering both constant and pulse-dosed oxygen. Simple switch settings allow alteration of the dose of oxygen delivered per breath. At lowest setting this

provides 16.5ml O2 per cycle, but this can be increased to 99ml O2 per breath on currently available systems. Using this pulse dose system, coupled with a 4L lightweight cylinder filled to 200 bar, this equates to a cylinder life of 40h at setting 1 (16.5ml O2/breath) or 6.7h at setting 6 (99ml O2/breath). If a patient is profoundly hypoxic, constant flow can be used initially to achieve SaO2 target and dose subsequently titrated to maximise cylinder life. This portable and versatile oxygen support solution makes cylinder-based oxygen support a potentially viable prehospital solution for the military scenario. Of course, the logistics of refilling cylinders with medical grade oxygen would need to be considered. Personal communication with the Managing Director of Summit Oxygen reveals that the product specifications could be tailored to the specific needs of the Armed Forces to ensure a rugged, simple and practical solution (286). While attractive options, there are concerns regarding safety of pressurised cylinder use in the battlefield environment and any procurement process would need to consider this aspect.

Another way of delivering oxygen is to use an oxygen generator system. Modern FDA- approved systems have been designed with military use in mind and come in both airframe/vehicle mounted format or in low-weight portable sizes. The 1Kg Battery has a continuous use life of 90 minutes and bolts onto the man-packable (4.5Kg) Saros^{TM} concentrator. 93% filtered oxygen can be delivered at flow rates of 3L min^{-1} continuous flow, or up to 96mL per pulsed dose (www.sequal.com). Oxygen concentrators do not require refilling and present no explosive risk to personnel. When vehicle mounted, power consumption is trivial (130-145 watt per minute at 3L flow), and battery life for the portable version is reasonable with capacity for spare battery carriage. The systems are usable up to 18,000ft altitude and from 0 to 43°C.

Both of these technologies could and should be exploited to develop an adaptable and integrated oxygen support capability across the battlespace.

7 Effects of a Single Intravenous Dose of Recombinant Activated Factor VII

7.1 Introduction

Recombinant activated Factor VII (rFVIIa) is an engineered protein structurally identical to the activated from of human coagulation factor VII (287). It was designed for and is licensed for use in bleeding haemophilia patients. Since development however, a number of preclinical studies in patients without pre-existing haematological pathology have shown it reduces haemorrhage from major blood vessel injury (263) and large clinical trials provide evidence of reduced transfusion requirement and organ failure rates after blunt trauma (42). Controversies persist however regarding its efficacy in penetrating injury and safety concerns remain, related to thrombotic and embolic sequellae (288). A more detailed discussion of the literature surrounding the efficacy of rFVIIa in major haemorrhage can be found in section 3.3.4.

Blast lung is associated with large areas of pulmonary contusion and intra-pulmonary haemorrhage. This is a picture similar to that seen in diffuse alveolar haemorrhage, which can occur as a result of various pathologies. The clinical data available regarding use of rFVIIa in controlling diffuse alveolar haemorrhage is scant, but suggests a potential effect (see section 3.3.6).

There is anecdotal data from ongoing operations in Afghanistan, that rFVIIa (albeit often in multiple doses) is proving useful as an adjunct to aggressive surgical and blood product (haemostatic) resuscitation in casualties critically wounded by explosion (personal experience Op HERRICK 10 and personal communication with

Defence Professor Anaesthesia (289). There is also anecdotal observation from Israel that rFVIIa improves outcome in blast lung (290). There is, however, no experimental data on rFVIIa use following a combined blast and haemorrhage insult.

7.2 Aims

The aim of the rFVIIa arm of this study is to establish whether a single intravenous dose of rFVIIa, which would be amenable to deployment in the far-forward military environment, can improve survival and/or physiology in the context of prolonged hypotensive resuscitation after combined blast and haemorrhage injury. The rationale underpinning this study was the possibility that administration of rFVIIa might attenuate the early blast-induced intrapulmonary haemorrhage and thereby, through limiting lung compromise, improve oxygenation of blood in the lungs. Since we have already shown that improvement of arterial oxygenation improves survival during prolonged hypotensive resuscitation (section **Error! Reference source not found.**) there is a possibility that rFVIIa may also improve survival after blast injury.

7.3 Methods

12 terminally anaesthetised pigs were exposed to a standardised injury model of primary blast insult, 30% (controlled haemorrhage) blood volume haemorrhage and grade IV liver laceration (uncontrolled haemorrhage). Methods of surgical preparation, blast injury and haemorrhage exposure, fluid therapy and physiological measurement were all identical to those described for the oxygen study and details of these methods can be found in Chapter 5 of this thesis.

7.3.1 rFVIIa administration protocol

Animals were randomly to receive either rFVIIa or placebo during the resuscitation phase. Following injury, all animals were resuscitated with aliquots of warmed normal saline to maintain a hypotensive systolic blood pressure of 80mmHg and the study was run to 8hr following onset of fluid therapy. After 30 minutes of fluid resuscitation, animals either received placebo or rFVIIa (Figure 26). The rFVIIa group received a single intravenous 180mcg/Kg dose of rFVIIa, while the placebo group received an equivalent volume of normal saline (0.18 ml/kg i.e. 9ml for 50Kg animal). All animals in this study breathed room air throughout the experiment. As mentioned in the methods section (5.5.9), the placebo group formed the control for both this study and the oxygen study (see Chapter 6).

7.3.2 Assessment of coagulation and inflammatory responses

Arterial blood samples were collected into citrated Vacutainers (9NC 0.105M Vacutainer 367691, Beckton Dickinson, UK), centrifuged at 1500 x g for 10 min and the plasma separated and stored at - 80°C. Prothrombin time (PT) was determined by turbidimetry using the ACL Elite (Beckman Coulter (UK) Ltd).

In each individual animal, prothrombin times are expressed as a proportion of that measured from an initial blood sample taken at start of surgery.

7.4 Results

Three animals died before T30 and separation of treatment strategies[5]. These were excluded from the study and are not presented, nor included in statistical analysis.

Table 12 illustrates pre-injury baselines for rFVIIa and placebo control groups.

	Blast rFVIIa		Blast Placebo		rFVIIa vs. Placebo
	Mean	SEM	Mean	SEM	(t-test)
n	6		6		
Wt (kg)	53	0.6	50.8	1.2	0.127
PaO2 (kPa)	9.9	0.4	8.9	0.3	0.073
PaCO2 (kPa)	6.2	0.2	6.6	0.2	0.2544
Art pH	7.41	0.03	7.42	0.02	0.9147
ABE (mM)	4.3	1.2	6.1	1	0.2798
SaO2 (%)	93	1.5	88.3	1.2	0.0339*
SvO2 (%)	68.1	1.2	62.1	1.8	0.0211*
CaO2 (ml/dl)	14.8	0.4	14.2	0.6	0.495
OER	0.23	0.02	0.3	0.02	0.0158*
T Hb (g/dl)	11.8	0.3	11.6	0.4	0.7319
Hct (%)	38.2	1	37.8	1.1	0.784
Art K+ (mM)	3.7	0.1	3.9	0.1	0.1702
T (oC)	38.3	0.3	38.7	0.3	0.3493

Table 12 Baselines variables for rFVIIa and Placebo animals. There were small, statistically significant, differences in arterial and mixed venous oxygen saturation and oxygen extraction ratio between the rFVIIa and placebo groups, although this did not translate to a difference in arterial oxygen content. Although statistically significant, the differences are small and unlikely to be of physiological consequence. There were no significant differences in the other parameters examined.

[5] Two animals were destined to be given rFVIIa after 30 min of resuscitation: one died of ventricular fibrillation at the end of the controlled haemorrhage and the other died after 15 minutes of resuscitation. Post-mortem examination of this latter animal revealed a substantially greater volume of intra-abdominal haemorrhage due to transaction of unusually large hepatic vessels on creation of the Grade IV liver injury.

One animal, destined for the control group, failed to respond to fluid resuscitation and died after 10 minutes of resuscitation.

7.4.1 Survival

Survival was the primary endpoint of this study. Survival times in the rFVIIa group were not significantly different from the blast air control group (P=0.649) (Figure 47).

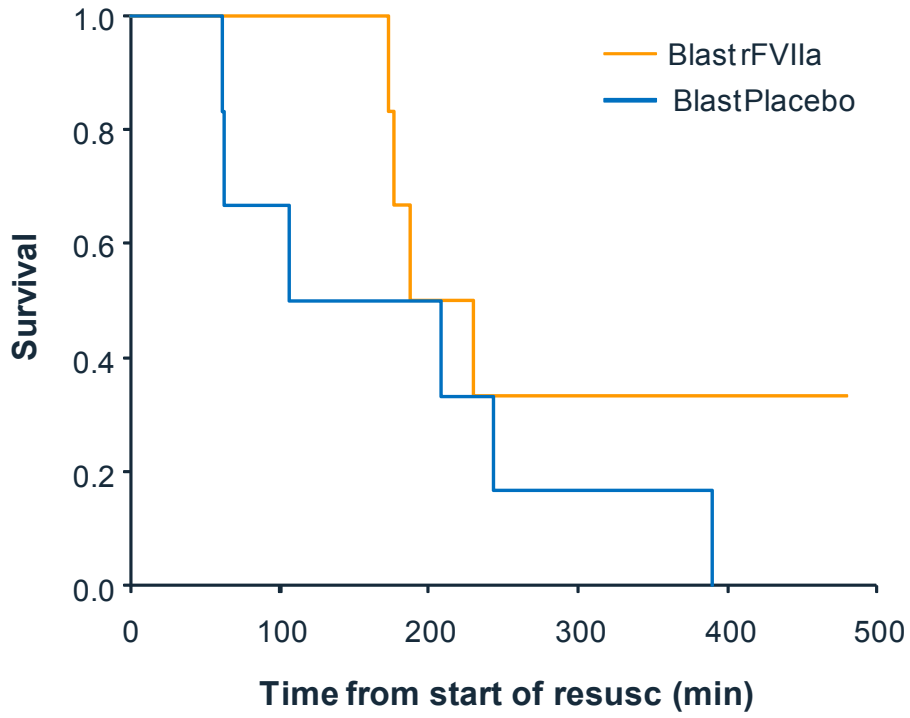

Figure 47 Kaplan-Meier survival plot for two groups of animals subjected to blast injury, haemorrhage and hypotensive resuscitation. Treatment with either rFVIIa or placebo (0.9% saline) was administered 30 min after onset of hypotensive resuscitation. Both groups breathed air throughout.

However, inspection of mean survival times: rFVIIa 253min (95%CI 167-339) vs. Placebo 195 min (95%CI 85-306) might suggest that treatment with rFVIIa did confer a small survival advantage compared with placebo. A *post hoc* power calculation (Power 0.8, alpha 0.05, Log rank test) suggested that 23 animals per group would be required to exclude a Type II error and determine whether there is indeed a true survival effect from rFVIIa therapy.

7.4.2 Oxygen Saturation

Prior to T30, oxygen tension and saturation levels were similar between groups (Figure 48). After administration of rFVIIa, arterial oxygen saturation was higher in

the rFVIIa group than in placebo control group and this achieved statistical significance at T45 (15 min after drug administration).

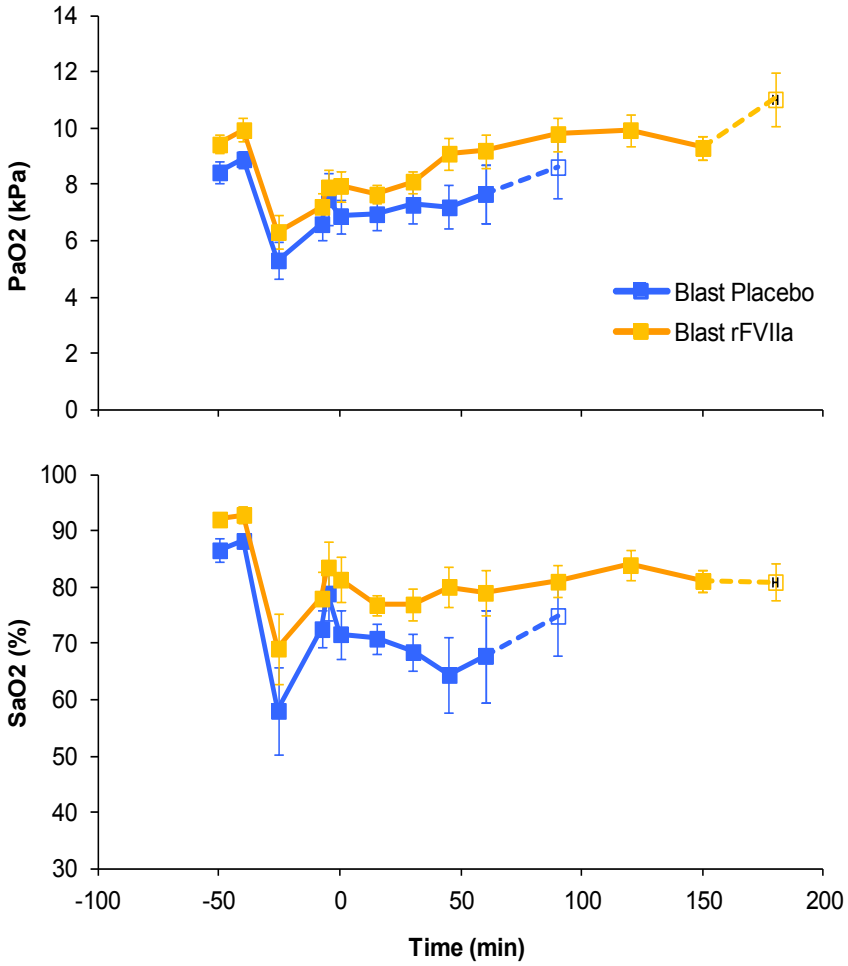

Figure 48 Arterial oxygen tension (PaO2) and saturation (SaO2) in two groups of animals subjected to blast injury, haemorrhage and hypotensive resuscitation. Time (min) indicates time from onset of resuscitation (T0). T30 represents the divergence of treatment groups, with rFVIIa (180 µg/kg iv) being given to the Blast rFVIIa group, and placebo (0.18ml/Kg saline) to the placebo control group. First two values represent Baseline, followed by Post Blast, Pre and Post Haemorrhage and then onset of resuscitation. Open symbols indicate 66% of animals surviving. No data plotted when proportion surviving fell to 50% or below. Mean values ± SEM.

7.4.3 Physiology

The injury model (blast injury plus haemorrhagic shock) induced a significant elevation of oxygen extraction ratio (OER), from baseline of approximately 0.25, to the physiological maximum of 0.80 in all groups by the onset of fluid resuscitation (Figure 49). OER in both rFVIIa and control groups remained at almost maximum

levels and the base deficit in both groups continued to increase over time until animals succumbed. There was no significant difference between rFVIIa and placebo control groups in ABE or OER.

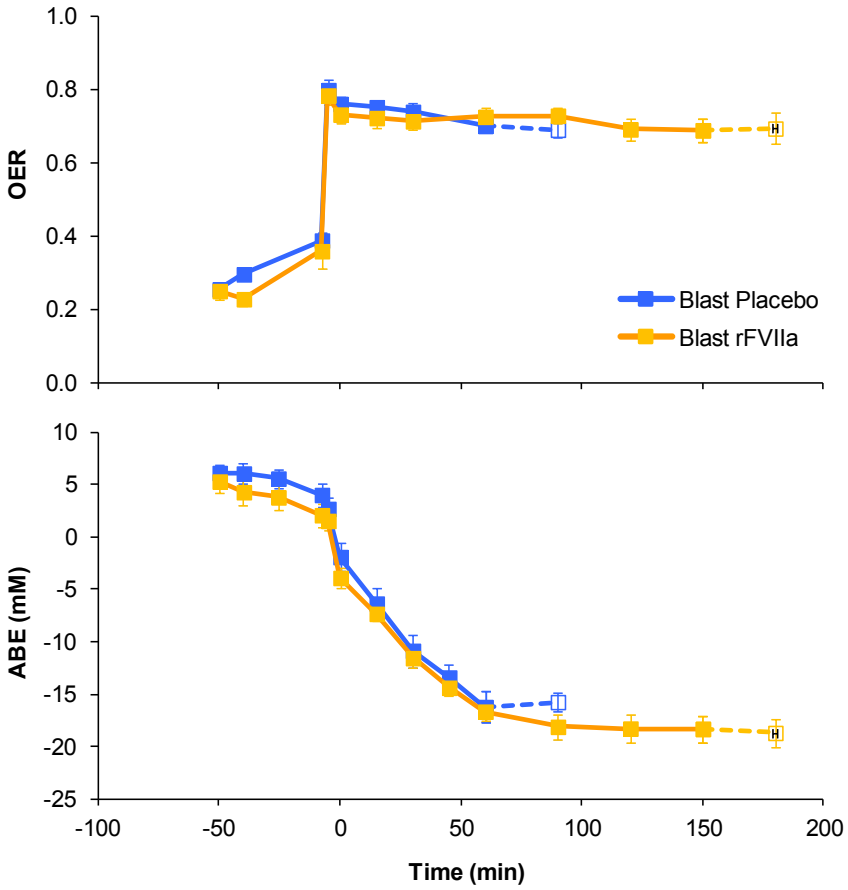

Figure 49 Arterial base excess (ABE) and Oxygen Extraction Ratio (OER) in animals subjected to blast injury, haemorrhage and hypotensive resuscitation.

7.4.4 Coagulation and Haemorrhage

Prothrombin time (PT) showed a small but statistically significant increase (P=0.043) following combined blast injury and haemorrhagic shock, but there was no difference between treatment groups from baseline to 30 min into the fluid resuscitation phase of the study (P=0.558, two way ANOVA, Figure 50). rFVIIa (or placebo) was administered immediately after the blood sample representing 30 min of resuscitation was taken. By 60 min after the onset of resuscitation, PT had fallen to

below baseline in the rFVIIa group, in contrast to the placebo-treated group where there had been a marked increase in PT coincident with the development of a shock state (Figure 51). By 60 min after the onset of resuscitation there was a significant difference in PT between rFVIIa and placebo-treated groups (P=0.001).

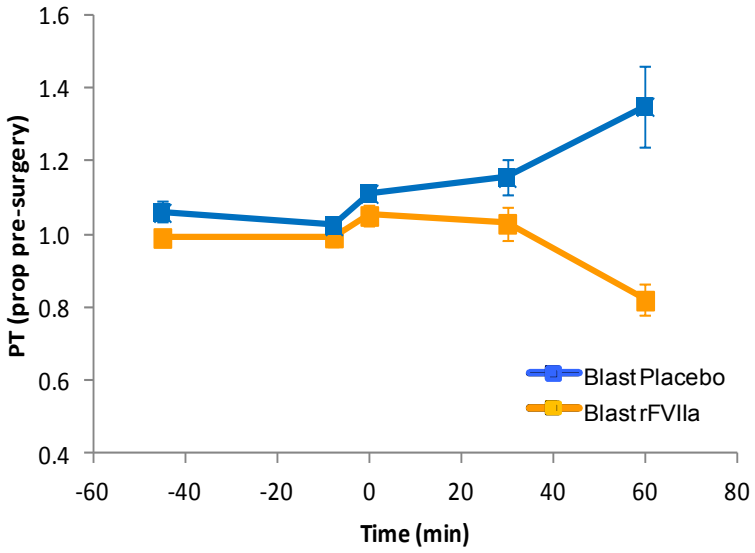

Figure 50 Prothrombin time (PT) normalised in each individual animal to the level seen immediately after implantation of the first intravascular cannula (value pre-surgery) in two groups of animals treated with either rFVIIa or placebo immediately after the taking of the blood sample representing the 30 min time point. Time 0 min represents the onset of fluid resuscitation. Mean values ± SEM. Further analysis found that the *change* in PT between 30 and 60 min after onset of resuscitation was significantly different between treatment groups (P=0.009, Mann Whitney U test, Figure 51).

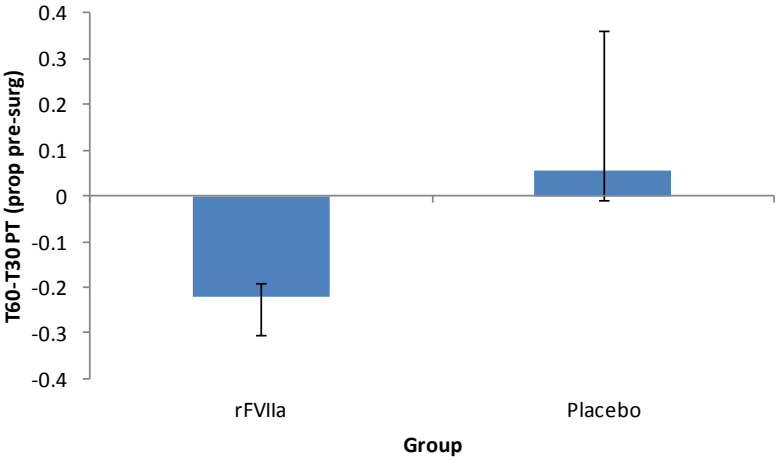

Figure 51 Change in normalised PT (for explanation see legend to Figure 50) between 30 and 60 min (T30 and T60, respectively) after onset of resuscitation. rFVIIa or placebo was administered immediately after the T30 sample was taken. Negative value indicates fall in PT between T30 and T60. Median, interquartile range.

Beyond T60, PT increased in individual animals in both groups (Figure 52). Due to progressive loss of animals in both groups beyond this time-point, no further statistical analysis was performed on this data.

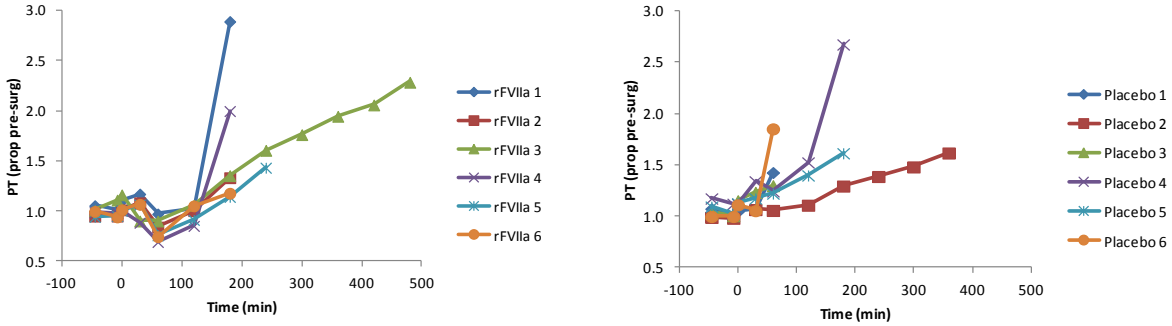

Figure 52 Normalised PT (for explanation see legend to Figure 51) in individual animals from the rFVIIa (left) and placebo (right) treated groups.

7.4.5 Histopathology and tissue microthrombi

Two independent Histopathologists, blinded to the treatment status of the animals, analyzed tissue sections. There was no evidence of identifiable micro-thrombus formation in any of the sections. All sections of (non-contused) lung did show positive staining for fibrin within some small-calibre vessels; however this was interpreted as post-mortem clot formation rather than ante-mortem thrombus formation on basis of morphology.

7.4.6 Lung Inflammation and pulmonary oedema

7.4.6.1 ICAM and VCAM

There were no significant differences in expression of ICAM or VCAM in lung tissue when the rFVIIa group was compared to that treated with placebo (P=0.65, ICAM; P=0.29, VCAM)

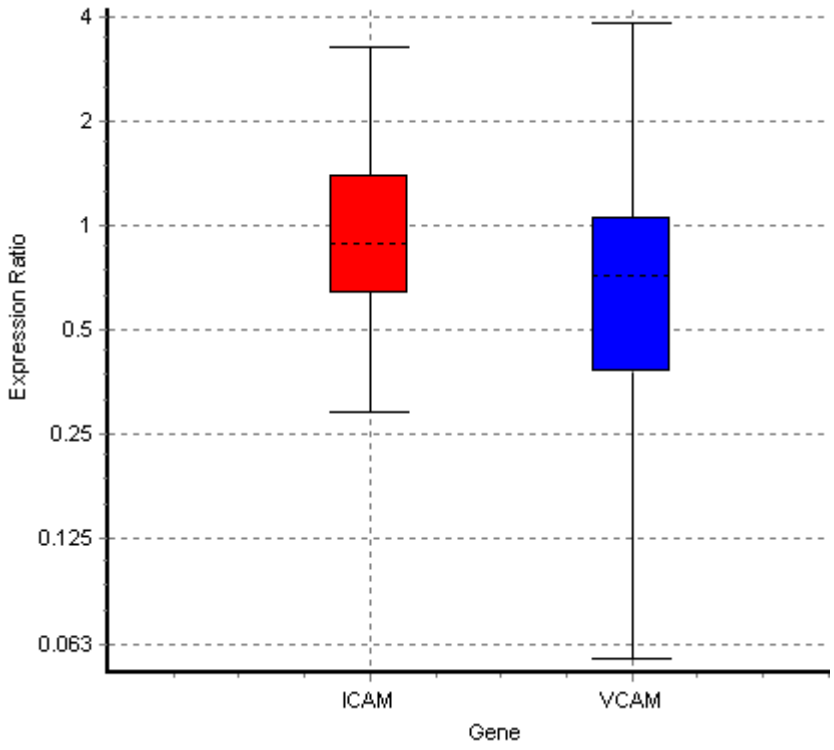

Figure 53 Expression of two inflammatory genes ICAM (Intercellular Adhesion Molecule 1) and VCAM (vascular cell adhesion molecule 1) in lung tissue from animals treated with rFVIIa; expressed as a proportion of levels seen in the placebo-treated group. Boxes represent interquartile range. The dotted line represents the median gene expression. Whiskers represent the minimum and maximum observations.

7.4.6.2 Lung weight indices

There was a trend towards a reduced lung weight index in the group given rFVIIa, compared to placebo, however this did not achieve statistical significance (P=0.07) (Figure 54).

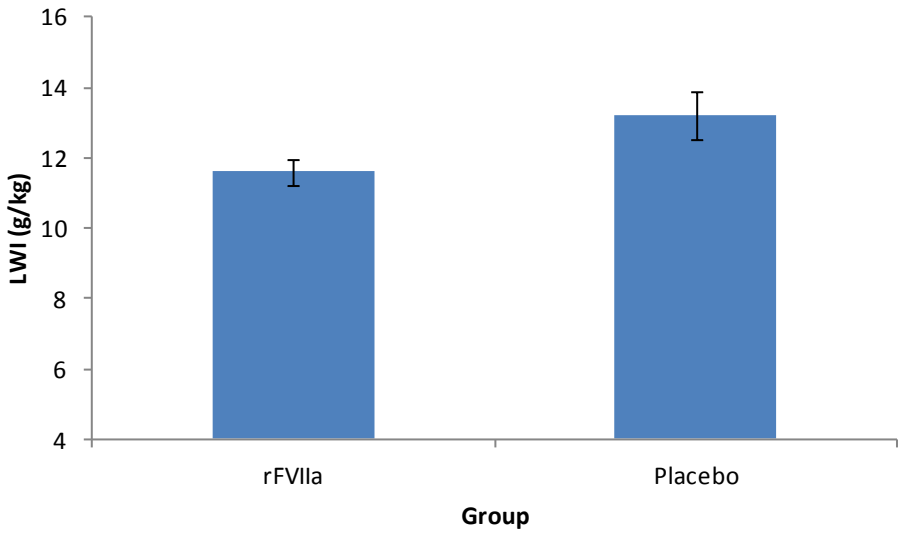

Figure 54 Lung weight index (LWI) in two groups of pigs treated with a single intravenous dose of either rFVIIa or placebo (saline) 30 min after the onset of resuscitation. Mean ±SEM.

7.5 Discussion of rFVIIa Results

This study has demonstrated no significant survival advantage from a single dose of rFVIIa given intravenously after 30 minutes of hypotensive-target saline resuscitation (Figure 47), compared to placebo given at the same time.

There is however the possibility that the lack of significance reflects too small a study group (Type II error), rather than a true failure of the intervention. The survival time showed a trend towards improvement and there was marginal improvement in oxygenation of the rFVIIa animals after treatment (Figure 48). Based on these results, a post hoc power calculation indicated that 23 animals per group would be required to establish whether there is indeed a small survival advantage. However, the clinical impact of such a small increase does not justify sacrificing the number of animals required (34 extra), nor the expense of completing the work. This is particularly pertinent given the efficacy of the other adjunct assessed in this thesis,

supplemental oxygen, which resulted in both statistically and clinically significant survival improvement (Figure 34).

7.5.1 Dosing

It is important to be confident that the dose of rFVIIa administered was sufficient to be expected to produce a clinical effect. There are three points that support this assumption.

First, Prothrombin time reduced significantly after drug administration in the rFVIIa group compared to placebo controls (Figure 51). This reduction in PT was accompanied by a slight but significant improvement in oxygenation of the animals (Figure 48).

Secondly, a previous study at DSTL; using the same breed of anaesthetised swine and an identical intravenous dose; demonstrated a significantly prolonged survival time in an aortotomy model of major arterial haemorrhage (263).

Third, reports of rFVIIa use in DAH have shown significant clinical improvement following intravenous doses of only 60 and 100mcg/kg (291;292) which is considerably less than the dose used in this study.

It is therefore concluded that a single intravenous dose of rFVIIa given 30 min after the onset of resuscitation does not produce a significant improvement in survival time, or in the physiological status associated with oxygen delivery to tissues, in our model of combined blast injury and haemorrhagic shock. This is in marked contrast to the beneficial effects of rFVIIa seen in large vessel bleeding, where single dose

rFVIIa was shown to produce a clinical and statistically significant improvement in survival in a porcine model of haemorrhagic shock and incompressible arterial bleeding (263). Furthermore, the lack of efficacy of rFVIIa in blast lung contrasts with anecdotal reports of a beneficial effect in diffuse alveolar haemorrhage from a variety of disease states (see Table 7).

7.5.2 Physiology

7.5.2.1 Oxygenation

The slight rise in oxygen saturations in the rFVIIa group was statistically significant 15min after drug administration (Figure 48). All animals however remained hypoxic, with the SaO2 recovering only to 80% (PaO2 below 10KPa). The improvement in oxygen saturation occurs very quickly (within 15 min) after drug administration, which makes it unlikely that the difference is due to an anti-inflammatory effect of rFVIIa. One must conclude therefore that the marginal improvement is due to a reduction in pulmonary haemorrhage following administration of a haemostatic agent.

7.5.2.2 Acid Base and Oxygen Extraction

Despite the slight improvement in oxygenation, there was no difference in the progression of metabolic acidosis, or the maintenance of maximal oxygen extraction ratio (Figure 49).

The failure to halt the progression of metabolic acidosis in the rFVIIa group reflects insufficient improvement in oxygen delivery to address the gross inadequacy of perfusion generated by the injury model and hypotensive fluid resuscitation. Any marginal improvement in arterial oxygen content due to rFVIIa was not enough to cross the threshold into acidosis stabilisation and survivability. This is in contrast to

the oxygen group discussed in Section 6, where metabolic acidosis progression was rapidly arrested by oxygen support.

7.5.3 Coagulation

rFVIIa administration reduced PT from elevated levels, due to the injury model and hypotensive resuscitation, to below baseline. Control group animals showed no such decrease in PT. Beyond 60min however, PT in all rFVIIa animals again increased without further recovery (Figure 50).

Recombinant Factor VII has been shown to have a half-life of 2.4hrs in bleeding trauma patients (293). Effective rFVIIa dosing regimens in major trauma haemorrhage studies have involved an initial 200mcg/Kg dose, followed by repeat boluses (100mcg/kg) at one and 3 hours as required (42). It is likely that the short term effect on PT of rFVIIa represents clearance and reduced active concentrations, but it is also possible that ongoing hypoperfusion (from the injury model and hypotensive fluid therapy) drives coagulopathy to such an extent that rFVIIa activity on blood coagulation is rapidly overwhelmed.

The principal aim for assessing rFVIIa in this study was not, however, to look at major haemorrhage control as this has been addressed in other preclinical and clinical studies (see section 3.3.4). The target site for drug action was the pulmonary haemorrhage and subsequent lung inflammation generated by blast lung. We have not directly determined the duration of any haemostatic effect on coagulation in the lungs from intravenous rFVIIa in this study, but we have seen that the systemic PT effect is short-lived, while the improvement in oxygen saturation after rFVIIa administration is maintained for the duration of the experiment. We have already

mentioned that haemorrhage control in the lungs is responsible for the improvement in arterial oxygen saturation as it occurs too early for an anti-inflammatory effect to have made an impact. However, ongoing improvement in oxygen saturation does not mean that there is ongoing rFVIIa activity: once bleeding points in the lungs have been controlled with stable clot, re-bleeding in the lungs should not occur in this model as all animals are resuscitated to a hypotensive 80mmHg SBP target

Repeat dosing would generate higher plasma drug concentrations and should prolong the PT effect seen initially in this study. It could also augment the beneficial effect on arterial oxygenation saturation after rFVIIa. Both of these features would potentially improve outcome, but there are logistic and practical issues related to the intended military role of the prehospital adjuncts we assess. It is very unlikely that repeat dosing would occur in the field setting.

7.5.4 Inflammatory markers and lung weight index

In order to gauge potential differential inflammatory responses, we have focussed on gene expression of the inflammatory markers, ICAM and VCAM. Both were quantitatively measured from non-contused lung post-mortem harvest. Their expression is upregulated very early in shock states - raised ICAM and VCAM have been shown almost immediately in lung tissue in rat models of haemorrhage and resuscitation (133). Circulating ICAM can be detected in blood within 4h of shock onset (132) and VCAM levels on hospital admission after trauma are predictive of outcome (137). Gene expression of these markers would be more likely to show a difference than circulating cytokine levels such as Interleukins, given the short survival times of some animals. Neither ICAM nor VCAM expression was altered compared to placebo by rFVIIa single dose administration in this study (Figure 53).

The trend towards reduced lung weight index in the rFVIIa group (Figure 54) could be due to an anti-inflammatory effect, but this is unlikely in the context of no change in inflammatory marker gene expression. It is more likely that the trend is a result of reduced bleeding from the blast lung injury caused by the administration of the potent haemostatic agent, rFVIIa.

Overall, there is no evidence from this study that a single intravenous dose of rFVIIa attenuates the lung or systemic inflammatory response following blast injury with haemorrhage.

7.5.5 Safety of rFVIIa

rFVIIa requires expression of tissue factor to generate thrombin so its action should be confined to sites of vessel injury. However, tissue factor can be expressed in other sites, such as atherosclerotic plaques or in the microcirculation in gram negative sepsis. There is a theoretical risk of thrombotic complications following rFVIIa therapy and adverse events have been reported. It is important therefore to consider the safety implications of this 180mcg/kg intravenous dose in the context of its use as a potential pre-hospital intervention following blast injury and haemorrhage.

As mentioned in section 5.6, organ tissue samples were harvested at post mortem and inspected for evidence of microthrombi formation. In order to prevent bias, two independent Histopathologists were asked to assess the samples and both were blinded to the treatment status of animals. There was no evidence of any pre-mortem thrombi in any harvested tissue sections. It is concluded therefore, that there

was no evidence of any safety issue with this single dose of 180mcg/Kg rFVIIa in our study.

7.5.6 Future work with rFVIIa in blast and haemorrhage

rFVIIa was not an effective adjunct as a single intravenous dose in this study and we cannot recommend its use in the prehospital environment in this form.

Another route of administration of rFVIIa may however produce an effect in a similar injury context. While the pathologies of blast lung and DAH have not been directly compared, there are similarities that make comparison of therapies relevant. Both conditions result in pulmonary alveolar haemorrhage and an inflammatory response (Sections 2.2.4 and 3.3.5). Both result in significant lung function compromise and potentially recover with supportive treatment. High elevation of Tissue Factor Pathway Inhibitor (TFPI) has been demonstrated in DAH and impedes normal function of the TF/FVIIa complex. This renders the lungs more vulnerable to ongoing haemorrhage. Although not assessed in blast lung, TFPI is also likely to be raised. Pharmacological doses of rFVIIa have been shown to generate thrombin via a TF-independent pathway in the presence of raised TFPI (Figure 20). Of course, blast lung injury can also include events such as pneumothorax and large vessel rupture, but these are both rare and victims with these features are likely to suffer overwhelming injury and succumb. Intrapulmonary administration, via broncho-alveolar-lavage or nebulizer, has been reported in only a very few cases of DAH. Heslet's report of 6 cases noted good or excellent efficacy of rFVIIa in all cases (257). In one case, intra-pulmonary administration succeeded, where intravenous rFVIIa had failed to work. There is no randomized data to confirm this effect, but

perhaps the intra-pulmonary route represents a more potent route for rFVIIa in blast lung?

The use of intra-pulmonary rFVIIa will be assessed in future work to clarify this point and a similar injury model, or small animal study might be employed to achieve this. From this study, however, there is no data to support field use of rFVIIa as a resuscitation adjunct.

8 Overarching Discussion

Modern combat trauma is characterized by injuries from explosive devices. Haemorrhage from devastating tissue injury is the primary cause of death on the battlefield, but the added effects of primary blast injury are evident in over 10% of wounded casualties (186). The combined insults of blast and haemorrhage invoke a 'double hit' on oxygen delivery and present significant challenges to prehospital life support algorithms. This randomised controlled trial assessed two adjuncts to fluid resuscitation for their potential to improve survival and maintain physiology for a prolonged period following a clinically relevant injury model of combined blast and haemorrhage (see section 3.4).

Supplemental oxygen significantly improved survival compared to control group, but rFVIIa did not (Figure 55). Oxygen significantly improved physiology compared to control groups, arresting the deterioration of base deficit (Figure 56). Both adjuncts significantly improved arterial oxygenation, but the rFVIIa animals remained hypoxic and the improvement was not sufficient to improve base deficit compared to control group.

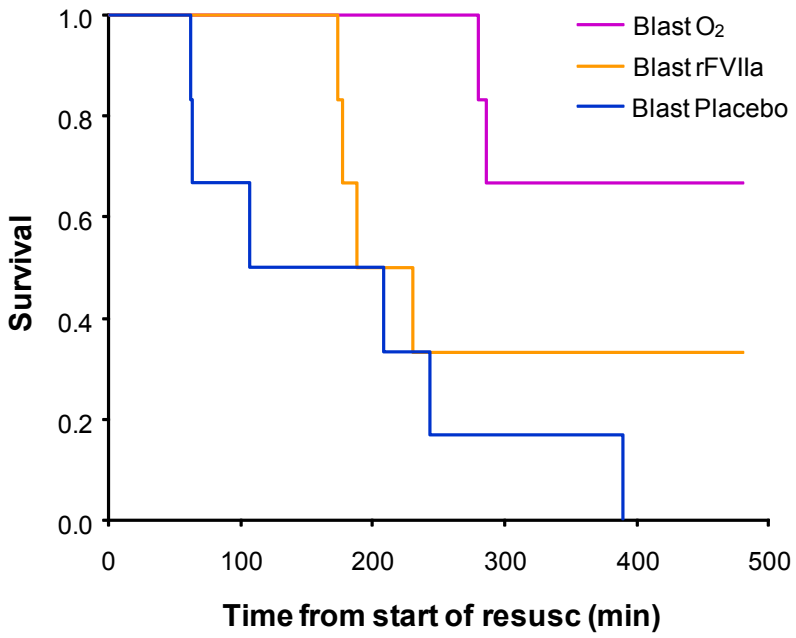

Figure 55 Kaplan-Meier survival plot for three groups of animals subjected to blast injury, haemorrhage and hypotensive resuscitation. The Blast rFVIIa and O2 groups were respectively given rFVIIa (180 µg/kg) or supplemental oxygen 30 min after the onset of resuscitation. Placebo breathed air throughout and received equivalent volume normal saline at T30 (0.018ml/kg).

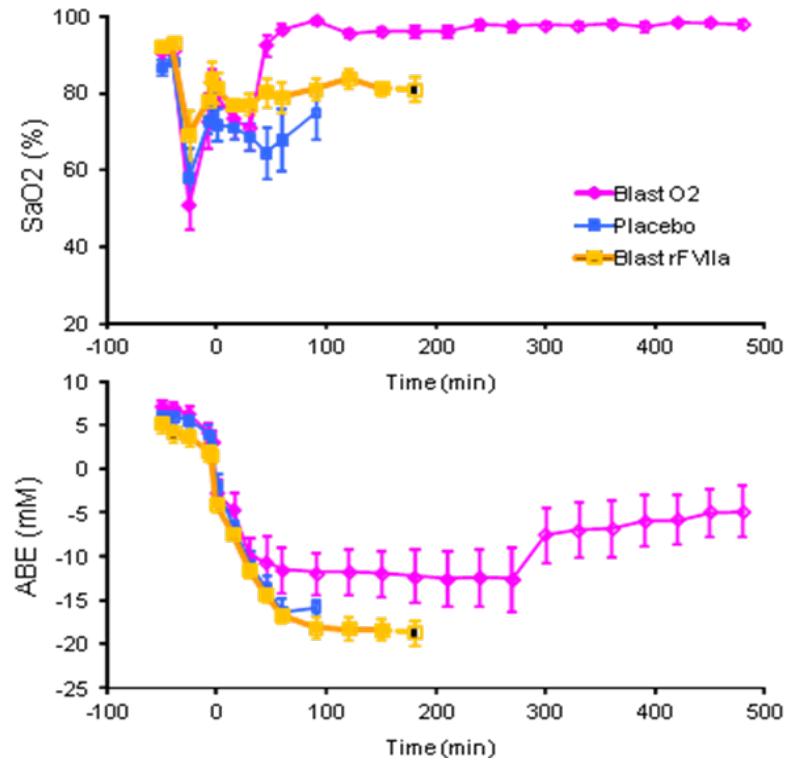

Figure 56 Arterial oxygen saturation and adjusted base excess plots for the three groups of animals. All groups received the same injury model and fluid therapy throughout. At T30, the oxygen group began to receive supplemental oxygen min (FiO2 0.3) to maintain SaO2 >95%. The rFVIIa group received a single intravenous dose of 180mcg/kg and the placebo control group received an identical volume of normal saline.

Supplemental oxygen is therefore a potent adjunct to fluid resuscitation following blast and haemorrhage injury. For the same scenario, rFVIIa, as a single intravenous dose given at 30 minutes after onset of resuscitation, cannot be recommended for use in the field.

It is however possible that any pro-coagulation effect of rFVIIa on pulmonary haemorrhage might be greater if the drug were given even earlier. This could improve the efficacy of the drug by reducing the hypoxic penalty of blast lung. Realistically however, an agent such as rFVIIa is unlikely to be administered any sooner than 30min following injury in the battlefield environment, so this avenue will not be pursued further in this programme of work. Intrapulmonary administration of rFVIIa is however a relevant concept to explore and has shown promise in clinical cases of DAH; a similar condition in terms of haemorrhage and hypoxia to blast lung. As discussed earlier (see section 7.5.6), this will be assessed in future work.

Previous work at Dstl Porton Down has identified a 'Novel Hybrid' (NH) fluid resuscitation strategy that is able to maintain life, and enable physiological recovery after a similar injury model (39). Although the study did not demonstrate any evidence of increased rebleeding with this NH fluid strategy, there will be casualties for whom the risk of rebleeding is simply too great for clinicians to consider increasing blood pressure to normal levels in the prehospital environment. For these patients, supplemental oxygen might offset the penalties of the reduced flow imposed by hypotensive therapy and enable prolonged prehospital survival.

In conjunction with NH, oxygen therapy should improve further the overall oxygen delivery (DO2) and optimise prehospital resuscitation. It can be considered that oxygen therapy essentially 'negates' the hypoxia effect of blast lung and leaves behind only the challenge of haemorrhage for the fluid therapy strategy to overcome.

8.1 Limitations of the Study

This randomised controlled large animal study has assessed two adjuncts to fluid therapy in a challenging injury model of primary blast injury and haemorrhage. Overall, the research questions have been answered and we have been able to make recommendations regarding potential clinical deployment of oxygen and suggest future work that might clarify whether or not there is any role for rFVIIa in the management of blast lung injury. Of course, there are areas where alterations in study design or future work might improve upon the clarity of our findings.

8.1.1 Recombinant FVIIa group

As mentioned in the Method Development Section (4.1.6), there was insufficient data to inform an initial power calculation with respect to group size required to show a survival effect of rFVIIa. The planned interim analysis at n=6 for each group allowed a timely re-evaluation of group size required to determine whether indeed rFVIIa had a positive effect on survival in this injury model. This post-hoc power calculation demonstrated that a further 23 animals per group would be required. This number of animals not only represents a large cost to the programme, but also a large number of animals that would need to be sacrificed. Given the success of supplemental oxygen in the same injury model, and of the novel hybrid fluid strategy in a very similar model, it was felt that this investment and sacrifice was unjustified, as it would

be unlikely to translate into clinical practice given such a marginal effect, if indeed any were shown to exist.

It might also be argued that inclusion of the liver snare potentially reduced any activity that intravenously-administered rFVIIa might have in blast-injured lungs. However large-scale tissue destruction is normally present in combat trauma victims. An injury model, which excluded any tissue injury, would therefore not be representative of the 'real world'. If rendered ineffective in addressing blast lung because of the diversion of a relatively small area of tissue injury, then an intravenous dose of rFVIIa is likely to be ineffective in the clinical setting.

rFVIIa treatment in this study did produce an increase in oxygenation, an improvement in clotting parameters to beyond baseline and a trend towards increased survival time. This suggests that the dose given was sufficient to ensure systemic effect even if some of the drug was being 'used up' at the site of the liver injury.

If one were determined to establish whether or not the liver snare had impacted on the efficacy of rFVIIa in addressing the blast lung, it would of course be possible to repeat the experiment without the liver snare, (the degree of controlled haemorrhage may need to be increased to maintain the severity of injury). It may be more relevant however, to assess the administration of rFVIIa via a more targeted route, so it is delivered directly to the area where its action is required: this work is already underway in a small animal model of blast lung and haemorrhage at Dstl Porton Down (see 7.5.6).

8.1.2 Oxygen Supplementation Group

We chose to use hypotensive fluid therapy as the baseline therapy control for this study. The Novel Hybrid programme recently completed at Dstl demonstrated significant survival and physiologic advantage of the Novel Hybrid fluid therapy strategy over prolonged hypotensive resuscitation following blast injury and haemorrhage - without increasing rebleeding (39). It might be argued that, for military trauma prehospital management, this Novel Hybrid fluid strategy represents the new standard, against which all future strategies need testing. However, the NH strategy has only recently been adopted into clinical practice, so the evidence as yet remains experimental. Furthermore, in the civilian setting, hypotensive therapy remains the standard of care. Another reason to persist with hypotensive therapy in this study is that we were seeking to address not the flow component, but the arterial oxygen content component of the oxygen delivery equation. The appropriate control is therefore one in which we know that oxygen delivery is unsurvivably low – this will best demonstrate any positive survival effect of our adjunct. It is logical to expect that a combination of novel hybrid resuscitation and supplemental oxygen would achieve excellent survival and physiological recovery in a similar injury model. We have shown no detrimental effects of oxygen and demonstrated improved DO2 (and a trend to improved VO2) by improving CaO2. NH improved DO2 by increasing the flow component. To assess both together, one could employ a similar animal model and compare combined therapy against NH alone. Given high NH survival however, it may require large numbers of animals to demonstrate a survival advantage of combined therapy.

8.2 Future Research

Dstl Porton Down has already studied interventions which address the flow component of DO2 by assessing fluid therapy strategies (39). The current study has approached the issue from the oxygen content point of view and clear answers to the research questions have been achieved: oxygen is effective; single dose intravenous rFVIIa is not. Another aspect of DO2 and oxygen content is oxygen carrying capacity. Future work could examine the effects of administering doses of packed red cells and/or HBOC compounds in the context of a similar combined blast and haemorrhage model and assessed against hypotensive fluid therapy controls. Both of these strategies should increase oxygen delivery to the organs and significantly improve the potential benefit of supplementary oxygen. Consequently, where supplementary oxygen by itself (current study) was able to arrest the physiological deterioration, a combination of supplementary oxygen and packed red cells (or HBOCs) might reverse the physiological deterioration. This combination clearly merits investigation since currently the only way shown to reverse the physiological deterioration has been to improve blood flow (novel hybrid resuscitation) by increasing blood pressure with the attendant risk of 'popping clots' and causing re-bleeding. A combined treatment of packed red cells (or HBOCs) and supplementary oxygen might therefore prove to be a safer alternative to novel hybrid resuscitation in casualties at particular risk of re-bleeding.

However, with respect to red cell transfusion, it is important to appreciate that stored blood behaves differently to fresh blood. Some features of stored blood are presented in Table 13 below.

Storage Lesion	**Clinical Effect**

Up to 25% of PRBC rapidly cleared by reticulo-endothelial system	Stress on R-E system Reduced impact of PRBC on CaO2
ATP depletion (>50% at 6 weeks)	Impaired NO-mediated vasodilation in response to hypoxia: microcirculatory problem
2,3 DPG levels negligible beyond 2 weeks (Takes 7hrs to regenerate 50% normal levels)	Reduced unloading of oxygen in tissues
Reduced deformability of erythrocytes	RBC less able to enter capillaries: microcirculatory problem
Increased free radicals	Transfusion related tissue damage and inflammation

Table 13 Storage lesion of blood

The net effect of this 'storage lesion' is that stored red cells are both less effective at delivering oxygen to the microcirculation and carry significant risks: in SIRS patients, red cells transfusions increase mortality and morbidity, independent of shock severity (294). Shah's study of red cell transfusion in 8 critical care patients after trauma demonstrated that; although transfusion increased Hb and hence CaO2; it did not increase oxygen consumption (295). The P50 of haemoglobin dropped significantly following two units of transfusion and was cited as the primary reason that VO2 was not improved.

HBOCs may however prove effective in increasing the oxygen content and consumption, while avoiding the problems associated with stored red cell transfusion.

Oxygen support capability is not currently available in forward military echelons. New technologies minimise the previously prohibitive logistic burdens of maintaining such a capability. Investment in these technologies will potentially improve survivability from modern combat wounds and is recommended.

9 References

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