Inhaled Recombinant Activated Factor VII (rFVIIa) in the Management of Blast Lung Injury

Thesis for the Degree of Doctor of Medicine

University of Newcastle upon Tyne

Jason Edward Smith

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List of Abbreviations

ADMEM	Academic Department of Military Emergency Medicine
AIS	Abbreviated Injury Scale
ARDS	acute (sometimes adult) respiratory distress syndrome
CCAST	critical care air support team
CGOs	Clinical Guidelines for Operations
CO2	carbon dioxide
CT	computed tomography
CXR	chest X-ray
DAH	diffuse alveolar haemorrhage
dstl	Defence Science and Technology Laboratory
ED	emergency department
EPR	electron paramagnetic resonance
ETCO2	end tidal carbon dioxide
ETT	endotracheal tube
FAST	focussed assessment with sonography in trauma
FiO2	inspired oxygen fraction
FDA	Food and Drug Administration (USA)
FITC	fluorescein isothiocyanate
HVGSW	high velocity gun shot wound
ICU	intensive care unit
IED	improvised explosive device
ISS	Injury Severity Score
IV	intravenous
JTTR	Joint Theatre Trauma Registry
KIA	killed in action
KNEA	killed (non-enemy action)
Kg	kilogram
mcg	microgram
MERT	Medical Emergency Response Team
NISS	New Injury Severity Score
PEEP	positive end expiratory pressure
PM	post mortem
pRBCs	packed red blood cells
RBCs	red blood cells
RCDM	Royal Centre for Defence Medicine
rpm	revolutions per minute
rFVIIa	recombinant activated factor seven
RSI	rapid sequence induction of anaesthesia and intubation
SPC	Summary of Product Characteristics
TAFI	thrombin activatable fibrinolytic inhibitor
TEG	Tris-HCl, EDTA - ethylenediaminetetraacetic acid - and glycerol
UK	United Kingdom

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Abstract

Injuries caused by explosions are an increasing threat. Primary blast lung injury, caused by the blast wave, precipitates intrapulmonary haemorrhage and subsequent oedema and inflammation. Treatment options are currently limited to supportive measures including mechanical ventilation. Recombinant activated factor VII (rFVIIa) promotes coagulation and reduces bleeding. It has been used to manage patients with traumatic bleeding that is not amenable to surgical haemorrhage control.

Retrospective database analyses were undertaken to establish the epidemiology of blast lung injury in the military patient population, and the use of rFVIIa within military hospitals. These showed that the incidence of blast lung injury in those injured by explosion in the most recent conflict in Afghanistan is 11%. Use of intravenous rFVIIa in military hospitals is declining, due to a combination of factors including the introduction of bespoke targeted resuscitation, although rFVIIa may still have a role in the management of patients with haemorrhage not amenable to conventional treatments, such as blast lung injury.

A programme of work was undertaken to develop an animal model of blast lung injury, in which a trial of nebulised rFVIIa could be undertaken. This involved the development of a reliable and reproducible model of blast lung injury in the rabbit, involving a shock tube. Work was also undertaken to establish that rFVIIa remained active after nebulisation, to define the distribution in the lungs following nebulisation, and to determine the amount deposited following nebulisation. Results showed that rFVIIa remains active following nebulisation, is widely distributed, and 10-15% is deposited in the lungs.

The clinical problem of blast lung injury has therefore been defined in the population of interest, and the experimental work has established the methodology enabling a proof of principle study to be undertaken to determine whether rFVIIa, delivered by nebulisation, attenuates the haemorrhagic phase of blast lung injury.

Section 1. Introduction and Background.

Chapter 1. Introduction.

Blast injuries are an increasing problem in both military and civilian practice. Primary blast lung injury is caused by the shock wave, which precipitates rupture of alveolar capillaries and subsequent intrapulmonary haemorrhage and oedema. This has some features in common with pulmonary contusion from blunt chest trauma. For casualties surviving the initial blast, depending on the distance from the blast and the protection worn, the most common cause of injury in an open environment following an explosion is secondary injury from fragmentation. Combat body armour protects individuals from torso fragmentation wounds, but affords little protection against the blast wave and primary injury such as blast lung. In an enclosed environment, such as inside a vehicle or building, the primary blast wave is reflected and amplified by the surfaces causing greater risk of primary injury.

The management of blast lung currently is predominantly supportive, with mechanical ventilation being the mainstay in severe cases. A significant number of patients with blast lung could easily overwhelm a medical treatment facility with limited critical care resources such as a deployed field hospital. Treatment that is targeted at reducing intrapulmonary haemorrhage may reduce the severity of blast lung.

Recombinant activated factor VII (rFVIIa) was developed in the 1980s as a treatment for patients with haemophilia, as it plays a role in the activation of coagulation and produces a thrombin burst to initiate blood clotting. It has both haemostatic and anti-inflammatory properties, and has been used to control haemorrhage in a variety of medical conditions. Over the last decade the use of rFVIIa in trauma patients has become widespread, although remains controversial.

Initial anecdotal reports from Israel have suggested that intravenous rFVIIa can reduce the severity of blast lung injury and associated clinical problems (personal communication Martinowitz - Kirkman, 2008). An effective and safe early treatment that attenuates intrapulmonary haemorrhage would have a significant impact on the resources necessary to deal with blast lung, in particular where there are multiple casualties. It may also have benefit

potentially optimising patient condition prior to and during aeromedical transfer back to the UK from theatres of operations.
Intravenous administration of rFVIIa carries the potential risk of thromboembolic complications, hence caution continues to be employed when prescribing for off-label indications. If rFVIIa could be delivered by inhalation, it may be limited to local distribution and not absorbed systemically, and this would reduce the potential for adverse events. There have been a few reports of the use of intravenous rFVIIa to mitigate the effects of intrapulmonary haemorrhage (diffuse alveolar haemorrhage) caused by other aetiologies (Heslet et al., 2006). To date, there have been no controlled human studies to determine the effects of rFVIIa, delivered either intravenously, or by inhalation, on blast lung.

This body of work explores the background of blast lung injury and recombinant activated FVII, then aims to define the current problem of blast lung injury within the service population. It then describes the current use of rFVIIa in deployed military hospitals, and a recent case of its use in a military patient with pulmonary haemorrhage. It goes on to describe a programme of work undertaken at the Defence Science and Technology Laboratory [dstl] Porton Down, where an animal model has been developed to investigate the effects of nebulised rFVIIa on blast lung injury. The thesis then explores the animal preparation, initial efforts to establish a reliable and reproducible level of blast injury in the rabbit, experiments to determine whether rFVIIa can be effectively nebulised, and estimates of the dose needed to effect a therapeutic response. The ultimate aim was to develop an animal model that would support a proof of principle study, to determine whether inhaled rFVIIa can attenuate the consequences of blast lung.

This work has been approved by the Stakeholders for the Combat Casualty Care research programme and endorsed by the Surgeon General's Research Strategy Group. The study successfully completed an approved Ethical Review Process.

Chapter 2. Blast Lung Injury.

2.1 Background

Blast injuries are an increasing problem in both military and civilian practice (Champion et al., 2009; Nelson et al., 2006). The recent conflicts in Iraq and Afghanistan, and terrorist bombings over the last few years, have brought the subject to the front door of many hospitals throughout the world that previously had not encountered such injuries. An understanding of the physics and pathophysiology of blast injury is essential if the injuries are to be optimally managed. However, the management options for pulmonary blast injury are currently limited to supportive management, with little in the way of therapeutic options for stopping or reversing the underlying pathological processes.

2.2 Classification

Blast injuries are usually divided into four and occasionally five main categories (Kirkman et al., 2011; Champion et al., 2009); primary, secondary, tertiary, quaternary and quinary.

Primary injuries result from the effects of the shock wave, which travels through the tissues depositing energy particularly where there is a gas-liquid interface. Because of their air-filled alveoli with a single layer of epithelial cells and delicate vascular structure, the lungs are particularly vulnerable to this form of injury, the consequences of which are termed blast lung. Other organs affected by primary injury are the gut, ear and sinuses. Solid organs including the skin are resistant to energy transfer from blast and a seriously blast-injured casualty may have no outwardly visible stigmata of injury. The incidence and extent of primary blast injury depends on both the level of peak overpressure experienced and the duration of exposure.

Secondary injuries result from fragmentation (mostly penetrating but some blunt) injury. These typically cause tissue destruction and loss, and penetrating injury from missile fragments and other objects that have acquired kinetic energy as a result of the blast.

Tertiary injuries are a result of the blast wind, which causes sudden and dramatic movement of air. This causes the body to be thrown against obstacles causing predominantly blunt injury.
Quaternary injuries are a result of burn or crush resulting from the blast, while quinary effects are those clinical consequences of environmental contaminants present as a result of the blast, such as radiation and bacterial exposure.

2.3 Physics of blast injury

An explosion is a process that rapidly liberates vast amounts of energy in the form of heat, kinetic energy and high-pressure shock waves. Energy is released when the chemical bonds within the explosive are broken down, resulting in detonation. This causes an exponential increase in heat production within the explosive, resulting in further increase in pressure, and as the pressure wave almost instantaneously passes through the rest of the material it rapidly releases energy in the form of heat, gaseous products and a blast wave.
The blast wave is defined as a combination of the shock wave and the dynamic overpressure (the latter is sometimes referred to as the blast wind).
Therefore the leading edge of the blast wave is made up of a rim of compressed air under high pressure surrounding the expanding ball of explosive material. Within 1-2 metres of a conventional explosion, the dynamic overpressure slows down while the shock wave detaches and continues to travel faster than the speed of sound (Figure 1).

Figure 1: An explosive detonation, demonstrating shock wave. Reproduced with permission from the Journal of the Royal Army Medical Corps.

If the pressure is measured at a specific point in space over time, there is a rapid (almost instantaneous) increase in pressure, which reaches a peak (overpressure) before falling to below normal atmospheric pressure (underpressure) and then returning to normal (Figure 2). As the blast wave moves through the surrounding atmosphere the magnitude of the wave decreases in proportion to the cube of the radius of its sphere of expansion. This waveform is known as a Friedlander wave, and represents a simple blast in open space that is not influenced by interactions with other structures, such as when a blast occurs inside a building or vehicle. As the shock wave decreases exponentially with distance away from the blast, so the immediate threat to life in an open environment following an explosion is usually secondary injury from fragmentation (Dearden, 2001). If an individual is close enough to the explosion to sustain primary blast injury, they are also likely to sustain serious secondary fragmentation injuries (unless they are wearing protection against fragmentation injury such as combat body armour).

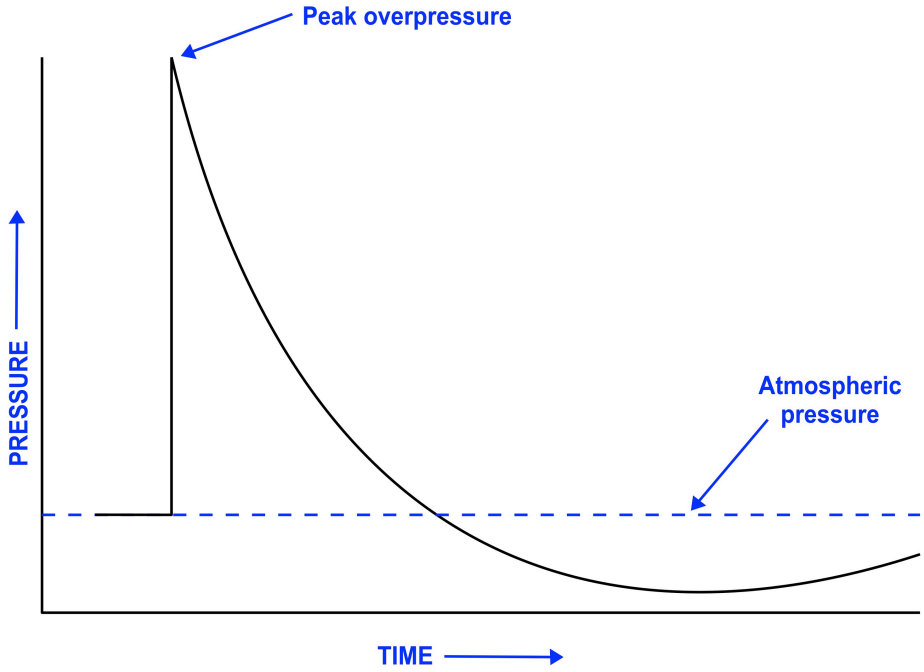

Figure 2: Schematic representation of Friedlander wave, showing peak overpressure, positive phase and sub-atmospheric phase.

When blast occurs in an enclosed space, more complex waveforms are seen, with typically a series of peaks in pressure over a more prolonged time (Figure 3). This can result in a different pattern of injury, which is dependent on the relative position of the subject to the reflecting surfaces and the explosive device (Axelsson and Yelverton, 1996).

When a shock wave comes into contact with a human body, several different mechanisms may cause injury. Stress waves of high amplitude and velocity pass through the body at speeds greater than the speed of sound, along with shear waves of lower velocity and longer duration.

The stress waves result in microscopic damage rather than visible lacerations, as they pass across interfaces between substances of different density, such as tissue – air borders. This causes the surface of the tissue to break up, a phenomenon known as spalling. Hollow gas-filled structures may implode as the pressure wave passes through them, and as the wave passes across delicate structures such as the tympanic membrane, pressure differentials across the boundaries may cause the structure to rupture.

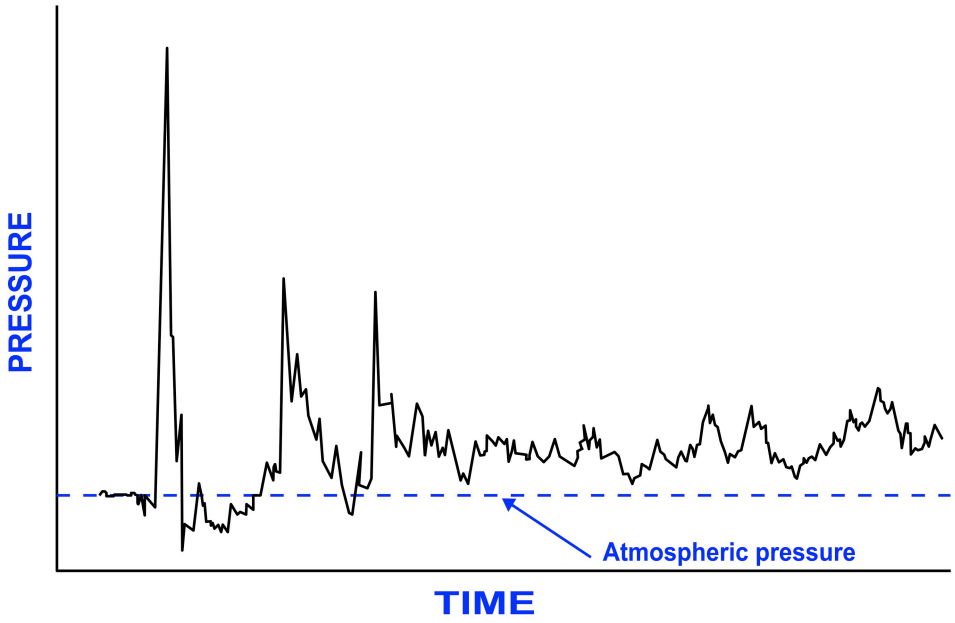

Figure 3: Complex waveform from a blast in an enclosed space.

The shear waves cause movement of tissues, but if there are tissues of different densities these will move at different rates causing tearing of delicate attachments, such as the attachments of bowel mesentery, and solid organs at the attachments of their capsules.

Combat body armour protects individuals from torso fragmentation wounds, and in the process may lead to a reduction in fatal wounds from secondary injury, but it delivers relatively little protection against the shock wave and primary injury. In addition, more modern weapon systems may produce fewer fragments but a more enhanced blast wave, carrying significantly higher risk of primary injury (Dearden, 2001). In an enclosed environment, such as inside a vehicle, the primary blast wave is reflected and amplified by the surfaces causing greater risk of primary injury (Marti et al., 2006; Chaloner, 2005). This risk is confirmed by data from civilian terrorist attacks, which indicate that blast lung is a common feature under these enclosed space circumstances (Marti et al., 2006; Avidan et al., 2005; de Ceballos et al., 2005).

2.4 Incidence

The incidence of primary blast injury is difficult to determine as it often fails to be recognised by clinicians. There are anecdotal reports of men dying after shell

bursts in the First World War without any external evidence of injury, which was probably a result of primary blast injury (Hooker, 1924). The incidence of explosions of all types increased throughout the twentieth century, due to the increased availability of explosives and the legacy of landmines throughout the world. In 1994, at the height of the problem, the international committee of the Red Cross estimated that 2000 people were being killed every month by landmines, predominantly civilians. This figure has been reduced dramatically due to several international agreements and subsequent demining; however, in 2007, the Landmine Monitor identified 5426 casualties from mine strikes across the world (although this is subject to gross underestimation from under-reporting of incidents) [Landmine Monitor Report 2008, Toward a Mine-Free World, p. 51].

There are many causes of explosion, military and civilian, intentional and accidental. Injuries may be caused by an industrial chemical explosion as well as by premeditated terrorist bombings. While the terrorist activities of the last decade and the wars in Iraq and Afghanistan have brought injuries from explosion to the forefront of our minds, injuries caused by explosion have been evident throughout the last century and beyond. In the UK, the IRA bombing campaign of the late twentieth century brought explosions to our doorstep, and in the USA, the bombings of the World Trade Centre in 1993, Oklahoma City in 1995, and the bombing of the US Embassies in Kenya and Tanzania were an ever-present reminder of the dangers of terrorism. In the Middle East attacks on civilian targets brought hundreds of casualties to the hospitals of Israel, many with evidence of primary blast injury.

With regard to the incidence of blast lung within groups of patients injured by explosions, there are large variations in reported incidence, and some even question whether blast lung injury is a significant problem at all. Different recent incidents and theatres of military operations have been reported separately, and these will therefore be considered in turn.

2.4.1 Northern Ireland

There have been two reports from the British Army's experience in Northern Ireland. In one study, 4 of 34 cases who had died as a result of blast injury and who had a limb amputation had either gross or microscopic evidence of blast lung injury (Hull et al., 1994). Mellor reviewed a database of injuries sustained

by 828 servicemen from 1970-84, and found a low incidence of *isolated* blast lung, although many patients had combined injury (Mellor, 1992). He found that 2 patients had isolated blast lung necessitating ventilation, although many more had combined injuries; 24 patients who died had severe chest injury alone, and 46 had combined head and chest injuries.

2.4.2 Balkans Conflict 1991-4

During the Balkans conflict, Cernak *et al.* reported an incidence of primary blast lung injury of 28% among 1300 survivors of explosions, using a combination of clinical, radiographic and blood gas analysis findings (Cernak et al., 1999). This is a relatively high rate of blast lung compared to other large series of survivors; this may be due in part to the criteria used to diagnose the condition, which included clinical symptoms and signs as well as radiological features and injury coding.

2.4.3 Oklahoma City

The Oklahoma City bomb in April 1995 killed 168 people of whom 13 had internal lung injury with no external signs of injury at post mortem. A further 13 survivors required admission to hospital with pulmonary contusion, pneumothorax or early acute respiratory distress syndrome (ARDS), suggestive of primary blast lung injury (Mallonee et al., 1996).

2.4.4 Israel

One case series from Jerusalem describes the findings of blast lung injury in patients injured in two terrorist bomb attacks on civilian buses in Jerusalem (Pizov et al., 1999). 47 patients were dead at scene, and of the 18 survivors being treated at the institution in question, 15 had evidence of blast lung injury. Half of these had evidence of severe hypoxaemia on arrival at hospital, although some had co-existing pneumothorax, which responded to appropriate treatment.

2.4.5 Madrid

Following the Madrid train bombs in 2004, where simultaneous explosions detonated in four commuter trains, 177 people were killed and more than 2000 injured. The incidence of primary blast lung injury was estimated to be 7%

among the total patient population, but 63% - 94% amongst the critically ill immediate survivors (Marti et al., 2006; de Ceballos et al., 2005).

2.4.6 Iraq and Afghanistan

Recent military interventions in Iraq and Afghanistan have seen a high proportion of casualties sustaining injury as a result of explosion. This is highlighted by recent published data from the conflicts. In one study, 46% of combat casualties treated by the American forward surgical teams between 2001-2004 had suffered injuries as a result of blast (Rush et al., 2005). Other studies found that 31% of US combat casualties repatriated to the Walter Reed Centre in Washington had sustained blast injuries (Montgomery et al., 2005), and 55% of severely injured patients had received injuries in explosions (Plotkin et al., 2008). An analysis of UK operational mortality during the period April 2006 – March 2007 showed that 66% of deaths were caused by blast and fragmentation injuries (Hodgetts et al., 2007).

One US study has looked at the incidence of blast lung among US servicemen injured by explosion between 2003 and 2006, and found that the incidence increased from 3.1% in 2003-4, to 4.6% in 2005-6 (Ritenour et al., 2010; Champion et al., 2009). One potential reason for the variation in incidence is the difference in inclusion and exclusion criteria employed by different authors.

As part of this thesis I performed a retrospective database review of patients on the UK Joint Theatre Trauma Registry during the period 2003-9, described in Chapter 4, which found that the incidence of blast lung within the group injured by explosion in Iraq was 7% and in Afghanistan was 11% (Smith, 2011).

2.5 Pathophysiology

The pathophysiology of blast lung injury has been previously investigated using animal models, which have demonstrated that there are immediate and delayed effects. The immediate respiratory response may include apnoea (which lasts from a few seconds to about a minute), and rapid, shallow breathing (Sawdon et al., 2002; Ohnishi et al., 2001). From the results of these animal experiments, the apnoea seems to last for less than 30 seconds (Guy et al., 1998), although it is possible that a proportion of the immediate deaths following blast exposure, when it is noted that there is no external evidence of injury, are due to a prolonged period of apnoea (Jaffin et al., 1987; Krohn et al., 1942).

There are also cardiovascular effects, with evidence of immediate hypotension and a reduction in cardiac index, suggesting global myocardial impairment and potentially a reduction in peripheral vascular resistance that may last several hours after exposure to blast (Irwin et al., 1997; Cernak et al., 1996; Barrow and Rhoads, 1944; Krohn et al., 1942). The cardiovascular response may also include bradycardia from initiation of a vagal reflex. The bradycardia typically returns to normal within 15 minutes, but the hypotension may take much longer to resolve (90 minutes to 3 hours) (Guy et al., 1998).

The blast wave causes immediate lung injury, characterised by rupture of alveolar capillaries and subsequent intrapulmonary haemorrhage and oedema, similar to pulmonary contusion from blunt chest trauma (Gorbunov et al., 1997; Brown et al., 1993). Intrapulmonary haemorrhage, with free blood and haemoglobin in the alveoli, leads to the formation of free radicals, oedema, and an augmented early inflammatory response, with accumulation of inflammatory mediators and chemotaxis, as well as free radical reactions causing oxidative damage (Chavko et al., 2009; Gorbunov et al., 2005). This in turn leads to leucocyte accumulation (this can be demonstrated within 3 hours of injury) and subsequently epithelial cell damage (at 12-24 hours), endothelial cell damage (24-56 hours) and the late oedema typical of adult respiratory distress syndrome (ARDS). Histological examination reveals prominent perivascular oedema and extensive alveolar haemorrhage during the first 12 hours, and thereafter, epithelial cell damage with detachment from the basement membrane (Gorbunov et al., 2005).

The extent of lung damage is related to the magnitude of the blast exposure, with effects ranging from sub-clinical microscopic petechiae to large areas of confluent frank haemorrhage. Delayed effects include the progression of an inflammatory response leading to worsening of the initial lung injury over the first few hours and days after blast exposure (Seitz et al., 2008; Gorbunov et al., 2006; Knoferl et al., 2003). Inflammation affecting the lungs develops over the first 24-48 hours, to peak at approximately 48 hours. This is thought to be due to the accumulation of reactive oxygen species and an imbalance between these and endogenous antioxidants. It has been shown that the inflammatory response can be influenced by the administration of exogenous antioxidants such as N-acetyl cysteine (NAC) (Chavko et al., 2009).

Hypoxaemia develops as a result of a variety of mechanisms including V/Q mismatch (Ohnishi et al., 2001; Pizov et al., 1999), and a reduction in surface area available for gas exchange. Other pulmonary effects include pneumothorax or haemothorax (or a combination), which may occur due to the shearing force of the primary blast injury causing damage to the peripheral alveoli (causing pneumothorax) and pulmonary vessels (causing haemothorax). These complications may also be related to secondary or tertiary blast injury. This has been confirmed by both animal models and retrospective human case series (Pizov et al., 1999; Irwin et al., 1997; Huller and Bazini, 1970). Damage to the pulmonary blood vessels may also cause traumatic broncho-venous or alveolo-venous fistulae, resulting in air embolism, another possible early cause of death following blast exposure. Air emboli may cause ECG abnormalities if they reach the coronary circulation, or a cerebral air embolus resulting in the clinical presentation of stroke. Bubbles have been shown in the circulation of animal subjects exposed to blast up to 30 minutes following blast exposure (Mason et al., 1971).

2.6 Clinical Features

The clinical features of primary blast lung injury are shortness of breath, cough, haemoptysis, cyanosis, and tachypnoea. Hypoxia is an almost universal finding, although this may develop later over the first few hours following injury, and may not be evident initially on presentation. In one series, 28% of patients surviving to a medical facility with blast lung injury had evidence of severe hypoxia on initial presentation (Smith, 2011). Pulmonary barotrauma may result in pneumothorax, haemothorax, pneumomediastinum or combination injuries, giving their associated clinical findings.

Related clinical findings are tympanic membrane rupture, and other manifestations of primary blast injury such as intestinal injury. Tympanic membrane rupture was traditionally thought to be a good biomarker of significant blast exposure, but this has been brought into question by several sources (Peters, 2011; Harrison et al., 2009; de Ceballos et al., 2005; Cohen et al., 2002; Leibovici et al., 1999; Cooper et al., 1983). If, following blast exposure, tympanic membrane rupture is present it is indicative of significant risk of primary blast injury to other organs, but if it is absent, this does not rule out significant blast exposure.

2.7 Imaging

Radiological manifestations of blast lung injury include bilateral perihilar pulmonary infiltrates, typically in a ‘bat-wing’ or ‘butterfly-wing’ pattern. Other findings are similar to pulmonary contusion, which present as areas of increased opacification on the chest radiograph, but in isolated primary blast lung injury there will be no evidence of concurrent chest wall injury on X-ray. This may be confirmed on computed tomography, where a pattern of intraparenchymal haemorrhage similar to pulmonary contusion may be present. In the current conflict in Afghanistan, many patients sustaining blast injury undergo CT to define their injuries (Peramaki, 2011; Smith et al., 2010) and evidence of blast lung injury is often revealed at this stage of the patient’s management even in the presence of a normal chest X-ray (see Figure 4).

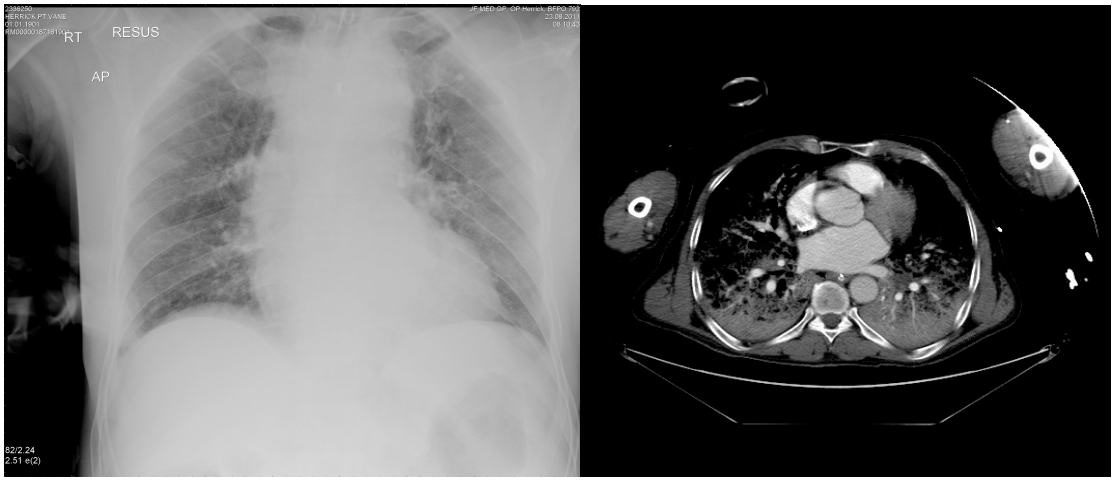

Figure 4: CXR and CT demonstrating severe blast lung injury. This Afghan local national was the victim of an improvised explosive device, and sustained a combination of severe primary and secondary blast injuries. His CT scan shows evidence of severe blast lung injury.

2.8 Management

The evidence to support one management strategy over another is poor, with the majority of published work being level 4 or 5 evidence made up of expert opinion and case reports (Cook et al., 1995). However, the nature of the injury makes a robustly designed randomised controlled trial in human patients almost impossible.

Treatment is predominantly supportive, with mechanical ventilation being the mainstay in severe cases (Avidan et al., 2005). However, the management of blast lung injury is often a challenge because of other complex injuries in the same patient, and these may necessarily dictate the initial management of the patient, for example the need to proceed to theatre for surgical haemorrhage control.

Management of the lung injury often involves mechanical ventilation, but this in itself is not without difficulty, as there is significant risk of barotrauma when ventilating lungs that have reduced compliance due to the nature of their injury. Ventilation may also cause further complication from air embolism, originating from damaged capillaries and traumatic fistulae in blast lung. Specific modes of ventilation have been employed in an attempt to reduce the pressure-related injury associated with high-pressure ventilation in friable lung, such as high-velocity ventilation and the inhalation of nitric oxide (Avidan et al., 2005). Other strategies include the use of low tidal volumes, permissive hypoxia (maintaining oxygen saturation at around 90%) and hypercapnia, limiting positive end-expiratory pressure (PEEP), and the use of pressure-controlled ventilation (Camporota and Hart, 2012).

One of the main problems with the management of blast lung injury is that the lung is susceptible to the effects of intravascular fluid loading, which may be necessary to treat other injuries. In the presence of haemodynamic compromise, fluid resuscitation and transfusion of blood and blood products is essential to maintain end organ perfusion, but will worsen the cascade of capillary leak and inflammation in the lungs, due both to the primary lung injury and secondary effects of systemic injury. The role of haemostatic resuscitation with aggressive replacement of blood, plasma and other blood products has a place in the management of these patients, as this has been shown to reduce the amount of crystalloid used in resuscitation.

The critical care transport of acute lung injury patients is just as great a challenge (Dorlac et al., 2009), and may necessitate prophylactic use of intercostal drains and conservative ventilatory strategies that may reduce the risk of deterioration during transport. This is reflected in the current management of combat casualties, who are typically evacuated from Afghanistan back to the UK within 24-48 hours, when the effects of blast lung injury may be at their worst.

The role of novel therapies to reduce the haemorrhage associated with blast lung injury have not yet been evaluated, but anecdotal reports of the use of intravenous recombinant activated factor VII (rFVIIa) to limit the extent of pulmonary haemorrhage have been encouraging, and this was endorsed by the UK Advisory Group on Military Medicine in August 2010.

2.9 Long-term Outcome

It has been suggested that the outcome from blast lung injury is good, if the patient survives the initial insult. In one report, a patient had persistent hypoxaemia for 4 months following blast lung injury, but lung function testing had returned to normal by 10 months (Caseby and Porter, 1976). In another study, 11 survivors of blast lung injury were followed up for one year, and all recovered their lung function, measured by lung function testing and exercise testing, and none had any respiratory symptoms (Hirshberg et al., 1999). All had normal chest radiographs at one year.

2.10 Summary

Blast lung injury is an increasing problem for UK forces in Afghanistan, but is not a new phenomenon, with evidence that it has been increasing in incidence over the last century. Management strategies are currently limited to supportive measures including protective ventilatory strategies.

Chapter 3. Recombinant Activated Factor VII (rFVIIa).

3.1 Introduction

Factor VII (FVII) is a plasma protein that is produced in the liver, and is involved in the coagulation process. FVII plays a role in the activation of coagulation by its interaction with another protein that is normally attached to blood vessel walls, tissue factor. Activated FVII (FVIIa) is present in the normal circulation, and represents about 1% of the total plasma FVII (Hedner, 2006). Novo Nordisk (Maaloev, Denmark) commercially developed recombinant activated factor VII (rFVIIa) in the 1980s to treat haemophilia patients with inhibitors to FVIII and FIX (Hedner, 2007; Hedner and Kisiel, 1983). Initially FVIIa was purified from plasma, although this was time-consuming, risked transmission of infectious agents, and only produced small quantities of the protein, so recombinant technology using baby hamster kidney (BHK) cells was then employed to produce substantial quantities for therapeutic use (Burnouf, 2011).

3.2 Normal haemostasis

Our understanding of haemostasis has developed over the last two decades from the traditional cascade model to a cell-based theory of coagulation. The traditional model described intrinsic and extrinsic pathways ending in a common endpoint, resulting in the production of thrombin from prothrombin, and ultimately fibrin from fibrinogen. However, this model suggested that the two pathways were essentially distinct, and only converged at the final stage.
It has become apparent since then that *in vivo* coagulation is dependant on a variety of factors, all of which seem to be interlinked, and seems to occur in stages; first the initiation phase, then the amplification phase and finally the propagation phase (Hoffman, 2003). The amplification phase only happens in the presence of a trigger such as damage to a vessel wall.
Integral to the role of FVII is tissue factor, a protein that is expressed by cells located within blood vessel walls, and that is not normally exposed to the circulation. Tissue factor, especially cell-bound, when exposed to the circulation (for example following injury), binds to circulating FVII; the resulting complexes of [tissue factor – FVII] produce small amounts of thrombin (the priming phase

of haemostasis), resulting in activation of other co-factors such as FV, FVIII, FX and FXI as well as activation of platelets, as shown in Figure 5 (Hoffman and Monroe, 2007).

FX, once activated, and in the presence of activated FV, cleaves prothrombin (FII) to form active thrombin molecules, feeding the propagation of clot. Platelets are vital in the production of effective clot, with phospholipids on the surface of the platelet membrane playing a key role in activation of other clotting factors and the production of a thrombin burst. Platelets play a key role in the propagation phase, where the majority of prothrombin is produced on the surface of the platelets. Factor XIII, once activated by thrombin, enhances clot stability and results in cross linkage of the fibrin strands.

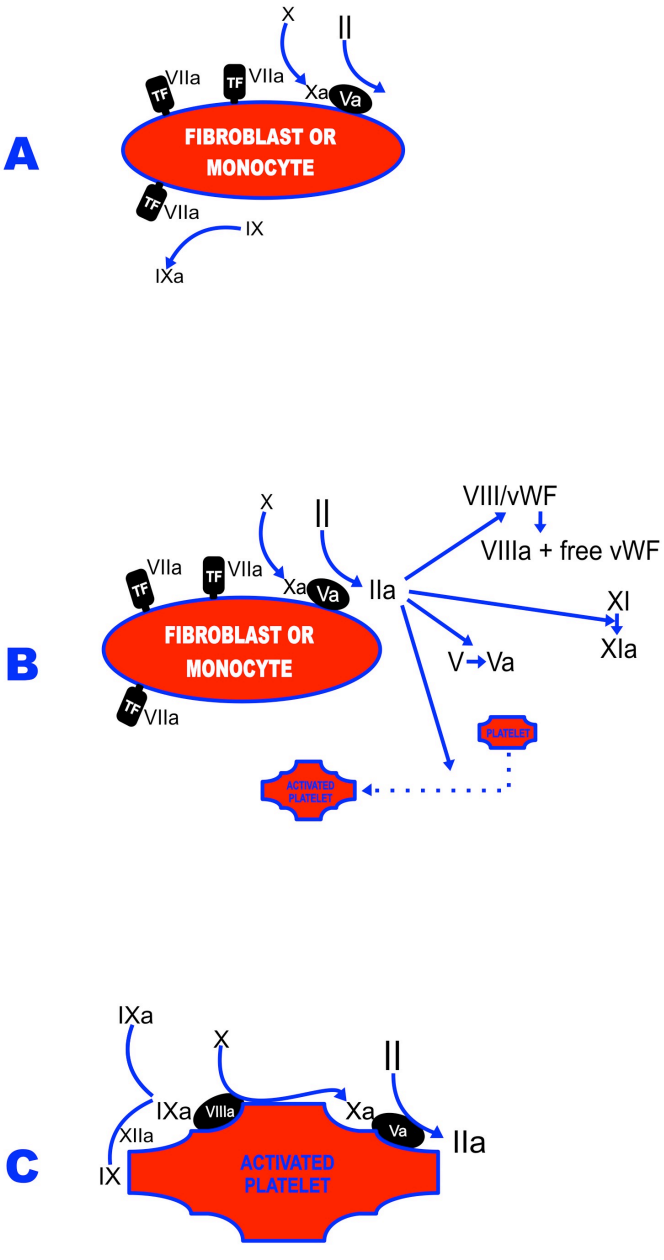

Figure 5: Diagrammatic representation of coagulation, from Hoffman and Monroe (2007), showing initiation phase on TF-bearing cell (A), as activated FX combines with its cofactor, activated FV, to activate small amounts of thrombin. (B) The small amount of thrombin generated on the TF-bearing cell amplifies the response by activating cofactors, factor XI, and platelets. (C) The propagation phase, involving activated platelets.

3.3 Haemophilia

Haemophilia patients lack FVIII (haemophilia A) or FIX (haemophilia B). While these factors are not involved in the initiation of clot production, they play a role in full activation of thrombin generation and therefore ultimate clot stability. These factors form complexes on the surface of activated platelets that enhance FX activation and thrombin generation. They also play a role in activation of thrombin activatable fibrinolytic inhibitor (TAFI), which helps to keep the fibrinolytic process in check. In its absence, the clot is susceptible to being broken down by fibrinolysis.

Some haemophilia patients develop inhibitors to FVIII and FIX, thereby making the treatment of their conditions more complex, as they do not respond to simple replacement of their deficient factors. The development of rFVIIa was initially targeted at these patients, in an attempt to bypass the FVIII/FIX system. FVIIa was first used in haemophilia patients in 1988. The administration of therapeutic levels of rFVIIa results in the production of a stable clot independent of FVIII or FIX. Its mechanism is likely to involve binding to the surface of the activated platelet, resulting in activation of FX and generation of thrombin.

3.4 Mechanism of action

In patients with normal coagulation, rFVIIa binds to tissue factor exposed at the site of vessel injury, to initiate coagulation (ten Cate et al., 1993). It also has an effect independent of tissue factor, thought to be due to its binding to activated platelets at the site of vessel injury, again resulting in activation of coagulation (Roberts et al., 2004; Hoffman et al., 2002). The addition of rFVIIa enhances thrombin generation and full FX activation. In the absence of other clotting factors, such as FVIII or FIX (haemophilia A and B respectively), or acquired factor deficiency (for example following trauma and subsequent resuscitation), rFVIIa binds to the activated platelet surface independent of the presence of FVIII or FIX, causing activation of FX. In the presence of FIX, thrombin generation may be mediated either through activation of FIX or FX (Friederich et al., 2001).

Platelet bound rFVIIa activates FIX and FX on the surface of platelets leading to local thrombin formation (Hoffman et al., 1998). This results in the formation of a tight fibrin clot resistant to breakdown by fibrinolysis (He et al., 2003; Monroe et al., 1997).

FVII clotting activity is dose dependant, as shown by a study on normal individuals, and is not related to race (Fridberg et al., 2005). Giving a patient rFVIIa therefore results in a thrombin 'boost' (and enhanced clot production) independent of the presence of other clotting factors. However, to work it does need adequate functioning platelets; it also needs exposed tissue factor, and therefore in theory should only promote clotting at the site of injury.

3.5 Current licence for use

Current European Union Licences for use of recombinant FVIIa are:

- Congenital haemophilia with inhibitors
- Acquired haemophilia
- Glanzmann's thrombasthenia
- Congenital FVII deficiency

However, in one study of 12,644 hospitalisations where rFVIIa was used, 97% were for reasons outside these licensed indications (Logan et al., 2011).

3.6 Preparation

rFVIIa (eptacog alpha [activated], Novoseven®) is available as a white lyophilised powder that should be reconstituted with diluent for injection, in single use vials. According to manufacturer's recommendations, following reconstitution it should be used within 3 hours. The reconstituted solution has a pH of around 6.0. It should be stored below 25 degrees Celsius, and protected from light. Contained within Novoseven® packaging there is a vial with active substance in powder form, and a vial for reconstitution containing sterile water, sodium hydroxide, hydrochloric acid, and histidine. Following reconstitution, 1ml of the solution contains 1mg eptacog alpha. 1mg equals 50 KIU or 50,000 IU (international units).

3.7 Dose and pharmacokinetics

The appropriate intravenous dose of rFVIIa has been the subject of several clinical studies. Some have used low dose, designated at less than 80mcg/kg, and some have used higher doses at more than 80mcg/kg. None have proven the efficacy of higher dose over lower dose, as shown by a Cochrane meta-analysis of 5 studies (Stanworth et al., 2007). A study comparing escalating doses of rFVIIa to control traumatic intracranial haemorrhage (from 40 to

200mcg/kg) showed no improvement in outcome measures with increasing dose, when compared to placebo (Narayan et al., 2008).

Clearance of rFVIIa is via the liver, with evidence of active endocytosis of FVII molecules into the hepatocytes as shown by radioisotope studies and electron microscopy (Seested et al., 2011). There is evidence that as well as binding to vascular endothelium, rFVIIa also enters the extravascular spaces bound to tissue factor (Gopalakrishnan et al., 2010).

The half-life of rFVIIa has been estimated to be 120-170 minutes (Friederich et al., 2001; Hedner, 1999), implying that repeated doses may be necessary in prolonged episodes of haemorrhage. This is slightly shorter than that quoted in the manufacturer's Summary of Product Characteristics, where in healthy subjects the mean half-life is described as between 3.9 and 6.0 hours.

3.8 Safety

The theoretical potential for thrombotic events following systemic activation of clotting mechanisms has been reported as low, but still contributes to the reluctance of clinicians to use rFVIIa in trauma. O'Connell *et al.* analysed adverse event reports to the US Food and Drug Administration (FDA) over a five year period between 1999-2004, and found 168 reports detailing thromboembolic events such as pulmonary embolus and thromboembolic stroke, with 50 reported deaths (O'Connell et al., 2006). Although there are some methodological flaws with this paper, and causality is at best tenuous in some of the cases (particularly when the increased risk of thromboembolic disease in trauma and post-surgical patients is considered), it would suggest that the use of rFVIIa is not benign, particularly in patients who do not suffer from haemophilia.

Ranucci *et al.* performed a meta-analysis of 7 randomised trials involving 772 patients undergoing major surgical procedures, examining safety in terms of thromboembolic complications and mortality rates (Ranucci et al., 2008). They found that there was no statistical difference in complication or mortality rates between those receiving rFVIIa and those receiving placebo, although in absolute terms there was a slight increase for each in the rFVIIa group (7.1% versus 5.3% for thromboembolism, and 2.8% versus 2.3% for mortality).

Analysis of data from the CONTROL study showed no significant difference in

adverse event rate, including the incidence of thromboembolism, between the treatment and placebo groups (Dutton et al., 2011).

However, in 2009, Novo Nordisk conducted their own internal meta-analysis of trials that reported use of rFVIIa as an off-licence treatment, and found that the incidence of arterial thromboembolic adverse events was 5.6% versus 3% in the placebo group (personal communication, Smith – Jackie Gilbart, Senior Medical Information Officer, Novo Nordisk, 10 Aug 2010). This led them to change the advice in the Summary of Product Characteristics to the following statement:

'Safety and efficacy of NovoSeven have not been established outside the approved indications and therefore NovoSeven should not be used.' This prompted a review of its use in several clinical contexts by numerous medical bodies.

3.9 Cost-effectiveness

The cost of a single dose of rFVIIa (NovoSeven® 5mg vial, equivalent to 70mcg/kg for a 70kg adult) is £2626.00 (personal communication, Smith – Novo Nordisk, 18 Aug 2010).

The cost effectiveness of the use of rFVIIa has been studied in a variety of situations. In a study of the use of rFVIIa in intracerebral haemorrhage it was found that rFVIIa was cost effective and indeed at a dose of 80mcg/kg was cost saving in terms of cost per quality-adjusted life year (QALY) (Earnshaw et al., 2006). However, cost remains a significant barrier to the more extensive use of rFVIIa.

3.10 Use of rFVIIa to control bleeding

The fact that rFVIIa enhances coagulation has led to its use in the management of haemorrhage in a wide range of circumstances, including control of bleeding from thombocytopathies (Peters and Heijboer, 1998), during cardiac and thoracic surgery (Felten and Fischler, 2010; Zangrillo et al., 2009), following post-partum haemorrhage (Ahonen et al., 2007; Alfirevic et al., 2007), and following spontaneous intracerebral haemorrhage (Mayer et al., 2008; Mayer et al., 2005).

In a study of 399 patients with intracerebral haemorrhage, Mayer *et al.* randomised patients to receive either placebo, or rFVIIa at three different doses (40mcg/kg, 80mcg/kg, or 160mcg/kg). The primary outcome measure was the

percentage change in haematoma size at 24 hours after the initial scan (Mayer et al., 2005). All patients who received rFVIIa had a smaller increase in size of haematoma at 24 hours, suggesting the rFVIIa is effective in reducing haematoma expansion following haemorrhage. There was a dose-response effect with a greater reduction in haematoma expansion with the higher dose patients. There was also a reduction in adverse clinical outcomes at 90 days in the rFVIIa group, although they also experienced an increase in thromboembolic adverse events (7% versus 2%). However, another larger phase 3 study by the same author involving 841 patients with intracranial haemorrhage showed that while again the volume of bleeding was less, 90 day clinical outcomes were no better (and if anything worse) with patients who had received rFVIIa (Mayer et al., 2008). There was also an increase in significant adverse thromboembolic episodes in the rFVIIa group (24% with placebo; 26% in patients receiving 20mcg/kg rFVIIa; 29% in those receiving 80mcg/kg rFVIIa), especially arterial thromboses (4% v 9%).

Lin *et al.* reviewed the evidence for the use of rFVIIa to prevent bleeding and treat non-haemophilia patients with bleeding (Lin et al., 2011). They looked at 25 randomised trials, of which 11 were specifically looking at treatment rather than prevention. 2366 patients were recruited to these studies, of whom 1507 received rFVIIa. There was a trend towards better survival in the rFVIIa group (RR 0.89; 95% CI 0.77 to 1.03), but this was balanced by an increased risk of thromboembolic adverse events (RR 1.21; 95% CI 0.93 to 1.58). There was no statistically significant difference in either positive or negative outcomes. They concluded that at present the use of rFVIIa outside its licensed recommendations should be limited to clinical trials.

3.11 Use of rFVIIa in trauma

The ability of rFVIIa to enhance thrombin generation on the surface of activated platelets has resulted in its use in other situations where the production of a stable blood clot is essential, the most intuitive of which is trauma with uncontrolled haemorrhage. In theory, rFVIIa will only provoke formation of clot where there is exposure of tissue factor, for example in vessels damaged by trauma. The first use of rFVIIa to control traumatic haemorrhage was reported in 1999; it was used to treat uncontrolled haemorrhage from a gunshot wound causing injury to the inferior vena cava in an Israeli soldier (Kenet et al., 1999).

Since then its use has become widespread in the management of traumatic haemorrhage, although it remains an off-licence indication.

After a few case reports of its successful use in trauma patients (Dutton et al., 2004; O'Neill et al., 2002; Martinowitz et al., 2001), and some promising results from retrospective reviews (Rizoli et al., 2006) two simultaneous randomised trials were conducted on blunt and penetrating trauma patients, with the administration of rFVIIa (or placebo) after 8 units of blood, followed by subsequent doses 1 and 3 hours later (Boffard et al., 2005). 143 blunt trauma patients and 134 penetrating trauma patients were randomised. There was a significant reduction in the amount of blood used in transfusion in the rFVIIa group for blunt trauma patients, and a non-statistically significant trend for penetrating trauma patients. However, there was no difference in mortality or other clinical endpoints (days ventilated, days on ICU), and no difference in thromboembolic adverse events between the two groups.

The use of rFVIIa has also been investigated in traumatic intracerebral haemorrhage (ICH) (Narayan et al., 2008). The study excluded patients with subdural and extradural haemorrhage (as the management of these cases is predominantly surgical). 97 patients with traumatic ICH received either placebo, or increasing doses of rFVIIa (40mcg/kg to 200mcg/kg). There was no difference in survival between the two groups, although there was a trend towards a reduction in expansion of haematoma at 24 hours post injury in the rFVIIa group. There was also a trend towards an increase in thromboembolic adverse events in the rFVIIa group, although this was not statistically significant, and difficult to attribute to the rFVIIa; for example, one case of deep venous thrombosis in the treatment group was in a patient with an open-book pelvic fracture, which in itself carries a considerable risk of thromboembolism. A further small series of 15 patients was described by Bartal *et al.*, where co-existing coagulopathy was managed by rFVIIa administration in consecutive patients with traumatic brain injury (Bartal et al., 2007).

Following these inconclusive studies, further retrospective database analyses were undertaken to investigate outcome of patients who had received rFVIIa for the management of traumatic haemorrhage. A retrospective database analysis of patients receiving massive transfusion (>10 units packed RBCs in 24 hours) for traumatic haemorrhage in a combat field hospital suggested a decrease in mortality in the group that received rFVIIa (Spinella et al., 2008). Out of 124

patients undergoing massive transfusion, 49 received rFVIIa. The rFVIIa group had a significantly lower mortality at 24 hours and 30 days, with no increase in adverse effects, although the population were more severely injured than in other studies involving civilian patients.

In another retrospective database review of injured military patients receiving a blood transfusion from 2003-2009, 25% received rFVIIa (Wade et al., 2010). In general, those who received rFVIIa were sicker (according to injury severity and physiological scores), but when cohorts were matched for age and injury severity, there was no difference in survival or adverse events.

A meta-analysis of results from studies in trauma patients showed a slight reduction in the incidence of acute respiratory distress syndrome (ARDS) following trauma in those receiving rFVIIa, but no difference in mortality or adverse events (Yank et al., 2011).

Following these studies, a large international multi-centre trial (the CONTROL trial) was then conducted to attempt to define the role of rFVIIa in traumatic haemorrhage (Hauser et al., 2010). This aimed to recruit 1502 adult patients with blunt or penetrating trauma and continuing bleeding despite resuscitation and transfusion of 4 units of packed red blood cells (RBCs). Patients were given either placebo, or rFVIIa (200mcg/kg initially, then 100mcg/kg at 1 hour and repeated at 3 hours). The trial was powered for, and designed around a mortality of approximately 27.5% in this group. However, the study was stopped early following an interim analysis of results, due to a lower than expected mortality (10.8%) across the study participants. It was therefore felt that it was futile to continue the study, as the primary outcome was all cause 30-day mortality, and a significant result was unlikely to be reached. In the participants recruited, the mortality was not significantly different although there was a reduction in blood product use in the rFVIIa group (mean 7.8 RBC units over 48 hours compared to 9.1 RBC units in the placebo group). There was no statistically significant difference in adverse events between the two groups, although there were 16 arterial thromboses in the rFVIIa group (n=224) and 11 in the placebo group (n=250).

There is, therefore, no class 1 evidence to support the use of rFVIIa in all-comers with traumatic haemorrhage, although there is some evidence that it reduces transfusion requirements and may reduce the incidence of ARDS.

3.12 Use of intravenous rFVIIa in lung haemorrhage

One previous case has been described of a patient with severe traumatic intrathoracic haemorrhage who had diffuse bleeding not amenable to surgical control, who was given two doses of rFVIIa (60mcg/kg), which appeared to control the bleeding and result in a positive outcome in terms of survival (Kamphuisen et al., 2002). A further case of its use in traumatic intrapulmonary haemorrhage is described in Chapter 6. The use of intravenous rFVIIa to control non-traumatic lung haemorrhage in the form of diffuse alveolar haemorrhage (DAH) or haemoptysis has been reported in several case series and studies.

DAH affects 1-21% of patients undergoing bone marrow transplantation for leukaemia and has a significant mortality rate (Hicks et al., 2002). Several cases of use of rFVIIa in this condition have been reported, following failure of conventional therapeutic options. This includes one case where 4 doses of 90mcg/kg were used to effectively stop intra-alveolar haemorrhage (Hicks et al., 2002), and in another, doses of 120mcg and 180mcg/kg were used successfully to abate the haemorrhage in a patient following bone marrow transplantation (Henke et al., 2004). In another case of DAH following stem-cell transplantation, Pastores describes resolution of symptoms following 2 doses of 90mcg/kg, after conventional therapy had failed (Pastores et al., 2003). Shenoy also reports success of rFVIIa in attenuating haemorrhage in a case of DAH following stem-cell transplantation (Shenoy et al., 2007). In another case of DAH caused by microscopic polyangiitis, rFVIIa stopped the haemorrhage and resulted in resolution of life-threatening pulmonary haemorrhage (at a dose of 80mcg/kg) (Betensley and Yankaskas, 2002). Another case of polyangiitis responded to three doses of intravenous rFVIIa (120mcg/kg), resulting in full recovery (Henke et al., 2004). A recent report also described a case of bleeding caused by vasculitis responding to rFVIIa (Dabar et al., 2011).

One case has been described of pulmonary haemorrhage complicating pulmonary metastases from choriocarcinoma responding to a single dose (100mcg/kg) of intravenous rFVIIa, which resulted in rapid resolution of the haemoptysis and subsequent recovery (Wheater et al., 2008).

In cystic fibrosis, severe lung haemorrhage resulting in massive haemoptysis is usually managed by bronchial artery embolisation, but if this fails, treatment options are limited. About 5% of cystic fibrosis patients will have at least one

episode of massive haemoptysis, with a recurrence rate of up to 26%. Lau *et al.* present 4 such cases where rFVIIa was used (2 received single doses of 90mcg/kg, one received two doses of 120mcg/kg, and the other presented twice, on both occasions receiving 90mcg/kg) (Lau et al., 2009).

Chronic necrotising aspergillosis can cause massive haemoptysis, and one such case has been described that responded to four doses of 30mcg/kg over two days, resulting in complete resolution of the bleeding (Samarzija et al., 2008). Another case of haemoptysis secondary to pulmonary aspergillosis was managed by administration of rFVIIa, with 3 doses of 90mcg/kg. Haemorrhage was controlled and the patient responded well to subsequent antifungal treatment (White et al., 1999).

Another case of pulmonary haemorrhage, due to bronchial bleeding following cardiac surgery and bypass, has been described where the use of rFVIIa allowed visualisation of the bleeding point and subsequent repair (Courtney, 2009). In another where haemoptysis was caused by pulmonary-renal syndrome, resolution of bleeding was achieved by 3 doses of 90mcg/kg, although the patient subsequently died of a further episode of haemoptysis (Yildirim et al., 2006). Another case of haemoptysis, this one secondary to pneumonia, was successfully managed by administration of a single dose of 90mcg/kg IV rFVIIa (Macdonald et al., 2006).

There have also been reports of the use of intravenous rFVIIa intra-operatively in lung transplantation surgery, after severe bleeding was present in four patients even after surgical haemorrhage control techniques, allowing chest closure and transfer to the ICU without further haemorrhage (Felten and Fischler, 2010).

The use of rFVIIa has been reported in infants and neonates, with particular relevance to pulmonary haemorrhage. One paper describes resolution of pulmonary haemorrhage in 2 infants, both two days old (among nine described with various aetiologies of bleeding), with a dose of 90-100mcg/kg of rFVIIa (Brady et al., 2006). Another paper describes the use of rFVIIa in 8 children with pulmonary haemorrhage of various aetiologies, and notes that while bleeding may be controlled, mortality is still high as a result of multi-organ failure in this group of patients (Yilmaz et al., 2008). Olomu *et al.* report similar success in the treatment of two cases of premature infants with pulmonary haemorrhage, using repeated doses of rFVIIa (50mcg/kg, every 3 hours) (Olomu et al., 2002).

3.13 Use of intra-pulmonary rFVIIa in pulmonary haemorrhage

There have also been limited case reports of intrapulmonary administration of rFVIIa for pulmonary pathologies such as DAH. Estella *et al.* described the pulmonary administration of rFVIIa in two cases of DAH, resulting in significant clinical improvement. One was a case of acute promyelocytic leukaemia complicated by haemoptysis and intrapulmonary haemorrhage, and the second was a case of haemorrhage complicating thrombolysis for myocardial infarction (Estella et al., 2008). rFVIIa was delivered via a bronchoscope directly into the lungs, at a dose of 50mcg/kg in 50mls of normal saline. Immediate cessation of bleeding was noted in both cases, and over the next 12 hours, oxygenation improved such that the FiO_2 could be reduced from 1.0 to between 0.3 and 0.4. Heslet *et al.* (2006) took 6 patients with diffuse alveolar haemorrhage and locally instilled rFVIIa by bronchoalveolar lavage. The dose used was 50mcg/kg, dissolved in 50mls normal saline, with 25mls being instilled into each main bronchus. 3 patients had an excellent response (haemostasis was achieved following a single dose of rFVIIa), but in the remaining 3 patients a repeat dose of rFVIIa was necessary before haemostasis was achieved. For one patient, this was after intravenous administration of rFVIIa had been unsuccessful. In another patient, following extubation, a repeat dose was administered via a nebuliser (again, at a dose of 50mcg/kg). Oxygenation was measured by the ratio of PaO_2 to FiO_2, before and after administration of rFVIIa. In all patients, haemostasis was achieved and oxygenation improved following administration of intra-pulmonary rFVIIa (Heslet et al., 2006). Broncho-alveolar lavage was repeated following administration of rFVIIa, and this confirmed cessation of bleeding (personal communication Heslet – Watts, 2009). This was the first reported use of nebulised rFVIIa to treat alveolar haemorrhage or acute lung injury.

3.14 Potential future uses

The holy grail of trauma therapy is to achieve a reduction in mortality. To prove this takes, in many cases, huge numbers of patients in a well-conducted randomised trial. It appears from the current evidence that rFVIIa is effective in achieving improvement in clinician-oriented outcomes but not the ultimate patient-oriented outcome of survival. However, the increasing incidence of blast lung and the lack of an effective treatment strategy have led to novel uses of

rFVIIa in the military operational environment. This is in the form of repeated doses of intravenous rFVIIa in an attempt to interrupt the haemorrhagic phase of blast lung development. The UK Advisory Group on Military Medicine endorsed this off-label use in August 2010.

Section 2. Novel Data from the Joint Theatre Trauma Registry.

Chapter 4. The Epidemiology of Blast Lung Injury During Recent Military Conflicts: a Retrospective Database Review of Cases Presenting to Deployed Military Hospitals, 2003-2009.

4.1 Introduction

Since April 2003 the UK armed forces have been deployed overseas in combat operations with field hospital medical support. Data on all seriously injured patients (including UK military, coalition forces, detainees, and local civilians) treated by UK Defence Medical Services in these facilities are collected by the deployed clinical team and returned to the UK Joint Theatre Trauma Registry (JTTR). This was formerly maintained by the Academic Department of Military Emergency Medicine (ADMEM) at the Royal Centre for Defence Medicine (RCDM) in Birmingham, and latterly Defence Analytical Services and Advice (DASA). Data are prospectively collected from clinical notes, trauma charts and in the case of death, post mortem findings. The JTTR holds continuous data on this cohort from 2003, coinciding with the start of hostilities in Iraq. Returns are electronic (where deployed IT systems allow), with hard copy accompanying UK military patients evacuated to RCDM for definitive care. The default entry criterion for UK JTTR is a casualty who triggers trauma team activation in a deployed field hospital or Primary Casualty Receiving Facility afloat. Trauma team activation criteria are described in Figure 6. The entry criteria were expanded in 2007 to include all trauma patients returned to RCDM for definitive treatment, irrespective of whether a trauma team response was mandated. All UK Service deaths from trauma are subject to post mortem examination on repatriation, and a representative from ADMEM attends all examinations and records the detailed findings within JTTR.

Injury patterns have changed during the conflicts in which we have been engaged. Hostile forces have changed their tactics, from direct confrontation to guerrilla style warfare and ambush with an increase in the use of improvised explosive devices and mines. There has been evidence from previous studies that the ratio of gun shot wounds (GSWs) to wounds caused by explosion (blast injuries) has been steadily reducing (Plotkin et al., 2008; Hodgetts et al., 2007;

Nelson et al., 2006; Montgomery et al., 2005; Rush et al., 2005). Combat body armour protects individuals from torso fragmentation wounds, and in the process may lead to a reduction in fatal wounds from secondary injury, but it delivers little protection against the blast wave and primary injury.

The aim of this study was to establish the epidemiology of blast injuries since the onset of hostilities in 2003 among patients presenting to UK field hospitals, and within the group injured as a result of explosion establish the incidence of blast lung injury. A secondary aim was to examine the use of rFVIIa in this group.

Trauma team activation criteria

2–3
Preparation

Preparation **3**

Mechanism/History

Penetrating trauma

- Gunshot or shrapnel wound
- Blast injury (mine/IED/grenade)
- Stab wound

Blunt trauma

- Motor vehicle crash with ejection
- Motorcyclist or pedestrian hit by vehicle >30km/h
- Fall >5 metres
- Fatality in the same vehicle
- Entrapment and/or crush injury
- Inter-hospital trauma transfer meeting activation criteria

You may only receive a triage category and a mechanism of injury.

For T1 casualties activate the Trauma Team

and

Anatomy

- Injury to two or more body regions
- Fracture to two or more long bones
- Spinal cord injury
- Amputation of a limb
- Penetrating injury to head, neck, torso, or proximal limb
- Burns >15% BSA in adults **or** >10% in children **or** airway burns
- Airway obstruction

or

Physiology

- Systolic blood pressure <90mmHg or pulse >120bpm (adults)
- Respiratory rate <10 or >30 per minute (adults); SpO_2 <90%
- Depressed level of consciousness or fitting
- Deterioration in the Emergency Department
- Age >70 years
- Pregnancy >24 weeks with torso injury

Figure 6: Trauma team activation criteria (extract from Clinical Guidelines for Operations, Joint Service Publication 4-03.1)

4.2 Methods

A retrospective database review was performed, using the JTTR database from 01 January 2003 to 01 October 2009. Inclusion criteria were all trauma patients in the JTTR from 1 January 2003 to 1 October 2009. This included UK military, coalition military, civilians, and local security forces. Exclusion criteria were disease (non-injury) classifications. Anonymised data were supplied from the JTTR database, and therefore ethical approval was not required.

From the overall database, filters were applied to limit the search to only those injured by explosive blast, then those with coding evidence of thoracic injury from blast. Finally the search was limited to those with injury coding of blast lung or pulmonary contusion caused by blast. All cases in the subsequent group were examined individually to ascertain if they had pulmonary blast injury. All thoracic injury categories were therefore examined for relevant cases, but those with an obvious alternative cause for their thoracic injuries, such as penetrating cardiac wounds, were excluded from the analysis.

Limits to the database search were applied sequentially:

1. Mechanism - Explosion
2. Thoracic injury
3. Coded for blast lung or pulmonary contusion
4. Use of rFVIIa within this group

4.3 Results

Overall during the study period there were 3,109 cases in the JTTR, of whom 1,678 suffered injuries as a result of an explosion. Of this group, 233 patients sustained thoracic injuries as a result of blast injury, and when the search was further limited to those coded (using AIS 2005 military) as blast lung or pulmonary contusion the final study group was made up of 113 cases (Figure 7). The mean ISS of this group was 52.6, with 92% of cases having an ISS>15 (95% of cases had a NISS>15).

Figure 8 shows the mechanism of blast injury; by far the most common weapon used was the improvised explosive device (IED). The number of cases of major trauma presenting to UK military hospitals rose during the study period, with peaks in Iraq in 2007, corresponding to Operation TELIC 9, and in Afghanistan in 2009, corresponding to Operation HERRICK 10 (Figures 9 and 10).

From Table 1 it is clear that 50 of these cases made it to a medical facility alive, with 63 patients dying before reaching a medical facility.

Outcome	Number
Killed in action	60
Died of wounds	12
Killed (non-enemy action)	3
Wounded (non-enemy action)	1
Wounded in action	37

Table 1: Outcome of patients with blast lung.

14 patients had evidence of severe hypoxia (oxygen saturation <92%) on arrival at a medical facility suggesting significant early respiratory compromise, in the pre-hospital phase of their blast injury.

Of the 50 patients surviving to medical support, 8 (16%) underwent rapid sequence induction of anaesthesia and intubation (RSI) pre-hospital (performed by the Medical Emergency Response Team, MERT), 25 (50%) underwent RSI in the Emergency Department (ED), 7 (14%) were intubated and ventilated in the operating theatre and 10 (20%) did not require ventilatory support. The majority of those undergoing RSI and ventilation underwent surgery and were subsequently transferred for ongoing respiratory support to the intensive care unit (ICU). Many of these patients required surgery for other injuries sustained from secondary fragmentation injuries, particularly limb injuries.

20 patients received intravenous rFVIIa as part of their management in the field hospital, 13 of these during a peak in use in 2009 (described in more detail in Chapter 5).

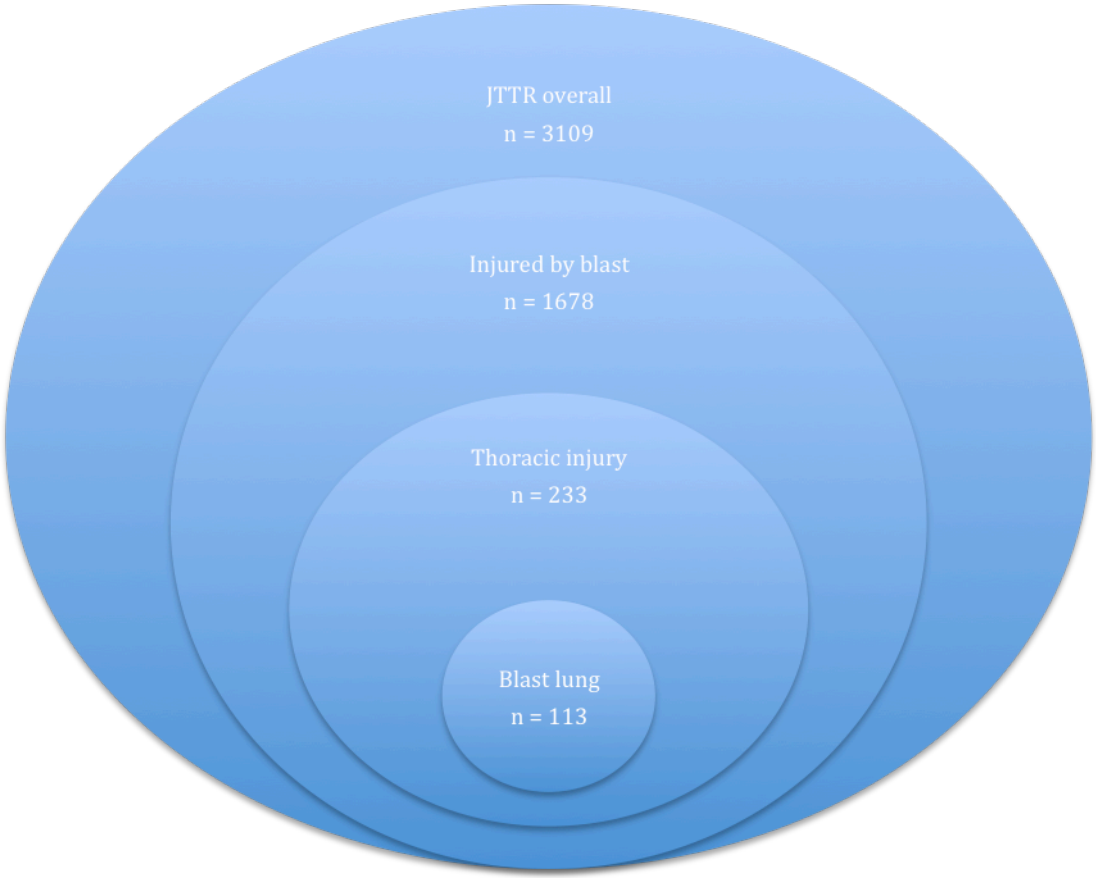

Figure 7: Breakdown of numbers of cases.

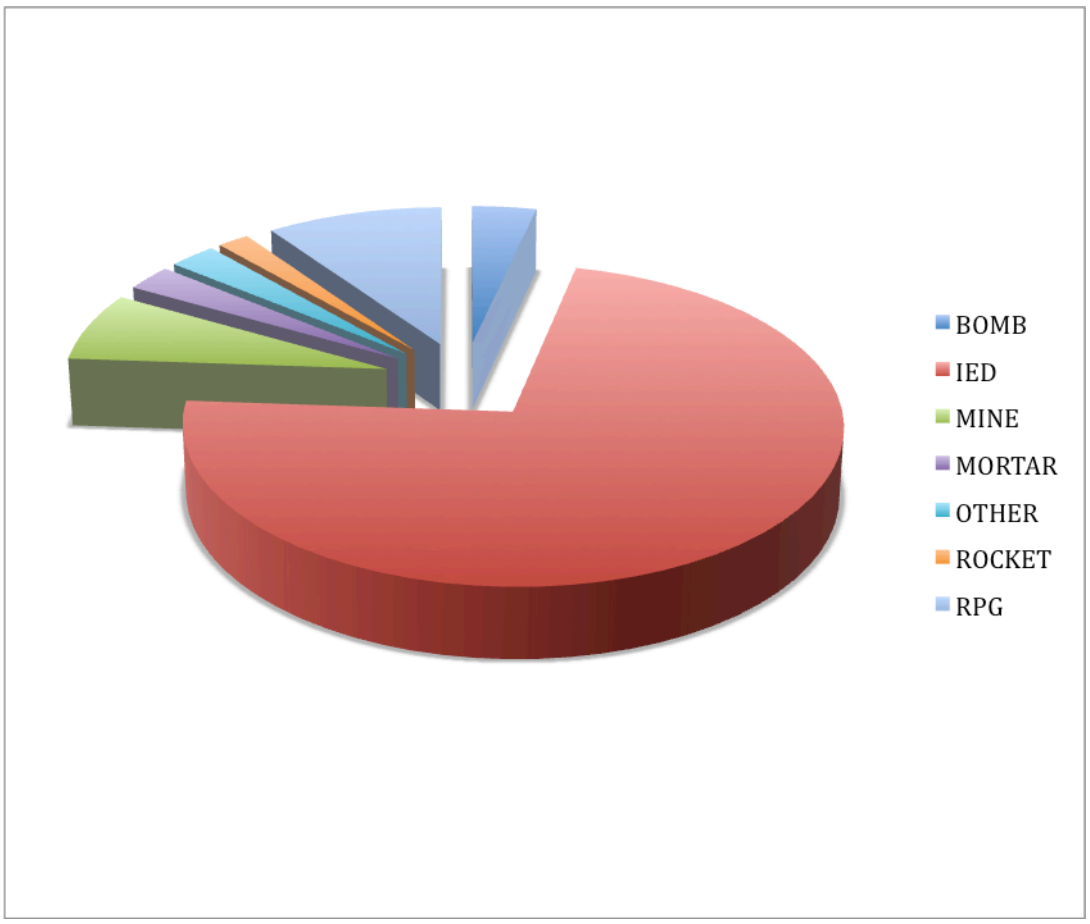

Figure 8: Mechanism of injury causing blast lung.

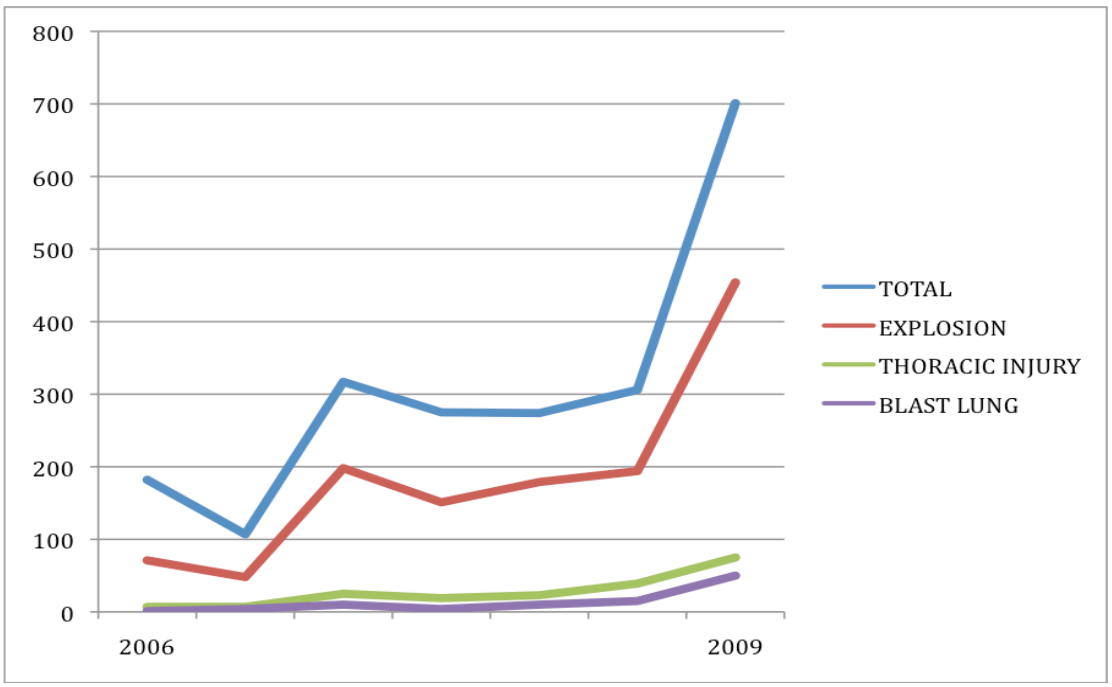

Figure 9: Numbers of casualties by year of operation in Afghanistan (total, injuries caused by explosion, thoracic injuries, and blast lung injury).

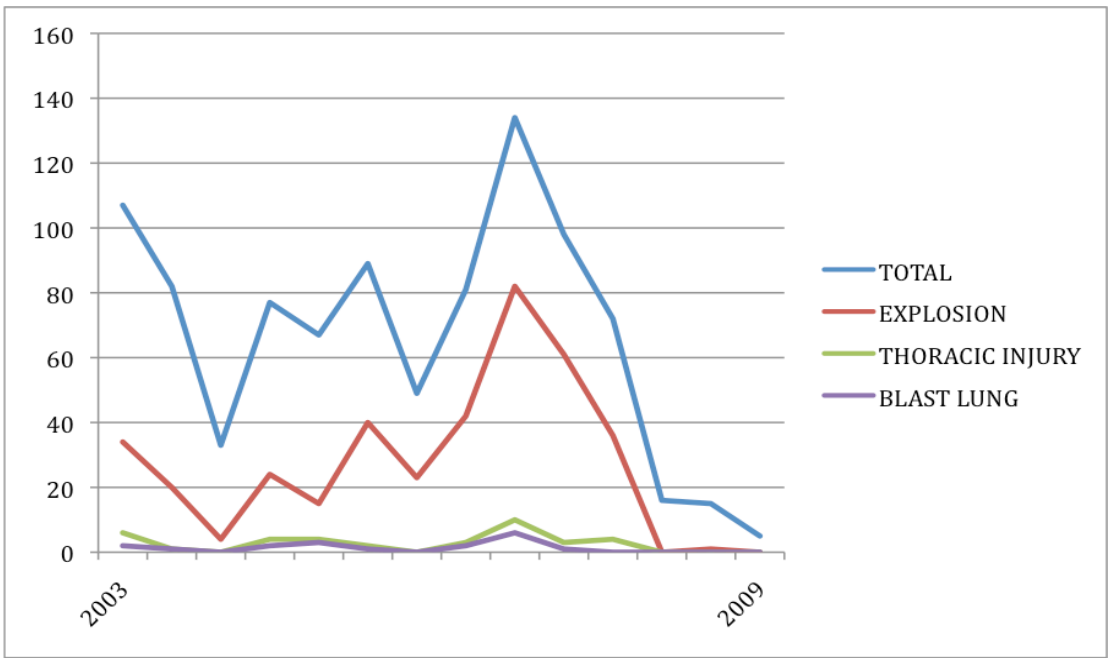

Figure 10: Numbers of casualties by year of operation in Iraq (total, injuries caused by explosion, thoracic injuries, and blast lung injury).

4.4 Discussion

This study highlights that injuries caused by explosion, and in particular blast lung, are an increasing problem for UK forces deployed overseas on military operations. There is a substantial fatality rate among those subjected to blast injury, which has led to calls for a review of the management of such patients and optimisation of their treatment from point of wounding to critical care. This study indicates that blast injuries, and blast lung in particular, are proving to be a significant clinical issue for deployed UK field hospitals during the current conflict. By far the commonest cause of these explosions was the IED, which is being used more frequently by insurgents in Afghanistan in particular.

A comparison of cases from Iraq and Afghanistan shows that there were peaks of injuries caused by explosion in Iraq in 2007, and Afghanistan in 2009. The incidence of blast lung during the Iraq peak was 7.3%. This figure is slightly higher than that quoted in a series of patients injured by explosions on recent US military operations, which showed an increase in the incidence of blast lung between 2003 and 2006 (Ritenour et al., 2010; Champion et al., 2009; Mora et al., 2009).

The incidence of injuries consistent with blast lung during the Afghanistan peak was found to be 11%, showing an increase (from 7.3% in Iraq) although this was not statistically significant ($p=0.31$, Chi-squared test); this was twice that reported in a previous series (Ritenour et al., 2010). Although it is impossible to ascribe a definite reason for this change, it is clear that the clinical burden is increasing, and that treatment strategies need to take this into account. The difference in reported incidence may be related to a difference in inclusion and exclusion criteria applied during retrospective analysis of data. For example some studies have excluded patients with rib or scapular fractures from the analysis of blast lung injury (Ritenour et al., 2010); while this would give a safe definition it does run the risk of excluding those with blast lung and concomitant injuries thereby potentially underestimating incidence.

The clinical features of primary blast lung injury are shortness of breath, cough, haemoptysis, cyanosis, and tachypnoea. Hypoxia is a universal finding, although this may develop later over the first few hours following injury, and may not be evident initially, as demonstrated by our findings that only 28% had hypoxia at initial presentation.

Management of patients with blast lung is mainly supportive, with limited scope for mediation of symptoms caused by pulmonary haemorrhage. If patients survive the initial phase of injury, outcome has been reported as good, with 76% of patients in one study reporting no symptoms at follow up telephone interview (Avidan et al., 2005). Novel strategies for the management of blast lung are needed if these patients, who as demonstrated are becoming increasingly common in deployed medical units, are to be treated optimally. Limitations of this study are evident from the method of data collection, and the problems with the definition of blast lung. The JTTR only captures those patients who have data submitted from the field hospitals, or who have post-mortem data from repatriation and coroners post mortem findings in the UK. No data is collected on civilians or other non-UK patients who die at scene or in the pre-hospital stages of their resuscitation, as post mortem examination is not routinely carried out on this group of patients. The incidence of blast lung injury may therefore be higher than quoted in the broader population undergoing medical treatment.

4.5 Conclusion

Blast injuries are an increasingly prevalent cause of considerable morbidity and mortality, particularly (although not exclusively) in the forward military environment. The sequelae of such injuries have been felt not only in Iraq and Afghanistan, but also the hospitals in the UK that receive such patients through the evacuation chain. Optimising management of these patients should be one of the priorities for further research and investigation.

Chapter 5. The Use of Recombinant Activated Factor VII (rFVIIa) in the Management of Patients with Major Haemorrhage in Military Hospitals over the Last 5 Years.

5.1 Introduction

Since the first reports of rFVIIa being used to control traumatic haemorrhage from Israel in 1999 (Kenet et al., 1999), its use in military patients has had a particular resonance. As rFVIIa emerged as a potential treatment for haemorrhage, the conflicts in Iraq and Afghanistan resulted in hundreds of patients with severe haemorrhage that required complex management, and many patients had ongoing bleeding despite resuscitation and attempted surgical haemorrhage control. The use of rFVIIa therefore flourished.

The UK Clinical Guidelines for Operations include guidance on when to use rFVIIa, and in particular, for use in life-threatening haemorrhage (Figure 11). These guidelines specify that rFVIIa should not be used in cases where the patient is expected to be unsalvageable despite administration of rFVIIa. As already described, the Summary of Product Characteristics for Novoseven® were changed in 2009 to reflect evidence of an increase in adverse events. At around the same time, haemostatic resuscitation and the use of bespoke transfusion of blood and blood products came into practice in the deployed military medical setting, with the use of targeted blood products guided by bedside thromboelastometry.

Anecdotally, the use of rFVIIa has decreased in the last few months. The aim of this study was to define the use of rFVIIa to establish a trend in use since the change in licensing, manufacturer advice and development of clinical practice.

5.2 Methods

The Joint Theatre Trauma Registry (JTTR) is described in Chapter 4. A database review, using the JTTR, was undertaken to produce a dataset of patients presenting to a military medical facility who received rFVIIa as part of their management. Until 2006, rFVIIa was not recorded as a separate treatment intervention, and therefore the search was conducted from the time it started to

be recorded. Data collected included use of rFVIIa, injury severity score (ISS), survival, and injury pattern. Within the JTTR, the injuries are defined by Abbreviated Injury Scale codes (AAAM, 1994), reflecting increasing severity of injury for a given body region. The highest injury code for each patient was also recorded. To produce a temporal trend in use of rFVIIa, an eligible population was defined as seriously injured (ISS>15), and having vital signs on arrival at hospital. Patients were therefore excluded if they were killed in action (KIA) or killed non-enemy action (KNEA), defined as the absence of vital signs on arrival at a medical treatment facility.

5.3 Statistical analysis

The proportion of treated patients from eligible patients was used as a marker of rFVIIa use. The temporal trend was then analysed using a test for equality of different proportions over time to see if there was a significant difference in use.

Recombinant factor VIIa

Treatment guidelines **2e**

Indications

- Life-threatening haemorrhage where conventional resuscitation and/or surgical techniques have failed. Life-threatening haemorrhage is defined as:
  - Loss of entire blood volume within 24 hours
  - Loss of 50% of blood volume within 3 hours
  - Blood loss at a rate of 150ml/min
  - Blood loss at a rate of 1.5m/kg/min for 20 minutes or more.
- In practical terms, rFVIIa should be considered if there is evidence of continued bleeding after 6–8 units of packed red blood cells and correction of coagulopathy with fresh frozen plasma.
- **The prescription of this drug is restricted to consultants only.**

Contraindications

- Do not use if the patient is expected to be unsalvageable despite rFVIIa.
- Known or suspected ischemic heart disease.
- A history of thromboembolic event in the preceding 6 months.

Dose

- 100mcg/kg IV bolus (a dose of 80mcg/kg has been used by Israelis for intraalveolar haemorrhage in blast lung, **but evidence is only anecdotal.**)
- A second bolus of 100mcg/kg IV may be given after ~20 minutes.
- Further doses are unlikely to be beneficial.

Adverse effects

- Thromboembolic events are a theoretical risk, but there has been no increased incidence within the available published literature when used in the trauma population.
- Disseminated intravascular coagulopathy.
- For a full description of potential adverse effects see product data sheet.

Follow up action

- All uses of rFVIIa will be tracked on the Joint Theatre Trauma Registry at ADMEM, Royal Centre for Defence Medicine. Ensure all documentation for the Trauma Nurse Coordinator is completed.

Policy

- This guideline is in accordance with DMSD/05/01/02 dated 14 June 2007 and DGAMS Policy Letter 12/05.

Figure 11: Guidelines for the use of rFVIIa (extract from Clinical Guidelines for Operations).

5.4 Results

During the period January 2006 to June 2011, 5170 injured patients presented to deployed medical facilities, of whom 156 received rFVIIa. 146 of these (94%) had an ISS>15; there were 45 fatalities. The median ISS among the group receiving rFVIIa was 30 (IQR 24-41). 20 patients had an ISS in the range 60-75. rFVIIa was given to 3 patients who were classified as KIA, or in whom vital signs were absent on arrival in the medical treatment facility. The trend of rFVIIa use during this period is shown in Figure 12. There was a statistically significant reduction in the use of rFVIIa in the second half of 2010 and first half of 2011, compared to the previous 12 month period (difference in proportion treated -15.8%, 95% CI -20.0%, -11.4%, p<0.0001).

The mechanism of injury of those receiving rFVIIa is shown in Figure 13. Lower extremity injury was the commonest body region to have the highest AIS score, reflecting the fact that lower extremity amputation is the signature injury caused by the IED.

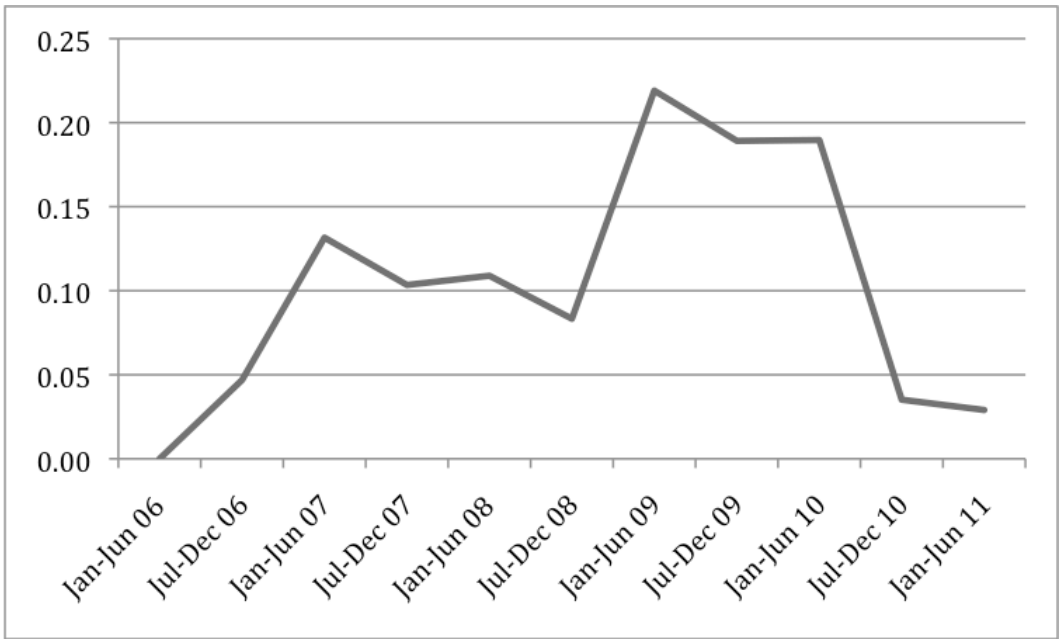

Figure 12: A temporal trend of rFVIIa use in deployed UK military hospitals from 2006-2011 (y axis shows proportion of severely injured patients, defined as ISS>15, receiving rFVIIa).

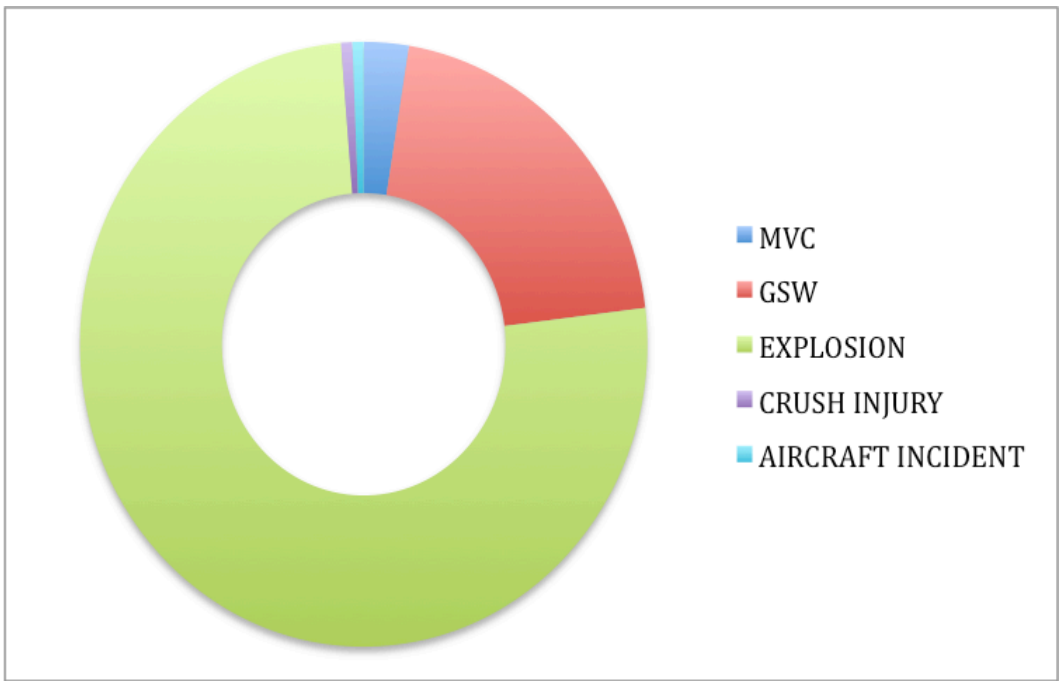

Figure 13: Mechanism of injury of patients given rFVIIa. GSW = gunshot wound; MVC = motor vehicle crash; other includes aircraft crash and crush injury.

5.5 Discussion

The use of rFVIIa in military medical treatment facilities has reduced significantly since 2009. This may be multifactorial, as there have been several factors that may have influenced its use over the last couple of years.

The first is that the manufacturers recommendations for the use of rFVIIa have been reviewed and revised in light of the adverse event profile of the drug. It has now been emphasised in the Summary of Product Characteristics that it should only be used within its licensed indications.

The second major factor is that in 2009, thromboelastometry (ROTEM) was introduced into clinical practice at Camp Bastion (Doran et al., 2010), which has allowed bespoke replacement of blood and blood products depending on the specific deficiencies according to the ROTEM result. This has allowed therapy targeted at the source of coagulopathy, in contrast to the blanket approach of giving rFVIIa and generating a thrombin burst, with all its potential difficulties. Rather than being forced to avoid the use because of the risk of adverse events, the current practice in Bastion has therefore allowed resuscitation without the need for the use of rFVIIa in the frequency previously recorded. However, it is still used for certain indications, such as continuing haemorrhage

despite adequate replacement of blood products, clotting factors and platelets. Anecdotally, during the author's period in Camp Bastion between August and October 2011, it was used for one patient who was a triple amputee with a large area of soft tissue injury to both buttocks, who was continuing to ooze despite resuscitation, and another patient who sustained a gun shot wound to the chest, and had uncontrolled haemorrhage from his endotracheal tube (see Chapter 6). Both patients survived to aeromedical evacuation to their host country.

The Clinical Guidelines for Operations include guidance on when to use rFVIIa, and in particular, for use in life-threatening haemorrhage (Figure 9). These guidelines suggest that rFVIIa should not be used in cases where the patient is expected to be unsalvageable despite administration of rFVIIa. During the period studied, rFVIIa was given to three patients who were killed in action, in other words had absent vital signs on arrival at the hospital. These were all double or triple amputees, who arrived at the medical facility with absent vital signs but who received rFVIIa as part of their attempted resuscitation. The reason for use in these circumstances is likely to be that it is felt to be worthwhile if there is any chance of survival of the patient, and with the increase in unpredicted survivors it is difficult to predict which patients will not survive even in the presence of traumatic cardiac arrest (Russell et al., 2011).

It is possible that rFVIIa use will in the future be targeted at specific injury patterns that are not amenable to surgical haemorrhage control, and those patients who are resistant to conventional haemostatic resuscitation. An example of this may be the use of rFVIIa in blast lung injury. As already stated, the use of intravenous rFVIIa was specifically endorsed for use to mitigate the haemorrhagic phase of blast lung injury by the UK Advisory Group on Military Medicine in August 2010; indeed, several patients during the peak in 2009 were given rFVIIa for this indication.

Limitations of this study are inherent from the method of data collection that makes up the JTTR, which only captures those patients who have data submitted from the field hospitals, or who have post-mortem data from repatriation and coroners post mortem findings in the UK. For patients who are local civilians, coalition forces, or other non-UK patients who die prior to arrival at a medical facility, no data is collected other than a body map of injuries. Detailed AIS scoring is therefore not possible on these patients. To define an appropriate denominator in order to assess a trend, patients who were KIA

were excluded from the denominator figure (for example, in months where a multiple casualty incident resulted in numerous KIA patients with ISS>15, this would have skewed the results). However, three patients were given rFVIIa during their attempted resuscitation despite being classified as KIA according to the strict database criteria.

5.6 Conclusion

The use of rFVIIa in UK deployed military hospitals has declined since the first half of 2010, which is likely due to a combination of factors, including a change in emphasis of manufacturers guidance, but also a change in resuscitation practice in these units.

Chapter 6. The Use of Recombinant Activated Factor VII in a Patient with Penetrating Chest Trauma and Ongoing Pulmonary Haemorrhage: a Case Report.

6.1 Introduction

As discussed in the previous chapter, the use of rFVIIa is decreasing in deployed medical treatment facilities. However, there may still be occasions where rFVIIa has a role in the management of haemorrhage that is not amenable to surgical control and haemostatic resuscitation.

Pulmonary haemorrhage, in particular bleeding into the alveolar space, is difficult to control, and conventional treatment involves positive pressure ventilation and adjustment of ventilatory settings including the use of positive end expiratory pressure. A case is described where rFVIIa was used to control pulmonary haemorrhage in the deployed medical setting allowing onward transfer of the patient to the UK.

6.2 Case Report

A 28-year old UK serviceman sustained a high velocity gunshot wound (HVGSW) to the left side of his chest, and was brought to the Role 3 Medical Treatment Facility at Camp Bastion, Helmand Province, Afghanistan. Pre-hospital management included high flow oxygen, intravenous (IV) access, needle decompression of the left side of the chest, and a Bolin chest seal over a wound to the left scapula.

The patient arrived conscious and talking. His vital signs were normal other than his oxygen saturations, which remained 88% despite high flow oxygen. He reported that he had received the injury while bending over to tie his bootlace. Examination revealed a wound to the lateral aspect of his left shoulder, and a wound over his left scapula.

A portable chest X-ray showed increased opacity over the left side of the chest (Figure 14). Focussed assessment with sonography in trauma (FAST) examination was normal. The patient underwent rapid sequence induction of anaesthesia and intubation, had a 32F intercostal drain inserted, and went to

the operating theatre via a CT scan (Figure 15), where his wounds were debrided. A thoracotomy was not performed. During the operation there was an episode where a significant quantity of blood came up his endotracheal tube (ETT). He required an inspired oxygen fraction (FiO_2) of 80% to maintain a PaO_2>9kPa. By the end of his initial operation he had received 2 units of packed red blood cells (pRBCs) and 1 unit of plasma.

The patient was admitted to the intensive care unit (ICU) in view of his hypoxaemia where he remained sedated and ventilated. An ARDSnet ventilatory strategy was adopted in view of his lung injury (Brower et al., 2000). Initially it was noted that the patient continued to have small amounts of blood coming up the ETT. Five hours later it was noted that the amount of blood coming up the ETT was increasing, and his oxygen saturations dropped to 74% despite an FiO_2 of 100%. Thromboelastometry was performed, which showed mild derangement of coagulation (the EXTEM showed low normal range values, and the FIBTEM trace suggested fibrinogen deficiency). The patient received 2 units of plasma, 1 bag of platelets, 1 unit of pRBCs, and 1 unit of cryoprecipitate. Due to ongoing haemorrhage and continuing hypoxia despite this treatment he was then given 10mg of intravenous rFVIIa (110 mcg/kg). His ventilatory parameters improved (his oxygen requirement fell from 100% to 40%, his requirement for PEEP fell from 12 to 10cm water) and clinically the blood stopped coming up the ETT. He then remained stable, with no further evidence of active bleeding, allowing transfer by Critical Care Air Support Team (CCAST) back to the UK, where he underwent further management in the ICU at the Queen Elizabeth Hospital, Birmingham. He subsequently recovered from his injuries and was successfully discharged home.

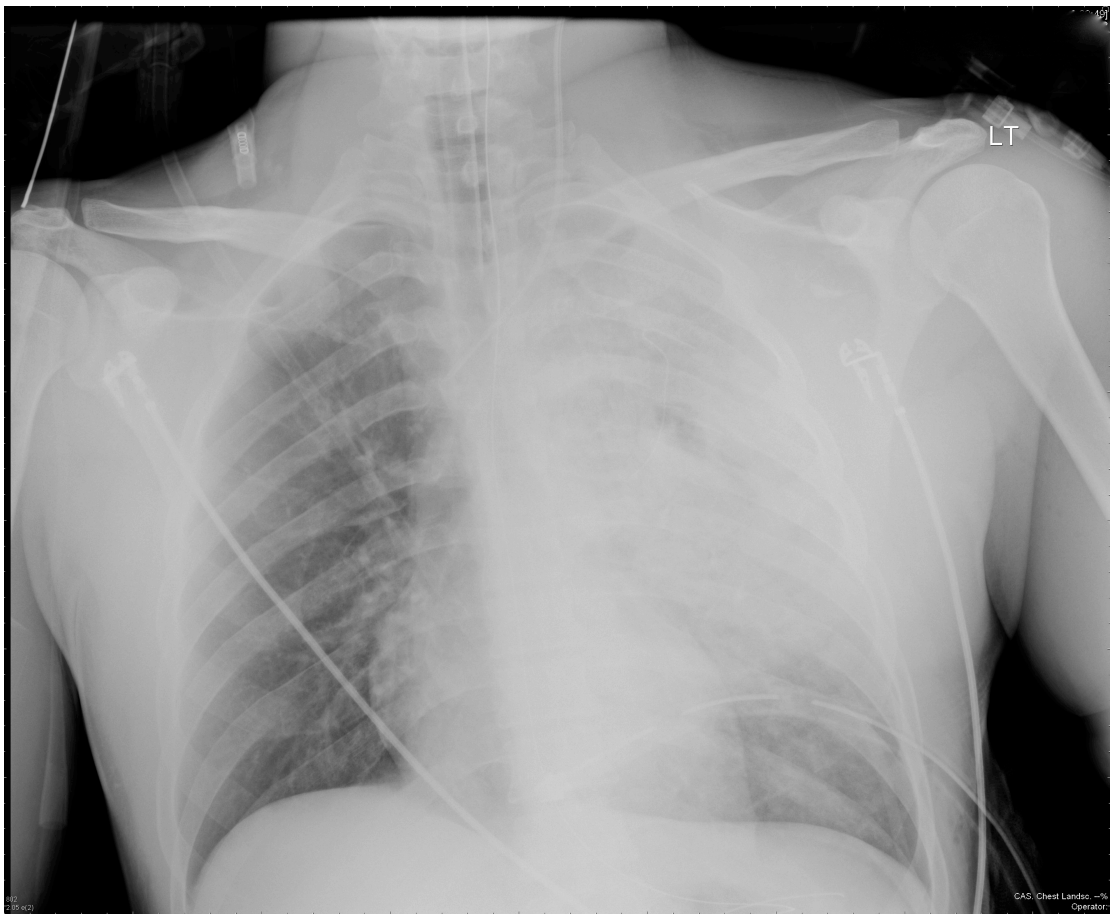

Figure 14: CXR showing increased opacity affecting the left upper and mid zones suggesting blood either within the lung or a haemothorax.

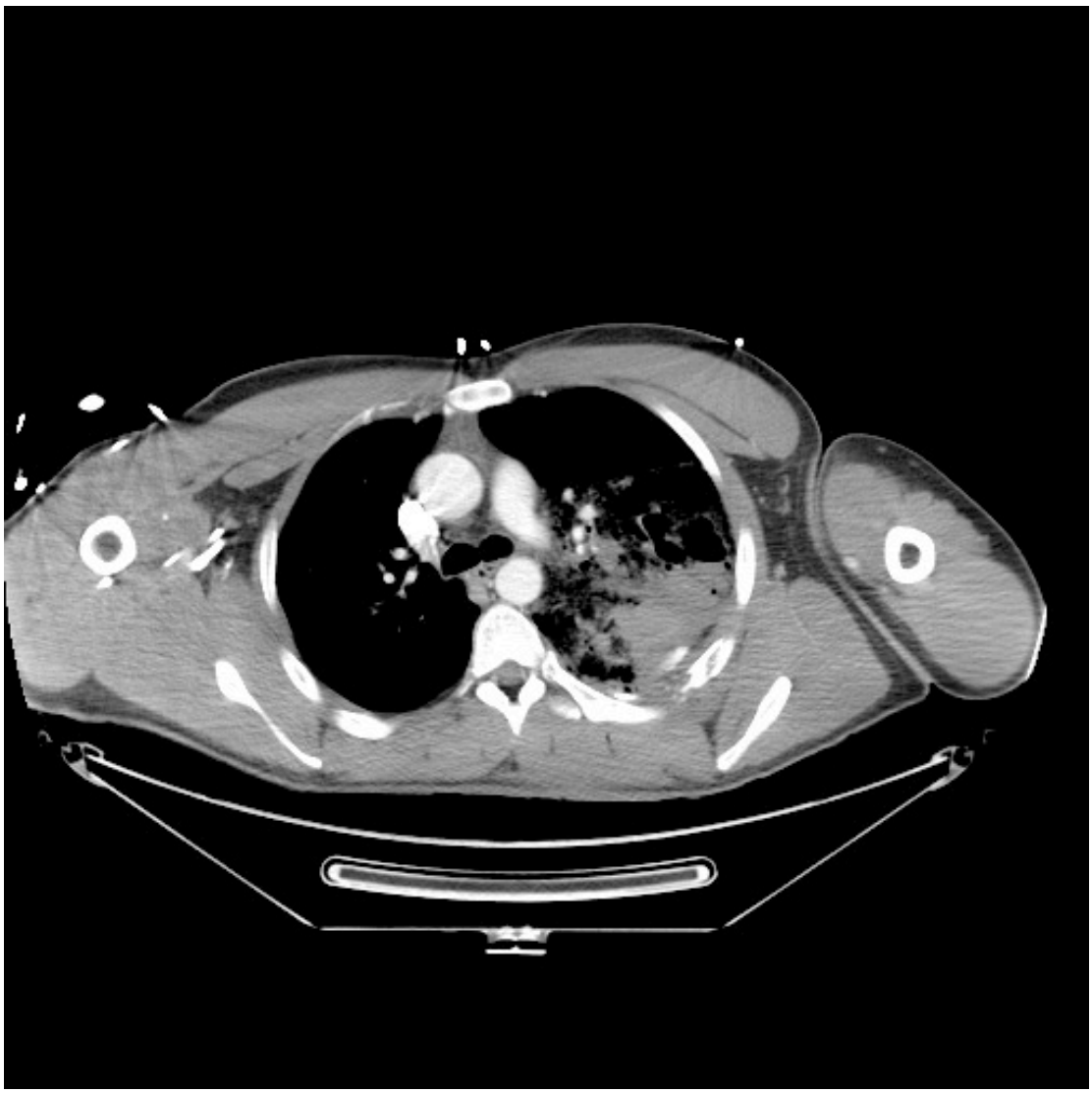

Figure 15: CT showing large lung laceration in the left upper lobe containing blood, with surrounding contusion and a tiny amount of pleural fluid but no pneumothorax. There is also a fracture to the left scapula, and fractures to the left 4th, 5th and 6th ribs.

6.3 Discussion

This case describes the use of rFVIIa for the management of pulmonary haemorrhage following HVGSW to the chest in a deployed military setting. Management options for pulmonary haemorrhage are limited, and usually consist of surgical haemorrhage control and ventilatory strategies to maintain adequate oxygenation.

The first use of rFVIIa to control traumatic haemorrhage was reported in 1999; it was used to treat uncontrolled haemorrhage from a gunshot wound causing injury to the inferior vena cava in an Israeli soldier (Kenet et al., 1999). Since

then its use has become widespread in the management of traumatic haemorrhage, although it remains an off-licence indication. One previous case has been described of a patient with severe traumatic intrathoracic haemorrhage from blunt trauma who had diffuse bleeding not amenable to surgical control, who was given two doses of rFVIIa (60mcg/kg), which appeared to control the bleeding and result in a positive outcome in terms of survival (Kamphuisen et al., 2002).

The UK Clinical Guidelines for Operations state that rFVIIa should be used in instances of life-threatening haemorrhage where conventional resuscitation and surgical techniques have failed to address the problem. While this is an unusual example of this situation it fulfils the requirement and proved useful in the management of this case. There is also anecdotal evidence that rFVIIa may be useful in the management of haemorrhage associated with blast lung injury, another cause of pulmonary haemorrhage, and a recent addition to CGOs includes guidance on management in this circumstance.

6.4 Conclusion

rFVIIa was successfully used to treat a patient with pulmonary haemorrhage following HVGSW to the chest, allowing CCAST transfer back to the UK for ongoing care.

Section 3. Model Development.

Chapter 7. Development of a Model of Blast Lung in the Rabbit.

7.1 Introduction

There is no doubt that blast lung injury is a significant problem in some patients injured by explosion, and the treatment options are currently limited to conservative management with mechanical ventilation.
As described, rFVIIa has been shown to enhance coagulation, and in some case reports to arrest pulmonary haemorrhage. The aim of this programme of research was to investigate whether the delivery of rFVIIa to the lungs by nebulisation could attenuate the effects of blast lung injury.

7.2 Ethics and licences

The research was subject to dstl Porton Down's Ethical Review Process (ERP), prior to submission to the United Kingdom Home Office Inspectorate and the submitted plan included all recommendations from that ERP. Experiments were conducted under terminal anaesthesia, under a United Kingdom Home Office Licence and in accordance with the Animals (Scientific Procedures) Act, 1986. All investigators, including this author, were in possession of an appropriate Home Office licence. All experiments were conducted with the assistance of, and under the supervision of a qualified veterinary surgeon.

7.3 Background

In order to investigate the effects of a drug on blast lung injury, it was necessary to develop an animal model giving a reliable and reproducible amount of blast lung injury for a given blast exposure. Based on previous work with rats, the aim was to achieve approximately 40% lung volume contusion, in order to establish an initial reduction in PaO_2 to approximately 7.5KPa, while ensuring survival to 24 hours post blast exposure. The intended primary outcome measure for the final study was oxygenation, measured by arterial blood gas analysis, at intervals throughout the 24-hour period.
Previous work has identified that reliable blast lung injury can be induced in mice (Knoferl et al., 2004; Knoferl et al., 2003) and rats (Sawdon et al., 2002; Ohnishi et al., 2001; Guy et al., 1998; Jaffin et al., 1987) using a compressed air

blast wave generator. One of these blast wave generators has been developed at dstl Porton Down (see Figure 1) (Sawdon et al., 2002; Ohnishi et al., 2001; Guy et al., 1998).

Unfortunately mice and rats lack sensitivity to commercially available human rFVIIa. In contrast, the rabbit has excellent cross-species interaction with human rFVIIa (and human tissue factor interacts well with rabbit FVII) (Knudsen et al., 2012). Rabbits have been used in blast research previously (Cernak et al., 1996), and have been used in blast lung injury research where blast injury was induced with a compressed air shock tube (Zunic et al., 2000). The use of the rabbit would also enable a more comprehensive list of samples to be taken for analysis, due to the larger blood and tissue volume. It was therefore decided that the rabbit would be used for development of this model.

However, the technique of delivering blast injury using the compressed air blast wave generator outlined above has not to date been described in larger animals such as the rabbit. Larger animal models of blast exposure have been described, using for example the pig. In these models, the pig is exposed to an explosive charge on an outdoor range while secured to a platform and protected from secondary and tertiary blast effects by Kevlar blankets (Garner et al., 2010; Garner et al., 2009).

Other techniques by which a blast wave can be generated include the use of shock tubes, which will be described in detail later in this chapter. These have the potential disadvantage of not allowing focusing of the blast wave on the thorax, causing additional unpredictable and unwanted extrathoracic injuries. It was therefore decided that the initial phase of model development should attempt to use the same compressed air blast wave generator as had been used previously in the rat, with appropriate modifications to the technique to allow its use on the rabbit.

The aim of this series of initial experiments was to demonstrate that blast lung injury could be achieved in the rabbit with *ex vivo* models using the compressed air blast wave generator.

7.4 Compressed Air Blast Wave Generator

7.4.1 Methods – ex vivo

The compressed air blast wave generator involves pressurising a cylinder to a pre-determined level, from a compressed air source. A solenoid is then

operated to release this pressure into a chamber, which is sealed with a metal disc. This ruptures the metal disc generating a blast wave, which is then directed towards the target (Figure 16).

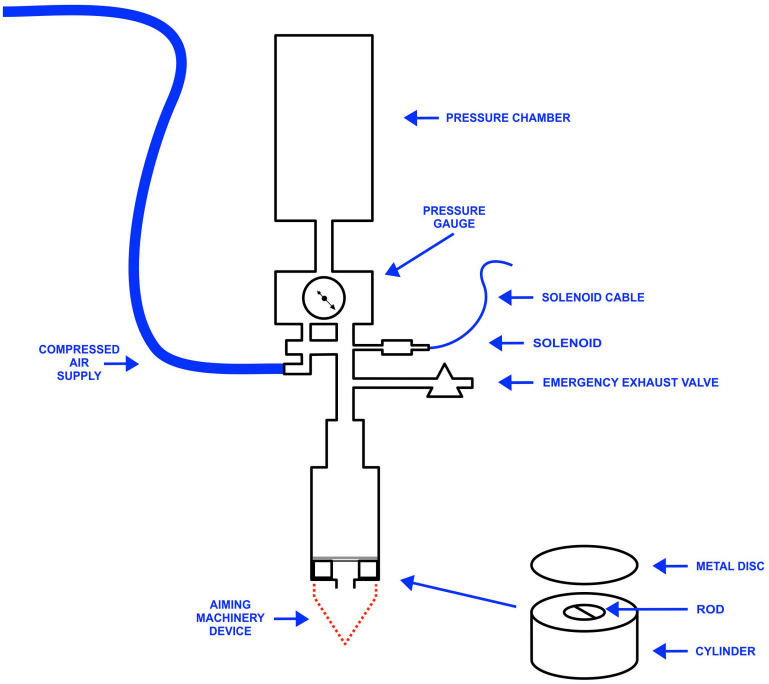

Figure 16: Compressed air blast wave generator.

The main and obvious difference between using a rat and a rabbit is the size of the animal; the average rat weighs approximately 250g, and the average rabbit weighs approximately 2.5kg. Using the same compressed air equipment as previously described in work with rodents, it was initially estimated that the dose would need to be multiplied by a factor of 10 to give the equivalent lung injury. It was therefore deemed probable that achieving the desired level of blast exposure using the blast wave generator in rabbits would require multiple exposures.

The exposure to a compressed air blast wave generator has few variables that could be altered to increase the amount of blast lung, namely the number of exposures, the distance from nozzle to skin, and position of the device in relation to the animal's chest.

A preliminary proof of principle experiment was performed on a freshly killed rabbit. The blast wave generator was used to give 4 blast exposures, at 2cm distance, to one side of the thorax.

7.4.2 Results

At post mortem there appeared to be significant visible pulmonary contusion to the lung on the exposed side. The principle that the blast wave generator could cause blast lung injury in the rabbit was therefore confirmed.

7.4.3 Methods – in vivo

Subsequent experiments were performed on live animals. All animals were prepared as described in the following section and maintained under terminal anaesthesia until the experiment was complete, at which point a lethal dose of intravenous anaesthetic was administered. Post mortem examinations were then undertaken to assess the degree of injury.

Animals were exposed to varying numbers of blast exposures, at different distances, to attempt to consistently achieve the desired degree of injury. Evaluation of gross evidence of blast lung injury was carried out at post mortem. A scoring system has been described previously to estimate the blast effect on gross physical examination of the lungs as part of an overall severity of injury index at post mortem (Yelverton, 1996). This identifies injury as negative (for no trace of injury); trace (for scattered surface petechiae); slight (for extensive petechiae or ecchymosis involving <10% of the lungs); moderate (10-30% involvement of lungs); and severe (>30% of the lungs involved with parenchymal contusion and hepatisation).

7.4.4 Instrumentation and preparation

The aim was to develop a model in which a rabbit could be maintained under anaesthesia and ventilated for 24 hours following blast exposure, with the ability to take regular blood samples for analysis of arterial blood gases and other blood tests. To enable this, a reliable and reproducible model of instrumentation and ventilation needed to be developed.

This model included the following elements:

- Induction of anaesthesia
- Intravenous access
- Intra-arterial access

- A tracheal tube to enable positive pressure ventilation
- Maintenance of anaesthesia
- Ventilation and measurement of lung function

7.4.5 Animals

Female New Zealand white rabbits were used. The average weight of the animals was 2.5kg. The animals were fed ad libitum with a commercial rabbit diet and allowed free access to water.

7.4.6 Induction of anaesthesia

The rabbits were given an intramuscular injection of midazolam (as a premedication) at a dose of 1-2mg/kg (see Table 2). They were left in a warmed environment (with overhead heater) for 15-30 minutes until they appeared comfortable and mildly sedated.

They were then transferred to an operating table, where the lateral ear vein was cannulated with a 24G cannula. Animals requiring euthanasia by a schedule 1 technique were at this stage given an overdose of intravenous pentobarbitone. For *in vivo* experiments, induction of anaesthesia was by intravenous injection of alfaxalone, at 2-3mg/kg, following a period of pre-oxygenation via a facemask.

7.4.7 Endotracheal intubation and initial maintenance of anaesthesia

An uncuffed endotracheal tube was then inserted into the trachea. This was performed by inserting the tube while listening for breath sounds at the opening. Anaesthesia was maintained using a volatile anaesthetic agent, isoflurane, delivered through an anaesthetic circuit along with nitrous oxide and supplemental oxygen.

7.4.8 Central vessel cannulation

Once surgical anaesthesia was established, and the animal was monitored using a Propaq® monitor with end tidal CO_2 and pulse oximetry, the neck was surgically dissected and cannulae placed into the common carotid artery and the jugular vein. These were secured by sutures, and the wound closed. This allowed central venous access, blood sampling and continuous invasive blood pressure monitoring via a transducer connected to a computer (Apple iBook G4), using LabChart software (ADInstruments, USA).

7.4.9 Ventilation

A ventilator designed for use with small animals was sourced to enable positive pressure ventilation of the rabbit. A ventilator that also allows lung function testing during ventilation was utilised, with a built in nebuliser in the inspiratory limb of the circuit. The equipment procured was a SCIREQ flexiVENT ventilator. The settings and functions were displayed using the flexiVENT software programme, on a Dell computer. Initial ventilatory parameters used were a tidal volume of 7-8ml/kg, pressure limited to 30cm water (in practice, the peak pressure was considerably below this level), a positive end-expiratory pressure (PEEP) of 5cm, and the rate was adjusted to maintain a $PaCO_2$ of approximately 5.2KPa.

Inhalational anaesthesia was not possible while this ventilator was being used, so the intravenous route had to be used to maintain anaesthesia once central venous access was established.

The ventilator had an Aeroneb laboratory nebuliser (SCIREQ) connected to the inspiratory limb of the ventilation circuit, and this was used to deliver nebulised material to the animal during experiments requiring this procedure.

7.4.10 Intravenous anaesthesia

A surgical plane of anaesthesia was maintained using alfaxalone at 1-2ml/kg/hour (10mg/ml solution), and midazolam 0.08-0.24mg/kg/hour (5mg/ml solution). Intermittent boluses of alfentanil (50mcg/ml solution, 5mcg/kg) were used to induce apnoea during lung function testing as appropriate.

Mode	Drug	Dose	Volume/infusion
Sedation	Midazolam	1-2mg/kg	0.2-0.4ml/kg
Induction	Alfaxalone	2-3mg/kg	0.2-0.3ml/kg
Maintenance	Alfaxalone Midazolam		1-2ml/kg per hour (10mg/ml solution) 0.03-0.06mg/kg/hour (5mg/ml solution)
Bolus	Alfentanil	5mcg/kg	0.2-0.3ml (0.1ml alfentanil in 0.9ml normal saline)

Table 2: Anaesthesia protocol; midazolam 5mg/ml solution made up to 5mg in 50mls normal saline (0.1mg/ml solution); alfentanil 50mcg/ml solution.

7.4.11 Results

The results are summarised in Table 3.

Animal ID	Intervention	PM result	Comments
P1	4 exposures to L side at 2cm, then 4 at 1.5cm. 4 exposures to R side at 1.5cm, then 2 at 1cm.	Gross severe lung contusion on both sides, worse on the left. There was also evidence of haemoperitoneum and a small liver laceration.	Physiological evidence of blast exposure – hypotension, bradycardia – but hypoxia resolved until final exposures produced terminal decline.
P2	6 exposures to R side at 1.5cm, 4 exposures to L side at 1.5cm (see Figure 17a).	Gross severe lung damage with 90% contusion to both lungs (see Figure 17b). No haemoperitoneum.	Small amount of bruising to chest wall. Similar physiological response to above.
P3	3 exposures to each side, all at 1.5cm.	Right lung lower lobe 30% contused (moderate), left lung lower lobe minor contusion. No haemoperitoneum.	Coughing spots of blood at end of tube after left sided blasts.
P4	5 exposures both sides, distance of 1.5cm.	Focal area of contusion R lower zone (moderate), with more diffuse changes in L lung.	Leak around ETT solved by tying trachea around tube at instrumentation.
P5	5 exposures both sides, at 1.5cm.	Left lung had little macroscopic evidence of contusion (trace). The right lung appeared to have significant lung contusion.	Episode of desaturation prior to blast exposure.

P6	5 exposures to each side, all at 1.5cm.	There was little evidence of blast lung at post mortem (trace), but 1 fractured rib was noted.	The arterial blood gases were roughly normal throughout. At no stage did the animal develop significant hypoxia.
P7	5 exposures to the left lung and two to the right, all at 1.5cm distance.	Severe blast lung, but also evidence of 4 fractured ribs on the left side, and 2 on the right.	The animal became hypoxic and hypotensive following the right sided blasts, with the blood pressure dropping to 60/20. It was transferred to the ventilator but despite resuscitative attempts died shortly afterwards.
P8	6 blast exposures to L side, 5 on the R side, all at 2cm	Small amount of blast lung evident (slight).	Little effect on oxygenation measured by arterial blood gas.
P9	7 blast exposures to the right side (4 at 1.5cm, 3 at 2cm), then 9 exposures to the left side at 1.5cm. No change in oxygenation was observed so further 5 exposures to the right side at 1.5cm.	Evidence of severe blast lung on both sides (estimated from gross examination at 85% lung volume on the left, and 50% on the right).	2 hours following the blast exposure, the blood gases showed normal oxygenation (PO_2 11.0KPa).
P13	7 blast exposures were delivered to	Very little evidence of blast lung injury to the	No lung recruitment was performed for 4

	each side, all at 1.5cm distance.	naked eye, with estimated 10% contusion to the right lung (slight).	hours, during which time the PO_2 dropped from 13 to 7.5KPa. Following recruitment the PO_2 rose to 13.7KPa.
P14	9 blast exposures on each side at 1.5cm. A further 3 on each side at 1.5cm. Further 3 exposures to each side at 2cm, then 3 exposures to R side at 1cm.	At post mortem little evidence of blast lung (trace). Superficial bruising noted on chest wall.	No effect on oxygenation and therefore a further 3 on each side. Still no effect on oxygenation so further 3 delivered to each side. No physiological change.

Table 3: Results from compressed air blast wave generator. ID = identification number; PM = post mortem; R = right; L = left.

Figure 17: (a) Marked position of blast exposures demonstrated at post mortem. (b) Severe blast lung injury evident at post mortem in animal ID P2.

7.4.12 Discussion

Having established that this device could induce blast lung injury, the aim was to refine the technique in order to produce a reproducible amount of blast lung injury, to the extent required.

Following these initial experiments, it was evident that 7 of these 11 animals had significant blast lung injury evident at post mortem examination, but the degree of blast lung injury did not seem to correlate with a reduction in oxygenation evident on physiological monitoring. However, despite receiving similar amounts of blast exposures, 4 of these 11 animals had very little gross macroscopic evidence of blast lung injury at post mortem.

Several problems were encountered during this series of experiments that were addressed as we continued through the series. Early on it was recognised that incidental intra-abdominal injury (particularly liver injury) would be a potential problem, and that positioning would be key to avoiding collateral injury. After the first animal, efforts were made to concentrate the blast exposures higher, around the scapula, to avoid intra-abdominal injury. It was also quickly apparent that to obtain the required amount of lung injury, exposures to both sides of the chest would be necessary. The linear pattern of exposures was then adapted to a square pattern to expose more of the lung to the blast, while keeping the exposures as cranial as possible to minimise the chance of inadvertent abdominal injury. The other element that could potentially be influenced was the position of the exposures in relation to the lungs by moving the forelimb into extension to displace the scapula (as in theory the bony scapula would reflect some of the blast wave). This manipulation of the forelimb was attempted from pilot 9 onwards.

Another unwanted collateral injury encountered was rib fracture, present in two of the animals. It was felt that this may be related to the distance of the nozzle from the skin, as in both of the animals it was related to exposures at 1.5cm, so initially the exposures were limited to 2cm to avoid this complication, although this then resulted in reduced blast lung injury on subsequent experiments, so we then reverted to 1.5cm distance for the latter experiments. It was also felt that the attitude of the nozzle in relation to the chest might be contributing to the variable picture of blast lung, so efforts were then made to ensure that the

nozzle was perpendicular to the chest wall, to reduce any possible reflection of the blast wave.

During the initial experiments it was noticed that a significant air leak was evident around the uncuffed endotracheal tube. For subsequent experiments, at the time of surgical dissection to place the intravascular cannulae, the trachea was dissected out and a ligature of suture material placed around the trachea (with the endotracheal tube inside the lumen) to minimise the air leak and provide a reliable seal. However, during the parallel work on nebulisation it was found that the endotracheal tube was causing other problems with increased ventilatory dead space and the potential for a large surface area for the nebulised substance to stick to, so this was further modified by the placement of a short metal tracheal cannula, performed at the time of the initial surgical dissection. The tracheal cannula was tied in place with a ligature of suture material to minimise air leak. This necessitated a switch from inhalational anaesthesia to the intravenous route before this could be performed. The drawback of the tracheal cannula was that end tidal CO_2 could no longer be monitored during the blast exposure. The sequence of intervention was therefore induction of anaesthesia, maintenance of anaesthesia using volatile anaesthetic, surgical dissection and insertion of intravascular catheters, then a switch to maintenance intravenous anaesthesia, followed by dissection of the trachea (with endotracheal tube in situ), and removal of the endotracheal tube immediately prior to insertion of the tracheal cannula. This sequence was performed from pilot 13 onwards.

Several unexpected findings were encountered during this series of experiments. In pilot 5, it was noted that when lung recruitment manoeuvres were undertaken prior to lung function testing, this had a marked effect on lung compliance, despite the presence of blast injury. This was further explored in subsequent animals, and seemed to be a consistent finding, with improved lung compliance and an improvement in oxygenation. In pilot 13, no lung recruitment or lung function testing was undertaken for 4 hours following blast exposure, to assess the effect of recruitment manoeuvres on gas exchange and the implications for study outcomes. Without lung recruitment, the PO_2 dropped from 13 to 7.5KPa. Following recruitment manoeuvres, the PO_2 rose to 13.7KPa, suggesting that the recruitment procedure had reversed the underlying problem causing the hypoxia. This lung recruitment seemed to

reverse any decrease in oxygenation that was produced, despite the presence or absence of macroscopic blast lung injury at post mortem. However, translation to a clinical setting would be difficult to justify as the mainstay of current management is pressure and volume controlled ventilation, and avoidance of barotrauma that might precipitate pneumothorax or air embolism. The other unexpected finding was that in animals with severe blast lung injury evident at post mortem, oxygenation measured by both non-invasive pulse oximetry and on arterial blood gas analysis remained normal, or if there was a transient period of hypoxia following blast exposure this corrected after a short period of time (up to 2 hours post blast exposure). At post mortem, pilot 9 displayed evidence of severe blast lung injury, and yet still displayed normal oxygenation up to 2 hours post blast exposure. In pilots 8 and 9 histological analysis was performed to confirm that blast lung injury was present, and these showed significant oedema and white blood cell infiltration, with blood in the alveoli, suggesting that the desired level of blast exposure was being achieved, but was not evident on clinical parameters immediately following blast exposure (Figure 18). There may be several possible explanations for this, including vascular shunting (more severely injured areas of lung having poor flow therefore shunting blood to other areas not so badly affected). However, it led to other primary outcome measures being considered for the final therapeutic trial; if it were not possible to detect the severity of blast lung injury by monitoring the clinical state of the animal, it would not be possible to measure the effect of an intervention.

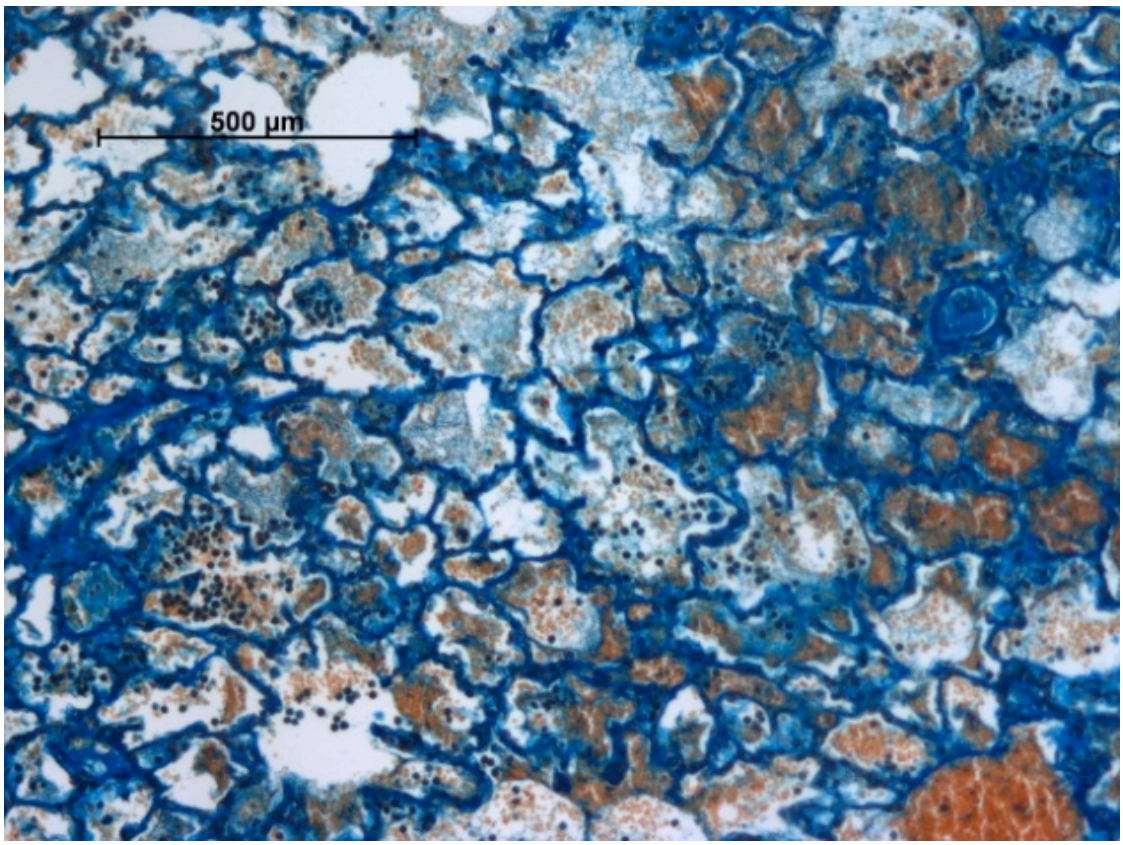

Figure 18: Histological specimen, with H&E (haematoxylin and eosin) stain, showing white cell infiltration and intra-alveolar red blood cells.

The main issue that was evident at the end of this series of experiments was that it was not possible to produce a consistent amount of blast lung injury at post mortem. When pilots 6 and 7 are compared, similar amounts of blast exposure were administered to both animals, but very different post mortem pictures were evident, with more blast lung on the animal with fewer exposures.

To ensure consistency of output from the blast apparatus, calibration exposures were performed daily prior to animal exposure to check peak overpressure. As a result of the inconsistent blast lung findings the blast apparatus was thoroughly re-evaluated at the end of this series of experiments to ensure that this was not the cause of the inconsistency of findings. This showed that the blast wave generation was consistent.

Having attempted to produce a reliable and reproducible amount of blast injury using this technique, the results indicate that this was not possible. This was due to the amount of exposures necessary to induce significant blast lung injury, and also the variability in injury seen in the larger animal. What had

worked well in the smaller rodent models had failed to achieve conversion to the larger animal model.

Other options for delivering blast injury were then considered including the use of a baton gun firing blank rounds, using a blast tube, or delivering blast injury by an explosion on a firing range.

7.5 Baton Gun

A modified firearm with an explosive primer has been used to deliver a blast wave in previously described work (Courtney and Courtney, 2010). Following preliminary experiments to ascertain the pressure characteristics of the baton gun round, a baton gun blank (an L4 cartridge case with blank 0.44 Magnum round with 1g of WPT 109) was used to produce a blast wave. After initial firings against a pressure transducer confirmed that it produced a shock wave with the relevant peak overpressure, the baton gun was rigged within the internal firing range, and a sling was constructed at the end of the gun, in which the animals were placed (Figure 19).

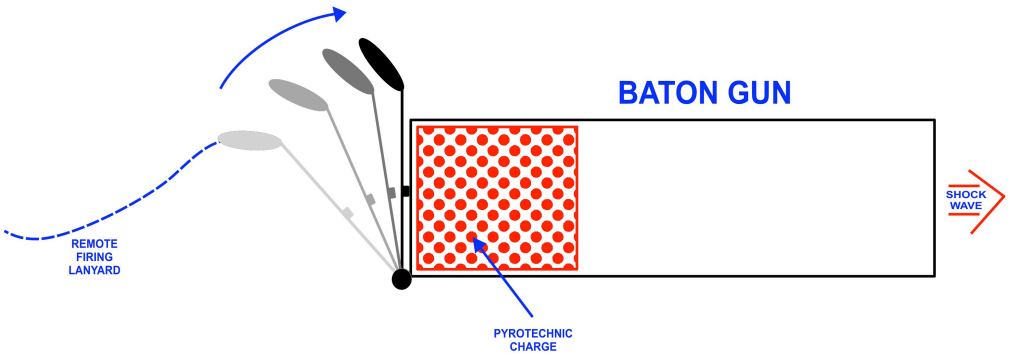

Figure 19: Baton gun system.

7.5.1 Aims

To assess the feasibility of using blank baton gun rounds fired from a baton gun to produce the desired degree of blast lung injury.

7.5.2 Methods – ex vivo

3 freshly killed animals were used to test the possibility that the baton gun could be used to induce an appropriate amount of blast lung injury. All animals were killed by a schedule 1 technique (lethal dose of intravenous anaesthetic) before

transfer to the indoor range. Following the test firings, post mortem examinations were carried out to assess the degree of injury.

7.5.3 Results

The first animal was exposed to blast at a distance of 10 cm from the nozzle of the baton gun. After a single firing, at post-mortem there was peppering of fragments in the skin and superficial muscle of the left side of the chest, although this did not penetrate the chest cavity. When the lungs were dissected there appeared to be a small area on the left lung of contusion or blast lung injury, not extending all the way through when sectioned.

The next animal was exposed to a blast using the same baton gun but this time at a distance of 2 cm. The chest was shaved and marked. At post-mortem, on gross appearance there was a large defect in the left side of the chest with visible macerated liver through the wound. There was definite evidence of lung contusion in the left lung, particularly the lower zone, and also the right lung to a lesser degree. Rib markings were visible on the left lung. This was deemed to be an unacceptable level of injury.

The final animal was subjected to the same blast at 4 cm distance. At post-mortem the gross appearance showed superficial peppering of the skin on the left side of chest. There was a minor degree of contusion to the lung particularly at the edge of the left lower zone.

7.5.4 Discussion

This proved that blast lung injury could be generated using this model, but indicated that 2 cm is too close a distance to avoid collateral injury. In addition, technical difficulties of positioning the animal, and monitoring the animal in the indoor range, meant that further experiments to refine this technique were thought not to be beneficial. This technique was therefore not pursued further.

7.6 Shock Tube

Shock tubes have been used in previous studies to induce a blast wave (Chavko et al., 2006; Gorbunov et al., 2004; Wang et al., 1998). The construction of the apparatus used is shown in Figure 20. The driving section (left side of the diagram) is enclosed when a diaphragm made of acetate sheets is inserted and the pressurised locking device employed to close the system. Once the driving section is pressurised to the desired level, the firing pin is deployed (by remote firing switch) to puncture the acetate sheets, creating a shock wave. The test section (right side of the diagram) then carries the shock wave to the end, where the animal is subjected to the effects of the shock wave. Due to the nature of the construction of the tube, the variable elements under our control were the pressure to which the compressed air compartment was filled (the compartment on the left side of the diagram), and the distance from the end of the tube that the animal was placed. The amount of acetate sheets forming the end of the enclosed compartment could also be varied, along with the volume of the compartment.

The peak overpressure of the shock wave used in previous rabbit blast lung injury experiments was 304KPa (Zunic et al., 2000).

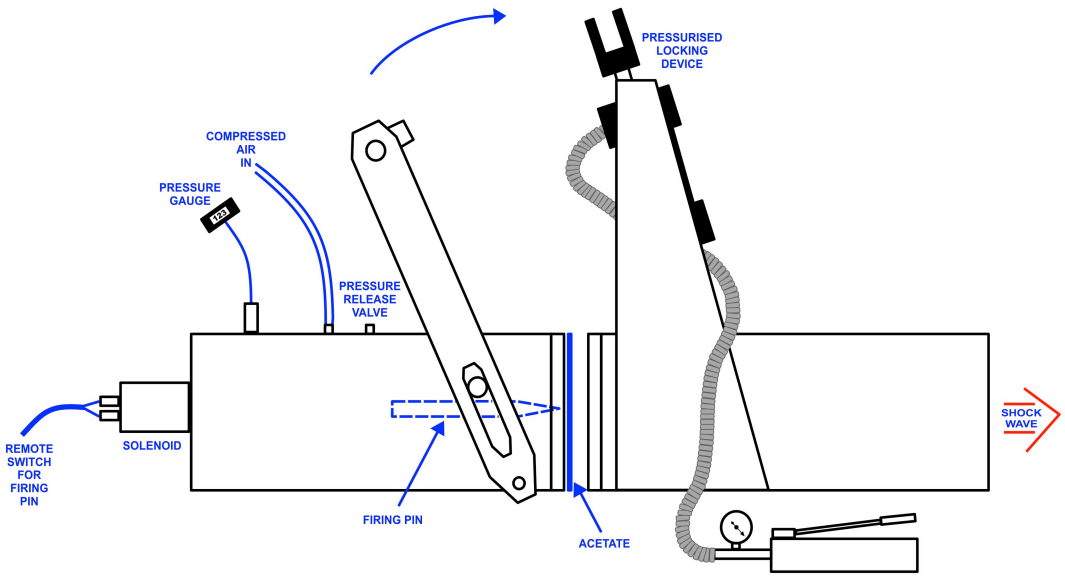

Figure 20: Schematic diagram of the shock tube.

7.6.1 Aim

The aim was to develop a reproducible model of blast lung injury using the shock tube apparatus.

7.6.2 Methods – ex vivo

Following initial firings against a pressure transducer to confirm the physical nature of the blast wave, finding that a peak overpressure of between 250-300KPa was achievable, experiments were commenced using killed animals to prove the principle that blast lung injury could be precipitated, before moving on to live animal models.

The first experiments on animals were performed at 46mm distance from the end of the shock tube, with the compartment pressurised to 850KPa, then 750KPa. The animals were held in a fabric sling, in front of the end of the shock tube. Single blasts were delivered to the left side of the chest. Post mortem examinations were then carried out to establish the degree of injury.

7.6.3 Results and progress

At the initial post mortem examinations, a large degree of liver damage and other intraperitoneal collateral injury was present, as well as severe blast lung injury. There was also evidence of fragmentation injury, caused by pieces of the acetate sheets being carried by the blast wind.

Ideas to minimise the amount of collateral injury and develop this model were then borrowed from the larger animal models. The shock tube was rigged with a metal plate or screen at the end, to shield the majority of the animal from the blast, with a window cut in the metal plate to allow the shock wave to pass unimpeded to the animal's chest. The animals were placed in a single layer of protective Kevlar, to reduce injury from fragmentation, initially within a Kevlar sling suspended from the plate, but subsequently within a tray (caged support), arranged on rollers to minimise acceleration and deceleration injury from swinging in the harness (see Figure 21). The chest was then aligned with the hole in the end plate, and foam blocks and wedges used to ensure a good fit in the tray, allowing for differences in animal size and weight. The animal was positioned slightly 'head-up' in an effort to allow the abdominal organs to fall with gravity away from the area of exposure on the chest. Some movement is

inevitable as a result of the blast wave exposure but the deleterious effects were minimised using these measures. By these means, secondary and tertiary injuries were prevented while the primary blast lung injury was still delivered. Animal positioning allowed monitoring to be maintained during the blast exposure.

The next experiment was conducted at 68 mm distance (the addition of two locking nuts to the rig increased the distance), pressurising the compartment to 600KPa. From the previous experiments using the pressure transducer this equates to approximately 300KPa overpressure of the shock wave. A single blast was delivered to the left side of the chest. At post-mortem there was minimal evidence of liver injury (a small peripheral liver laceration), with some evidence of blast lung injury (but less than that found in previous experiments).

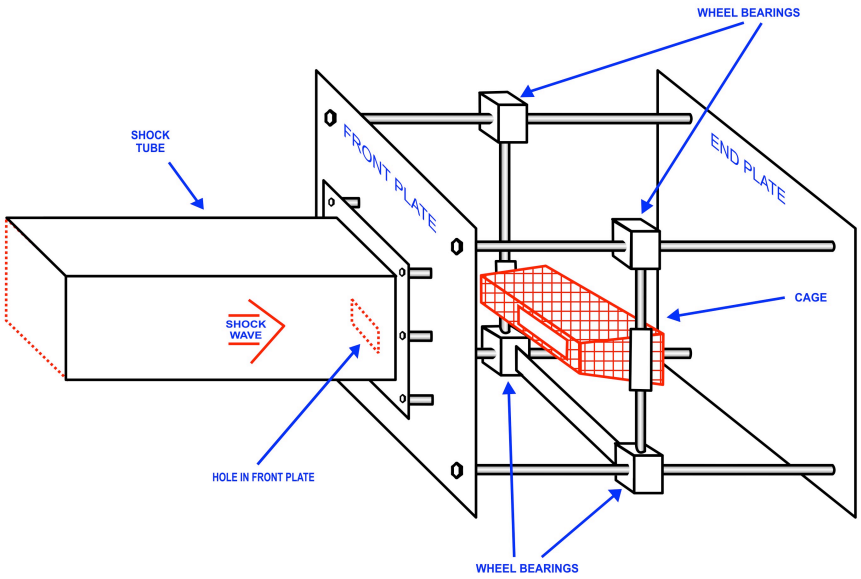

Figure 21: Roller system rig at the end of the shock tube. This system allowed the blast wave to be focussed on the thorax of the animal, with minimal movement of the animal within the caged support during the blast exposure.

These trials on killed animals were successful, proving that severe blast lung could be delivered using this model, and allowing us to proceed to live animal experiments.

7.6.4 Methods – in vivo

A further three experiments were performed on live animals under terminal anaesthesia. The animals were prepared as described earlier in this chapter. The only difference from previous preparation was that the endotracheal tube was left in place during the blast exposure, to allow monitoring of end tidal CO_2, and the tracheal cannula was then placed following blast exposure. Both invasive and non-invasive monitoring were difficult with this method of delivery of blast exposure, but end tidal CO_2 is a simple and effective way of monitoring respiratory function and was therefore chosen as an appropriate method of monitoring during the blast exposure.
The animals were kept alive for between 4-6 hours, after which post mortem examinations were carried out.

7.6.5 Results

The results of these experiments are shown in Table 4 and Figure 22. Following blast exposure, the animals had a degree of hypoxia but this seemed to recover over the following few hours. At post mortem all animals had evidence of blast lung injury, and no animal displayed evidence of collateral intraperitoneal injury.

	PO_2 (KPa) measured on arterial blood gas								
ID	Pre blast	+15 mins	+30 mins	+ 1 hour	+2 hours	+3 hours	+4 hours	+5 hours	+6 hours
04	13.7	7.9	8.8	9.2	9.6	8.9	9.6	-	-
05	7.5	8.1	9.3	9.9	10.7	10.5	9.2	12.4	12.3
06	8.4	9.6	9.5	10.1	12.1	11.6	11.5	13.6	13.5

Table 4: Arterial PO_2 measured by arterial blood gas, pre and post blast exposure

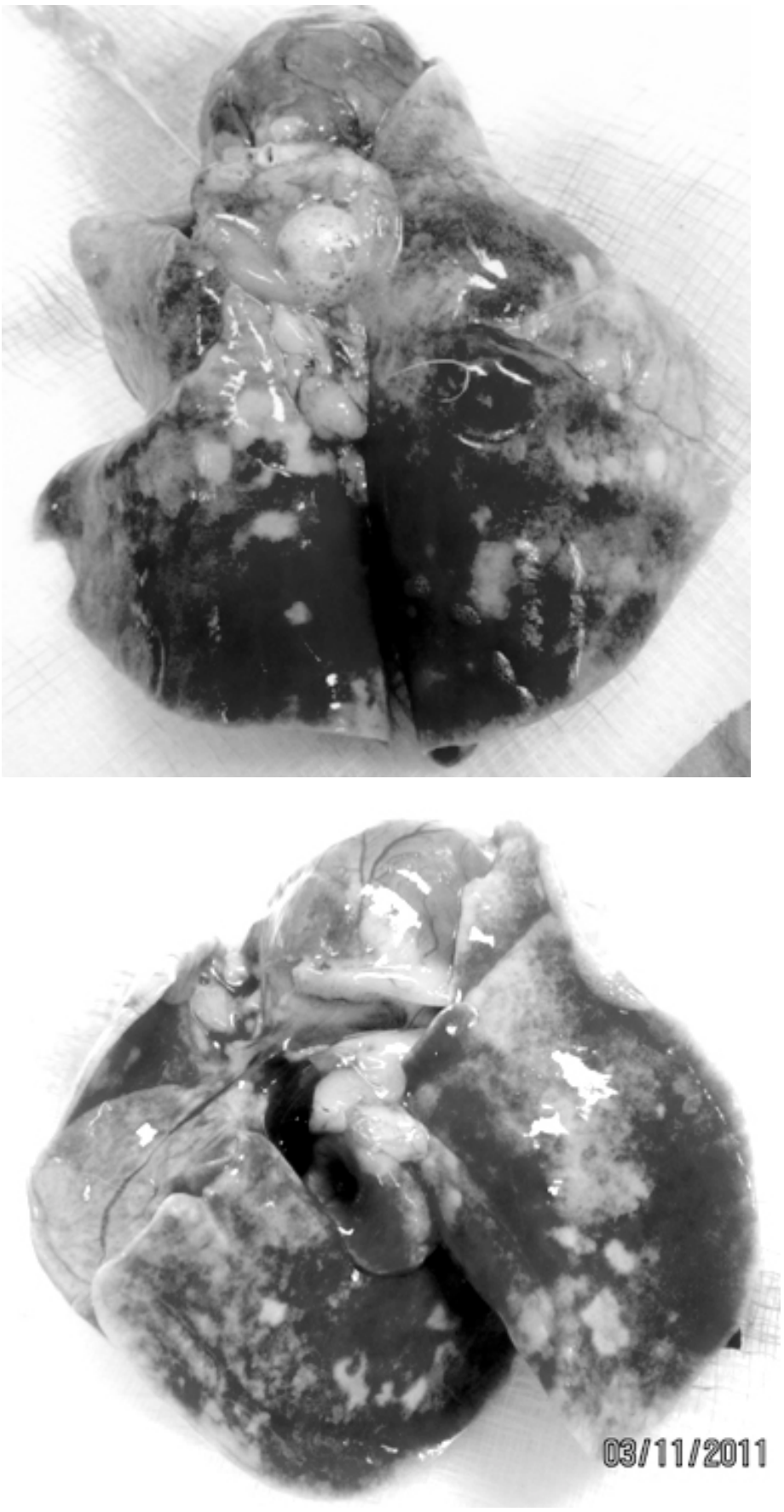

Figure 22 (a and b): Post mortem appearance of blast lung (animal 06). In black and white to highlight the proportion of lung affected by haemorrhage.

7.6.6 Discussion

This series of experiments proved that severe blast lung was demonstrated at post mortem following blast exposure with the shock tube, but that this did not correspond to a decrease in oxygenation measured either by peripheral oxygen saturations or arterial blood gas analysis. It did, however, demonstrate that blast lung injury was present, and that it was possible to maintain the animals to 6 hours post blast injury on the ventilator. This allowed progression to longer experiments out to 24 hours, in preparation for the final phase of the study. This will be described in Chapter 9.

Chapter 8. Preliminary Modelling of the Use of Nebulised rFVIIa.

8.1 Introduction

It has been established that blast lung is a significant problem in a minority of patients injured by explosion; it manifests in the form of pulmonary haemorrhage, which initiates a cascade of inflammation and oedema. Recombinant activated FVII has been used off-label for control of numerous different types of haemorrhage, including traumatic haemorrhage and more specifically in pulmonary haemorrhage (diffuse alveolar haemorrhage) of other aetiologies. A case report has been presented of the use of intravenous rFVIIa in a patient with intrapulmonary haemorrhage caused by high velocity gun shot wound to the chest.

rFVIIa is manufactured for use in solution for intravenous administration, although its use via the intrapulmonary route has been described. This has been delivered to subjects via a bronchoscope (Estella et al., 2008; Heslet et al., 2006), and its delivery by nebulisation has also been described (Heslet et al., 2006) although its use in this form has not been fully evaluated and reported.

Nebulisation involves conversion of a solution into aerosol form by using gas under pressure being forced through the solution. When nebulised, droplets of rFVIIa solution must be deposited in the lungs to have the desired therapeutic effect. Deposition in the lungs following nebulisation of a substance is dependent on several factors, including droplet size (dependant on the method of nebulisation), and air flow velocity, which together determine how much of the substance is deposited on the delivery tubing and upper airway (Dolovich and Dhand, 2011). Some will also be exhaled and lost to the exhalation limb of the ventilation circuit, possibly as much as 50% (O'Callaghan and Barry, 1997). It is also not clear how much of the nebulised rFVIIa remains biologically active following nebulisation and delivery through an endotracheal tube, and whether a significant proportion of the rFVIIa may be deposited on the internal surfaces of the endotracheal tube.

The ultimate aim of this programme of research was to define whether rFVIIa, delivered directly to the lungs via nebulisation, could attenuate the

haemorrhagic effects of blast lung injury. The intention to use rFVIIa in nebulised form necessitated a series of experiments to prove that this was possible in an animal model, both from a physical perspective and in terms of pharmacological activity.

The aims of this series of experiments were therefore to answer the following questions:

- would rFVIIa retain its pharmacological activity following nebulisation?
- what is the distribution of rFVIIa in the lungs following nebulisation?
- how much of the drug reaches the target organ (in this case the lungs) following nebulisation?

8.2 Does rFVIIa retain its pharmacological activity following nebulisation?

To define whether rFVIIa remains active following nebulisation, an experiment was undertaken to nebulise rFVIIa into a buffer solution, and measure the rFVIIa activity in the recipient buffer solution. However, it was not known how much of the start solution would finish in the recipient buffer (for example, some may be retained in the nebuliser, and some would be lost on the tubing), so to quantify the amount of activity retained following nebulisation, fluoroscein was added to the solution in a known concentration. The ratio of rFVIIa to fluoroscein in the start solution was calculated, and following nebulisation, the ratio of rFVIIa to fluoroscein in the recipient buffer was then calculated. Fluorescein is not known to be degraded by nebulisation, and therefore a decrease in the ratio of rFVIIa:fluorescein concentrations in the recipient buffer would indicate a loss of active rFVIIa, giving an idea of how much of the rFVIIa is destroyed by nebulisation.

The buffer solution used in this series of experiments was TEG; a mixture of Tris-HCl, EDTA - ethylenediaminetetraacetic acid - and glycerol. This closely resembled the soluent provided by the manufacturer of rFVIIa, which is provided in powder form and reconstituted with soluent prior to administration. The aim of this initial experiment was therefore to determine what proportion of rFVIIa remains active following nebulisation.

8.2.1 Methods

A standard curve for fluoroscein was constructed using serial dilutions of a known concentration of solution (6.5mg Na fluorescein + 6.5ml distilled water = 1.0mg/ml solution) (see Figure 20). rFVIIa solution was prepared according to the manufacturer's guidance, and a solution of fluorescein dye in Na_2CO_3 added to give a concentration of rFVIIa of 33.3mcg/ml, and fluorescein 120mcg/ml. This start solution was nebulised and passed into a recipient buffer solution (TEG). Following nebulisation rFVIIa activity was measured by bioassay (HemosIL®, Intrumentation Laboratory, USA). Fluorescence in the recipient buffer solution was measured in a Perkin-Elmer LS-5B fluorimeter versus a 0.1M Na_2CO_3 solution as a blank.

The ratio of concentrations of rFVIIa to fluoroscein was calculated in the original solution, and the recipient buffer solution, and these ratios compared to give a percentage recovery of activity.

8.2.2 Results

The standard curve is shown in Figure 23.

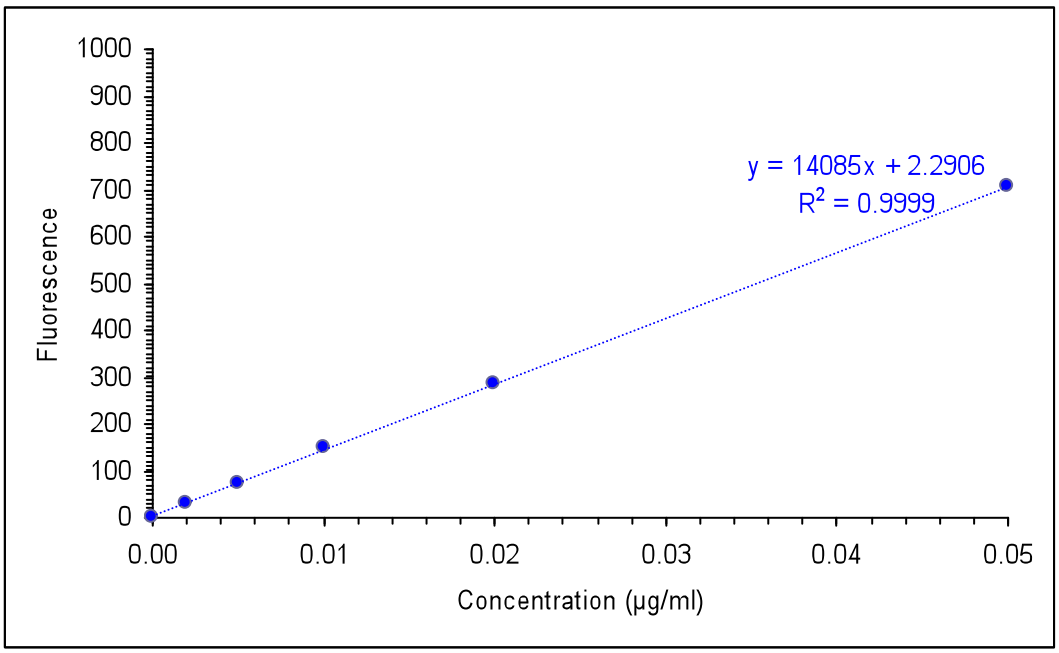

Figure 23: Fluoroscein standard curve.

In the start solution the concentration of rFVIIa was 33.3mcg/ml, and of fluoroscein 120mcg/ml, giving a ratio of rFVIIa:fluoroscein of 0.2775. The concentration in the recipient buffer solution of rFVIIa was 0.047mcg/ml, and of fluoroscein 0.17mcg/ml, giving a ratio of 0.2764. A comparison of these ratios showed that 99% rFVIIa activity was therefore recovered following nebulisation.

8.2.3 Conclusion

This experiment suggests that rFVIIa retains almost all of its bioactivity following nebulisation.

8.3 What is the distribution of rFVIIa in the lungs following nebulisation?

Having established that rFVIIa remains biologically active following nebulisation, the next stage was to define the distribution of the nebulised rFVIIa in the lungs. Immunohistological analysis was performed to qualitatively map the distribution of rFVIIa in the lungs, using a labelled antibody to rFVIIa. This was initially undertaken *ex vivo*, and then once the concept had been proven, in an *in vivo* model.

The aim of this series of experiments was therefore to define the distribution of nebulised rFVIIa in the lungs.

8.3.1 Methods

Qualitative analysis of distribution was performed by immunohistochemical staining. Two freshly killed animals were ventilated through a tracheal cannula, and a known dose of rFVIIa (200mcg/kg for the first, and 500mcg/kg for the second) was nebulised into their lungs. The lungs were then recruited by total lung capacity manoeuvre, and the trachea clamped to maintain inflation. The lungs were harvested at post mortem, and were transferred to 10% neutral buffered formalin for fixation. Samples remained immersed in fixative for seven days, following which the lungs were removed and divided into left and right prior to histochemical processing using standard wax embedding techniques. Sections of lung tissue were taken at 25, 50 and 75% of total wax block thickness from ventral to dorsal aspect of embedded lung tissues from both lungs and mounted on glass slides, before being exposed to goat IgG anti human FVII antibody (RD Systems BAF2338) overnight, then incubated at room temperature with rabbit IgG polyclonal anti-goat antibody (Abcam ab6740) for one hour. Final incubation in diaminobenzidine (DAB) and urea hydrogen peroxide produced the staining visible on light microscopy. Control sections were stained without the primary anti-FVII antibody to ensure non-specific staining did not interfere with the analysis.

A control set of lungs was also taken from an animal used for another experiment to identify whether any naturally occurring FVIIa is present in lung tissue.

The experiment was then repeated on a live animal. The animal was anaesthetised and instrumented as already described. A tracheal cannula was placed by dissection and a tracheal tie placed around the trachea and cannula.

The animal was then ventilated using normal ventilatory settings. 600mcg/kg of rFVIIa was nebulised into the animal (the animal weight was 3kg, so 1.8mg rFVIIa was used). The lung samples were then collected and processed at post mortem as above.

8.3.2 Results

The results of this experiment showed that rFVIIa is distributed widely throughout the lungs following nebulisation. No DAB staining was observed in sections where FVII antibody was omitted indicating that non-specific antibody binding could be discounted as a confounding factor when interpreting the staining results. No rFVIIa was visible in the control lungs.

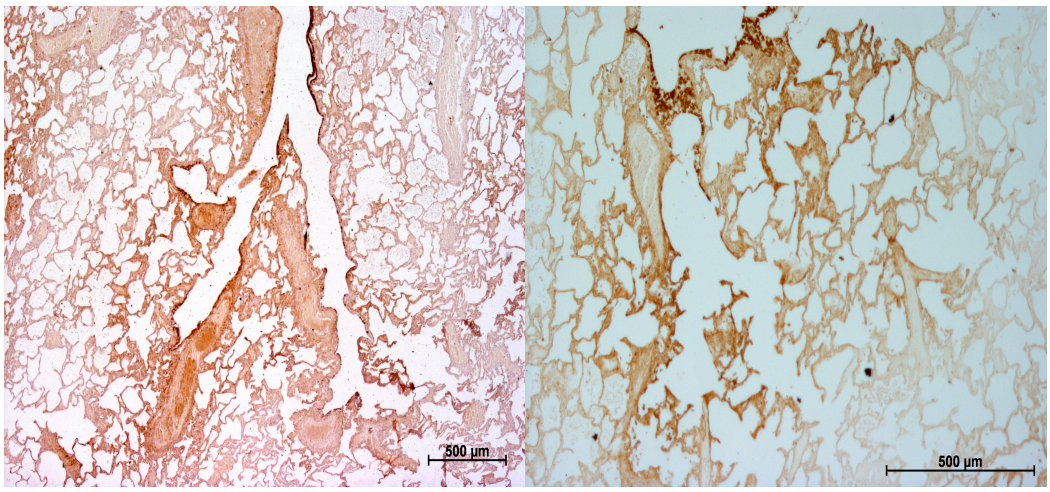

Figure 24: From *ex vivo* model, intense DAB staining observed throughout respiratory tree from main primary bronchioles (left) down to individual alveolar air spaces (right).

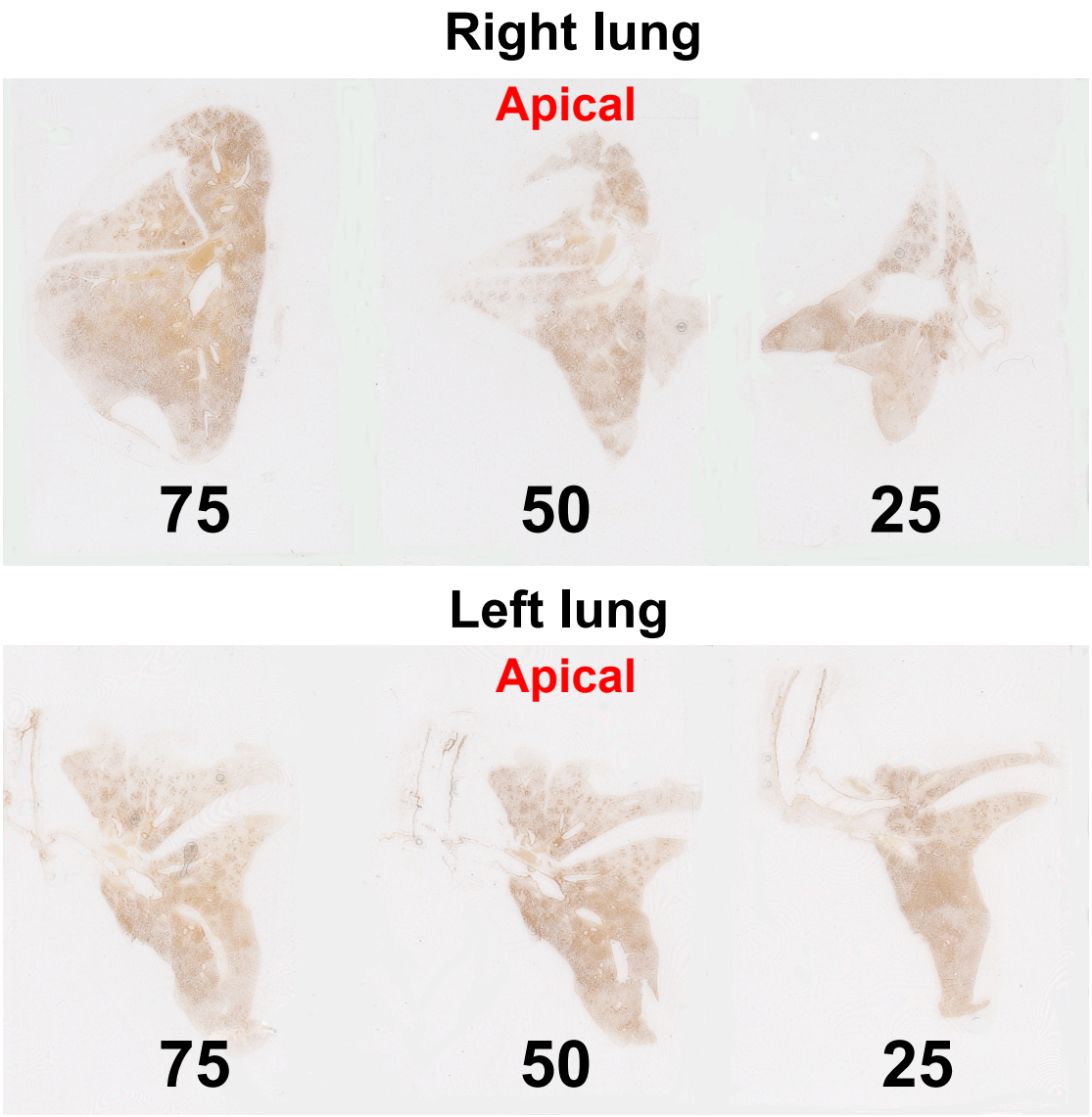

Figure 25: From *in vivo* experiment, distribution of FVII in right and left lung lobes demonstrated with DAB staining (x1.4mag).

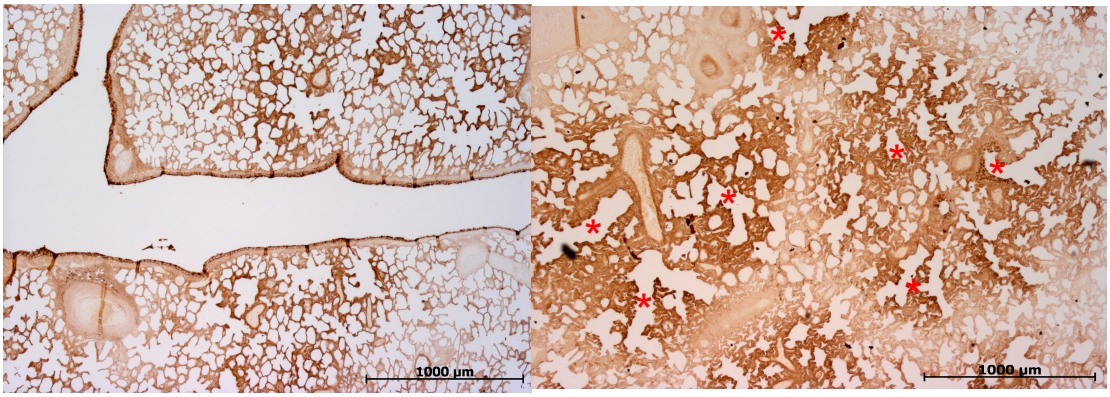

Figure 26: Brown DAB staining indicating FVII distribution in lung sections from (left) bronchiolar epithelial surface and alveolar spaces and (right) terminal bronchioles (*) and alveoli (x25 magnification).

8.3.3 Discussion

These results indicated a relatively even distribution of rFVIIa between the right and left lungs, with rFVIIa present in the deep lung alveoli. Alveolar collapse was not seen in any of the tissue sections indicating that maximum total lung volume was available for gaseous exchange and delivery of rFVIIa during the drug administration. rFVIIa staining was observed in all lung lobes (Figure 25) and while most intense in and surrounding the larger conducting airways was also seen to extend into the terminal bronchioles and alveolar spaces (Figure 26). This would suggest that this method of nebulisation produces particles of various sizes that are deposited throughout the respiratory tree.

Some focal areas of non-staining were seen at the peripheral edges of all lung lobes but were judged to represent only a small percentage of the total lung area present in each stained sections.

Immunohistochemical labelling of rFVIIa therefore showed that it is widely distributed throughout the lungs following nebulisation, suggesting that nebulisation may be an effective method of delivery of rFVIIa to target respiratory pathology.

8.4 How much rFVIIa is deposited in the lungs following nebulisation?

The experiments above showed that bioactivity is still maintained following nebulisation, and distribution of rFVIIa following nebulisation is widespread. Having proven that rFVIIa remains active and is distributed throughout the lungs, the final stage was to quantify how much rFVIIa is deposited in the lungs following nebulisation.

A series of experiments was conducted to attempt to measure how much rFVIIa was present in lung tissue after nebulisation. This is dependent on several factors including droplet size, the method of nebulisation, and how much is exhaled with the particular method of ventilation used (Dolovich and Dhand, 2011).

Different methods were considered to attempt to answer this question. One method that was discounted early on was to lavage the lungs, and measure the rFVIIa captured by this method. It has been shown that the rFVIIa reaches the small airways and alveoli, and therefore it was felt that lavage was not a practical way to measure the deposition, as it would not be effective in washing out the smaller airways. An in-out analysis was considered and piloted (measuring the rFVIIa left in the nebuliser, stuck to the tubing and in the exhaled limb of the ventilation circuit), but this ran into technical problems with increased pressure in the circuit caused by the distal filter in the exhalation limb tubing, so was abandoned. This left the option of a direct analysis of the amount of rFVIIa present in lung tissue following nebulisation. However, a preliminary experiment was performed to gauge a rough estimate of how much may be deposited, using a surrogate physical lung model.

rFVIIa has a molecular weight of around 50,000 Daltons. FITC dextran is an inert molecule of 40,000 Daltons, which is made up of dextran attached to fluorescein isothiocyanate (FITC), a fluorescent dye.

The aim of these experiments was to estimate how much rFVIIa is deposited in the lungs following nebulisation.

8.4.1 Methods

A preliminary simple experiment was performed to gauge very roughly the amount of nebulised solution ending up in the lungs, using the same nebuliser employed for subsequent experiments, and a physical lung model. It was accepted that this does not mimic the structure of the lungs in any way, but was

intended to give a rough guide as to what may be expected in later animal experiments. It was accepted that some solution would be retained in the nebuliser, some lost on the tubing, and some lost in the expiration phase.
To avoid using an animal for this simple experiment, a condom was used as a surrogate physical lung model, and TEG buffer solution was nebulised into the condom to replicate a solution being nebulised into the lungs (see Figure 27). 2mls of TEG was nebulised using the ventilator and an Aeroneb laboratory nebuliser until the nebuliser appeared dry, and the condom was weighed before and after nebulisation to identify how much solution, by weight, remained in the condom at the end of the process. The difference in weight of the condom before and after nebulisation was 0.6mg, suggesting that 0.6ml of the nebulised solution was present in the condom, in turn suggesting that approximately 30% of the volume of solution ends up in the target organ following nebulisation.

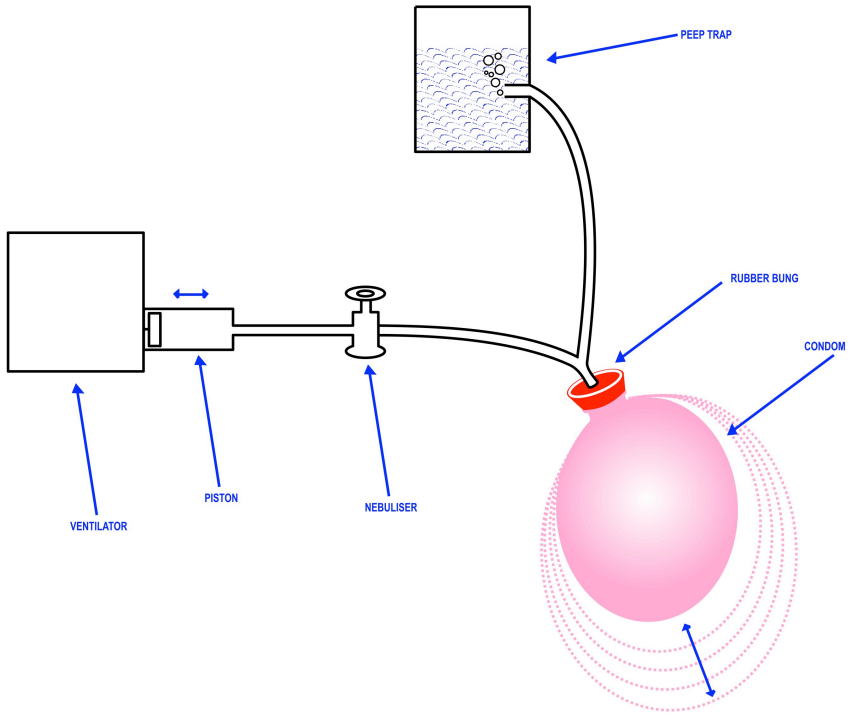

Figure 27: Surrogate lung apparatus

This experiment was followed by attempts to measure rFVIIa directly following nebulisation.
An animal was killed using a schedule 1 technique, then instrumented and prepared as described in the previous chapter. Once established on the

ventilator, a known quantity of rFVIIa (600mcg/kg) was nebulised into the animal. The lungs were harvested at post mortem, weighed, and mixed with TEG buffer solution (1g tissue to 4mls buffer). The whole lungs were homogenised (using a Kenwood CH180 'mini chopper' blender), then samples spun at 4500 rpm for 5 minutes, and the supernatant stored at -80°C before FVII activity assay analysis (HemosIL®, Intrumentation Laboratory, USA).

As will be discussed later, the results from the rFVIIa assay suggested an interaction between rFVIIa and lung tissue, potentially leading to an underestimation of the amount of rFVIIa deposited. Consequently, further studies were conducted with an inert substance, FITC dextran, which has similar physical properties and behaviour when nebulised as rFVIIa. FITC dextran is a dextran molecule attached to a fluorescent dye, so measurement of this molecule was therefore possible using fluorescence.

Further problems encountered with the colorimetric analysis of FITC dextran included the effect of pH on fluorescence, and interference between the red colour of the supernatant and FITC dextran. These were addressed by producing a standard curve at the pH of the lung homogenate, and by determining an appropriate dilution of the supernatant to reduce the intensity of the red colour.

Increasing dilutions of supernatant (1 in 10, 1 in 20, 1 in 30, 1 in 40, 1 in 50, 1 in 60) were made up from the solution, and colorimetry assays performed to see at what dilution the level of colour remained the same, or at what point the concentration of solution had no effect on the colorimetry. This occurred at dilutions below 1 in 40.

A standard curve was therefore constructed for the 1 in 50 dilution, adding different amounts of FITC dextran to a standard amount of solution, to effect a standard curve of luminescence (Figure 28).

Four animals were used to confirm the validity of the results. A known amount of FITC dextran (2mls of 10mg per ml solution) was nebulised into the lungs of four freshly killed rabbits via a tracheal cannula; the lungs were harvested at post mortem, and homogenised. Following centrifugation at 3000rpm for 10 minutes, the supernatant was diluted to abolish red colour interference (250µl of supernatant was added to 12.5ml TEG to achieve 1:50 dilution), and fluorescence was measured. The results were compared to the standard curve of 1 in 50 dilution solution.

8.4.2 Results

The results of the initial rFVIIa nebulisation experiment are shown in Table 5.

Sample ID	% predicted activity					
	Sample 1	Repeat 2	Repeat 3	Repeat 4	mean	SD
Lung homogenate 1:400	0.7	0.8	1.1	0.9	0.9	0.2
Lung homogenate 1:200	0.5	1.0	1.1	0.6	0.8	0.3
Lung homogenate 1:100	0.5	0.7	1.1	1.2	0.9	0.3
Lung homogenate 1:50	1.1	1.1	1.6	1.0	1.2	0.3
Lung homogenate	8.4	5.0	5.5	3.5	5.6	2.0

Table 5: Results of rFVIIa nebulisation experiment showing % predicted activity of FVII on direct assay

The standard curve for FITC dextran, applicable to all 4 animals, is shown in Figure 28. The results of the FITC dextran experiments are shown in Table 6. These show that there are consistent measurements of between 10-15% recovery.

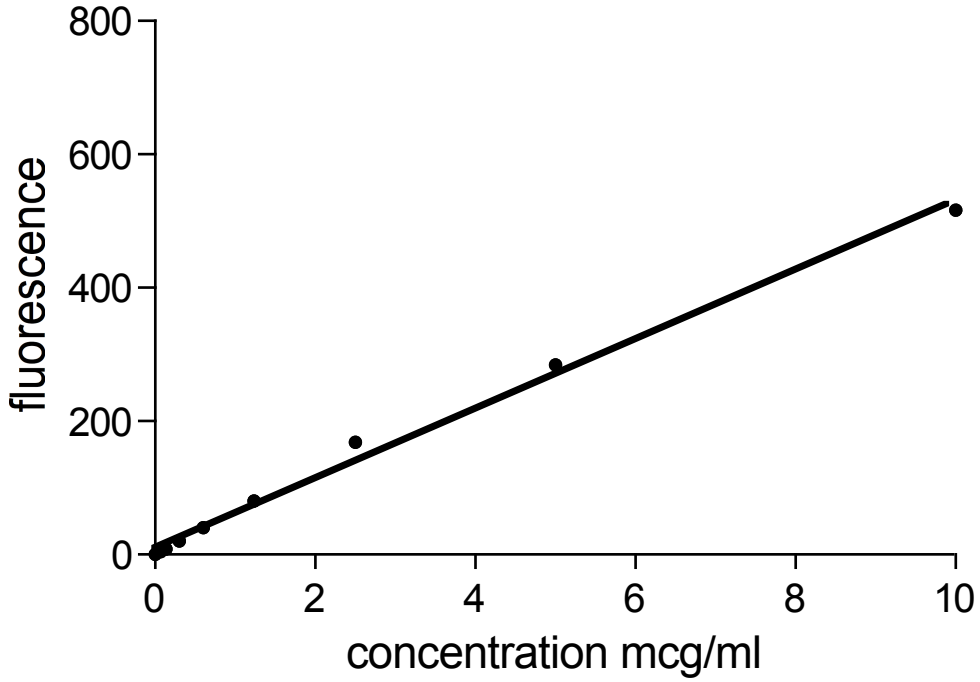

Figure 28: Standard curve of fluorescence for FITC-dextran

Animal ID	Fluorescence 513nm	Concentration mcg/ml	mcg/whole lung	% FITC-dextran recovered
F7-17	35.7	0.60	2141.7	10.7%
F7-18	49.8	0.77	2392.1	12.0%
F7-19	43.7	0.65	2458.0	12.3%
F7-20	58.3	0.93	2893.7	14.5%

Table 6: Results of FITC-dextran recovery from nebulisation. Data presented are means of 4 results for each animal.

8.4.3 Discussion

These experiments, using firstly rFVIIa and subsequently an inert substitute, gave very different results (approximately 1% recovery versus 10-15% recovery), and there may be several reasons for this.

It was not known whether the process of homogenising the lung tissue would affect rFVIIa activity due to the release of intracellular contents including proteases that could reduce the rFVIIa activity. The release of proteases following trauma that may affect FVII activity has been previously described (Kanse et al., 2012).

rFVIIa may be bound to molecules or taken up into cells. It has been postulated this might be due to binding of the rFVIIa molecule (possibly by tissue factor), or internalisation into cells. This has previously been described by Hansen *et al.*, who noted that FVIIa, bound to tissue factor, undergoes endocytosis (Hansen et al., 2001). This leant weight to the possibility of active uptake or binding of the rFVIIa molecule, affecting both the activity assay and an ELISA assay (which was used when the activity assay gave unexpectedly low results). This would not necessarily mean that the rFVIIa would fail to have the desired effect when nebulised into the lung, but it would affect any assay designed to test the presence of free rFVIIa.

It was evident from the initial experiments that when rFVIIa was nebulised into rabbit lungs, the activity levels were markedly reduced compared to the predicted values. The question of how much nebulised rFVIIa reaches the lungs had still not been answered. Some residual solution would be left in the nebuliser (approximately 300µl when measured), and some would be lost in the expiratory limb of the ventilation circuit. However, due to the problems measuring rFVIIa in the lungs described above, it was not possible to define how much of the nebulised drug reached the lungs and remained active. If rFVIIa could be substituted for an inert molecule of similar size, which is not bound or internalised into cells, and which produces similar droplet size when nebulised, it may be possible to extrapolate the results to estimate the proportion of rFVIIa reaching the lungs following nebulisation, hence experiments with FITC dextran were performed.

Using FITC dextran as an inert molecule substitute for rFVIIa, it was possible to measure how much of the FITC dextran is present in the lungs following nebulisation (and by extrapolation, estimate how much nebulised rFVIIa reaches the lungs). Consistent results of 10-15% recovery were obtained leading to the conclusion that approximately 10-15% of the start solution is deposited in the lungs. This would seem to be consistent with radio-isotope studies examining the deposition following nebulisation of other drugs such as salbutamol in asthma patients (Zainudin et al., 1990; Zainudin et al., 1988) and gentamicin in cystic fibrosis patients (Ilowite et al., 1987).

The results described demonstrate that a much higher dose of rFVIIa is required if delivered by nebulisation than by a standard intravenous route (due to loss or non-deposition of approximately 85-90% of the start solution).

8.5 Conclusions

From this series of experiments it had been established that rFVIIa activity is preserved following nebulisation, and it is widely distributed throughout the lungs, including down to the smaller airways and alveoli. It is likely, through extrapolation from the measurement of an inert surrogate, that approximately 10-15% of the starting nebulised solution is deposited in the lungs. This facilitated the final series of experiments in preparation for the randomised trial of nebulised rFVIIa for the treatment of blast lung injury.

Chapter 9. Subsequent Development of the Model.

9.1 Introduction

The aim of this programme of research was to develop an animal model in which the effects of rFVIIa, delivered by nebulisation, could be compared to placebo in animals with blast lung injury. The intention was to use oxygenation as the primary outcome measure, with secondary outcomes including the effects on lung function, the inflammatory response, and gross appearance of intrapulmonary haemorrhage at post mortem. It was also important to establish whether inhaled rFVIIa is absorbed systemically; if the rFVIIa delivered by nebulisation remains confined to the lungs, then concerns over systemic adverse effects may be assuaged. Coagulation would be monitored using rotational thromboelastometry (ROTEM), a sensitive near patient test of coagulation, as described in Chapter 5.

Previous chapters describe studies where animals had been subjected to blast exposure using a shock tube, and ventilated for up to 6 hours following blast exposure. It had been established that rFVIIa remains active following nebulisation, is widely distributed throughout the lungs, and 10-15% is deposited in the lungs by nebulisation. The intention for the final proof of principle study was to maintain ventilated animals for 24 hours following blast exposure, to allow a comparison of outcome measures that may develop over this period (as well as oxygenation, secondary outcomes including markers of inflammation and other indicators of acute lung injury that take some time to develop). It was necessary to establish whether it was possible to maintain this rabbit model anaesthetised and ventilated for 24 hours, to ensure the planned blood sampling did not adversely effect the animal, and whether there were any effects of prolonged ventilation on lung function and pathology.

The aim of this final phase of model development was therefore to extend the experiments to 24 hours, incorporating the elements that would be employed in the subsequent randomised trial.

9.2 Methods

4 animals were prepared and instrumented as described in Chapter 7. In addition to the interventions already described, animals were provided with maintenance intravenous fluids (6ml/kg/hour of 0.9% saline).

2 animals were exposed to blast using the shock tube, and 2 animals were exposed to sham blast (all interventions other than the blast exposure). All 4 animals were then ventilated and maintained under intravenous anaesthesia for 24 hours (Figure 29). Blood samples were taken at intervals, for arterial blood gas analysis, rotational thromboelastometry (ROTEM), electron paramagnetic resonance (EPR) and cytokine analysis. At 24 hours, the animals were humanely killed by intravenous injection of pentabarbitone, following which post mortem examination was undertaken.

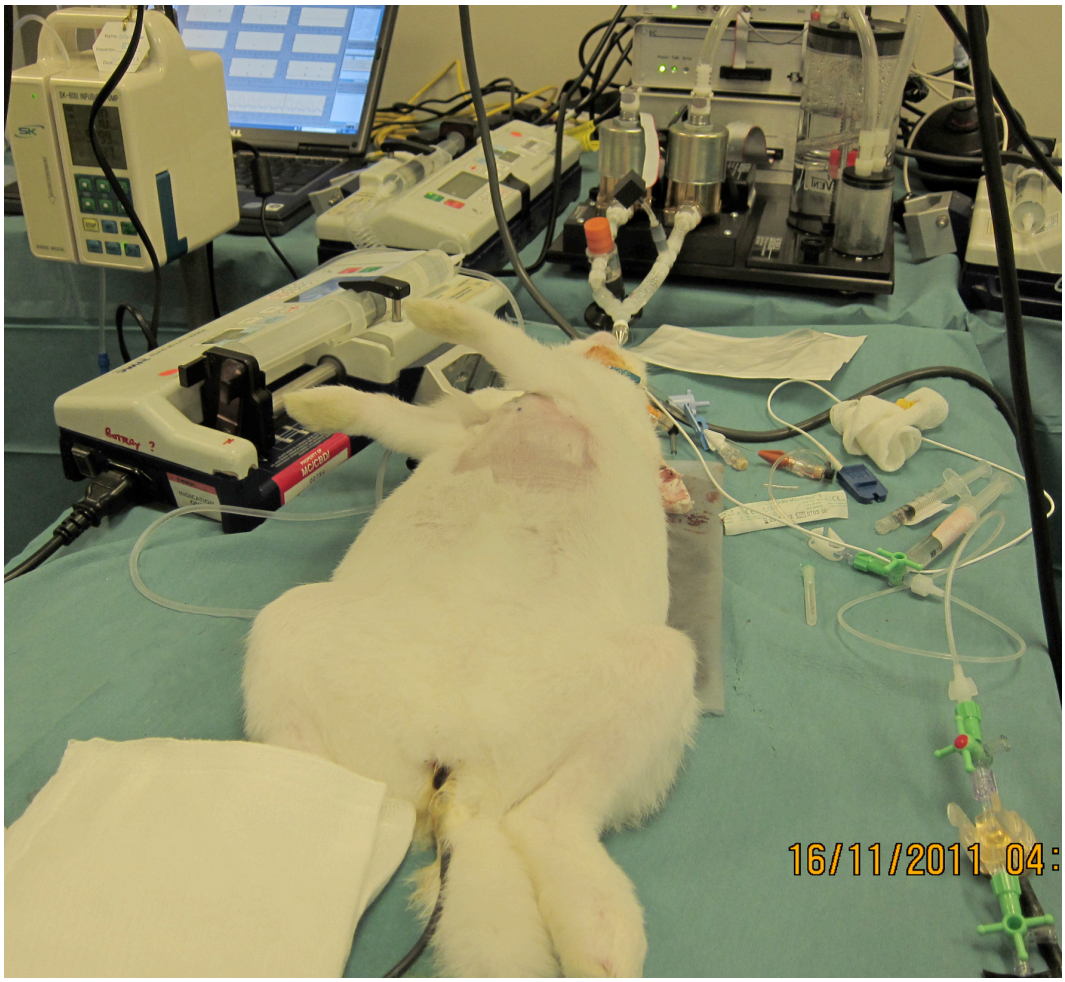

Figure 29: Photograph showing rabbit with ventilator (top of picture) and other equipment visible.

9.3 Results

All animals survived the 24-hour study period. Oxygenation, measured by arterial blood gas analysis, is shown in Figure 30. The ROTEM data is presented in Table 7. Post mortem findings confirmed evidence of severe blast lung injury in the animals exposed to blast, and evidence of post mortem superficial dependant change in the animals that were not exposed to blast (Figures 31 and 32). The results of the EPR and cytokine analysis are still awaited.

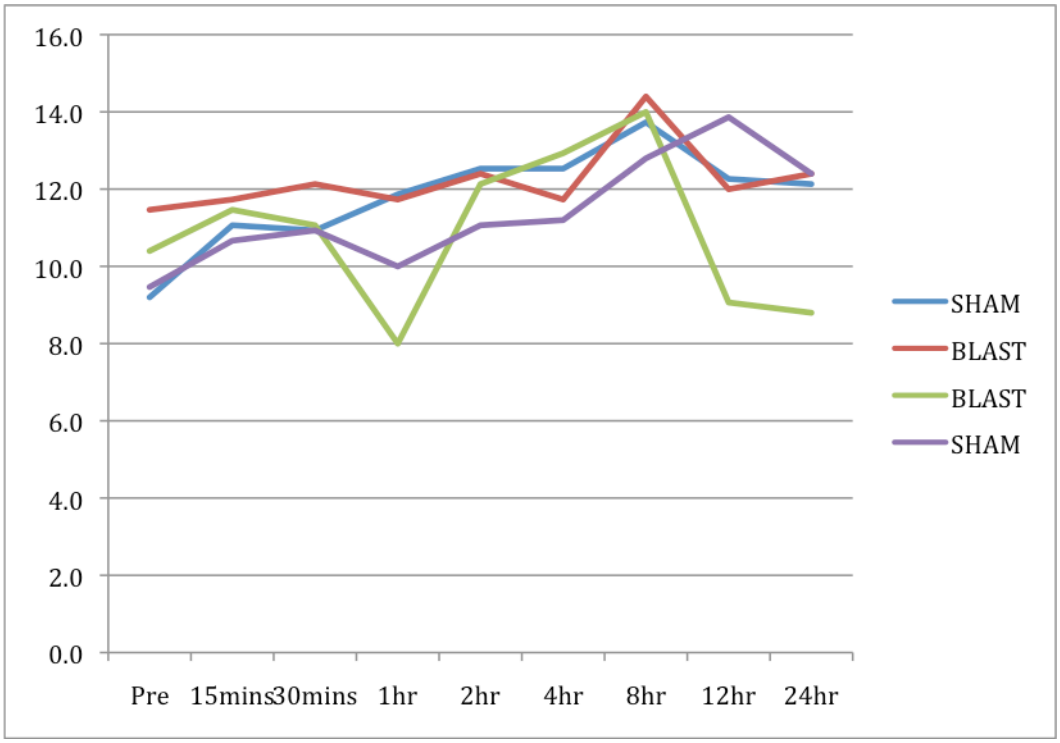

Figure 30: PO_2 measured by arterial blood gas, shown in KPa, against time.

Sample ID	Comment	Test name	CT	A5	A10	CFT	MCF
F7-07	Baseline	Extem	44	26	33	181	41
F7-07	T15	Extem	43	26	33	172	41
F7-07	1 HR	Extem	41	29	35	155	43
F7-07	3 HRS	Extem	35	28	36	150	45
F7-07	6 HRS	Extem	31	29	37	150	45
F7-07	12 HRS	Extem	31	33	41	114	49
F7-07	24 HRS	Extem	35	37	46	96	54
F7-08	Baseline	Extem	41	42	50	69	57
F7-08	T15	Extem	43	36	44	99	52
F7-08	1 HR	Extem	30	44	53	70	58
F7-08	3 HRS	Extem	36	39	47	85	55
F7-08	6 HRS	Extem	30	37	46	86	54
F7-08	12 HRS	Extem	33	42	50	73	56
F7-08	24 HRS	Extem	30	48	57	63	63
F7-09	Baseline	Extem	35	18	23	393	31
F7-09	T15	Extem	44	30	38	133	46
F7-09	1 HR	Extem	32	34	42	104	49
F7-09	3 HR	Extem	31	28	36	150	44
F7-09	6 HR	Extem	26	25	34	182	44
F7-09	12 HRS	Extem	17	16	19	697	24
F7-09	24 HRS	Extem	44	40	49	88	54
F7-10	Baseline	Extem	37	27	34	175	42
F7-10	T15	Extem	52	30	37	136	42
F7-10	1 HR	Extem	46	30	37	130	43
F7-10	3 HR	Extem	44	32	39	132	45
F7-10	6 HR	Extem	63	25	32	198	38
F7-10	12 HRS	Extem	25	29	36	145	43
F7-10	24 HRS	Extem	21	34	42	102	50

Table 7: Table showing ROTEM data for all 4 animals. CT = clotting time; CFT = clot formation time; MCF = maximum clot firmness; A5 and A10 = clot strength at 5 and 10 minutes.

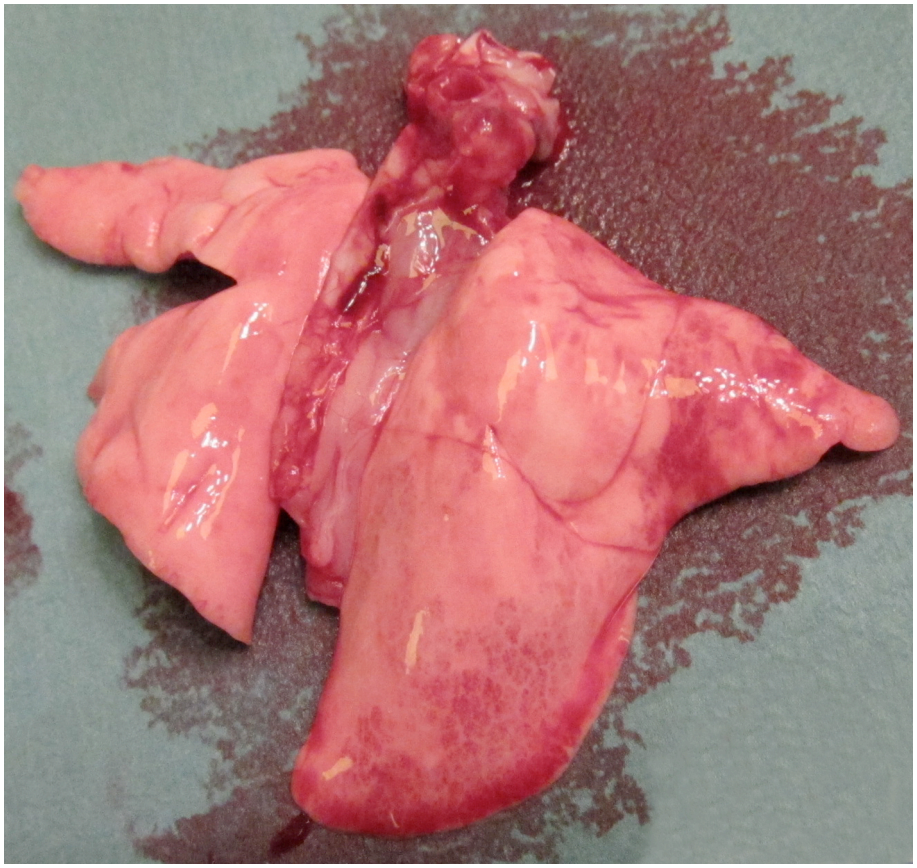

Figure 31: Post mortem findings, showing minor dependant areas of petechiae in an animal exposed to sham.

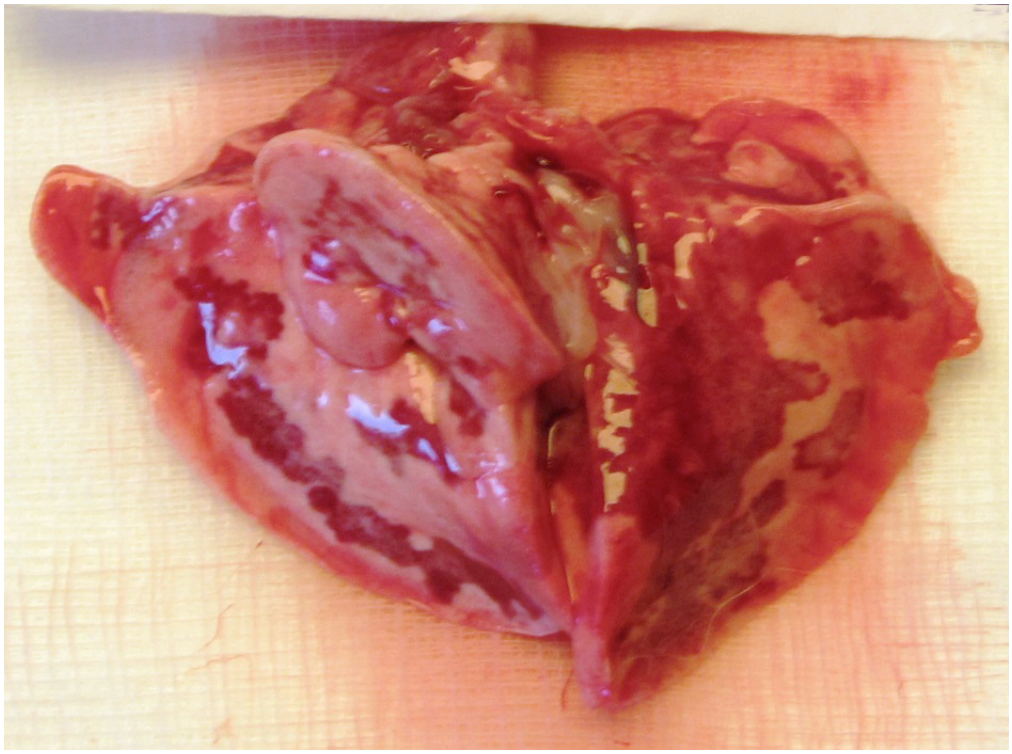

Figure 32: Post mortem findings, showing areas of established haemorrhage in an animal exposed to blast.

9.4 Discussion

This series of experiments demonstrated that anaesthetised animals could be ventilated for 24 hours, and that there was no obvious adverse effect of prolonged ventilation on the animals exposed to sham. The effects of prolonged ventilation that were noted, such as the development of atelectasis giving reduced lung compliance, could be reversed by simple lung recruitment manoeuvres, a finding that was consistent between animals exposed to sham and blast. In the two animals exposed to blast, there was no evidence of consistent hypoxia, despite the presence of severe blast lung injury on post mortem examination.

The ROTEM results showed that clotting time was not significantly different between the animals, although one of the animals subjected to blast had a wide variation in maximum clot firmness and other parameters, although this was not consistent throughout the experimental period. The reduction in maximum clot firmness may represent a transient coagulopathy in this animal exposed to blast. This was not related to any other physiological abnormality such as

acidosis or haemodilution that may have explained the coagulopathy. However, due to blood sample volumes it was not possible to perform these tests in duplicate, therefore it is possible that the variation is an artefact.

Post mortem examination of the animals exposed to sham revealed minimal dependant changes (areas of petechiae in the dependant areas), which developed in the few minutes during processing of the post mortem samples, but no evidence of significant lung damage was visible to the naked eye.

On post mortem examination of those exposed to blast, it was evident that there was severe blast lung injury, with areas of confluent organised haemorrhage visible within the substance of the lung, suggesting significant haemorrhage had occurred at the time of the initial blast.

The results of the EPR and cytokine analysis are still awaited, but should inform the utility of these measures in the final randomised trial.

Section 4: Discussion and Conclusions.

Chapter 10. Discussion.

Having demonstrated that blast lung injury is a significant problem affecting those injured by explosions, and that rFVIIa may be a potential treatment for blast lung injury, a programme of research was undertaken to develop an experimental model that could test this theory. The experiments described have confirmed that it is possible to precipitate blast lung injury in the rabbit model, keep it alive and ventilated for 24 hours, and deliver rFVIIa by nebulisation into the lungs. The animal model development is now complete, allowing continuation of the main experimental work to ascertain the effects of nebulised rFVIIa compared to placebo.

It was the original intention to perform a proof of principle study that would investigate whether rFVIIa, delivered directly to the lungs by nebulisation, could attenuate the haemorrhagic effects of blast lung injury. The initial proposed methodology was to use oxygenation as the primary outcome measure, measured by arterial blood gas analysis. The aim was to generate sufficient blast lung injury to cause systemic hypoxaemia following blast exposure, with a PO_2 of approximately 7.5KPa. This had been possible in the previous animal work using a rat model, with the compressed air blast wave generator, and has been demonstrated in pigs following exposure to blast wave from high explosives.

During the model development phase it became evident that despite severe blast lung injury being present at post mortem, hypoxia was not evident during the *in vivo* phase of the experiments. The reasons for this are not immediately evident, but the phenomenon has been described before. In Cernak's paper of 1996, she describes the 'oxygen saturation unexpectedly remaining close to the control values, despite severe tissue damage' (Cernak et al., 1996), although further details are not given. This may be a phenomenon that is species-specific, as it has not been encountered to this degree in other animal models. One possible explanation is that of ventilation and perfusion shunting away from areas of damaged tissue. This occurs in many forms of acute lung injury, in a physiological response to optimise ventilation when there are areas of damaged lung tissue. Shunting has previously been described in blast lung injury, but this was in the context of explaining why there is a reduction in oxygenation

following blast lung injury rather than a compensatory mechanism to optimise ventilation and perfusion (Irwin et al., 1997).

Another interesting finding was that in animals that did become hypoxaemic following blast exposure, this could be reversed by performing lung recruitment manoeuvres; a phenomenon that was noted while performing assessments of lung function. Prior to lung function testing, recruitment was performed to standardise the pre-test state of the lungs. As well as optimising lung compliance, this improved oxygenation measured by non-invasive pulse oximetry and arterial blood gas analysis, both in control animals and those exposed to blast. This has been described before in animal models of acute lung injury (Kloot et al., 2000), in healthy subjects under general anaesthesia (Tusman et al., 1999; Rothen et al., 1995), and in patients with ARDS (Foti et al., 2000; Lapinsky et al., 1999; Pelosi et al., 1999). However, the pathophysiology of atelectasis (and ARDS) is different to the acute intrapulmonary haemorrhage seen in blast lung injury, although the subsequent inflammatory phase of the injury bears more similarity to ARDS.

Anecdotally, ventilators that are pre-programmed to deliver pressure-limited ventilation, have been noted to be ineffective at maintaining adequate oxygenation in some patients following blast exposure prior to arrival at a definitive medical facility. The underlying concern is that barotrauma or air embolism may be precipitated if ventilation is overzealous. In intensive care units this has led to the employment of novel methods of ventilation to reduce volume and pressure related injury, such as high frequency oscillatory ventilation (HFOV), where small tidal volumes are delivered at a high rate, while maintaining a constant mean airway pressure during inspiration and expiration. The use of HFOV has been described in other forms of acute lung injury (Siau and Stewart, 2008), and blunt chest trauma (Funk et al., 2008), but has also been successfully used in military patients repatriated to the UK with blast lung injury (Mackenzie and Tunnicliffe, 2011). However, HFOV still tends to be used as a rescue technique when other methods have failed, and is not possible in the forward military environment due to logistical equipment constraints.

In clinical practice, therefore, caution is advised in cases of blast lung injury, with pressure and volume limitation being the mainstay of ventilatory management. However, based on the findings in this series of experiments, further work to define the effects of lung recruitment manoeuvres on

oxygenation (including an assessment of adverse effects) following blast lung injury is warranted.

The finding that oxygenation did not show a predictable reduction following blast exposure in the rabbit model has led to consideration of other outcome measures that may prove valuable as indicators of the severity of blast lung injury in the final study. One possible surrogate would be a volume estimation of intrapulmonary haemorrhage, which would be possible with three-dimensional computed tomography (CT) reconstruction, but this may not be able to distinguish haemorrhage from oedema. A better solution may be magnetic resonance imaging. In clinical practice the evaluation of blast lung injury is now routinely undertaken with CT reconstruction, as part of a trauma CT protocol, as this modality affords definition of other potentially life-threatening injuries, including occult spinal injury following exposure to blast (Comstock et al., 2011; Gay and Miles, 2011). However, CT and MRI were not available at the time of this research programme.

It was therefore necessary to identify other markers of severity of blast lung injury. It has been established that blast injury induces activation of inflammation and alteration in the levels of cytokines in the blood and tissues. Plasma interleukin 6 (IL-6), a pro-inflammatory cytokine, has been shown to increase following blast exposure to the chest in a murine animal model (Perl et al., 2006; Knoferl et al., 2003). This work suggested that there was a direct correlation between local and systemic concentrations of these cytokines, allowing serum sampling rather than necessitating broncho-alveolar lavage. There is little evidence to define the levels of these cytokines in human patients with blast lung injury, although there is some information available for other forms of blunt chest injury. Previous work has shown that in human trauma patients, the ratio of IL-6 to interleukin-10 correlates well with severity of injury measured by injury severity score (ISS), and may be used as a predictor of severity of injury (Taniguchi et al., 1999). However, in another study of blunt chest trauma patients on an intensive care unit, the levels of IL-6 (and procalcitonin) in serum and bronchoalveolar lavage fluid failed to show good correlation with the extent of lung contusion (Stiletto et al., 2001), although they were significantly elevated.

Macrophage inflammatory protein-2 (MIP-2), a lung specific chemokine, shows promise as a marker of severity of lung injury (Perl et al., 2006). It is released

due to the activation of alveolar macrophages, and signifies the release of inflammatory mediators from immune cells local to the site of injury. It has been measured in animal models of lung contusion (Jarrar et al., 2002), showing levels that are elevated as early as 10 minutes following blunt chest trauma (Liener et al., 2011; Seitz et al., 2011), although no studies have looked specifically at this as a marker of severity of blast lung injury.

Perhaps the most promising marker that has been shown to correlate with the severity of lung injury is the iron-transferrin complex. Transferrin is the iron binding protein carrier that facilitates transport of iron around the body, and is capable of reversibly binding two iron molecules (Mateos et al., 1998).

It has been demonstrated that iron is sequestrated into the tissues as a result of trauma (Walsh et al., 1996). This initial study examined 36 patients with traumatic injuries to determine the effect on iron metabolism over the days following injury, and found that significant hypoferraemia was present, and was associated with reduced levels of transferrin. These findings precipitated further studies that sought to identify the effects of blast injury on iron metabolism. One parameter that it is possible to measure is plasma iron, and in particular the iron-transferrin complex, which can be analysed using low temperature electron paramagnetic resonance (EPR) spectroscopy techniques (Svistunenko et al., 1997).

Nikolai Gorbunov and his colleagues at the Walter Reed Institute of Research in Maryland, USA undertook a series of studies to define the role of iron and iron-transferrin in blast injury, as part of a broader programme looking at oxidative stress and free radical damage precipitated by blast injury. It has been demonstrated that iron is sequestrated into the tissues as a result of trauma, as mentioned above, and of further relevance this has been demonstrated in blast injury (Gorbunov et al., 2003). Gorbunov *et al.* (2003) showed that different models of injury, including exposure to blast overpressure in a rat, produced a reduction in the amount of iron-transferrin in the blood, and that this reduction was inversely correlated with the severity of injury.

A further report detailed that iron-transferrin was normal at 1 hour following blast exposure, but was significantly reduced compared to sham controls at 3 hours, a reduction that persisted for the 24 hour observation period (Gorbunov et al., 2004). The group then went on to suggest that the degree of iron sequestration

correlated well with the degree of blast lung injury as measured by estimation at post mortem (Gorbunov et al., 2005).

It would seem, therefore, that an estimation of iron-transferrin over a 24 hour period may give an indication of therapeutic benefits of an intervention, possibly in conjunction with measurement of inflammatory cytokines such as IL-6 and MIP-2. This approach has been used in investigation of other potential treatments that may ameliorate the inflammatory process precipitated by blast injury, such as the use of anti-oxidants (Elsayed and Gorbunov, 2003). It has been established that as a result of blast wave exposure, oxidative stress may cause further damage to the lungs due in part to a reduction in antioxidants such as ascorbate and glutathione. The possible therapeutic options of delivery of antioxidants to patients with blast injuries have only started to be explored (Chavko et al., 2009; Chavko et al., 2008; Chavko et al., 2006), and may form the basis of future research in this area.

Measurement of IL-6 and MIP-2, along with EPR measurement of the iron-transferrin complex, was therefore undertaken as part of the final 24-hour experiments in this series, to validate the processing of samples. Samples were collected and results are still awaited. These markers could be used as an appropriate surrogate to measure the impact of delivering rFVIIa by nebulisation on animals with blast lung injury.

Another potentially important aspect of research that was outside the constraints of this programme would be to define the effects of blast lung injury on lung function over 24 hours following blast exposure. This has not previously been defined in any detail, but would potentially be important in informing the management of patients with blast lung injury over the initial phase of their treatment, particularly if they remain ventilated for a prolonged period. This could be performed using the same equipment that has been utilised at dstl Porton Down and would be a simple comparison of lung function tests, measured at intervals throughout the 24-hour period. The SCIREQ ventilator would be used to compare function between sham and blast exposed specimens.

If rFVIIa is shown to be effective at attenuating blast lung injury, the main barrier to its use in the clinical setting will be concern over adverse events, and cost. The revised Summary of Product Characteristics for rFVIIa that were published in 2009, highlighting the increased incidence of thromboembolic adverse

events, has raised concern over its continued use for unlicensed indications, and has probably contributed to a decrease in its use in deployed military hospitals. However, delivery of rFVIIa by the pulmonary route to target local pathology may eliminate concerns. Nebulised rFVIIa may not cross into the systemic circulation to any degree, thereby lessening the risk of systemic adverse events. In the final study, circulating levels of rFVIIa will be determined by direct plasma FVIIa assay and its clinical effects will be monitored via coagulation assay, by ROTEM analysis. Further cost effectiveness analysis will be necessary once the required dose of nebulised rFVIIa has been fully established before such treatment can be recommended.

Chapter 11. Summary and Conclusions.

This work has successfully defined the incidence of blast lung injury in the modern-day military patient population, and the use of rFVIIa in deployed military hospitals. Blast lung injury is a significant problem affecting approximately 11% of those injured by explosion in Afghanistan. The use of rFVIIa has declined over the last 12 months, but may still have a role in the management of patients with haemorrhage that is not controllable by surgical means.

The development of an animal model of blast lung injury first necessitated the establishment of a ventilated rabbit model, maintained on total intravenous anaesthetic. A model of nebulisation of rFVIIa has also been established, which has led to successful nebulisation of rFVIIa into the respiratory tree (with the distribution identified by staining), and retention of activity on bioassay. We have estimated the appropriate nebulised dose of rFVIIa to provide an equivalent dose to the systemic therapeutic range.

Further laboratory work has established an appropriate and reproducible model of blast lung injury in the rabbit, using a shock tube. This will allow a definitive programme of research to define whether rFVIIa, delivered by nebulisation, can attenuate the effects of blast lung injury.

Chapter 12. Publications and presentations arising from this work.

12.1 Publications

Smith, J. E. (2011) 'The epidemiology of blast lung injury during recent military conflicts: a retrospective database review of cases presenting to deployed military hospitals, 2003-2009', Phil Trans R Soc B, 366, (1562), pp. 291-4.

Smith, J. E. (2011) 'Blast lung injury', J R Nav Med Serv, 97, (3), pp. 99-105.

Smith, J. E., Fawcett, R. and Randalls, B. (2012) 'The use of recombinant activated factor VII in a patient with penetrating chest trauma and ongoing pulmonary hemorrhage', Mil Med, 177, (5), pp. 614-6.

Smith, J. E. (2012) 'The use of recombinant activated factor VII (rFVIIa) in the management of patients with major haemorrhage in military hospitals over the last 5 years', Emerg Med J. Published online first (10.1136/emermed-2012-201334).

12.2 Presentations

Smith, J. E. The epidemiology of blast lung injury during recent military conflicts: a review of cases presenting to deployed military hospitals, 2003-2009.
College of Emergency Medicine Conference, Birmingham, September 2010.

Smith, J. E., Mackenzie, I., Tunnicliffe, B., Mahoney, P., Watts, S. and Kirkman, E. Blast lung injuries: a clinical perspective from current operations.
NATO conference (HFM 207 symposium), Halifax, Canada, October 2011.

Smith, J. E. The use of recombinant activated factor VII (rFVIIa) in the management of patients with major haemorrhage in military hospitals over the last 5 years.
International Conference on Emergency Medicine, Dublin, June 2012.

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