In-Vivo Models of Blast Injury

13

Theofano Eftaxiopoulou

13.1 Introduction

Over the years, several *in-vivo* injury models have been developed to study the effects of blast injuries to experimental animals, in order to identify the injury mechanisms involved in the pathobiology of blast injury. This review provides an overview of the most commonly used blast injury models and the local and systemic changes induced in a wide range of tissues following blast.

13.2 Injury Models of Blast

13.2.1 Shock Tube

The Shock tube is a device able to generate pressure waves of varying intensity and duration. Because of its ability to produce repeatable blast waveforms that resemble the shock waves seen in free field blasts (Friedlander curve) as described in Chap. 1, it is by far the most common experimental design employed in studies involving *in-vivo* models, in order to study the effects of primary blast waves [1–6]. A shock tube is usually comprised of two chambers separated by one, two or multiple diaphragms. Compressed gas (air or helium) is loaded into the first chamber (often referred to as the overpressure chamber or the driver section), causing the diaphragm to deform plastically and fail [1, 2, 7]. This sudden rupture of the diaphragm releases the pressure into the low-pressure section forming a shock wave that travels along the tube [1]. Recently, more complex shock tubes have been designed, capable of reproducing complex shockwave signatures [8]. By a careful change of the volume and the pressure on the driver section, the output pulse of the system can be changed to vary from the ideal 'Friedlander' curve to a flat, long-duration pressure pulse corresponding to that seen inside vehicles subjected to an external blast (Fig. 13.1).

Animals in *in-vivo* studies are placed either within the main section [2, 4, 6, 10] or across the outlet of the shock tube [3, 5, 11]. In both set ups animals can be in either a supine or prone position [2] facing away, towards or on the side of the pressure wave. However, in all cases the animals are fixed in custom made holders or platforms, that prevent any potential movement of their bodies during blast, in order to minimise any tertiary blast effects [12].

13.2.2 Blast Tubes

A blast tube is a device that produces shock waves with a short duration of the primary peak [8]. Blast tubes were originally designed to study how

T. Eftaxiopoulou, PhD
Department of Bioengineering, Royal British Legion Centre for Blast Injury Studies, Imperial College London, London, UK
e-mail: a.bull@imperial.ac.uk

A.M.J. Bull et al. (eds.), *Blast Injury Science and Engineering: A Guide for Clinicians and Researchers*,
DOI 10.1007/978-3-319-21867-0_13

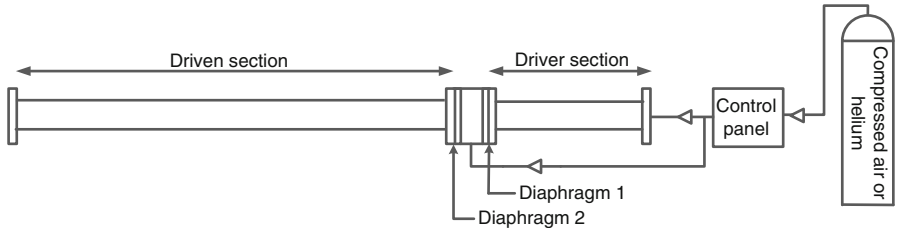

Fig. 13.1 Simplified schematic of a shock tube. Different designs exist in the literature. The length of the driver section can vary significantly, with values ranging from 0.76 m [3] to 1.22 m [1] reported in the literature. The two sections can be separated from each other by either one [2], two [1] or multiple diaphragms [9]. The driven section has also been seen to vary in length from 2.45 m [1] to 6.225 m [9]. In addition, a shock tube can have either a circular cross section (varying from 5.9 cm [1] to 30.5 cm [3] in diameter) or a square cross section (of 23 × 23 cm for instance [9])

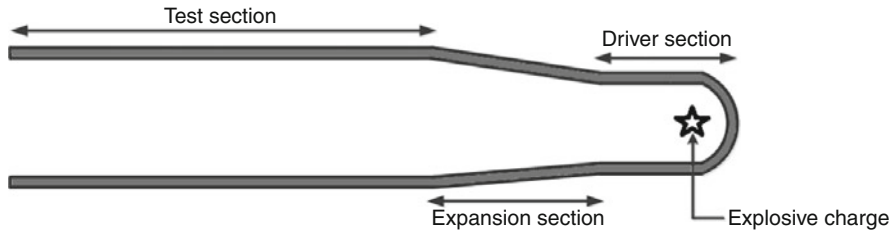

Fig. 13.2 Simplified schematic of a blast tube. Usually 1–2 g of pentaerythritol tetranitrate (PETN) explosive are used [16]. Saljo et al. and Risling et al. used a 1.54 m long blast tube with a 40 cm diameter [16, 17]. Bauman et al. used a much larger blast tube. The dimensions of the driven section were 2.44 m in length and 60.96 cm in diameter. The expansion section was about 3.05 m in length whilst the test section was 15.24 m long and 180.34 cm in diameter [15]

construction parts withstand shock waves of varying intensities [8]. However, during the 1950s these were modified by Clemedson and Jonsson to investigate vascular, respiratory and nervous effects of blast waves in rabbits [8, 13, 14].

A blast tube commonly comprises three chambers. The first chamber consists of a heavy-walled driver chamber in which explosives are placed [15]. The middle segment is called the expansion section and is connected to the third chamber called the test section where the animal is placed. An increase in the charge has been found to lead to a proportional increase in the peak pressure, but to have a small effect in the duration of the wave [8]. Similar to the shock tube, animals need to be restrained inside the blast tube to minimise any secondary or tertiary blast effects. Even though, blast tubes can produce repeatable and controlled waves, the smoke and gas emissions from the detonation of the explosives can lead to the development of quaternary blast effects, thus limiting their use in *in-vivo* studies [8] (Fig. 13.2).

13.2.3 Open/Free Field Blasts

In open/free field blast tests, shock waves are generated using explosives in an open field or concrete pad [18]. Very few studies have used chemical explosives to recreate battlefield injuries in a controlled environment to study their effects on animals. Most of these experiments were carried out primarily to determine thresholds for mortality and severity of injury [8]. More recently, Cheng et al. used an electric detonator with the equivalent of 400 mg TNT to develop a rat model to simulate blast injuries that occur in the battlefield [19] whilst, Rubovitch et al. used 500 g TNT, elevated 1 m above

ground, to replicate a low-level blast trauma using a murine model [20]. Open field blasts allow for more realistic experiments and for the investigation of the poly-traumatic nature of blast injuries, however, outdoor conditions in combination with the large number of animals needed are often too expensive. This, in addition to some lack of control variables, renders their use limited [8]. Similarly to the blast tubes, smoke/gas emissions from the explosives can cause quaternary blast effects (see Chap. 6, Sect. 6.2.4) whilst, isolating the blast effects into a particular organ/tissue is challenging.

13.2.4 Cranium Only Blast Injury Apparatus (COBIA)

The Cranium Only Blast Injury Apparatus (COBIA) was employed by Kuehn et al. in order to isolate the effect of direct cranial blast injury (dcBI) from the indirect blast injury to the brain mediated by thoracic transmission of the blast wave which can affect all the previous *in-vivo* models [21]. The experimental set up delivers blast overpressures generated by detonating cartridges of smokeless powder [21]. The peak pressure from the blast wave can reach up to 1000 kPa and the pressure traces show a large brief transient overpressure, followed by smaller slower transient under and overpressures, fully damped within 2 s [21]. This model could potentially be useful in isolating direct from indirect effects of blast. However, at this stage further validation is required to ensure that the pressure waves produced are related to the ones seen by conventional blast waves.

13.2.5 Laser-Induced Stress Waves (LISWs)

Laser-induced stress waves (LISWs) can be generated through the irradiation of a laser target with a laser source [22]. With respect to blast, LISWs have been used to investigate traumatic brain injury and pulmonary blast injury in rodent models. In both models the experimental animal is anaesthetised and fixed on a plate whilst, the region of interest is positioned in the focal area of a LISW and exposed to the stress waves which are described in detail in Chap. 1. In order to match the peak pressures seen in blast conditions, Hatano et al. and Satoh et al. used a natural black rubber disk covered and bonded with a transparent polyethylene terephthalate (PET) sheet as their laser target to generate waves with peak overpressures up to 604 MPa [23, 24] .

Laser-induced stress waveforms are dominated by a positive stress component that lasts for about 1 microsecond (μs) [22]. This duration is significantly shorter than that of a blast wave from a conventional weapon, which usually ranges between 2 and 6 millisecond (ms) [25]. Even though LISWs can reproduce some characteristics of shock waves and isolate effectively blast effects onto a particular tissue/organ, further investigation is needed to compare injuries induced by LISWs to the ones induced by conventional blast waves [23].

13.2.6 Secondary Blast Injury Models

Many explosive devices contain metallic and other fragments that along with the disintegrated munition casing can cause penetrating wounds [26]. Penetrating injuries can result either from fragments that are part of the device (primary fragments) or from the explosion (secondary fragments) [26] as previously discussed in Chap. 6. Few studies have addressed high-speed penetrating objects that produce shockwaves such as missiles and cause injury in large animal models for example in primates [27], sheep [28], pigs [29], cats [30] and dogs [31]. More recently, Plantman et al. (2009) recreated a penetrating traumatic brain injury to a rat model in the laboratory, using a modified air rifle that initially accelerated lead pellets that then impact a small probe that penetrated the surface of the brain with a speed ranging between 1 and 100 m/s [32]. Animals used in this work need to be

anaesthetised and fixed in a frame so as to avoid any acceleration injuries [16].

13.2.7 Tertiary Blast Injury Models

Proctor et al. used a rat model to investigate the effect of blast-induced acceleration on the brains of laboratory animals, in the absence of exposure to blast waves and of secondary impacts. In this model anaesthetised animals are secured to a metal platform and wrapped in a thick cotton "blanket" to minimise secondary movement. This platform is then accelerated vertically at either 20 or 50 G. What causes the acceleration is the detonation of pentaerythritol tetranitrate (PETN) placed in the water precisely under the centre of the plate [33]. This is the only study so far to have developed an underbody blast induced hyper-acceleration trauma model on the brains of laboratory animals [33]. However, to this point only two maximal G forces have been used, much lower than the survivable G forces experienced by service personnel within vehicles [33].

Another model developed to look at the effects of acceleration – deceleration due to blast, has been described by Risling et al. (2011). In this model the skull of an anaesthetised rat is tightly secured to a bar. An air rifle is used to accelerate a striker that is then used to impact the bar causing the head to rotate rearward [16]. By changing the air pressure in the rifle acceleration ranging between 0.3 and 2.1 Mrad/s^2 can be achieved. Following impact, the acceleration phase lasts 0.4 ms and then the head rotates at a constant speed and finally decelerates.

13.2.8 Underwater Blast Models

When an explosive is detonated under water, it produces a large volume of gaseous by-products in the form of an underwater bubble. The denser water spalls into the less dense air, causing fragmentation [34]. Underwater explosions are generally characterised by a much higher shock speed and a greater range of various effects than air blasts with primary blast injury and mortality rate being greater when the blast is under water [35]. However, Philips and Richmond submerged dogs in water and exposed them to underwater blast showing that the animals experienced internal injuries pathologically identical to that of air blast [36]. In the majority of these models, anaesthetised animals are submerged in water and exposed to under water blast [17, 36, 37]. One different approach, is the blast-amputation model developed by Tannous et al., whereby the animals were not submerged in water but secured on an aluminium platform with a hole, elevated above the surface a water-filled steel tank. Under water detonation of PETN led a column of water to rise at a maximum speed of 534 m/s through the hole in the platform [38].

13.3 *In-Vivo* Models

13.3.1 Traumatic Brain Injury

Blast waves generated by conventional and improvised explosive devices (IEDs) cause traumatic brain injury (TBI) in military personnel and civilians. Blast TBI is generally characterised by a primary injury that occurs at the time of exposure due to the immediate mechanical disruption of brain tissue followed by a secondary injury that develops hours to months after the initial trauma. Traumatic brain injury specific to blast is classified into three types:

- mild, whereby loss of consciousness for less than 1 h and posttraumatic amnesia for less than 24 h are noted,
- moderate, whereby loss of consciousness is less than 24 h and posttraumatic amnesia lasts anywhere up to 7 days, and
- severe, where patients exhibit loss of consciousness for time periods longer than 24 h and amnesia persistent for longer than 7 days [39, 40].

In-vivo animal models have been used to simulate blast conditions in an attempt to identify the mechanisms of TBI in a controlled environment

and to develop injury thresholds and therapeutic interventions. Primary blast-induced brain injury in rodents classified as mild usually shows no signs of structural damage at gross pathological examination [2], however, several authors have reported signs of limited neuronal/axonal injury in the cortex, corpus callosum, and periventricular areas [41–43]. Cernak et al. found that a single, mild blast in exposed mice induced glial activation, whilst Goldstein et al. showed that their histopathology 2 weeks after the blast event was similar to chronic traumatic encephalopathy (CTE), exhibiting signs of phosphorylated tauopathy, myelinated axonopathy, microvasculopathy, chronic neuroinflammation, and neuro-degeneration [2, 4].

Behavioural and functional changes associated with mild blast TBI (bTBI) often include weight loss, motor deficits, memory decline and impaired spatial learning [2, 15, 17, 41]. Cernak et al. (2011) showed that even though most of these symptoms were normalised 1 month after the exposure, some behaviour characteristics remained changed. Goldstein et al. showed that immobilisation of the head during the blast prevented associated learning and memory deficits [4], suggesting that head acceleration and subsequent deceleration may be critical factors in the development of bTBI [4, 44].

Fewer studies have focused on the effects of moderate and severe blasts on *in-vivo* models. Cernak et al. (2011) showed that moderate levels of blasts caused memory deficits and increased stress/anxiety in mice, whilst Svetlov et al. (2009) showed that head acceleration and deformation after severe blast trauma to the head of rats, was accompanied by typical focal and massive intracranial hematomas and brain swelling [5]. Changes on β-amyloid (Aβ) peptide that have been reported to occur rapidly after acute TBI in humans, as early as 2 h after a severe TBI [45, 46], have been seen to decrease acutely following injury in rodent models [47]. In addition, some authors have reported levels of the amyloid precursor protein (APP) to be increased following blast exposure [21, 47] whilst others [7, 42, 48], noted no APP accumulation in axons of rats exposed to over pressure waves ranging from 130 to 260 kPa. *In-vivo* animal models of primary blast-induced brain injury (bTBI) will be reviewed further in Chap. 14, Sect. 14.4, focusing on the effects of repetitive blast-induced TBI and acceleration – deceleration injury on animal models.

A large number of models also exist that describe the effect of penetrating traumatic injury, although very few of them are clinically relevant to blast conditions. Most notably, Plantman et al. (2009) recreated a penetrating traumatic brain injury to a rat model that caused tissue destruction such as white matter degeneration, haemorrhage, oedema, and gliosis accompanied by impairment of reference memory function. Long et al. (2009) [3] compared neuro-pathological changes evoked by blast to those described following controlled cortical contusion or fluid percussion injuries [49, 50] finding significant differences between the models and showing that exposure to airblast elicits fibre degeneration without being associated with obvious cell loss or injury. Similarly, Singleton et al. found that fluid percussion injury caused traumatic axotomy which also did not result in neuronal cell death [51, 52].

In summary, the existing literature on the pathobiology of blast-induced TBI presented is contradictory [12] and only partially imitates real life conditions. These variations in the models reported are often due to the broad range of experimental animals and blast injury models being used [12]. The shape and size of different brain structures can also influence the response under blast loading. Another severe limitation in developing animal models of TBI is that the classification of human blast TBI is based on the behavioural symptoms of injury [39]. Animal welfare regulations require that animals are anaesthetised when subjected to procedures that can potentially cause stress or pain thus, rendering diagnosis a challenging task [12]. Finally, the position and orientation of the experimental animal within the injury model and the presence or absence of noise stressors also play a crucial role in the biomechanical loading on the animal, the

type of injury that it sustains as well as the severity [12].

13.3.2 Blast Lung

Exposure to blast overpressures has been found to result in contusion or barotrauma-like injury mainly to air-filled organs such as lungs [53]. Indeed, exposure to blast pressure waves can result in cardiovascular and respiratory impairment because of the disruption of the alveolar septa and pulmonary capillaries, resulting in acute pulmonary haemorrhage [6]. *In-vivo* studies of blast TBI have identified that significant damage is observed in the lungs regardless of the body position of the experimental animal [2, 3, 54, 55]. In fact it has been suggested that there is an indirect thoracic mechanism of mild traumatic brain injury due to blast pressure waves [35, 54, 56, 57]. In addition, it has also been suggested that blast injury to the lower extremities may lead to systemic inflammatory changes affecting the limbs in addition to distal sites such as the lungs [58, 59].

Delius et al. implanted pressure probes into dogs to determine the conditions leading to lung damage. They found that shock wave pressures over 10 MPa caused bleeding [60] and attributed this to vessel rupture. Chavko et al. (2006) placed anaesthetised rats into a shock tube and exposed them to blast waves of a mean peak overpressure of 140 kPa. Characteristic landmarks of lung contusion such as intra-alveolar and subpleural haemorrhage, massive infiltration of neutrophils, and activation of macrophages in the lung parenchyma were noted [6, 24, 61]. More interestingly, administration of the antioxidant NACA prior to blast was seen to facilitate lung recovery from inflammatory damage [6]. Skotak et al. defined a lower peak overpressure of 100 kPa as the threshold for 'blast lung' injury that is characterised by pulmonary haemorrhage, vascular damage, direct alveolar injury, and oedema [10, 62]. In addition, extensive release of cytokines IL-1, IL-6, MCP-1, and MIP-2 have been observed in the Bronchoalveolar Lavage (BAL) fluid and blood plasma [63].

Rafaels et al. and Bass et al. developed curves for the assessment of the risk of fatality from primary pulmonary injury for long-duration (>10 ms positive overpressure phase) and short-duration blast waves respectively. They outlined the differences in the injury mechanisms from the two types of blast stating that for long durations the injury risk had little dependence on the duration parameter [64, 65].

Chai et al. investigated lung injury induced by a combined burn–blast trauma. They showed that rats with burn-blast combined injury had more severe lung injuries and abnormal coagulation and fibrinolytic function than those induced by either a blast or a burn only injury [66, 67]. Elsayed et al. (1997) investigated the effects of multiple low level shock waves (62 ± 2 kPa) in the lungs of rats and showed that repeating blasts did not significantly add to the effect of the first one [68].

13.3.3 Heterotopic Ossification

It has often been hypothesised that Heterotopic Ossification (HO) is caused by a combination of systemic and wound specific responses to trauma [69]. Whilst, there are several *in-vivo* models that reproduce HO in a laboratory environment, [70–72] the majority of these models use injections of bone morphogenetic proteins BMPs to induce HO, thus not replicating the conditions under which HO is formed in blast injuries. Nevertheless, through these studies a significant correlation between injury to the peripheral nervous system (PNS) and HO formation has been made attributed to the decreased expression of substance P (SP) and calcitonin gene-related peptide (CGRP) [69, 70]. Tannous et al. used a blast-amputation model to produce HO in rat residual limbs. Heterotopic bone was then radiographically classified as periosteal growth (Type A) or noncontiguous growth (Type B) in the rats. Whilst this is a very promising technique, relevant to blast scenarios,

limitations such as the high mortality rates and the variations in the blast overpressure delivered to the animals still need to be addressed [38, 69].

More recently, Polfer et al. established a rat HO model consisting of full body blast exposure, controlled femur fracture, crush injury and transfemoral amputation through the zone of injury [73]. In detail, they divided rats into three groups: animals exposed to a full body blast overpressure (120 ± 7 kPa), animals that sustained only a crush injury and femoral fracture followed by amputation through the zone of injury and animals exposed all insults. HO developed in all the rats in the third group and in about 65 % of the animals in the second group. Exposure to blast waves was seen to increase the prevalence of HO in this model [73] and the genes that regulate this early chondrogenic and osteogenic signalling and bone development (COL1a1, RUNX-2, OCN, PHEX, and POU5F1) were found to be induced early during the tissue reparative/healing phase [74]. This model simulates quite closely a combat-related extremity injury and can be used to further investigate the effects of different blast pressures and durations and provide an insight into the cellular and molecular pathways that lead to HO development [73, 74].

13.3.4 Hearing Loss

Blast overpressure can produce injury to the ears resulting in rupture of the tympanic membrane, dislocation or fracture of the ossicular chain, and damage to the sensory structures on the basilar membrane [75]. Animal studies have demonstrated that trauma to the auditory system induces hyperactivity in the inferior colliculus which may occur immediately after noise exposure and last for up to 3 months following exposure or cochlear ablation [76]. Mao et al. (2012) exposed rats to a single 10 ms blast at 14 psi and with a sound pressure level of 194 dB. Blast exposure induced early onset of tinnitus and central hearing impairment due to significant damage to certain auditory brain regions, in particular the inferior colliculus and medial geniculate body [77]. Absence of microstructural changes in the corpus callosum, led the authors to suggest that primary blast mainly exerts effects through the auditory pathways [77].

Kurioka et al. used LISW generated overpressures up to 400 MPa, applied to the cochlea of rats through bone conduction that revealed that the presence of an inner ear dysfunction is proportional to the peak overpressure [78]. In addition, severe oxidative damage accompanied by a lower survival rate of hair cells and spiral ganglion neurons were observed in the inner ear. Newman et al. also reported extensive loss of cochlear hair cells and a reduced cochlear outer hair cell function of rats exposed to three low level blast waves (of a 50.4 kPa peak pressure and a sound pressure level of 188 dB) separated by approximately 5 min using a blast tube [79].

Wu et al. used a D-86 spark pulse generator that caused deafness to rats when exposed to a 172 dB sound pressure level for 30 times with 2 s intervals and 0.5 ms pulse width [80]. The study then showed that adenovirus-mediated human β-nerve growth factor has a protective effect on rat cochlear spiral ganglion cells after blast exposure.

13.3.5 Skeletal Blast Trauma & Nerve Injuries

Blast injuries as a result of conventional and improvised explosive devices (IEDs) account for 75 % of modern war injuries. Over 70 % of these injuries involve the limb [81] (see Chap. 21, Sect. 21.1). However, very few animal models have been developed to look at the effects of blast injuries to the skeletal and peripheral nervous system. Christensen et al. exposed cadaveric pigs to semi-controlled free field blast events of varying explosive type charge size, and distance, including some cases with shrapnel. They found extensive skeletal trauma and amputation of the limbs and cranium. Usually, long bone shafts were the most severely fracture, whilst transverse and oblique fractures were commonly noted in the head, neck, and

shaft of numerous ribs. What is of interest is that specimens exposed to blasts that included shrapnel displayed even greater fracture severity, with extreme bone fragmentation of the long bones [82]. This study showed that primary and secondary blast mechanisms can produce traumatic amputations and skeletal fractures, although it is fairly limited in scope. One of its limitations is the fact that only small blast distances and open-air settings were studied. Data from blasts occurring in a confined space would add significant information to this work.

An interesting poly-traumatised model was developed by Claes et al. to investigate the effect of a thoracic trauma and an additional soft-tissue trauma on fracture healing in a rat tibia model. The tibial fracture was created using a 3-point bending guillotine device and a drop tower was used to create additional soft tissue-trauma. Finally, the thoracic trauma was induced by a single blast wave centered on the thorax with a modified blast wave generator. Results confirmed that fracture healing was increasingly impaired with increasing severity of trauma, especially when a soft tissue trauma was applied in addition to the thoracic trauma [83]. The authors explained this effect reporting that in the poly-traumatised animals there was reduced callus formation in comparison to animals with isolated fractures. Although only the thoracic trauma was created using a primary blast set up, such models could be further improved and become very useful in understanding the nature of human polytrauma from blast injuries.

Contrary to central nervous injury that has been extensively studied in blast conditions, peripheral nerve injury has not been addressed despite the significant burden of peripheral nerve damage seen following exposure to blast [84]. Suneson and Seeman used a high-energy missile that impacted to the left thigh of a large animal creating a short lasting shockwave. This shockwave caused immediate contralateral sciatic nerve dysfunction, as revealed by the decreased number of microtubules and the Schwann cells exhibiting signs of damage and swelling, despite demonstrating no haemorrhage or major tissue deformation [85]. In addition, similar changes were noticed in the phrenic nerves as well as in unmyelinated axons in both sciatic and phrenic nerves.

13.4 Summary

Based on the work presented in this chapter it can be seen that *in-vivo* models are widely used to study several aspects of the blast injuries, especially blast traumatic brain injury and blast lung. However, one fundamental question that arises is how can we compare the existing models, the findings from which are often contradictory? When looking into the effects of primary blast for instance, there is significant variability among researchers in the peak overpressures and the duration of the waves used. The majority of the existing models are often vague about the characteristics of the shockwaves produced, in some cases reporting only the peak overpressure and thus limiting comparability between studies [2]. Furthermore, there is also significant variability in the position and orientation of the experimental animal during blast which has an important role in the biomechanical loading on the animal, the injury sustained and its severity [4, 12].

Another significant limitation is associated with the species of the experimental animals used in these studies. Existing large animal models require large scale settings and are often too expensive, thus their use is limited. Large animal models also can have difference ethical considerations associated with their use. More often rodent models, rats and mice in particular, are used. However, even in these cases it has been argued that different strains may exhibit different inflammatory responses to blast [86]. In addition, researchers have also suggested that the rodent's lissencephalic cortex makes them inappropriate for modelling changes in cognition and behaviour after bTBI [12].

A second key question is how do we validate these *in-vivo* models? Due to differences in properties, size and mass between humans and animals, scaling has been proposed and used in several studies. Panzer et al. (2014) recently reviewed a number of different approaches to scaling the dose and response of animal models

to humans. In the majority of the existing scaling techniques the animal's blast duration is scaled to the equivalent human duration while the amplitude of the overpressure remains unchanged [87]. During IED explosion shockwaves with peak pressures from 50 to 1000 kPa and 2–6 ms duration have been measured, whereas most of the experimental models involve blast waves with durations between 4 and 8 ms [2, 4, 17] and some with durations longer than 10 ms [25]. Without considering scaling, the shock wave characteristics of most of the animal models developed are comparable to what has been reported during actual blast conditions. However, when scaling is considered, then these scaled durations are much longer than the ones reported during real blast events [25]. Researchers still debate as to whether scaling methods should be used and if so which are appropriate. To this end more data from real blast events are needed. Finally, the majority of *in-vivo* models tend to replicate only single factors involved in pathology of specific tissue/organs, simplifying the clinical problem. Despite these limitations, animal models have contributed substantially in the interpretation of some of the key injury mechanisms involved in blast injuries. However, more complex models are needed to gain a better understanding of the highly heterogeneous nature of blast injuries.

References

1. Nguyen TTN, Wilgeroth JM, Proud WG. Controlling blast wave generation in a shock tube for biological applications. In: 18th APS-SCCM and 24th AIRAPT, Seattle/Washington, DC.
2. Cernak I, et al. The pathobiology of blast injuries and blast-induced neurotrauma as identified using a new experimental model of injury in mice. Neurobiol Dis. 2011;41(2):538–51.
3. Long JB, et al. Blast overpressure in rats: recreating a battlefield injury in the laboratory. J Neurotrauma. 2009;26(6):827–40.
4. Goldstein LE, et al. Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. Sci Transl Med. 2012;4:134ra60.
5. Svetlov S, et al. Morphological and biochemical signatures of brain injury following head-directed controlled blast overpressure impact. J Neurotrauma. 2009;26(8):A75.
6. Chavko M, Prusaczyk WK, McCarron RM. Lung injury and recovery after exposure to blast overpressure. J Trauma. 2006;61(4):933–42.
7. Risling M, et al. Experimental studies on mechanisms of blast induced brain injuries. J Neurotrauma. 2009;26(8):A74.
8. Risling M, Davidsson J. Experimental animal models for studies on the mechanisms of blast-induced neurotrauma. Front Neurol. 2012;3(30).
9. Chandra N, et al. Evolution of blast wave profiles in simulated air blasts: experiment and computational modeling. Shock Waves. 2012;22(5):403–15.
10. Skotak M, et al. Rat injury model under controlled field-relevant primary blast conditions: acute response to a wide range of peak overpressures 7. J Neurotrauma. 2013;30(13):1147–60.
11. Mohan K, et al. Retinal ganglion cell damage in an experimental rodent model of blast-mediated traumatic brain injury. Invest Ophthalmol Vis Sci. 2013;54(5):3440–50.
12. Cernak I. Animal models of head trauma. NeuroRx J Am Soc Exp Neurother. 2005;2(3):410–22.
13. Clemedson CJ, Jonsson A. Effects of frequency content in complex air shock-waves on lung injuries in rabbits. Aviat Space Environ Med. 1976;47(11):1143–52.
14. Clemedson CJ. Shock wave transmission to the central nervous system. Acta Physiol Scand. 1956;37(2–3):204–14.
15. Bauman RA, et al. An introductory characterization of a combat-casualty-care relevant swine model of closed head injury resulting from exposure to explosive blast. J Neurotrauma. 2009;26(6):841–60.
16. Risling M, et al. Mechanisms of blast induced brain injuries, experimental studies in rats. Neuroimage. 2011;54:S89–97.
17. Saljo A, et al. Mechanisms and pathophysiology of the low-level blast brain injury in animal models. Neuroimage. 2011;54:S83–8.
18. Bass CR, et al. Brain injuries from blast. Ann Biomed Eng. 2012;40(1):185–202.
19. Cheng JM, et al. Development of a rat model for studying blast-induced traumatic brain injury. J Neurol Sci. 2010;294(1–2):23–8.
20. Rubovitch V, et al. A mouse model of blast-induced mild traumatic brain injury. Exp Neurol. 2011;232(2):280–9.
21. Kuehn R, et al. Rodent model of direct cranial blast injury. J Neurotrauma. 2011;28(10):2155–69.
22. Ogura M, et al. In vivo targeted gene transfer in skin by the use of laser-induced stress waves. Lasers Surg Med. 2004;34(3):242–8.
23. Hatano B, et al. Traumatic brain injury caused by laser-induced shock wave in rats: a novel laboratory model for studying blast-induced traumatic brain injury. Proc SPIE. 2011;7897:78971V.
24. Satoh Y, et al. Pulmonary blast injury in mice: a novel model for studying blast injury in the laboratory using

laser-induced stress waves. Lasers Surg Med. 2010;42 (4):313–8.
25. Panzer MB, Wood GW, Bass CR. Scaling in neurotrauma: how do we apply animal experiments to people? Exp Neurol. 2014;261:120–6.
26. DePalma RG, et al. Current concepts: blast injuries. N Engl J Med. 2005;352(13):1335–42.
27. Crockard HA, et al. An experimental cerebral missile injury model in primates. J Neurosurg. 1977;46:776–83.
28. Finnie JW. Pathology of experimental traumatic craniocerebral missile injury. J Comp Pathol. 1993;108: 93–101.
29. Suneson A, Hansson HA, Seeman T. Peripheral high-energy missile hits cause pressure changes and damage to the nervous-system – experimental studies on pigs. J Trauma. 1987;27(7):782–9.
30. Carey ME, et al. Experimental missile wound to the brain. J Neurosurg. 1989;71(5):754–64.
31. Tan YH, et al. A gross and microscopic study of cerebral injuries accompanying maxillofacial high-velocity projectile wounding in dogs. J Oral Maxillofac Surg. 1998;56(3):345–8.
32. Plantman S, Davidsson J, Risling M. Characterization of a novel model for penetrating brain injury. J Neurotrauma. 2009;26(8):A86.
33. Proctor JL, et al. Rat model of brain injury caused by under-vehicle blast-induced hyperacceleration. J Trauma Acute Care Surg. 2014;77:S83–7.
34. Elder GA, et al. Blast-induced mild traumatic brain injury. Psychiatr Clin North Am. 2010;33(4):757–81.
35. Courtney MW, Courtney AC. Working toward exposure thresholds for blast-induced traumatic brain injury: thoracic and acceleration mechanisms. Neuroimage. 2011;54:S55–61.
36. Phillips YY, Richmond DR. Primary blast injury and basic research: a brief history. In: Textbook of military medicine, Part I, Conventional warfare: ballistic, blast and burn injuries. Washington, DC: Office of the Surgeon General of the US Army; 1990.
37. Andersen P, Loken S. Lung damage and lethality by underwater detonations. Acta Physiol Scand. 1968;72 (1–2):6–14.
38. Tannous O, et al. Heterotopic ossification after extremity blast amputation in a Sprague–Dawley rat animal model. J Orthop Trauma. 2011;25(8):506–10.
39. de Lanerolle NC, et al. Characteristics of an explosive blast-induced brain injury in an experimental model. J Neuropathol Exp Neurol. 2011;70(11):1046–57.
40. Warden D. Military TBI during the Iraq and Afghanistan wars. J Head Trauma Rehabil. 2006;21 (5):398–402.
41. Park E, et al. Electrophysiological white matter dysfunction and association with neurobehavioral deficits following low-level primary blast trauma. Neurobiol Dis. 2013;52:150–9.
42. Pun PBL, et al. Low level primary blast injury in rodent brain. Front Neurol. 2011;2:1–15.
43. Park E, et al. A model of low-level primary blast brain trauma results in cytoskeletal proteolysis and chronic functional impairment in the absence of lung barotrauma. J Neurotrauma. 2011;28(3): 343–57.
44. Gao W, et al. Association between reduced expression of hippocampal glucocorticoid receptors and cognitive dysfunction in a rat model of traumatic brain injury due to lateral head acceleration. Neurosci Lett. 2013;533:50–4.
45. Ikonomovic MD, et al. Alzheimer's pathology in human temporal cortex surgically excised after severe brain injury. Exp Neurol. 2004;190(1):192–203.
46. DeKosky ST, et al. Association of increased cortical soluble A beta(42) levels with diffuse plaques after severe brain injury in humans. Arch Neurol. 2007;64 (4):541–4.
47. De Gasperi R, et al. Acute blast injury reduces brain abeta in two rodent species. Front Neurol. 2012;3 (177):1–17.
48. Garman RH, et al. Blast exposure in rats with body shielding is characterized primarily by diffuse axonal injury. J Neurotrauma. 2011;28(6):947–59.
49. Lighthall JW. Controlled cortical impact: a new experimental brain injury model. J Neurotrauma. 1988;5(1):1–15.
50. Hall KD, Lifshitz J. Diffuse traumatic brain injury initially attenuates and later expands activation of the rat somatosensory whisker circuit concomitant with neuroplastic responses. Brain Res. 2010;1323: 161–73.
51. Singleton RH, et al. Traumatically induced axotomy adjacent to the soma does not result in acute neuronal death. J Neurosci. 2002;22(3):791–802.
52. Singleton RH, Povlishock JT. Diffuse brain injury-mediated neuronal somatic plasmalemmal wounding: a study of the effects of membrane disruption on neuronal reaction and fate. J Neurotrauma. 2003;20 (10):1125.
53. Chavko M, Prusaczyk WK, McCarron RM. Protection against blast-induced mortality in rats by hemin. J Trauma. 2008;65(5):1140–5.
54. Koliatsos VE, et al. A mouse model of blast injury to brain: initial pathological, neuropathological, and behavioral characterization. J Neuropathol Exp Neurol. 2011;70(5):399–416.
55. Rafaels K, et al. Brain injury from primary blast. Brain Inj. 2012;26(4–5):745–6.
56. Cernak I, et al. Involvement of the central nervous system in the general response to pulmonary blast injury. J Trauma. 1996;40(3):S100–4.
57. Bhattacharjee Y. Neuroscience – shell shock revisited: solving the puzzle of blast trauma. Science. 2008;319(5862):406–8.
58. Ning JL, et al. Lung injury following lower extremity blast trauma in rats. J Trauma Acute Care Surg. 2012;73(6):1537–44.

59. Ning JL, et al. Transient regional hypothermia applied to a traumatic limb attenuates distant lung injury following blast limb trauma. Crit Care Med. 2014;42(1): E68–78.
60. Delius M, et al. Biological effects of shock-waves – lung hemorrhage by shock-waves in dogs – pressure-dependence. Ultrasound Med Biol. 1987;13(2): 61–7.
61. Seitz DH, et al. Pulmonary contusion induces alveolar type 2 epithelial cell apoptosis: role of alveolar macrophages and neutrophils. Shock. 2008;30 (5):537–44.
62. Sasser SM, et al. Blast lung injury. Prehosp Emerg Care. 2006;10(2):165–72.
63. Gorbunov NV, et al. Pro-inflammatory alterations and status of blood plasma iron in a model of blast-induced lung trauma. Int J Immunopathol Pharmacol. 2005;18(3):547–56.
64. Rafaels KA, et al. Pulmonary injury risk assessment for long-duration blasts: a meta-analysis. J Trauma. 2010;69(2):368–74.
65. Bass CR, Rafaels KA, Salzar RS. Pulmonary injury risk assessment for short-duration blasts. J Trauma. 2008;65(3):604–15.
66. Chai JK, et al. Role of neutrophil elastase in lung injury induced by burn-blast combined injury in rats. Burns. 2013;39(4):745–53.
67. Chai JK, et al. A novel model of burn-blast combined injury and its phasic changes of blood coagulation in rats. Shock. 2013;40(4):297–302.
68. Elsayed NM. Toxicology of blast over-pressure. Toxicology. 1997;121(1):1–15.
69. Alfieri KA, Forsberg JA, Potter BK. Blast injuries and heterotopic ossification. Bone Joint Res. 2012;1 (8):174–9.
70. Salisbury E, et al. Sensory nerve induced inflammation contributes to heterotopic ossification. J Cell Biochem. 2011;112(10):2748–58.
71. Apel PJ, et al. Effect of selective sensory denervation on fracture-healing an experimental study of rats. J Bone Joint Surg Am. 2009;91A(12): 2886–95.
72. Yano H, et al. Substance-P-induced augmentation of cutaneous vascular-permeability and granulocyte infiltration in mice is mast-cell dependent. J Clin Invest. 1989;84(4):1276–86.
73. Polfer EM, et al. The development of a rat model to investigate the formation of blast-related post-traumatic heterotopic ossification. Bone Joint J. 2015;97-B (4):572–6.
74. Qureshi AT, et al. Early characterization of blast-related heterotopic ossification in a rat model. Clin Orthop Relat Res. 2015;473(9):2831–9.
75. Patterson JH, Hamernik RP. Blast overpressure induced structural and functional changes in the auditory system. Toxicology. 1997;121(1):29–40.
76. Luo H, et al. Blast-induced tinnitus and spontaneous firing changes in the rat dorsal cochlear nucleus. J Neurosci Res. 2014;92(11):1466–77.
77. Mao JC, et al. Blast-induced tinnitus and hearing loss in rats: behavioral and imaging assays. J Neurotrauma. 2012;29(2):430–44.
78. Kurioka T, et al. Characteristics of laser-induced shock wave injury to the inner ear of rats. J Biomed Opt. 2014;19(12):125001.
79. Newman AJ, et al. Low-cost blast wave generator for studies of hearing loss and brain injury: blast wave effects in closed spaces. J Neurosci Methods. 2015;242:82–92.
80. Wu JA, et al. Study of protective effect on rat cochlear spiral ganglion after blast exposure by adenovirus-mediated human beta-nerve growth factor gene. Am J Otolaryngol. 2011;32(1):8–12.
81. Birch R, et al. Nerve injuries sustained during warfare part II: outcomes. J Bone Joint Surg Br. 2012;94B (4):529–35.
82. Christensen AM, et al. Primary and secondary skeletal blast trauma. J Forensic Sci. 2012;57(1):6–11.
83. Claes L, et al. The effect of both a thoracic trauma and a soft-tissue trauma on fracture healing in a rat model. Acta Orthop. 2011;82(2):223–7.
84. Birch R, et al. Nerve injuries sustained during warfare part I – epidemiology. J Bone Joint Surg Br. 2012;94B (4):523–8.
85. Suneson A, Seeman T. Pressure wave injuries to the nervous-system caused by high-energy missile extremity impact.1. Local and distant effects on the peripheral nervous-system – a light and electron-microscopic study on pigs. J Trauma. 1990;30(3):281–94.
86. Bellander BM, et al. Genetic regulation of microglia activation, complement expression, and neurodegeneration in a rat model of traumatic brain injury. Exp Brain Res. 2010;205(1):103–14.
87. Panzer MB, Bass CRD. Human results from animal models: scaling laws for blast neurotrauma. J Neurotrauma. 2012;29(10):A151.