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Prolonged but not short-duration blast waves elicit acute inflammation in a rodent model of primary blast limb trauma

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ABSTRACT

Background: Blast injuries from conventional and improvised explosive devices account for 75% of injuries from current conflicts; over 70% of injuries involve the limbs. Variable duration and magnitude of blast wave loading occurs in real-life explosions and is hypothesised to cause different injuries. While a number of *in vivo* models report the inflammatory response to blast injuries, the extent of this response has not been investigated with respect to the duration of the primary blast wave. The relevance is that explosions in open air are of short duration compared to those in confined spaces.
Methods: Hindlimbs of adult Sprauge-Dawley rats were subjected to focal isolated primary blast waves of varying overpressure (1.8–3.65 kPa) and duration (3.0–11.5 ms), utilising a shock tube and purpose-built experimental rig. Rats were monitored during and after the blast. At 6 and 24 h after exposure, blood, lungs, liver and muscle tissues were collected and prepared for histology and flow cytometry.
Results: At 6 h, increases in circulating neutrophils and CD43Lo/His48Hi monocytes were observed in rats subjected to longer-duration blast waves. This was accompanied by increases in circulating pro-inflammatory chemo/cytokines KC and IL-6. No changes were observed with shorter-duration blast waves irrespective of overpressure. In all cases, no histological damage was observed in muscle, lung or liver. By 24 h post-blast, all inflammatory parameters had normalised.
Conclusions: We report the development of a rodent model of primary blast limb trauma that is the first to highlight an important role played by blast wave duration and magnitude in initiating acute inflammatory response following limb injury in the absence of limb fracture or penetrating trauma. The combined biological and mechanical method developed can be used to further understand the complex effects of blast waves in a range of different tissues and organs *in vivo*.

Introduction

Blast injuries from conventional and improvised explosive devices (IEDs) account for 75% of modern war injuries, over 70% of these involve the limbs [1]. Blast injuries remain a threat to civilians too; detonation of IEDs in the recent Boston marathon bombings in 2013, together with industrial accidents such as the 2013 west Texas fertilizer plant explosion caused many injuries with a similar pattern to that seen amongst military casualties [2].

Blast trauma may occur by four discrete mechanisms: primary injuries are due to the interaction between the blast wave and the human body, secondary injuries are caused by the impact of fragments thrown and energised during the explosion, tertiary injuries result from acceleration of the body against an obstruction, and quaternary injuries include other physical insults, such as burns and smoke inhalation [3,4,5]. The type and severity of the injury sustained depends on the explosive system and the environment of the blast, the size of explosive device, the distance between the person and the explosion and the presence of

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obstacles or reflections. In an open space (free-field blast), a blast wave spreads radially from its origin and quickly dissipates as a function of the cube of the distance [3]. In these cases, the blast wave consists of a rapid rise to a positive overpressure followed by a negative under-pressure and return to ambient pressure. In an enclosed space, the explosive energy is contained leading to rises in both the peak overpressure and the duration of the positive-pressure phase of the blast wave [3]. Previous studies have shown that explosions within enclosed spaces are associated with a higher incidence of primary blast injuries and more severe injuries compared to open-air explosions [6].

In both the civilian and military settings, blast-injured patients are often poly-traumatised with the head, torso and soft tissues commonly affected [7,8]. The understanding within the trauma community of the relationship between injury, inflammation, sepsis and clinical outcome is growing [9,10,11]. It is important to note that blast injury survivors may suffer shock or hypoxemia in the absence of external signs of injury [12]. The onset of inflammation following injury is a common phenomenon; however, in severely compromised patients (particularly those with sepsis), systemic inflammation may contribute towards deleterious and life-threatening changes, such as multi-organ failure, which are difficult to manage clinically [11].

In vivo animal models are often used with simulated blastconditions in a controlled environment to investigate the mechanisms of blast injury. These studies enable greater understanding of the nature of the injury, including mechanical failure thresholds of tissues, physiological or inflammatory responses and the effect of therapeutic interventions. However, given the clinical burden of lower limb blast injuries [13], few experimental live models have been reported relating to blast limb trauma; those undertaken show that explosive limb injury may lead to systemic inflammatory changes affecting the limbs as well as distal organs [14,15,16,17]. However, the injury documented in these models is severe and encompasses several blast injury mechanisms. It is recognised that further research is needed to closely examine the interplay of different blast mechanisms in limb injury [18].

During IED explosion, blasts with peak pressures from 50 to 1000 kPa and 2–6 ms duration have been measured [19]. However, the majority of the experimental animal models involve blast waves with longer durations between 4 and 8 ms [20,21,22] and some with durations longer than 10 ms [23]. A large number of existing models also lack detail when reporting the pressure histories of the blasts produced, with many reporting only the peak overpressure or the distance from the outlet of the shock tube, often without details or schematics of animal positioning and orientation, thus limiting comparability between studies [24,25].

In this study, we develop a model to investigate the inflammatory response to primary blast wave application to the limb, investigating the effect of changing the magnitude or duration of the blast wave, thus permitting the controlled delivery of primary blast to replicate durations associated with a range of open-field and enclosed environments.

Materials and methods

Characterisation of the injury device

A shock tube (Fig. 1A) was employed in this study to generate pressure pulses of controlled intensity and duration. The shock tube used is a stainless steel tube that is 3.8 m long and 59 mm

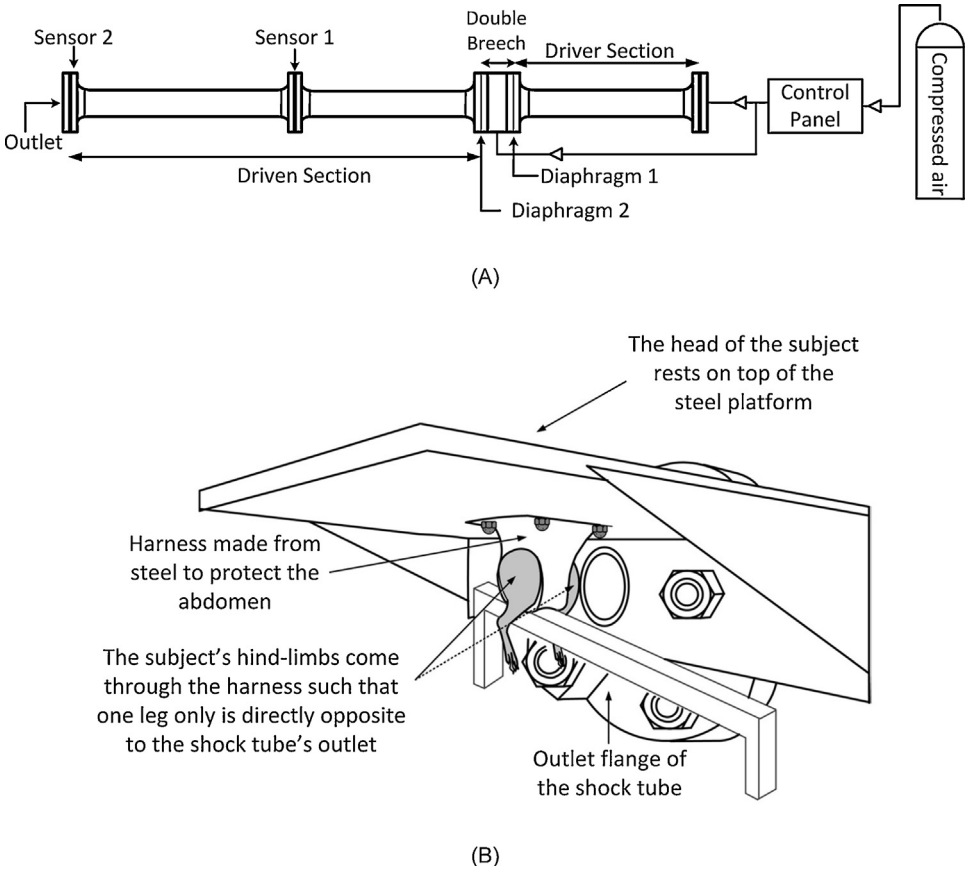

Fig. 1. (A) Shock tube schematic. (B) Experimental rig mounted at the outlet of the shock tube to isolate the blast to the animal's left hindlimb. The distance from the shock tube's outlet to the left thigh is 5 cm. The left leg is exposed to the shock wave from the pelvis to the ankle joint.