Environmental toxicology of blast exposures: injury metrics, modelling, methods and standards

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Introduction

Blast injuries are significant problem for the military and an increasing problem for.[1] This has contributed to an increase in research in blast effects (a PubMed search using the term 'blast injury' found over 40 primary research papers for 2016 using animal models versus less than 5 papers for 2006). Blast injury research is required to enable improvements in body armour and post-injury therapies for example; this will save lives as well as reduce the impact of such injuries on quality of life.

Blast injuries are those caused by an explosive event and results in a wide range of injuries therefore it is important that those involved in research in this area as well as those involved in the treatment of patients are united with respect to the classification of blast injuries to avoid misunderstanding and inappropriate interpretation of results. This NATO panel has used the classification of blast injuries according to DoD Directive 6025.21E.

- Primary blast injury: the result of pressure waves acting on air-tissue interfaces, which cause shearing and spalling on tissues.
- Secondary blast injury: caused by projectiles from the explosive device itself, intentionally included items, or exploded items in the surrounding area.
- Tertiary blast injury: from the high-velocity blast wind propelling victims into objects or objects into victims, as well as injuries from structural collapse.
- Quaternary blast injury: other injuries from explosive effects, or exacerbations of existing conditions or illnesses.
- Quinary blast injury: the morbidity and injuries resulting from non-projectile additives to explosives, as well as any environmental contamination.

Discussions at the NATO Health Factors and Medicine (HFM) Symposium 207 highlighted the importance of a systematic approach to understanding blast injuries. One of the recommendations from this symposium was the need to explore the concept of "the Toxicology of Blast Injury" with a focus on several difficult problems which included the relevancy and commonality of animal models. The NATO HFM Research Task Group 234 was established following the Symposium 207 and Guidelines for Using Animal Models in Blast Injury Research have been produced.

The Need For The Guidelines

Blast injury is a very complex phenomenon and frequently results in multiple injuries.[2] Whilst data gathering from those casualties injured by an explosive event is extremely valuable this does not provide all the answers and due to the sporadic nature of events evaluation of potential therapies in the target population for example is not a viable option. One method to investigate the consequences of blast injuries is with the use of living systems (animal models).[3-5] The use of animals allows the examination and evaluation of injury mechanisms in a more controlled manner, allowing variables such as primary or secondary blast injury for example, to be isolated and manipulated as required. Animal experiments can control for age, gender, and other genetic parameters not possible when examining data from human subjects exposed to blast.

The use of animals in blast injury research presents many challenges. Due to the complexity of blast injury it is unlikely that one model will be able to replicate all the relevant injuries and post-injury consequences therefore it is highly likely that several models will be required. To ensure a degree of standardisation across the blast research community a set of guidelines which helps researchers navigate challenges of modelling blast injuries in animals is required. Existing guidelines for animal studies, for example, traumatic brain injury are not designed for blast exposure.[6]

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Challenges that impact on blast injury modelling in living systems

The injuries sustained from a blast is influenced by a number of factors including the following:

- the physical loads from the explosive event, therefore the impact of any scaling issues need to be addressed;
- biological effects from the initial response in tissues as well as secondary effects (the final injury depends on the initial trauma, secondary responses and then any treatment effects);
- and finally species effects that can result in a failure to replicate the features of interest for human casualties (e.g. PTSD).

In addition researchers using animals for blast injury research have an ethical obligation to ensure that the research has scientific and clinical validity and thus ensure that no animals are used unnecessarily.

The complexity of blast injuries and the challenges of modelling such injuries in living system highlights the importance of the experience of the research team and any research group undertaking blast research using living animals must have the appropriate capability, knowledge, skills and expertise to address the intended research questions.

The Guidelines

The aim of the guidance is to ensure that experiments are validated and replicate the human condition or aspects of the human condition to enable the translation of the results.

The Guidelines are intended to provide a framework for scientifically valid methodological approaches to address the pathological consequences of blast exposures, and assist researchers during all stages of blast trauma animal experiments. The intension is that this will reduce inter-laboratory variability and allow valid comparisons of results to be made.

The Guidelines are aimed at research scientists when planning, executing and reporting animal experiments for blast trauma; funding bodies when evaluating a proposed plan of work; and journal editors and reviewers to determine validity and relevance of research presented.

At the heart of these guidelines is good experimental design as this is fundamental to the translation of results from animal studies to clinical practice. The general principals of good experimental design in this context are listed below:

1. Study Aim – what problem does the study address?
2. Study Hypothesis
3. Study methodology – how the experiment addresses the hypothesis.
4. Relationship to real world operational conditions – appropriate levels of ‘blast’ exposure
5. Choice of model
    I. Which blast effect is being modelled e.g. primary, secondary etc. This will determine the choice of exposure environment e.g. primary blast requires one of the following: open field exposure, shock tube or blast tube.
    II. Exposure conditions: exposure level and target positioning.
    Special consideration is needed in terms of positioning of specimen in the shock/blast tubes as well as orientation in relation to incident shock wave.
    Positioning the animal outside the shock tube results in exposure to a subsonic jet wind, this results in effects that are significantly different from those generated by a shock wave.
    It has been shown that both the pattern and severity of organ damage caused by blast depends on the orientation of the body toward the shock wave front. In addition, cognitive and behavioral responses in animals to blast are also dependent on the orientation of the animal.

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The choice of the animal holder is another important component in shock/blast tube experiments.

III. Species selection
Consideration needs to be given to the physiological responses to blast injury for the chosen species; the similarity of anatomical properties to humans must be considered; study feasibility must be considered; and model limitations must be acknowledged and discussed to ensure that results are neither misinterpreted nor over-interpreted.

All these factors need to be considered to ensure reported results are appropriate and not misleading.

6. Data collection - it is not possible to provide a comprehensive list of parameters to be measured, and the exact data collected will depend on the actual research question. The choice of data collected as well as omitted data will need to be described and justified by the research team.
   I. The method of data collection must be described, e.g. frequency of sampling must be appropriate to the parameter being assessed
   II. Sample timing must be justified, e.g. the time course of disease process is likely to be species dependent and therefore needs to be appropriate.
   III. Post-experimental analysis must be described and the statistical plan must be appropriate.
7. Limiting variability – not all variability can be eliminated but steps must be taken to reduce and limit its impact. This can be achieved by measuring as many critical parameters as possible and controlling the animal species across laboratories.

In addition to experimental design validation is a critically important aspect of animal models and regardless of the research questions to be addressed the criteria every clinically and militarily relevant blast injury model should fulfill are the following:

- The injurious component of the blast should be clearly identified and reproduced in a controlled, reproducible, and quantifiable manner (see the Guidelines for Reproducing Blast Exposures in the Laboratory);
- The inflicted injury should be reproducible, quantifiable, and mimic components of human blast injury;
- The injury outcome established based on morphological, physiological, biochemical, and/or behavioral parameters should be related to the chosen injurious component of the blast;
- The mechanical properties (intensity, complexity of blast signature, and/or its duration) of the injurious factor should predict the outcome severity.

One aspect of validation may be dose-response relationships demonstrating that with the increasing intensity of blast exposure the biological responses will be more pronounced and the pathological consequences more severe. Dose-response studies are necessary to determine injury threshold and saturation values. Bowen curves, for example, provide excellent framework for experiments analyzing the relationship between primary blast exposure(s) and tissue / organ damage.[7] The Bowen curves have their limitations such as limited usefulness for chronic biological or psychological outcomes, as well as non-primary blast effects. Thus, new dose-response curves are needed, based on appropriate scaling laws that would establish the relationship between individual blast components and biological / psychological outcome measures, while taking into account the size, composition, and geometry of the exposed body and/or organ.

Studies undertaken with good experimental design using validated animal models will improve the state-of-the-science for blast injury.

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Conclusion

In an ideal world there would be no requirement for the use of living animals in blast injury research however there are currently no non-living models that can integrate all the biological (cardiovascular effects, immunological effects etc.) responses seen post-injury. Therefore animal experiments are necessary and they can generate valuable data regarding blast injury not only the disease process but also for the investigation of potential therapies. Animal models allow for a more controlled examination of blast injuries. One particular advantage of animal models is that results can be achieved from a relative small number of animals.

No animal model can replicate all the conditions of a blast event and the human's response to blast exposure and decisions made by the researcher regarding the nature of each experiment has the potential to reduce the relevance of a study.

Limited reproducibility can be a concern in animal studies. This leads to translational problems, both between animal data and real life blast events but also between different animal studies. Good monitoring of experiments and adherence to guidelines are important ways to decrease such problems. However, multicenter studies could also be an effective way to increase the usefulness of animal studies.

This guidance document provides a framework for the research community with the aim of improving experimental quality. It is anticipated that adherence to this guidance document will help reduce the following; the uncertainly regarding the nature of the blast injury being modeled; the variability and quality in study outcomes; and finally enhabce the impact and translation of results such that patient outcomes will be improved.

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