

Burns as a model of SIRS

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1. ABSTRACT

Thermal injury following burns is a common clinical condition. Excessive systemic inflammatory response syndrome (SIRS) following burns leads to distant organ damage and multiple organ dysfunction syndrome (MODS). Development of *in vivo* experimental models of burns over the past 50 years have facilitated the study of the effects of thermal injury on physiological and immunological parameters in the pathogenesis of burns and associated systemic organ damage. Using these models, researchers have established the critical role played by inflammatory mediators such as TNF- α , IL-1 β , IL-6, IL-2 and substance P in burns and associated systemic organ damage. The rationale of this chapter is to present an overview of different experimental animal models, both rodents as well as large animals, of burns and associated SIRS and the role of inflammatory mediators in the pathogenesis of this condition as well as in pathogenesis of the resultant MODS.

2. INTRODUCTION

Despite improved prognosis, increased morbidity and mortality still remain major concerns in burns. Not only do they cause accidental death, they also result in considerable morbidity and disfigurement leading to significant functional and social impairment. The result of multiple clinical and animal studies have established that the initial immune response to serious injury is inflammatory, often referred to clinically as the systemic inflammatory response syndrome (SIRS) (1-3). The activation of a pro-inflammatory cascade after burn injury appears to be important in the development of subsequent immune dysfunction, susceptibility to sepsis, development of SIRS and multiple organ dysfunction syndrome (MODS), which primarily contribute to morbidity and mortality in severe burn injuries.

Over the years, researchers have developed a wide variety of animal models (in rodents as well as in

large animals) and using these models; have identified several inflammatory mediators that contribute to burn associated SIRS. These models have enabled us to study the effect of burn injury on physiological and immunological parameters. Burn injury induces immune dysfunction and impacts numerous physiological parameters. Burn injury of sufficient magnitude causes extensive tissue damage resulting in many physiological alterations including hepatic synthesis of acute phase protein, dysfunctional temperature regulation, fever in the absence of infection, hemostatic changes, muscle wasting associated with negative nitrogen balance and hyperglycemia (4). This hypermetabolic response, often called the systemic inflammatory response syndrome (SIRS), concerns excessive whole body inflammation (5) and is considered a major determinant in the development of multiple organ dysfunction, often with a lethal result (6). Burn injury of the skin results in a local release of inflammatory mediators that might initiate the systemic inflammatory cascade (7).

The complete pathophysiology of burn injury is not completely understood. To gain a better understanding of the mechanisms of altered tissue responses in thermal injury and associated SIRS, there is a need of an animal model that adequately reflects the various aspects of burn pathology besides being reliable and reproducible. A standardized animal model of burns is important to study the indicators of mortality and morbidity. Previous burn injury studies have suggested different methods of creating a burn model using various animal species.

Majority of what is known about burn injury and associated SIRS has been learned from studies with animal models, primarily mice, rats and pigs. Animal models play a pivotal role in the discovery of mediators of systemic organ damage and potential therapeutic targets for this condition thereby furthering biomedical advances. Success of any research endeavor depends to a great extent on the proper selection of the animal model for these studies.

3. ANIMAL MODELS OF BURNS AND ASSOCIATED SIRS

Choice of species can have a major impact on outcomes. Selection of an animal model depends on a number of factors including availability, cost, ease of handling, investigator's familiarity and anatomical/functional similarity to humans. Many models of burn injury are described in literature but each model is ultimately judged by its ability to predict how a treatment will behave in a human burn injury (under clinical conditions). A good burn model is one that is simple, safe, reproducible and reliable. It should create burns that are consistent in their extent and depth. For an experimental burn model to be homologated and considered comparable, it should be reproducible by other researchers, consistent in results, simple in performance and if possible, low in cost. Any description of an animal model (8) should include the instruments used to create the burns, the temperature and duration of exposure and the method of applying thermal injury. For researchers to compare various new therapies for burns, it is important to ensure that the agents are being used on similar types and extents of injury. In experimental

animals, burn injury models make it possible to control the extent of tissue damage by adjusting the area and depth of burn injury (9). Using these basic criteria, several models of burns injury and associated SIRS both in rodents and large animals have been described in the literature. An overview of these methods is outlined below.

3.1. Rodent models (mice, rats)

Small mammals like mouse, rat, guinea pig and rabbit are frequently used in burn studies, as they are inexpensive and easy to handle. Despite these advantages, these small mammals differ from humans in a number of anatomical and physiological properties. For example, these mammals have a dense layer of body hair, thin epidermis and dermis and the main difference is that they heal primarily through wound contraction as opposed to reepithelialisation.

Mouse is an excellent model for studying the immune dysfunction post-burn as its immune system is well characterised. Rodent burn injury models have varied significantly with regard to the source as well as size of the injury. Clinical studies have shown a strong correlation between burn size and mortality, with increasing burn size being associated with higher mortality rates, irrespective of age. Studies using a murine system have continually demonstrated suppression of cell-mediated immune responses post-burn and increased susceptibility to subsequent septic complications and mortality (10-13). Murine models have also helped demonstrate that expression of a hyperactive macrophage phenotype, which is associated with the increased productive capacity for inflammatory mediators, has been implicated in burns and subsequent sepsis (14-16).

The most frequently used animal for the study of burns is Wistar rat due to its availability, low cost, resistance to infections and the feasibility of reproducing different types of burns. The Sprague Dawley (SD) rat, a common choice for other studies, is more susceptible to respiratory tract infections than the Wistar rat. Rabbits have a higher risk of infection than rats and therefore require much more care in their manipulation including sterile conditions. The price of rabbits is approximately ten times higher than rats, which increase the burden on limited financial resources.

Scalding is the easiest mechanism of provoking an experimental dermal burn. The possibility of varying water temperature, time of exposure and the burned area makes this method ideal for reproducing almost every kind of thermal aggression. Electrical burns usually require higher animals like monkeys to achieve lesions comparable to those observed in humans. Constant temperature water scald burn models have been created in several strains of mice (17).

3.2. Large animal models {porcine (pig, miniature pig)}

Pigs are now being used with increased frequency as experimental animals and play a vitally important role in burn studies. Biomedical research requires a suitable animal model that allows for human-related

validation of valuable research information gathered from experimentation with lower mammals. The pig has been favored over other animals for studying cutaneous burns due to several reasons. First, of all mammals, the skin of the pig most closely resembles that of humans. The cornified layer and epidermis of the pig is relatively thick, similar to that of the human (18, 19). Pig epidermis ranges from 30 to 140 μm and the human's from 50 to 120 μm with a dermal-epidermal thickness ratio in pigs ranging from 10:1 to 13:1 which is similar to comparable measurements of human skin (20). Its sparsely haired coat is also similar to that found in most humans, although the hair shafts are coarser than typical terminal hair in humans. Other similarities between porcine and human skin include epidermal enzyme patterns, epidermal tissue turnover time, the keratinous proteins, and the composition of the lipid film of the skin surface.

Secondly, pigs are of a large enough size to allow creation of multiple burn sites on the same pig, thus increasing the sample size without significantly increasing the cost. The large size also allows creation of multiple burns in each individual pig without resulting in a systemic stress response. Thirdly, young pigs are highly resistant to contamination and infection. This quality offers an advantage specifically for burn studies, as the administration of antibiotics might affect the results of the treatments given. Also, porcine dermal collagen is biochemically similar to human dermal collagen. Both man and pig heal through physiologically similar processes (21) and close partial thickness burns through reepithelialisation.

Despite many similarities between humans and pigs, certain facts relating to pigs cannot be overlooked while choosing the animal model for your research. Pigs are comparatively expensive and require additional manpower necessary during procedures to administer general anesthesia. Generally they do not form blisters between the epidermis and dermis as in humans making it difficult to recognize second-degree burns. The pigs tend to gain weight very fast. The study of burns is consummated normally over a period of two weeks (reepithelialisation period of partial-thickness burns) and by that time the management of the grown pigs becomes quite difficult.

As a biomedical research model, the domestic pig has physiological similarities to man, but the size and weight of this animal often makes it difficult to handle and house for laboratory purposes. To overcome this drawback, miniature pigs have been developed. The miniature pig with its great anatomical and physiological similarities to humans, offers several breeding and handling advantages, compared to dogs and non-human primates, making it a preferred choice for pre-clinical experimentation. The adult mini-pig weighs approximately 70 kilograms, about the size of an average person. Its body size, skeletal size, skin, teeth, gastrointestinal tract, heart position and blood supply are strikingly similar to humans and can be handled under laboratory conditions, in appropriate cages and climate controlled facilities. However, because of their high cost, special anesthetic and post-operative care requirements, mini-pigs are not considered ideal research animals. Studies

performed on mini-pigs are accepted by most of the regulatory authorities. They are mentioned in the guidelines of the OECD, Japanese Ministry of Health and Welfare, Canadian Health and Welfare and United States FDA.

3.3. Standardized experimental burn injury models

3.3.1. Scalding in mice (22)

Under general anesthesia the mouse, the dorsum is shaved with electric clippers to ensure even burn wounding. The mouse is placed on its back in a template constructed of plastic and a metal screen. The mouse and template are immersed together into a 100°C water bath for 8 sec to inflict full thickness burn in the area of the metal screen.

3.3.2. Scalding in rats (23)

After anesthetization of the rat, an electric shaver is used to expose a cutaneous surface on the back constituting 30% of total body surface area (TBSA). The rat is then placed on its back in a moldable metal wire cage which is a modification of that described by Walker (24). The shaved dorsal area is submerged for 12 sec in water at 70°C to inflict a deep dermal burn in the entire cutaneous area exposed.

3.3.3. Scalding in pig

Heated or boiling water is circulated over the area to be injured. The advantage of this method is its ability to cover uniformly an entire area of skin. The main disadvantage is that it is technically more challenging and poses a risk of burning to the researcher. Constant temperature water scald burn models have been created in pigs (25).

3.3.4. Thermal burn by direct contact in rats (26)

After shaving the back of the animal, a copper disk (diameter 4 cm) heated to 250°C is applied to the skin as many times as necessary to burn the desired surface area. The drawback of this method being that it is almost impossible to obtain a burn of uniform depth.

3.3.5. Thermal burn based on skin contact with a glass chamber in rats (27)

In this method water circulates at a predetermined temperature through the glass chamber. This allows application at a constant pressure of 10 g/CM². The advantage of this method is the possibility of varying temperature and exposure time as required.

3.3.6. Thermal burn by direct contact in pig (28)

After anesthetization of the pig, the dorsum is shaved with electric clippers. The burns are created with a 2.5 cm x 2.5 cm by 7 cm, 150-g aluminium or brass bar equilibrated in 80°C water for 5 minutes and then applied to the skin for 20 seconds to create partial thickness burns.

4. MEDIATORS OF BURNS-ASSOCIATED SIRS IDENTIFIED USING ANIMAL MODELS

Although it is known that cytokines tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and interleukin 6 (IL-6) are involved in the hypermetabolic host

response to thermal injury, the exact cytokine response is poorly understood. Various kinds of trauma are influenced by various kinds of stress (29) resulting in a wide-ranging post-injury cytokine response at various times. Due to this reason, despite multiple investigations, no consistent pattern of cytokine response is available. Also, there is sparse information concerning the early development of acute response phase under thermal injury stress (30)

Various inflammation models have shown that inflammation-related cytokines work in sequence (31,32) and IL-1 is the first cytokine to appear in systemic circulation in the inflammatory cascade followed by TNF- α and IL-6. Potential sources of cytokines in thermal injury include neutrophils (33), phagocytic cells (34), lymph nodes (35,36), hepatocytes (36) and uninjured skin (37,38).

Dynamic changes in the circulating levels of TNF- α , IL-1 β and IL-6 implicate the role of these cytokines in the early response to thermal injury. Burn injury has been shown to cause augmented TLR2- and TLR4-induced TNF- α , IL-1 β and IL-6 production by splenocytes and in particular, macrophages in C57BL/10SnJ mouse (39).

In one study, sham- or burn-injured mice were treated with various doses of staphylococcal enterotoxin A (SEA), and then observed for their cytokine response. The assessment of serum cytokine levels demonstrated significantly elevated IL-2 and TNF- α levels when compared to sham mice. *In vitro* studies confirmed *in vivo* results and also demonstrated elevated levels of interferon gamma (IFN γ). These authors also observed a novel injury-dependent switch from CD4+ to CD8+ T-cells as the dominant T-cell type producing TNF- α and IFN γ in response to SEA stimulation *in vitro*. Taken together, these findings indicated that injury primes the immune system for an augmented early T-cell response that can result in a lethal shock-like syndrome (40).

In another study in the mouse, topical p38 MAPK inhibition was shown to significantly reduce burn wound inflammatory signaling and subsequent systemic expression of proinflammatory cytokines and chemokines. *In vitro* macrophage functional assays demonstrated a significant attenuation in serum inflammatory mediators from animals receiving the topical inhibitor. Topical p38 MAPK inhibition resulted in significantly less pulmonary inflammatory response via reduction of pulmonary neutrophil sequestration, pulmonary cytokine expression, and a significant reduction in pulmonary microvascular injury and edema formation. Although dermal activating transcription factor-2, a downstream p38 MAPK target, was significantly reduced, there was no reduction in pulmonary activating transcription factor-2 expression, arguing against significant systemic absorption of the topical inhibitor. These experiments demonstrated a strong interaction between dermal inflammation and systemic inflammatory response. Attenuating local inflammatory signaling appears effective in reducing SIRS and subsequent systemic complications after burn injury (41).

In a recent study, the tachykinin peptide substance P (SP) has been identified as a key mediator of burn injury associated SIRS. Results in this study show that burn injury in male BALB/c mice subjected to 30% total body surface area full thickness burn augments significant production of SP, preprotachykinin-A gene expression, which encodes for SP, and biological activity of SP-neurokinin-1 receptor (NK1R) signaling. Furthermore, the enhanced SP-NK1R response correlates with exacerbated lung damage after burn as evidenced by increased microvascular permeability, edema, and neutrophil accumulation. The development of heightened inflammation and lung damage was observed along with increased proinflammatory IL-1 β , TNF- α , and IL-6 mRNA and protein production after injury in lung. Chemokines MIP-2 and MIP-1 α were markedly increased, suggesting the active role of SP-induced chemoattractants production in trafficking inflammatory cells. More importantly, administration of L703606, a specific NK1R antagonist, 1 h before burn injury significantly disrupted the SP-NK1R signaling and reversed pulmonary inflammation and injury. These findings show for the first time the role of SP in contributing to exaggerated pulmonary inflammatory damage after burn injury via activation of NK1R signaling (42).

5. SUMMARY AND PERSPECTIVE

Recent reports have identified various inflammatory mediators that contribute to SIRS following burn injury. Pharmacological modulation with anti-inflammatory drugs may serve as an effective strategy for prevention of tissue injury and organ dysfunction as a result of burn injury. Animal models of burn injury and associated SIRS are playing a key role in the identification of these mediators and potential therapeutic targets. It is appropriate to say that some of the medical advances that we take for granted today, would not have been possible without animal models.

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