Heat Shock Proteins and the Maintenance of Gut Barrier Following Burn Injury

Abigail R. Cannon¹,²,⁴, Michael J. Javorski¹,⁴ and Mashkoor A. Choudhry¹,²,³,⁴*

¹ Burn & Shock Trauma Research Institute, Loyola University Chicago Health Sciences Division, Maywood, IL 60153
² Integrative Cell Biology Program, Loyola University Chicago Health Sciences Division, Maywood, IL 60153
³ Department of Surgery, Loyola University Chicago Health Sciences Division, Maywood, IL 60153
⁴ Stritch School of Medicine, Loyola University Chicago Health Sciences Division, Maywood, IL 60153

Received May 2017, Accepted May 2017, Published Online June 2017

Abstract
Traumatic injury, specifically burn injury, remains a prominent medical problem due to the prevalence of secondary, burn-induced complications of sepsis and multiple organ dysfunction syndrome. These complications arise as a result of gut barrier breakdown following injury. As the gut harbors trillions of resident bacteria, any compromise in barrier integrity would allow for bacteria or bacterial products to gain access to extraintestinal sites potentiating systemic infections and inflammation associated with burn injury. Both experimental and human data provide evidence linking heat shock proteins with gut barrier maintenance under various pathological conditions. This article highlights the intestinal pathophysiologies associated with burn injury while proposing a potential cytoprotective role of heat shock proteins of the intestine in the innate immune response to injury.

Keywords: Heat shock proteins; Gut barrier; Burn injury; Immune barrier

1 Introduction
As estimated by the American Burn Association 450,000 individuals succumb to burn injuries every year, with approximately 40,000 of those individuals requiring subsequent hospitalization¹. Furthermore, sepsis and multiple organ dysfunction syndrome (MODS) continue to be the leading causes of death in burn patients who survive the initial insult of injury². Over 75% of all burn related deaths occur as a result of sepsis or infectious complications³. Immediately following injury patients experience a systemic inflammatory response, which presents as a surge of pro-inflammatory cytokine release⁴. This shift toward a pro-inflammatory environment from one of a healthy balance of pro- and anti-inflammatory cytokines manifests not only at the site of burn injury, but in extraneous sites of the lung, liver, and intestinal tract⁵. Coupled with the suppression of the immune system seen after serious burn injury, this inflammation increases patient susceptibility to sepsis and subsequent multiple organ failure⁴−⁶. Intestinal bacteria are likely to be the main source of infection following burn injury⁵,⁷−⁹. The presence of over 100 trillion microbes in the intestine under normal conditions creates a symbiotic relationship benefiting the host by aiding in metabolism nutrients, protection from invading pathogens, and immune system development and function¹⁰−¹³. In a healthy individual, the intestine maintains a physiological and immunological barrier sequestering both commensal and pathogenic bacteria to the luminal space¹¹,¹⁴,¹⁵. After burn trauma intestinal epithelial cells undergo intense cellular stress, which contributes to the barrier breakdown following injury¹⁶−¹⁸. This perturbation in gut barrier integrity could result in bacterial translocation out of the luminal space into extraintestinal sites ending in SIRS, sepsis, and MODS⁵−⁷,⁹,¹⁶,¹⁷,¹⁹. However, the exact mechanism of gut barrier breakdown and subsequent burn related pathophysologies remains largely unknown. The intestinal barrier consists of an immune barrier and a physical barrier made up of an epithelial cell lining, T and B cells, macrophages, dendritic cells of the Peyer’s patches (PP), mesenteric lymph nodes (MLN), and lamina propria (LP) of the gut associated lymphoid tissue (GALT) make up the in...
intestinal immune barrier. The key components of the physical intestinal barrier include tight junctional complexes, adherens junctions, and desmosomes between intestinal epithelial cells (IECs). In particular, tight junctional complexes of the small and large intestine are made up of the proteins: claudin, occludin, and zonula-occludin. These proteins are imperative to the maintenance of the physical intestinal barrier prohibiting translocation of bacteria out of the lumen while allowing the selective absorption of critical nutrients required by the host. Many studies have suggested a role of heat shock proteins (HSPs) in maintenance of tight junction integrity. However, such roles of HSPs in the context of maintaining tight junction integrity in intestinal epithelial cells after burn injury remains largely unexplored. Understanding the interplay between a burn related breakdown of the intestinal barrier and its reliance on the HSP response could open doors for novel therapies in the treatment of burn patients. This article reviews studies published in the area of gut barrier maintenance after burn injury.

1.1 Burn injury, hypovolemia, and gut barrier integrity
Burn trauma is clinically associated with significant edema formation, which can extend to sites beyond that of the injury. Damage to the microvasculature along with the large release of vasoactive inflammatory mediators leads to increased capillary permeability, allowing plasma proteins to leak out of the blood vessels and into the surrounding interstitium, thereby increasing the oncotic pressure of the interstitium. The increasing level of proteins in the interstitium draws plasma fluid into the interstitium, causing large edema formation and a loss of blood plasma volume. With a relatively large total body surface burn, the body loses a significant amount of plasma, and therefore becomes hypovolemic. Clinical shock is reached once 40% of the vascular fluid volume is lost, which is the point where the sympathetic nervous system can no longer provide adequate compensation. Shock seen after trauma, such as burn trauma, is a type of hypovolemic shock that is characterized by a significant vascular volume loss along with a systemic inflammatory response. If the hypovolemic status becomes too great, vasoconstriction of the mesenteric regions can become great enough to cause insufficient blood flow and nutrient delivery to those tissues, resulting in a hypoxic environment. Intestinal tissues undergo a classic example of ischemia/reperfusion injury after burn incurring damage caused by induction of the hypoxic state along with reperfusion of the tissue causing massive oxidative damage and stress. As the largest mucosal surface in the human body, the gastrointestinal tract comes into contact with the most dietary antigens and largest diversity of microbial organisms. Therefore, any damage to the intestinal barrier as a result of hypovolemia, could disrupt the balance between intestinal barrier and the diverse community of microorganisms residing in a healthy gut. The intestinal barrier can be understood in two main parts: the immune barrier and the physical barrier.

1.2 Immune Barrier
The immune barrier consists of cells such as dendritic cells (DCs), macrophages, T cells, and B cells. DCs will constantly sample the intestinal lumen and present antigen to cells of both the adaptive and innate immune system. This, in turn, prompts proper immune responses restricting commensal microbes to the lumen via secretion of cytokines by activated epithelial or T cells and/or release of opsonizing antibodies such as IgA from plasma cells. After a severe burn injury, trauma from the cutaneous burn injury and the damage from hypoxia-induced tissue damage triggers the release of many pro-inflammatory cytokines and other inflammatory mediators leading to SIRS. The body tries to compensate for this by upregulating a state of immunosuppression, termed CARS. It may seem paradoxical to have an excess of both pro-inflammatory and anti-inflammatory signals. However, in general it can be explained as an over-activated innate immune response with a suppressed adaptive immune response. Macrophages, the resident tissue phagocytes, release massive amounts of pro-inflammatory signals creating the overactive innate immune response in SIRS. In the adaptive immune system post-burn injury, there is a reduction of T cell proliferation along with a reduction of TH1 responses and an increase in TH2 responses. The reduction in T cell proliferation is indicative of a suppressed adaptive response, however the increase in TH2 responses compared to TH1 responses may not be an aberrant response, considering that TH2 responses are best suited for handling an extracellular bacterial pathogen. Impaired functioning of dendritic cells after burn injury also leads to a suppressed immune response, since the dendritic cells have a major role in communicating between the innate and adaptive immune systems. Suppression of the adaptive immune defense reduces the capacity for the body to mount a specific response against invading pathogens. This along with a breakdown in gut barrier integrity could allow for the invasion of resident bacteria into the epithelium or to extraintestinal sites resulting in systemic infections and inflammation in the injured host.

1.3 Physical Barrier
The physical barrier of the gastrointestinal tract mainly consists of intestinal columnar epithelial cells,
which adjacently associate, creating an epithelial cell lining of the intestinal lumen. Covering the epithelial cell lining is the layer of mucus secreted by specialized epithelial cells called goblet cells. The mucus layer prevents most bacteria residing in the lumen of the intestine from direct contact with the epithelial cells, and therefore, restricting improper immune responses to resident intestinal bacteria. Critical to the maintenance of intestinal homeostasis and the integrity of the physical intestinal barrier are a class of proteins known as tight junction (TJ) proteins. Even in an intact intestinal epithelial cell layer, there still exists a paracellular pathway allowing for transepithelial transport between adjacent cells, however this does not allow bacterial transport outside of the intestinal lumen. The claudins and occludins are two types of transmembrane TJ proteins, which are associated with zonula-occludin-1,2 (ZO1, ZO2) proteins. ZO1 and ZO2 anchor the claudin and occludin into the intestinal epithelial cell’s cytoskeletal component F-actin. Proper formation and integrity of tight junction complexes is required for a healthy gut. Earsley et al. showed that in mice 1 day after burn injury, there is a 40% decrease in the gene expression of claudin 4 and 8 in the small intestine. This decrease in claudins may disrupt the junction complex which can then lead to increased intestinal permeability as observed after burn injury. Tight junctional complexes are not limited to the make-up of the intestinal barrier, but exist in many other natural barriers of the body such as the lungs, kidney, and the blood brain barrier, etc. Many studies on tight junction proteins at other barrier sites have implicated the role of heat shock proteins (HSPs) as support for tight junction protein integrity. HSPs are classified as small cytoprotective proteins, which are induced after stresses such as heat, cytotoxic drugs, and bacterial endotoxins. Cell stress is alleviated by HSPs, which function to chaperone denatured proteins back to the endoplasmic reticulum allowing for correct refolding. Burn injury can result in intense cellular stresses, which can consequently lead to an accumulation of denatured proteins. However, there exists a gap in the knowledge of the potential role HSPs could play in upholding the integrity of tight junction proteins in intestinal epithelial cells and, therefore, proper maintenance of the physical barrier of the intestine following burn injury. Understanding the interplay between a burn related breakdown in the intestinal barrier and its reliance on the HSP response could open doors for novel therapies in the treatment of burn patients.

1.4 Heat Shock Proteins (HSPs)

HSPs are highly conserved stress proteins, which are expressed ubiquitously across all organisms from humans to bacteria to yeast. First discovered in 1962, HSPs are characterized by and subsequently named by their molecular weights, which range from approximately 15 to 110 kDa. The distribution of HSPs in different cellular compartments is widespread as it includes the cytoplasm, endoplasmic reticulum, and nucleus. The precise mechanistic function of HSPs has yet to be determined, but it is well known they are essential for survival at normal or elevated temperatures and in response to ischemia, cytokines, and energy depletion. Although exact mechanisms are still not understood, researchers have determined that HSPs have strong cytoprotective effects, are critical to many regulatory pathways, and act as molecular chaperones for other proteins. Mammalian HSPs have historically been termed as a chaperone, however, it can also be involved with anti-inflammatory processes, proliferation, and prevention of apoptosis. HSP70 has at least two regulatory sequences that interact with the major heat shock protein transcription factor, HSF1. The two sequences of HSP70, HSPA1A and HSPA1B, will code for almost identical amino acid sequences generating nearly indistinguishable proteins, which results in some redundancy. In particular, HSP72 was shown to be the most temperature sensitive and highly conserved out of all the HSPs. In contrast to others in the HSP family, HSP72 is highly inducible in response to a variety of stressors, such as hypoxia, ischemia with over-expression of HSP70 protecting from ischemic heart injury by enhancing post-ischemic contractile function reactive oxygen species, and pro-inflammatory cytokines like TNF-α. Furthermore, heat induction of HSP72 in rats has been correlated with the protection of neurons from oxidative injury. HSP25 belongs to a group called the small heat shock proteins. In addition to its role as a chaperone, HSP25 also plays a role in F-actin cytoskeleton remodeling, cell migration, and anti-apoptotic processes. Unlike the ATP-dependent HSP72, HSP25 acts completely independently of ATP, as do many of the other small molecular weight HSPs. However, many of the actions of HSP depend upon its phosphorylation and oligomerization state. Mammalian HSP25 proteins have the ability to dimerize under conditions of stress. It is theorized that a unique cysteine residue on HSP25 gives it its ability to act as an anti-apoptotic protein under threats of apoptosis due to injury. Lastly, HSP90 comprises approximately 1-2% of all
cellular proteins in a cell, as it is so ubiquitously expressed. One of the main roles of HSP90 is to properly fold and activate client proteins. A HSP90 client protein is a protein that uses HSP90 in order to mature or activate. Due to the vast array of HSP90 client proteins, HSP90 influences many cellular processes such as intracellular signaling, cell survival, and proliferation. Like HSP25, HSP90 requires dimerization to properly function, but it differs in its dependency on ATP, similar to that of HSP72. Unique to HSP90 is its ability to bind more than one naïve or stress-induced mis-folded protein in order to aid its proper folding/re-folding.

1.5 HSPs and the Intestine
In the context of the gastrointestinal tract, there exists a fine-tuned relationship between the resident microflora, intestinal epithelial cells, and HSP induction. It is of interest to note that HSP25 and 72 are present only at low basal amounts in the healthy distal small and large intestine. Kojima et. al. found that this distribution of HSP25 and 72 was due to the differential amounts of bacteria as one descends down the GI tract, with greater number and diversity of bacteria from small to large intestine. A healthy human gut requires the presence of the resident microflora, but under normal physiologic conditions microbial overgrowth is restricted, in part, by innate immune responses from intestinal epithelial cells. Their study, and others, have provided evidence for the fact that potential pathogenic bacteria or bacterial products could upregulate the HSP25 and 72 response. For example, the bacterial products butyrate, a short chain fatty acid, and LPS, the outer membrane of Gram-negative bacteria, may both be stimuli inducing expression of HSPs in intestinal epithelial cells. As both HSPs, HSP25 and HSP72, are known to be cytoprotective, their induction would allow for intestinal epithelial cell protection of critical cellular functions and viability. Compromised expression of either HSP25 or 72 in the small or large intestine, either by a change in bacterial diversity of the gut or by some other mechanism, could potentially increase susceptibility to invading pathogens and subsequent systemic complications.

1.6 HSPs and Disease
As HSPs are renowned for their cytoprotective roles, it follows that they have been implicated in the protection from various diseases, including Amyotrophic lateral sclerosis (ALS), cardiovascular disease, and Inflammatory Bowel Disease (IBD). ALS is a progressive paralysis disease characterized by the death of motoneurons in spinal cord and motor cortex. Researchers found that treatment with a broad-spectrum inducer of HSPs could drastically slow the progression of ALS. In the context of cardiovascular disease, induction of HSPs by thermal stress can significantly improve the outcome of ischemic heart disease. Ischemic heart disease results in an accumulation of circulating leukocytes, leading to increased T-cell and macrophage presence in the arterial way releasing pro-inflammatory cytokines of TNF, IL-6, and IFN-80. As a consequence of increased inflammation, endothelial cells undergo severe cellular stresses, which lead to tissue damage and necrosis. Currie et. al. found that hearts with HSP over-expression had improved contractile functioning in response to ischemic conditions. Additionally, the reperfusion damage was significantly lower in hearts with basal levels of HSPs. IBD is described as a chronic inflammatory state of the gastrointestinal tract. This pro-inflammatory state in IBD can be characterized not only by elevated production of the pro-inflammatory cytokines TNF-alpha, IL-6, and IL-1beta, but also by elevated levels of cell adhesion molecules (CAMs), which are crucial to the infiltration of leukocytes into the bowel. Leukocyte infiltration and chronic intestinal inflammation results in severe intestinal epithelial cell damage, which can proceed to colonic bleeding and intense discomfort in patients. However, Tanaka et. al. have shown that the presence of HSPs can significantly reduce IBD symptoms of intestinal epithelial cell damage and leukocyte infiltration compared to that of an HSF1 null mouse, i.e. mice producing no or very low levels of HSPs. Furthermore, transgenic mice expressing the human HSP70 were found to have lower clinical scores of IBD symptoms, less intestinal epithelial damage, and reduced levels of the pro-inflammatory cytokines TNF-alpha, IL-6, and IL-1beta. From this they concluded that HSP70 was essential in protection from symptoms of IBD.

1.7 HSPs and Burn Injury
Although HSPs have been extensively studied in other diseases, their role in gut barrier maintenance after burn injury has yet to be fully elucidated. Findings from our laboratory have shown dramatic increases in neutrophil migration to the intestine, a site of increased inflammation after alcohol and burn injury. This accumulation of neutrophils in the intestine promotes release of reactive oxygen species resulting in severe intestinal epithelial tissue damage and subsequent intestinal permeability in injured rats. These results correlated with significant decreases in HO-1, an HSP commonly known as HSP32. HSP32 functions as a potent antioxidant alleviating oxidative stress through the production of bilirubin, CO, and iron. Therefore, upon up-regulation of HSP32 in the model...
of burn and alcohol, neutrophil production of reactive oxygen species was attenuated resulting in significant decreases in intestinal epithelial cell damage and intestinal permeability\(^{42}\). Additional findings from our laboratory have shown that burn injury significantly increased apoptosis in the small intestine. This increase in apoptosis correlated with significant increases in intestinal inflammation and a dysbiosis of the resident microbial communities of the gut; two intense intestinal cellular stresses\(^{42}\). Mosser et al. found that HSP70 over-expression protected cells from stress driven apoptosis by inhibiting the processing of procaspases 9 and 3, two enzymes in the signal transduction pathway towards apoptotic cell death\(^{40,91}\). Alterations in the heat shock response of HSP70 along with the other two most studied HSPs, HSP25 and HSP90, after burn could potentially drive intestinal epithelial cell damage, increased gut leakage, and septic shock. Together, these studies show strong evidence for HSPs as potential targets for protection from various diseases. Therefore, it is of utmost importance to understand how pharmacological upregulation of HSPs could be a new, viable therapeutic option for the maintenance of gut barrier integrity in acute injury conditions such as burn and trauma.

1.8 Future Directions and Perspectives

The subjects reviewed herein, show how the induction of the heat shock response is of critical importance to the maintenance of the intestinal barrier, under both normal homeostatic conditions and under times of stress or trauma such as burn injury. However, the exact mechanisms by which HSPs contribute to barrier integrity after such injury remain largely unexplored. Therefore, further study needs to be conducted to elucidate whether the dysregulation of HSPs following burn injury could compromise intestinal barrier integrity resulting in burn related complications of sepsis and subsequent multiple organ dysfunction syndrome. Future studies of the interplay between the heat shock protein response and intestinal barrier integrity after thermal injury could reveal potential therapeutic interventions not yet discovered.

Acknowledgements

Supported by NIH R01 AA01573, R21AA022324 and T32AA013527.

Author details

1 Burn & Shock Trauma Research Institute, Loyola University Chicago Health Sciences Division, Maywood, IL, USA. 2 Integrative Cell Biology Program, Department of Surgery, Loyola University Chicago Health Sciences Division, Maywood, IL, USA. 3 Department of Surgery, Loyola University Chicago Health Sciences Division, Maywood, IL, USA. 4 Stritch School of Medicine, Department of Surgery, Loyola University Chicago Health Sciences Division, Maywood, IL, USA.

References


Akira T, Kawai S. TLR signaling. Cell Death and Differentiation. 2006;816-825.
How to cite this article:

The “Journal of Nature and Natural Sciences (JNNSci)” is an international, peer-reviewed journal that is published bi-annually. Published articles are FREE to view, download and to print. The journal is a “Barkat Ali Firaq Trust for Education and Research (B.A.F.T.E.R)” publication. For more information, please visit www.bafter.org.

Your research contributions are invited at editor@jnnsci.com.