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Melatonin protection against burn-induced liver injury. A review

Review Article

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Abstract: Severe thermal injury may be complicated by dysfunction of organs distant from the original burn wound, including the liver, and represents a serious clinical problem. Although pathophysiology of burn-induced liver injury remains unclear, increasing evidence implicate activation of inflammatory response, oxidative stress, endothelial dysfunction and microcirculatory disorders as the main mechanisms of hepatic injury. Several studies suggest melatonin as a multifunctional indolamine that counteracts some of the pathophysiologic steps and displays significant beneficial effects against burn-induced cellular injury. This review summarizes the role of melatonin in restricting the burn-induced hepatic injury and focuses on its effects on oxidative stress, inflammatory response, endothelial dysfunction and microcirculatory disorders as well as on signaling pathways such as regulation of nuclear erythroid 2-related factor 2 (Nrf2) and nuclear factor-kappaB (NF-kB). Further studies are necessary to elucidate the modulating effect of melatonin on the transcription factor responsible for the regulation of the pro-inflammatory and antioxidant genes involved in burn injuries.

Keywords: *Melatonin • Liver injury • Oxidative stress • Inflamamation • Endothelial dysfunction • Microcirculatory disorders • Burns*

1. Introduction

A thermal injury represents one of the most severe forms of trauma and clinical problems in emergency medicine. A severe burn is a devastating injury affecting every organ system and leads to complications with and poor outcome. Pathophysiology of burn-induced liver injury includes many mechanisms and is not yet entirely defined. Various cellular and molecular interactions between neutrophils and macrophages, oxygen radicals, cytokine overproduction, depletion of glutathione and mitochondrial dysfunction may be involved in these processes [1]. These complex mechanisms cause liver dysfunction, damage of hepatic parenchyma and, eventually, cell death [2]. Liver plays an important role in metabolism, inflammation, homeostasis and host defense mechanisms [3] and is a major organ responsible for initiating multi-organ dysfunction syndrome (MODS) following burn injury. Taking into consideration the role of lipid peroxidation in burn-induced hepatic injury, the interference with hepatic oxidative status and inflammatory response, it is logical to hypotesize that therapy with substances possessing antioxidant, antiinflammatory and anti-apoptotic effects may be beneficial in reducing these complications [4,5].

Melatonin is very attractive from this point of view as it is a powerful antioxidant and scavenger, especially concerning reactive oxygen and nitrogen species by restricting their proinflammatory and membrane-damaging effects [6]. It stimulates the activity of a variety of antioxidant enzymes [7] and reduces cytokine production

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[8-10]. Melatonin could modulate inflammation by decreasing the NF-κB activation cascade and oxidative stress by increasing the nuclear expression of erythroid Nrf2 which might be responsible, at least in part, for its hepato-protective effect [11]. Furthermore, because of its high lipid and water solubility, it may enter every cell of the body, whereas other 'classical' antioxidants such as vitamin A and vitamin E are located in cell membranes, and vitamin C is found primarily in the cytosol. Another advantage of melatonin over classical antioxidants is its lack of prooxidative actions. Melatonin sacrifices itself and does not participate in redox cycling after scavenging the free radicals. Combining the characteristics of an antioxidant, a membrane stabilizer and an antiinflammatory agent, melatonin administration ameliorates liver damage induced by ischemia reperfusion and other toxic substances [12,13]. The present review focuses on the role of melatonin as a factor restricting the burn-induced liver injury.

2. Oxidative stress in the pathogenesis of liver injury and melatonin as factor restricting the burn-induced hepatic injury

Burns are a common traumatic injury that result both in local tissue damage and systemic mediator-induced response. There is evidence of both local and systemic oxidant changes manifested by increased free radical activity and lipid peroxidation [4,14]**.** At the same time, a burn injury causes a remarkable reduction of total antioxidant status and scavenging capacity.

Severe burn trauma is associated with high oxidative stress. Reactive oxygen species (ROS) are first generated from the burned skin. After major burns, ischemia due to vasospasm in the splanchnic area and inadequate perfusion may disturb the cell metabolism with local release of tissue-damaging mediators such as oxygen-derived free radicals. The xanthine/xanthine oxidase system enzyme is the main source of free radicals or ROS, superoxide radicals and more toxic radicals such as hydroxyl radical (OH-) and peroxinitrite anion (ONOO-) [15]. Activated neutrophils and macrophages sequestrated in liver generate massive ROS production that involves either myeloperoxidase (MPO) or NADPH oxidase in an inflammatory environment is called the 'oxidative burst'**.** It has been reported that hepatic MPO activity as an index of tissue neutrophil infiltration is markedly elevated after a burn injury [16]. Cytokines such as tumour necrosis factor alpha (TNF-α) induce iNOS synthesis which further produces large NO amounts. In the presence of abundant O_2^- and eNOS-derived NO, the biochemical interaction of these two substances inevitably produces vast amounts of cytotoxic peroxinitrite [17]. It may oxidize tetrahydrobiopterin (BH4), a co-factor of eNOS_which in turn can uncouple eNOS to form a dysfunctional superoxide-generating enzyme that contributes further to oxidative stress. Mitochondrial cytochromes and hemoglobin are other main hepatic source of ROS. After thermal injury, the release of free reactive iron from erythrocytes might also implicate an important contributor to ROS production [18].

ROS can function in activities such as cell growth and cell adaptation responses, whereas at higher concentrations, ROS can cause cellular injury and death. Free radicals are strongly reactive and may attack highly unsaturated fatty acids in cell membranes to induce lipid peroxidation as well membrane proteins and DNA. These events would activate signal redox-sensitive transcription pathway that in turn might cause mobilization of the body's antioxidant defense. Several studies associated an increased antioxidant activity to counteract the oxidative stress-induced by thermal injury [19]. CuZnSOD expression as front-line antioxidant increases as compensatory reaction early post-burn when oxidative stress increases [20,21].

Ding et al. (2002) show an increased glutathione-perroxidase (GSH-Px) activity 24 h after thermal injury that may result from the activation of oxygen responsive element (ORE) in GSH-Px gene by high oxygen level. The increased GSH-Px activity suggests enhanced GSH levels to provide an adaptive response against oxidative stress in liver tissue post-burn. Severe thermal injury gives rise to oxidative stress and dramatically enhanced expression of liver metallothionein could be one of the important compensatory mechanisms of natural defense system under conditions of oxidative stress post-burn trauma [22].

The excessive production of ROS and reactive nitrogen species (RNS) leads to glutathione depletion due to its increased consumption. Overproduction of free radicals along with unbalanced hepatic antioxidant defense capacity initiates lipid peroxidation and directly damages plasma and intracellular membranes which alters membrane-bound proteins. Both ROS and RNS enhance the production of reactive aldehydes such as MDA**,** an end product of lipid peroxidation with potent pro-inflammatory and pro-apoptotic properties [23]. Activation of peroxidative mechanisms results in cellular dysfunction, apoptosis or necrosis in hepatocytes with the release of the hepatic enzymes into circulation. Serum ALT and AST levels increase markedly 24 h after burn injury and these elevations attenuate with melatonin (10 mg/kg), thus indicating melatonin's role in

preservation of the structural integrity of hepatocellular membranes and protection against burn-induced hepatic oxidant injury [24-26] as evidenced by increased lipid peroxidation in the liver, lung and gut. The increased generation of ROS and RNS along with unbalanced hepatic antioxidant defense capacity contributes to liver oxidative injury.

In terms of its scavenger activity, melatonin quenches the superoxide anion and hydroxyl radical, single oxygen, peroxyl radical and peroxynitrite anion [27]. Moreover, melatonin inhibits the activity of xanthine xanthine oxidase (XO) and iNOS as well along with its NO and peroxinitrite scavenging activity [7]. Melatonin as a direct free radical scavenger and an indirect antioxidant ameliorates the systemic oxidative stress. Its administration results in a significant reduction of lipid peroxidation product MDA in plasma and hepatic tissue in animals with thermal trauma [23,25]. These observations point to effictiveness of melatonin to ameliorate local and systemic stress.

By lowering MDA levels and lipid peroxidation and reducing the augmentation of sulphydril levels, melatonin influences cell membrane stabilization and protects against burn-oxidative damage in remote organs [25]. Furthermore, it attenuates the hepatic glutathione depletion after burns [24]. Melatonin stimulates gamma glutamyl cystein synthetase and GSH recycling in the cell, it influences upon *de novo* GSH synthesis, thus playing an important role in the maintenance of this crucial antioxidant. Besides direct neutralization of several free radicals, ROS, RNS and MDA melatonin stimulates several antioxidative enzymes such as SOD, GSH-Px, GSH- reductase, which increases its efficiency as antioxidant [29]. Additionally, nanoparticles containing melatonin provides an important increase in its antioxidant effect against lipid peroxidation [30]. Being amphiphilic, melatonin can protect against free-radical damage through the cell and limits the breakdown of membrane lipids and proteins in the cytosol and oxidation of DNA in the nucleus [31]. Another advantage of melatonin over the classical antioxidants is its lack of prooxidative actions. As mentioned above, melatonin does not only consume cellular GSH but also preserves or even increases GSH tissue content.

Oxidative stress can cause inflammation by direct toxic effect of free radicals and by inducing the activation of redox sensitive pronflammatory transcription factors and signal transcription parthways. Therefore, oxidative stress and its constant companion, inflammation, play an important role in the pathogenesis of hepatic injury progression.

3. Early inflammatory response post-burn, liver injury and apoptosis and melatonin as anti-inflammatory and anti-apoptotic agent

Burns initiate systemic inflammatory response syndrome (SIRS) where the generation of pro-inflammatory cytokines and ROS causes a progressive distant organs dysfunction [32]. Liver is the central organ involved in this response because it is a primary source of inflammatory cytokines as well as a target organ for the damaging effects of these mediators. Liver plays a pivotal role in a thermal injury by modulating the immune function, inflammatory processes, and the acute-phase response.

TNF-α is one of the first-wave cytokines secreted by macrophages in response to an injurious stimulus which results in the multiple organ damage following burns [33-36]. TNF-α elicits synthesis and release of a cascade of cytokine mediators such as IL-1, IL-6, IL-8, and platelet activating factor (PAF). TNF-α itself playing an essential role in triggering the systemic inflammatory cascade as an important pathophysiological mechanism in liver injury [26,37]. TNF-α plays an important role in the occurrence and development of hepatic and cardiac injury in experimental model of thermal trauma [26,36]. Furthermore, hepatocytes are much more sensitive to TNF-α damaging effect when their antioxidant capacity is low. In addition, TNF-α can induce NO production by iNOS and cytotoxic peroxintrite. The latter and TNF-α are described as apoptotic signals [38]. Thermal skin injury causes hepatic NF-κB activation that can mediate the release of hepatic TNF-α and other pro-inflammatory mediators contributing to liver damage. Melatonin protects against burn-induced hepatic injury as to a certain extent this effect may result from the suppression of NF-κB-mediated inflammatory response [26].

Experimental thermal trauma enhances plasma CPR levels, an effect that is abolished by melatonin administration [25]. CRP produced by activated leukocytes itself stimulates the expression of cytokines and free radical production which can lead to progression of tissue oxidative damage [39,40]. Protective effect of melatonin is related not only to the inhibition of inflammatory cytokines but also to the reduction of the expression of adhesion molecules after burns [8]. Exogenous melatonin protects the liver by ischemia/reperfusion injury inhibiting the production of free radicals, reducing TNF-α concentration in systemic circulation and suppressing intercellular adhesion molecule-1 (ICAM-1) liver expression [41]. These data suggest a bidirectional

and synergistic linkage of inflammation and oxidative stress contributing to burn-induced hepatic injury.

Certain melatonin's protective mechanism of action includes is decreasing activity of the multiple pro-apoptotic factors and apoptosis [42]. Apoptosis of hepatocytes and endothelial cells is considered important in pathological hepatic changes and is a result of systemic apoptotic response occurring after burns [43]. Apoptosis plays an important role in inflammatory responses. Hypoperfusion, ischemia and elevated cytokines IL-6 and TNF-α after burns exert a direct damaging effect to hepatocytes (mitochondria) and can induce apoptotic or also necrotic death. The increased hepatic programmed cell death is compensated for by an increased hepatic cell proliferation suggesting that liver attempts to maintain homeostasis [44].

Oxidative stress is recognized as an important mechanism of apoptosis. The pro-apoptotic activity of free radicals is mainly mediated by the loss of mitochondrial potential, release of cytochrome C and activation of caspases 3 [45,46]. The latter induces mitochondrial damage with cytochome C release and apoptosis. Melatonin is a known powerful antioxidant and anti-inflammatory agent. Increasing experimental and clinical evidence shows its beneficial effects against mitochondrial dysfunction, the major source of ROS and RNS [47]. Most reports indicate that the anti-apoptotic effect of melatonin occurs by acting on the abundance of two main membranes of Bcl/Bax family, namely the pro-apoptotic Bax and the antiapoptotic Bcl-2 protein [48]. Bcl-2 exerts a well-known anti -apoptotic effect by inhibiting the action of Bax which induces apoptosis by forming pores in the outer mitochondrial membrane thus permeabilizing it to the passage of pro-apoptotic factors such as cytochome C [49]. Indeed, many studies demonstrate that its anti-apoptotic effect involves Bax down-regulation and/or Bcl-2 up-regulation that occurs through NF-κB activation [50]. The balance between Bax and Bcl-2 determines the propensity of cells to respond to a given insult by apoptosis and survival. Melatonin is capable of shifting the balance towards a cell-protective anti-apoptotic Bcl-2 protein [Bekyarova, unpublished data]. Therefore, anti-apoptotic effects of melatonin are related to the reduced mitochondrial ROS production and the activated anti-apoptotic and redox-sensitive Bcl/Bax system [48].

Melatonin protects against burn-induced liver damage through its strong scavenging, anti-inflammatory and anti-apoptotic effect. By means of pleotropic function of an antioxidant, anti-inflammatory and anti-apoptotic mediator, melatonin protects against burn-induced liver damage and improves survival of rats after burns [8-10].

4. Melatonin and NF-κB

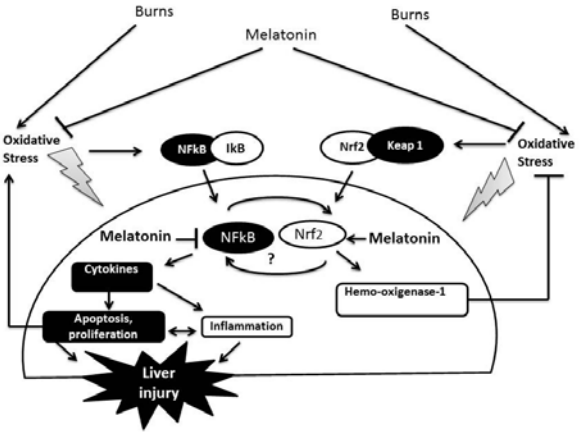
NF-κB is a ubiquitous transcription factor responsible for oxidative stress, pro-inflammatory mediator release and hepatocellular dysfunction after burn trauma [26,51]. NF-κB is critical in the regulation of liver apoptosis and proliferation (Figure 1) [44]. NF-κB is implicated in the up-regulation of various cytokines and chemokines, including TNF-α and IL-8 [52]. CRP itself can potently enhance NF-κB expression, thereby contributing to a positive feedback loop that causes tissue injury. NF-κB activation is a necessary step in the transcriptional induction of cell adhesion molecules and growth factors [53].

The basis for the protective effects of antioxidants such as melatonin is consistent with interference at the level of NF-κB transcriptional pathway. Melatonin inhibits NF-κB translocation to the nucleus and its binding to DNA [54], thereby reducing the up-regulation of the pro-inflammatory cytokines and CRP**.** It restricts burn-induced hepatic oxidative injury by inhibiting the elevated TNF-α levels and NF-κB expression in the liver [26]. Melatonin diminishes not only pro-inflammatory cytokine expression but also ICAM in a rat model of colitis through NF-κB inhibition [54]. These findings suggest that melatonin protects against burn-induced hepatic injury as to a certain extent this effect could result from the suppression of NF-κB-mediated inflammatory response.

5. Melatonin's protection of endothelial dysfunction, microcirculatory disorders and early post-burn liver injury

ROS production in the early post-burn period can provoke disturbances in microcirculation [55]. ROS stimulating secretion of cytokines such as TNF-α and IL-1 can increase vascular endothelial permeability and thus promote endothelial adhesion for leukocytes by increased ICAM-1 expression, two critical factors governing endothelial dysfunction. Oxidant-mediated increased polymorphonuclear leukocyte adhesion to endothelium is coupled with alterations in endothelial signal transduction and redox-regulated transcription factors such as activator protein-1 and NF-κB [56]. Intermediate ROS and intermediate RNS are the final common pathways leading to endothelial cell dysfunction, premature endothelial cell senescence and apoptosis. Antioxidants represent an important therapeutic strategy for the prevention and correction of endothelial dysfunction [57].

Figure 1. Possible mechanisms of burn-induced hepatic injury and melatonin protection. Thermal trauma can induce liver injury by activating NF-κB-mediated pro-inflammatory pathway. Melatonin can considerably ameliorate burn-induced hepatic injury either by the induction of Nrf2-mediated antioxidant response to attenuate oxidative stress and/or by suppressing NF-κB-mediated inflammatory response.



Burn-induced endothelial dysfunction may results from diminished production of eNOS NO, or NO inactivation by oxygen free radicals. Our results clearly indicate a decreased eNOS expression, most probably, due to free radical-mediated eNOS inhibition Bekyarova, unpublished. The reduced eNOS-derived NO production, or NO inactivation decrease its bioavailability that may also contribute to an increased platelet aggregability and expression of inflammatory cytokines, all of which are involved in the pathogenesis of microcirculatory disorders in liver and other organs after burns.

Melatonin inhibits leukocyte activation and adhesion, decreases pro-inflammatory cytokine expression during an inflammatory response by interfering with NF-κB activation, therefore, suppressing endothelial cell pro-adhesive phenotype [54]. Similarly, it protects rat liver from intestinal ischemia/reperfusion injury, with a marked reduction of ICAM-1-positive hepatic cells [41]. Exogenous melatonin protects liver from burn and I/R injury by inhibiting free radical production, reducing TNF-α concentration in systemic circulation, and suppressing ICAM-1 expression in liver.There is indirect evidence that melatonin inhibits the production of adhesion molecules such as selectins, VCAM-1 and ICAM-1 [10]. These findings suggest that its administration can interrupt the endothelial-leukocyte interactions and abolish local inflammatory processes and blood coagulation [28].

CRP may interfere with the inflammatory process of endothelial cells and the mechanism may be to induce expression of adhesion proteins. Besides, CRP promotes expression of tissue factor by mononuclear cells and also contributes to the pro-coagulant state [58]. CRP plays a direct role in blood coagulation by suppressing NO expression, thrombomodulin and protein C and increasing the expression of plasminogen activator inhibitor-1 (PAI-1) and ROS [59]. Experimental burns increase the levels of acute-phase proteins and blood coagulation evidenced by the elevated PA, activated partial thromboplastin time (aPTT) and fibrinogen and this effect is abolished by melatonin administration [60]. Melatonin treatment normalizes the levels of skin lipid peroxidation as well as prothrombin time (PT) and fibrin degradation products (FDP) in the blood of rats with thermal injury [61]. As an antioxidant and anti-inflammatory factor it suppresses the burn-induced coagulation disorder and could prevent the disseminated intravascular microthrombosis following thermal injury.

Burn-induced hypercoagulability with thrombosis further impairs blood flow, while oxidative stress damages endothelial cells and compromises vascular patency. Increased endothelial permeability and elevated interstitial hydrostatic pressure may cause edema with vascular congestion, microcirculatory disorders and tissue damage. These disorders following burns can be associated with decreased erythrocyte deformability and increased RBS and platelet aggregability [62,63]. Elevated fibrinogen levels, and especially oxidized fibrinogen, can increase burn-induced spontaneous platelet aggregation [64]. Oxidative damage of erythrocyte membrane can decrease RBC deformability and increase platelet and red blood cell aggregability [81-62-63]. Melatonin is a potent protector of erythrocytes from impaired deformability because of its restrictive effect on the lipid peroxidation under *in vitro*, however, we fail to find any effect on burn-induced erythrocyte deformability during early stages following burns. There is evidence of platelet aggregates in burned animals which disappear after melatonin treatment [60]. Besides, melatonin inhibits oxidative damage of platelet membranes**.** This is, probably, another means of melatonin protection against elevated burn-induced platelet aggregation and coagulation disorders.

Burn-induced down-regulation of eNOS expression may also contribute to increased platelet aggregability and expression of inflammatory cytokines all of which are involved in the pathogenesis of hepatic injury. When preventing eNOS down-regulation melatonin may avoid the cascade of events resulting from decreased NO production, thereby exerting a protective effect [Bekyarova, unpublished]. Melatonin benefits correlate with NO generation through constitutive e-NOS activation and prevention of oxidative stress and inflammatory cytokine release including TNF-α, respectively. Melatonin provides a significant microvascular protection by means of eNOS activation [65].

In conclusion, these observations collectively suggest that oxidative stress, inflammation and endothelial dysfunction are all causally and synergistically linked to the pathogenesis of burn-induced oxidative injury. Melatonin is a multifunctional indolamine which counteracts several pathophysiologic steps and displays significant beneficial effects against burn-induced cellular injury. The cytoprotective effect of melatonin in burn-induced hepatic injury includes an antioxidant, anti-inflammatory and anti-apoptotic function as well as the role in maintaining microcirculation.

6. Burn-induced hepatic morphological and functional changes in liver and melatonin protection

Clinical studies have shown that impaired liver function and morphological damages are critical for survival in critically ill and severely burned patients [66,67]. Many factors such as free radicals, cytokines released by inflammatory cells, damaging hepatocytes and vascular endothelial cells can cause liver injury after burns [51].

Our own data shows that burn-induced activation of the inflammatory response (i.e., TNF-α) and oxidative stress (i.e., MDA) is accompanied by histopathological changes, vascular congestion, leukocyte infiltration around the central veins, intracellular vacuolization, hepatic cell degeneration and, eventually, apoptosis. There are apoptotic bodies (Councilman's bodies) occurring in rats after burns [Bekyarova unpublished data]. Melatonin prevents these morphological impairments [26].

Clinical investigations show that burn-induced hepatic injury is accompanied by impairment of hepatic parenchymal cell function [14,68]. Plasma levels of aminotransferases such as AST and ALT, as indicators of hepatic injury and functional disorders, are higher after burns [44]. After melatonin inhibition of increased MDA, TNF and CRP, there is only restriction of liver injury evidenced by increased plasma AST and ALT activities suggesting that other mechanisms take part in burn-induced liver injury [25,26].

After thermal trauma, the activity of specific markers of hepatic dysfunction such as gamma glutamyl transpeptidase (GGT) and serum cholinesterase (ChE) changes. The decreased GhE levels known as an indicator of the synthetic capacity of hepatic parenchyma and GGT related with antioxidant defense and oxidative stress reflect the impaired hepatic function. Burn-induced hepatic damage associated with increased hepatic apoptosis impairs hepatic protein production. Despite the attempt to compensate increased apoptosis by increased proliferation, liver cannot regain hepatic mass and thus hepatic protein concentration decreases following burn trauma [68].

After severe burns, hepatic protein production shifts from predominantly constitutive hepatic proteins to acute-phase proteins such as CRP [25]. This can release survival signals to counteract increased hepatic apoptosis by inhibiting the apoptosis or stimulating the proliferation. Melatonin restricts burn-induced reduction of plasma GGT and ChE activity and hepatic proteins as well as an increase of acute-phase protein production. Given the hypothesis that a decrease of acute-phase proteins and an increase of constitutive hepatic proteins restricts hepatic parenchyma injury and improves liver function, we suggest that melatonin treatment may be beneficial after a burn injury [26].

7. The role of heme-oxigenase-1 (HO-1) in the protection of burn-induced hepatic injury

Heme oxygenase-1 (HO-1) enzyme catalyzes the rate-limiting reaction in the catabolism of heme yielding products with pleiotropic, but ultimately, cytoprotective activities. HO-1 is not only a key enzyme in the heme metabolism but also a heat shock protein (HSP 32) in rats [69]. HO-1 is induced by its substrate heme as well as by hypoxia, ischemia, hyperthermia and inflammation which facilitate ROS generation. HO-1 is expressed constitutively in synosoidal cells but its induction represents an important defensive cellular mechanism in liver against oxidative stress. High HO-1 levels are frequently detected in various pathological states and, generally, under conditions of cellular oxidative stress. In liver and other organs, HO-1 is strongly up-regulated by stress and, in general, is considered to be cytoprotective in experimental models of hepatic injury including ischemia/reperfusion, hemorrhage/resuscitation, heat shock, and against hepatoxins such endotoxin and acetaminophen [65,70-72]. HO-1 is one of the most sensitive and reliable indicators of cellular oxidative stress.

Nakae et al. [73] reported that thermal skin injury results in a time-dependent increase of HO-1 activity in the liver and lung which reaches a maximum at 24 h. We found that HO-1 expression is significantly increased not only in sinusoidal cells, but also in hepatocytes [Bekyarova unpublished data]. HO-1 induction is accompanied by the oxidative stress and pro-inflammatory mediators (i.e. TNF-α) that are concurrently increased by thermal injury at 24 h. Interestingly, HO-1 expression in hepatocytes of thermally injured rats with melatonin

administration is more significanly increased compared to that of burned untreated rats. The results indicate that a thermal trauma not only might induce HO-1 expression, but the increased expression could be further enhanced by melatonin administration. Parallelly with HO-1 induction, there is a significant inhibition of increased lipid peroxidation (i.e. MDA) and TNF-*α* level by melatonin (10 mg/kg). HO-1 induction by melatonin administation is important in prevention of reactive immediate-induced oxidative stress and burn-induced liver injury.

The cellular mechanism by which this occurs remains to be established. However, there is evidence that protection by HO-1 is largely attributable to its end product CO and bile pigments, biliverdin (BVD) and bilirubin [74]. Bilirubin represents the highest endogenous antioxidant among the constituents of normal human plasma. Bilirubin can scavenge peroxyl radicals as effectively as α-tocopherol which is the most potent antioxidant against lipid peroxidation [74]**.** CO may also contribute to the antioxidant actions of HO-1 by stimulating the expression of antioxidant genes and inhibiting the activity of prooxidant enzymes [75]. Free iron sequestration by ferritin lowers the prooxidant state of the cell. HO-1-dependent release of iron also results in ferritin up-regulation. Recent experimental evidence shows that HO-1-deficient cells and mice are susceptible to the accumulation of free radicals and to oxidative injury after endotoxin administration [71]. Therefore, HO-1 is an important enzyme antioxidant system. Its induction might provide cytoprotection against oxidative stress.

HO-1 expression and concomitant production of its metabolites, CO and BVD, present with anti-inflammatory consequences [76]. CO possesses anti-inflammatory and anti-apoptotic properties [77]. CO, a newly identified signaling molecule participates in many biological events and plays a role in mediating the cytoprotection against oxidant-induced injury [69]. CO-releasing molecules (CORM) significantly modulate the protection of liver injury in burned mice by increasing HO-1 expression, down-regulating iNOS expression and NO production, and inhibiting the production and secretion of the pro-inflammatory mediators TNF-α and IL-1β after severe burn injury [78]. BVD stimulates IL-10 production while suppressing synthesis of inflammatory cytokines [79].

HO-1 induction inhibits microvascular endothelial cell leukocyte adhesion through the action of its metabolites, bilirubin and CO [80]. CO improves microcirculation through vasodilation and inhibition of platelet aggregation [81]. Additionally, it modulates vascular tone which reduces platelet aggregation and vascular dysfunction through the induction of a soluble guanylyl cyclase similar to NO [82]. Both eNOS-derived NO and CO from HO-1 take part in non-specific host defense and maintain cellular and vascular homeostasis providing potent cytoprotection. Our data show that decreased hepatic eNOS expression is, most likely, due to elevated ROS and TNF-*α* generation after burns [83]. Melatonin as an antioxidant prevents the decrease of eNOS expression and through up-regulation of eNOS and HO-1 expression it might exert protection of hepatocytes against oxidative stress after burns.

HO-1 possesses antioxidant, anti-inflammatory and anti-apoptotic properties and plays an important protective role against oxidative injury [77,84]. We propose that HO-1 induction by melatonin could be a novel mechanism for protecting the burn-induced vascular and hepatocellular injury. However, the mechanism involved in the activation of HO-1 protection by melatonin remains to be further investigated. Given the multifactorial cytoprotective properties of the HO-1 system, it would highlight potential therapeutic strategies targeting HO-1 in the treatment or prevention of vascular disorders and restoration of the hepatic damage following thermal skin injury.

Stress-activated transcription factors such as nuclear factor (NF) erythroid2-related factor-2 (Nrf2), activator protein-1, and NF-κB play predominant roles and mediate potent HO-1 induction by agents that cause cellular stress [85].

8. Nuclear factor erythroid2-related factor-2 (NrF2)

Nuclear factor erythroid2-related factor-2 (Nrf2) is another defense system involved in combating oxidative stress and modulating inflammatory processes [86]**.** Nrf2 is a major defense mechanism that plays an important role in HO-1 induction. It is a transcriptional factor that regulates expression of a number of detoxifying antioxidant genes such as glutathione transferase (GST) and anti-inflamatory cytokines such as IL-10. Nrf2 plays a key role in the induction of anti-inflammatory cytokine suppression and as well as pro-inflammatory signaling [87]. Additionally, it up-regulates anti-apoptotic Bcl-xL protein and enhances cell survival [88]**.**

The hepatoprotective role of melatonin is partially mediated through the abrogation of oxidative stress and the prevention of the diminished activity of antioxidant enzymes through Nrf2 pathways. Melatonin prevents the decrease of antioxidant enzymic activity and activates Nrf2 signaling. Melatonin administration activates Nrf2 signaling in a rabbit model of hepatic failure of viral origin which normalizes antioxidant enzyme expression and supports the potential hepatoprotective role of this indole [11]. Melatonin up-regulates Nrf2 expression,

increases antioxidant activities, reduces NF-κB activation and inhibits iNOS and pro-inflammatory cytokine expression with subsequent reduction of vascular oxidative stress, inflammation and dimethylnitrosamine-induced liver injury.

NF-κB and Nrf2 are major transcription factors involved in regulating the pro-inflammatory, anti-inflammatory, and antioxidant genes. Melatonin increases antioxidant enzymes and oxidative stress by Nrf2 activation and decreases inflammatory mediators by NF-κ B inhibition in rats with dimethylnitrosamine (DMN)-induced liver injury [11]. It could modulate inflammation by decreasing NF-κB activation cascade and oxidative stress by increasing Nrf2 expression that might be responsible, at least in part, for its hepatoprotective effect (Figure 1). These data imply that melatonin induces antioxidant defense via the Nrf2 pathway and reduces inflammation via NF-κB inhibition.

9. Conclusion

Melatonin administration attenuates oxidative stress, inflammaton, microcirculatory disorders and delays deterioration of liver structure and function in rats during early response following burn trauma. Further studies would be directed to the possible useful applications of melatonin in specific, pharmacologically tested dosages to patients after severe acute thermal injury. If proven effective, melatonin may be an attractive adjiuctive therapy, since it is a natural, inexpensive, widely available, orally applicable, and relatively safe product.

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