Preinduction of heat shock proteins protects cardiac and hepatic functions following trauma and hemorrhage

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Mizushima, Yasuaki, Ping Wang, Doraid Jarrar, William G. Cioffi, Kirby I. Bland, and Irshad H. Chaudry. Preinduction of heat shock proteins protects cardiac and hepatic functions following trauma and hemorrhage. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R352–R359, 2000.—Although studies have shown that induction of the heat shock proteins (HSPs), such as HSP-70, has various beneficial effects after ischemia-reperfusion, it remains unknown whether prior induction of HSP-70 has any salutary effects on cardiovascular and hepatocellular functions after trauma-hemorrhage and resuscitation. Male rats were exposed to heat stress (41°C, 15 min) and then allowed to recover for 24 h at room temperature (21°C). The rats then underwent laparotomy (i.e., trauma induced) and were bled to and maintained at a mean arterial pressure of 40 mm Hg until 40% of the maximal shed blood volume was returned in the form of Ringer lactate. Animals were then resuscitated with four times the volume of shed blood with Ringer lactate over 60 min. The maximal rate of the left ventricular pressure increase or decrease was measured up to 4 h after resuscitation. Cardiac output, hepatocellular function, plasma levels of tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were determined at 4 h after resuscitation. Cardiac and hepatic tissue were examined for HSP-70 by Western blot analysis. Left ventricular performance, cardiac output, and hepatocellular function decreased significantly following trauma-hemorrhage. Plasma levels of TNF-α and IL-6 were also significantly increased. However, prior heat stress attenuated cardiovascular and hepatocellular dysfunction, decreased circulating levels of proinflammatory cytokines following trauma-hemorrhage, and was associated with an increased abundance of HSP-70 in the heart and liver. Our data, therefore, suggest that preinduction of HSP-70 protects cardiovascular and hepatocellular functions following trauma-hemorrhage and resuscitation.

heat shock protein-70; cardiac performance; cardiac output; hepatocellular function; hemorrhagic shock; tumor necrosis factor-α; interleukin-6

SEVERE HEMORRHAGE is a prevalent complication in trauma patients (3). Hemorrhage also is frequently encountered during complex surgical procedures, such as aneurysm resection. Studies have shown that patients who survive the initial fluid resuscitation have increased risk of sepsis and septic shock, which in turn would lead to multiple organ system failure and ultimately to death (3). It is encouraging, however, that the pathophysiology of trauma and hemorrhagic shock is becoming better understood. To this end, studies have examined the myriad of mechanisms to preserve cellular structure and function under various noxious stimuli. One area of focus for such a protective mechanism is the highly conserved cellular proteins classified as heat shock proteins (HSPs) (15). Among them, 70-kDa HSP-70 is the most abundant and best-characterized HSP. The expression of the HSPs such as HSP-70 was first observed with hyperthermia; however, subsequently it was shown that stress such as ischemia, hypoxia, oxygen radicals, and proinflammatory cytokines are also able to induce them (7, 18). Although a number of studies have shown that preinduction of HSP-70 by heat stress has various beneficial effects after ischemia-reperfusion injury (6, 8, 25), it remains unknown whether preinduction of HSP-70 has any salutary effects on organ function following hemorrhagic shock.

HSP-70 may protect the cell by processing denatured proteins and by protecting RNA processing and translation (13). HSP-70 is also involved in antigen processing and presentation as well as in the acute phase response and cytokine production (13, 17). Studies have shown that HSP-70 inhibits the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1β in different cell populations (27, 29). However, few in vivo animal studies have tried to determine the role of HSP-70 in regulating cytokine production. Previous studies from our laboratory have demonstrated that circulating levels of proinflammatory cytokines are significantly elevated following trauma-hemorrhage and resuscitation (2). However, it remains unknown whether preinduction of HSP-70 alters such cytokine production after trauma and hemorrhage. Therefore, the aims of this study were to determine whether preinduction of heat stress 1) has any beneficial effects on cardiovascular and hepatocellular functions and 2) decreases circulating levels of proinflammatory cytokines after trauma-hemorrhage and crystalloid resuscitation.

MATERIALS AND METHODS

Experimental model of trauma-hemorrhage and resuscitation. As previously described, a nonheparinized model of
trauma-hemorrhage in the rat (4, 33) was used in this study with some modification. Male Sprague-Dawley rats (body wt 291 ± 16 g; Charles River Laboratories, Wilmington, MA) were fasted overnight (~16 h) prior to trauma-hemorrhage but allowed water ad libitum. The animals were then anesthetized with methoxyflurane, and a 5-cm ventral midline laparotomy was performed to induce tissue trauma before the onset of hemorrhage. The abdominal incision was closed in layers. Catheters were placed into the right carotid artery and both femoral arteries with PE-50 tubing (Becton Dickinson, Sparks, MD). The carotid catheter was used for the measurement of cardiac performance. Mean arterial pressure (MAP) and heart rate (HR) were monitored by attaching a femoral catheter to a blood pressure analyzer (Micro-Med, Louisville, KY). PE-50 tubing was also inserted to the level of the right atrium via the right jugular vein for the injection of indocyanine green (ICG) and crystalloid resuscitation. The wounds were bathed with 1% lidocaine to reduce postoperative pain. Immediately after recovery from anesthesia, the animals were rapidly bled to a MAP of 40 mmHg within 10 min. The MAP of 40 mmHg was maintained by further withdrawing blood until the animal could no longer maintain that pressure unless Ringer lactate was infused. This time was defined as maximum bleedout, and the amount of withdrawn blood was noted. The MAP of 40 mmHg was maintained until 40% of the shed blood volume was returned in the form of Ringer lactate. At that time (~90 min from the initiation of hemorrhage), the rats were resuscitated with four times (~45 ml/rat) the volume of maximal bleedout over 60 min with Ringer lactate. Sham-operated animals underwent the same surgical procedure but were neither bled nor resuscitated. The experiments described in this paper were performed in adherence with the guidelines from the National Institutes of Health for the use of experimental animals. This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital (Providence, RI).

Introduction of heat stress. Rats were anesthetized with methoxyflurane 24 h before trauma-hemorrhage. Body core temperature was monitored with a thermistor inserted into the rectum, and rats were placed between heating pads until their rectal temperature reached 41°C. After the body temperature was maintained at 41°C for 15 min, the rats were withdrawn blood was noted. The MAP of 40 mmHg was defined as maximum bleedout, and the amount of

Western blot analysis of HSP-70. Immediately after blood sampling, heart and liver samples were harvested and snap frozen in liquid nitrogen. Frozen samples of ~300 mg were homogenized in phosphate-buffered saline (pH 7.40, 0°C), 1% Nonidet P, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate with protease inhibitors (10 mg/ml phenylmethylsulfonyl fluoride and 30 µl/ml aprotinin). Samples were drawn from the femoral arterial catheter into a heparinized syringe following the measurement of hepatocellular function. The plasma was separated by centrifugation at 12,000 g for 15 min at 4°C and stored at −70°C until assayed. Plasma IL-6 and TNF-α levels were measured using an enzyme-linked immunosorbent assay kit specific for rat IL-6 and TNF-α (Biosource, Camarillo, CA).

Results

The mean time to reach the maximum bleedout was 54 ± 2 min in hemorrhaged animals and 53 ± 2 min in hemorrhaged animals that received prior heat stress. The average shed blood volume was 10.0 ± 0.1 ml in the hemorrhage group and 10.1 ± 0.2 ml in the heat shock plus hemorrhage group. Systemic hematocrit was 45.3 ± 0.3% and 44.8 ± 0.3% in sham-operated and heat-shock
sham-operated animals, respectively. These values decreased to 21.7 ± 0.6% and 21.5 ± 0.6% in hemorrhage and heat-shock plus hemorrhage rats, respectively, after hemorrhage and crystalloid resuscitation. There was no significant difference in the above parameters between hemorrhaged animals with or without prior heat stress.

Effects of hemorrhagic shock on $+\frac{dP}{dt_{\text{max}}}$ and $-\frac{dP}{dt_{\text{max}}}$. As shown in Fig. 1A, $+\frac{dP}{dt_{\text{max}}}$ decreased at the end of hemorrhage in hemorrhaged animals that did or did not undergo prior heat stress. After resuscitation, $+\frac{dP}{dt_{\text{max}}}$ in both groups returned to a level that was not significantly different from sham-operated values. However, in hemorrhaged animals that were not pretreated with heat stress, $+\frac{dP}{dt_{\text{max}}}$ decreased significantly at 30 min after the completion of resuscitation and remained decreased throughout the study period of 240 min. In contrast, animals that underwent heat stress prior to hemorrhage showed a significant increase in $+\frac{dP}{dt_{\text{max}}}$ compared with nonstressed hemorrhaged animals, and the values remained similar to the sham-operated animals during the 240-min study period. The $-\frac{dP}{dt_{\text{max}}}$ also decreased at the end of hemorrhagic shock in both hemorrhaged groups (Fig. 1B). Resuscitation increased $-\frac{dP}{dt_{\text{max}}}$ in both hemorrhaged groups, but the values were not restored to sham-operated values. However, induction of heat stress before hemorrhage resulted in a significantly increased $-\frac{dP}{dt_{\text{max}}}$ from 60 to 240 min after resuscitation compared with the nonheat stress hemorrhaged group.

Effects of hemorrhagic shock on MAP and HR. In both groups of hemorrhaged animals MAP decreased significantly compared with sham-operated animals at the end of resuscitation, irrespective of heat stress, and remained depressed throughout the study period (Fig. 2A). Similarly, the HR in both hemorrhaged groups were significantly decreased at the end of hemorrhage compared with sham-operated animals (Fig. 2B). Although HR remained depressed in hemorrhaged animals throughout the entire study period, prior heat stress restored and maintained HR at a level that was not significantly different compared with sham-operated animals after the end of resuscitation (Fig. 2B).

Comparison of heart performance in sham-operated animals and heat-shocked sham-operated animals. There was no significant difference between sham-operated animals either with or without heat stress in any heart performance parameters (i.e., $+\frac{dP}{dt_{\text{max}}}$, HR, MAP; Table 1).

Effects of hemorrhagic shock on cardiac output and stroke volume. As shown in Fig. 3A, cardiac output was significantly decreased after hemorrhage and resuscitation. Prior heat stress, however, significantly increased cardiac output to a level that was not different from sham-operated values. Similarly, stroke volume decreased in hemorrhaged animals, whereas heat-stressed rats increased stroke volume to values that were not significantly different from sham-operated animals.

Fig. 1. Maximal rate of pressure increase ($+\frac{dP}{dt_{\text{max}}}$, A) and decrease ($-\frac{dP}{dt_{\text{max}}}$, B) in left ventricle up to 240 min after completion of resuscitation. Animals underwent sham operation (Sham), trauma-hemorrhage and resuscitation without heat shock (Hem) or with heat shock (H-Hem). There were 7 animals in each group. Data are expressed as means ± SE and compared by one-way ANOVA and Tukey’s test. *P < 0.05 vs. sham-operated animals; #P < 0.05 vs. hemorrhaged rats.

Fig. 2. Mean arterial pressure (MAP, A) and heart rate (HR, B) up to 240 min after completion of resuscitation. Animals underwent sham operation (Sham), trauma-hemorrhage and resuscitation without heat shock (Hem) or with heat shock (H-Hem). There were 7 animals in each group. Data are expressed as means ± SE and compared by one-way ANOVA and Tukey’s test. *P < 0.05 vs. sham-operated animals; #P < 0.05 vs. hemorrhaged rats.
Moreover, heat stress in sham-operated animals had no effect on cardiac output or stroke volume.

Effects of hemorrhagic shock on hepatocellular function. The maximal velocity of ICG clearance ($V_{\text{max}}$) and the transport efficiency of ICG ($K_m$) were significantly depressed by 76 and 55%, respectively, compared with sham-operated animals following trauma-hemorrhage and resuscitation (Fig. 4). Prior heat stress, however, significantly improved both $V_{\text{max}}$ and $K_m$ at 4 h after the completion of resuscitation compared with hemorrhaged animals (Fig. 4). Prior heat shock in sham-operated animals did not affect $V_{\text{max}}$ or $K_m$.

Effects of hemorrhagic shock on plasma levels of TNF-α and IL-6. Plasma TNF-α and IL-6 levels were significantly increased at 4 h after resuscitation in hemorrhaged animals (Fig. 5). Prior heat stress, however, significantly decreased plasma TNF-α to levels comparable to sham-operated animals. Heat stress also decreased IL-6, although the reduction was not significant. Heat stress in sham-operated animals did not affect plasma TNF-α or IL-6.

HSP-70 expression in liver and heart. Pretreatment with heat stress (41°C, 15 min) induced the abundance of HSP-70 in the liver and heart in both sham-operated and hemorrhaged animals (Fig. 6, A–B). Although expression of HSP-70 could be detected after trauma-hemorrhage alone, there was no significant difference compared with sham-operated animals in densitometric analysis (Fig. 6, C–D).

DISCUSSION

It is well known that heat shock induces the synthesis of HSPs (15). The stress-induced induction of HSPs gives the cell resistance against subsequent and potentially lethal challenge by a phenomenon known as

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<th>Table 1. Comparison of heart performance parameters between sham-operated rats with and without prior heat stress</th>
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Values are means ± SE; n = 7 animals in each group. Sham, sham-operated rats without prior heat stress; H-Sham, sham-operated rats with prior heat stress; $+dP/dt_{\text{max}}$, maximal rate of pressure increase in left ventricle; $-dP/dt_{\text{max}}$, maximal rate of pressure decrease in left ventricle; HR, heart rate; MAP, mean arterial pressure. There were no significant differences between both groups.

Fig. 3. Cardiac output (CO, A) and stroke volume (SV, B) at 4 h after completion of resuscitation. Animals underwent sham operation without heat shock (Sham) or with heat shock (H-Sham), traumahemorrhage and resuscitation without heat shock (Hem) or with heat shock (H-Hem). There were 7 animals in each group. Data are expressed as means ± SE and compared by one-way ANOVA and Tukey’s test. *P < 0.05 vs. sham-operated animals; #P < 0.05 vs. hemorrhaged rats.

Fig. 4. Maximum velocity of ICG clearance ($V_{\text{max}}$, A) and efficiency of ICG transport ($K_m$, B) at 4 h after completion of resuscitation. Animals underwent sham operation without heat shock (Sham) or with heat shock (H-Sham), trauma-hemorrhage and resuscitation without heat shock (Hem) or with heat shock (H-Hem). There were 7 animals in each group. Data are expressed as means ± SE and compared by one-way ANOVA and Tukey’s test. *P < 0.05 vs. sham-operated animals; #P < 0.05 vs. hemorrhaged rats.
Heat shock proteins and organ function after hemorrhage

Thermotolerance (15). This phenomenon has been closely linked to the induction of proteins belonging to the HSP family and most notably the highly stress-inducible HSP-70 (7, 18). A number of studies have shown that an increased synthesis of HSP-70 induced by hyperthermia plays a protective role against ischemia-reperfusion injury in the heart, lungs, and liver (6, 8, 14, 25). Induction of HSP-70 by heat stress has also been shown to reduce organ damage and enhance survival rates in a rat model of sepsis (12, 31). Furthermore, recent studies have shown that increased expression of inducible HSP-70 in transgenic mice protects myocardial function following ischemia-reperfusion injury (30). Thus it is becoming increasingly clear that expression of HSP-70 may play an important protective role following various adverse circulatory conditions. However, it remains unknown whether HSP-70 induction prior to hemorrhage has any protective effects on cardiac and hepatocellular functions following hemorrhage and resuscitation.

The results of this study indicate that left ventricular performance was significantly depressed after trauma-hemorrhage and resuscitation, as demonstrated by marked depression in \( +\frac{dP}{dt_{\text{max}}} \) and \( -\frac{dP}{dt_{\text{max}}} \). These findings confirm our previous results, which showed that cardiac function was significantly depressed after trauma-hemorrhage (33, 35). Prior heat stress, however, restored \( +\frac{dP}{dt_{\text{max}}} \) and significantly improved \( -\frac{dP}{dt_{\text{max}}} \) following trauma-hemorrhage. The improvement in left ventricular function was also reflected by the restored cardiac output and stroke volume. Furthermore, hepatocellular function was also significantly improved as evidenced by restoration of \( V_{\text{max}} \) and \( K_{m} \) of ICG clearance following trauma-hemorrhage in animals that underwent prior heat stress. Thus thermal preconditioning restores and maintains cardiovascular and active hepatocellular functions following trauma-hemorrhage and resuscitation.

Whereas systemic hyperthermia in itself may lead to injury, results from this study indicate that after a 24-h recovery period there was no difference in cardiovascular and hepatocellular functions between sham-operated animals that did or did not undergo a transient heat stress (41°C, 15 min). Studies have shown that a temperature increase of 4 to 5°C above the normal body temperature is necessary to induce the production of HSPs in mammalian cells (23). Previous studies have also shown that a temperature of 41°C is sufficient to trigger the stress response in the rat, as assessed by HSP-70 induction (5). Although it was previously unknown whether HSP-70 has any protective role after trauma-hemorrhage and resuscitation, the present study indicates that transient heat stress maintains...
cardiovascular and hepatocellular functions under such conditions. Moreover, animals in the heated group had evidence of abundant HSP-70 expression detected in the liver and heart, thus indicating adequate HSP induction.

HSP-70 is known to be upregulated by hemorrhagic shock itself. Previous studies of Schoeniger et al. (28) have shown that hepatic HSP-70 mRNA induction occurs in resuscitated hemorrhagic shock. Other studies have demonstrated that the degree of HSP-70 expression is dependent on the severity of shock (1). Those observations therefore raise the question of whether HSP-70 production following hemorrhagic shock also has any cytoprotective role on organ function or is only the result of the stress response because organ function is significantly decreased after hemorrhagic shock despite small accumulation of HSP-70. In this regard, recent studies by Nakano et al. (19) have shown that blockade of the endogenous increase in HSP-70 using antisense oligonucleotides has deleterious effects under hypoxic stress. Furthermore, reduction of HSP-70 following stress, as found in aging, diabetes, and Cushing syndrome, subjects, has also been implicated in decreased organ function and tissue repair (11, 16, 20). Thus expression of HSP-70 under stress could not only serve as a marker of stress but may also contribute to the mechanism of cell adaptation to a subsequent stress. Although we observed a slight but insignificant increase in HSP-70 induction in heart tissue at 4 h following trauma-hemorrhage and resuscitation, heart performance and cardiac output were still significantly depressed compared with animals undergoing prior induction of HSP-70. In view of this, the protective effects against organ dysfunction following trauma-hemorrhage may require a certain degree of HSP-70 expression. The exact amount of HSP-70 expression required to protect the organ following adverse circulatory conditions, however, remains unknown.

Although regulation of HSP production appears critically important to cell survival, the mechanism regarding how these proteins function in protecting cellular integrity is not yet fully understood. Studies have suggested that heat shock and HSP-70 are able to protect cells and tissues by regulating cytokine production (27, 29). It also has been shown that HSP decreases the release of TNF-α in an in vivo model of endotoxemia (24). Riberio et al. (24) have suggested that the decrease in TNF-α protein release by heat stress is attributed to posttranscriptional control of TNF-α (24). A study by Feinstein et al. (9) demonstrated that HSP-70 may reduce inflammation by decreasing nuclear factor (NF)-κB activation, preventing intranuclear translocation of NF-κB and thereby also accomplishing pretranscriptional inhibition of TNF-α production. Studies have also shown that HSP-70 binds to NF-κB in the cytosol and prevents its translocation to the nucleus (9). Moreover, it has been shown that NF-κB appears to have a major role in the transcriptional activation of several cytokines involved in hemorrhagic shock (26). Thus heat stress induction of HSP-70 appears to provide its protective effects by binding cytosolic NF-κB, thereby limiting destructive inflammation through downregulation of proinflammatory cytokines. Previous studies from our laboratory have demonstrated that circulating TNF-α as well as IL-6 was significantly elevated following trauma and hemorrhage (2). The present results also show a significant increase in plasma levels of TNF-α and IL-6 at 4 h after hemorrhage and resuscitation. Previous studies have shown that circulating levels of TNF-α may contribute to the pathogenesis of depressed left ventricular contractility and hepatocellular function (32, 36). Walley et al. (32) demonstrated a significant decrease in left ventricular performance following TNF-α infusion. Moreover, it has been demonstrated that administration of TNF-α at a dose that did not significantly affect cardiac output and blood pressure produced hepatocellular dysfunction and increased circulating level of IL-6 (36). Heat stress prior to hemorrhage shock, however, downregulated TNF-α and IL-6 to values that were not significantly different from those of sham-operated animals. Thus it appears that the beneficial effects of transient preheating on cardiovascular and hepatocellular functions after trauma-hemorrhage may be through the downregulation of proinflammatory cytokines TNF-α and IL-6.

Although we have shown that circulating levels of IL-6 after trauma-hemorrhage were decreased by prior heat stress, other studies have shown that IL-6 production was upregulated in vitro by induction of HSP-70 (10, 22). To this end, Parikh et al. (22) suggested that IL-6 production may be differentially regulated in different cell populations. In this regard, our previous studies have shown that Kupffer cells are the major source for the release of IL-6 following trauma-hemorrhage (21). Thus it is possible that induction of HSP-70 reduces the production of IL-6 in Kupffer cells following trauma-hemorrhage. However, further studies are required to evaluate this possibility and to determine the precise mechanism of the salutary effects of HSP-70 on organ function following trauma-hemorrhage. It should be mentioned that we utilized a model of trauma (i.e., laparotomy) and hemorrhage to have a more clinically relevant model, because most trauma victims sustain a combination of soft tissue injury and major blood loss.

In summary, our results indicate that transient prior heat stress significantly attenuates cardiac and hepatic dysfunction following trauma and hemorrhage and fluid resuscitation. These salutary effects were associated with decreased circulating levels of TNF-α and IL-6. Western blot analysis also demonstrated that heat stress increased the abundance of HSP-70 in the liver and heart. Our data therefore suggest that prior induction of HSP-70 protects cardiovascular and hepatocellular functions following trauma-hemorrhage and resuscitation.

Perspectives

Cells and organisms are equipped with primitive mechanisms to carry out cellular protection against
exogenous stress. In this regard, it has been shown that one of the most conserved mechanisms is the expression of a family of polypeptides, called HSPs. In the present study, we have shown that preinduction of HSP-70 by brief hyperthermia conferred significant protection against organ dysfunction following trauma and severe hemorrhagic shock. Although the exact mechanism responsible for the protective effects of HSPs remains to be determined, the present and other studies indicate that this family of polypeptides has important endogenous protective capacity. Although it remains unknown whether induction of HSPs following trauma-hemorrhage and other adverse circulatory conditions has any salutary effects, it appears that induction of such proteins prior to complicated elective surgery should decrease the incidence of postoperative complications.

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