Effects of heat shock, stannous chloride, and gallium nitrate on the rat inflammatory response

S.D. House,¹ P.T. Guidon Jr,¹ G.A. Perdrizet,³ M. Rewinski,³ R. Kyriakos,¹ R.S. Bockman,⁴ T. Mistry,¹ P.A. Gallagher,¹ and L.E. Hightower²

¹Department of Biology, Seton Hall University, South Orange, NJ 07079, USA

²Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA

³Hartford Hospital, Hartford, CT 06102, USA

⁴Hospital for Special Surgery, Cornell University Medical College, New York, NY 10028, USA

Abstract Heat and a variety of other stressors cause mammalian cells and tissues to acquire cytoprotection. This transient state of altered cellular physiology is nonproliferative and antiapoptotic. In this study, male Wistar rats were stress conditioned with either stannous chloride or gallium nitrate, which have immunosuppressive effects *in vivo* and *in vitro*, or heat shock, the most intensively studied inducer of cytoprotection. The early stages of inflammation in response to topical suffusion of mesentery tissue with formyl-methionyl-leucyl-phenylalanine (FMLP) were monitored using intravital microscopy. Microvascular hemodynamics (venular diameter, red blood cell velocity [V_{rbc}], white blood cell [WBC] flux, and leukocyte-endothelial adhesion [LEA]) were used as indicators of inflammation, and tissue levels of inducible Hsp70, determined using immunoblot assays, provided a marker of cytoprotection. None of the experimental treatments blocked decreases in WBC flux during FMLP suffusion, an indicator of increased low-affinity interactions between leukocytes and vascular endothelium known as rolling adhesion. During FMLP suffusion LEA, an indicator of firm attachment between leukocytes and vascular endothelial cells increased in placebo and gallium nitrate-treated animals but not in heat- and stannous chloride-treated animals, an anti-inflammatory effect. Hsp70 was not detected in aortic tissue from placebo and gallium nitrate-treated animals, indicating that Hsp70-dependent cytoprotection was not present. In contrast, Hsp70 was detected in aortic tissues from heat- and stannous chloride-treated animals, indicating that these tissues were in a cytoprotected state that was also an anti-inflammatory state.

INTRODUCTION

The heat shock response protects cells from exposures to temperatures lethal to naïve animals, a phenomenon known as thermotolerance (Gerner and Schneider 1975; Mitchell et al 1979; Li and Werb 1982; Landry and Chretien 1983). Reciprocal and nonreciprocal cross-protection has been observed between pairs of a variety of stressors in addition to heat, and the altered physiological state induced by these stressors has been termed more generally cytoprotection. The existence of cross-protection has suggested a medical application of cytoprotection in which this state is induced pharmacologically prior to

elective surgery to protect tissues against ischemia/reperfusion injury. Ischemic injury *per se* induces cytoprotection in several animal model systems (Currie and White 1981; Dillman et al 1986; Mehta et al 1988), and the heat shock response protects against ischemic injury (Barbe et al 1988; Currie et al 1988) as well as against other forms of metabolic injury (Chopp et al 1989; Yellon and Latchman 1992; Currie et al 1993; Das et al 1995; Knowlton 1995).

Inflammation is a major component of ischemia/reperfusion injury, and previous studies suggest an anti-inflammatory role for Hsp70 in blood vessels. For example, the induction and accumulation of Hsp70 in vascular endothelial cells accompanies the prevention of necrosis induced by activated human polymorphonuclear leukocytes (Wang et al 1995). Furthermore, induction of Hsp70 accompanies a reduction in the number of adherent and

Received 22 February 2000; Revised 16 February 2001; Accepted 20 February 2001.

Correspondence to: Dr Steven D. House, Tel: 973 761-7947; Fax: 973 761-9596; E-mail: houseste@shu.edu.

migrated leukocytes measured using intravital microscopy in an ischemia/reperfusion model system (Chen et al 1996). This same group showed that lipopolysaccharide-induced microvascular injury was reduced in thermotolerant rats in which leukocyte-endothelial adhesion (LEA) and migration decreased (Chen et al 2001).

To test the hypothesis that cytoprotection is an anti-inflammatory state and that stress proteins, particularly Hsp70, may play a role, rats were stress conditioned using either heat shock or stannous chloride or gallium nitrate, each of which has immunosuppressive activity. The accumulation of Hsp70, measured using Western blots, was used as a marker for the cytoprotected state. Inflammatory responses in blood vessels were studied using intravital microscopy to monitor hemodynamic parameters after addition of formyl-methionyl-leucyl-phenylalanine (FMLP) to exposed mesentery tissue, which stimulates the initial stages of inflammation. A preliminary report of these studies has been published (Hightower et al 2000).

In vivo studies of the microcirculation indicate that white blood cells (WBCs) may roll along the venular wall at a constant velocity or endure brief intermittent and multiple contacts under physiological conditions in all tissues (gastrointestinal mucosa, skin, lung) that are continually exposed to external inflammatory stimuli (physical and/or chemical) (Grant 1973; House and Lipowsky 1987, 1988; Perry and Granger 1991; Nazzola and House 1992; Granger and Kubes 1994). During more severe inflammatory reactions, leukocytes may adhere firmly to the venular endothelium and migrate across the endothelium into the traumatized tissue (Grant 1973; House and Lipowsky 1987). Leukocyte-endothelium interactions are regulated by flow dynamics (Perry and Granger 1991; Nazzola and House 1992) and by various adhesion receptors found on both leukocytes and vascular endothelium (Tonnesen et al 1989; Lawrence and Springer 1991; Smith 1993).

MATERIALS AND METHODS

Experimental animals

Adult male Wistar rats, ranging from 200–250 g at the time of the experiment, were obtained from Charles River Laboratories (Kingston, NY, USA) 1 week before the experiment and maintained on a 12:12-hour light:dark cycle (lights on 600–1800).

Animal preparation and intravital microscopy

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (45 mg/kg). A tracheostomy was performed to facilitate respiration. The carotid artery and

jugular vein were cannulated for blood pressure measurements.

Rats were placed on a heating pad, and mesentery tissue was exposed, pulled through a midsagittal abdominal incision, and draped over a transparent Plexiglas shelf. Preparations were continuously suffused with an isotonic bicarbonate ringer-gelatin solution buffered to pH 7.4 and maintained at 37°C. The intestine was covered with moist cotton, and areas outside the region of interest were covered with Saran Wrap (Dow Corning, Corning, NY, USA).

The rat mesentery was observed with a Nikon UM3 metallurgic microscope adapted for intravital microscopy. Either a Nikon 10× water immersion lens (N.A. 0.3) providing a field of view 195 µm wide on a Panasonic TR-930A high-resolution video monitor (heat shock experiments) or a Nikon 20× water immersion lens (N.A. 0.4) providing a field of view 138 µm wide on the same monitor (metal salts experiments) was used. All microcirculatory events were recorded using a Panasonic VWCD-52 video camera and a Mitsubishi videocassette recorder.

Microvessel diameter and length were measured using the video image shearing technique (IPM Model 908). V_{rbc} along the centerline of venules was measured using the 2-slit techniques (Johnson and Wayland 1967), using a self-tracking velocity correlator (IPM Model 102B-C). Measurements of marginating leukocyte flux and WBC velocity were obtained through variable-speed playback of video recordings. Flux was determined by counting the number of leukocytes rolling past a line drawn perpendicular to the vessel of interest during a 30-second interval. Leukocytes traveling at a velocity less than that of the erythrocytes in the same flow stream were included. Leukocytes sticking to the endothelium for more than 5 seconds during the duration of the hemodynamic measurements were counted as adhering WBCs and were expressed as number per 100 µm vessel length. Previous experiments in our laboratory indicate that greater than 94% of the cells that adhere for more than 5 seconds adhere for at least 30 seconds (House et al 1997). The shorter time period was selected because LEA for even 1 second has a significant impact on vascular resistance (House and Lipowsky 1987, 1988).

Microvascular hemodynamics were measured during a 2-minute control period of suffusion with ringer-gelatin solution and during a 2-minute suffusion period with 10^{-7} M FMLP (Sigma, St Louis, MO, USA) to induce adhesion and the initial stages of acute inflammation. FMLP was prepared daily by dissolving in dimethyl sulfoxide (DMSO) and diluting in ringer-gelatin. The concentration of DMSO in the suffusion was found to have no effect on leukocyte-endothelium interactions or microhemodynamics (House and Lipowsky 1987). A period of 10–15

minutes was allotted between successive runs with a maximum of 4 runs per animal.

Heat shock treatment

Animals were anesthetized as described previously and were placed on a heating pad set on the "medium" setting. A rectal thermometer was inserted into the animal, and the temperature of the animal was monitored. Once the core temperature of the animal reached 42°C, the heating pad was either switched to "low" or shut off in order to keep the animal as close to 42°C as possible for 15 minutes (Guidon and Hightower 1986). The animal's temperature did not exceed 42.5°C, a temperature that results in an increased mortality. After a 2-day recovery period, the animals were subjected to analysis using intravital microscopy as described previously. Sham-treated animals were anesthetized and placed in a heating pad without application of heat.

Gallium nitrate treatment

Gallium nitrate (Sigma) was administered via subcutaneous injections above the abdomen once a day for 5 days, including the day of the experiment. The dose of each injection was 50 mg/kg, a dose that was chosen to maximize any potential effects of gallium nitrate. Phosphate buffered saline was substituted for gallium nitrate in control animals (placebo group).

Stannous chloride treatment

Stannous chloride (Sigma) was administered via an intraperitoneal injection 16 hours prior to experimentation. The dose of the injection was 0.15 mg/kg, a dose that had previously been demonstrated to elicit anti-inflammatory activities in rabbits (Williams et al 2001). Water for injection was substituted for stannous chloride in control animals (placebo group).

Gel electrophoresis and immunoblotting

Rat tissue was recovered, immediately snap-frozen in liquid nitrogen, and stored at -80°C until homogenization was performed as described previously (Hotchkiss et al 1993). Approximately 75–100 mg of rat tissue were homogenized in 2 mL buffer (40 mM Tris-HCl pH 6.8, 10% glycerol, 2% sodium dodecyl sulfate [SDS] + protease inhibitors [iodoacetamide, phenylmethylsulfonyl fluoride, and leupeptin]) using a VirTishear Tempest Homogenizer (The VirTis Company, Gardiner, NY, USA), heated at 95°C for 4 minutes and then centrifuged for 10 minutes at 3000 × g. After determining protein concentration of the homogenates using the BCA Protein Assay (Pierce, Rock-

ford, IL, USA), homogenate protein concentrations were normalized to 2 mg/mL in 6× sample buffer (Tris-HCl, SDS, 2-mercaptoethanol, glycerol, and bromophenol blue). After boiling tissue homogenates in sample buffer for 3 minutes, 10 µg/5 µL/lane were loaded onto 10% or 12% acrylamide separating gels using the Mighty Small II mini-gel system (Hoefer, Amersham Pharmacia Biotech Inc, Piscataway, NJ, USA). The proteins in the separating gel were then transferred onto an Immobilon-P PVDF membrane (Millipore, Bedford, MA, USA) using a semi-dry electroblotter (Alltech, Deerfield, IL, USA). The blots were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS, 0.1M Tris/0.15M NaCl, pH 7.4) for 1 hour at room temperature and washed 3 times in 0.1% Tween-20/TBS (TTBS). Mouse monoclonal antibody to inducible Hsp70 (SPA810, StressGen, Victoria, BC, Canada), at a dilution of 1:1000, was then incubated with the blots for 1 hour at room temperature. After 3 washes in TTBS, the blots were incubated with horseradish-peroxidase conjugated goat anti-mouse antibody (Accurate Chemical, Westbury, NY, USA) for 1 hour at room temperature. Following 3 final washes in TTBS, the blots were processed with an enhanced chemiluminescence reagent (Amersham) and exposed to film.

Statistical methods

Statistical analyses for differences were performed by paired and unpaired *t*-tests and the nonparametric Wilcoxon and Mann-Whitney tests. In all tests, significance was assessed at the 95% confidence level ($P < 0.05$).

RESULTS

Hemodynamic studies

The effects of either heat shock or gallium nitrate treatment or stannous chloride treatment on leukocyte-endothelial interactions were determined using intravital microscopy. Placebo animals were analyzed for all 3 test groups: heat shock, gallium, and SnCl₂. Because there were no significant differences between the 3 placebo groups, these data are combined into 1 placebo group ($n = 42$). The average venular diameter for the placebo group maintained under control conditions was 24.4 ± 0.9 (SE) µm. No significant differences were observed among the 4 groups of animals under control conditions. FMLP treatment had no significant effect on average venular diameter compared to control conditions for any of the groups (data not shown).

The average V_{rc} in the placebo group was 2.2 ± 0.2 mm/s under control conditions. Neither heat shock nor gallium nitrate treatment nor stannous chloride treatment had a significant effect under control conditions. Also,

Fig. 1. Effects of heat shock ($n = 34$), gallium nitrate treatment ($n = 44$), and stannous chloride treatment ($n = 28$) on white blood cell (WBC) flux compared to placebo group ($n = 42$) during control conditions (unfilled columns) and during exposure to formyl-methionyl-leucyl-phenylalanine (FMLP) (filled columns). All values are 30-second averages. Vertical bars represent mean $\pm$ standard error. * denotes significantly different ($P \leq 0.05$) from control conditions.

Fig. 2. Effects of heat shock, gallium nitrate treatment, and stannous chloride treatment on leukocyte-endothelial adhesion (LEA) compared to placebo group during control conditions (unfilled columns) and exposure to formyl-methionyl-leucyl-phenylalanine (FMLP) (filled columns). Vertical bars represent mean $\pm$ standard error. * denotes significantly different ($P \leq 0.05$) from control conditions. # denotes significantly different ($P \leq 0.05$) from placebo group.

there were no significant differences between control conditions and FMLP treatment observed between groups (data not shown).

The effects of either heat shock or gallium nitrate treatment or stannous chloride treatment on WBC flux compared to the placebo group under control conditions (unfilled columns) and during FMLP suffusion (filled columns) are shown in Figure 1. The average WBC flux in the placebo group was 17.2 ± 1.8 cells/30 s under control conditions. No significant differences were observed between groups under control conditions, indicating that the stress conditioning regimens did not affect WBC flux in the absence of a proinflammatory stimulus. However, in all 4 groups of animals, FMLP suffusion resulted in a significant decrease in WBC flux (columns marked by *), which was 7.8 ± 1.2 cells/30 s for the placebo group. No significant differences in the magnitude of this reduced flux were observed between groups. It was concluded that WBC flux in the stress-conditioned animals decreased during the FMLP proinflammatory stimulus in a fashion similar to that of placebo animals.

The effects of either heat shock or gallium nitrate treatment or stannous chloride treatment on LEA compared to the placebo group under control conditions (unfilled columns) and during FMLP suffusion (filled columns) are shown in Figure 2. The average LEA in the placebo group was 3.1 ± 0.4 cells/100 μm under control conditions. No significant differences were observed between groups under control conditions, indicating that the stress-conditioning regimens did not affect LEA in the absence of the

FMLP proinflammatory stimulus. During FMLP suffusion, average LEA increased significantly in the placebo group (6.4 ± 0.6 cells/100 μm) and in the gallium nitrate-treated group (columns marked by *). It was concluded that these groups mounted a proinflammatory response characterized by increased numbers of leukocytes firmly attached to vascular endothelium. In contrast, LEA did not increase in either heat-shocked or stannous chloride-treated animals during FMLP suffusion (columns marked by #, indicating significantly different from FMLP-suffused placebo group).

Immunoblot assays for inducible Hsp70

The presence of Hsp70 in stress-conditioned tissues was used as a marker for the cytoprotected state. For each stress-conditioning protocol, the sampling time for immunoblot analysis was chosen to correspond to the times used in the hemodynamic studies. These times were chosen on the basis of previous studies involving each of the treatments indicating effects on immune responses for gallium nitrate and cytoprotective effects for heat shock and stannous chloride. Monoclonal antibody SPA 810, specific for inducible Hsp70, was used to detect rat Hsp70 on these blots. Inducible Hsp70 was not detected in samples of aortic tissue taken from either placebo or gallium nitrate-treated animals (data not shown).

The results of immunoblot analysis of the induction of Hsp70 in vivo by whole-body hyperthermia in tissue samples from lung, liver, kidney, and aorta are shown in

Fig. 3. Induction of Hsp70 in vivo by whole-body hyperthermia. Rats were subjected to heat shock or given sham treatments as described in the Materials and Methods section. Inducible Hsp70 was identified in samples from a variety of rat tissues using SDS-PAGE and immunoblotting as described in the Materials and Methods section. The portion of the immunoblot containing the 70-kDa region of the gels is shown here. Lanes 2–5 contain samples from the same heat-shocked animal, and lanes 10–13 contain samples from a second heat-shocked animal. Lanes 6–9 contain samples from the same sham-treated animal. Lane designations: lane 1, purified recombinant human inducible Hsp70-1 used as a marker; lanes 2, 6, 10, lung; lanes 3, 7, 11, liver; lanes 4, 8, 12, kidney; lanes 5, 9, 13, aorta.

Figure 3. No Hsp70 was detected in liver, kidney, and aorta, and only a trace amount was detected in lung tissue (lane 6) from a sham animal. Substantial amounts of Hsp70 were detected in all the sampled tissues from heat-shocked animals (lanes 2–5 and lanes 10–13 are data sets from separate animals). Based on the presence of Hsp70, it was concluded that these tissues are likely to be in a cytoprotected state.

The results of immunoblot analysis of the induction of Hsp70 in vivo by stannous chloride treatment in tissue samples from lung, liver, kidney, and aorta are shown in Figure 4. No Hsp70 was detected in liver, kidney, and aorta from a placebo animal, but, as in the sham animal described previously, a small amount was detected in lung tissue (lane 10). Increased levels of Hsp70 were detected in lung (lanes 2 and 5) and aorta (lanes 6 and 9) from stannous chloride-treated rats. Unlike the heat-shocked tissues, elevated levels of Hsp70 were not detected in liver and kidney samples. Lanes 2–5 and lanes 6–9 are data sets from separate treated animals. The immunoblot data for heat-shocked and stannous chloride-treated animals have been replicated independently in 2 different laboratories.

DISCUSSION

Using the technique of intravital microscopy, the real-time effects of exogenously added agents on the inflammatory

response can be quantified. The inflammatory agent FMLP causes a rapid and dramatic change in WBC flux and leukocyte adhesion in rats. Specifically, transient addition of FMLP causes a reduction of WBC flux and more than doubles the number of adherent leukocytes (LEA) in venules. This is consistent with previous experiments in our laboratory (Nazziola and House 1992; House et al 1997). The increased adhesion after exposure to the chemoactivator FMLP has been attributed to conformational changes in or increased surface expression of the integrins CD11/CD18 on polymorphonuclear leukocytes (Tonnesen et al 1989; Graham and Brown 1991). FMLP also stimulates the production of LTB_4 and PAF from leukocytes and endothelial cells, both of which stimulate adhesion receptors (Tonnesen et al 1989; Graham and Brown 1991).

The results presented here indicate that exposing an animal either to a heat shock (42°C for 15 minutes) followed by 2 days of recovery or to stannous chloride for 16 hours prior to exposure to FMLP eliminated the increase in LEA. Interestingly, the decrease in WBC flux was almost identical after FMLP treatment either with or without stress conditioning by either of these stressors. This result suggests that although the WBCs did respond to the FMLP challenge, probably by undergoing increased rolling along vascular endothelium, subsequent firm adherence to blood vessel walls (LEA) in the mesentery was

Fig. 4. Induction of Hsp70 in vivo by $SnCl_2$. Rats were treated with stannous chloride or sham-treated as described in the Materials and Methods section. Inducible Hsp70 was identified in samples from a variety of rat tissues using SDS-PAGE and immunoblotting as described in the Materials and Methods section. The portion of the immunoblot containing the 70-kDa region of the gels is shown here. Lanes 2–5 contain samples from the same stannous chloride-treated animal, and lanes 6–9 contain samples from a second stannous chloride-treated animal. Lanes 10–13 contain samples from the same sham-treated animal. Lane designations: lane 1, purified recombinant human inducible Hsp70-1 used as a marker; lanes 2, 6, 10, lung; lanes 3, 7, 11, liver; lanes 4, 8, 12, kidney; lanes 5, 9, 13, aorta.

attenuated or blocked. The mechanism by which heat shock and stannous chloride treatment attenuates LEA is unknown, but 2 reasonable hypotheses are that upregulation of adhesion molecules on the surface of leukocytes and/or vascular endothelial cells may be blocked and/or that production of the mediators that stimulate this upregulation may be attenuated.

In a recent review, Perdrizet (1997) reached the conclusion that the heat shock proteins themselves are clearly proinflammatory, whereas the heat shock response appears to be anti-inflammatory in nature. Inside cells, heat shock proteins contribute to the cytoprotected state, but when they are released from cells, they become proinflammatory cytokines (see *Cell Stress & Chaperones* 5[4] and 5[5] for recent papers and abstracts). Polla and co-workers emphasized the potential protective intracellular effects of Hsps against the cytotoxic effects of mediators such as TNF α and reactive oxygen species (reviewed in Polla and Cossarizza 1996). It has been previously demonstrated that the heat shock response inhibits the systemic in vivo inflammatory responses of rats treated with endotoxin (Klosterhalfen et al 1997), whole lungs of rats treated with endotoxin (Hauser et al 1996), and the proinflammatory responses in alveolar macrophages (Ribeiro et al 1996). In addition, while LEA is partially affected by changes in leukocytes after exposure to an inflammatory agent, vascular endothelial cells also play a critical role in the interaction, and the heat shock response influences/changes endothelial cell function (Conway et al 1994; DeMeester et al 1997).

Much less is known about the effects of stannous chloride on inflammatory responses. In the stress response field, stannous chloride is best known as an inducer of heme oxygenase, the rate-limiting enzyme in heme degradation (Kappas and Maines 1976; Neil et al 1995). It was reported previously that stannous chloride induces Hsp70 mRNA in human hepatoma cells (Mitani et al 1993), but to our knowledge this is the first report of the induction of Hsp70 protein by stannous chloride. Perdrizet and colleagues have obtained evidence that stress-conditioning rabbits and rats with stannous chloride induces cytoprotection and an anti-inflammatory condition (Perdrizet, unpublished observations; Williams et al 2001).

Regarding the use of Hsp70 as a marker for cytoprotection, we acknowledge that not all forms of inducible cytoprotection depend on Hsp70 induction (reviewed in Hightower et al 1999). Thus, we must qualify its use here to indicate that Hsp70-dependent mechanisms of cytoprotection are induced by heat shock and stannous chloride treatment. In the case of gallium nitrate treatment, we can rule out the presence of this form of cytoprotection in the time frame sampled, but the existence of an Hsp70-independent form of cytoprotection cannot be ruled out.

Numerous studies, mostly using cultured mammalian cells, have shown that cells in the cytoprotected state are at least transiently nonproliferative. More recent studies have shown that this is also an antiapoptotic state. Combined with the data reported here that the heat shock responses establishes an anti-inflammatory state, all 3 of these characteristics suggest a state of unresponsiveness in cytoprotected cells. Two possible mechanisms for this unresponsiveness, not mutually exclusive, have found experimental support recently. One possibility is that Hsps block signal transduction pathways involved in these responses. In support of this possibility, Sherman and colleagues have found that apoptosis is suppressed in cells overexpressing Hsp70 and that this suppression can be fully explained by a rapid inactivation of stress kinase JNK in the apoptotic signaling pathway (Volloch et al 2000). A second possibility is that the production of proinflammatory mediators such as chemokines that activate inflammatory responses of monokines and endothelial cells have been inhibited. To this end, Wong and coworkers obtained data suggesting that heat induction of the inhibitory regulator I- κ B inhibits the activation of the proinflammatory transcription factor NF- κ B (Wong et al 1999). Also in support of this latter possibility, heat shock transcription factor Hsf1 acts as a transcriptional repressor for genes encoding several proinflammatory cytokines, including IL1 β and TNF α (Xie et al 1999), adding to a previous study showing that inducers of the heat shock response downregulate IL1 β synthesis (Schmidt and Abdulla 1988).

Gallium nitrate treatment presented an interesting negative control for the Hsp70-dependent cytoprotective and anti-inflammatory effects observed in heat-shocked and stannous chloride-conditioned rats. Gallium nitrate has a range of positive effects in immunological settings, the mechanisms of which are unclear. Based on the studies presented here, we conclude that they do not involve the induction of Hsp70-dependent cytoprotection and the inhibition of adhesion of leukocytes to the endothelium during the early stages of inflammation. Gallium nitrate inhibits the release of the inflammatory mediators IL-6, TNF- α , and nitric oxide from activated macrophage-like RAW 264 cells (Makkonen et al 1995). When coupled to transferrin, gallium inhibits the mitogen-induced proliferative response of peripheral blood mononuclear cells; in the same study, administration of gallium significantly prolonged survival in mice undergoing severe graft-vs-host disease (Drobyski et al 1996). Similar to the effects seen with administration of the anti-CD4 monoclonal antibody, gallium nitrate promotes long-term survival in murine cardiac allograft recipients, although the allografts display histopathologic signs of ongoing inflammation (Orosz et al 1996, 1997). Also, gallium nitrate stimulates the expression of intracellular adhesion mole-

cule 1 on gonadal vein endothelial cells but has no direct suppressive effects on endothelial cells. These results (Huang et al 1994) and those presented here suggest that inflammatory responses associated with endothelial cell function, such as tissue repair, remain intact in the presence of gallium nitrate.

It is interesting to note that blood vessels are returning to center stage in studies of cytoprotection in intact animals and tissues. 'Returning' is the appropriate word choice because the studies of Fredric White done 20 years ago (White 1980a, 1980b) showed that cells associated with the brain microvasculature are among the most stress-responsive cells in explants and in heat-shocked rats. Based in part on these studies, it was suggested that inflammatory responses could be a major venue in mammals for the response known historically as the heat shock response (Hightower and White 1981).

ACKNOWLEDGMENTS

This research was funded by Public Health Service Grants AR41581-01 to P.T.G. and R29 HL-44914 for S.D.H. and by a contract from StressGen Biotechnologies Corp.

REFERENCES

- Barbe MF, Tytell M, Gower DJ, Welch WJ. 1988. Hyperthermia protects against light damage in the rat retina. *Science* 241: 1817-1820.
- Chen G, Kelly C, Chen H, Leaky A, Bouchier-Hayes D. 2001. Thermotolerance protects against endotoxin-mediated microvascular injury. *J Surg Res* 95: 79-84.
- Chen G, Kelly C, Stokes K, Leahy A, Bouchier-Hayes D. 1996. Induction of heat shock protein 72 attenuates ischemia/reperfusion-induced microvascular injury. Association for Academic Surgery, 30th Annual Meeting, Chicago, IL. November 13-16, 1996. Abstract 77.
- Chopp M, Chen H, Ho IK, Dereski MO, Brown E, Hetzel FW, Welch KMA. 1989. Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat. *Neurology* 39: 1396-1398.
- Conway EM, Liu I, Nowakowski B, Steiner-Mosonyi M, Jackman RW. 1994. Heat shock of vascular endothelial cells induces an up-regulatory transcriptional response of the thrombospondulin gene that is delayed in onset and does not attenuate. *J Biol Chem* 269: 22804-22810.
- Currie RW, Karmazyn M, Kloc M, Mailer K. 1988. Heat-shock response is associated with enhanced post-ischemic ventricular recovery. *Circ Res* 63: 543-549.
- Currie RW, Tanguay RM, Kingma JG. 1993. Heat-shock response and limitation of tissue necrosis during occlusion/reperfusion in rabbit hearts. *Circulation* 87: 963-971.
- Currie RW, White FP. 1981. Trauma-induced protein in rat tissues: a physiological role for a "heat shock" protein? *Science* 214: 72-73.
- Das DK, Maulik N, Moreau IL. 1995. Gene expression in acute myocardial stress. Induction by hypoxia, ischemia, reperfusion, hyperthermia and oxidative stress. *J Mol Cell Cardiol* 27: 181-193.
- DeMeester SL, Buchman TG, Qiu Y. 1997 Heat shock induces IkB α and prevents stress-induced endothelial cell apoptosis. *EMBO J* 12: 2773-2779.
- Dillman WH, Mehta HB, Barrieux A, Guth BD, Neeley WE, Ross J. 1986. Ischemia of the dog heart induces the appearance of a cardiac mRNA coding for a protein with migration characteristics similar to heat-shock/stress protein 71. *Circ Res* 59: 110-114.
- Drobyski WR, Ul-Haq R, Majewski D, Chitamber CR. 1996. Modulation of in vitro and in vivo T-cell responses by transferrin-gallium and gallium nitrate. *Blood* 88: 3056-3064.
- Gerner EW, Schneider MJ. 1975. Induced thermal resistance in HeLa cells. *Nature* 256: 500-502.
- Graham LL, Brown EJ. 1991. Extracellular calcium results in a conformational change in Mac-1 (CD11b/CD18) on neutrophils: differentiation of adhesion and phagocytosis functions of Mac-1. *J Immunol* 146: 685-691.
- Granger DN, Kubes P. 1994. The microcirculation and inflammation modulation of leukocyte-endothelial cell adhesions. *J Leuk Biol* 55: 662-675.
- Grant L. 1973. The sticking and emigration of white blood cells in inflammation. In: *The Inflammatory Process*, ed Zweifach BW, Grant L, McClusky R. Academic Press, Orlando, FL, 205-249.
- Guidon PT Jr, Hightower LE. 1986. Purification and initial characterization of the 71 kilodalton heat shock protein and its cognate from rat liver. *Biochemistry* 25: 3231-3239.
- Hauser GJ, Dayao EK, Wasserloos K, Pitt BR, Wong HR. 1996. Heat shock protein induction inhibits inducible nitric oxide synthetase mRNA expression and attenuates hypotension in endotoxin-challenged rats. *Am J Physiol* 271: H2529-H2535.
- Hightower LE, Brown MA, Renfro JL, Perdrizet GA, Rewinski M, Guidon PT Jr, Mistry T, House SD. 2000. Tissue-level cytoprotection. *Cell Stress Chaperones* 5: 412-414.
- Hightower LE, Norris CE, diIorio PJ, Fielding E. 1999. Heat shock responses of closely related species of tropical and desert fish. *Am Zool* 39: 877-888.
- Hightower LE, White FP. 1981. Cellular responses to stress: comparison of a family of 71-73 kilodalton proteins rapidly synthesized in rat tissue slices and canavanine-treated cells in culture. *J Cell Physiol* 108: 261-275.
- Hotchkiss R., Nunnally I, Lindquist S, Taulien J, Perdrizet G, Karl I. 1993. Hyperthermia protects mice against the lethal effects of endotoxin. *Am J Physiol Regul Integrative Comp Physiol* 265: R1447-R1457.
- House SD, Lipowsky HH. 1987. Leukocyte-endothelium adhesion: microhemodynamics in mesentery of the cat. *Microvasc Res* 34: 363-379.
- House SD, Lipowsky HH. 1988. In vivo determination of the force of leukocyte-endothelium adhesion in the mesenteric microvasculature of the cat. *Circ Res* 63: 658-668.
- House SD, Ruch S, Koscienski WF III, Rocholl CW, Moldow RL. 1997. Effects of the circadian rhythm of corticosteroids on leukocyte-endothelium interaction in the AM and PM. *Life Sci* 60: 2023-2034.
- Huang EH, Gabler DM, Krecic ME, Gerber N, Ferguson RM, Orosz CG. 1994. Differential effects of gallium nitrate on T lymphocyte and endothelial cell activation. *Transplantation* 58: 1216-1222.
- Johnson PC, Wayland H. 1967. Regulation of blood flow in single capillaries. *Am J Physiol* 212: 1405-1415.
- Kappas A, Maines MD. 1976. Tin: a potent inducer of heme oxygenase in kidney. *Science* 192: 60-62.
- Klosterhalfen B, Hauptmann S, Offner F-A. 1997. Induction of heat shock protein 70 by zinc-bis-(DL-hydrogenaspartate) reduces cytokine liberation, apoptosis, and mortality rate in a rat model of LD100 endotoxemia. *Shock* 7: 254-262.

- Knowlton AA. 1995. The role of heat shock proteins in the heart. *J Mol Cell Cardiol* 27: 121–131.
- Landry J, Chretien P. 1983. Relationship between hyperthermia induced heat shock proteins and thermotolerance in Morris hepatoma cells. *Can J Biochem* 61: 428–437.
- Lawrence MB, Springer TA. 1991. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell* 65: 859–873.
- Li GC, Werb Z. 1982. Correlation between the synthesis of heat shock proteins and the development of thermotolerance in Chinese hamster fibroblasts. *Proc Natl Acad Sci U S A* 79: 3918–3922.
- Makkonen N, Hirvonen MR, Savolainen K, Lapinjoki S, Monkkinen J. 1995. The effect of free gallium and gallium in liposomes on cytokine and nitric oxide secretion from macrophage-like cell *in vitro*. *Inflamm Res* 44: 523–528.
- Mehta HB, Popovitch BK, Dillmann WH. 1988. Ischemia induces changes in the level of mRNAs coding for stress protein 71 and creatine kinase M. *Circ Res* 63: 512–517.
- Mitani K, Fujita H, Fukuda Y, Kappas A, Sassa S. 1993. The role of inorganic metals and metalloporphyrins in the induction of haem oxygenase and heat-shock protein 70 in human hepatoma cells. *Biochem J* 290: 819–825.
- Mitchell HK, Moller G, Petersen NS, Lipps-Sarmiento L. 1979. Specific protection from phenocopy induction by heat shock. *Dev Genet* 1: 181–192.
- Nazziola E, House SD. 1992. Effects of hydrodynamics and leukocyte-endothelium specificity on leukocyte-endothelium interactions. *Microvasc Res* 44: 127–142.
- Neil TK, Abraham NG, Levere RD, Kappas A. 1995. Differential heme oxygenase induction by stannous and stannic ions in the heart. *J Cell Biochem* 57: 409–414.
- Orosz CG, Wakely E, Bergese SD, VanBuskirk AM, Ferguson RM, Mullet D, Apseloff G, Gerber N. 1996. Prevention of murine cardiac allograft rejection with gallium nitrate: comparison with anti-CD4 monoclonal antibody. *Transplantation* 61: 783–791.
- Orosz CG, Wakely E, Sedmak DD, Bergese SD, VanBuskirk AM. 1997. Prolonged murine cardiac allograft acceptance: characteristics of persistent active alloimmunity after treatment with gallium nitrate versus anti-CD4 monoclonal antibody. *Transplantation* 63: 1109–1117.
- Perdrizet GA. 1997. *Heat Shock Response and Organ Preservation: Models of Stress Conditioning*. RG Landes Co, Austin, TX.
- Perry MA, Granger DN. 1991. Role of CD11/CD18 in shear rate-dependent leukocyte-endothelial cell interactions in cat mesenteric venules. *J Clin Invest* 87: 1798–1804.
- Polla BS, Cossarizza A. 1996. Stress proteins in inflammation. In: *Stress-Inducible Cellular Responses*, ed Feige U, Morimoto RI, Yahara I, Polla BS. Birkhauser Verlag, Basel, 375–392.
- Ribeiro SP, Villar J, Downey GP, Edelson JD, Slutsky AS. 1996. Effects of the stress response in septic rats and LPs-stimulated alveolar macrophages: evidence for the TNF- α post-translational regulation. *Am J Respir Crit Care Med* 154: 1843–1850.
- Schmidt JA, Abdulla E. 1988. Down-regulation of IL-1 β biosynthesis by inducers of the heat shock response. *J Immunol* 141: 2027–2034.
- Smith CW. 1993. Endothelial adhesion molecules and their role in inflammation. *Can J Physiol Pharmacol* 71: 76–87.
- Tonnesen MG, Anderson DC, Springer TA, Knedler A, Avdi N, Henson PM. 1989. Adherence of neutrophils to cultured human microvascular endothelial cells: stimulation by chemotactic peptides and lipid mediators and dependence upon the Mac-1, LFA-1, p150,95 glycoprotein family. *J Clin Invest* 83: 637–646.
- Volloch V, Gabai VL, Rits S, Force T, Sherman, MY. 2000. Hsp72 can protect cells from heat-induced apoptosis by accelerating the inactivation of stress kinase JNK. *Cell Stress Chaperones* 5: 139–147.
- Wang JH, Redmond HP, Watson RW, Condron C, Bouchier-Hayes D. 1995. Induction of heat shock protein 72 prevents neutrophil-mediated human endothelial cell necrosis. *Arch Surg* 130: 1260–1265.
- White FP. 1980a. Differences in protein synthesized *in vivo* and *in vitro* by cells associated with the cerebral microvasculature: a protein synthesized in response to trauma? *Neuroscience* 5: 1793–1799.
- White FP. 1980b. The synthesis and possible transport of specific proteins by cells associated with brain capillaries. *J Neurochem* 35: 88–94.
- Williams V, Lena C, Grady-Benson J, Perdrizet G. 2001. Acute lung injury in the fat embolism syndrome: attenuation by stannous chloride pretreatment. *Conn Med* (in press).
- Wong HR, Ryan MA, Menendez IY, Wispé JR. 1999. Heat shock activates the I- κ B α promoter and increases I- κ B α mRNA expression. *Cell Stress Chaperones* 4: 1–7.
- Xie Y, Cahill CM, Asea A, Auron PE, Calderwood SK. 1999. Heat shock proteins and regulation of cytokine expression. *Infect Dis Obstet Gynecol* 7: 26–30.
- Yellon DM, Latchman DS. 1992. Stress proteins and myocardial protection. *J Mol Cell Cardiol* 24: 113–124.