Lung (2007) 185:287–293
DOI 10.1007/s00408-007-9018-x

INTERSTITIAL LUNG DISEASE

Association Between Heat Stress Protein 70 Induction and Decreased Pulmonary Fibrosis in an Animal Model of Acute Lung Injury

Satoshi Hagiwara · Hideo Iwasaka · Shigekiyo Matsumoto · Takayuki Noguchi · Hidekatsu Yoshioka

Received: 12 March 2007 / Accepted: 3 June 2007 / Published online: 16 July 2007

Abstract The hyperthermia-induced activation of the stress protein response allows cells to withstand metabolic insults that would otherwise be lethal. This phenomenon is referred to as thermotolerance. Heat shock protein 70 (HSP70) has been shown to play an important role in this hyperthermia-related cell protection. HSP70 confers protection against cellular and tissue injury. Our objective was to determine the effect of heat stress on the histopathology of pulmonary fibrosis caused by the administration of lipopolysaccharide (LPS) in Wistar rats. The rats were randomly divided into three groups. In the control group, rats were heated to 42°C for 15 min. In the LPS group, rats were given LPS in 0.9% NaCl solution (10 mg/kg body weight). In the WH (whole-body hyperthermia) +LPS group, rats were heated to 42°C for 15 min, and 48 h later they were injected with LPS dissolved in a 0.9% NaCl solution (10 mg/kg body weight). We investigated lung histopathology and performed a Northern blot analysis daily. Hyperthermia was shown to reduce tissue injury caused by the administration of LPS. Pulmonary tissue HSP70 mRNA was found to be elevated at 3 h after heating. HSP70 protein levels in the serum increased after whole-body hyperthermia. However, neither the expression of HSP47 mRNA nor the expression of type I or type III collagen mRNA was induced by the administration of LPS after whole-body hyperthermia. These data indicate that thermal pretreatment is associated with the induction of HSP70 protein synthesis, which subsequently attenuates tissue damage in experimental lung fibrosis.

Keywords Heat shock protein · Pulmonary fibrosis · Lipopolysaccharide · Cytoprotection · Collagen

S. Hagiwara (✉) · H. Iwasaka · S. Matsumoto · T. Noguchi
Department of Brain and Nerve Science, Anesthesiology, Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasamamachi, Yufu City, Oita 879-5593, Japan
e-mail: saku@med.oita-u.ac.jp

H. Yoshioka
Department of Anatomy, Biology and Medicine, Biochemistry, Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasamamachi, Yufu City, Oita 879-5593, Japan

Introduction

Heat shock proteins (HSPs), commonly called stress proteins, are a group of proteins induced when living cells are exposed to temperatures 5°C higher than their optimum growth temperature [1]. Stress proteins are also induced in response to environmental stressors such as the exposure to heavy metals, arsenate, and oxidants. Pathophysiologic stressors such as microbial infections, ischemia, tissue trauma, and genetic damage also induce the stress proteins. Furthermore, even under normal conditions (such as cell cycle progression, embryonic development, cell differentiation, hormonal stimulation in vertebrate cells, and growth in microorganisms) stress proteins are induced [2, 3]. The major families of mammalian stress proteins, HSP90 and HSP70, as well as the smaller HSP28, have all been well characterized [4, 5].

Acute respiratory distress syndrome (ARDS) is a common cause of morbidity and mortality. Late ARDS refers to the clinical stage when the lung attempts to repair an initial or persistent injury to the endothelial and epithelial linings of the respiratory apparatus [6]. Histologically, it is characterized by the replacement of damaged epithelial cells and the accumulation of mesenchymal cells. During this re-epithelialization, connective tissue products and matrix deposit in

the air spaces and walls [7, 8]. Unfortunately, this healing process is frequently ineffective because it often results in a worsening of both lung compliance and oxygenation [9]. The fibroproliferative phase of ARDS results in a restrictive ventilatory defect and impaired alveolar membrane function characterized by a prolonged reduction in the diffusing capacity for carbon monoxide. This ultimately leads, either directly or indirectly, to the patient's death [10].

In this study we used whole-body hyperthermia to increase the expression of HSP70 before the administration of lipopolysaccharide (LPS). We then investigated whether the expression of HSP70 inhibits LPS-induced pulmonary fibrosis by inducing the expression of collagen mRNAs.

Methods

Animals

All protocols conformed to National Institute of Health (NIH) guidelines and all animals received care in compliance with the Principles of Laboratory Animal Care. Male Wistar rats weighing 250–300 g (7 weeks old; Kyudou, Saga, Japan) were used in all experiments. All animals were housed with access to food and water *ad libitum*.

Experimental Protocols

Animals were randomly assigned to one of three groups. In the control group, the whole bodies of rats were heated to 42°C for 15 min, and saline was administered intratracheally after 48 h. In the LPS group, rats received intratracheal administration of LPS (Sigma, St. Louis, MO) dissolved in a 0.9% NaCl solution (10 mg/kg body weight). In the heat and endotoxin (WH [whole-body hyperthermia] + LPS) group, the whole bodies of rats were heated to 42°C for 15 min, and LPS dissolved in 0.9% NaCl solution (10 mg/kg body weight) was administered intratracheally after 48 h.

Body warming was performed on rats anesthetized under 3% sevoflurane with continuous monitoring of rectal temperature. The rats were heated with a warm water blanket and the trachea was exposed through an anterior midline neck incision. The LPS solution was injected into the trachea using 24-gauge needles. The animals were sacrificed by clipping the vena cava, transecting the aorta, and then injecting normal saline intra-atrially. Lung tissue specimens were thereafter quickly removed and processed as indicated below.

Histologic Examination

After the LPS injection, the rats were sacrificed by exsanguination under 3% sevoflurane anesthesia. The lung was inflated and instilled with 10% formalin. Sections were cut at 3 μm and embedded into paraffin prior to staining with hematoxylin and eosin. We compared the severity of interstitial fibrosis among the groups using the Ashcroft score. Briefly, paraffin sections of the lung were systematically scanned by microscopy. Each successive field was individually assessed for severity of interstitial fibrosis and given a score from 0 to 8 using a predetermined scale of severity [11]. Azan staining was then performed to visualize the collagen fibrils in the tissue.

Northern Blot Analysis

Blood was washed out via a saline perfusion of the right ventricle. Afterward the lung was homogenized with a polytron homogenizer (IKA Labortechnik, Staufen, Germany) in isogen regent (Nippon Gene Co., Tokyo, Japan). After the total cellular RNA was isolated, the precipitated total RNA pellet was washed with 1 ml of 75% ethanol, air-dried, and then resuspended in sterile water. The RNA concentration was measured by absorbance of light at 260 nm.

The total RNA was separated on a 1% agar gel containing formaldehyde. It was then transferred onto nylon membranes (Amersham, Buckinghamshire, England) as recommended by the manufacturer. The blotted filters were prehybridized for 6 h at 42°C in hybrizol (Interogen Co., Purchase, NY). The filter was hybridized in hybridization solution containing P-labeled DNA at 42°C for 16 h. It was then washed several times with 2×SSC at room temperature, 2×SSC with 0.5% SDS at 65°C, and 2×SSC with 0.5% SDS at 60°C. The filter was exposed at –80°C overnight using an RXO-H film with HR-X intensifying screens (Fuji Photo Film Co., Ltd., Tokyo, Japan).

The following P^{32} multiprime-labeled probes (Amersham, Buckinghamshire, England) were used. We used a fragment from mouse HSP47 cDNA, a fragment from mouse HSP70 cDNA, a fragment from mouse type I collagen cDNA, and a fragment from mouse type III collagen cDNA.

Immunoblot Analysis

Lungs were obtained from each group of rats. Blood was cleared with saline. The lung was then homogenized and centrifuged at 10,000 rpm for 5 min. Thereafter, the supernatant was collected and concentrations were measured using the BCA Protein Assay Reagent (Pierce, Rockford, IL).

Proteins were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gel. After running, proteins were immediately transferred to a PVDF (polyvinylidene difluoride) membrane (Millipore, Bedford, MA) at 30 mA for 2 h. The membrane was blocked with 5% nonfat dry milk in TBS/Tween (25 mM

Tris-HCl, 0.14 M NaCl, 2% Tween 20) at 4°C overnight. Membranes were incubated with monoclonal anti-HSP70 antibody (Stressgen Biotechnology, Temecula, CA), diluted 1:1000, for one hour. The secondary antibody against rabbit IgG was diluted 1:1000 for one hour. Washing between and after antibody incubation steps was performed three times for 10 min each with TBS/Tween buffer. Proteins were revealed by enhanced chemiluminescence (ECL) (Amersham, Buckinghamshire, England) and exposed to film (Hyperfilm ECL; Amersham). The band concentration was calculated by quantifying the integrated optical density using the NIH Image software program (National Institutes of Health, Washington, DC) The expression intensities of HSP70 were shown as percentages of the control.

Statistical Analysis

All data were presented as the mean ± standard error of the mean (SEM). The data were analyzed using a variance analysis and Scheffe's post-hoc test (for multiple comparisons) and the unpaired t test (for single comparisons). A $p < 0.05$ was considered to be statistically significant.

Results

Lung Histology of Rats Treated with Lipopolysaccharide

In the normal lung, fine fibrous connective tissue was observed in the alveolar wall (Fig 1A, D). After 14 days of LPS treatment (unheated rats), lung fibrosis was observed. Fibrotic lung sections showed inflammatory infiltrates and proliferated alveolar macrophages with alveolar edema around the fibrotic areas (Fig 1B, E). After 14 days of LPS in the WH + LPS group, lung fibrosis sections showed that the inflammatory infiltrates were significantly decreased compared to unheated rats. A pulmonary architecture with minimal cellular interstitial infiltrates and alveolar exudates was observed in the preheated animals (Fig. 1C, F). The Ashcroft scores in the WH + LPS group were significantly reduced compared to the LPS group (Fig. 1G). The azan-stained sections showed an increased amount of connective tissue in the lung of the LPS group compared with the WH+LPS group (Fig. 2).

Expression of HSP70 mRNA and Protein

The expression of HSP70 was analyzed by Northern blot analysis (Fig. 3). HSP70 mRNA levels increased after heat exposure. The increase was greatest after 3 h of heating. The HSP70 mRNA levels decreased thereafter. In addition, the expression of HSP70 was analyzed using immunoblotting after 48 h of heat exposure (Fig 4A, B). The HSP70 protein level in heat-exposed lung significantly increased after 48 h compared with the control lung.

Expression of HSP47, Type I Collagen, and Type III Collagen mRNA

The expression of HSP47 was analyzed by Northern blot analysis (Fig. 3). HSP47 mRNA levels increased after heat exposure, peaking at 6 h and subsequently decreasing. In the LPS treatment rats, the expression of HSP47 and type I and type III collagens in the lung was also analyzed using Northern blot analysis (Fig. 5). All three mRNAs were markedly increased in the lungs of unheated rats treated with LPS. In contrast, the preheated rats treated with LPS demonstrated significantly decreased expressions of HSP47 and type I and type III collagens in comparison to the preheated-only rats.

Discussion

The common pathologic features of pulmonary fibrosis following acute lung injury involve the production of collagen, the proliferation of elastic and smooth muscle elements, architectural remodeling, and the onset of chronic inflammation [12]. In addition, increased expression of proinflammatory mediators, expansion of the pulmonary extracellular matrix, and upregulation of genes induced by transforming growth factor-β (TGF-β) are all seen in pulmonary fibrosis [12].

Recently, HSP47 was discovered as a unique collagen-binding stress protein [13]. HSP47 is the only stress protein described to date that is capable of binding a specific substrate. HSP47 is localized to the endoplasmic reticulum (ER) and binds specifically to newly synthesized procollagen there [13–15]. In addition to these functions, HSP47 plays an important role in various fibrotic diseases by regulating the increased synthesis and assembly of collagens [16]. We have previously reported that the upregulation of HSP47 plays a critical role in the fibrotic process in an animal model of acute lung injury [17]. Thus, we surmise that the upregulation of this collagen-specific chaperone stress protein plays an important role in the fibrotic process. One possible explanation for this is that HSP47 regulates the increased synthesis and assembly of collagens [16].

The present study demonstrates that LPS significantly increases the expression of HSP47 and type I and type III collagens. We found that the increase in HSP47 is correlated with the presence of pulmonary fibrosis. On the other hand, exogenously administered pretreatment hyperthermia

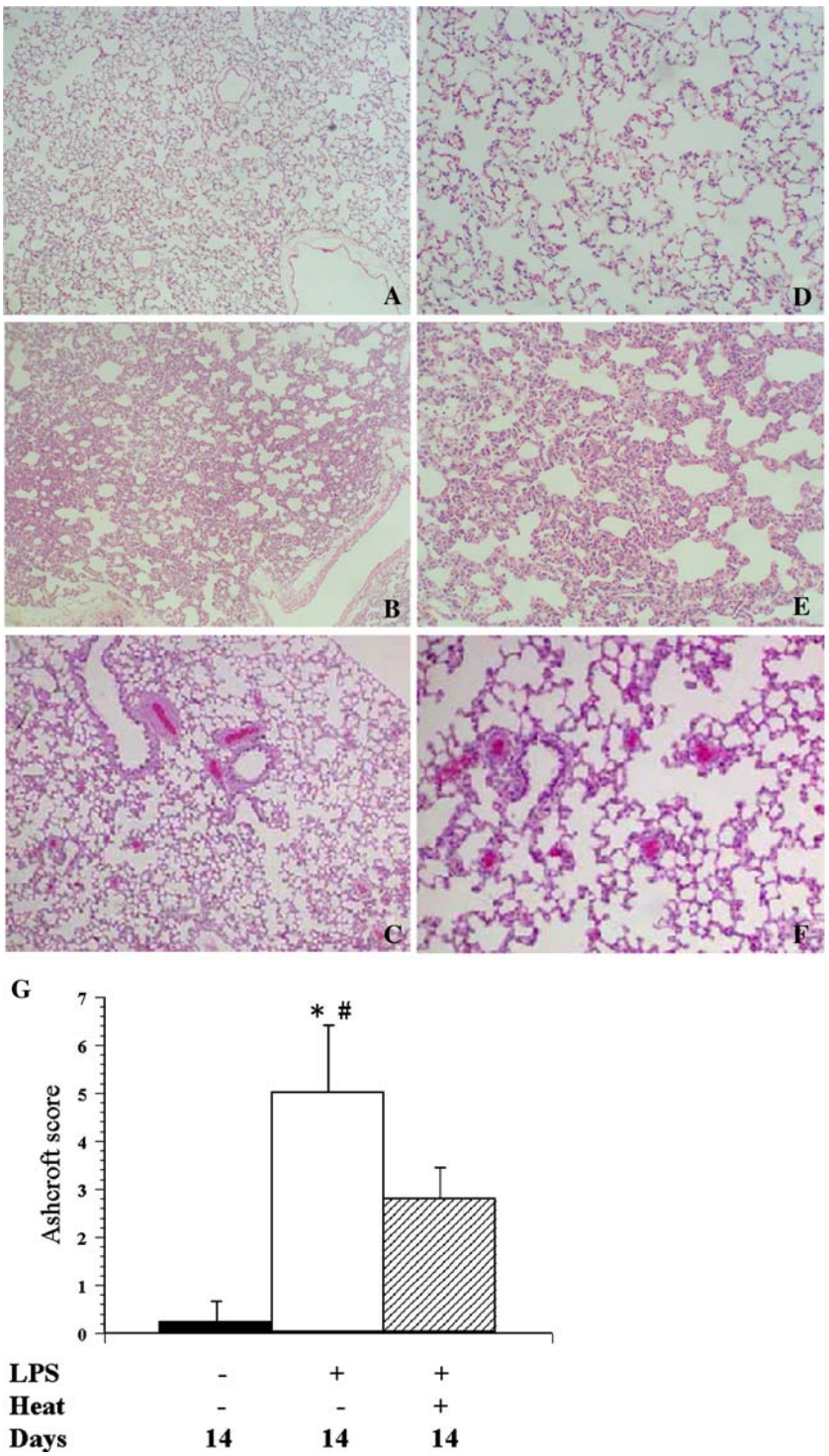

Fig. 1 **A, D** A micrograph of the lung from an animal killed 14 days after saline injection. Hematoxylin & eosin stain; original magnification: A: ×40, D: ×200. **B, E** A micrograph of the lung from an animal killed 14 days after injection with 10 mg/kg LPS, showing diffuse consolidation of the lung parenchyma with a loss of alveolar architecture and increased cellularity. Hematoxylin & eosin stain; original magnification: B: ×40, E: ×200. **C, F** A micrograph of the lung from an animal that was pretreated with whole-body heat and then killed 14 days after injection with 10 mg/kg LPS. This shows an improvement of the LPS-induced lung lesions. Hematoxylin & eosin stain; original magnification: C: ×40, F: ×200. **G** Histopathologic assessment of pulmonary fibrosis using the Ashcroft score. Comparison of lung tissue on day 14 for the control, LPS, and WH + LPS groups. Data are expressed as the mean (SEM. $^{*}p < 0.05$ vs. control group. $^{\#}p < 0.05$ vs. WH + LPS group

decreased the expression of HSP47 and type I and type III collagens following LPS administration. As such, the presence of an HSP might improve the pulmonary fibrosis induced by LPS. These results suggest that a transient hyperthermia induces biological changes that render lungs less susceptible to a highly toxic agent. This, in turn, may prevent the development of pulmonary fibrosis.

Furthermore, the exogenously administered pretreatment hyperthermia attenuated the toxicity of LPS despite the 48-h interval between hyperthermia exposure and LPS administration. This finding suggests that the protection afforded by hyperthermia does not reflect a direct thermal effect on any specific defense mechanisms. In addition, there was an association between thermal treatment and an

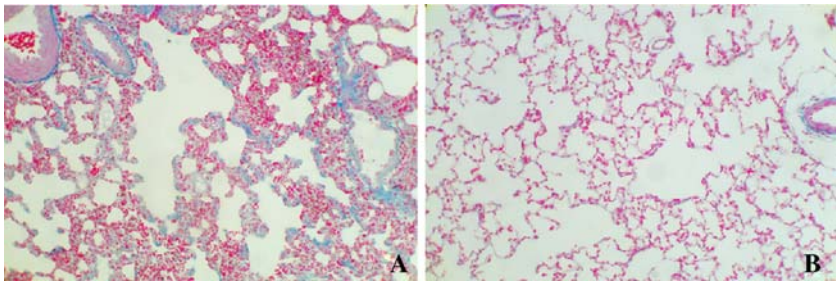

Fig. 2 **A** The lung of a rat treated with LPS for two weeks was stained with azan to visualize collagen fibrils (×200). **B** The whole body was heated before LPS administration. The lung of the rat was treated with LPS for two weeks and then the lung was stained with azan to visualize the collagen fibrils (×200)

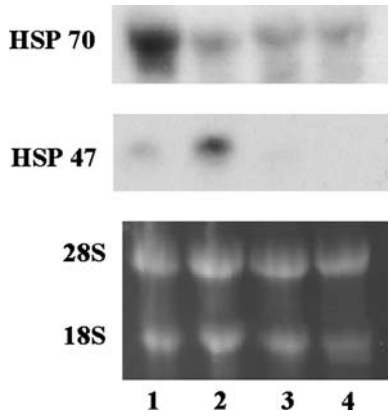

Fig. 3 Northern blot analysis of HSP70 and HSP47 mRNA from lungs of whole-body-heated rats. Ten micrograms of total RNA were loaded in each lane, electrophoretically resolved, transferred to nylon membranes, and hybridized with P^{32}-labeled probes for HSP70 and HSP47. 28S and 18S ribosomal RNA bands on ethidium bromide-stained gel were used to control the RNA loading. Lane 1: control; lanes 2–5: 3, 6, 12, and 24 h after LPS treatment, respectively

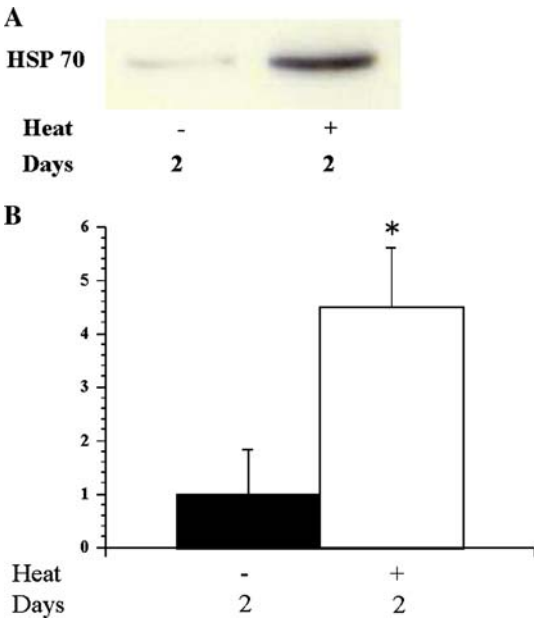

Fig. 4 **A** An immunoblot analysis of HSP70 protein in the LPS-treated rat lung. One hundred micrograms of protein in each lane was electrophoretically separated, blotted onto nylon membranes, and hybridized with anti-rat HSP70 antibody. Lane 1: control; lane 2: 2 days after LPS treatment. **B** The signal intensities for the density based on an immunoblot analysis of the HSP70 protein. HSP70 was quantified using an image analyzer. The expression intensities of HSP70 were shown as percentages of the control. Data are expressed as the mean (SEM. $^{*}p < 0.05$ vs. control group

increased transcription and translation of the HSP70 gene product. These both correlated with significant protection from subsequent lung injury after the administration of LPS. Furthermore, there are significant data that link *in vitro* HSP induction [18–20] and *in vivo* HSP induction with cytoprotection [21]. The results of this study suggest that the antifibrotic effects of hyperthermia observed herein are related to the induction of HSP.

The current study establishes a relationship between exogenously administered hyperthermia-induced HSP synthesis and protection from LPS. The main effects of HSP and other anti-inflammatory enzymes include exogenously administered hyperthermia-induced interactions with the host inflammatory response. Some of these interactions include, for example, glucocorticoid release or metabolism [22, 23], gut permeability in response to endotoxin [24], and the response of the antioxidant enzyme Cu, Zn superoxide dismutase [25].

The relationship between *in vivo* hyperthermia, HSP induction, and organ protection in mammals does require further investigation. However, the *in vitro* data strongly suggest a relationship between HSP induction and protection against subsequent cellular injury. *In vitro* thermotolerance can be reduced by blocking HSP70 synthesis at the level of gene transcription [26] or by microinjection with antibodies specific for HSP70 [27]. The mechanisms whereby HSP70 confers cytoprotection are still not clearly known. The role of HSPs in both normal and damaged cell function must be studied further. HSPs have been implicated in protein folding and assembly and in the stabilization of proteins. HSP70, in particular, has been implicated in the refolding and reassembly of damaged

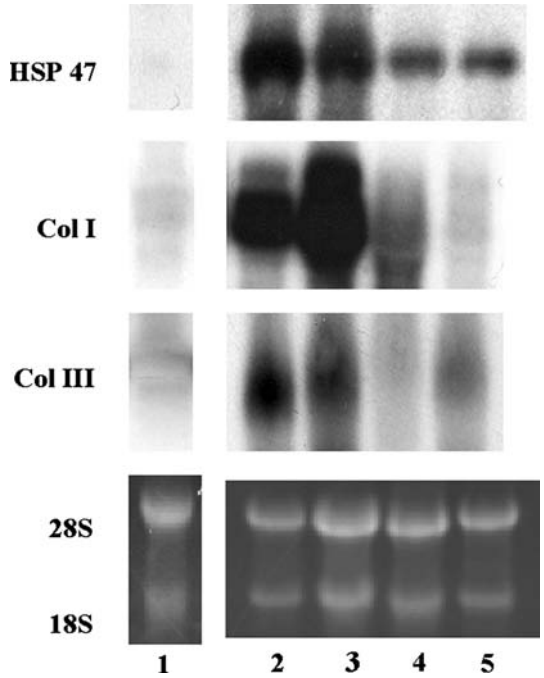

Fig. 5 Northern blot analysis of HSP47 and type I collagen and type III collagen mRNA in the lung of the LPS-treated rats. Ten micrograms of total RNA were loaded in each lane, electrophoretically resolved, transferred to nylon membranes, and hybridized with P^{32}-labeled probes for HSP47, type I collagen (Col I), and type III collagen (Col III). 28S and 18S ribosomal RNA bands on ethidium bromide-stained gel were used to control the RNA loading. Lane 1: 14 days after treatment of preheated alone; lanes 2 and 3: 7 and 14 days after LPS treatment; lanes 4 and 5: 7 and 14 days after LPS treatment of preheated rats

proteins and in the stabilization of damaged proteins [28]. HSP47, on the other hand, is known to be a collagen-specific molecular chaperone in the endoplasmic reticulum [29, 30]. We showed that the expression of HSP47 mRNA was different from HSP70 mRNA. The degree of HSP47 induction by heat stress was not as prominent as that of HSP70 [31]. Lele et al. [32] demonstrated that the different inducible regulatory mechanisms for these genes operate in a cell- and stress-specific manner. Further studies are necessary to examine the roles played by HSPs (HSP70 and HSP47) as well as the mechanism of the interaction with HSPs.

This study offers clear implications for the mechanism through which hyperthermia protects against injury caused by noxious exposure. However, these observations are also of interest from the perspective of the pathophysiology of fever. Core temperatures similar to the ones used in this study are seen in the upper range of fever in humans [33]. It remains unclear as to whether such temperatures benefit or harm the host. Exogenously induced hyperthermia may not be physiologically identical to endogenously derived fever. Nevertheless, the observed correlation between hyperthermia and cytoprotection is consistent with the observation that the failure to mount an appropriate febrile response to infection is associated with increased mortality [34]. The development of strategies to increase HSP concentrations might provide novel methods of minimizing the morbidity and mortality associated with various toxic exposures. Whether the therapeutic benefits of fever are mediated through HSP production remains to be seen, however. As such, further research is needed on the effects of HSP production in the various toxic exposures. Furthermore, both adenovirus-mediated delivery of the HSP70 gene and drug-induced HSP70 protein production are both expected to become useful therapies in the future.

The present study demonstrates that exogenously administered hyperthermia protects the lungs from LPS toxicity through the induction of HSPs. Exogenously administered hyperthermia may possibly lead to a systemic endotoxemia or similar effects which, in turn, precondition the host response to the subsequent challenge. This possibility remains unknown, however, and requires further study.

Acknowledgments The authors thank Professor K. Nagata (Kyoto University, Japan) for the plasmid with the HSP47 mRNA insert, which enabled us to perform the Northern hybridization analysis.

References

1. Pespeni M, Hodnett M, Pittet JF (2005) In vivo stress preconditioning. Methods 35:158–164
2. Kregel KC (2002) Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol 92:2177–2186
3. Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670–684
4. Schlesinger MJ (1990) Heat shock proteins. J Biol Chem 265:12111–12114
5. Welch WJ (1992) Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol Rev 72:1063–1081
6. Hudson LD, Hough CL (2006) Therapy for late-phase acute respiratory distress syndrome. Clin Chest Med. 27:671–677
7. Adamson IY, Young L, Bowden DH (1988) Relationship of alveolar epithelial injury and repair to the induction of pulmonary fibrosis. Am J Pathol 130:377–383
8. Pan T, Mason RJ, Westcott JY, Shannon JM (2001) Rat alveolar type II cells inhibit lung fibroblast proliferation in vitro. Am J Respir Cell Mol Biol 25:353–361
9. Shimabukuro DW, Sawa T, Gropper MA (2003) Injury and repair in lung and airways. Crit Care Med 31:524–531
10. Neff TA, Stocker R, Frey HR, Stein S, Russi EW (2003) Long-term assessment of lung function in survivors of severe ARDS. Chest 123:845–853
11. Ashcroft T, Simpson JM, Timbrell V (1988) Simple method of estimating severity of pulmonary fibrosis on a numerical scale. J Clin Pathol 41:467–470
12. Chua F, Gauldie J, Laurent GJ (2005) Pulmonary fibrosis: searching for model answers. Am J Respir Cell Mol Biol 33:9–13
13. Nagata K, Saga S, Yamada KM (1988) Characterization of a novel transformation-sensitive heat-shock protein (HSP47) that binds to collagen. Biochem Biophys Res Commun 153:428–434

14. Nakai A, Satoh M, Hirayoshi K, Nagata K (1992) Involvement of the stress protein HSP47 in procollagen processing in the endoplasmic reticulum. J Cell Biol 117:903–914
15. Satoh M, Hirayoshi K, Yokota S, Hosokawa N, Nagata K (1996) Intracellular interaction of collagen-specific stress protein HSP47 with newly synthesized procollagen. J Cell Biol 133:469–483
16. Razzaque MS, Taguchi T (1999) The possible role of colligin/HSP47, a collagen-binding protein, in the pathogenesis of human and experimental fibrotic diseases. Histol Histopathol 14:1199–1212
17. Hagiwara S, Iwasaka H, Matsumoto S, Noguchi T, Yoshioka H (2007) Coexpression of HSP47 gene and type I and type III collagen genes in LPS-induced pulmonary fibrosis in rats. Lung 185:31–37
18. Craig EA (1985) The heat shock response. Crit Rev Biochem 18:239–280
19. Subjeck JR, Shyy TT (1986) Stress protein systems of Mammalian cells. Am J Physol 250:1–17
20. Lindquist S (1986) The heat shock response. Annu Res Biochem 55:1151–1191
21. Kiang JG, Tsokos GC (1998) Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. Pharmacol Ther 80:183–201
22. Alexandrova M, Farkas P (1992) Stress-induced changes of glucocorticoid receptor in rat liver. J Steroid Biochem 42:493–498
23. Gathiram P, Wells MT, Brock-Utne JG, Gaffin SL (1988) Prophylactic corticosteroid increased survival in experimental heat stroke in primates. Aviat Space Eviron Med 59:352–355
24. Carter EA, Gonnella A, Tompkins RG (1992) Increased transcellular permeability of rat small intestine after thermal injury. Burns 18:117–120
25. Hass MA, Massaro D (1988) Regulation of synthesis of superoxide dismutase in rat lung during oxidant and hyperthermic stresses. J Biol Chem 263:776–781
26. Johnston RJ, Kucey BL (1988) Competitive inhibition of HSP70 gene expression causes thermosensitivity. Science 242:1551–1554
27. Riabowol KT, Mizzen LA, Welch WJ (1988) Heat shock is lethal to fibroblasts microinjected with antibodies against HSP70. Science 242:433–436
28. Beckman RP, Mizzen LA, Welch WJ (1990) Interaction of HSP70 with newly synthesized proteins. Science 248:850–854
29. Kazuhiro N (1996) HSP47: a collagen-specific molecular chaperone. Trends Biochem Sci 21:21–26
30. Kazuhiro N (1998) Expression and function of heat shock protein 47; a collagen-specific molecular chaperone in the endoplasmic reticulum. Matrix Biol 16:379–386
31. Kudo H, Hirayoshi K, Kitagawa Y, Imamura S, Nagata K (1994) Two collagen-binding proteins, osteonectin and HSP47, are coordinately induced in transformed keratinocytes by heat and other stresses. Exp Cell Res 212: 219–224
32. Lele Z, Engel S, Krone PH (1997) Hsp47 and hsp70 gene expression is differentially regulated in a stress- and tissue-specific manner in zebrafish embryos. Dev Genet 21:123–133
33. Shibolet S, Lancaster MC, Danon Y (1976) Heat stroke: a review. Aviat Space Environ Med 47:285–301
34. Styrt B, Sugarman B (1990) Antipyresis and fever. Arch Intern Med 150:1589–1597