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Expression of the 78 kD glucose-regulated protein is induced by endoplasmic reticulum stress in development of hepatopulmonary syndrome

Huiying Zhang^{1,*}, Minli Lv², Jiantao Jia¹, Zhongfu Zhao³, Lili Zhang¹, Lina Lai⁴, Yanjun Wu⁵, Baohong Li¹, Chen Li⁶, Yan Liu⁶, Xujiang Li⁶, Xiaoxia Tian¹, Hui Pang⁵, Jianhong Guo⁷, Limin Wang⁸, Yimin Fan⁸, Cuiying Zhang⁸, Jingquan Ji¹, Dewu Han⁷, and Cheng Ji⁹

¹Pathophysiology Department, Changzhi Medical College, Changzhi 046000, Shanxi, China

²ICU of the Second hospital of Shanxi Medical University, Taiyuan 030001, Shanxi, China

³Institute of Hepatology, Changzhi Medical College, Changzhi 046000, Shanxi, China

⁴Pharmacology Department, Changzhi Medical College, Changzhi 046000, Shanxi, China

⁵Laboratory of Microbiology and Immunology, Changzhi Medical College, Changzhi 046000, Shanxi, China

⁶Physiology Department, Changzhi Medical College, Changzhi 046000, Shanxi, China

⁷Institute of Hepatology, Shanxi Medical University, Taiyuan 030001, Shanxi, China

⁸Functional Laboratory, Changzhi Medical College, Changzhi 046000, Shanxi, China

⁹USC Research Center for Liver Disease, Department of Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

Abstract

Objective—This study is to explore the role of 78 kD glucose-regulated protein (GRP78) in development of hepatopulmonary syndrome (HPS) in rats.

Methods—The rat model of liver cirrhosis and HPS were induced with multiple pathogenic factors. Hematoxylin and eosin (H & E) staining was performed to detect the pathological changes of the lung and liver tissues. The levels of alanine transferase (ALT), endotoxin, and tumor necrosis factor- α (TNF- α) in plasma and TNF- α and malondialdehyde (MDA) in lung tissues were detected. RT-PCR and Western blotting were conducted to detect the mRNA and protein expression levels of GRP78 in lungs.

Results—The plasma endotoxin level was gradually increased as HPS developed, and the mRNA and protein expression levels of GRP78 in lungs were also increased as the disease progressed. The levels of ALT and TNF- α in plasma and the contents of TNF- α and MDA in lung tissues were gradually increased along with the disease progression, with a strong positive correlation. Compared with controls, the plasma TNF- α level and the mRNA and protein

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*Corresponding author: Huiying Zhang, Pathophysiology Department, Changzhi Medical College, Changzhi, 046000, Shanxi, China. Tel: 86-0355-3031235; Fax: 86-0355-3034065; zhanghy2001@163.com.

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expression levels of GRP78 in lung tissues were significantly higher in rats with HPS. The levels of endotoxin and ALT in plasma and the level of MDA in lungs were significantly higher in rats with HPS than controls.

Conclusions—The increased GRP78 expression is indicative of endoplasmic reticulum stress response during HPS, which may play an important role in the disease pathogenesis.

Keywords

hepatopulmonary syndrome; 78kD glucose-regulated protein; endoplasmic reticulum stress; intestinal endotoxemia

Introduction

Hepatopulmonary syndrome (HPS) refers to the respiratory dysfunction originating from acute or chronic liver diseases, especially liver cirrhosis, which has been pathologically characterized by the pulmonary vascular lesions and the subsequent hypoxemia. Endoplasmic reticulum (ER) is one of the important cellular organelles responsible for protein processing and calcium storage. The accumulation of misfolded and/or unfolded proteins and/or calcium dyshomeostasis in ER induces stress, which would in turn enhance the cell resistance and adaptability to injuries through the unfolded protein response pathway. Intestinal endotoxemia (IETM) plays a central role in HPS development (Zhang et al. 2005), in which endotoxin is the main factor triggering endoplasmic reticulum (ER) stress, as indicated by the elevated expression level of 78kD glucose-regulated protein (GRP78) (Hiramatsu et al. 2006). The increased ER stress actively participates in the pathogenesis of alcoholic and non-alcoholic liver injuries, as well as CCl₄-induced liver cirrhosis, with up-regulated GRP78 expression in the animal models (Ji, 2008). Based on these results, we wondered if GRP78 was involved in HPS pathogenesis, and if GRP78 expression is related with IETM.

In this study, we have detected the expression levels of GRP78 in lung tissues of model rats suffering from liver cirrhosis and HPS induced by multiple pathogenic factors. We have also investigated the relationship between the GRP78 expression level and the plasma endotoxin content.

Materials and methods

Animals and reagents

SD rats (clean grade) were obtained from the Animal Center of Shanxi Medical University. All the animal protocols have been approved by the Experimental Animal Care and Use Committee of Shanxi Medical University. Rabbit anti-rat polyclonal antibody against GRP78 was purchased from Sigma (St Louis, Missouri, USA). Horseradish peroxidase-conjugated goat anti-rabbit IgG was from ZSGB-BIO (Beijing, China). Mouse anti-rabbit monoclonal antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and horseradish peroxidase-conjugated goat anti-mouse IgG were from Beyotime Institute of Biotechnology (Haimen, Jiangsu, China). Super Enhanced chemiluminescence (ECL) detection reagents and BCA reagents for protein determinations were purchased from Applygen Technologies Inc (Beijing, China). Trizol reagent and RT-PCR kit were obtained from Gibco (Grand Island, NY, USA). Taq DNA polymerases were from Promega (Madison, WI, USA). Primers for GRP78 and GAPDH were synthesized by Zhongshan University Anda Gene Diagnostic Center (Guangzhou, Guangdong, China). Endotoxin chromogenic substrate limulus amoebocyte lysate kit was purchased from Xiamen LAL Experimental Factory Co., Ltd (Xiamen, Fujian, China). Alanine transferase (ALT) kit, malondialdehyde

(MDA) kit, and protein assay kit based on Coomassie brilliant blue method were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Tumor necrosis factor- α (TNF- α) radioimmunoassay kit was purchased from Beijing Sino-UK institute of Biological Technology (Beijing, China).

Animal grouping and Modeling

Male Wistar rats, weighing 200–240 g, were used for the present study. Liver cirrhosis and HPS were induced in these animals as described in our previously published study (Su et al. 2010; Zhang et al. 2007): rats in the HPS group were fed with a mixture of maize flour, 0.5% cholesterol, and 20% lard (lard used only for the first 2 wk), and they only drank 5%–15% (v/v) ethanol. These animals received subcutaneous injection of carbon tetrachloride (CCl₄) solution (0.5 ml/100 g bodyweight) on the first day of experiment and thereafter the CCl₄ solution was injected (0.3 ml/100 g bodyweight) at an interval of three days. The control rats were fed with standard diet and tap water. All the rats were randomly divided into 6 groups: (1) 4-wk control group (fed with standard diet for 4 wk), (2) 4-wk HPS group (fed with the complex diet and injected with CCl₄ for 4 wk), (3) 6-wk control group (fed with standard diet for 6 wk), (4) 6-wk HPS group (fed with the complex diet and injected with CCl₄ for 6 wk), (5) 8-wk control group (fed with standard diet for 8 wk), (6) 8-wk HPS group (fed with the complex diet and injected with CCl₄ for 8 wk).

Tissue preparation

Blood was collected from each group when the animals were anesthetized with 3% pentobarbital sodium (3 ml/kg body weight; Shanghai XiTang Biological Technology Co., Ltd., Shanghai, China), under a sterile and endotoxin-free condition. Then it was subjected to centrifugation at 3,000 rpm for 15 min. The plasma was collected and stored at –70 °C.

Parts of the right lobe of the liver tissue and the left lung tissue were harvested from these animals and then immediately placed in liquid nitrogen until further use. The remaining parts were fixed in 10% neutral formalin for histological processing.

Biochemical detection

For the plasma samples, the colorimetric method of Reitman and Frankel, the limulus test method, and the radioimmunoassay method were used to determine the ALT activities, the endotoxin levels, and the TNF- α levels, respectively, according to the manufacturer's instructions.

For the lung tissues, after homogenized by sonication, the tissues were subjected to the Coomassie brilliant blue staining, the thiobarbituric acid method, and the radioimmunoassay method to determine the protein levels, the MDA contents, and the TNF- α levels, respectively, according to the manufacturer's instructions.

H & E staining for liver and lung tissues

The liver and lung tissues were embedded with paraffin, and then cut into 4 μ m sections for H&E staining. These sections were observed with an optical microscope.

Western blot analysis

Protein (50 μ g) was subjected to 10% SDS-PAGE, and the transferred onto the nitrocellulose membrane. The membrane was incubated with rabbit anti-rat GRP78 polyclonal antibody (1:1,000 dilution) at 4°C overnight, and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1,000 dilution) at room temperature for 1 h. After washing, the membrane was subjected to ECL hypersensitive reagents before the

exposure to X-ray and the development. GAPDH worked as internal reference. The protein bands on the membrane were scanned and analyzed with a molecular biological image analysis system to determine the protein levels.

Real-time PCR

According to the manufacturer's instructions, 5 µg total RNA was used for the reverse transcription and amplification. The primers used were as follows: GRP78 5'-GGAGGATGTGGGCACGGTGGTC-3' and 5'-GTCATTCCAAGTGCGTCCGATGAGG-3' (385 bp); GAPDH 5'-GGTCATCAACGGGAAACCC-3' and 5'-TCTGAGTGGCAGTGATGGCA-3' (450 bp). The products were resolved by 2% agarose gel electrophoresis, and the DNA standard markers (D12000) was used to determine the molecular weight. These products were semi-quantitatively evaluated with Quantity One gel analysis system (Bio-Rad, USA), and normalized to GAPDH to obtain the relative mRNA levels.

Statistical analysis

Data were expressed as means $\pm$ SD. SPSS 13.0 software was used for statistical analysis. Analysis of variance (ANOVA), LSD-t test, and linear correlation method were used to conduct the multiple sample mean comparison, the pairwise comparison, and the correlation analysis, respectively. *P* values of less than 0.05 were considered statistically significant.

Results

Biochemical indexes in plasma and lung tissues in HPS models

To characterize the biochemical indexes of HPS models, the levels of endotoxin, TNF- α , ALT, and MDA in the plasma and lung tissues were investigated. As indicated in Table 1 and Table 2, the level of endotoxin in plasma was increased as the disease progressed in HPS groups, with significant differences between these groups ($P < 0.05$). The levels of TNF- α in plasma and lung tissues and the level of MDA in plasma were also increased with the progression of the disease in HPS groups, with significant differences between groups ($P < 0.05$). All the biochemical indexes in the 6-wk and 8-wk HPS groups were significantly increased from that in corresponding control groups ($P < 0.05$).

Pathological changes in liver and lung tissues in HPS models

To determine the pathological features of HPS models, H&E staining was performed both in liver and lung tissues in control and HPS groups. As shown in Fig. 1, HE staining indicated regular lobular structures and intact radial hepatic cord arrangement around the central vein, and the hepatic cells were rich in cytoplasm and revealed blue round nuclei in the control group (Fig. 1A). In the livers in 4-wk HPS group, the arrangement of hepatic cords was irregular, and the necrosis of central hepatic lobule was detected accompanied by inflammatory cell infiltration. Steatosis was found in some hepatocytes, and the interlobular fibroplasias was also observed (Fig. 1B). As shown in Fig. 1C, the hepatic steatosis and the inflammatory cell infiltration were aggravated, and the proliferating fibrous network took place in the 6-wk HPS group. Furthermore, pseudolobule was formed in the 8-wk HPS group (Fig. 1D).

For the lung tissues, H&E staining revealed that the alveolar structure was intact with uniform septum in control group (Fig. 1E). In 4-wk HPS group, the alveolar septal walls were slightly thickened, and the accumulation of macrophages and neutrophils was observed (Fig. 1F). When induced for 6 wk, the alveolar septal walls were moderately thickened, accompanied by telangiectasis and accumulated macrophages and neutrophils, and some of

the alveolar cavities became smaller (Fig. 1G). The alveolar septal walls were further thickened and the alveolar cavities became even smaller, and capillaries with increased lumen and plenty of accumulated macrophages were found in alveolar septal walls in 8-wk HPS group (Fig. 1H).

Elevated protein and mRNA expression levels of GRP78 in lung tissues in HPS models

To investigate the role of GRP78 in HPS pathogenesis, its protein and mRNA expression levels in lung tissues were detected. The results indicated GRP78 expression was elevated as the disease progressed, at both protein (Fig. 2) and mRNA (Fig. 3) levels, with significant differences between HPS groups ($P < 0.05$). At each time point, the protein and mRNA expression levels of GRP78 are both significantly higher than corresponding controls ($P < 0.05$) (Fig. 2B and 3B). These results suggest that the protein and mRNA expression levels of GRP78 are increased in lung tissues of the HPS models.

Correlation analysis between indexes in HPS models

To investigate whether these changed indexes in HPS models were related, correlation analysis was performed. The plasma endotoxin level was positively correlated with the levels of TNF- α and MDA, in both plasma and lung tissues ($P < 0.05$). There were also strong positive correlations between the protein expression level of GRP78 in lung and the level of endotoxin in plasma ($P < 0.01$). The protein expression level of GRP78 was also involved in strong positive correlations with MDA and TNF- α , in both plasma and lung tissues ($P < 0.01$) (Table 5).

Discussion

In the present study, we have successfully created the animal models suffering from HPS as indicated by the ALT level in plasma and the observations of morphological changes in liver and lung tissues. Increased GRP78 is a key event in ER stress, and is the primary regulator for ER homeostasis during this process. In addition, recent studies have found that GRP78 is also involved in the pathogenesis of various diseases (Gonzalez-Gronow et al. 2009; Ji et al. 2011). Our results showed that both the protein and mRNA expression levels of GRP78 started to increase in lung in the 4-wk HPS animal models, and continued to go up as the disease progressed, with a strong positive correlation, indicating that the up-regulation of GRP78 was related with HPS progression.

It has been demonstrated that endotoxin is an important trigger for ER stress (Ni and Lee, 2007), which could induce ER stress via the accumulation of unfolded proteins and/or calcium signaling (Endo et al. 2006; Kimura et al. 2008), as well as through oxidative stress, resulting in the elevated expression of GRP78. Studies have reported that oxidative stress would inhibit the calcium pump function, leading to the reduction in ER-stored calcium or the increase of abnormal proteins, and causes the ER stress (Ji and Kaplowitz, 2003; Nakayama et al. 2010). Our previous studies have also demonstrated the increased nitric oxide level in lungs during HPS pathogenesis, and together with endotoxin, stimulate the production of reactive oxygen species (ROS) (Güngör et al. 2010; Xu et al. 1999). This ROS elevation attack the molecular chaperones or accelerate the cellular calcium accumulation, inducing ER stress (Gorlach et al. 2006; Pacher et al. 2007), which further contributed to the disease progression. Our results herein show that the endotoxin level in plasma was increased as the disease progressed, and the pro-inflammatory cytokine TNF- α was also elevated in plasma and lung tissues, which was consistent with the change in endotoxin. Furthermore, they are both positively correlated with the expression of GRP78 in lungs in each model group, indicating that intestinal endotoxin and its related cytokines might be important factors inducing the ER stress and the elevated expression of GRP78. We also

found that the contents of MDA (an intermediate during lipid peroxidation, the levels of which reflect the impairing extent of oxygen free radicals to biomembrane) in plasma and lung tissues are increased with the disease progression, which was not only in line with but also correlated with the elevated endotoxin level, suggesting that oxidative stress might be the important intermediate link through which endotoxin caused ER stress.

In previously published work, we have demonstrated that intestinal endotoxin participated in HPS pathogenesis using animal models of liver cirrhosis and HPS (Zhang et al. 2005). When normal rats received low doses of endotoxin (0.5µg/g bodyweight/d) consecutively for 4 days, the protein expression level of GRP78 would be significantly elevated in lung tissue compared with controls, and the accumulation of large number of macrophage was observed in the alveolar cavity (unpublished data). All these results suggest an intimate relationship between endotoxin and up-regulated GRP78 expression and their important role in HPS pathogenesis. As GRP78 has been reported to be involved in a variety of diseases as a multifunctional receptor (Gonzalez-Gronow et al. 2009), it is suggested that intestinal endotoxin-induced up-regulated expression of GRP78 is most likely to be an important mechanism promoting HPS progression.

As intestinal endotoxemia (IETM) induced by liver cirrhosis is an important trigger for ER stress, we therefore conclude that the elevated expression of GRP78 is indicative of ER stress response during HPS, which may play a key role in the disease pathogenesis. Our finding reveals a novel mechanism for HPS pathogenesis, and provides a new insight into the related non-surgical treatment.

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Abbreviations

HPS	hepatopulmonary syndrome
ER	endoplasmic reticulum
IETM	intestinal endotoxemia
GRP78	78kD glucose-regulated protein
GAPDH	glyceraldehyde –3-phosphate dehydrogenase
ECL	enhanced chemiluminescence
ALT	alanine transferase
MDA	malondialdehyde
TNF-α	tumor necrosis factor-α
CCl₄	carbon tetrachloride
ROS	reactive oxygen species.

References

- Endo M, Mori M, Akira S, Gotoh T. C/EBP homologous protein (CHOP) is crucial for the induction of caspase-11 and the pathogenesis of lipopolysaccharide-induced inflammation. *J. Immunol.* 2006; 176:6245–6253. [PubMed: 16670335]
- Gonzalez-Gronow M, Selim MA, Papalas J, Pizzo SV. GRP78: a multifunctional receptor on the cell surface. *Antioxid. Redox. Signal.* 2009; 11:2299–2306. [PubMed: 19331544]
- Gorlach A, Klappa P, Kietzmann T. The endoplasmic reticulum: folding, calcium homeostasis, signaling, and redox control. *Antioxid. Redox. Signal.* 2006; 8:1391–1418. [PubMed: 16986999]
- Güngör N, Pennings JL, Knaapen AM, Chiu RK, Peluso M, Godschalk RW, Van Schooten FJ. Transcriptional profiling of the acute pulmonary inflammatory response induced by LPS: role of neutrophils. *Respir. Res.* 2010; 11:24. [PubMed: 20184723]
- Hiramatsu N, Kasai A, Hayakawa K, Yao J, Kitamura M. Real-time detection and continuous monitoring of ER stress in vitro and in vivo by ES-TRAP: evidence for systemic, transient ER stress during endotoxemia. *Nucleic. Acids. Res.* 2006; 34:e93. [PubMed: 16877567]
- Ji C. Dissection of endoplasmic reticulum stress signaling in alcoholic and non-alcoholic liver injury. *J. Gastroenterol. Hepatol.* 2008; 23:S16–S24. [PubMed: 18336657]
- Ji C, Kaplowitz N, Lau MY, Kao E, Petrovic LM, Lee AS. Liver-specific loss of glucose-regulated protein 78 perturbs the unfolded protein response and exacerbates a spectrum of liver diseases in mice. *Hepatology.* 2011; 54:229–239. [PubMed: 21503947]
- Ji C, Kaplowitz N. Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice. *Gastroenterology.* 2003; 29:235–243.
- Kimura K, Ito S, Nagino M, Isobe K. Inhibition of reactive oxygen species down-regulates protein synthesis in RAW 264.7. *Biochem. Biophys. Res. Commun.* 2008; 372:272–275. [PubMed: 18486599]
- Nakayama Y, Endo M, Tsukano H, Mori M, Oike Y, Gotoh T. Molecular mechanisms of the LPS-induced non-apoptotic ER stress-CHOP pathway. *J. Biochem.* 2010; 147:471–483. [PubMed: 19919955]
- Ni M, Lee AS. ER chaperones in mammalian development and human diseases. *FEBS. Lett.* 2007; 581:3641–3651. [PubMed: 17481612]
- Pacher P, Beckman JS, Liaudet L. Nitric Oxide and Peroxynitrite in Health and Disease. *Physiol. Rev.* 2007; 87:315–424. [PubMed: 17237348]
- Su AR, Zhang HY, Guan SD. Lung tissue NOS expression in the formation of HPS in rats. *Journal of Bengbu Medical College.* 2010; 35:340–342.
- Xu KY, Huso DL, Dawson TM, Bredt DS, Becker LC. Nitric oxide synthase in cardiac sarco plasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* 1999; 96:657–662. [PubMed: 9892689]
- Zhang HY, Han DW, Wang XG. The role of lipopolysaccharide in HPS development. *Chinese Journal of Pathophysiology.* 2005; 21:2238–2242.
- Zhang HY, Han DW, Zhao ZF, Liu MS, Wu YJ, Chen XM, Ji C. Multiple pathogenic factor-induced complications of liver cirrhosis in rats: A new model of hepatopulmonary syndrome with intestinal endotoxemia. *World J. Gastroenterol.* 2007; 13:3500–3507. [PubMed: 17659698]

Highlights

Serum levels of endotoxin, ALT and TNF- α were significantly increased in HPS rats.

Levels of GRP78 and MDA in lung tissues were significantly increased in HPS rats.

Serum ALT and TNF- α levels were correlated with TNF- α and MDA levels in lung tissues.

The increased GRP78 expression is indicative of ER stress response during HPS.

Figure 1. Pathological changes in liver and lung tissues in HPS models (H & E staining, $\times 100$). (A) Normal liver tissue in control group. (B) Liver cirrhosis in 4-wk HPS group. (C) Liver cirrhosis in 6-wk HPS group. (D) Liver cirrhosis in 8-wk HPS group. (E) Normal lung tissue in control group. (F) Lung tissues in 4-wk HPS group. (G) Lung tissues in 6-wk HPS group. (H) Lung tissues in 4-wk HPS group.

Figure 2.

The protein expression levels of GRP78 in lung tissues in HPS models. (A) The protein level of GRP78 in lungs was detected with Western blot, using the rabbit anti-rat polyclonal antibody against GRP78. (B) Statistical analysis of GRP expression levels in HPS models. *, $P < 0.05$ v.s. normal control; Δ, $P < 0.05$ v.s. 4-wk HPS group; #, $P < 0.05$ v.s. 6-wk HPS group.

Figure 3.

The mRNA expression levels of GRP78 in lung tissues in HPS models. (A) The protein level of GRP78 in lungs was detected with real-time PCR. (B) Statistical analysis of GRP expression levels in HPS models. *, $P < 0.05$ v.s. normal control; Δ, $P < 0.05$ v.s. 4-wk HPS group; #, $P < 0.05$ v.s. 6-wk HPS group.

Table 1

Levels of endotoxin, TNF-α, and ALT in the plasma in control and HPS groups.

	Endotoxin (EU/ml)		TNF-α (ng/ml)		ALT (IU/L)	
	Control (n=6)	HPS (n=11)	Control (n=6)	HPS (n=11)	Control (n=6)	HPS (n=11)
4	0.039±0.01	0.068±0.035	0.955±0.12	1.526±0.162 [*]	209.25±5.2	220.92±1.697
6	0.040±0.02	0.273±0.169 ^{*Δ}	1.025±0.24	1.728±0.416 [*]	207.05±1.8	311.04±2.758 ^{*Δ}
8	0.042±0.03	0.642±0.427 ^{*Δ#}	1.221±0.28	1.864±0.318 ^{*Δ}	199.90±2.4	253.66±3.765 ^{*Δ#}

Note:

^{*} *P* < 0.05 v.s. normal control;

^Δ *P* < 0.05 v.s. 4-wk HPS group;

[#] *P* < 0.05 v.s. 6-wk HPS group.

Table 2

Levels of TNF- α and MDA in the lung tissue in control and HPS groups.

	TNF- α (ng/ml)		MDA(nmol/mg)	
	Control (n=6)	HPS (n=11)	Control (n=6)	HPS (n=11)
4 wk	0.849 $\pm$ 0.071	1.219 $\pm$ 0.051 [*]	1.651 $\pm$ 1.432	2.463 $\pm$ 0.574
6 wk	0.941 $\pm$ 0.183	1.987 $\pm$ 0.772 ^{*Δ}	1.819 $\pm$ 1.157	3.214 $\pm$ 1.991 ^{*Δ}
8 wk	0.982 $\pm$ 0.108	2.018 $\pm$ 0.325 ^{*Δ}	1.686 $\pm$ 1.112	5.167 $\pm$ 0.455 ^{*Δ#}

Note:

^{*} $P < 0.05$ v.s. normal control;

^{Δ} $P < 0.05$ v.s. 4-wk HPS group;

[#] $P < 0.05$ v.s. 6-wk HPS group.

Table 3

Correlation analysis of changed indexes in HPS models.

Group	r	P value
Endotoxin level in plasma v.s. TNF- α level in plasma	0.658	< 0.01
Endotoxin level in plasma v.s. TNF- α level in lungs	0.681	< 0.01
Endotoxin level in plasma v.s. MDA level in lungs	0.796	< 0.01
GRP78 protein expression level v.s. Endotoxin level in plasma	0.833	< 0.01
GRP78 protein expression level v.s. GRP78 mRNA expression level	0.821	< 0.01
GRP78 protein expression level v.s. TNF- α level in plasma	0.693	< 0.01
GRP78 protein expression level v.s. TNF- α level in lungs	0.731	< 0.01
GRP78 protein expression level v.s. MDA level in lungs	0.621	< 0.01