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PROTEOMIC ALTERATION OF MITOCHONDRIAL ALDEHYDE DEHYDROGENASE 2 IN SEPSIS REGULATED BY HEAT SHOCK RESPONSE

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ABSTRACT—The present study was designed to investigate the proteomic alteration of hepatic mitochondria during sepsis and to explore the possible effects induced by heat shock treatment. Sepsis was induced by cecal ligation and puncture in Sprague-Dawley rats. Liver mitochondrial proteins were isolated and evaluated by 2-dimensional electrophoresis with broad pH–ranged (pH 3 – 10) immobile DryStrip and sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The protein spots were visualized with silver stain and analyzed by Bio-2D software. Results showed that around 120 dominant spots could be separated and visualized distinctly by 2-dimensional electrophoresis analysis. Among them, three spots with the same molecular weight (56.4 kd), mitochondrial protein 1 (MP1), MP2, and MP3, were significantly altered in septic specimens. When analyzed by liquid chromatography–tandem mass spectrometry, the three spots all revealed to be an identical enzyme: aldehyde dehydrogenase 2 (ALDH2, EC 1.2.1.3). During sepsis, MP1 and MP2 were downregulated, whereas MP3 was upregulated concomitantly. Interestingly, heat shock treatment could reverse this phenomenon. Phosphoprotein staining showed that the degree of phosphorylation is higher in MP1 and MP2 than that in MP3. The enzyme activity assay showed that ALDH2 activity was downregulated in nonheated septic rats of 18 h after cecal ligation and puncture operation, and preserved in heated septic rats. The results of this study suggest that posttranslation modification, highly possible the phosphorylation, in ALDH2 may play a functional role in the pathogenesis of sepsis and provide a novel protective mechanism of heat shock treatment.

KEYWORDS—2-Dimensional electrophoresis, cecal ligation and puncture, heat shock treatment, liver, rat

INTRODUCTION

Despite recent advances in modern medicine, sepsis and the subsequent syndromes remain one of the major causes leading to mortality in medical critical care unit. Clinically, sepsis causes adverse metabolic alterations and leads to cellular and tissue dysfunction. Subjects with sepsis also manifest variable stress conditions that contribute to multiple organ system failure (1, 2). Among them, liver failure is one of the characteristics in critically ill and patients with sepsis. Although the mechanisms of cellular dysfunction induced by sepsis is still a mystery, more and more published literatures have shown that it is linked to the mitochondrial disturbances (3–5). These disturbances include impaired mitochondrial energy production, generation of free radicals within the mitochondria, and activation of the mitochondrial apoptotic pathway. Because mitochondria play a crucial role in mammals, disturbances of mitochondrial function have been proposed as the possible explanation for some pathological developments and can be critical in leading to adverse cascade of sepsis (4, 6). Conversely, preservation of mitochondrial function during sepsis can lead to positive results. However, the pathological changes of mitochondria during sepsis were still incompletely known.

In clinical settings, the course of sepsis with similar foci infection can differ between individuals. This could be correlated with individual differences in expression of cytoprotective mechanisms. The heat shock response and its subsequent effects, such as cross-tolerance induction, are considered to be a type of mechanism of self-protection (3, 7). Induction of heat shock proteins (HSPs) is the major characteristic of the heat shock response (8). Recent researches have shown that HSPs are recognized as a family of endogenous protective proteins, induced by various cellular stressors, and may be attributed in part to their ability to stabilize intracellular protein structures (9). In addition, they are molecular chaperones that facilitate the biosynthesis and the folding of intracellular protein, assist in refolding or renaturing of the misfolded or denatured peptide in various cellular compartments, and contribute to the translocation of peptides across the membranes of organelles (9, 10).

Our previous studies showed that HSPs, induced by heat shock treatment, significantly reduced the mortality rate of rats with cecal ligation and puncture (CLP)–induced sepsis (11). The protective effects of heat shock response also have been confirmed in various animal models of sepsis (12–14). Specifically, heat shock response contributes to reversing the

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impediment of mitochondrial respiratory enzyme activities and the decrease of adenosine triphosphate content in the liver of experimental septic rats (3).

It is clear that heat shock treatment is beneficial to the outcome of sepsis, which is closely linked to mitochondrial disturbances (3), although the molecular mechanisms remain to be elucidated. The aim of this study is to investigate the proteomic alteration of hepatic mitochondria during sepsis. Three spots were expressed novelly and analyzed to reveal an identical enzyme: aldehyde dehydrogenase 2 (ALDH2, EC 1.2.1.3). The possible role of the enzyme during sepsis was discussed.

MATERIALS AND METHODS

Animals

Experiments were performed on adult male Sprague-Dawley rats (weighing 270 – 350 g) obtained from the National Experimental Animal Center (Nan-Kang, Taipei, Taiwan, ROC). The experiments conducted in this study were approved by the Animal Committee of Kaohsiung Medical University, and the authors adhered to the Guidelines of Animal Care laid down by the National Institutes of Health for use on experimental animals.

The animals were arranged in two experimental parts: CLP treatment part, which includes the groups: sham-operated control group (S group, n = 7); nonheated early stage of sepsis group, 9 h after CLP operation (E group, n = 7); nonheated late stage of sepsis group, 18 h after CLP operation (L group, n = 7); heated early stage of sepsis group, heat shock treatment 24 h before CLP operation (HE group, n = 7); and heated late stage of sepsis group, heat shock treatment 24 h before CLP operation (HL group, n = 7); and heat shock treatment parts, which include the groups: nonheated intact rats (NH group, n = 4); rats after 42 h of heat shock treatment (H42 group, n = 4).

Heat shock treatment

Rats of the preheated group were anesthetized with pentobarbital and then heated whole bodily 24 h before CLP operation with an automatic heating pad (3, 15). When the rectal temperature reached 41°C, it was maintained between 41°C and 42°C for 15 min. The rats of the nonheated group were also anesthetized, but no heating was applied.

Induction of sepsis

Sepsis was induced by CLP as described previously (16). Briefly, the animals were permitted nothing but drinking water 6 h before the operation. Under anesthesia, a laparotomy was performed, in which the cecum was pulled out and ligated just below the ileocecal valve. The ligated cecum was punctured twice at different sites, and the cecum was gently compressed until the feces were extruded. The bowel was then returned to the abdomen, and the abdominal incision was closed in two layers. Control animals underwent a sham operation.

Isolation of liver mitochondria

The experimental animals were killed under anesthesia, and the mitochondria of liver were isolated as described previously (3). Samples were finely minced and were homogenized in cold buffer A (100 mM Tris-HCl, 70 mM sucrose, 10 mM EDTA, 210 mM mannitol, pH 7.4) and then centrifuged twice at 700 *g* for 10 min at 4°C. The supernatant was centrifuged again at 8,000 *g* for 15 min, and the pellet was then washed with buffer B (10 mM Tris-HCl, 70 mM sucrose, 1 mM EDTA, 230 mM mannitol, pH 7.4). The mitochondria-rich pellet was collected, and the purity was assured by electron microscope in several randomly selected samples. The yield of mitochondria was stable, and the protein content ranged from 6 to 8 mg/g of liver. In addition, mitochondrial fractions were routinely monitored by Western blotting for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and α-tubulin to exclude the contamination of cytosolic fractions in the mitochondrial fraction and for cytochrome oxidase to be a mitochondrial fraction marker. The cytosolic fraction was also detected concomitantly.

Western blot analysis

Equal amounts (10 μg) of protein extract were loaded and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, the proteins on the gel were transferred to polyvinylidene difluoride membranes (NEN Life Science Products, Boston, Mass). Heat shock protein 72 (StressGen Biotechnologies, Victoria, British Columbia) was used as the primary antibody, whereas horseradish peroxidase conjugated antimouse was used as the secondary antibody. Target protein was detected by enhanced chemiluminescence. Actin was detected simultaneously and acts as an internal control.

Two-dimensional gel electrophoresis

Samples of 30-μg protein were contained in rehydration solution (2% immobilized nonlinear pH gradient [IPG] buffer; 8 M urea; 2% NP-40; 7 mg dithiothreitol per 2.5 mL) and were applied to rehydrate the immobilized pH 3 to 10 nonlinear IPG strip (Pharmacia Biotech, Piscataway, NJ). Focusing started at 200 V, and after gradually increasing to 3,500 V, the voltage was kept constant for another 1 h (approximately 6,500 Vh total; Multiphor II, Pharmacia Biotech). After focusing, the IPG strip was immersed in SDS–equilibration buffer (50 mM Tris-HCl/pH 8.8; 6 M urea; 30% glycerol; 2% SDS; 0.01% bromophenol blue) for 10 min, and the 2-dimensional separation was performed on 12% SDS-PAGE. After electrophoresis, the gels were stained with silver staining (Wako Pure Chemical Industries, Osaka, Japan). The molecular masses were evaluated by comparing with standard protein markers. The pI values were used as given by the supplier of the IPG strips. Protein spots were quantified by a densitometer and an analysis software (Bio-2D V.97 software, Vilber Lourmat, France).

Protein identification by liquid chromatography–tandem mass spectrometry

Target spots were cut out, and the peptides were digested in-gel with trypsin (cuts after lysine and arginine). The peptide digests were then analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) as described previously. After in-gel digest, peptides within the mass range of 500 to 1,500 d were subjected to MS/MS analysis, and the protein identification was performed with the MASCOT MS/MS Ion Search software (17).

Detection of ALDH2 activity

Enzyme activity was determined by monitoring nicotinamide adenine dinucleotide (NADH) formation at 340 nm using a spectrophotometer (Beckman, DU600, Fullerton, Calif), with slight modifications made to the previously described procedure (18). The incubation mixture was consisted of 3 mg of mitochondrial extraction, 100 mM sodium phosphate buffer (pH 7.4), 0.75 mM NAD^+, and 1 mM 2-butoacetaldehyde as a substrate in a total volume of 1.5 mL. The reaction was initiated by addition of the substrate and was detected continually for at least 30 min at 25°C. The result was calculated as unit NADH/min per mg protein (nmol NADH/min per mg protein).

Phosphoprotein detection

Sample was delipidated and desalted using methanol/chloroform precipitation by mixing with 600 μL of methanol, 150 μL of chloroform, and 450 μL of water, and centrifuging at 12,000 *g* for 5 min. The white precipitation disk between the upper and lower phases was collected and washed with 450 mL of methanol and vacuum-dried. Proteins were then resuspended in sample buffer and separated by 2-dimensional gel electrophoresis. After electrophoresis, staining of phosphorylated proteins was performed using the Pro-Q Diamond Phosphoprotein Stain Kit (Molecular Probes, Eugene, Ore) according to the manufacturer's protocol and imaged on an Amersham Typhoon laser scanner (Piscataway, NJ) at wavelengths of 530/580 nm for excitation/absorption. Subsequently, Pro-Q diamond-stained gels were washed in Milli-Q water and then fixed overnight in a methanol:acetic acid:water solution (4:1:5 vol/vol/vol) before counterstaining with silver stain.

Statistical analysis

The protein bands of the Western blot analysis were quantified by densitometer and analysis software (Bio-1D V.97 software, Vilber Lourmat). All data were expressed as mean ± SEs. Statistical analysis of the data was performed using 1-way analysis of variance followed by the Newman-Keuls test. A 95% confidence limit was accepted as statistically significant.

RESULTS

Compartmentalization of the HSP-72 expressions after heat shock treatment

The HSP-72 was significantly increased after heat shock treatment in rat liver, and it is a sustained expression until the time point that the animals are killed, at 9 or 18 h after CLP operation. Moreover, the expression of HSP-72 in the cytosolic fraction of the liver is numerous, while HSP-72 is undetected in the mitochondrial fraction (Fig. 1A). In the nonheated group, HSP-72 cannot be detected both in the cytosol fraction and in the mitochondrial fraction.

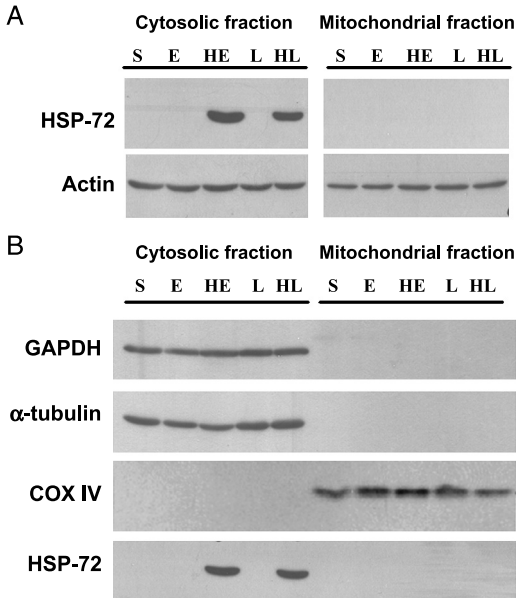

FIG. 1. **A, The HSP-72 expression induced by heat shock treatment in the cytosol and mitochondrial fractions.** One representative expression out of four was shown. Actin was detected simultaneously and acts as the internal control. B, Purity of mitochondrial fractions monitored by Western blotting. Detection of GAPDH and α-tubulin was performed to exclude cytosolic contamination in the mitochondrial fraction. The COX-IV served as a mitochondrial fraction marker. COX IV – cytochrome *c* oxidase subunit IV; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; S – sham-operated control group; E – nonheated early stage of sepsis group, 9 h after CLP operation; HE – preheated early stage of sepsis group; L – nonheated late stage of sepsis group, 18 h after CLP operation; and HL – preheated late stage of sepsis group.

Illustration of mitochondria proteomic alteration in septic liver and the possible effect of heat shock treatment

The quality of the mitochondrial fractions was monitored by detecting the amount of GAPDH and α-tubulin by Western blotting. The GAPDH and α-tubulin act as markers for cytosolic contamination of the mitochondrial fraction, and cytochrome *c* oxidase subunit IV (COX IV) was detected concomitantly as positive control. The almost undetectable GAPDH and α-tubulin in the mitochondrial fraction indicated that there was minimal cytosolic contamination of this fraction (Fig. 1B). The mitochondrial protein from liver of the septic rat was first separated on pH 3 to 10 IPG strips by isoelectric focusing and then separated based on mass by SDS-PAGE. After silver staining, the gels reproducibly revealed about 120 dominant spots. One representative picture of sham group was shown in Figure 2A. Spots of glucose-regulated protein 75 (Grp 75), HSP-60, actin, and protein disulfide isomerase, acting as indices of relative position, have been identified by immunochemical staining. Furthermore, the regions of the pH 5-9 and 37-65 kd gel were zoomed and shown in Figure 2B. There were three spots, mitochondrial protein 1 (MP1), MP2, and MP3, of similar molecular weights (56.4 kd), with notable different intensities between specimens. For quantification, the spots of actin were selected as the internal control.

The relative contents of various MPs (MP1, MP2, and MP3) and the sum of MP1, MP2, and MP3, representing the total MP, were compared between different groups (Fig. 2C). The results showed that the expression of MP1 and MP2 decreased significantly as compared with sham group in both early stage (9 h after CLP) and late stage (18 h after CLP) of sepsis ($P < 0.05$), whereas the expression of MP3 were induced during sepsis ($P < 0.05$). After heat shock treatment, it leads exactly to the opposite results. Heat shock treatment prevented the decrease of MP1 and MP2 and interfered with the induction of MP3 during sepsis. Interestingly, there are no differences in the total MP, the sum of MP1, MP2, and MP3 between all groups.

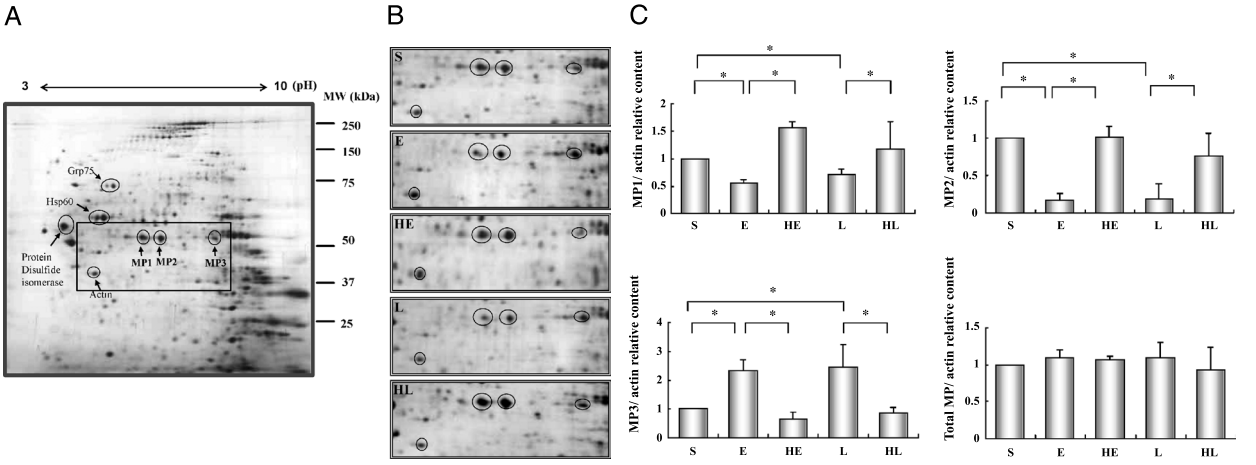

FIG. 2. **The 2-dimensional electrophoresis map of mitochondrial proteins from rat liver.** A, A representative image of liver mitochondrial proteins of rat separated by 2-dimensional electrophoresis. The gel was stained using silver staining. Over 120 spots were observed. Some proteins, such as protein disulfide isomerase, HSP-60, Grp75, and actin, were determined by Western blot analysis and are marked by circle and arrowhead. The three spots, named MP1, MP2, and MP3, are also marked. The numbers above the photograph indicate pI values, and the numbers on the right indicate molecular masses of standard proteins. B, Regions of representative 2-dimensional gels containing differentially expressed proteins in different groups. Equal amounts of liver mitochondrial extract from different groups were applied. One representative expression out of six or seven different experiments is shown. The regions of the pH 5 to 9 gel were expanded, and MP1, MP2, and MP3 are circled. Actin is circled simultaneously and acts as the internal control. C, Statistical analysis of relative content of MP1, MP2, MP3, and total MP in different groups. Values of relative intensities of a series of MPs to actin are shown. Data were expressed as mean ± SE in each group. S – sham-operated control group (n = 7); E – nonheated early stage of sepsis group, 9 h after CLP operation (n = 6); HE – preheated early stage of sepsis group (n = 7); L – nonheated late stage of sepsis group, 18 h after CLP operation (n = 6); and HL – preheated late stage of sepsis group (n = 7). *$P < 0.05$ vs. each other as indicated.

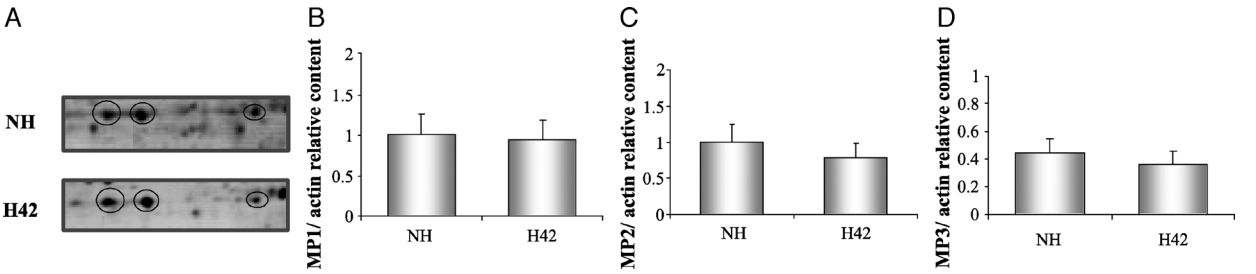

FIG. 3. **The effect of heat shock treatment for MP1, MP2, and MP3 in liver mitochondria of septic rats.** A, Regions of representative 2-dimensional gels containing MP1, MP2, and MP3. Equal amounts of liver mitochondrial extract from different groups were applied. One representative expression of four different experiments is shown. The regions of the pH 5 to 9 gel were expanded, and MP1, MP2, and MP3 are circled. Actin is circled simultaneously and acts as the internal control. B, C, and D, Statistical analysis of relative content of MP1, MP2, MP3 in different groups. Values of relative intensities of a series of MPs to actin are shown. Data were expressed as mean ± SE in each group. NH – nonheated intact rat (n = 4); H42 – 42 h after heat shock treatment rat (n = 4).

Effects of heat shock treatment in MP1, MP2, and MP3

The expressions of MPs (MP1, MP2, and MP3) in intact rat without heat shock treatment and 42 h after heat shock treatment were detected by the identical method described previously (Fig. 3A). The results showed that the expression of MP1, MP2, and MP3 were of no difference after heat shock treatment as compared with intact group (Fig. 3B).

Identification of MPs MP1, MP2, and MP3

Analysis of MP1, MP2, and MP3 by LC-MS/MS showed that the various three spots all matched to an identical enzyme and were highly homogeneous with ALDH2 (EC 1.2.1.3) (Fig. 4). Thus, the three variants of ALDH2 were temporarily termed as MP1, MP2, and MP3, respectively.

Mitochondrial ALDH2 activity in septic liver with or without heat shock pretreatment

The enzyme activity assay showed that mitochondrial ALDH2 activity was slightly decreased during nonheated early sepsis, whereas it was significantly decreased in nonheated late sepsis group ($P < 0.05$) (Fig. 5). With heat shock pretreatment, the mitochondrial ALDH2 activity of early and late sepsis showed no difference as compared with sham control group. This implies that heat shock treatment contributes to preserve the ALDH2 activity during sepsis, particularly in the late stage ($P < 0.05$).

Phosphoprotein detection

The degree of protein phosphorylation in MP1, MP2, and MP3 was detected by a phospho-specific protein dye (Fig. 6). In sham group, the MP1 and MP2 are highly stained by silver stain and they are also showed high intensity in phosphorylated protein staining. In the late stage of sepsis, the high expression of MP3 was observed by silver stain, while the intensity of MP3 phosphoprotein staining was weaker compared with MP2. The result showed that the degree of phosphorylation in MP1 and MP2 phosphorylation is obviously higher than that in MP3.

DISCUSSION

Using proteomic analysis, we investigated the alteration of liver mitochondrial proteins during sepsis, and the possible effects of heat shock treatment. The results showed that the

(1) MLRAALSTAR RGPRLSRLLS AAATSAVPAP NQQPEVFCNQ IFINNEWHDA VSKKTFPTVN PSTGEVICQV AEGNKEDVDK 80
(2) MLRAALSTAR RGPRLSRLLS AAATSAVPAP NQQPEVFCNQ IFINNEWHDA VSKKTFPTVN PSTGEVICQV AEGNKEDVDK 80
(3) MLRAALSTAR RGPRLSRLLS AAATSAVPAP NQQPEVFCNQ IFINNEWHDA VSKKTFPTVN PSTGEVICQV AEGNKEDVDK 80

(1) AVKAAQAAFQ LGSPWRRMDA SDRGRLLYRL ADLIERDRTY LAALETLDNG KPYVISYLVD LDMVLKCLRY YAGWADKYHG 160
(2) AVKAAQAAFQ LGSPWRRMDA SDRGRLLYRL ADLIERDRTY LAALETLDNG KPYVISYLVD LDMVLKCLRY YAGWADKYHG 160
(3) AVKAAQAAFQ LGSPWRRMDA SDRGRLLYRL ADLIERDRTY LAALETLDNG KPYVISYLVD LDMVLKCLRY YAGWADKYHG 160

(1) KTIPIDGDFF SYTRHEPVGV CGQIIPWNFP LLMQAWKLGP ALATGNVVVM KVAEQTPLTA LYVANLIKEA GFPPGVVNIV 240
(2) KTIPIDGDFF SYTRHEPVGV CGQIIPWNFP LLMQAWKLGP ALATGNVVVM KVAEQTPLTA LYVANLIKEA GFPPGVVNIV 240
(3) KTIPIDGDFF SYTRHEPVGV CGQIIPWNFP LLMQAWKLGP ALATGNVVVM KVAEQTPLTA LYVANLIKEA GFPPGVVNIV 240

(1) PGFGPTAGAA IASHEDVDKV AFTGSTEVGH LIQVAAGSSN LKRVTLELGG KSPNIIMSDA DMDWAVEQAH FALFFNQGQC 320
(2) PGFGPTAGAA IASHEDVDKV AFTGSTEVGH LIQVAAGSSN LKRVTLELGG KSPNIIMSDA DMDWAVEQAH FALFFNQGQC 320
(3) PGFGPTAGAA IASHEDVDKV AFTGSTEVGH LIQVAAGSSN LKRVTLELGG KSPNIIMSDA DMDWAVEQAH FALFFNQGQC 320

(1) CCAGSRTFVQ EDVYDEFVER SVARAKSRVV GNPFDSRTEQ GPQVDETQFK KILGYIKSGQ QEGAKLLCGG GAAADRGYFI 400
(2) CCAGSRTFVQ EDVYDEFVER SVARAKSRVV GNPFDSRTEQ GPQVDETQFK KILGYIKSGQ QEGAKLLCGG GAAADRGYFI 400
(3) CCAGSRTFVQ EDVYDEFVER SVARAKSRVV GNPFDSRTEQ GPQVDETQFK KILGYIKSGQ QEGAKLLCGG GAAADRGYFI 400

(1) QPTVFGDVKD GMTIAKEEIF GPVMQILKFK TIEEVVGRAN NSKYGLAAAV FTKDLDKANY LSQALQAGTV WINCYDVFGA 480
(2) QPTVFGDVKD GMTIAKEEIF GPVMQILKFK TIEEVVGRAN NSKYGLAAAV FTKDLDKANY LSQALQAGTV WINCYDVFGA 480
(3) QPTVFGDVKD GMTIAKEEIF GPVMQILKFK TIEEVVGRAN NSKYGLAAAV FTKDLDKANY LSQALQAGTV WINCYDVFGA 480

(1) QSPFGGYKMS GSGRELGEYG LQAYTEVKTV TVKVPQKNS
(2) QSPFGGYKMS GSGRELGEYG LQAYTEVKTV TVKVPQKNS
(3) QSPFGGYKMS GSGRELGEYG LQAYTEVKTV TVKVPQKNS

Mass (average): 56488.4

FIG. 4. **Graphical representation of the protein sequence and the location of the identified peptides.** The Figure shows the ordered arrangement of the amino acids in the protein of ALDH2. The sequence is written from the N-terminus at the left, to the C-terminus at the right using the 1-letter amino acid codes. The position of the last amino acid in each row is given on the right hand side. The sections of peptide matching MP1, MP2, or MP3, detected by LC-MS/MS peptide analysis, are underlined and bold. 1 – MP1; 2 – MP2; 3 – MP3.

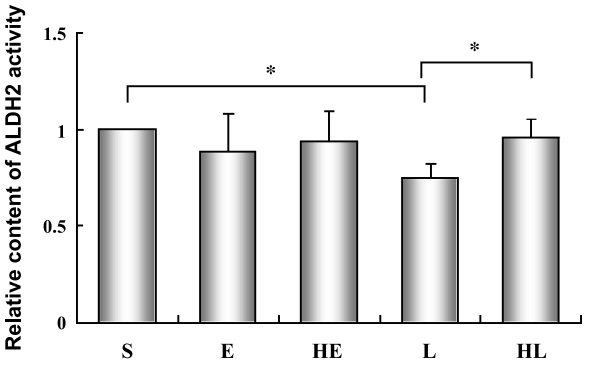

FIG. 5. **The ALDH2 activity of the liver mitochondria in various experimental groups.** The ALDH2 activity was determined using NADPH depletion detected by spectrophotometer. Relative activity ratio of experimental groups to the sham-operated control group was quoted. Data of seven samples were expressed as mean ± SE in each group. S – sham-operated control group; E – nonheated early stage of sepsis group, 9 h after CLP operation; L – nonheated late stage of sepsis group, 18 h after CLP operation; HE – preheated early stage of sepsis group; HL – preheated late stage of sepsis group. $*P < 0.05$ vs. each other as indicated.

three variants of mitochondrial ALDH2, termed MP1, MP2, and MP3, were detected by 2-dimensional electrophoresis in the liver mitochondria of septic rats. The contents of MP1 and MP2 were decreased, and the MP3 content was increased in both nonheated sepsis groups, whereas heat shock pretreatment can reverse this effect. Moreover, the activity of the ALDH2 in the liver mitochondria was inhibited significantly in nonheated sepsis 18 h after CLP operation, whereas heat shock treatment contributed to preserve the ALDH2 activity during sepsis. Interestingly, the total amount of ALDH2 in mitochondria, which is the sum of the three variants, was of no difference between each group. Moreover, the degree of protein phosphorylation in MP1 and MP2 was higher than that in MP3. The posttranslational modification, protein phosphorylation, may be involved in affecting the ALDH2 enzymatic activity, without modifying protein content. Heat shock treatment can prevent the decrease of ALDH2 activity through mediating the proportional content of the ALDH2 variants in mitochondria during sepsis.

The ALDHs are a group of $NAD(P)^+$-dependent enzymes responsible for oxidizing aldehydes to carboxylic acids (18, 19), and they are present in most tissues, particularly with a higher activity in the liver (20, 21). The ALDH2, one of the ALDH families, is mainly localized in the mitochondrial fraction (19). Being responsible for oxidation of acetaldehyde derived from ethanol metabolism, ALDH2 is correlated with the oxidation of various exogenous toxic aldehydes and endogenous aldehydes, such as those derived from lipid peroxidation (22, 23). Most of the authors suggest that ALDH2 acts as a protector against oxidative stress (22, 24, 25).

During sepsis, oxidative stress is a consequence of critical illness and may have an impact on survival. After the inappropriate production of reactive oxygen species during sepsis, the intermediary production, such as reactive aldehydes from lipid or protein oxidation, can lead to organelle damage or cell death (26, 27). A major source of reactive oxygen species is a superoxide anion radical derived from mitochondria (27) that results in peroxidation of the mitochondrial membrane. Mitochondrial inner membrane is rich in polyunsaturated fatty acids, and the toxic reactive aldehydes can be easily derived from the peroxided polyunsaturated fatty acids (22, 24). Toxic reactive aldehydes have the ability to readily adduct cellular proteins and may damage the proteins by interacting with lysine, histidine, serine, and cysteine residues (28). Thus, rapid detoxification of toxic reactive aldehydes of lipid peroxidation by ALDH2 of mitochondria is important to cell survival (24, 29). Indeed, mitochondrial structural damage induced by oxidative stress during sepsis, such as rupture of the outer membrane, budding, and extension of the inner and the voluminous vacuoles in the mitochondrial matrix, can be observed in our previous study (3). The ultrastructure alteration is compatible with the change of ALDH2 activity. In the present study, we showed for the first time that ALDH2 activity decreased significantly in the late stage of sepsis. We therefore suggest that the decrease in ALDH2 activity may contribute to oxidative stress by enhancing the accumulation of toxic acetaldehydes.

The three variants of ALDH2 had been described in previous literature and were considered as novel markers that are correlated to hepatocellular carcinomas (30). During sepsis, the downregulation of MP1 and MP2 and the upregulation of MP3 are observed concomitantly. To cross reference with the results of ALDH2 activity, change in amounts of ALDH2 variants induced by sepsis may correlate closely with ALDH2 activity. We suggest that MP1 and MP2 may be the activity-associated variants, and that MP3 is the inactive/de-active form. Based on this assumption, heat shock treatment can modulate the transformation of ALDH2 variants during sepsis by maintaining the

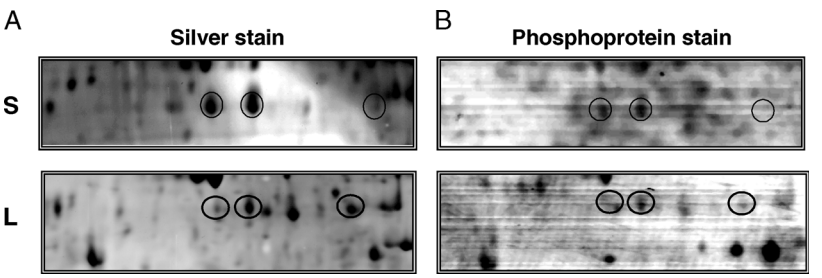

FIG. 6. **The degree of phosphorylated protein in the three variants of ALDH2.** One representative expression of three was shown in the sham group and the late sepsis group. A, The total protein was counterstained with silver stain. B, The phosphorylated proteins were stained with Pro-Q phosphoprotein gel stain, and the regions containing MP1, MP2, and MP3 were represented. S – sham-operated control group; L – nonheated late stage of sepsis group, 18 h after CLP operation.

expressions of the active form, MP1 and MP2, thus preventing the decrease of ALDH2 activity induced by sepsis. Therefore, we considered that the ALDH2 variants could be considered as a novel marker of the disease processes in sepsis.

Besides the protein content, the activity of enzyme protein is also affected with itself posttranslational modification, such as protein phosphorylation. We detected the protein phosphorylation with a commercial assay of Pro-Q Diamond Phosphoprotein Gel Stain. The result showed that the degree of protein phosphorylation in MP1 and MP2 are higher than that in MP3. We consider that MP1 and MP2, with higher phosphorylation, would contribute to higher enzyme activity than MP3, with lower phosphorylation. Without modifying total protein content, the shift of ALDH2 variants toward MP3 during sepsis may be a critical factor that leads to the decrease of ALDH2 activity.

Like most mitochondrial proteins, ALDH2 is a nuclear-encoded mitochondrial enzyme that is localized in the mitochondrial matrix (31). The ALDH2 precursor, transported from cytoplasm to the mitochondrial matrix, needs to associate with a series of chaperone molecules. The HSPs contribute in the peptide folding, renaturing, and transporting between different organelles (32, 33). Cytosolic HSP facilitates the transfer of ALDH2 precursor into the mitochondria, and mitochondrial HSPs help to drive the translocation of ALDH2 into the matrix (32, 33). In addition, HSPs in the mitochondria may influence the pattern of protein refolding and protect the protein from proteolytic degradation (33). During sepsis, the total ALDH2 content was not different between each group, although the activity of ALDH2 decreased in nonheated late sepsis. We suggest that ALDH2 should be readily transported from cytoplasm to mitochondria in both heated and nonheated sepsis groups, and that HSPs participate in maintaining the active form of ALDH2 variants, thus preserving the ALDH2 activity during sepsis. In the present study, HSP-72 induction can be detected in cytosol and can be regarded as a successful indicator for heat shock response. Successful heat shock treatment may contribute to maintain mitochondrial HSP-70 (Grp75) expression during sepsis, and that is correlated closely with mitochondrial functions (3). However, further studies are required to examine the characteristics of different ALDH2 variants and their interaction with HSPs.

Besides the direct effects of HSP described previously, the ALDH2 activity could be modulated indirectly through the heat shock response–related effects during sepsis. During sepsis, many endogenous or exogenous mediator substances may lead to cellular dysfunction or disturbance. The subsequent release of nitric oxide (NO) is considered an important candidate for disturbing mitochondrial function because NO, produced by inducible NO synthase (iNOS), is a potent inhibitor of ALDH (34). Heat shock response, however, contributes to inhibiting iNOS expression in septic rat hepatocytes and prevents the NO-induced oxidative stress (35, 36). In our previous study, we have also shown that heat shock treatment decreased the iNOS mRNA during sepsis (7). Thus, we suggest that the heat shock response–related effect, the iNOS/NO inhibition, also maintains the ALDH2 activity during sepsis. Although the mechanisms remain to be investigated, the role of ALDH2 in sepsis was highlighted in the present study.

In conclusion, we suggest that posttranslation modification, highly possibly the phosphorylation in ALDH2, may play a functional role in the pathogenesis of sepsis and provide a novel protective mechanism of heat shock treatment.

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