J Gastroenterol 2004; 39:724–733
DOI 10.1007/s00535-003-1380-8

Journal of Gastroenterology

Expression of a 72-kDa heat shock protein, and its cytoprotective function, in gastric mucosa in cirrhotic rats

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Background. Portal hypertensive gastropathy (PHG) is a clinical entity that is observed frequently in patients with liver cirrhosis. In PHG, gastric mucosa is highly susceptible to mucosal injury caused by noxious agents. Many studies, including ours, have reported that a 72-kDa heat shock protein (HSP72) has a crucial cytoprotective function in gastric mucosa. In this study, we investigated the expression and cytoprotective effect of HSP72 on gastric mucosa in portal hypertensive rats. ***Methods.*** PHG was produced by bile duct ligation (BDL) or carbon tetrachloride administration in male Sprague-Dawley rats. The expression of HSP72 in the gastric mucosa was evaluated by Western blotting. Induction of gastric mucosal HSP72 by 6-h water-immersion stress was compared between cirrhotic and control rats. Also, mucosal protective abilities against hydrochloric acid (HCl; 0.6 N) following pretreatment with water-immersion stress to induce HSP72 were studied in both groups. ***Results.*** Portal venous pressure was significantly higher in cirrhotic rats compared with control rats ($P < 0.05$). Baseline expression (before water-immersion stress) of mucosal HSP72 was significantly lower in cirrhotic rats compared with control rats. HCl-induced gastric mucosal lesions were significantly suppressed in control rats compared with cirrhotic rats, especially when HSP72 was preinduced by water-immersion stress. ***Conclusions.*** These findings suggest that HSP72 in the gastric mucosa plays a crucial role with respect to cytoprotection; the induction of HSP72 may provide therapeutic strategies for protection against mucosal injury in PHG.

Key words: heat shock protein, liver cirrhosis, portal hypertensive gastropathy

Received: September 1, 2003 / Accepted: December 19, 2003
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Introduction

The gastric mucosal changes associated with portal hypertension are termed "portal hypertensive gastropathy (PHG)".[1–3] PHG is a common feature of cirrhosis and its prevalence parallels the severity of liver dysfunction.[4,5] The prevalence of mild PHG is approximately 20% to 90%.[5–10] Previous studies have demonstrated that the portal hypertensive gastric mucosa has characteristic morphological and functional abnormalities.[11,12] In addition, clinical and experimental data indicate that the portal hypertensive gastric mucosa has increased susceptibility to mucosal injury by noxious agents such as alcohol, bile acid, and nonsteroidal anti-inflammatory drugs (NSAIDs) compared with normal gastric mucosa.[13–17] This increased susceptibility to mucosal damage is thought to be due to the impairment of mucosal defense mechanisms. Ultrastructural abnormalities of portal hypertensive gastric mucosal microvessels, and submucosal blood flow shunting, producing mucosal surface hypoxia, have been implicated in the increased susceptibility of portal hypertensive mucosa to damage.[17–19] This impairment of mucosal defense mechanisms results in the reduced synthesis of prostaglandin E_2, and in various ionic transport abnormalities, such as increased back-diffusion of hydrogen ions and reduced electronegativity of potential difference, in portal hypertensive gastric mucosa.[11,15,16] Further, other possible factors, such as excessive nitric oxide (NO) production and increased generation of oxygen free radicals and lipid peroxidation in the portal hypertensive gastric mucosa, have also been implicated in its increased susceptibility to injury.[20–22] Also, Kawanaka et al.[23] have reported the impaired signaling of mitogen-activated protein kinase (ERK2), which protects cells against cellular stress and induces cell proliferation, in gastric mucosa of portal hypertensive rats.

Heat shock proteins (HSPs), highly conserved and ubiquitous proteins synthesized by a variety of stresses,

including elevated temperature, exposure to toxins, or heavy metals, have been shown by many studies to be important for cell survival under stress conditions.[24–26] HSPs are classified into four families, HSP90, HSP70, HSP60, and the small HSP families, based on their molecular weight. One of the most comprehensively studied members of the HSP superfamily of proteins is the inducible form of HSP70, commonly referred to as HSP72.[27–29] Animals with elevated levels of HSP72 have been shown to be protected against ischemia-induced cerebral and myocardial infarctions, sepsis, and liver injury.[30–33] In gastric mucosa also, HSP72 has a crucial cytoprotective function mediated by its function as a "molecular chaperone".[34–36] We have shown that water-immersion stress induces HSP72 in gastric mucosa, and that the induction of HSP72 prevents HCl-induced gastric mucosal lesions in normotensive gastric mucosa.[37] However, the cytoprotective effect of HSP72 in portal hypertensive gastric mucosa has not yet been studied. Because PHG is a frequent, serious complication in patients with liver cirrhosis, we consider that this theme has to be investigated. In this study, we investigated the expression and cytoprotective effect of HSP72 in portal hypertensive gastric mucosa in cirrhotic rats.

Materials and methods

Animals

Male Sprague-Dawley rats (220–250 g) were fed a standard laboratory diet and water ad libitum, and kept in cages in a temperature (22 ± 2°C)- and humidity (55 ± 5%)-controlled room with a 12-h dark-light cycle before and the during experiment. This study was approved by the Akita University Animal Care Committee. Two experimental models of portal hypertension were used: one caused by bile duct ligation (BDL) and the other caused by carbon tetrachloride (CCl_4)-induced cirrhosis (CCl_4 model). In the BDL model, the surgical procedure was performed while rats were under pentobarbital anesthesia, as previously described.[38] Briefly, the bile duct was exposed after median laparotomy, and a 3-0 silk was used to ligate it in two places; then the bile duct was cut between both ligations. In sham-operated rats, the bile duct was exposed but not ligated. In the CCl_4 model, rats (150 g) were injected intraperitoneally with CCl_4 (diluted 1:1 with olive oil; Wako Chemical, Osaka, Japan) twice each week for 8 weeks. CCl_4 was administered at increasing dosages, of 0.5 ml/kg during the first week and 1.0 ml/kg from the second week until the eighth week. One week after the cessation of CCl_4 injections, CCl_4-treated rats were killed.

For the time course of liver cirrhosis, studies were performed at 1, 2, 4, and 7 weeks after the BDL or sham procedure, and four to six animals were used each week, as indicated in "Results." All animals later than 4 weeks after the BDL procedure had macroscopic evidence of liver cirrhosis at the time of the study, which was confirmed histologically. At weeks 1, 2, 4, and 7, the body, liver, and spleen weights of the rats were measured. For portal venous pressure measurements, intraluminal polyethylene (PE-50) tubing was inserted via a large pericolonic mesenteric tributary vein and gently advanced into the portal vein. The pressure was measured from the level of the vena cava to the tip of a column of saline within the tubing.[39] For light-microscopic study, part of the stomach tissue was fixed in 10% buffered formalin, embedded in paraffin wax, and stained with hematoxylin and eosin.

Immunohistochemistry

Part of the gastric mucosa of each rat was used for immunohistochemical analysis. Tissue fixation and immunoperoxidase staining were performed as described previously, with slight modification.[40] Briefly, tissue pieces were fixed with 10% formalin, and the tissue sections were sliced (5 μm) with a cryostat. After the blocking of endogenous peroxidase with 0.3% H_2O_2 in methanol, the tissue sections were incubated with anti-proton pump antibody or anti-HSP72 antibody (STRESSGEN, Victoria, BC, Canada), diluted 1:1000 in 10% normal pig serum, overnight at 4°C. Then sections were incubated with biotinylated anti-rabbit IgG (DakoCytomation, Kyoto, Japan), diluted 1:500, for 30 min at room temperature. The site of peroxidase activity was visualized with 0.02% 3,3-diaminobenzidine tetrahydrochloride, containing 0.0015% H_2O_2.

Parietal cell counts

The number of parietal cells was counted in a square-millimeter area under a light microscope in 400 high-power fields. Cells were counted in ten such random fields.

Measurement of gastric acid secretion

Gastric juice was collected using a pylorus-ligation method.[41] Briefly, rats were fasted for 24 h, placed in restraint cages, and then pylorus ligation was performed. Laparotomy and pylorus ligation was performed with the animals under light anesthesia, with the animals regaining the righting reflex 5–15 min after operation. The pylorus-ligated rats were reanesthetized with ether 6 h after pylorus ligation. The stomach was then removed from each rat, and the gastric contents were collected, and centrifuged; the supernatants were used. The volume (ml) and pH of the gastric juice was

Table 1. General characteristics of cirrhotic rats in the study

	Sham	BDL	CCl_4
n	6	5	5
Body weight (g)	298.00 ± 7.30	302.00 ± 12.00	294.50 ± 7.58
Liver weight (% body weight)	2.80 ± 0.10	6.30 ± 0.30*	3.20 ± 0.20
Spleen weight (% body weight)	0.20 ± 0.01	0.40 ± 0.02**	0.40 ± 0.01**
Portal pressure (cm H_2O)	12.66 ± 0.33	23.30 ± 2.07***	20.50 ± 1.32***

* $P < 0.05$ vs sham rats or CCl_4-treated rats; ** $P < 0.05$ vs sham rats; *** $P < 0.05$ vs sham rats
Data were recorded 4 weeks after the sham or bile duct ligation (BDL) procedure and 1 week after the cessation of CCl_4 injections, (which had been given for 8 weeks). Data values are expressed as means ± SEM

measured, the acid output (mEq/6 h) of gastric secretion was determined by titration with 0.1 mol/l NaOH to pH 7.0, using a pH meter (f50 pH Meter; Beckman, Tokyo, Japan), and acidity (mEq/l) was calculated by dividing the acid output by volume.

Western blot analysis

In each rat, part of the gastric mucosa in the pyloric and the fundic mucosal area was removed before formalin fixation, according to previously described methods.[42] Briefly, gastric mucosa was scraped off, using two glass slides, and frozen at −80°C until use for Western blot analysis. Each gastric mucosa sample was homogenized with 5 volumes of ice-cold 25 mM Tris-Cl buffer (pH 7.5). The homogenates were centrifuged at 18000 *g* for 20 min. The supernatants were collected, and protein concentration, measured by the method of Bradford,[43] was adjusted to 20 μg protein/lane. The expression of HSP72 was evaluated by a method which was previously reported.[44] Briefly, samples were electrophoresed on 9% sodium dodecyl sulfate (SDS)-polyacrylamide gels, transferred electrophoretically to a polyvinylidene difluoride (PVDF) membrane (Nihon Millipore Kogyo, Tokyo, Japan), and processed as described by Towbin et al.[45] The membrane was incubated with anti-HSP72 antibody and treated with horseradish peroxidase-conjugated anti-rabbit IgG (1:1000 dilution; Bio-Rad, Richmond, CA, USA). The peroxidase substrate was 3,3-diaminobenzidine tetrahydrochloride. The density of immunologically stained bands was analyzed by a scanning densitometer. The relative density of stained bands was calculated by the equation: relative density (%) = density (BDL rats or CCl_4-treated rats)/density (sham rats) × 100.

Induction of HSP72 in portal hypertensive gastric mucosa by water-immersion stress

Four weeks after the BDL or sham procedure, animals were placed in a restraint cage and immersed vertically, to the level of the xiphoid process, in a water bath (23°C, 6 h).[46] After 3-h recovery from the pretreatment (anesthesia with or without water-immersion stress), rats were killed to measure the expression of HSP72 in the gastric mucosa before HCl administration. Gastric mucosa was isolated, scraped off using two glass slides, and frozen at −80°C until use for Western blot analysis.

Evaluation of gastric mucosal damage induced by HCL administration

After 3-h recovery from the pretreatment (anesthesia with or without water-immersion stress), rats were administered hydrochloric acid (HCl, 0.6 N, 8 ml/kg) intragastrically. At 30 min after HCl administration, the stomach was excised and the extent of mucosal damage was scored by two individuals who were blind to the experiments. Total length of all mucosal lesions was measured according to a method described previously,[42,47] and the severity of the damage was assessed.

Statistical analysis

All the values for results are expressed as means ± SEM. Statistical analysis was performed using the Mann-Whitney *U*-test. *P* Values less than 0.05 were considered to be significant.

Results

General characteristics of rats in the study

As expected, rats with cirrhosis induced by BDL (4 weeks after the BDL procedure) and those with cirrhosis induced by CCl_4 treatment for 8 weeks had a significant increase in spleen weight and portal venous pressure ($P < 0.05$). All BDL rats had hepatomegaly ($P < 0.01$; Table 1). Elevation of portal venous pressure in BDL rats was dependent on the time course after the BDL procedure ($P < 0.05$ compared with sham rats at

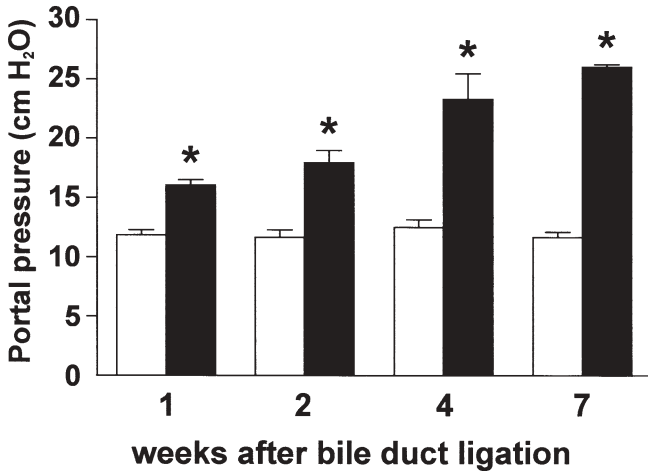

Fig. 1. Portal pressure in sham (*open bars*) and bile-duct ligation (BDL) rats (*closed bars*). Portal pressure was measured from the level of the vena cava to the tip of a column of saline within the tubing. Portal pressures after the sham procedure were 11.9 ± 0.4, 11.7 ± 0.5, 12.6 ± 0.5, and 11.6 ± 0.5 cmH_2O at 1, 2, 4, and 7 weeks, respectively. Portal pressures after the BDL procedure were 16.1 ± 0.2, 17.9 ± 1.0, 23.3 ± 2.1, and 26.0 ± 0.4 cmH_2O at 1, 2, 4, and 7 weeks, respectively. Data values are presented as the means ± SEM of four to five rats for each group. *$P < 0.05$ vs sham rat at the corresponding time point

the time points indicated in Fig. 1). In contrast, portal venous pressure was stable in sham rats during the experiment (11.6–12.6 cmH_2O; Fig. 1).

Immunohistochemical study of portal hypertensive gastric mucosa

Four weeks after the BDL or sham procedure, an immunohistochemical study showed that the expression of HSP72 in gastric mucosa was reduced in BDL rats compared with sham rats (Fig. 2A). The expression of HSP72 was localized not only in parietal cells but also all mucosal cells in BDL rats. Similarly, parietal cells were apparently decreased in BDL rats compared with sham rats (Fig. 2A). Quantitative evaluation of parietal cells showed that the number of parietal cells in gastric mucosa had decreased by 1.8-fold in BDL rats compared with sham rats ($P < 0.05$; Fig. 2B).

Gastric acid secretion in portal hypertensive rats

To evaluate gastric acid secretion in portal hypertensive rats, gastric juice was collected at 4 weeks after the BDL or sham procedure. Gastric juice volume, acidity, and acid output were significantly decreased in BDL rats compared with sham rats ($P < 0.05$). However, there was no difference in pH levels between BDL and sham rats (Fig. 3).

Expression of HSP72 in portal hypertensive gastric mucosa

Western blotting was used to determine the possibility of altered expression of HSP72 in BDL rats 4 weeks after the procedure and in CCl_4-treated rats after 8 weeks of the treatment. Expression of HSP72 in gastric mucosa in the cirrhotic rats was decreased to 15% in the BDL rats and to 34% in the CCl_4 model rats compared with 100% in sham rats ($P < 0.05$; Fig. 4). As shown in Fig. 1, portal venous pressure increased with time with increasing severity of liver cirrhosis. To further assess whether the variation of HSP72 expression in gastric mucosa was associated with the severity of liver cirrhosis, we investigated the expression of HSP72 at 1, 2, 4, and 7 weeks. As shown in Fig. 5, significant reduction of HSP72 was found at 1 week in BDL rats as compared with sham rats ($P < 0.05$). In addition, the expression of HSP72 decreased with time in BDL rats, whereas no significant difference was observed in sham rats during the experiment, even at 7 weeks.

Induction of HSP72 in portal hypertensive gastric mucosa and effect of HSP72 on gastric mucosal injury

To evaluate the induction of HSP72 in portal hypertensive gastric mucosa, rats were exposed to water-immersion stress at 4 weeks after the BDL or sham procedure. Without the pretreatment of water-immersion stress, the expression of HSP72 was significantly lower in BDL rats compared with sham rats ($P < 0.05$). However, 6-h exposure to water-immersion stress induced a 2.5-fold increase in HSP72 in gastric mucosa in both BDL and sham rats (Fig. 6A,B). In addition, to investigate the cytoprotective effect of HSP72 in portal hypertensive gastric mucosa, rats were given HCl intragastrically with or without the pretreatment of water-immersion stress. Although ulcers induced by the water-immersion pretreatment were significantly reduced relative to findings in animals without water-immersion pretreatment, in both BDL and sham rats, the ulcer index in animals without water-immersion pretreatment was significantly higher in BDL rats compared with sham rats (153.8 ± 15.6 mm vs 95.5 ± 13.7 mm; $P < 0.05$). Similarly, the ulcer index in animals with water-immersion pretreatment was also significantly higher in BDL rats compared with sham rats (101.0 ± 10.4 mm vs 48.5 ± 10.6 mm; $P < 0.05$; Fig. 7).

Discussion

Portal hypertensive gastropathy (PHG) is a frequent, serious complication of liver cirrhosis.[6,48] In PHG, the gastric mucosa has increased susceptibility to mucosal

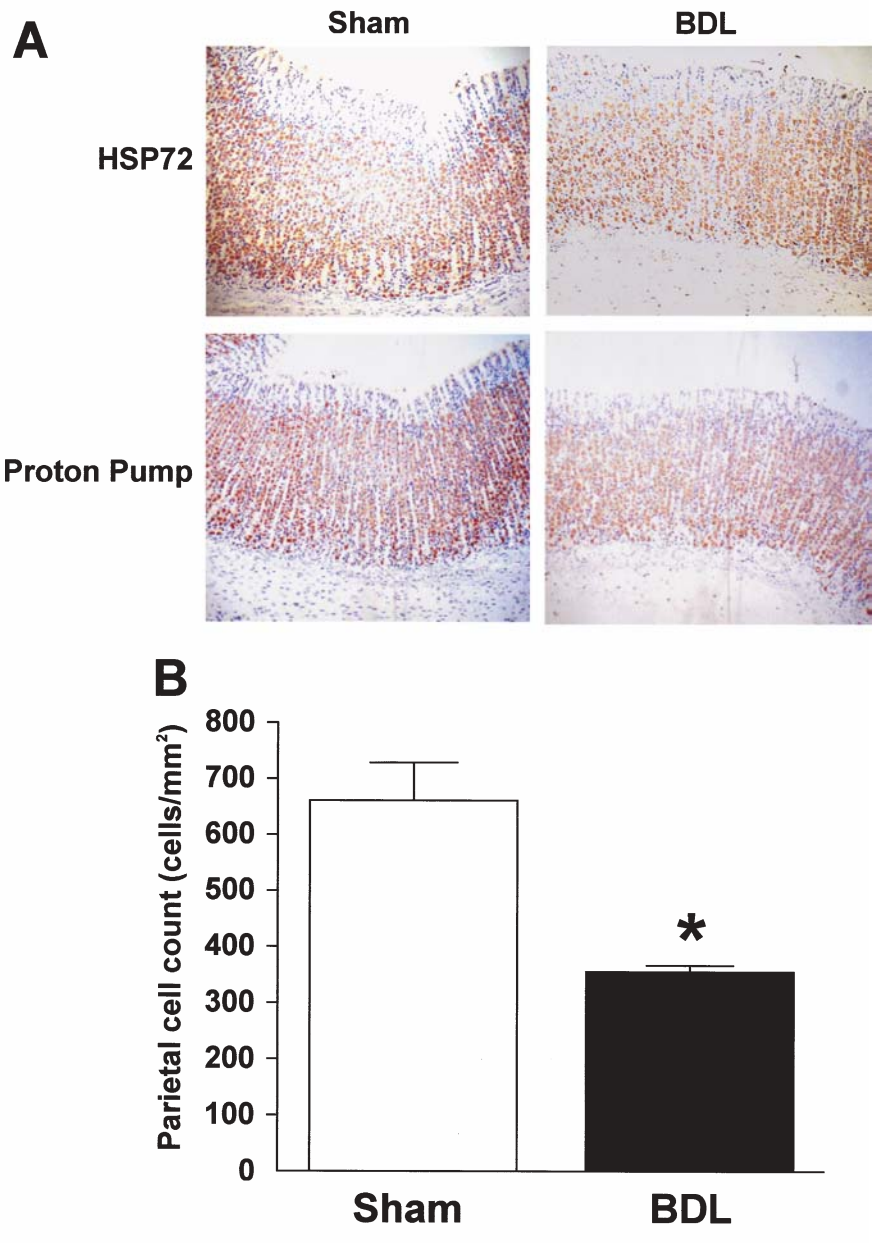

Fig. 2A,B. Immunohistochemistry of gastric mucosa in sham and BDL rats. **A** Expression of heat shock protein 72 (*HSP72*) in sham and BDL rats at 4 weeks after the operative procedure. Positive immunostaining was observed predominantly within the cytoplasm of the gastric mucosal cells, and the staining was decreased in BDL rats (×200). Parietal cells were examined by immunostaining for proton pump in sham and BDL rats (×200). The expression of HSP72 was localized not only in parietal cells but also in all mucosal cells in BDL rats. **B** The number of parietal cells was counted in a square-millimeter area in ten fields. Parietal cell counts were 660 ± 69 cells/mm^2 in sham rats (*open bars*) and 353 ± 13 cells/mm^2 in BDL rats (*closed bars*). Parietal cell numbers were significantly decreased in BDL rats compared with sham rats. Data values are presented as the means ± SEM of five rats in each group. *$P < 0.05$ vs sham rats

injury.[7,13,14] However, the pathogenesis of PHG is still under investigation.[49] Our results showed that the expression of HSP72 in the gastric mucosa was decreased in portal hypertensive rats. Interestingly, in cirrhotic rats, the expression of HSP72 in gastric mucosa was inversely related to the elevation of portal venous pressure with increasing severity of liver cirrhosis (Figs. 1 and 5). Moreover, the induction of HSP72 protected the portal hypertensive gastric mucosa against HCl-induced mucosal injury (Fig. 6). As far as we know, this is the first report of HSP72 in portal hypertensive gastropathy.

Many studies have shown that HSPs are essential for cell survival under stress conditions.[24–26] It has been revealed that members of the HSP70 family, including HSP72 (stress-inducible HSP70) and HSP73 (constitutive HSP70, HSC70), protect cells from sublethal stress, mediated by their function as a "molecular chaperone".[50,51] Furthermore, many studies have reported that overexpression of HSP72 protects various cells and organs from subsequent attacks by heat, hypoxia, ischemia, reperfusion, infection, and toxic agents.[52–54] Nakamura et al.[35] have reported that, in the stomach, HSP70 has important cytoprotective functions in cultured gastric mucosal cells. In our series of studies, we have reported that the preinduction of HSP72 in gastric mucosa by water-immersion stress prevents HCl-induced mucosal lesions.[37] In this model, mucosal

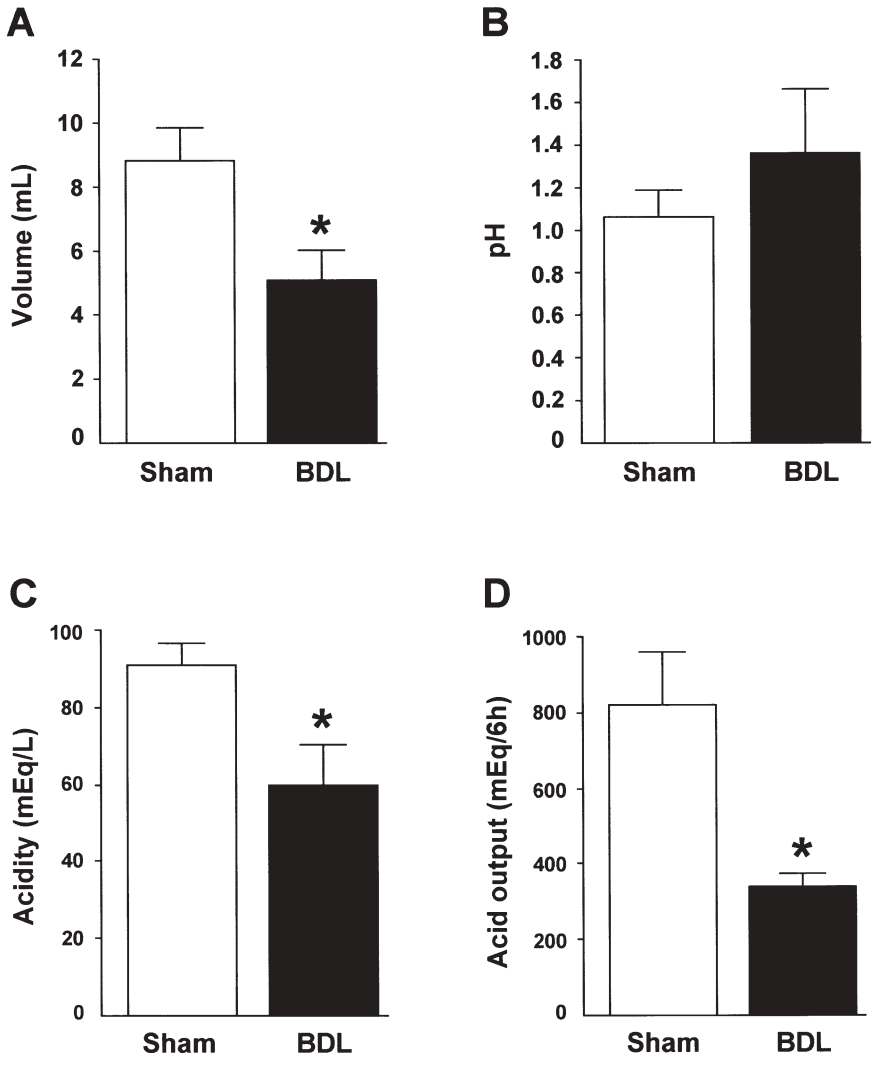

Fig. 3A–D. Gastric secretion in sham (*open bars*) and BDL rats (*closed bars*). At 4 weeks after the sham or BDL procedure, **A** gastric juice volume, **B** gastric pH, **C** gastric acidity, and **D** gastric acid output were examined by pylorus ligation in rats ($n = 6$ for each group). Gastric acid was obtained 6 h after pylorus ligation. Gastric juice volume, gastric acidity, and gastric acid output were significantly decreased in BDL rats compared with sham rats, but gastric pH was not decreased. Data values are presented as the means ± SEM of five rats in each group. $^*P < 0.05$ vs sham rats

HSP72 may be induced by the decrease in gastric pH and mucosal blood flow caused by the water-immersion stress. Also, we have reported that gastric mucosal adaptation was accompanied by an increase in HSP72 after long-term administration of a low dose of aspirin, which decreases mucosal prostaglandins.[55] These results indicate that HSP72 may have an important cytoprotective function in gastric mucosal cells. On the other hand, an impaired mucosal defense system plays an important role in the increased susceptibility of portal hypertensive gastric mucosa to damage.[11,12,15–17] The thickness of the gastric mucosal gel layer and the content of mucosal hexosamine are decreased in portal hypertensive gastric mucosa.[56,57] It has been reported that adaptive cytoprotection, with pretreatment of mild irritants in normotensive gastric mucosa, provides tolerance against gastric mucosal damage.[58,59] Similarly, Ninomiya et al.[60] reported that, in portal hypertensive rats, the impaired gastric mucosal defense system caused impairment of the adaptive cytoprotection against damage. They examined the adaptive cytoprotection to ethanol-induced damage in the gastric mucosa of portal hypertensive rats, in which gastric mucosal hexosamine was low compared with control rats. It has been shown that HSP72 contributes to adaptive cytoprotection.[35,37] We consider that a reduction of HSP72 is associated with susceptibility to mucosal damage in the portal hypertensive gastric mucosa.

Our previous study has shown that the inhibition of gastric acid secretion with histamine H2-receptor antagonists or proton pump inhibitors decreased the expression of HSP72 in normotensive gastric mucosa.[61] These results indicate that gastric acid may stimulate the synthesis of HSP72 in the gastric mucosa. Although gastric acid is one of the aggressive factors involved in the development of gastric mucosal injury, there is a good possibility that gastric acid continually modulates the expression of HSP72 in gastric mucosa. In other

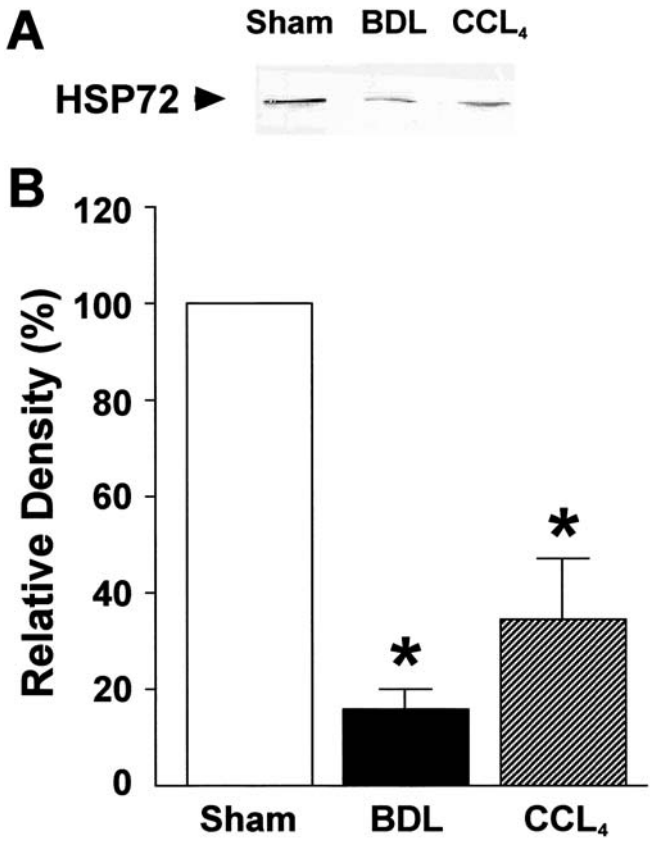

Fig. 4A,B. Expression of HSP72 in the gastric mucosa in cirrhotic rats. **A** Expression of HSP72 in sham rats, BDL rats (at 4 weeks after sham or BDL procedure), and CCl_4-treated rats (after 8 weeks' CCl_4 administration) was determined by Western blot analysis. **B** Relative density, presented as a percentage of the measured level in sham rats (*open bar*). Expression of HSP72 was significantly decreased in BDL rats (*closed bar*) and CCl_4-treated rats (*hatched bar*) compared with sham rats, but the expression was not different between BDL rats and CCl_4-treated rats. Data values are presented as the means ± SEM of five rats in each group. $*P < 0.05$ vs sham rats

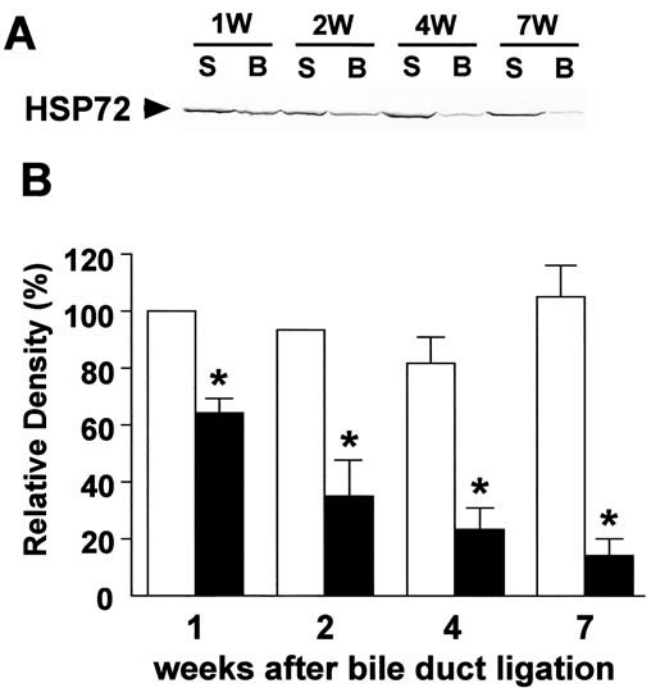

Fig. 5A,B. Expression of HSP72 in the gastric mucosa in sham and BDL rats. **A** Expression of HSP72 in sham (*S*) rats and BDL (*B*) rats was determined by Western blot analysis at 1, 2, 4, and 7 weeks (*W*) after the sham or BDL procedure. **B** Relative density, presented as a percentage of the measured level in sham rats (*open bars*) at 1 week. Data values are presented as the means ± SEM of four rats for each group. $*P < 0.05$ vs sham rat at the corresponding time point. *Closed bars*, BDL rats

words, mucosal injury is reduced because of the reduction of gastric acid, whereas the expression of HSP72 decreases with the ingestion of histamine H2-receptor antagonists or proton pump inhibitors. However, it takes a long time to restore the expression of HSP72 following discontinuation of the ingestion of these agents. Until HSP72 is restored, severe gastric mucosal injury may be induced by noxious agents. Generally, a variety of environmental stresses induce HSP synthesis. Therefore, we think that gastric acid induces HSP72 in gastric mucosa, in view of the hypothesis that gastric acid is considered to be a "stress substance" to the gastric mucosa. It has also been reported that there is an impairment in gastric acid secretion in patients with liver disease.[62] Agnihotri et al.[63] reported that parietal cell mass was decreased, and Agnihotri et al.[64] and Kaur et al.[65] reported that signal transduction in parietal cells was impaired in portal hypertensive gastric mucosa. They concluded that gastric mucosal hypoxia may have contributed to parietal cell loss in portal hypertensive rats. Furthermore, Pique et al.[39] have reported that there is an increased gastric mucosal blood flow and an impaired acid output response to pentagastrin stimulation in portal hypertensive rats, and these changes appear to be mediated by an increase in endogenous prostaglandin E_2. It is, therefore, conceivable that impairment in gastric acid secretion in portal hypertension, probably due to a decrease in parietal cells, is one of the important factors involved in diminishing the expression of HSP72 in gastric mucosa, as shown in the present study. It has been reported that nonselective β-blockers, such as propranolol and nadolol, reduce bleeding related to PHG.[66–68] Nonselective β-blockers have been shown to reduce portal venous pressure and gastric mucosal blood flow. However, other pharmacological treatments will be required for PHG in cirrhotic patients when nonselective β-blockers are not effective. Thus, from a clinical point of view, therapeutic agents, such as "chaperone inducers" that induce HSP72 in the portal hypertensive gastric mucosa without any toxic effect, are hoped for.

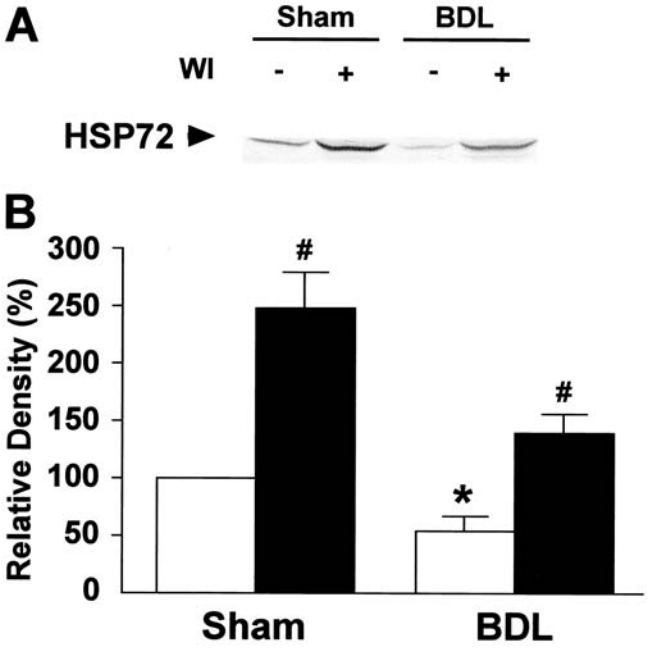

Fig. 6A,B. Expression of HSP72 after water-immersion stress in sham and BDL rats. **A** Expression of HSP72 was determined by Western blot analysis at 4 weeks after the sham or BDL procedure with or without water-immersion (*WI*) stress. **B** Relative density, presented as a percentage of the measured level in sham rats without water-immersion stress (*open bars*). The expression of HSP72 was increased in both the sham group and the BDL group with water-immersion (*closed bars*). Data values are presented as the means ± SEM of four rats for each group. $^{*}P < 0.05$ vs sham rats without water-immersion stress. $^{\#}P < 0.05$ vs corresponding rats without water-immersion stress

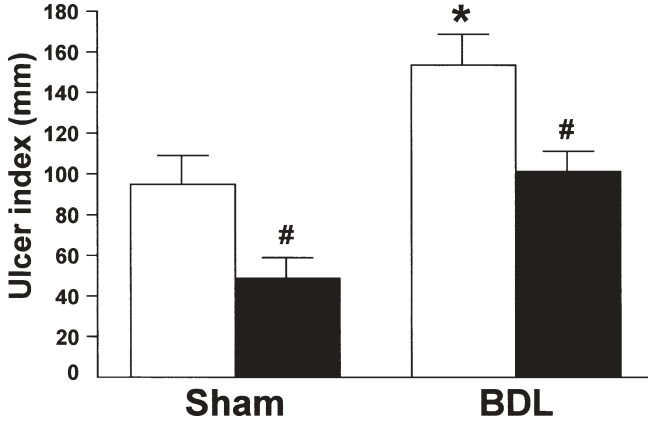

Fig. 7. Ulcer index (measurement of mucosal lesions induced by HCl administration) in sham and BDL rats. The ulcer index is presented as the total length of all mucosal lesions induced by HCl administration in rats with (*closed bars*) or without water-immersion stress (*open bars*). The ulcer index was decreased in the sham group and the BDL group with water immersion stress. Data values are presented as the means ± SEM of four rats for each group. $^{*}P < 0.05$ vs sham rats without water-immersion stress. $^{\#}P < 0.05$ vs corresponding rats without water-immersion stress

In conclusion, our results indicate that the expression of HSP72 in portal hypertensive gastric mucosa was decreased in cirrhotic rats with reduced gastric acid secretion. Although the exact mechanisms remain to be uncovered, the expression of HSP72 in gastric mucosa is associated with portal hypertension in cirrhotic rats. In addition, the preinduction of HSP72 prevented HCl-induced mucosal injury in the portal hypertensive gastric mucosa. One important result of our studies is that the mucosal defense system may be a target to modulate the severity of PHG. Although additional studies will be necessary to confirm whether this finding can be extrapolated to humans, the finding provides a rationale to develop new pharmacologic strategies for the clinical management of PHG in cirrhotic patients.

Acknowledgments. We thank Yuki Watanabe and Takako Sasaki for their excellent technical assistance.

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