Expression and Function of Stress (Heat Shock) Proteins in Digestive Organs

MICHIRO OTAKA *, SUMIO WATANABE

First Department of Internal Medicine, Akita University School of Medicine
1-1-1 Hondo, Akita City, Akita, 010-8543, Japan

Abstract: Our recent studies have been focused on the expression and cytoprotective function of heat shock proteins (HSPs) mediated by their function as a molecular chaperone in digestive organs. We have reported that HSP72 (72-kDa heat shock protein, stress-inducible HSP70) has crucial function in the gastric mucosa, colonic mucosa and the liver. In the pancreas, we proved that HSP60 (60-kDa heat shock protein, chaperonin homolog) has cytoprotective function. These evidences lead us to develop effective “chaperone-inducing therapy” including drugs, chemicals and gene therapies which might effective for disease therapy enhancing not only cytoprotective ability but also tissue restoration. In this review, we introduce our previous data.

Key Words: heat shock protein, molecular chaperone, stress, cytoprotection

Introduction

Many recent reports are indicating that heat shock proteins (HSPs) also called molecular chaperones, have important functions in response to stress-related events 1-7). HSPs are classified in some families by their molecular masses. Their structures are conserved from prokaryotic cells to eukaryotic cells 1-4). Therefore, it is considered that they have essential functions for survival of cells and developmental process. Some recent reports have proved the cytoprotective functions of HSPs against environmental stresses, and these functions are considered to be important for living cells to obtain tolerance to adapt environmental changes 1-5).

Our recent studies have been focused on the “chaperone” functions of HSPs mainly their cytoprotective functions in digestive organs and cells 6-32). In these series of studies, we have demonstrated that 1) HSPs are induced by several environmental stresses including heat, neuropeptide, neuroamine or drugs also in vivo. 2) Induced HSPs are different in each organ even by same stress. 3) In view of cytoprotection, important HSP is different in each organ. Some of these findings are not compatible with in vitro findings reported before in which almost all HSPs have cytoprotective function mediated by chaperone function. Based on our in vivo findings, chaperon-inducing therapy might be a candidate for new
therapeutic strategy enhancing cytoprotective ability. Therefore, our recent studies are designed to
develop chaperone-inducing drugs or chaperone-inducing gene therapy (mainly HSP70). In this review,
some of our findings are introduced and discussed.

Regulation and chaperone function of heat shock proteins

Regulation mechanism of HSP is summarized in
Fig. 1. Environmental stresses induce heat shock
transcription factor (HSF) mainly HSF-1.
Actually, accumulation of aggregated or denatured
proteins in cytosol is thought to be a trigger for the
induction of HSF-1 as the first step of stress
response. Trimer formation of phosphorylated
HSF-1 gets ability to move into the nucleus and
binds to stimulate promotor lesion (heat shock
element) to stimulate HSPs gene.

HSPs bind to degenerated or aggregated proteins.
It is thought that the folding of aggregated
denatured) proteins and many newly synthesized
polypeptides in the cell are assisted by a class of
proteins (HSPs, molecular chaperones) which
function mainly in preventing off-pathway folding
reactions that lead to aggregation. And HSPs are known to normalize their structure and maintain
intracellular environment. These chaperone functions are considered to increase protective ability
against cytotoxic agents or events.

(1) Stomach

The concept of so-called “adaptive cytoprotection” in the gastric mucosa was first reported by Robert
et al, demonstrating that when rats were treated with mild irritant such as 10-25% ethanol, 0.05-0.35 N
HCl, or 2-4% NaCl, the gastric mucosa increased its resistance to various necrotizing agents such as 100%
ethanol, 0.6N HCl, or 25% NaCl 33). In experimental animals and humans, it has been demonstrated that gastric mucosa could increase its resistance to damaging effect of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin when
NSAIDs were repeatedly administered 34-38). Some reports have suggested that prostaglandins (PGs),
which have been reported to contribute to acute phase mucosal adaptation, may not be important at least
in the case of long-term administration of NSAIDs 37)39).

On the other hand, many studies have shown the importance of heat shock proteins (HSPs) for cells
survival under stress conditions 40-42). A 70-kDa heat shock protein (HSP70) has been induced in
cultured gastric mucosal cells by heat stress, and this protein has a cytoprotective function in vitro as
mentioned above 1).

Therefore, we speculated that HSPs might be playing some roles in the mucosal adaptation also in vivo
not only in acute stress response mechanism but also in chronic stress exposure that is observed after long term administration of NSAIDs.

1) Acute stress model

As acute stress models, we used water-immersion stress (WI-stress) and administration of stress-related neuropeptide and neurotransmitter such as thyrotropin releasing hormone (TRH) or serotonin (5-HT).

We have reported that HSP72 and HSP60 were significantly induced to synthesize after WI-stress exposure \(^{12,14}\). In order to study the effects of pre-induction of HSP72 on HCl-induced gastric mucosal damage, we designed the experiment shown in Fig. 2a. In control group, rats were just fasted for 6 hr and then 0.1ml of 0.6N HCl was administered per orally. In stress group, rats were pre-treated with WI-stress for 6 h and HSP72 and HSP60 were pre-induced in the gastric mucosa before HCl administration. Then rats were sacrificed 3 hours after HCl-challenge followed by evaluation of the ulcer index.

As shown in Fig. 2b, HCl-induced gastric mucosal damage was significantly reduced in rats pre-treated with WI-stress to induce mucosal HSP72 and HSP60.

Are all HSPs important for cytoprotection? To examine which HSP is important for gastric mucosal protection, we have tried to find specific inducer of HSP60 and HSP72.

Finally, we have found that stress-related substances, TRH and 5-HT, are specific inducer of HSP72 and HSP60 respectively. As shown in Fig. 3a, TRH specifically induced the synthesis of HSP72. On the other hand, HSP60 was specifically induced by 5-HT administration. Then, we applied these specific induction methods to compare the functions of HSP60 and HSP72 for mucosal protection as designed in Fig. 3b. Rats were administered with TRH (20 mg/kg, i.p.) or 5-HT (30 mg/kg, i.p.) to pre-induce HSP72 and HSP60 respectively prior to 0.6N HCl-administration (1ml, p.o.) and the mucosal damage was compared. When rats were pretreated with TRH to pre-induce HSP72, the mucosal damage was significantly reduced compared with control pretreated with vehicle. When rats were pretreated with 5-HT to induce HSP60, mucosal damage was rather exacerbated compared with control (Fig. 3c).

These results could indicate that HSP72 plays an important role to protect gastric mucosa possibly mediated by the function of molecular chaperone as an acute stress response also in vivo.

2) Chronic stress model

In order to understand the condition under chronic stress, we studied the effect of long term administration of low dose-aspirin (Asp) on the expression of HSPs and mucosal protection \(^{25}\). Rats were perorally administered with 100 mg/kg of aspirin everyday until 20th day (This dose did not produce mucosal damage). And mucosal expression of HSPs and mucosal protection against high dose of Asp-challenge (250mg/kg) were evaluated (Fig. 4a).

Long-term administration of aspirin caused time-dependent increase in expression of HSP72 in the gastric mucosa. HSP72 tended to increase at day 10 and reached statistical significance after 20 days administration. The induction of HSP72 correlated with mucosal protection against high dose Asp-induced mucosal lesion (Fig. 4b).

We also measured mucosal PGE2 levels at 20th days. PGE2 level was decreased compared with control.
**Fig. 2a:** In control group, rats were just fasted for 6 hr and then 0.1ml of 0.6N HCl was administered per orally. In stress group, rats were pre-treated with WI-stress for 6 to induce HSP72 and HSP60 in the gastric mucosa before HCl administration. Then rats were sacrificed 3 hours after HCl-challenge followed by evaluation of the ulcer index.

**Fig. 2b:** Effect of pre-induction of HSP60 and HSP72 on HCl-induced gastric mucosal damage. HCl-induced gastric mucosal damage was significantly reduced in rats pre-treated with WI-stress to induce mucosal HSP72 and HSP60.

**Fig. 3.**

**a:** Expression of HSPs in the gastric mucosa after TRH (20 mg/kg, i.p.) or 5-HT (30 mg/kg, i.p.) injection. TRH specifically induced the synthesis of HSP72. On the other hand, HSP60 was specifically induced by 5-HT administration.

**b:** Rats were administered with TRH (20 mg/kg, i.p.) or 5-HT (30 mg/kg, i.p.) to pre-induce HSP72 and HSP60 respectively prior to 0.6N HCl-administration (1ml, p. o.) and the mucosal damage was compared.

**c:** Effect of specific pre-induction of HSP on HCl-induced mucosal damage. When rats were pretreated with TRH to pre-induce HSP72, the mucosal damage was significantly reduced compared with control pretreated with vehicle. When rats were pretreated with 5-HT to induce HSP60, mucosal damage was rather exacerbated compared with control.
HSPs in digestive organs • M. Otaka et al.

Therefore, PGE2 may not be associated with this adaptation at least in our model. This study indicated the first evidence that long-term administration of aspirin is associated with a significant increase in HSP72 in gastric mucosa, which correlates with a reduction in the extent of mucosal damage induced by higher dose of aspirin. These findings suggest that HSP72 may have an important role also in chronic gastric adaptation to aspirin.

### Table I

<table>
<thead>
<tr>
<th></th>
<th>PGE2 (ng/g)</th>
<th>LTB4 (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>519.3 ± 115.2</td>
<td>5.70 ± 0.92</td>
</tr>
<tr>
<td>Aspirin</td>
<td>65.6 ± 40.3</td>
<td>5.09 ± 0.73</td>
</tr>
</tbody>
</table>

Values are mean ± SE for four animals in each group.* P < 0.05

(2) Colon

In order to study the cytoprotective function of heat shock proteins (HSPs) in the colonic mucosa, the effect of pre-induction of HSPs by hyperthermia (42.5°C for 20 min.) on acetic acid-induced colitis was investigated. Expression of HSP60, HSP72 and HSP90 in rat colonic mucosa was investigated before and after hyperthermia. Following pre-treatment with or without hyperthermia, the rats received intrarectal infusion of 5% acetic acid (1 ml). The colonic mucosal damage was evaluated by macroscopic and microscopic assessments 24 hr after the intrarectal infusion of acetic acid (Fig. 5a). Expression of HSPs was significantly increased by hyperthermia in rat colonic mucosa although the pattern of each HSP expression was different (Fig. 5b). Acetic acid-induced colitis was dramatically prevented by pre-treatment with hyperthermia when HSP72 and HSP90 were pre-induced (Fig. 5c). On the other hand, induction of HSP60 did not correlate with mucosal protection. This data suggest that HSP72 and HSP90 may have cytoprotective function against acetic acid-induced mucosal damage. These results were also reproduced when HSPs are induced by intrarectal infusion of zinc derivatives.
(3) Pancreas

It has been demonstrated that water-immersion stress accelerates the activation of zymogen proteases induced by cerulein and suggested that stress could be an exacerbation factor of acute pancreatitis in rats when the stress was given after cerulein injection. The mechanism of this phenomenon is considered to be caused by microcirculatory disturbance in the pancreas. It has been well understood that stress is exacerbation factor of pancreatitis. On the other hand, we found that water-immersion stress induced the synthesis of a HSP60 (chaperonin homolog) and hyperthermia induced HSP72 in the pancreas. What are the meanings and roles of these HSPs in the pancreas? We have designed the experimental protocol to study the effect of specific pre-induction of HSP60 or HSP72 by WI-stress or hyperthermia (42.5°C for 20 min.) reversing the order of cerulein injection and stress exposure (Fig. 6b). In group A, rats were fasted for 6 hours, and injected with 40 μg/kg body weight of cerulein intraperitoneally. In group B, rats were preexposed to WI-stress for 6 hours, and injected with 40 μg/kg body weight of cerulein intraperitoneally (at the peak of HSP60 expression in the pancreas). In group C, rats were pre-exposed to hyperthermia for 20 minutes. Six hours after the end of hyperthermia (at the peak of HSP72 expression in the pancreas), rats were injected with 40 μg/kg body weight of cerulein. As shown in Fig 6c, in group A (control group) and C (pre-treated with hyperthermia), significant increment in serum amylase level and pancreatic weight was observed after cerulein injection. Also, severe edema, neutrophil infiltration, necrosis and bleeding were observed.
HSPs in digestive organs • M. Otaka et al.

Histologically in all rats of group A and C. In opposite, in group B, rats pre-treated with water-immersion stress, serum amylase and pancreas weight did not increase after cerulein injection. No pathologic alteration, indicating the development of pancreatitis, was observed in group B (Fig. 6d).

Our data suggests that cerulein-induced pancreatitis was clearly prevented by specific pre-induction of HSP60 by water-immersion stress whereas specific induction of HSP72 by hyperthermia had no preventive effect in rats. These findings could suggest that HSP60 but HSP72 play an important role for the cytoprotection in the pancreatic cells against cerulein-induced pancreatic cellular damage.

Fig. 6.

a: Expression of HSP60 and HSP72 in the pancreas after WI-stress and hyperthermia. HSP60 was induced by WI-stress and HSP72 was induced by hyperthermia (42.5°C for 20 min.).

b: Experimental protocol: In group A, rats were fasted for 6 hours, injected with 40 μg/kg body weight of cerulein intraperitoneally. In group B, rats were pre-exposed to WI-stress for 6 hours injected with 40 μg/kg body weight of cerulein. In group C, rats were pre-exposed to hyperthermia for 20 minutes before cerulein injection.

c: Effect of pre-induction of HSPs on cerulein-induced pancreatitis. Development of pancreatitis was prevented when HSP60 was pre-induced by WI-stress.

histologically in all rats of group A and C. In opposite, in group B, rats pre-treated with water-immersion stress, serum amylase and pancreas weight did not increase after cerulein injection. No pathologic alteration, indicating the development of pancreatitis, was observed in group B (Fig. 6d).

Our data suggests that cerulein-induced pancreatitis was clearly prevented by specific pre-induction of HSP60 by water-immersion stress whereas specific induction of HSP72 by hyperthermia had no preventive effect in rats. These findings could suggest that HSP60 but HSP72 play an important role for the cytoprotection in the pancreatic cells against cerulein-induced pancreatic cellular damage.

(4) Liver

In the hepatocytes, Andoh et al. have shown possible importance of HSP70 family for cytoprotection and hepatocytes regeneration during thioacetamide-induced hepatocellular necrosis. We undertook to examine the effects of pre-induction of HSP72 by hyperthermia on...
Fig. 7a. b Effect of systemic hyperthermia (42.5°C for 20 min.) to pre-induce HSP72 in the liver on thioacetamide-induced hepatic injury. Thioacetamide-induced hepatic injury was clearly prevented by pre-induction of HSP72 by hyperthermia (Fig 7a, 7b).

Fig. 7c. Effect of hyperthermia on HSP72 induction in cirrhotic liver. Hyperthermia preconditioning induced a 4-fold increase in HSP72 also in cirrhotic rat liver.

Fig. 7d. Effect of hyperthermia on plasma TNF-α level. Pre-induction of HSP72 reduced plasma TNF-α response after LPS-injection.

Table II
Liver and renal test before and LPS administration

<table>
<thead>
<tr>
<th>Time(h)</th>
<th>AST(IU/L)</th>
<th>ALT(IU/L)</th>
<th>T. Bil(mg/dl)</th>
<th>LDH(IU/l)</th>
<th>Cre(mg/dl)</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GroupC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>268±25</td>
<td>139±24</td>
<td>0.28±0.12</td>
<td>2205±780</td>
<td>0.26±0.02</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>1403±351*</td>
<td>584±234**</td>
<td>1.64±0.47**</td>
<td>6550±971**</td>
<td>0.43±0.01*</td>
<td>5</td>
</tr>
<tr>
<td>GroupHS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>203±22**</td>
<td>83±5**</td>
<td>0.18±0.03</td>
<td>1796±187</td>
<td>0.30±0.01</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>188±26</td>
<td>70±10</td>
<td>0.26±0.04</td>
<td>1681±177</td>
<td>0.37±0.06</td>
<td>5</td>
</tr>
</tbody>
</table>

*P<0.01 vs. baseline levels in group C.

**P<0.05 vs. baseline levels in group C.
thioacetamide-induced hepatic injury in vivo, in order to elucidate the function of HSP72 by pre-inducing excess amount of HSP in the hepatocytes. Rats were injected with thioacetamide (100 mg/kg, sc.) with or without pre-induction of HSP72 by hyperthermia. Serum AST and ALT concentrations were measured before and after thioacetamide injection in both groups. Systemic hyperthermia (42.5°C for 20 min.) significantly induced HSP72 in the liver. Thioacetamide-induced hepatic injury was clearly prevented by pre-induction of HSP72 by hyperthermia (Fig. 7a, 7b).

Also, we have recently reported that induction of HSP72 could prevent lipopolysaccharide-induced liver injury in cirrhotic rats. It had been reported that heat stress protected non-cirrhotic rats against endotoxemia. However, its cytoprotective effect against endotoxemia in cirrhotic rats has not yet been studied. Since clinically endotoxemia is frequent in liver cirrhosis, this theme has to be investigated. Cirrhotic rats (induced by eight-week intraperitoneal injection of CCl₄) were given an intraperitoneal injection of LPS (10 mg/kg) with or without hyperthermia preconditioning (42.5°C, 15 min) in order to pre-induce HSP72 in the liver. The degree of liver injury and plasma tumor necrosis factor (TNF-α level was determined. Hyperthermia preconditioning induced a 4-fold increase in HSP72 also in cirrhotic rat liver (Fig. 7c). Preinduction of HSP72 prevented LPS-induced liver injury evaluated by serum biochemical parameters and histology with reduced TNF-α response (Fig. 7d, Table II). These findings suggest that preinduction of HSP72 may provide therapeutic strategies for Gram-negative sepsis-induced liver injury also in liver cirrhosis.

### Discussion

Our series of in vivo studies have demonstrated that 1) HSPs are induced by several environmental stresses including heat (hyperthermia), neuropeptide, neuroamine or drugs also in vivo. 2) Induced HSPs are different in each organ even by same stress. 3) In view of cytoprotection, important HSP is different in each organ (Table III).

Some of these findings are not compatible with in vitro findings reported before in which almost all HSPs have cytoprotective function mediated by chaperone function. However, based on our data, induction pattern and mechanism of HSPs are different between in vivo and in vitro. Possibly, induction mechanism of HSPs is regulated by many physiological factors such as stress-related hormones, peptides and neuroamines in vivo compared with in vitro. We are thinking that to understand these differences is important when HSP (chaperone-inducing therapy) would be applied for disease treatment utilizing their unique characters as a nonspecific internal cytoprotectant. Actually, some diseases have considered to be caused by dysfunction or decrease of molecular chaperone (chaperone diseases). We also have reported that antibiotic gentamicin-induced renal toxicity could be caused by specific binding of this antibiotic to HSP73 to reduce its chaperone function. Some cause-unknown diseases including digestive diseases might be caused by this kind of mechanism. We are thinking that binding substances,
which reduce chaperon function, could be drugs, chemicals, toxins, denatured proteins or peptides in a category of “chaperone-diseases”.

Further, recently, we have established HSP-over-expressing gastric mucosal cells by transfecting full length of human HSP70 gene. This mucosal cell shows high protective ability against both necrosis and apoptosis. Therefore, our recent studies are designed to develop chaperone-inducing drugs or chaperone-inducing gene therapy as a new strategy for disease therapy.

Acknowledgement

Authors express great thanks to chief editor giving us an opportunity to submit a review article to this journal.

References


13) Kuwabara T., Otaka M., Itoh H., Zeniya A., Fujimori S., Otani S., Tashima Y., Masamune O.: Regulation of HSP60


消化器系臓器における熱ショック蛋白質
（分子シャペロン）の発現と機能

大高道郎・渡辺純夫

秋田大学第1内科

要　旨：これまでに我々はHeat Shock Protein（以下HSP）の有する分子シャペロン機能を介した細胞保護作用に着目し、研究を進めてきた。in vivoの実験系で、胃、大腸、肝臓ではHSP72（72-kDa heat shock protein, stress-inducible HSP70）が、腎臓ではHSP60（60-kDa heat shock protein, chaperonin homolog）が臓器保護的に機能していることを明らかにするとともに、これらのHSPを効率よく臓器で誘導する薬剤、物質に関して検討し、「シャペロン誘導療法」や、最近では遺伝子治療も視野に入れ、HSPの細胞保護作用、損傷修復能に関しても検討している。本稿ではこれまでの我々の知見を中心にとっていただき。