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# Is *Mongolian gerbil* really adequate host animal for study of *Helicobacter pylori* infection-induced gastritis and cancer? ☆

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**Abstract**

*Background:* Many researches have been published to understand the pathogenesis and mechanism of *Helicobacter pylori* (Hp)-associated diseases, including gastritis followed by gastric cancer, using *Mongolian gerbil* (MG) model because Hp could be hardly inoculated in other animal species. The aim of this study was to evaluate the induction ability of heat shock protein (HSP70) and protective ability in the gastric mucosa of MG comparing with those of Sprague–Dawley (SD) rats, since HSP70 is a key molecule known to be involved in important biological activities such as apoptosis, carcinogenesis, and cytoprotection from cytotoxic damage.

*Materials and methods:* Basal expression level and induction ability of gastric mucosal HSP70 were evaluated by immunoblotting and densitometric analysis in MG and SD rats before and after HSP-induction by zinc L-carnosine, gastric HSP70 inducer, administration. Mucosal protective ability against water-immersion stress-induced mucosal lesion was also compared.

*Results:* Basal expression level of HSP70 was not significantly different between MG and SD rats. However, HSP70-induction by zinc derivatives was not observed in MG. Mucosal lesion induced by water-immersion stress was significantly severe in MG compared with SD rats.

*Conclusions:* MG might be special (not ordinary) animal, in which HSP70-induction was absent and has extremely poor mucosal protective ability in view of HSP-dependent cytoprotection in the gastric mucosa. Our results may suggest that MG is not an adequate animal to evaluate the effect of Hp-infection-associated gastric inflammation followed by development of gastric cancer.


*Keywords:* *Mongolian gerbil*; *Helicobacter pylori*; Gastritis; Gastric cancer; Heat shock protein; HSP70; Molecular chaperone; Zinc

It is now widely accepted that *Helicobacter pylori* (Hp) infection leads to significant inflammations in the gastric mucosa, which is considered to be closely associated with development of gastric cancer [1–8]. Actually, discovery of Hp has changed the concepts of pathogenesis and therapy for peptic ulcer diseases, and might be going to change the strategy of disease prevention for gastric cancer.

Many researches have been published to understand the pathogenesis and mechanism of Hp-associated diseases using *Mongolian gerbil* model because Hp could be hardly inoculated in other animal species except Japanese monkey and human [6,7]. Hp infection has been reported to cause inflammation in the gastric mucosa, leading to gastritis, gastric ulcers, duodenal ulcer disease, and even gastric cancer in MG [7].

Heat shock proteins (HSPs) are crucial for the maintenance of cell integrity during normal cellular growth as well as during pathophysiological conditions. HSPs, also called molecular chaperones, have important functions in response to stress-related events [9–11]. HSPs are highly conserved proteins and rapidly induced in cells in response

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to abrupt and adverse change in their environment from prokaryotic cells to eukaryotic cells. Therefore, it is considered that they have essential functions for survival of cells and developmental process. Some recent reports, including ours, 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 to environmental changes also in digestive organs and cells [12–14].

*Helicobacter pylori* has high urease activity that results in the production of ammonia [1,15] and elicits oxidative burst of neutrophils [15]. *H. pylori*-activated neutrophils reduce $O_2$ to superoxide ($O_2^-$), and dismutation of $O_2^-$ yields hydrogen peroxide ($H_2O_2$). Myeloperoxidase-catalyzed oxidation of chloride ($Cl^-$) by $H_2O_2$ yields hypochlorous acid (HOCl), and the reaction of HOCl with ammonia ($NH_4^+$) yields monochloramine ($NH_2Cl$) that is known to be strongly cytotoxic [16]. These oxidative conditions are one of common and major pathogenic factors in Hp-infected gastric mucosa not depending on Hp genotype classified by toxin status (cag A or vac A).

Recently, we have reported the importance of HSP70 for the gastric mucosal cytoprotection against cytotoxic agent-induced cell damage including $H_2O_2$ or $NH_2Cl$-induced cell necrosis and apoptosis [17–19]. Therefore, to evaluate the basal expression level and induction ability of HSPs, that is a key molecule as an internal cytoprotectant in host animal, could be important and essential when a concept established in animal model is going to be applied to human. In this study, we evaluate the induction ability of HSP70 and protective ability in the gastric mucosa of MG comparing with those of Sprague–Dawley (SD) rats.

## Materials and methods

*Animals*. This study was approved by the Akita University Animal Care Committee.

Male *Mongolian gerbil* and Sprague–Dawley rats (8 weeks old) were fed on standard laboratory diet and water ad libitum, and kept in cages in temperature (22 ± 2 °C) and humidity (55 ± 5%) controlled room with a 12-h dark-light cycle before and during the experiment. Rats were deprived of food but were allowed access to water 24 h before experiment.

*Drugs*. Zinc L-carnosine, gastroprotective agent which is commercially available in Japan, is generous gift from Zeria Pharmaceutical Company (Tokyo, Japan) [20].

*Expression of HSP70 in the gastric mucosa in SD rats and MG.*

(1) Basal expression of HSP70 (without HSP-induction). After 24 h fasting, the stomach was removed from MG or SD rat ($n = 4$ in each animal species) and placed on dried ice immediately. The gastric mucosa was scraped with a slide glass, chopped finely with scissors, and homogenized using polytron homogenizer with five volumes of ice-cold 25 mM Tris–HCl buffer (pH 7.5). The homogenate was centrifuged at 18,000*g* for 20 min. The supernatant was collected and the protein concentration was measured by the method of Bradford et al. [21]. Expression of each HSP was evaluated by the method which we previously reported [13]. Briefly, samples (20 μg/lane) were electrophoresed on 9% SDS–polyacrylamide gels, transferred electrophoretically to a polyvinylidene (PVDF) membrane (Nihon Millipore Kogyo, Tokyo, Japan), and processed as described by Towbin et al. [22]. The membrane was incubated with anti-HSP70 antibodies (StressGen, Victoria, BC, Canada) (1:10,000 dilution) and treated with horseradish peroxidase conjugated anti-rabbit IgG (1:1500 dilution) (Bio-Rad Laboratories, Richmond, CA.). The peroxidase substrate was 3,3-diaminobenzidine-tetrahydrochloride. The density of the immunologically stained band was analyzed by scanning densitometer using National Institute of Health (NIH)-image program (version 1.59). The relative density of the stained band was calculated using the following formula. Relative density (%) = density (SD rats)/density (MG) × 100.

(2) Induction of HSP70 by HSP-inducer (zinc L-carnosine). In order to evaluate and compare the induction ability of HSP70 in both animals by zinc derivatives (HSP70 inducer) [20], 100 mg/kg of zinc L-carnosine was per-orally administered ($n = 4$ in each animal species). HSP70 expression in the gastric mucosa was measured using the same method as mentioned above and the relative density was calculated. The density of basal level expression in SD rat gastric mucosa (before zinc L-carnosine administration) was used as control (100%).

*Mucosal protective ability against short-term water-immersion-induced gastric mucosal damage in SD rat and MG*. Severity of gastric mucosal damage induced by water-immersion stress (6 h) was compared between SD rat and MG according to the method previously reported [23]. Briefly, following 24 h fasting, both animal species ($n = 4$ in each species) were placed in restraint cages and then immersed vertically in a water bath (23 °C) to the level of xiphoid process for 6 h. After the stomach was removed, the extent of mucosal damage was scored by ulcer ratio (ulceration area/total gastric mucosal area × 100), and the severity of the damage was assessed by two pathologists who were blinded to the animal group.

*Statistical analysis*. All results are presented as means ± SEM. Statistical comparisons were made by unpaired Mann–Whitney *U*-test. A *P*-value < 0.05 was considered to indicate significance.

## Results

### *Expression and induction ability of HSP70 in the gastric mucosa in SD rats and MG*

No significant difference was observed in basal protein expression level of gastric HSP70 between SD rats and MG (Fig. 1). Also, no significant difference was observed

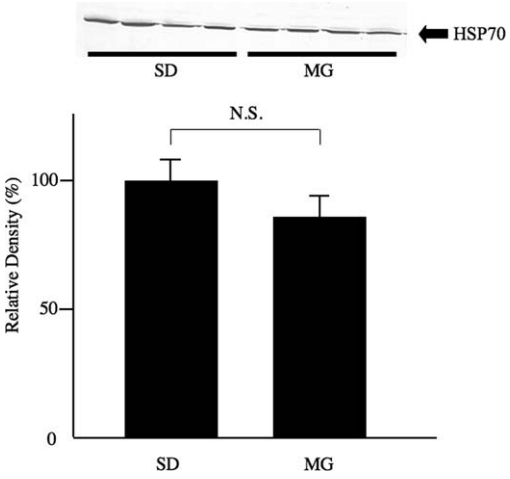


Fig. 1. Basal expression of HSP70 (without HSP-induction). No significant difference was observed in basal protein expression level of gastric HSP70 between SD rats and MG. Upper panel shows the results of immunoblotting and lower panel shows the relative density of stained bands ($n = 4$ in each species). Values are means ± SEM. NS, statistically no significant difference.

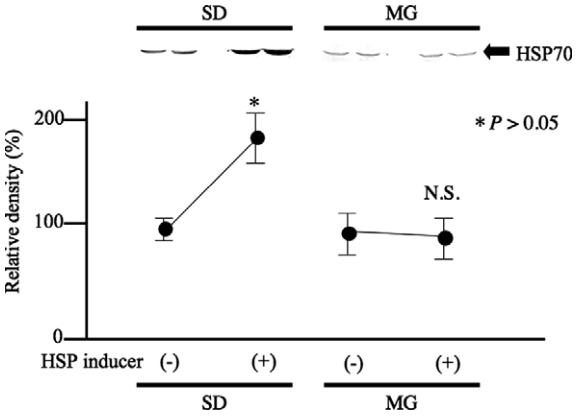


Fig. 2. Induction of HSP70 by HSP-inducer (zinc L-carnosine). HSP70 expression in the gastric mucosa was measured in each species before and after administration of zinc L-carnosine (100 mg/kg). Values are means ± SEM. $^{*}P < 0.05$, significant difference compared with control (before administration).

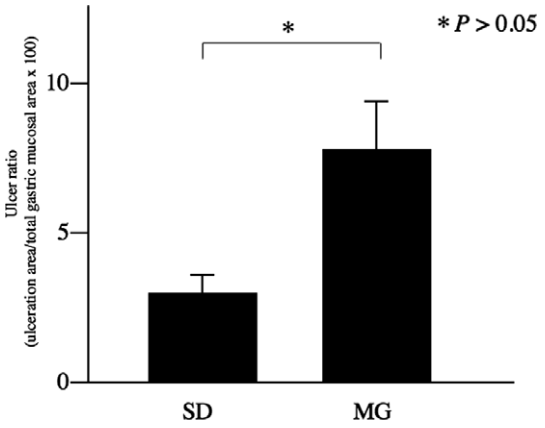


Fig. 4. Ulcer ratio after short-term (6 h) water-immersion stress. Severity of mucosal damage, expressed as ulcer ratio (ulceration area/total gastric mucosal area × 100), was significantly severe in MG compared with SD rats. Values are means ± SEM. $^{*}P < 0.05$, significantly different.

in HSP70 expression between male and female MG (data not shown).

As shown in Fig. 2, HSP70 was significantly induced in the gastric mucosa of SD rats 6 h after per-oral administration of zinc L-carnosine, HSP-inducer ($P < 0.05$). In opposite, HSP70 did not increase in the gastric mucosa of MG.

*Mucosal protective ability against water-immersion-induced gastric mucosal damage in SD rat and MG*

Short-term water-immersion stress developed dotted and linear erosions and shallow ulcers. Severity of mucosal damage was apparently severe in MG compared with SD rats as shown in Fig. 3. Ulcer ratio was significantly higher in MG compared with SD rats ($P < 0.05$) (Fig. 4).

## Discussion

Many large-scale clinical studies have demonstrated the significance of Hp eradication for prevention of recurrence of peptic ulcer diseases [3–5]. Thus, discovery of Hp has changed the concepts of pathogenesis and therapy for peptic ulcer diseases. Also, based on animal studies, the strategy of disease prevention for gastric cancer might be going to be changed [7]. However, many researches have been published to understand the pathogenesis and mechanism of Hp-associated diseases using *Mongolian gerbil* because Hp could be hardly inoculated in other animal species [6,7]. Hp infection has been reported to cause inflammation in the gastric mucosa, leading to gastritis and gastric cancer in MG [7]. Since carcinogenesis, cancer development or tumor growth in Hp-infected MG is considered to be caused by chronic inflammation, protective ability of gastric mucosa from inflammation is extremely important factor if the results would be going to reflect to understand the pathogenesis in human.

It has been reported that high urease activity of Hp results in the production of ammonia [1] and elicitation of oxidative burst of neutrophils [7] (Suzuki et al. [15]) as the pathogenesis of Hp-induced inflammation. Also, as a result, activation of neutrophils produces superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$), that is, dismutation

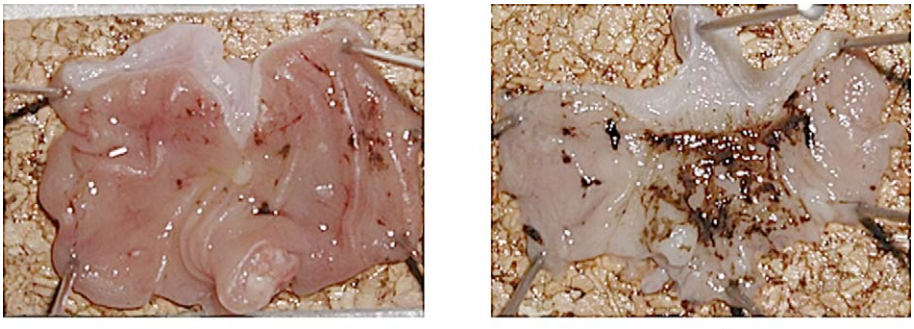


Fig. 3. Mucosal protective ability against short-term (6 h) water-immersion-induced gastric mucosal damage in SD rat and MG. Short-term water-immersion stress developed dotted and linear erosion and shallow ulcers. Severity of mucosal damage was apparently severe in MG compared with SD rats.

product of $O_2^-$. Further, myeloperoxidase-catalyzed oxidation of chloride ($Cl^-$) by $H_2O_2$ yields hypochlorous acid (HOCl), and the reaction of HOCl with ammonia ($NH_4^-$) yields monochloramine ($NH_2Cl$) that is known to be strongly cytotoxic to epithelial cells [16]. Therefore, protective ability of the gastric mucosa against these toxic materials could provide the severity or development of gastric inflammation and carcinogenesis in the host animal. As an internal cytoprotective factor in the gastric mucosa, importance of heat shock proteins has been reported in various cells and organs. We have reported that overexpression or pre-induction of HSP70 protects gastric mucosal cells from $H_2O_2$ or $NH_2Cl$ by suppressing both apoptotic and necrotic pathways [17,19]. These facts lead us to investigate the expression of HSP70 in MG comparing with other animal species. As demonstrated in the present study, although basal expression level of HSP70 was not significantly different between MG and SD rats, HSP70-induction by zinc derivatives, HSP70-inducer [20], was not observed in MG. Mucosal lesion induced by water-immersion stress was significantly severe in MG compared with SD rats.

As conclusions, MG might be special animal in which HSP70-induction was absent and protective ability is extremely poor in view of HSP-dependent cytoprotection in the gastric mucosa. Our results may suggest that MG may not be an adequate animal to evaluate the effect of Hp-infection-associated gastric inflammation and development of gastric cancer. Considering that MG is widely used for Hp-infection experiments now, we should be more careful for evaluating the results from animal experiments especially when we reflect the concept to human.

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