Effect of pre-induction of heat shock proteins on indomethacin-induced small-intestinal lesion in rats

MARIO JIN,1 MICHIRO OTAKA,1 SETSUYA OTANI,1 ATSUSHI OKUYAMA,1 SATOSHI ITOH,1 AKIRA IWABUCHI,1 HIDEAKI SASAHARA,1 SHUSEI FUJIMORI,1 HIDEAKI ITOH,2 YOHTALOU TASHIMA,2 and OSAMU MASAMUNE1

1First Department of Internal Medicine and 2Department of Biochemistry, Akita University School of Medicine, 1-1-1 Hondo, Akita City, Akita 010, Japan

Abstract: Systemic hyperthermia induces the synthesis of heat shock proteins (HSPs) in several organs. However, the mechanism of induction and the functions of HSPs in the small-intestinal mucosa have not been established. We examined the expression of HSPs in the small-intestinal mucosa after systemic hyperthermia, and evaluated the cytoprotective function of pre-induced HSPs on experimentally induced mucosal damage. HSP expression was investigated by Western blot and densitometric analysis before and after hyperthermia (42.5°C; 20 min). Expression of a 72-kDa heat shock protein (HSP72) and a 73-kDa heat shock protein (HSP73), both of which are endogenous cytoprotectants in vitro significantly increased, peaking 6–9 h after hyperthermia, without any pathologic alterations, whereas the expression of a 60-kDa heat shock protein (HSP60) did not increase. To investigate the influence of pre-induction of HSPs on small-intestinal damage, rats received indomethacin (10 mg/kg; orally) with or without pre-treatment with hyperthermia. Small-intestinal damage caused by indomethacin was not influenced by pre-induction of HSP72 and HSP73. We demonstrated that systemic hyperthermia induced HSP72 and HSP73, although pre-induction of these proteins did not have a cytoprotective function in the small-intestinal damage caused by indomethacin.

Key words: heat shock protein, small-intestinal mucosa, cytoprotection, indomethacin

Introduction

Many studies have shown the importance of heat shock proteins (HSPs) for cells survival under stress conditions.1–3 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.4 We have reported that specific pre-induction of HSP60 by water-immersion stress clearly prevents pancreatitis induced by cerulein in rats.5,6 Further, we demonstrated that the HSP70 family had a crucial cytoprotective function in the gastric mucosa in vivo.7 The cytoprotective functions of this family of proteins are considered to be mediated by their functions as “molecular chaperons”. However, little is known about the expression and function of HSPs in the small-intestinal mucosa under stress conditions. We studied the influence of hyperthermia on the expression of HSP60, HSP72, and HSP73 (these being, quantitatively, the major HSPs in mammalian tissue) in rat small-intestinal mucosa. We also investigated the effect of pre-induction of HSPs on indomethacin-induced small-intestinal damage.

Materials and methods

Animals

Male Sprague-Dawley rats, weighing 250–300 g, received a standard laboratory diet and water ad libitum, and were kept in cages in a temperature (22 ± 2°C) and humidity (55 ± 5%)-controlled room with a 12-h dark-light cycle before and during the experiment.

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

Expression of HSPs after hyperthermia

To study the expression of HSP60, HSP72 and HSP73 after hyperthermia, rats were placed in restraint cages.
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and then vertically immersed in a hot water bath (42.5 °C) to the level of the xyphoid process for 20 min. Rats were killed by stunning and cervical dislocation before and 3, 6, 9, 12, 15, 18, 21, 24 h after the end of hyperthermia (n = 4 at each time point). The small-intestinal mucosa in the middle of the small intestine of each rat was quickly removed and homogenized with five volumes of ice-cold 25 mM Tris-Cl buffer (pH 7.5). The homogenates were centrifuged at 18,000 g, for 20 min. The supernatants were collected and the protein concentration was measured by the method of Lowry et al. Expression of each HSP was evaluated by a method that we previously reported. Briefly, samples (20 μg/lane) were electrophoresed on 9% sodium dodecyl sulfate (SDS)-polyacrylamide gel, transferred electrophoretically to a polyvinylidene difluoride (PVDF) membrane (Nihon Millipore Kogyo, Tokyo, Japan), and processed as described by Towbin et al. The membrane was incubated with anti-HSP60, anti-HSP72, or anti-HSP73 antibodies (1:1000 dilution) and treated with horseradish peroxidase-conjugated anti-rabbit IgG (1:1500 dilution) (Bio-Rad, Richmond, CA, USA). The peroxidase substrate was 3,3'-diaminobenzidine tetrahydrochloride. The density of the immunologically stained band was analyzed by scanning densitometer, using a National Institutes of Health (NIH) image program (http://rsb.info.nih.gov/nih-image/). The relative density of the stained band was calculated by the formula: Relative density (%) = density (after hyperthermia)/density (before hyperthermia) × 100. The results of densitometric analysis were analyzed using two-tailed unpaired Student’s t-test.

For light microscopy, the small intestine was fixed in 20% formalin and embedded in paraffin wax. The tissue section was stained with hematoxylin and eosin.

Effect of pre-induction of HSPs on indomethacin-induced mucosal damage in the small intestine

To study the effect of pre-induction of HSP on indomethacin-induced small-intestinal mucosal damage, we assigned eight rats to two groups (Fig. 1). All received food ad libitum during the experiment. In group A, rats received indomethacin (10 mg/kg, orally) at 9 p.m. without any pre-treatment, and were placed into a cage. In group B, rats were pre-treated with hyperthermia (42.5 °C) for 20 min 6 h before receiving oral administration of indomethacin, and then placed into a cage. Both groups of rats were killed 12 h after the administration of indomethacin. The small intestine was then removed and the extent of mucosal damage was scored by an individual who was blinded to the groups. An ulcer index was determined, based on the total longitudinal length of all mucosal lesions, according to a method described previously. Differences in the ulcer index between the two groups were analyzed by two-tailed unpaired Student’s t-test.

### Results

**Specificity of anti-HSP antibodies and expression of HSPs after hyperthermia**

The specificity of each anti-HSP antibody is shown in Fig. 2. Only the 60-kDa, 72-kDa, and 73-kDa bands were stained.

Expression of HSPs after heat stress is shown in Fig. 3. HSP72 and HSP73 increased significantly 3 h after the end of hyperthermia and peaked 6–9 h after hyperthermia. The relative density of HSP72, evaluated by densitometry after the end of hyperthermia, compared with the density at 0 h (before hyperthermia) was 161 ± 50.7% (3 h, NS), 247 ± 27.3% (6 h, P < 0.01), 311 ± 30.2% (9 h, P < 0.01), 199 ± 24.9% (12 h, P < 0.01), 284 ± 61.0% (15 h, P < 0.05), 263 ± 40.4% (18 h, P < 0.01), 299 ± 37.9% (21 h, P < 0.01), and 224 ± 23.8% (24 h, P = 0.05). The relative density of HSP73 was 178 ± 42.8% (3 h, NS), 260 ± 29.3% (6 h, P < 0.01), 257 ± 29.2% (9 h, P < 0.01), 189 ± 23.9% (12 h, P < 0.05), 212 ± 27.8% (15 h, P < 0.01), 196 ± 26.3% (18 h, P < 0.05), 228 ± 10.4% (21 h, P < 0.01), and 138 ± 15.5% (24 h, P < 0.05) (means ± SEM) (Fig. 4). No significant increase was observed in HSP60. No pathologic alterations were observed in the small-intestinal mucosa 12 h after hyperthermia, based on histologic