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Specific induction of a 72-kDa heat shock protein protects esophageal mucosa from reflux esophagitis

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ABSTRACT

Aims: The aim of this study is to investigate the expression and cytoprotective function of a 72-kDa heat shock protein (HSP72) using a reflux esophagitis model in rats.
Main methods: Expression of HSP60, HSP72, and HSP90 in rat esophageal mucosa was evaluated by Western blot analysis before and after hyperthermia (42.5 °C, 20 min). Rats received the operation to produce reflux esophagitis with or without pretreatment with hyperthermia to induce HSPs. The esophageal mucosal damage was evaluated 12 h after the operation.
Key findings: Expression of HSP72 was significantly increased by hyperthermia in rat esophageal mucosa. Reflux esophagitis was dramatically prevented when HSP72 was preinduced by hyperthermia. Furthermore, activation of TNF-α and IL-1β in esophageal mucosa was also suppressed.
Significance: These results suggested that hyperthermia protects the esophageal mucosa in reflux esophagitis model by inducing HSP72 and suppressing proinflammatory cytokine activation. These findings might suggest that HSP-inducing therapy could be a novel and unique therapy for reflux esophagitis.

Introduction

Heat shock proteins (HSPs), also called as molecular chaperones, are highly conserved proteins in all organisms and are rapidly synthesized in cells in response to a variety of stresses (Itoh and Tashima 1991; Hightower 1980). It is therefore suggested that these proteins may have important roles in a basic cellular defense mechanism against environmental stresses. Many studies have shown the importance of HSPs for survival of cells under stress conditions (Itoh and Tashima 1991; Hightower 1980; Tissieres et al. 1974).

HSPs are classified into some families by their molecular masses. It has been demonstrated that the synthesis of a 72-kDa heat shock protein (HSP72) is induced in cultured gastric mucosal cells by heat stress (hyperthermia), and this protein has cytoprotective functions *in vitro* (Nakamura et al. 1991; Otaka et al. 2006a,b; Oyake et al. 2006; Odashima et al. 2007; Matsuhashi et al. 2007). We demonstrated that the HSP70 family had a crucial function as an endogenous cytoprotectant in the gastric mucosa also *in vivo* (Zeniya et al. 1995; Watanabe et al. 2004; Mikami et al. 2006; Wada et al. 2006). In the colonic mucosa HSP72 has a crucial cytoprotective function mediated by its function as a "molecular chaperon" (Otani et al. 1997; Otaka et al. 2006a,b). Further, we have reported that specific preinduction of HSP60 by exposure to water-immersion stress dramatically prevents cerulein-induced pancreatitis in rats (Otaka et al. 1994, 1997).

However, little is known about the expression and function of HSPs in the esophageal mucosa under stress conditions. The limited data available indicate that high levels of HSP27 are found in the human esophagus, while its expression is markedly decreased in individuals with Barrett's esophagus and adenocarcinoma (Soldes et al. 1999). HSP72 expression is reported to be decreased after exposure to hyperthermia in organ culture of biopsied specimen (Hopwood et al. 1997). In acid-induced esophagitis in opossum, HSP90 and HSP60 expressions are increased, while HSP72 expression is decreased (White et al. 2002).

However, expression or induction mechanisms of HSPs in esophagus have been still controversial. Prevalence of reflex esophagitis is increasing. It is known that reflux esophagitis could be a cause of Barrett's esophageal mucosa followed by adenocarcinoma (Anandasabapathy et al. 2007; Zhang et al. 2009; Pondugula et al. 2007; Modiano and Gerson 2007). Therefore, to understand the defensive mechanisms of esophageal mucosa is clinically important.

In this paper, we studied the influence of hyperthermia on the expression of HSP60, HSP72, and HSP90, which are quantitatively major HSPs in mammalian tissue, in rat esophageal mucosa. The effect of preinduction of these HSPs on reflux esophagitis was also investigated.

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Materials and methods

Animals

Nine-week-old male Sprague–Dawley rats (weight: 300–350 g) were fed on standard laboratory diet and water ad libitum, and kept in cages in a temperature (24 ± 2 °C)-controlled room with a 12 h-dark-light cycle before and during experiments. This study is approved by the Juntendo University School of Medicine Animal Care Committee.

Hyperthermia

Rats were placed in a restraint cage and treated for 20 min by immersing them vertically to the level of the xyphoid process in a hot water bath (42.5 °C).

Expression of HSPs after hyperthermia

Rats were exposed to hyperthermia for 20 min using the same method described above. Rats were not given free access to food and water during experiments.

Rats were killed before and 1.5, 3, 6, 9, 12, or 15 h ($n = 4$ at each time point) after the end of hyperthermia (Fig. 1A). The esophageal mucosa was quickly removed and was used for Western blot analysis.

Each esophageal mucosa was homogenized with ice-cold lysis buffer containing 50 mmol/l Tris-HCl, pH 8.0, 150 mmol/l NaCl, 1 mmol/l ethylene diamine tetraacetic acid (EDTA), 1% TritonX-100, and Complete Mini according to manufacturer's recommendation (EDTA-free, Protein inhibitor cocktail tablets, Mannheim, Germany). The homogenates were centrifuged at 600 *g* for 5 min at 4 °C and the supernatant was stored at −80 °C until Western blotting was performed. Protein concentrations were measured with a protein assay kit (Bio-Rad, Richmond, California), using bovine serum albumin as a standard. Equal total protein content was applied for 9% SDS–polyacrylamide gel electrophoresis (Laemmli 1970). Gels were stained with 0.1% Coomassie Brilliant Blue (R-250) in a mixture of 25% isopropyl alcohol–10% acetic acid and destained with 10% isopropyl alcohol–10% acetic acid.

Expression of each HSP was evaluated by the method that we previously reported (Otaka et al. 1994). Briefly, samples (30 μg/lane) were electrophoresed on 9% SDS–polyacrylamide gels, transferred electrophoretically to PVDF membrane (removal rating, 0.45 μm; Amersham Pharmacia Biotech, Bockinghamshire, England), and processed as described by Towbin et al. (Towbin et al. 1979). The membrane was incubated with rabbit anti-HSP60 antibody (Otaka et al. 1993), rabbit anti-HSP72 antibody (Wakui et al. 1991), or rabbit anti-HSP90 antibody (Itoh et al. 1990) and treated with horseradish peroxidaseconjugated anti-rabbit IgG (1:1000). After preparation of the washing solution, the stained bands were visualized with 0.02% 3, 3-diaminobenzidine tetrahydrochloride containing 0.005% H_2O_2. The intensity of the visualized band was quantified using Quantify One.4.0 software (Bio-Rad, Richmond, California).

Reflux esophagitis production

A reflux esophagitis model was created in rats by modifying the method reported by Omura et al. (Omura et al. 1999; Asaoka et al. 2005). Briefly, anesthesia was performed by inhalation anesthesia using isoflurane. After laparotomy, duodenal stenosis was created by wrapping the duodenum near the pylorus with a piece of 16-Fr Nelaton catheter (diameter 5.3 mm; Terumo, Tokyo, Japan), and the width of the catheter is 2.0 mm. The thickness of the catheter was 18-Fr (diameter 6.0 mm) in Omura's method. However it is difficult to induce reflux esophagitis in a stable manner at acute stage using the method reported by Omura et al. (Omura et al. 1999). We changed the thickness of the catheter from 18-Fr to 16-Fr (diameter 5.3 mm) (Fig. 2). To prevent catheter dislodgement, we sutured the edge of the catheter to the serosa of the pylorus, using a 6-0 nylon thread. The

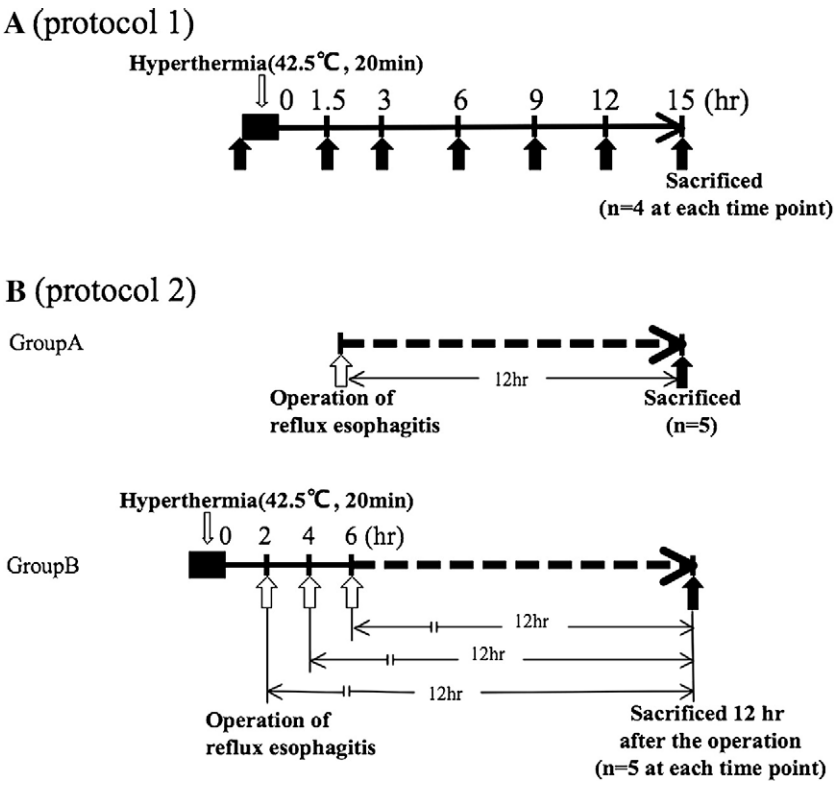

Fig. 1. (A) Protocol 1: Rats were treated with hyperthermia (42.5 °C, 20 min) and were killed before (control (0 h)) and 1.5, 3, 6, 9, 12, or 15 h after the end of hyperthermia. (B) Protocol 2: Rats received the operation of reflux esophagitis without pretreatment with hyperthermia (group A) or 2, 4, or 6 h after the end of hyperthermia (group B). All rats were sacrificed 12 h after the operation.