Feeding induces expression of heat shock proteins that reduce oxidative stress

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Abstract Heat shock proteins (Hsps) are induced in response to various kinds of environmental and physiological stresses. However, it is unclear whether Hsps play roles in protecting cells in the digestive organs against xenobiotic chemicals. Here, we found that feeding induces expression of a set of Hsps specifically in the mouse liver and intestine by activating heat shock transcription factor 1 (HSF1). In the liver, HSF1 is required to suppress toxic effects of electrophiles, which are xenobiotic chemicals causing oxidative stress. We found that overexpression of Hsp27, which elevates cellular glutathione level, promotes survival of culture cells exposed to electrophiles. These results suggest a novel mechanism of cell protection against xenobiotic chemicals in the food.

Keywords: Electrophile; Glutathione; Heat shock protein; Heat shock transcription factor; Liver; Oxidative stress

1. Introduction

Heat shock response, which is characterized by the induction of a set of heat shock proteins (Hsps), is a fundamental response in all organisms to protect themselves from environmental stresses such as heat, oxidative stress, ischemia, inflamation and exposure to toxic chemicals [1]. This response is regulated mainly at a transcription level by heat shock transcription factor 1 (HSF1) in mammals [2]. There are many Hsps belonging to diverse families and Hsps act coordinately to assist the folding of cellular proteins [3]. Hsps also bind to denatured proteins, prevent misaggregation and facilitate renaturation. In addition to the "foldase activity", each Hsp has a unique role. For example, Hsp27 reduces oxidative stress by raising the pool of reduced glutathione (GSH) in the cells [4].

In addition to environmental stresses, heat shock response is induced in response to physiological stresses such as exercise [5,6] and restraint stress [7,8]. Here, we found that feeding induces expression of Hsps selectively in the liver and intestine among digestive organs. Digested elements such as oligosaccharides, proteins, lipids and nucleic acids are adsorbed mostly in the intestine, and blood containing these elements is directly transported to the liver through the portal vein. To protect themselves from xenobiotic chemicals such as electrophiles in the food, detoxifying enzymes are rich in the liver [9,10]. We found that electrophiles induce heat shock response in HeLa and Jurkat cells, probably by causing oxidative stress. Furthermore, induction of at least Hsp27 reduced toxic effects of electrophiles on culture cells.

2. Materials and methods

2.1. Animals, food and injection of DEM

Mice of ICR background were maintained at 24 °C with light on from 8 to 20 h. 6-week-old mice had free access to water and diet (bleeding grade F-1, Oriental Yeast Co., Ltd, Tokyo, Japan). Food deprivation was performed for 48 h and then the same diet was fed for indicated periods. Digestive organs were immediately dissected and stored at −80 °C until use. To examine the expression of Hsps in the absence of HSF1, HSF1-null mice were used [11]. Diethyl maleate (DEM) (Wako, Osaka, Japan) prepared in sesame oil (5.3 mmol of DEM/kg of body weight) was injected intraperitoneally and serum alanine aminotransferase (ALT) levels were measured (SRL Co., Tokyo, Japan). All experimental protocols were reviewed by the Committee for Ethics on Animal Experiments of Yamaguchi University School of Medicine.

2.2. Western blot analysis, Gel shift assay and Northern blot analysis

Tissues were dissected, immediately frozen and stored at −80 °C until use. The extracts were prepared with NP-40 lysis buffer [150 mM NaCl, 1.0% Nonidet P-40, 50 mM Tris (pH 8.0), 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin and 1 μg/ml pepstatin]. HeLa cell extracts were also prepared with NP-40 lysis buffer. Western blot analysis was performed as described previously [12] using mouse monoclonal IgG for Hsp70 (W27, Santa Cruiz), and antiserum for human Hsp70 (Fujimoto, unpublished), rat Hsp27 (a kind gift from K. Kato), and human GST-pi (Novocastra Lab. Ltd., UK). To detect Hsp90, we generated a specific antiserum. Recombinant human Hsp90α (amino acids 333–732) fused to glutathione-*S*-transferase was immunized in rabbits in a TiterMax (CrtRx Co., Georgia) water-in-oil emulsion. The levels of Hsp70 protein in various tissues were estimated by using NIH image.

To perform gel shift assay, mice were systematically anesthetized with ketamine (16 mg/kg, i.p.) and xylazine (16 mg/kg, i.p.) and perfused with phosphate-buffered saline. Whole tissue extracts were prepared from the liver, and gel shift assay and supershift experiments were performed as described previously [12].

Northern blot analysis was performed essentially as described previously [13].

2.3. Cells and the treatment with reagents

HeLa cells were maintained at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium containing 10% fetal calf serum at 37 °C in

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5% CO_2. HeLa cells stably expressing mouse Hsp27 were described previously [14]. To prepare HeLa cells expressing human GST-pi (pcDNA3.1/GST-pi), GST-pi cDNA [15] was cloned by RT-PCR and was inserted into pcDNA3.1(+) (Invitrogen). The DNA was transfected into HeLa cells by a calcium-phosphate method and cells grown in the presence of 1.5 mg/ml of G418 were selected as described previously [14]. Jurkat cells were maintained in RPMI containing 10% fetal calf serum. To block de novo synthesis of glutathione, cells were treated with 0.1 mM L-buthionine-(*S,R*)-sulfoximine (BSO) (stock, 100 mM in H_2O) (Sigma, St. Louis, MO) until 48 h. Cells were also treated with electrophile, 1-chloro-2,4-dinitrobenzene (CDNB) (stock, 10 mM in 100% ethanol) or DEM (stock, 10 mM in 100% ethanol) at a concentration of 10 to 40 μM for 8 h.

To examine cell survival, HeLa cells were treated with CDNB for 24 h and were replated in dishes containing normal medium for 10 days. Cells were fixed in 70% ethanol for 30 min and then stained with Giemsa's stain solution (Muto Pure Chemicals, Tokyo, Japan). Colonies counted, and means and S.D. were calculated from three independent experiments.

2.4. Determination of glutathione levels

Total cellular glutathione concentrations (reduced plus oxidized forms) were measured as described previously [16,17] and showed as nanomoles per 1×10^6 cells.

3. Results

3.1. Feeding induces heat shock response specifically in the liver and intestine

To reveal whether feeding induces expression of Hsps in various tissues, mice starved for 48 h were fed for 3 h and expression of Hsp70 protein was determined. We found that Hsp70 expression was induced in the liver and the intestine after feeding, but was not induced in the colon, stomach, brain and muscle (Fig. 1A). As the liver is important for detoxifying chemicals, we further analyzed the response in the liver. Inductions of proteins and mRNAs of Hsp70 and Hsp90 were observed even 1 h after feeding, and induction of Hsp27 protein was particularly strong although it was detected firstly at 6 h after feeding (Fig. 1B). Induction of Hsp70 was low probably due to unique regulation of Hsp70 in the liver [18].

3.2. HSF1 plays roles in suppressing toxic effects of electrophiles in the liver

We next examined HSF1 activation in the liver. Weak, but distinct HSE-binding activity of HSF was induced 1 h after feeding in the liver and then the activity attenuated (Fig. 2A). Supershift experiment using specific antiserum showed that the HSE-binding activity was composed of HSF1 (data not shown). We next examined the induction of Hsp90, Hsp70 and Hsp27 expression after feeding in HSF1-null mice. We observed no induction of Hsps in HSF1-null mice (Fig. 2B), indicating that HSF1 is required for the feeding-induced induction of Hsps in the liver.

To examine whether HSF1 plays roles in rendering toxic effects of xenobiotic chemicals in the liver, an electrophilic reagent DEM was injected intraperitoneally. We determined toxic effects of DEM on liver cells by estimating levels of serum ALT that is released from damaged liver cells. We found that serum ALT levels did not increase in both wild-type starved and feeding mice injected with 5.3 mmol of DEM/kg of body weight (41.7 and 30.7 IU/L, respectively), but increased in DEM-injected starved HSF1-null mice (69.7 IU/L) (Fig. 2C). Furthermore, ALT level in DEM-injected feeding HSF1-null mice (174.3 IU/L) was much higher than that in starved mice.

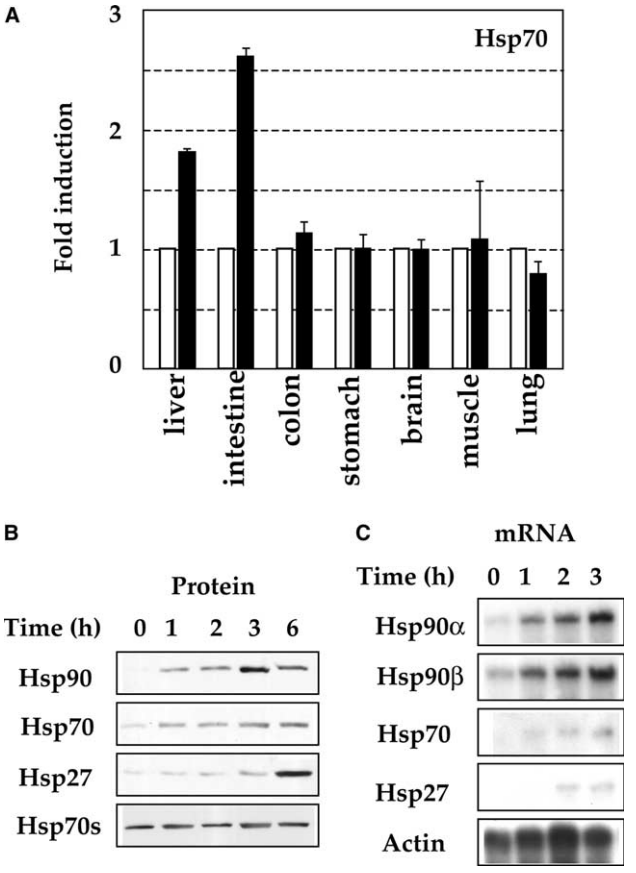

Fig. 1. Feeding induces heat shock response in the liver and intestine. (A) 6-week-old mice were starved for 48 h (open bars) and then fed for 3 h (closed bars). Expression levels of Hsp70 in various tissues were determined by Western blot analysis and were normalized by expression levels of β-actin. Means and S.D. of fold inductions from three independent experiments are shown. (B) Starved mice for 48 h (0) were fed for 1, 2, 3 and 6 h. Expression levels of Hsps in the liver were determined by Western blot analysis using each specific antibody and antibody recognizing Hsp70 and Hsc70 (Hsp70s). Representative data are shown from three experiments. (C) Starved mice for 48 h (0) were fed for 1, 2 and 3 h. Expression levels of Hsps in the liver were determined by Northern blot analysis using each specific cDNA probes. Representative data are shown from three experiments.

These results suggest that HSF1 plays roles in suppressing toxic effects of electrophiles in the liver.

3.3. Electrophiles induce heat shock response

As we found a little induction of Hsps in the liver by injection with 5.3 mmol of DEM/kg of body weight (data not shown), we analyzed the response of cultured HeLa and Jurkat cells to electrophiles. We found that electrophiles, both DEM and CDNB, induced activation of HSF1 and expression of Hsp70 (Fig. 3A and B, data not shown). BSO reduces de novo synthesis of cellular glutathione level (Fig. 3C), and Hsp90, Hsp70, Hsp40 and Hsp27 were induced at lower concentrations of CDNB when the level of glutathione was reduced (Fig. 3D). These results suggest that electrophiles cause oxidative stress that induces heat shock response.

3.4. Hsp27 reduces toxic effects of electrophiles on culture cells

Because Hsp27 expression is markedly induced by the feeding and was too low to be detected in HSF1-null liver

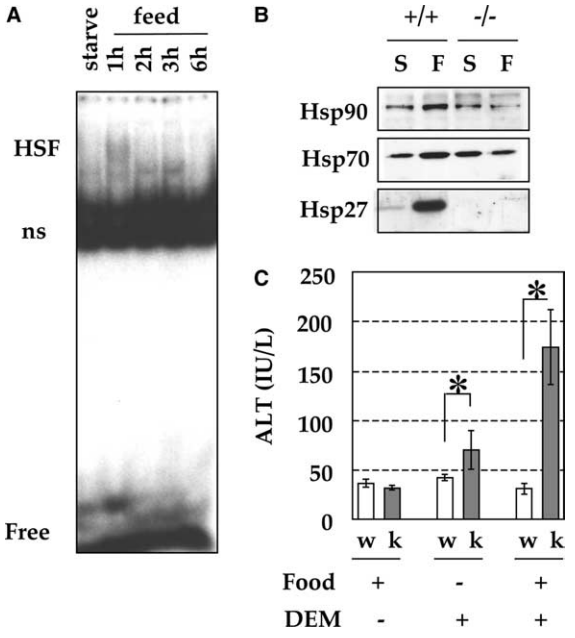

Fig. 2. HSF1 is required for feeding-induced induction of Hsps and protection from an electrophile in the liver. (A) Starved mice for 48 h (starvation) were fed for 1, 2, 3 and 6 h. HSE-binding activities in the liver extracts were determined by gel shift assay using a ^{32}P-labelled HSE-oligonucleotide. HSF, complexes of HSF and HSE-probe; Free, free oligonucleotide; ns, non-specific binding. (B) 6-week-old wild-type (+/+) and HSF1-null (−/−) mice were starved for 48 h (S), and then fed for 6 h (F). Expression levels of Hsp90, Hsp70 and Hsp27 were determined by Western blot analysis. (C) In a starved mouse group (food −), wild-type (w) and HSF1-null (k) mice were starved for 42 h and were injected with DEM. At 6 h after the injection, mice were fed for 24 h and serum ALT levels were determined. In a fed mouse group (food +), mice were starved for 42 h. After they were fed for 6 h, mice were injected with DEM. Serum ALT levels were determined 24 h after the injection. Serum ATL levels were also determined without DEM injection (DEM −). Means and S.D. from three experiments are shown. Stars indicate $P < 0.05$. Representative data are shown from at least three experiments.

(Fig. 2B), we examined whether overexpression of Hsp27 into HeLa cells [14] reduces toxic effects caused by electrophiles. As a control, we also generate HeLa cells overexpressing GST-pi (GST/HeLa), which promotes conjugation of glutathione to chemicals. Glutathione level was increased twofold in Hsp27/HeLa cells. Induction of Hsp70 was observed in response to 40 μM of CDNB in HeLa and GST/HeLa cells, whereas we detect no induction of Hsp70 in Hsp27/HeLa cells even in the presence of 40 μM of CDNB. Overexpression of Hsp27 prevented the induction of HSE-binding activity (data not shown). Furthermore, we found that survival of cells exposed to CDNB was significantly promoted in the presence of Hsp27 (Fig. 4D). These results indicate that induction of Hsp27 reduces toxic effects of electrophiles on culture cells.

4. Discussion

Digestive organs are frequently exposed to xenobiotic chemicals in food. These chemicals are adsorbed in the small intestine and then transported into the liver, where xenobiotic chemicals are mainly detoxified [9,10]. Therefore, it is interesting to show that feeding induces the heat shock response only in the liver and small intestine among digestive organs. Previously, it was reported that feeding induces molecular chaperones located in the endoplasmic reticulum in the liver [19,20]. However, this is the first demonstration that feeding induces a set of cytoplasmic Hsps. Furthermore, we found that feeding induces heat shock response by activating HSF1, which is also activated by heat shock [2]. Although heat shock-induced activation of HSF1 is triggered by denaturation of cellular proteins, it is unlikely that normal food induces denaturation of proteins. HSF1 may be activated through an unknown pathway to cope with unexpected toxicants such as electrophilic agents in the food.

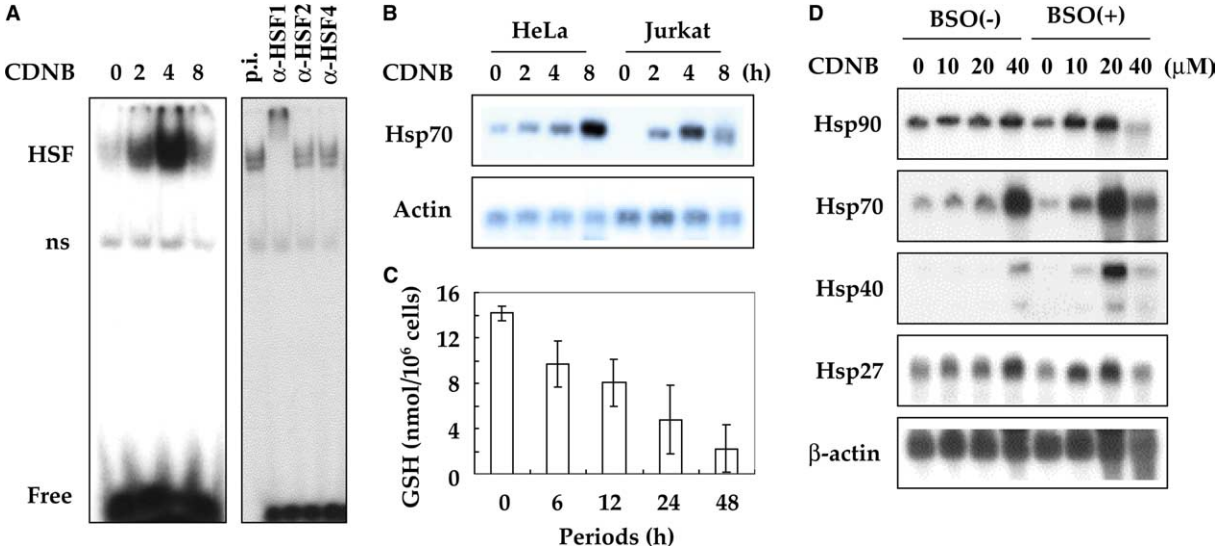

Fig. 3. CDNB induces heat shock response. (A) HeLa cells were treated with 40 μM of CDNB for indicated periods and gel shift assay was performed using a ^{32}P-labelled HSE-oligonucleotide (left). A supershift experiment of extract prepared from cells treated with CDNB for 4 h using preimmune serum (p.i.) or each specific antiserum. (B) HeLa and Jurkat cells were treated with CDNB as described in A. Northern blot analysis was performed using a cDNA probe specific for Hsp70 or β-actin. (C) HeLa cells were treated with 0.1 mM BSO for indicated periods. Levels of GSH were measured. Means and S.D. of three independent experiments are shown. (D) HeLa cells were treated with 0.1 mM BSO for 40 h and further incubated in the presence of 0, 10, 20 or 40 mM CDNB for 8 h. Total RNAs were isolated and Northern blot analysis was performed.

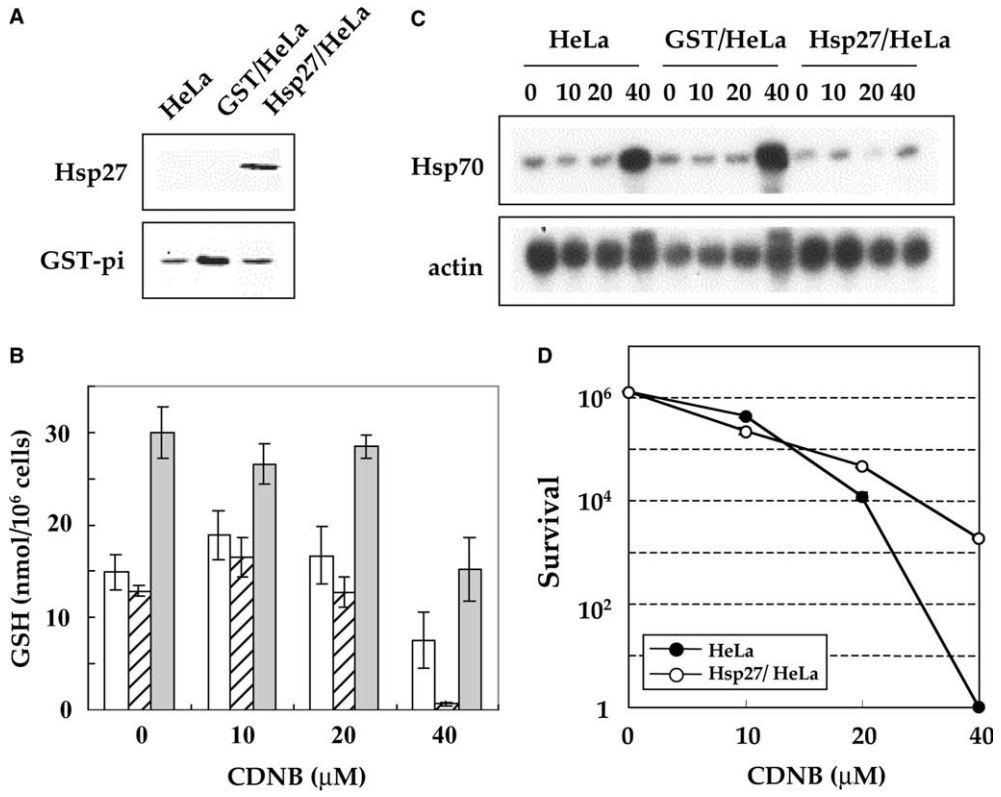

Fig. 4. Hsp27 reduces oxidative stress caused by CDNB. (A) HeLa cells were stably transfected with GST-pi expression vector or Hsp25 expression vector. Levels of Hsp25 and GST-pi were determined by Western blot analysis. (B) Cells were treated with 0, 10, 20 and 40 μM of CDNB for 8 h, and levels of GSH were measured. Means and S.D. of three independent experiments are shown. Open bar, HeLa; hatched bar, GST/HeLa; gray bar, Hsp27/HeLa. (C) Cells treated as described in B were harvested, and total RNAs were isolated from the cells and Northern blot analysis was performed. Representative data are shown from three experiments. (D) HeLa and Hsp27/HeLa cells were incubated in the presence of 0, 10, 20 or 40 μM CDNB for 24 h, and replated in dishes containing normal medium for 10 days. Means of three independent experiments of cell survivals are shown. S.D. are too small to be shown.

Electrophiles cause oxidative stress with rapid reduction of cellular glutathione level [21]. To cope with electrophiles and oxidants, cells induce phase 2 enzymes such as glutathione *S*-transferase and NAD(P)H:quinone oxidoreductase [22,23] by activating Nrf2 transcription factor [24,25]. Induction of these enzymes is accompanied by elevation of intracellular glutathione levels [26,27]. In addition to Nrf2 activation, we showed here that HSF1 is activated by electrophiles. This result might be expected because diamide and menadione, which induce oxidation of protein thiols by rapidly forming GSSG with subsequent GSH depletion, activate HSF1 [28,29]. The oxidation of protein thiols may cause denaturation of proteins, resulting in the formation of a state with similar properties to the thermally denatured state [30]. Here, we directly showed that overexpression of Hsp27 reduces toxic effects on cells, probably by increasing cellular GSH level. Because Hsp27 is a major inducible protein after feeding, it may play significant roles in suppressing toxic effects of xenobiotic chemicals in food.

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