Large Intestine and Heat Shock Protein (HSP)

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Summary All diseases in the large intestine represented by inflammatory bowel disease develop due to destruction of the balance between aggressive factors inducing lesions and defensive factors protecting the mucosa or the body itself. This mechanism is similar to that for the development of gastric lesions. However, compared with gastric disease, the mechanism of large intestinal disease has been studied mainly in terms of aggressive factors inducing mucosal injury but rarely in terms of cytoprotection or mucosal repair. Heat shock protein (HSP) in the large intestine is induced by external stimulation, inflammatory cell infiltration, and stimulation of the intestinal bacterial flora. The induced HSP cytoprotectively acts as a molecular chaperon or by other mechanisms. Therefore, the effective induction of HSP expression has a potentiality for the development of new treatment methods for inflammatory intestinal disease.

Key Words: Heat shock protein, inflammatory bowel disease, cytoprotection, hyperthermia

Introduction

In all organisms from bacteria to humans, heat shock protein (HSP) synthesis is transiently induced in the cells in response to heat stress, and defensively acts on this stress [1]. The history of studies on heat stress and HSP is long, starting in the 1930s. In the 1960s, the exposure of Drosophila larvae to high temperature was reported to induce the formation of a large amount of mRNA of HSP70 and HSP90 in the puff region of the salivary chromosomes [2]. Subsequent studies have shown that HSP genes are stored in the cells of all organisms from bacteria to animals and induced by not only heat stress but also other stresses such as deleterious substances including heavy metals, arsenic, ethanol, reactive oxygen, and amino acid derivatives [3–7]. In this study, we briefly summarize the roles of HSP expressed in the large intestine in the healthy state or pathologic conditions.

Expression of a large amount of HSP

The molecular weight of HSP widely varies from less than 10,000 to 110,000. HSP can be classified into the constitutive and inducible forms. Constitutive HSP is expressed in the cells and plays an important physiological role as a molecular chaperon. The well-known roles of HSP as a molecular chaperon are folding and assembly of new peptides, and its major roles are as follows. (1) HSP binds to proteins immediately after synthesis, preventing misfolding, and dissociates for protein maturation. (2) HSP binds to proteins as potential subunits for the completion of the unit. (3) HSP dissociates from proteins, and other HSPs bind to the proteins for maturation. (4) HSP binds to proteins, transports them to the endoplasmic reticulum, binds to accessory factors such as glucose-regulated protein 78.
heat shock protein (GRP78/BiP), forming the final form of proteins. (5) HSP binds to many mitochondria proteins coded by nuclear genes, transports them to the mitochondria, and dissociates, and the proteins are completed by other HSPs. Concerning inducible HSP, HSP expression induced by stresses such as heat stress is regulated at the transcription level by heat shock factors (HSF) [8]. In the HSF family, HSF-1 has been studied most extensively. HSF-1 is present in the cytoplasm in the absence of stimulation but forms trimers in response to stress, is transferred to the nucleus, binds to the promoter region of the HSP gene, initiating transcription. This promoter region of the HSP gene has a specific sequence called the heat shock element (HSE) upstream from the basic promoter region. The HSE has 5 bp XGAAX as a basic unit and a sequence consisting 3 units or more such as XGAAX-XTTCCX(reverse XGAAX)-XGAAX, and the repeated sequence regulates the intensity of promoter activity [9]. Thus, HSP induced via the HSF-1-HSE system prevents irreversible changes in intracellular proteins (cytoprotection) due to stress as a cause of its induction or other completely different stresses. In the healthy large intestine, HSP is induced in the mucosa by stimuli such as intestinal bacterial flora or fermentation products of digested foods [10–13]. It has been shown that HSP can be upregulated in vitro in intestinal epithelial cell lines by bacterial products [17]. Recently R. Medzhitov et al. [12] reported that the steady-state expression of hsp25 and hsp72 are constitutively induced by commensal products through Toll-Like Receptors. HSP70 and HSP27 are expressed in the mucosal epithelium of the large intestine and interstitial cells but not in the small intestine in the absence of stimulation [14]. HSP60 is expressed in the mucosal epithelium of the large intestine and monocytes [15, 16] while HSP90 is expressed in the mucosal epithelium of the large intestine, monocytes, and polymorphonuclear leukocytes [17]. In general, a large amount of HSP is expressed in the mucosal epithelium in the healthy large intestine (Table 1). These HSPs may have cytoprotection actions. In the healthy large intestinal mucosa, intestinal bacteria and HSP induced by stimulation with fermentation products of digested foods may be important in maintaining the function and structure of the mucosal epithelium of the large intestine and also have defensive actions on damage due to reactive oxygen. This speculation has been confirmed by animal experiments. A study showed decreases in the expressions of HSP70 and HSP25 and an increase in mucosal damage due to Clostridium difficile toxin A in mice in which intestinal bacterial flora was reduced by metronidazole [18, 19]. Interestingly, in contrast to the role of intestinal bacteria on the induction of HSP, H. pylori infection cancelled the expression of HSP70 and HSP27 in gastric mucosa. Though the mechanisms by which H. pylori infection cancelled the expression of HSP and the relationship between H. pylori infection and host HSP response have not been fully clarified, a recent study has shown that H. pylori infection phosphorylated HSP90 [20] and cancelled the induction of HSP70 and HSP27 in gastric mucosa [21] for perpetuating gastric inflammation and disrupting defensive homeostasis.

### Large intestinal inflammation and HSP

Oxidation stress in the large intestine is a cause and an aggravation factor not only of ulcerative colitis and Crohn disease but also of ischemic enteritis, infectious enteritis, and radiation colitis [22–26]. In the local inflammatory area of the large intestine, inflammatory infiltrative cells such as neutrophils produce various reactive oxygen species, which may damage the large intestinal mucosa. Animal experiments have shown that HSP70 and HSP32 inhibit mucosal injury due to reactive oxygen [27]. However, its mechanism remains largely unclear, and further studies are necessary to determine whether HSP induced before stimulation acts only as a molecular chaperon, and also to evaluate the possible involvement of other actions such as the scavenger action of reactive oxygen, anti-apoptosis, or the inhibitory effects of transcription factor nuclear factor-κB (NF-κB), which regulates inflammatory cytokine production.

### Inflammatory bowel disease and HSP

There have been some studies on the HSP expression pattern in the intestinal mucosa of patients with inflammatory bowel disease such as ulcerative colitis and Crohn disease. HSP70 expression is increased in both inflammatory and non-inflammatory areas in patients with ulcerative colitis compared with healthy subjects, but is not increased in Crohn disease [28]. HSP60 expression is increased in lesions of ulcerative colitis and Crohn disease [29, 30]. HSP90 expression is not increased in either disease [17]. (Table 2) Interestingly, the degree of inflammation is not

<table>
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<tr>
<th>Family</th>
<th>type of HSPs</th>
<th>localization</th>
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<tbody>
<tr>
<td>Hsp90</td>
<td>Hsp90</td>
<td>cytosol</td>
</tr>
<tr>
<td></td>
<td>GRP94</td>
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<td>Hsp70</td>
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<td>Hsc73</td>
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<td>Hsp60</td>
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<td>Hsp58</td>
<td>mitochondria</td>
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<td>Hsp47</td>
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<td>Hsp27</td>
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<td>cytosol, nucleus</td>
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<td>Hsp10</td>
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<td></td>
<td>Ubiquitin</td>
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associated with the intensity of HSP expression in either disease [28]. However, this finding does not negate the cytoprotection effects of HSP induction. The absence of their association may be a result of the complicated involvement of various factors such as a complicate spontaneous course with repeated remission/aggravation, treatment contents, a time lag between HPS expression and the subsidence of inflammation. However, the possibility that HSP expression represents the aggravation of pathogenic factors or disease activity can not be excluded. When the living body immunologically responds to HSP expressed in intestinal bacteria, there is a possibility that HSP induced in the mucosa epithelium of the large intestine by these pathogenic bacteria immunologically responds to HSP in the mucosal epithelium of the large intestine due to its high homology with HSP in intestinal bacteria as is observed in autoimmune disease. This possibility has also been suggested in rheumatoid arthritis and SLE [31, 32]. However, there is much controversy regarding this possibility at present [33]. One reason is the inconsistency between the localization pattern of anti-HSP antibody and pathological inflammatory findings. The other reason is no association between quantitative changes in anti-HSP antibody and treatment effects on inflammatory colitis.

**Treatment attempts using HSP for inflammatory bowel disease**

All diseases in the large intestine represented by inflammatory bowel disease and including cancer develop due to destruction of the balance between aggressive factors inducing disease and defensive factors protecting the mucosa or the body itself. This mechanism is similar to that of the development of gastric lesions. However, compared with gastric disease, the mechanism of large intestinal disease has been studied mainly in terms of aggressive factors inducing mucosal injury but rarely in terms of cytoprotection or mucosal repair. This tendency is particularly marked in treatment strategies for inflammatory bowel disease, and treatment methods such as inhibition of the activity of immunocytes including neutrophils or inhibition of inflammatory cytokine production are frequently performed. Of course, the effects of these methods have scientifically been confirmed. However, on the other hand, we consider that further studies on defensive factors are necessary. Therefore, as a treatment strategy with consideration given to both aggressive and defensive factors, we have evaluated HSP induction therapy. Our previous studies have shown that HSP70 and HSP32 inhibit inflammatory cytokine expression in epithelial cells, and heat shock reactions inhibit NF-κB activation by tumor necrosis factor α (TNFα) in MKN45 human gastric cancer cells (Fig. 1), suggesting the involvement of HSP (particularly HSP70) in the mechanism of the inhibition of NF-κB activation. In addition, HSP70 has been reported to inhibit IKK activation, resulting in inhibition of NF-κB activation [34–36]. Therefore, we have attempted to inhibit inflammatory reactions of the living body and protect mucosal epithelial cells by HSP70 induction. In vitro HSP70 induction is performed by heat treatment at 42°C for 60 minutes, and its in vivo induction using a far-infrared ray whole body heating apparatus we developed. Evaluation using human large intestinal cancer cells (HT29) showed inhibition of NF-κB activation by TNFα and a decrease in IL-8 production in HT29 cells after

<table>
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<th>Ulcerative Colitis</th>
<th>Crohn disease</th>
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<tr>
<td><strong>Inflammatory area</strong></td>
<td><strong>Non-Inflammatory area</strong></td>
</tr>
<tr>
<td>HSP70</td>
<td>↑</td>
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Table 2. Expression of HSPs in the patients with UC and Crohn disease (as compared with normal mucosa in healthy volunteer)

Fig. 1. Electrophoretic mobility gel shift assay for the NF-κB binding site. MKN45 cells were preincubated at 37.0°C or 42.0°C for 1 h and stimulated with 100 ng/ml TNF-α for 4 h. Lane 1, untreated MKN45 cells; lane 2, MKN45 cells treated with hyperthermia; lane 3, MKN45 cells treated with TNF-α; lane 4, MKN45 cells treated with hyperthermia plus TNF-α.
Heat treatment followed by addition of TNFα compared with addition of TNFα without heat pretreatment (unpublished data). These results suggest that HSP70 induced by heat treatment has anti-inflammatory effects. Therefore, we evaluated the treatment effects of whole body hyperthermia in rats as a model of inflammatory bowel disease. 2,4,6-Trinitrobenzene Sulfonic Acid (TNBS) enema was performed in rats, and the deep temperature was increased to 42°C, which was maintained for 20 minutes. Enteritis in the rats improved in terms of both macroscopic and pathological findings. These results suggest that HSP induction therapy can be a treatment method for inflammatory bowel disease.

Conclusion

In response to certain stimuli in the large intestine, various proteins are expressed, and each of them has certain functions. Even for HSP, some types of HSP are expressed, and therefore, the clarification of the mechanisms of responses (particularly cytoprotection action) is difficult. As a mechanism of cell responses to external stimulation, the concept of molecular chaperons was proposed in 1989 [35] and has attracted attention since the early 1990s. At present, the importance of molecular chaperons has increasingly been recognized in terms of molecular biology, signal transmission, and clinical medicine. Therefore, research on HSP as a molecular chaperon may be a promising field when evaluating the physiological function of the large intestine and the pathology and treatment of various large intestinal diseases.

References

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