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Preinduced intestinal HSP70 improves visceral hypersensitivity and abnormal intestinal motility in PI-IBS mouse model

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ABSTRACT

Objective: To investigate the impact of the preinduced intestinal heat shock protein 70 (HSP70) on the visceral hypersensitivity and abnormal intestinal motility in a post-infectious irritable bowel syndrome (PI-IBS) mouse model.

Methods: Eighty-four female C57BL/6 mice were randomly assigned to four groups: control group ($n = 21$) and induction + PI-IBS group ($n = 21$), PI-IBS group ($n = 21$) and induction group ($n = 21$). The mice in PI-IBS group were infected *in vivo* with *Trichinella spiralis* by oral administration. The visceral hypersensitivity and intestinal motility were evaluated respectively with abdominal withdrawal reflex and colon transportation test. The intestinal HSP70 protein and mRNA level was measured by Western blot and real-time PCR. Meanwhile, the intestinal proinflammatory cytokines IL-10 and TNF- α level was detected by ELISA.

Results: Compared with their counterparts in PI-IBS group, the animals in the Induction + PI-IBS group show significantly increased intestinal level of HSP70 and obviously ameliorative clinical figures, including abdominal withdrawal reflex score, intestine transportation time and Bristol scores ($P < 0.05$). Meanwhile, the intestinal post-inflammatory cytokines remarkably changed, including increased IL-10 level and decreased TNF- α level ($P < 0.05$).

Conclusions: Intestinal HSP70 may play a potential protective role through improving the imbalance between the intestinal post-inflammatory and anti-inflammatory cytokines in PI-IBS.

1. Introduction

As a kind of clinical syndrome characterized by abdominal pain, discomfort and bloating accompanied with abnormal defecation, the precise pathological mechanism of irritable bowel syndrome (IBS) remains unclear [1–3]. During the last two decades, abundant clinical and experimental research focused on the role of infection and inflammation in the pathogenesis of IBS, called as post-infectious irritable bowel syndrome (PI-IBS) [4–6]. Recently, it was reported that heat shock protein 70

(HSP70) has an unique capability of regulating the protein misfolding, aggregation and serves critical roles in some diseases [7,8]. Thus the aim of the current study is to investigate the potential role of HSP70 in PI-IBS.

2. Materials and methods

Eighty-four female 57BL/6 mice with (6–8) weeks and (13–15) g were purchased from Kunming Institute of Zoology, Chinese Academy of Science. All animals were housed in sterile, pathogen-free, temperature controlled facility on normal 12-h light/dark cycle, and standard diet and water were provided *ad libitum*. The experiment was carried out in accordance with the Chinese guidelines for animal welfare. Experimental protocol was approved by the Animal Care and Use Committee of the Hainan Provincial General Hospital. The animals were randomly assigned into four groups: control group, induction + PI-IBS

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group, PI-IBS group and induction group ($n = 21$ in each group). In each group, 7 mice were sacrificed for the detection of the intestinal HSP70 protein level and 7 mice for that of the intestinal HSP70 mRNA level. The other 7 mice were examined for the visceral hypersensitivity and the intestinal motility. *Trichinella spiralis* (*T. spiralis*) were purchased from Lanzhou Animal Medical Institute.

2.1. Main reagents

Pepsin (Invitrogen Corporation, U.S.A); histochemistry and Western blot agents (Wuhan Boster Corporation, China); antibodies (BDBioscience Co., U.S.A).

2.2. Modeling of PI-IBS

The mice were infected with *T. spiralis* as described previously [9]. Briefly, the *Trichinella spiralis* larvae were separated from Sprague–Dawley rats 60 d after infection of *Trichinella spiralis*'s cyst by digestion with 1.5% gastric pepsin. The mice were fed with the larvae in 0.2 mL saline (300 larvae per mouse). The animals in the control group were fed with only 0.9% saline.

2.3. Abdominal withdrawal reflex (AWR)

AWR was used to evaluate the visceral hypersensitivity [10]. The anesthetized animals were inserted via their anus with air chamber and catheter. The air chamber was distended at volume of 0.25/0.5/0.75 mL \times 15 min \times 3 times. Between each distending time, the animals were permitted to have a rest for 30 s. The AWR scoring standard: when stimulated, the animals are in stable mood, 0 point; if the animals are in unstable mood, twisting their heads once in a while, 1 point; slightly contracting their abdomen and back muscles, 2 points; intensively contracting their abdomen muscles and uplifting the abdomen from the ground, 3 points; intensively contracting abdomen muscles, bowing abdomen and uplifting the abdomen and perineum, 4 points.

2.4. Colon transportation test

Colon transportation test was used to evaluate the status of the intestinal motility. After filled into stomach with 0.4 mL active carbon, the first black stool time was recorded. The total stool within 8 h was collected and evaluated by Bristol stool grade [11]: normal shaped stool, 1 point; soft or deformed stool, 2 points; water-like stool, 3 points.

2.5. Preinduction of HSP70

Expression of HSP70 in mice was induced by heat treatment according to previous reports [12]. Briefly, mice were anesthetized with sodium pentobarbital (50 mg/kg). Rectal temperature was monitored with a thermistor inserted into the rectum in a baking oven with constant temperature 50 °C. After the body temperature was maintained at 41 °C for 20 min, the mice were return to their cages at room temperature and allowed water and food *ad libitum*. Nonheated mice were only anesthetized but received no hyperthermic stress.

2.6. Determination of intestinal HSP70

HSP70 protein level and mRNA expression was measured by Western blot and real-time PCR respectively. The tissue sample was grinded and cracked with RIPA. The homogenate was centrifuged for 30 min. The protein concentration in the supernatants was measured by Bradford Assay. Tissue sample of 40 g was separated by SDS page gel electrophoresis and transferred to the PVDF membrane. The membrane was blotted with TBST for 1 h, then was added with goat-anti-mouse HSP70 multiple clone antibodies (1:1 000) and rabbit-anti-mouse β -actin multiple clone antibodies (1:1 000) at 4 °C for 12 h. One day later, the membrane was washed in TBST and autographed by ECL chemiluminescent assay. The gray-scale value of HSP70/ β -actin represented the relative expression level of HSP70.

Total RNA was isolated from the terminal ileum tissue with Trizol liquid and treated with DNAaseI. Primer was designed according to mouse gene sequence. β -actin was used as an internal control.

HSP70 gene primer: F: 5'-GAAGGTGCTGGACAAGTGC-3' [(1903–1921) bp], R: 5'-GCCAGCAGAGGCCTCTAATC-3' (2120–2139) bp]. β -actin gene primer (470 bp): F: 5'-AGGCTGTGCTGTCCCTGTATG-3', 5'-GAGGTCTTTACG-GATGTCAACG-3'.

Real-time PCR was operated with following protocol: 1. Pre-denaturation program (5 min at 94 °C); 2. Denaturation program (1 min at 94 °C); 3. Amplification and qualification program, repeated 30 cycles (50 s at 57 °C, 20 s at 60 °C); 4. Prolonging program (7 min at 72 °C). The relative expression was expressed as a ratio of the target gene to the control gene.

2.7. Determination of proinflammatory cytokines

The tissue sample was ultrasonically shivered and centrifuged at 4 °C. The concentration of cytokines IL-10 and TNF- α in the supernatants was measured by ELISA.

2.8. Statistics analysis

Data were analyzed using Student's *t*-test (SPSS 17.0 software). Data were expressed as the mean \pm SE. Values in the same row with different superscripts are significant ($P < 0.05$), while values with same superscripts are not significantly different ($P > 0.05$).

3. Results

3.1. Expression of intestinal HSP70

Western blot and RT-PCR show that the intestinal HSP70 protein and mRNA level in Induction + PI-IBS mice was far more than that in PI-IBS mice ($P < 0.05$) (Table 1).

Table 1

HSP70 protein and mRNA level in heat pretreated PI-IBS mice ($n = 7$).

Group ($n = 7$)	Protein level	mRNA level
Control	0.27 \pm 0.04	0.44 \pm 0.04
PI-IB	0.66 \pm 0.04	0.76 \pm 0.05
Induction + PI-IBS	1.03 \pm 0.06 ^a	1.22 \pm 0.10 ^b
Induction	0.33 \pm 0.07	0.57 \pm 0.06

^a Compared with the control group, $P < 0.05$. ^b Compared with the PI-IBS group, $P < 0.05$.

Table 2

AWR score in heat pretreated PI-IBS mice.

Group (n = 7)	Distending air volume (mL)			
	0.25	0.35	0.5	0.65
Control	0.00 ± 0.00	1.43 ± 0.53	2.57 ± 0.53	3.57 ± 0.53
PI-IBS	0.00 ± 0.00	2.57 ± 0.53	3.57 ± 0.53	3.71 ± 0.49
Induction + PI-IBS	0.00 ± 0.00	2.14 ± 0.38 ^a	2.71 ± 0.49 ^b	3.86 ± 0.38
Induction	0.00 ± 0.00	1.14 ± 0.69	1.86 ± 0.69	3.71 ± 0.49

^a Compared with the PI-IBS group, $P < 0.05$. ^b Compared with the PI-IBS group, $P < 0.05$.

Table 3

Changes of the intestinal mobility in heat pretreated PI-IBS mouse.

Group	First black stool time (min)	Bristol stool grade
Control (n = 7)	104.30 ± 11.05	0.97 ± 0.10
PI-IBS (n = 7)	67.97 ± 5.94	2.76 ± 0.42
Induction + PI-IBS (n = 7)	86.74 ± 5.90 ^a	2.07 ± 0.19 ^b
Induction (n = 7)	101.42 ± 12.38	1.05 ± 0.12

^a Compared with the PI-IBS group, $P < 0.05$. ^b Compared with the PI-IBS group, $P < 0.05$.

3.2. AWR

At the distending air volume of 0.25 mL and 0.65 mL, there was no significant difference between the AWR score of the Induction + PI-IBS mice and the PI-IBS mice ($P > 0.05$); but at the distending air volume of 0.35 mL and 0.5 mL, the AWR score of Induction + PI-IBS mice was obviously lower than that of PI-IBS mice ($P < 0.05$) (Table 2).

3.3. Intestinal motility

The colon transport time of the mice in Induction + PI-IBS group was significantly longer than that of the mice in the PI-IBS group ($P < 0.05$); the Bristol score of the 8 h stool from the PI-IBS mice was obviously less than their counterparts in Induction + PI-IBS group ($P < 0.05$) (Table 3).

3.4. Expression of intestinal proinflammatory cytokines

The intestinal post-inflammatory cytokines remarkably changed, with decreased IL-10 level and increased TNF- α level ($P < 0.05$) (Figure 1 and Figure 2).

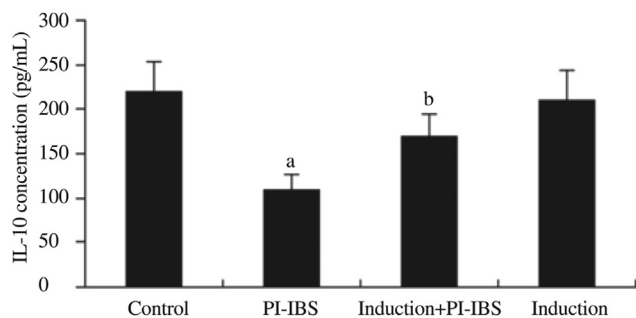


Figure 1. Intestinal IL-10 level in heat-pretreated PI-IBS mouse. a: compared with the control group, $P < 0.05$; b: compared with the PI-IBS group, $P < 0.05$.

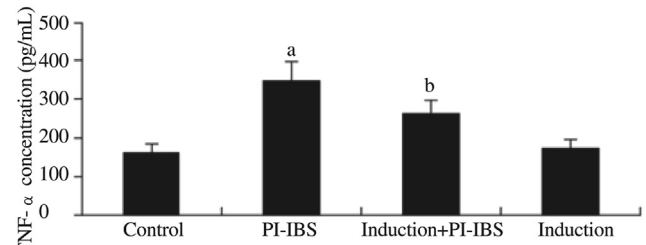


Figure 2. Intestinal TNF- α level in heat-pretreated PI-IBS mouse. a: compared with the control group, $P < 0.05$; b: compared with the PI-IBS group, $P < 0.05$.

4. Discussion

With high incidence rate and refractory history, IBS seriously impact the patients' life quality, exerting a huge burden on the patients. There is no effective therapy for this kind of disease up to now [13].

It has been proved that IBS results from a synergy of multiple etiological factors including the visceral hypersensitivity, the abnormal intestinal motility, local immune response and social-psychological factors [14–17]. Recently, researches mainly focus on two aspects: the neuropeptides induced visceral hypersensitivity and abnormal intestinal motility, the low grade intestinal inflammation and continuous immune activating process. The concept of PI-IBS was considered as concurs [6,18,19]. The current study was to investigate the role of the intestinal mucosa immune system using a *T. spiralis* infected mice model.

HSP70 is a kind of chaperons whose structures remain unchanged, structurally expressed or induced by heat shock, trauma and other stress stimulation. HSP70 could help the essential proteins to refold their structure, whose folding structure were changed by the stress and inflammatory injury, thus fixed the lesion [17]. Furthermore, the inflammatory cells expressing HSP70 could be recognized and eradicated by immunocytes [18,19]. PI-IBS is regarded as a disorder induced by the intestinal infection and inflammation. Our previous study found that the intestinal HSP70 level increased during PI-IBS. Thus HSP70 could exert a negative modulating role in PI-IBS. In the current study, heat pre-treatment significantly increased the intestinal HSP70 level in normal mice, but did not induced viceroy hypersensitivity and abnormal intestinal motility. Interestingly, heat pre-treatment up-regulated the increasing HSP70 level in PI-IBS mice. Simultaneously, viceroy hypersensitivity and abnormal intestinal motility in PI-IBS were improved after heat pretreatment. These results suggest that HSP70 could protect mice against PI-IBS. On the other hand, heat pre-treatment could not wholly reverse the clinical symptoms, suggesting that there could be other pathway through which PI-IBS occurred.

Furthermore, the inflammatory cytokines level was detected to explain how HSP70 exerted its impact on the intestinal inflammation during PI-IBS. IL-10 was a kind of cytokine inhibiting inflammation [20]. TNF- α was considered as an important pro-inflammatory cytokines in some intestinal inflammatory diseases [21]. We found that during PI-IBS, the IL-10 level decreased and the TNF- α increased significantly. Whereas preinduction of HSP70 obviously improved the changes of these cytokines. These results suggest that HSP70 could exert its protective role via pro-inflammatory cytokines pathway.

In conclusion, our results indicated that preinduction of HSP70 could protect the mice against PI-IBS via improving the

imbalance of the intestinal pro-inflammatory cytokines. Our finding could help to optimize the treatment for PI-IBS, although the precise underlying cellular and molecular mechanism of the regulation remains to be explored.

Conflict of interest statement

We declare that we have no conflict of interest.

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