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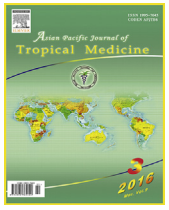

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Preinduced intestinal HSP70 improves visceral hypersensitivity and abnormal intestinal motility in PI-IBS mouse model

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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 spirali lavace were separated from Sprague–Dawley rats 60 d after infection of Trichinella spirali's cryst by digestion with 1.5% gastric pepsin. The mice were fed with the lavarce in 0.2 mL saline (300 lavarce 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 × 15 min × 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 autographied 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′ [(1 903–1 921) bp, R: 5′-GCCAGCAGAGGCCTCTAATC-3′ (2 120–2 139) bp]. *β-actin* gene primer (470 bp): F: 5′-AGGCTGTGCTGTCCCTGTATG-3′, 5′-GAGGTCTTTACG-GATGTCAACG-3′.

Real-time PCR was operated with following protocol: 1. Predenaturation 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 ± 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 ± 0.04	0.44 ± 0.04
PI-IB	0.66 ± 0.04	0.76 ± 0.05
Induction + PI-IBS	1.03 ± 0.06[a]	1.22 ± 0.10[b]
Induction	0.33 ± 0.07	0.57 ± 0.06

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