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ORIGINAL ARTICLE

Preinduction of heat shock protein 70 protects mice against post-infection irritable bowel syndrome via NF-κB and NOS/NO signaling pathways

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Abstract This study aimed to investigate the protective effects of preinduction of heat shock protein 70 (HSP70) on Trichinella spiralis infection-induced post-infectious irritable bowel syndrome (PI-IBS) in mice. Trichinella spiralis infection significantly reduced HSP70 abundance, ileal villus height and crypt depth, expression of tight junctions, serum lysine and arginine concentrations, and ileal SCL7A6 and SCL7A7 mRNA levels, induced inflammatory response, and activated NF-κB signaling pathway. Meanwhile, the heat treatment upregulated HSP70 expression, and then reversed intestinal dysfunction and inflammatory response. Preinduction of HSP70 enhanced serum arginine and intestinal SCL7A7 expression and inhibited NF-κB activation compared with PI-IBS model. Treatment with pyrrolidine dithiocarbamate (PDTC, an NF-κB inhibitor) and *N*-nitro-L-arginine methyl ester hydrochloride (L-NAME, a nitric oxide synthase inhibitor, NOS) further demonstrated that preinduction of HSP70 might inhibit NF-κB and activated NOS/nitric oxide (NO) signaling pathways. In conclusion, preinduction of HSP70 by heat treatment may confer beneficial effects on Trichinella spiralis infection-induced PI-IBS in mice, and the protective effect of HSP70 may be associated with inhibition of NF-κB and stimulation of NOS/NO signaling pathways.

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Introduction

Irritable bowel syndrome (IBS) is a highly prevalent gastrointestinal disease characterized by abdominal pain, discomfort and bloating (Vaiopoulou et al. 2014). 3.7–36 % IBS patients develop into post-infectious IBS (PI-IBS) due to acute gastrointestinal infection (Longstreth et al. 2006; Wang et al. 2014a). The pathological mechanism is still obscure, while recent evidences suggested that low-grade inflammation and increased permeability in the intestine associate with the development of PI-IBS (Spiller and Garsed 2009; Wang et al. 2014a). Intestine is the main site for amino acids absorption and metabolism and functional amino acids play important roles as metabolic intermediates in nutrition, immune response, inflammation, signaling transduction, and protein synthesis (Wu et al. 2014; Yin et al. 2015c). However, the amino acids' absorption and metabolism in PI-IBS model are still unclear.

The HSP70 family of heat shock proteins consists of molecular chaperones of approximately 70 kDa in size that serve critical roles in survival function in the cell (Murphy 2013). Compelling evidences have shown that preinduction of HSP70 by heat stress has various beneficial effects in different pathological injury (Dong et al. 2013; Fleming et al. 2002; Lee et al. 2014a; Yang et al. 2009). Meanwhile, overexpression or recombinant HSP70 exhibits an anti-inflammatory property in various pathological models (Doeppner et al. 2013; Vinokurov et al. 2012). However, the effects of HSP70 on PI-IBS have not been reported. Thus, the aim of this study was to investigate the protective

role of preinduction of HSP70 in Trichinella spiralis infection-induced PI-IBS in mice.

Methods and materials

Animals

Forty female C57BL/6 (B6) mice (12–14 weeks old) were purchased from Kunming Institute of Zoology, Chinese Academy of Sciences (China). All animals were housed in a sterile, pathogen-free, temperature controlled facility on a 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 First Affiliated Hospital of Chongqing Medical University.

Study design

Firstly, 40 mice were included to investigate the protective role of preinduction of HSP70 on PI-IBS mice. Mice were randomly divided into four groups ($n = 10$): a control group, a PI-IBS group (PI-IBS), an HSP70 preinduction group (HSP70), and a PI-IBS + HSP70 preinduction group (PI-HSP). To induce PI-IBS model, mice were infected with Trichinella spiralis larvae (260–300 larvae per mouse) by oral gavage (0.2 ml in 0.9 % saline) according to previous report (Wang et al. 2014b). Sham-infected animals were administrated 0.9 % saline. Before slaughter, blood was collected from eyes in mice. Ileal samples were harvested at day 14 infection according to our previous study (unpublished data).

In addition, we used pyrrolidine dithiocarbamate (PDTC, NF-κB inhibitor; obtained from Sigma Bio. Tech.) and *N*-nitro-L-arginine methyl ester hydrochloride (L-NAME, NOS inhibitor; obtained from Sigma Bio.Tech.) to clarify the role of NF-κB and NOS activity in the protective role of HSP70 in PI-IBS model. 60 mice were randomly divided into six groups ($n = 10$): a PI-IBS model group (PI-IBS), a PI-IBS + HSP70 preinduction group (PI-HSP), a PI-IBS + PDTC treatment group (PDTC), a PI-IBS + HSP70 preinduction + PDTC group (HSP-PDTC), a PI-IBS + L-NAME treatment group (NAME), a PI-IBS + HSP70 preinduction + L-NAME treatment group (HSP-NAME). PDTC (Sigma), dissolved in distilled water, was administered intraperitoneally to mice at dose levels of 50 mg/kg every day in the morning (Zhai et al. 2012). L-NAME (90 mg/kg of body weight) was administered with drinking water during the whole experimental period (Ocsan et al. 2013). Before slaughter, blood was collected from eyes in mice. Ileal samples were harvested at day 14 infection.

Preinduction of HSP70

Expression of HSP70 in mice was induced by heat treatment according to previous reports (Mizushima et al. 2000). 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 centigrade. 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 as libitum. Nonheated mice were only anesthetized but received no hyperthermic stress.

Morphological analyses

For light microscopic observation, the ileal tissues were fixed with 10 % formalin in PBS at 4 °C, dehydrated in a graded series of ethanol, and then embedded in paraffin wax. The tissues were sectioned at 5 μm thick and mounted on slides. They were dewaxed, hydrated, and then stained with Hematoxylin–Eosin (HE). Villus height and crypt depth were measured using an image-analysis system. The HE staining was further used for evaluating inflammatory score basing previous scoring system (Suzuki et al. 2005).

Serum diamine oxidase activity, lipopolysaccharide, amino acid profile, and NO

Serum samples were separated from blood by centrifugation at $3500\times g$ for 15 min. Serum diamine oxidase (DAO) activity, lipopolysaccharide (LPS), and nitric oxide NO concentration were measured using assay kits in accordance with the manufacturer's instructions (BioVision Inc., USA). NO was measured as released NO metabolites (nitrates and nitrites). Amino acids in serum were determined by LC–MS/MS (HPLC Ultimate 3000 and 3200 QTRAP LC–MS/MS) using standards from Sigma Chemicals (St. Louis, MO, USA) according to previous report (Yin et al. 2014).

Histomorphometry determination

Samples from ileal middle section (3 cm) were kept in 4 % neutral buffered 10 % formalin for H&E staining. Villus height and crypt depth were measured using an image-analysis system (Yin et al. 2014).

Ileal NF-κB and NOS activity

Ileal samples were homogenized (1 g tissue in 9 mL saline) and then centrifuged at $3500\times g$ for 15 min. The supernatant was collected. Ileal NF-κB activity was measured using an ELISA kit according to the manufacturer's

instructions (Shanghai Yaji Bio. Tech., China). Ileal NOS activity was detected using an ELISA kit according to the manufacturer's instructions (Shanghai Meilian Bio. Tech., China).

Western blot

Western blot analysis was conducted according to a previous study (Yin et al. 2013b). Briefly, the equal amounts of proteins obtained from cytoplasmic or nuclear fractions were separated by a reducing SDS-PAGE electrophoresis. Cytoplasmic and nuclear protein extraction used nuclear and cytoplasmic extraction reagents in accordance with the manufacturer's instructions (Thermo Fisher Scientific Inc., Waltham, MA, USA). The proteins were transferred onto PVDF membranes (Millipore, MA, USA) and blocked with 5 % non-fat milk in Tris-Tween buffered saline buffer (20 mM Tris, pH 7.5,150 mM NaCl, and 0.1 % Tween-20) for 3 h. The primary antibodies HSP70 (ab2787), ZO1 (ab61357), occludin (ab168957), claudin1 (ab15098), NF-κBp65 (ab86299), and inducible nitric oxide synthase (iNOS, ab129372) were obtained from Abcam Bio. (USA) and incubated overnight at 4 °C; the HRP-conjugated secondary antibodies were subsequently incubated for 1 h at room temperature before developing the blots using Alpha Imager 2200 software (Alpha Innotech Corporation, CA, USA). We digitally quantified the resultant signals and normalized the data to the proliferating cell nuclear antigen (PNCA, ab29) or actin (ab8226) abundance. PCNA or actin was used as an internal loading control for nuclear and cytoplasmic protein fractions, respectively.

Real-time quantitative (RT-PCR)

Total RNA was isolated from liquid nitrogen frozen and ground ileum with TRIZOL regent (Invitrogen, USA) and then treated with DNase I (Invitrogen, USA) according to the manufacturer's instructions. Primers (Table 1) were designed with Primer 5.0 according to mouse gene sequence. β-actin was used as an internal control to normalize target gene transcript levels. Real-time PCR was performed according to the following steps. Briefly, 1 μl cDNA template was added to a total volume of 10 μl containing 5 μl SYBR Green mix, 0.2 μl Rox, 3 μl ddH2O, and 0.4 μl each of forward and reverse primers. We used the following protocol: (1) pre-denaturation program (10 s at 95 °C); (2) amplification and quantification program, repeated 40 cycles (5 s at 95 °C, 20 s at 60 °C); (3) melting curve program (60–99 °C with a heating rate of 0.1 °C/s and fluorescence measurement). The relative expression was expressed as a ratio of the target gene to the control gene using the formula $2^{-(\Delta\Delta Ct)}$, where $\Delta\Delta Ct = (Ct_{Target} - Ct_{\beta\text{-actin}})_{treatment} - (Ct_{Target} - Ct_{\beta\text{-actin}})_{control}$. Relative expression was normalized and expressed as a ratio to the expression in the control group.

Table 1 Primers used in this study

Gene	Primer squence (5–3′)
β-Actin	F: GTCCACCTTCCAGCAGATGT
	R: GAAAGGGTGTAAAACGCAGC
IL-1β	F: ATGAAAGACGGCACACCCAC
	R: GCTTGTGCTCTGCTTGTGAG
IL-10	F: ACAGCCGGGAAGACAATAAC
	R: CAGCTGGTCCTTTGTTTGAAAG
IL-17	F: TACCTCAACCGTTCCACGTC
	R: TTTCCCTCCGCATTGACAC
IFN-γ	F: AAATCCTGCAGAGCCAGATTAT
	R: GCTGTTGCTGAAGAAGGTAGTA
SCL7A6	F: TACATCCTGACCAACGTGGC
	R: ATGCCGAATGTCTGGTCAGC
SCL7A7	F: ATCTTCCTGGTGGCTGTTC
	R: TGCTCTGGCACTCTGATGA
SCL7A9	F: AGCCTCCTGCTGTGGTAGTG
	R:GCTGCCGTGAAGACATTC
SCL7A8	F: GGTGGAGGCGATCTGTTTCA
	R: AGTGCGCTCCTACCCTCTAA
SCL6A14	F: GGGTCACTTTGGGAGGTAGC
	R: TCGGGCACTTCAATCTGTCC
SCL6A20	F: GTCATCAACAGCTCCACCTC
	R: ATGGCCGCTGTATTTCCAAG

Statistical analysis

All data were analyzed using Grubbs' test and then performed using the one-way analysis of variance (ANOVA) to test homogeneity of variances via Levene's test and followed with Ducan's multiple comparison test (SPSS 17.0 software). Data are expressed as the mean ± standard error of the mean. Values in the same row with different superscripts are significant ($P < 0.05$), while values with same superscripts are not significantly different ($P > 0.05$).

Results

The effect of heat treatment on HSP70 expression

Upregulation of HSP70 was induced by heat treatment according to previous report (Mizushima et al. 2000). Thus, the primary focus was to monitor the protein abundance of HSP70 after heat treatment. As shown in Fig. 1, ileal HSP70 abundance was significantly higher in HSP70 group than that in control group ($P < 0.05$), suggesting that preinduction of heat upregulates HSP70 expression. Meanwhile, Trichinella spiralis infection in IP-IBS group markedly inhibited ileal HSP70 expression ($P < 0.05$), while

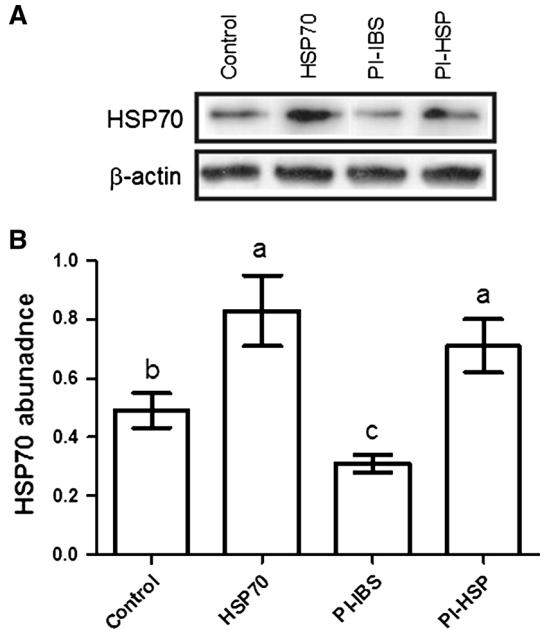

Fig. 1 HSP70 expression in four groups. Data are presented as mean ± SEM, $n = 5$. *a–c* For each variable, means with *different letters* differ ($P < 0.05$)

preinduction of heat reversed the HSP70 expression in IP-HSP group ($P < 0.05$).

Morphological analyses

The results of macroscopic observations of ileal morphology are shown in Fig. 2a, Compared with control group and HSP70 group, the villus was scattered and desquamated seriously in the ileum after Trichinella spiralis infection. We further investigated the ileal villus height and crypt depth and found that ileal villus height and crypt depth were significantly lower in IP-IBS model group compared with the control and HSP70 groups ($P < 0.05$) (Fig. 2b). Preinduction of HSP70 tended to alleviate the effects of Trichinella spiralis infection on ileal villus height and crypt depth, but the difference was insignificant ($P > 0.05$). Furthermore, we evaluated the inflammatory scores from the HE staining according to previous reports. In the present study, Trichinella spiralis infection in PI-IBS group significantly increased inflammatory scores compared with the control and HSP70 groups ($P < 0.05$), while preinduction of HSP70 in PI-HSP group markedly reversed inflammatory scores compared with PI-IBS group ($P < 0.05$) (Fig. 2c).

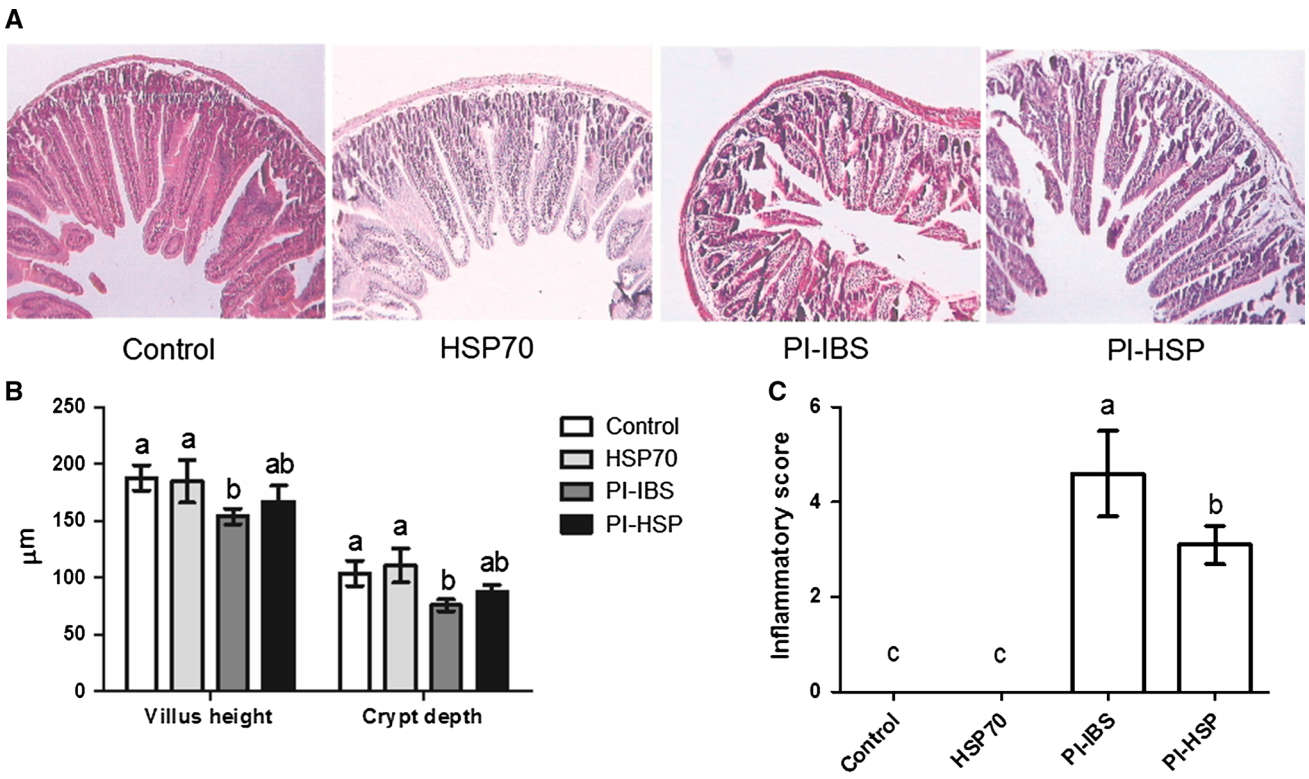

Fig. 2 Effects of preinduction of HSP70 on ileal morphology (HE × 400) and ileal inflammatory scores after exposure to Trichinella spiralis infection. Data are presented as mean ± SEM, $n = 5$. *a–c* For each variable, means with *different letters* differ ($P < 0.05$)

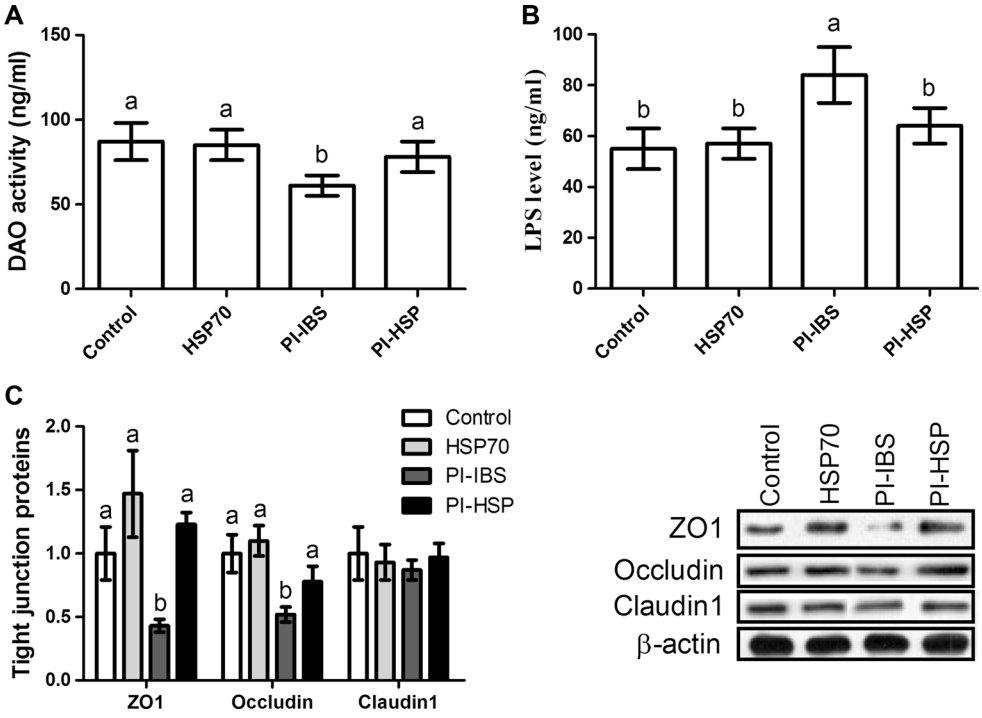

Fig. 3 Effects of preinduction of HSP70 on intestinal permeability and ileal expression of tight junctions after exposure to Trichinella spiralis infection. Data are presented as mean ± SEM, $n = 5$. *a–c* For each variable, means with *different letters* differ ($P < 0.05$)

Intestinal permeability and tight junction expression

We next measured intestinal permeability and ileal expression of tight junctions, including ZO1, occludin, and claudin1. Serum DAO activity and LPS level are two major makers for intestinal permeability (Yin et al. 2015a); in the present study, we found that Trichinella spiralis infection in PI-IBS group significantly lowered serum DAO activity and enhanced LPS concentration compared with the control and HSP70 groups ($P < 0.05$). Meanwhile, preinduction of HSP70 significantly reversed the effects of Trichinella spiralis infection on intestinal permeability evidenced by the increased DAO activity and reduced LPS levels ($P < 0.05$) (Fig. 3).

Tight junctions play a key role in maintaining the homeostasis in gastrointestinal tract. In the present study, tight junctions (i.e., ZO1, occludin1, and claudin1) were determined by western blot. The results showed that Trichinella spiralis infection in PI-IBS group significantly reduced ZO1 and occludin1 abundances and preinduction of HSP70 markedly enhanced ZO1 and occludin1 expressions ($P < 0.05$) (Fig. 3).

Proinflammatory cytokines

Expression of proinflammatory cytokines is shown in Fig. 4. Trichinella spiralis infection markedly enhanced IL-10, IL-17, and IFN-γ mRNA levels in ileum ($P < 0.05$). Preinduction of HSP70 reduced IL-10 and IL-17 expression compared with PI-IBS model group ($P < 0.05$) but failed to inhibit the overexpression of IFN-γ($P > 0.05$).

Serum amino acid profile and amino acid transporters

After Trichinella spiralis infection, serum amino acid concentrations in mice are summarized in Table 2. Although most serum amino acids were not changed ($P > 0.05$), lysine and arginine were significantly lower in PI-IBS model group compared with the control and HSP70 groups ($P < 0.05$), and preinduction of HSP70 markedly increased serum arginine level after exposure to Trichinella spiralis ($P < 0.05$).

Intestinal amino acid transporters mainly contribute to amino acids absorption. Thus, we further investigated several amino acid transporters, including SLC7A1, SCL7A7, SLC7A9, SLC7A10, SLC1A5, and SLC38A1 (Fig. 5). The results showed that Trichinella spiralis infection significantly reduced SCL7A6 (0.52-fold) and SCL7A7 (0.43-fold) mRNA levels compared with control group ($P < 0.05$). Although preinduction of HSP70 failed to increase SCL7A6 expression, SCL7A7 mRNA abundance was significantly higher than that in PI-IBS model group ($P < 0.05$).

NF-κB

NF-κB signaling pathway is involved in various inflammations. So, we measured NF-κB activity by ELISA kits and western blot (Fig. 6a, b). The ELISA kits revealed that Trichinella spiralis infection significantly increased ileal NF-κB activity and preinduction of HSP70 inhibited NF-κB activity ($P < 0.05$). The western blotting analysis indicated that Trichinella spiralis infection markedly

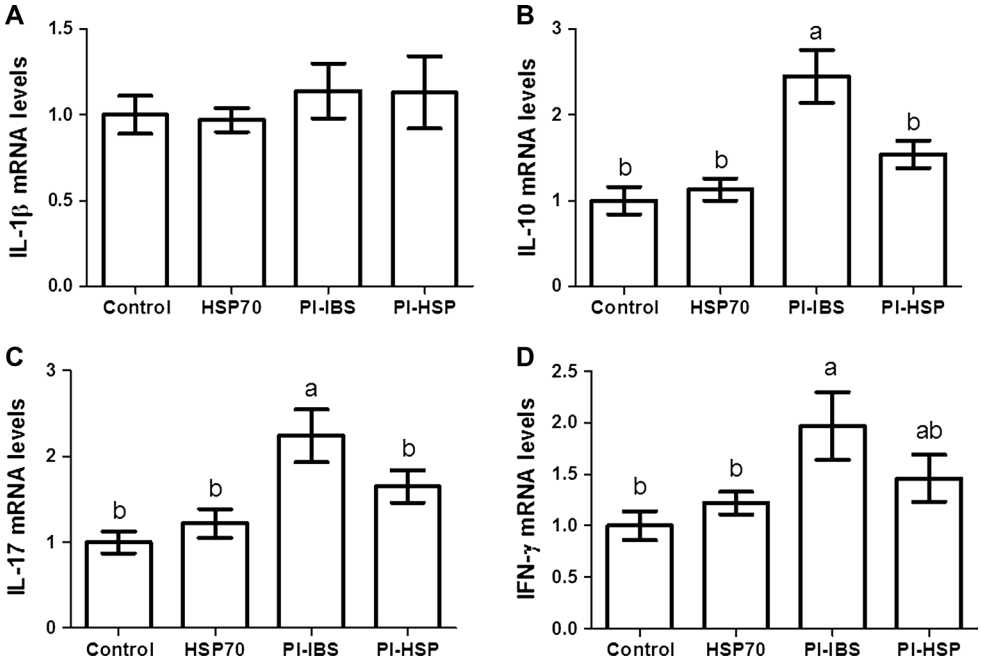

Fig. 4 Effects of preinduction of HSP70 on expression of ileal proinflammatory cytokines after exposure to Trichinella spiralis infection. Data are presented as mean ± SEM, $n = 5$. *a–c* For each variable, means with *different letters* differ ($P < 0.05$)

Table 2 Effects of preinduction of HSP70 on serum amino acids profiles (mg/L) after exposure to Trichinella spiralis infection

Item	Control	HSP70	PI-IBS	PI-HSP
Aspartate	154.78 ± 16.87	161.43 ± 13.43	156.42 ± 15.58	163.87 ± 19.26
Threonine	612.13 ± 31.19	569.00 ± 95.32	593.88 ± 58.23	547.99 ± 90.53
Serine	187.65 ± 23.75	199.56 ± 17.53	212.00 ± 20.31	201.59 ± 13.12
Glutamate	821.79 ± 45.15	783.63 ± 43.37	832.23 ± 74.85	767.58 ± 38.95
Glycine	1453.84 ± 74.12	1345.86 ± 148.54	1482.86 ± 162.42	1398.69 ± 85.12
Alanine	952.77 ± 30.34	976.18 ± 92.57	1032.52 ± 93.76	977.15 ± 144.32
Cysteine	45.35 ± 2.76	49.34 ± 3.34	47.55 ± 4.38	45.26 ± 6.22
Valine	165.19 ± 23.23	174.78 ± 16.76	195.52 ± 28.34	146.80 ± 29.76
Methionine	65.57 ± 9.32	51.19 ± 7.65	76.89 ± 11.32	60.78 ± 2.12
Isoleucine	100.83 ± 8.12	113.14 ± 8.97	112.55 ± 7.34	113.66 ± 11.56
Leucine	214.20 ± 13.76	244.83 ± 10.43	261.60 ± 24.34	231.67 ± 15.12
Tyrosine	64.49 ± 9.12	47.92 ± 5.44	58.77 ± 5.65	44.25 ± 4.65
Lysine	376.52 ± 56.37^{a}	399.02 ± 49.12^{a}	229.18 ± 55.23^{b}	312.37 ± 28.82^{ab}
Histidine	92.60 ± 12.51	83.18 ± 6.54	109.84 ± 11.89	101.12 ± 8.86
Arginine	189.58 ± 17.12^{a}	205.50 ± 15.34^{a}	103.23 ± 19.34^{b}	168.10 ± 27.65^{a}
Proline	343.12 ± 19.23	331.23 ± 34.22	421.34 ± 37.12	306.43 ± 33.24

Data are presented as mean ± SEM, $n = 5$. For each variable, means with different letters differ ($P < 0.05$)

enhanced nuclear NF-κBp65 protein and preinduction of HSP70 reduced nuclear NF-κB abundance ($P < 0.05$). Thus, the current study demonstrated that NF-κB signaling pathway involves in the protective mechanism of HSP70 in PI-IBS model.

Furthermore, we used PDTC, an NF-κB inhibitor, to clarify the role of native NF-κB activity in the PI-IBS model. The results showed that preinduction of HSP70 exhibited a similar function with NF-κB inhibitor and significantly inhibited NF-κB activity ($P < 0.05$) (Fig. 6c). Furthermore, the combination of HSP70 induction and PDTC largely reduced NF-κB activity compared with other groups ($P < 0.05$). We further investigated the ileal expression of cytokines and the results revealed that preinduction of HSP70 might exhibit anti-NF-κB property, which is similar with PDTC.

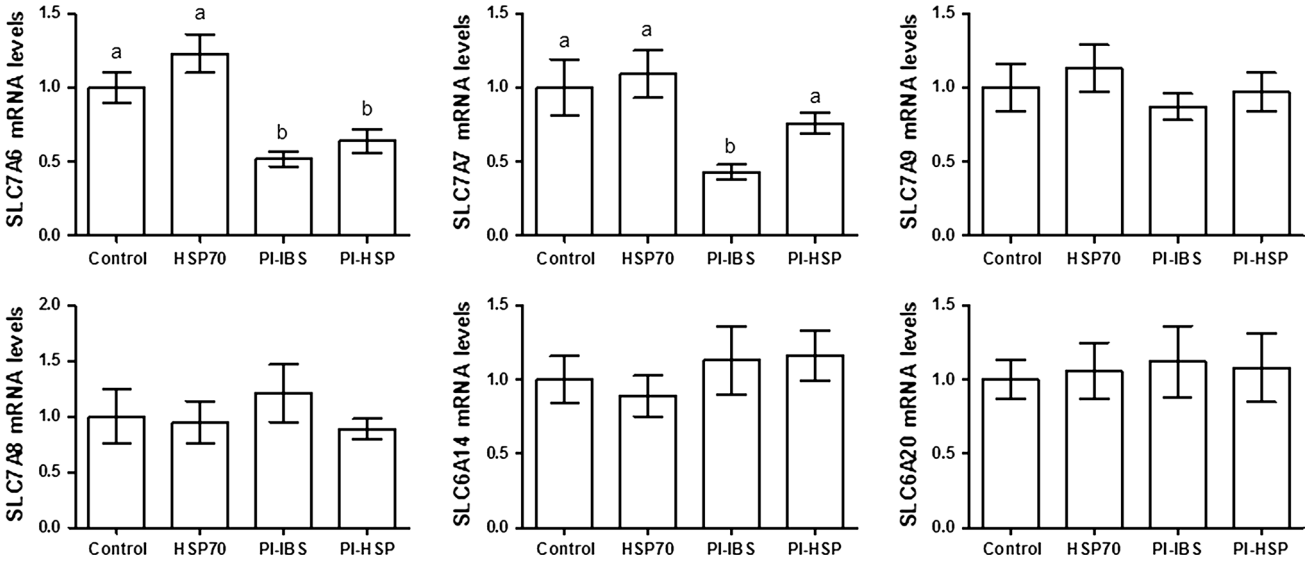

Fig. 5 Effects of preinduction of HSP70 on expression of ileal amino acids transporters after exposure to Trichinella spiralis infection. Data are presented as mean ± SEM, $n = 5$. *a–c* For each variable, means with *different letters* differ ($P < 0.05$)

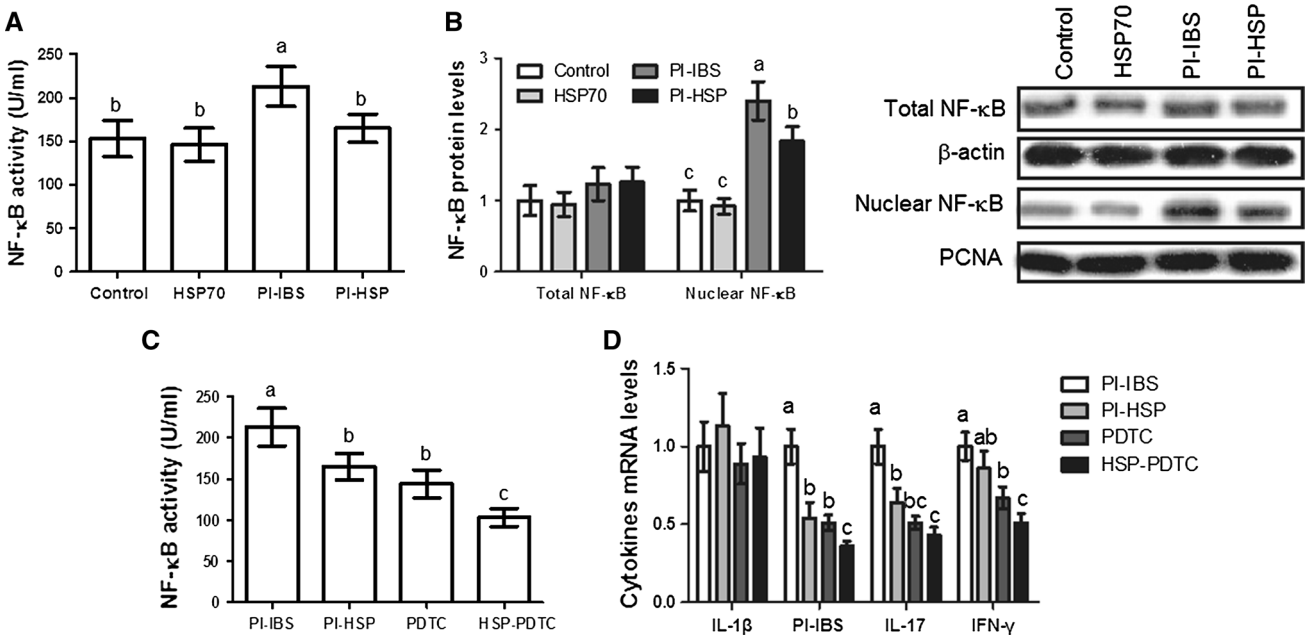

Fig. 6 Effects of preinduction of HSP70 on NF-κB signaling pathway after exposure to Trichinella spiralis infection. Data are presented as mean ± SEM, $n = 5$. *a–c* For each variable, means with *different letters* differ ($P < 0.05$)

Arginine/NOS/NO signaling pathway

The current study showed that preinduction enhanced serum arginine, which may serve as a protective mechanism in PI-IBS model. Thus, we further investigated the NOS/NO signaling pathway after Trichinella spiralis infection using L-NAME, an NOS inhibitor. We, firstly, determined serum arginine and NO concentrations after L-NAME treatment; the results showed that L-NAME failed to affect serum arginine, but it significantly decreased NO concentration in NAME and HSP-NAME groups ($P < 0.05$) (Fig. 7a, b). Meanwhile, the western blotting and ELISA data indicated that L-NAME markedly reduced ileal iNOS abundance and NOS activity compared with PI-HSP group ($P < 0.05$) (Fig. 7). The expression of ileal cytokines further indicated that preinduction of HSP70 mitigated

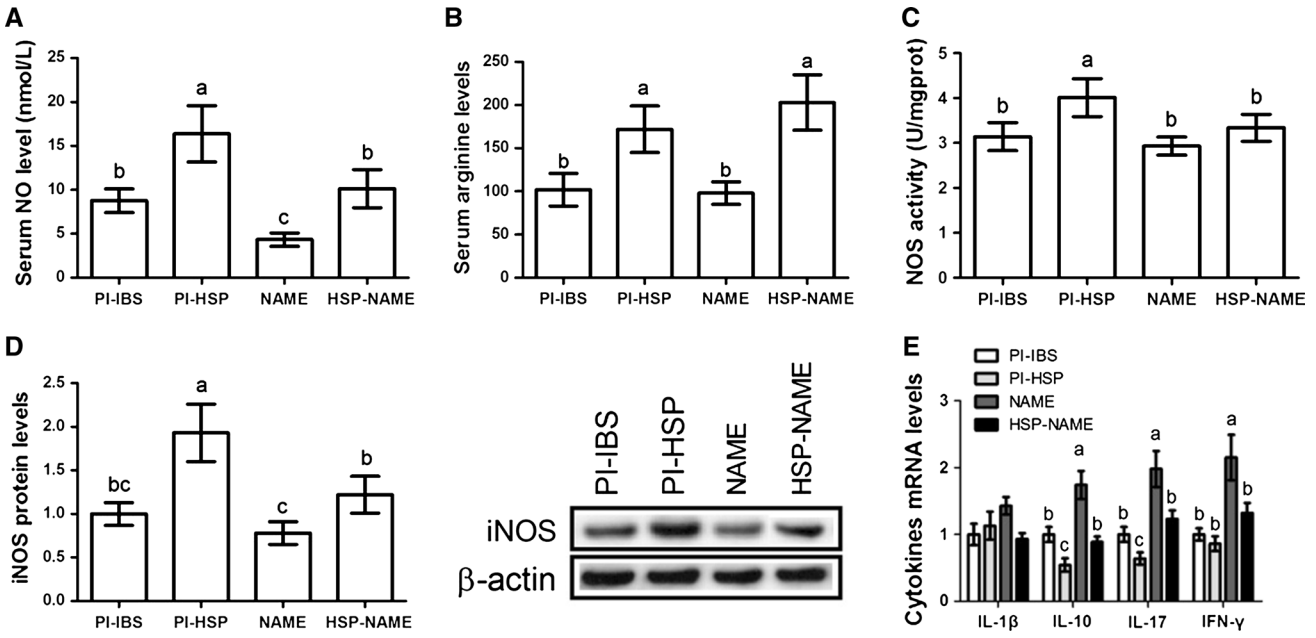

Fig. 7 Effects of preinduction of HSP70 on NOS/NO signaling pathway after exposure to Trichinella spiralis infection. Data are presented as mean ± SEM, $n = 5$. *a–c* For each variable, means with *different letters* differ ($P < 0.05$)

Trichinella spiralis-induced inflammation, while inhibition of iNOS reversed the protective function of HSP70 ($P < 0.05$) (Fig. 7), suggesting that preinduction of HSP70 mediates arginine/NOS/NO signaling pathway in PI-IBS model.

Discussion

Our previous data demonstrated that Trichinella spiralis infection induces serious inflammatory response at day 14 and the ileum is the main target in mice (unpublished data). Thus, we mainly investigated the effect of Trichinella spiralis infection on intestinal integrity, nutrients absorption, and inflammation, and the protective role of preinduction of HSP70 in the ileum at day 14. In the current study, upregulation of HSP70 was induced by heat treatment according to previous report (Mizushima et al. 2000). It has been demonstrated that HSP70 expression continues to increase with duration of exposure, while HSP90 and HSP110 increase to a much higher level and then decrease and HSP27 and HSP47 fail to be affected under heat stress (Purohit et al. 2014). Meanwhile, we also found that Trichinella spiralis infection inhibited HSP70 expression, indicating that HSP70 plays a key role in post-infection irritable bowel syndrome.

Compelling evidences have shown that low-grade inflammatory response involves in the occurrence and persistence of the symptoms in PI-IBS patient (El-Salhy 2012; Schmulson et al. 2014). In the present study, we found that Trichinella spiralis infection increased ileal inflammatory score and expression of proinflammatory cytokines, including IL-10, IL-17, and IFN-γ,while upregulation of HSP70 significantly inhibited ileal inflammatory response, indicating a protective role in Trichinella spiralis-induced inflammation in PI-IBS model. Meanwhile, several studies also demonstrated that HSP70 inhibits the production of proinflammatory cytokines in different cell populations (Schmidt and Abdulla 1988; Simon et al. 1995). More recently, Muralidharan et al. reported that association of HSP70 with NF-κB subunit p50 in alcohol-treated macrophages correlates with reduced NF-κB activation and downstream TNF-α, IL-6 and IL-1β production (Muralidharan et al. 2014).

HSP70 protects intestinal integrity in Trichinella spiralis-induced PI-IBS model. Intestinal mucosal barrier function is the capacity of the intestine to provide adequate containment of luminal microorganisms and molecules while preserving the ability to absorb nutrients. Alteration of the mucosal barrier function with accompanying increased permeability and/or bacterial translocation has been linked with a variety of conditions, including inflammatory bowel disease (Sanchez de Medina et al. 2014). The present data indicated that Trichinella spiralis infection increased intestinal permeability evidenced by the reduced DAO and increased LPS concentrations. Serum DAO activity and LPS concentration are associated with the maturation and integrity of small intestinal mucosa (Guo et al. 2013; Namikawa et al. 2012). Previous reports revealed that the

increased permeability partially corresponded to down-regulated tight junction (Ruan et al. 2014). In the current study, Trichinella spiralis infection reduced ileal ZO1 and occludin1 abundances, which may contribute to the alteration in intestinal permeability. Liedel et al. reported that mother's milk-induced HSP70 expression preserves intestinal epithelial barrier function in an immature rat pup model (Liedel et al. 2011), which is similar with our results that preinduction of HSP70 significantly reversed the intestinal dysfunction caused by Trichinella spiralis infection. There are also a large number of studies demonstrating a protective role of HSP70 in intestinal barrier, including ischemia–reperfusion (I/R) mucosal injury (Fleming et al. 2002), ethanol-induced intestinal damage (Lee et al. 2014a), and chronic water avoid stress (Yang et al. 2009).

Intestine is the main site for nutrients absorption and metabolism (Yin et al. 2015c). The present study demonstrated that Trichinella spiralis infection induces intestinal dysfunction, while the nutrients' absorption and metabolism in PI-IBS model are still unclear. In this study, we firstly reported that ileal villus height and crypt depth, serum lysine and arginine, and ileal SLC7A1 and SLC7A7 transporters are significantly reduced in PI-IBS model. Upregulation of HSP70 markedly reversed the reduced serum arginine and ileal SCL7A7 mRNA. Arginine plays important roles as metabolic intermediates in nutrition, immune response, inflammation, and protein synthesis (Yin et al. 2014, 2015b). Several reports indicated that arginine serves as a mediator for HSP70 expression (Wu et al. 2013b) and dietary supplementation with arginine upregulates HSP70 expression in animals (Wu et al. 2013a) and cells (Pedrycz and Siermontowski 2013). SCL7A7 mainly contributes to the absorption of arginine and lysine (Yin et al. 2014). The present study suggested that upregulation of HSP70 enhances SCL7A7 expression subsequent with arginine absorption. But further mechanical research between HSP70 and SCL7A7 should be investigated.

It is widely confirmed that NF-κB signaling pathway plays a key role in inflammatory response and mediates various proinflammatory cytokines generation in different pathologies (Nguyen et al. 2014; Ruan and Chen 2012; Yin et al. 2015a). Symeonidou et al. investigated 128 genes' expression profiles from NF-κB signaling pathway in Trichinella spiralis-infected mice via microarray analysis and found that various gene expressions were affected between the specific points of Trichinella spiralis infection (Symeonidou et al. 2010). Similarly, the present results revealed that Trichinella spiralis infection activated NF-κB and induction of HSP70 significantly inhibited NF-κB activation, which is similar with the role of PDTC, an NF-κB inhibitor. HSP70 has been described as a Toll-like receptor 4 (TLR4) ligand and TLR4 contributes NF-κB activation (Enomoto et al. 2006; Vabulas et al. 2002). TLR4/NF-κB axis has been demonstrated to be involved in various infection-induced inflammations (Liu et al. 2013; Shen et al. 2014; Yin et al. 2013a). Thus, we speculated that induction of HSP70 plays a protective role in PI-IBS mice via inhibiting NF-κB activation, following with the lower inflammation. This hypothesis has been validated in a mouse model of lethal oxidant lung injury which indicated that HSP70 mediated NF-κB activation to protect against hyperoxia-induced lung injury and the protective property of HSP70 is TLR4 dependent (Zhang et al. 2013).

NO is the main metabolite from arginine and NOS/NO signal modulates downstream proteins via specific post-translational modifications (Pigott et al. 2013). Dysfunction of NOS/NO (i.e., altered expression, location, coupling, activity, etc.) exists in various pathological conditions (Tang et al. 2014). iNOS and endothelial NOS (eNOS) are two major isoforms in the macrophage inflammatory response, which is the source of NO that is potently induced in response to proinflammatory stimuli (McNeill et al. 2015). The current study demonstrated that arginine is involved in the protective function of HSP70 in PI-IBS, so we further investigated NOS/NO signal and the results showed that upregulation of HSP70 activated NOS/NO signal in Trichinella spiralis-induced PI-IBS model and mitigated ileal inflammation, while the beneficial effect was blocked by NOS inhibitor. Various reports indicated that iNOS promotes inflammatory response (Lee et al. 2014b), while our data suggested that iNOS is involved in the protective function of HSP70 in Trichinella spiralis-induced PI-IBS model. Huang et al. reported that NF-κB activation can induce hyper-expression of iNOS in inflammation (Huang et al. 2010), so we speculated that the difference may be NF-κB dependent or the different inflammatory model. Although we failed to determine eNOS expression after Trichinella spiralis infection, other studies further demonstrated that eNOS is involved in various inflammatory diseases, including ulcerative colitis, ischemia/reperfusion injury, and acute coronary syndrome (Gomaraschi et al. 2013; Okaniwa et al. 2015; Zhu et al. 2015). Meanwhile, HSP70 has been reported to be involved in NOS signal activation against seizure-induced neuronal cell death (Chang et al. 2014). Thus, we concluded that HSP70 might activate arginine/iNOS/NO signal pathway in PI-IBS.

In conclusion, Trichinella spiralis infection in PI-IBS model inhibits HSP70 expression and caused intestinal inflammation and injury. Heat-induced HSP70 expression enhances intestinal integrity, inhibits the expression of proinflammatory cytokines, and augments serum arginine concentration and intestinal SCL7A7 mRNA abundance. Thus, heat-induced HSP70 upregulation may exert a protective role against Trichinella spiralis infection-induced PI-IBS in mice and the mechanism may be associated with NF-κB and arginine/NOS/NO signal pathways.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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