International Journal for Parasitology 44 (2014) 23–35

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Involvement of heat shock protein 47 in *Schistosoma japonicum*-induced hepatic fibrosis in mice

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ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form 14 August 2013
Accepted 15 August 2013
Available online 1 December 2013

Keywords:
Hepatic fibrosis
Schistosomiasis japonica
Heat shock protein 47
Short hairpin RNA
Cytokines

ABSTRACT

Chronic infection with the blood fluke *Schistosoma japonicum* is associated with both liver cirrhosis and liver cancer. Previously, heat shock protein 47, a collagen-specific molecular chaperone, was shown to play a critical role in the maturation of procollagen. However, less is known about the role of heat shock protein 47 in *S. japonicum*-induced hepatic fibrosis. We therefore investigated the expression of heat shock protein 47 in *S. japonicum*-induced liver fibrosis and attempted to determine whether inhibition of heat shock protein 47 could have beneficial effects on fibrosis in vitro and in vivo. In this study, we found that the expression of heat shock protein 47 was significantly increased in patients with *Schistosoma*-induced fibrosis, as well as in rodent models. Immunohistochemistry revealed heat shock protein 47-positive cells were found in the periphery of egg granulomas. Administration of heat shock protein 47-targeted short hairpin (sh)RNA remarkably reduced heat shock protein 47 expression and collagen deposition in NIH3T3 cells and liver tissue of *S. japonicum*-infected mice. Life-table analysis revealed a dose-dependent prolongation of survival rates with the treatment of heat shock protein 47-shRNA in murine fibrosis models. Moreover, serum alanine aminotransferase and aspartate transaminase activity, splenomegaly, spleen weight index and portal hypertension were also measured, which showed improvement with the anti-fibrosis treatment. The fibrosis-related parameters assessed were expressions of Col1a1, Col3a1, TGF-β1, CTGF, IL-13, IL-17, MMP-9, TIMP-1 and PAI-1 in the liver. This study demonstrated that heat shock protein 47-targeted shRNA directly reduced collagen production of mouse liver fibrosis associated with *S. japonicum*. We conclude that heat shock protein 47 plays an essential role in *S. japonicum*-induced hepatic fibrosis in mice and may be a potential target for ameliorating the hepatic fibrosis caused by this parasite.

1. Introduction

Schistosomiasis, caused by blood-dwelling trematode helminths, is a transmissible parasitic disease of worldwide significance. Second only to malaria, it affects more than 200 million people living in tropical and subtropical regions (McManus and Loukas, 2008). In China, one of the areas endemic for *Schistosoma japonicum*, there are still 360,000 patients per year suffering from this disease burden, due to its chronic effects, e.g., liver cirrhosis or liver cancer (Gryseels et al., 2006; McManus and Loukas, 2008; McManus et al., 2010). The difficulty in preventing *S. japonicum* prevalence and clinical consequences is mainly dependent on the rapid progression of the disease course, long-standing infections, complex host–parasite interactions and drug-resistance (Biempica et al., 1993; Takemura et al., 1998; McManus et al., 2010).

Unlike other types of hepatic fibrosis caused by long-term alcohol abuse (Breitkopf et al., 2005), non-alcoholic steatohepatitis (NASH) (Cortez-Pinto et al., 2001) or viral hepatitis (Tomanovic et al., 2009), liver damage caused by *S. japonicum* results from granuloma formation around schistosome eggs and the subsequent immune response of the host. A massive deposition of extracellular matrix (ECM) in the periportal spaces leads to blockage of the portal veins and characteristic pipe-stem fibrosis, followed by portal hypertension, splenomegaly, portocaval shunting and gastric varices (McManus and Loukas, 2008). Moreover, cytokines, which communicate between the fibrotic areas and the immune system form a network of host–parasite responses. Nevertheless, the mechanisms involved in the pathogenesis and progression of hepatic fibrosis in patients with schistosomiasis have not yet been fully elucidated (Andrade, 2008; Anthony et al., 2012). Although chemotherapy can effectively target and kill schistosomes, the progression of hepatic fibrosis persists. Since the anti-fibrotic therapies of schistosomiasis have been neglected, new prospective drugs are urgently required that can reverse the fibrosis itself.

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0020-7519/$36.00
http://dx.doi.org/10.1016/j.ijpara.2013.08.009

Liver fibrosis represents the final common pathway of all chronic inflammatory injuries, including those stemming from infectious agents. Fibrosis is a reversible and highly dynamic process involving both matrix synthesis and degradation (Friedman, 2010). Hepatic stellate cells (HSC), one of the resident liver cells, play a key role in liver fibrosis (Ueno et al., 1997; Friedman, 2008). Activated HSCs adopt a myofibroblast-like phenotype, secreting collagen, matrix metalloproteinase (MMPs) and their inhibitors (e.g. tissue inhibitor of metalloproteinase, TIMP) to remodel the tissue matrix. Thus, manipulation of matrix synthesis and degradation may result in the development of effective antifibrotic therapies.

Heat shock protein 47 (HSP47) is a stress protein that binds to collagen types I through V (Natsume et al., 1994) and plays a critical role as a molecular chaperone for accumulation of collagen around fibrotic areas. Additionally, selective blocking of HSP47 may reduce collagen accumulation and delay the progression of fibrotic diseases (Masuda et al., 1994; Kawada et al., 1996; Sunamoto et al., 1998). It has been unclear, however, whether HSP47 is involved in hepatic fibrosis associated with *S. japonicum*. The aim of this study was to investigate the role of HSP47 in liver fibrosis of human schistosomiasis and the murine model, as well as to analyse whether administration of HSP47-short hairpin (sh)RNA could prevent the progression of liver fibrosis as a potential therapeutic tool.

2. Materials and methods

2.1. Patients with S. japonicum-induced hepatic fibrosis and liver histology

Liver tissue and serum samples were obtained from five healthy volunteers and 72 inpatients at Tongji Hospital (Tongji Medical College Huazhong University of Science and Technology, Wuhan, China) from October 2008 to September 2012. All enrolled patients had chronic schistosomiasis, as proven by positive schistosomal serology. Study exclusion criteria included patients with other types of hepatitis and liver disease associated with drugs or alcohol. Patients in this study were given antischistosomal treatment with a single dose of praziquantel (PZQ) at 40 mg/kg of bodyweight (World Health Organization (WHO), 2012). The study protocol was approved by the Human Research Ethics Committee of Tongji Medical College. Written informed consent was also obtained from healthy volunteers and from all patients who were involved in this project.

Liver fibrosis (Stage) and inflammatory activity (Grade) of all biopsies were assessed separately according to the Scheuer scoring system (Scheuer, 1994). Fibrosis stages were evaluated on a scale of S0–S4, as follows: S0: no fibrosis, S1: enlarged portal tracts, S2: periportal or portoportal septa, S3: fibrosis with architectural distortion and S4: cirrhosis. The inflammatory activity was determined by combining scores for portal inflammation (0–4) and lobular necrosis (0–4). In these patients, the level of infection directly correlated with the grade of inflammation. The majority of the biopsies showed moderate inflammation (G0: 0%, G1: 60% (43/72 cases), G2: 29% (21/72 cases), G3: 11% (8/72 cases), G4: 0%). Patient characteristics are summarised in Table 1.

2.2. Animal models of S. japonicum-induced hepatic fibrosis

Six-week-old female BALB/c mice, each weighing 14–18 g, were purchased from Hubei Center for Disease Control and Prevention, Wuhan, China. All animals received standard laboratory animal care, including free access to food and water, at the center for Animal Experiment/Animal Biosafety Level III Laboratory (A3 Lab), Wuhan University. The protocols for all animal experiments were approved by the Ethics Committee of Tongji Medical College.

A Chinese wild strain of *S. japonicum* cercariae, maintained in infected *Oncomelania hupensis*, was purchased from the Hunan Institute for Schistosomiasis Control, Yueyang, China, and used to induce liver fibrosis. Each mouse was infected percutaneously with 16 ± 1 *S. japonicum* cercariae and sacrificed at 6, 8, 12 or 14 weeks p.i. Liver tissue samples were removed under sevoflurane anesthesia.

To evaluate the liver worm burden, the number of eggs per gram of hepatic tissue was counted. One gram of liver tissue was removed from each mouse in the HSP47-shRNA injected group and the control at 14 weeks p.i. Samples were digested in 20 ml of 5% potassium hydroxide solution at 37 °C overnight (Cheever, 1968). An average of three counts per 20 μl of mixture was taken to estimate the number of eggs, and this count was converted to eggs per gram.

2.3. Cell lines

Mouse foetal fibroblasts, NIH/3T3, were purchased from the China Center for Type Culture Collection (CCTCC, Wuhan University) and maintained in DMEM (Life Technologies, Inc., USA) containing 10% (wt/vol) FBS at 37 °C in a 5% CO_2 atmosphere.

2.4. Preparation and transfection of HSP47-targeted shRNA

RNA interference (RNAi) was used to block translation of HSP47 mRNA. Three specific RNAi plasmids targeting HSP47 (HSP47-shRNA) and a non-targeting shRNA control (negative control-shRNA) were constructed and verified by the Shanghai GeneChem Co., Ltd. (Shanghai, China). The sense strands of insert sequences were: (sequence A, beginning at nucleotide (nt) 255): 5′-CGA ACACTCCAAGATCAACTTTTCAAGAGAAAGTTGATCTTGGAGTGTTCG-3′; (sequence B, beginning at nt 544): 5′-GACAAGAACAAGGCAGACC TATTCAAGAGATAGGTCTGCCTTGTTCTTGTC-3′; (sequence C, beginning at nt 593): 5′-CTTCAGCCTATATCAGGCGATTTCAAGAGAATCG CCTGATATAGGCTGAAG-3′ and the non-targeting shRNA control sequence was: 5′-GATCCCCGAACACT CCAAGATTTCAAGAGAAAGTT GATCTTGGAGTGTTCG-3′. Each shRNA sequence was inserted into

Table 1
Characteristics of patients with *Schistosoma japonicum* according to the Scheuer fibrosis stages.

Characteristic	Patients with *S. japonicum*					Control (n = 5)
	S0 (n = 8)	S1 (n = 18)	S2 (n = 15)	S3 (n = 17)	S4 (n = 14)	
Sex (M/F)	6/2	14/4	13/2	14/3	11/3	4/1
Age (years)	33 ± 5	35 ± 7	41 ± 11	36 ± 14	43 ± 13	40 ± 12
ALT (IU/L)	27.2 ± 3.6	54.9 ± 9.8[a]	78.1 ± 34.5[a]	48.8 ± 9.8[a]	39.3 ± 7.9	29.0 ± 6.3
AST (IU/L)	31.2 ± 7.5	38.2 ± 4.8	66.6 ± 23.1[a]	66.3 ± 27.8[a]	53.8 ± 14.7	31.2 ± 5.4

Values are mean ± S.D. where applicable.
ALT, alanine aminotransferase; AST, aspartate aminotransferase.
[a] $P < 0.05$ compared with the control group.