International Journal for Parasitology: Drugs and Drug Resistance 6 (2016) 35–43

Contents lists available at ScienceDirect

International Journal for Parasitology: Drugs and Drug Resistance

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

Taurine drinking ameliorates hepatic granuloma and fibrosis in mice infected with *Schistosoma japonicum*

Yan-Rong Yu [a, *], Xian-Qiang Ni [a], Jie Huang [a], Yong-Hong Zhu [a], Yong-Fen Qi [a, b, c, **]

[a] Department of Pathogen Biology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China
[b] Laboratory of Cardiovascular Bioactive Molecule, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China
[c] Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, Beijing 100191, China

ARTICLE INFO

Article history:
Received 17 October 2015
Received in revised form
12 January 2016
Accepted 12 January 2016
Available online 14 January 2016

Keywords:
Schistosomiasis
Schistosoma japonicum
Granuloma
Fibrosis
Taurine

ABSTRACT

In schistosomiasis, egg-induced hepatic granuloma formation is a cytokine-mediated, predominantly $CD4^+$ Th2 immune response that can give rise to hepatic fibrosis. Hepatic fibrosis is the main cause of increased morbidity and mortality in humans with schistosome infection. Taurine has various physiological functions and hepatoprotective properties as well as anti-inflammatory and immunomodulatory activity. However, little is known about the role of taurine in schistosome egg-induced granuloma formation and fibrosis. We aimed to evaluate the therapeutic potential of taurine as preventative treatment for *Schistosoma japonicum* infection. Mice infected with *S. japonicum* cercariae were supplied with taurine drinking water (1% w/v) for 4 weeks starting at 4 weeks post-infection. Taurine supplementation significantly improved the liver pathologic findings, reduced the serum levels of aminotransferases and area of hepatic granuloma, and prevented fibrosis progression. In addition, taurine decreased the expression of the granulomatous and fibrogenic mediators transforming growth factor β1, tumor necrosis factor α, monocyte chemotactic protein 1α and macrophage inflammatory protein 1α as well as the endoplasmic reticulum stress marker glucose-regulated protein 78. Thus, taurine can significantly attenuate *S. japonicum* egg-induced hepatic granuloma and fibrosis, which may depend in part on the downregulation of some relevant cytokine/chemokines and reducing the endoplasmic reticulum stress response.

1. Introduction

Schistosomiasis is one of the most widely occurring neglected tropical diseases, with high incidence in Asia, Africa and Latin America. About 207 million people have been infected worldwide and 780 million people are at risk of infection, and more than 240 million patients require treatment each year (Beckmann et al., 2014; Guimarães et al., 2015). *Schistosoma japonicum* (*S. japonicum*) is the major causative agent in Southeast Asia and China. The presence of eggs from *S. japonicum* in the host liver and intestinal tissue is the major cause of pathologic schistosomiasis. During infection, schistosome eggs are trapped in the host liver and stimulate the granulomatous response. Subsequent significant fibrosis and circulatory impairment can develop in a subset of individuals with extensive or repeated infection and/or lack of treatment (Liu et al., 2013). Hepatic fibrosis is the principal cause of morbidity and mortality in humans with schistosome infection. However, effective medical interventions to control and treat granuloma and fibrosis in schistosomiasis are lacking.

Granulomatous reaction of schistosome is the $CD4^+$ T cell-mediated delayed-type hypersensitivity induced by soluble egg antigen secreted from viable miracidium within eggs trapped in host tissues. The formation of granulomas is a dynamic process that involves the accumulation of inflammatory and immune cells at the site of antigen release, leading to the confinement of the eliciting agent (Chuah et al., 2014). Toward the late stage, fibroblasts are stimulated by egg products and by T-lymphocyte cytokines to proliferate, replacing most of the cellular elements and mediating fibrotic collagenous material deposition around the portal vein tributaries (Olveda et al., 2014). Many factors are involved in regulating the immunopathogenesis of schistosomiasis. T helper 1 cell (Th1) and Th2 cytokines determine the hepatic granuloma size (Liu et al., 2013; Chuah et al., 2014), and Th17 responses have

* Corresponding author. Department of Pathogen Biology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China.
** Corresponding author. Department of Pathogen Biology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China.
E-mail addresses: yuyr@bjmu.edu.cn (Y.-R. Yu), yongfenqi@163.com (Y.-F. Qi).

http://dx.doi.org/10.1016/j.ijpddr.2016.01.003

been linked with severe hepatic inflammation in schistosomiasis (Chuah et al., 2014). Chemokines, particularly macrophage inflammatory protein 1α (MIP-1α) and monocyte chemotactic protein 1α (MCP-1α), play major roles in the formation of hepatic granuloma. Mice deficient in MIP-1α show decreased hepatic granuloma size and both fibrosis and eosinophil peroxidase activity (Souza et al., 2005). MCP-1α, can be produced by activated hepatic stellate cells (HSCs) following liver injury, showed increased transcriptional levels during *S. japonicum* infection correlates with peak fibrosis (Bartley et al., 2006). Transforming growth factor-β1 (TGF-β1) is the most potent fibrogenic cytokine in the liver. TGF-β1 activates and transforms HSCs into myofibroblast-like cells, which express a-smooth muscle actin (a-SMA) and secrete collagens containing hydrohyproline that form extracellular matrix (ECM) fibrosis (Dooley and ten Dijke, 2012; Sun et al., 2015). In addition, pro-inflammatory stimulation, oxidative stress and tissue damage may play important roles in schistosomiasis (Cunha et al., 2012; de Oliveira et al., 2013).

Taurine (2-aminoethane sulfonic acid), a sulfur-containing β-amino acid, is ubiquitously distributed in animal tissues and cells, accounts for approximately 0.1% of total human body weight. It is both synthesized endogenously from cysteine and methionine and ingested directly with certain foodstuffs. According to the European Food Safety Authority, taurine (3–6 g) has been administered daily to a large number of patients (including adults, children and even infants). No adverse health effects have been noted (Schaffer et al., 2014). In recent years taurine has been widely used as a performance-enhancing ingredient in energy drinks (Luckose et al., 2015). In general, oral taurine can be absorbed by gastrointestinal tract, plasma proteins combination with taurine are fewer. Taurine discharges mainly in prototype and kidney can adjust the content of taurine in the body. The normal concentration of taurine in the plasma is very low (e.g.<60 μM in cat) but most tissues contain very high taurine levels (mM range), creating a substantial concentration gradient across the cell membrane (Schaffer et al., 2014). The half-life of turnover of taurine in the mouse was 18.6 days (Huxtable and Lippincott, 1982). Taurine has various physiological functions and protective properties including protection against various types of hepatic damage (Gentile et al., 2011). In addition, taurine possesses anti-inflammatory and immunoregulatory properties (De Luca et al., 2015). Previous studies have demonstrated that exogenous supplementation with taurine can prevent liver injury caused by different harmful substances as well as inhibit ECM deposition on the damaged liver and stop the process of liver fibrosis (Miyazaki et al., 2005; Devi et al., 2009, 2010; Gentile et al., 2011). Mice with hetero- and homozygous knockout of the taurine transporter show chronic liver disease characterized by fibrosis, inflammation, and hepatocyte apoptosis (Warskulat et al., 2006). The hepatoprotective effects of taurine are often accompanied by reduced endoplasmic reticulum (ER) stress, oxidative stress, production of inflammatory and fibrogenic mediators and activation of stellate cells (Erman et al., 2004; Devi et al., 2010; Gentile et al., 2011). However, whether taurine supplementation can affect the pathological processes of hepatic granulomas and fibrosis elicited by *S. japonicum* infection is not known.

In this paper, we aimed to determine the effect of taurine supplementation on granuloma formation and the fibrosis process in an animal model of *S. japonicum* infection to assess its potential as preventative and therapeutic treatment for schistosomiasis. Miyazaki et al. (2004) reported that the effective and optimal doses of oral taurine administration for two weeks on a transient exercise performance in rat were between 100 and 500 mg/kg/day. Various reports have described the experimental use of taurine supplemented in drinking water in mice over the concentration range of 0.05%~5% (Ribeiro et al., 2010; Santora et al., 2013; Santos-Silva et al., 2015). Hence, we used 1% taurine supplementation in our experiment. We found that taurine supplementation could suppress *S. japonicum* egg-induced liver granuloma and fibrosis in mice.

2. Materials and methods

2.1. Parasite and animals

S. japonicum (Chinese mainland strain)-infected *Oncomelania hupensis* snails were purchased from the Jiangsu Institute of Parasitic Diseases (Wuxi, Jiangsu, China). Female ICR mice, 6–8 weeks old, were from the Department of Laboratory Animal Science of Peking University Health Science Center (Beijing). All animal care and experimental protocols complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011) and were approved by the Animal Care Committee of Peking University Health Science Center.

2.2. Infection of mice with S. japonicum

S. japonicum cercariae were shed in a beaker after exposing infected *Oncomelania* to light for 6 h in 24–28 °C. The mice were infected percutaneously with 30 ± 2 freshly shed cercariae after they had been anesthetized by intraperitoneal injection of ketamine.

2.3. Experimental group and taurine treatment

Mice were randomly divided into three groups for treatment (10 mice per group): 1) control (Con), mice not infected with *S. japonicum* and fed standard chow; 2) infected (Inf), mice infected with *S. japonicum* and fed standard chow; 3) infected/taurine (Tau), mice infected with *S. japonicum*, fed standard chow and 1% taurine (Sigma, MO, USA) in drinking water for 4 weeks starting at 4 weeks post-infection. At 8 weeks post-infection, the body weight of each mouse was weighed and serum was separated from blood taken from the mouse eye socket, then mice were killed by cervical dislocation, and liver tissue and serum samples were collected for analysis.

2.4. Liver and spleen indexes, egg burden

The liver and spleen tissue was weighed. Liver and spleen indexes were calculated as ratios of liver to body weight and spleen to body weight, respectively.

To determine the egg burden, 1 g of each liver was digested with 5% KOH at 37 °C overnight. After centrifugation, released eggs in the liver were then determined by microscopic examination.

2.5. Analysis of liver transaminase activity

Liver injury was assessed by measuring serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by use of an Olympus AU5800 automatic biochemical analyzer (Olympus, Japan).

2.6. Histology and immunohistochemistry (IHC) of liver sections

Excised livers were instantly fixed in 10% neutral formalin overnight and embedded in paraffin. For histology, according to standard procedures, tissue sections (4 μm) were stained with hematoxylin and eosin (H&E) to examine the area of the granulomas or with Masson trichrome to evaluate the extent of hepatic