Glutathione protects against hepatic injury in a murine model of primary Sjögren's syndrome

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ABSTRACT

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease which may cause complications such as hepatic dysfunction and injury. As an important antioxidant, reduced glutathione (GSH) has been reported protecting against hepatic injury induced by some diseases, but the role of GSH in pSS is poorly understood. This study aims at investigating the role of GSH in hepatic injury during pSS. A murine model of pSS, non-obese diabetic (NOD) mice, was used for GSH administration via tail intravenous injection. Enzyme-linked immunosorbent assay (ELISA) was performed to detect serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as the levels of GSH, tumor necrosis factor, interleukin (IL) 10, integrin alpha M, IL1B, malondialdehyde, nicotinamide adenine dinucleotide phosphate oxidase 4, and superoxide dismutases in hepatocyte homogenates. Hematoxylin-eosin staining was performed to observe hepatic histology. The results showed that serum AST and ALT levels were up-regulated in the NOD mice (p = 0.0021 and 0.0048), but were significantly recovered after the GSH administration (p = 0.0081 and 0.0263). The NOD mice exhibited disturbed hepatic tissue structure, which was attenuated by GSH. The GSH administration could also promote the production of GSH in the hepatocytes (p = 0.0264), and control the levels of inflammatory factors and oxidative stress-related factors. These results indicate that GSH has significant effects on protecting against the hepatic injury during pSS, which may be associated with its regulation of the inflammatory factors and oxidative stress-related factors. This study suggests that GSH is a promising therapeutic strategy for controlling hepatic injury during pSS and offers valuable information for further research.

KEY WORDS: Primary Sjögren's syndrome; glutathione; hepatic injury; inflammation; oxidative stress
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INTRODUCTION

Primary Sjögren's syndrome (SjS) (pSS) is a systemic autoimmune disease characterized by dryness such as xerophthalmia and xerostomia, and even fatigue and pain. pSS may be caused by a combination of genetic, environmental, and other factors. Women are more vulnerable to SjS as they are prone to have local androgen deficiency in the salivary gland [1]. In pSS patients, exocrine gland dysfunction can generate from the activation of the innate immune system, and some studies have also discovered that exocrine dysfunction may exacerbate the autoimmunity and inflammation response of pSS [1]. pSS in some cases increases the risk of other complications such as ocular lesions [2] and neuropathy [3]. Molecular research has revealed key genes for SjS regulation including factors implicated in cell apoptosis, interferon signaling, and innate and adaptive immune responses [4-6]. Human studies have uncovered the beneficial effects of immune suppressing drugs and the potential of gene transfer approaches in the management of SjS [7], but further investigation is still required.

Among the complications, injury-like chronic active hepatitis is a symptom that bothers pSS patients [8]. Studies showed that SjS patients with anticentromere antibody are more sensitive to autoimmune hepatic diseases [9]. pSS patients also show a higher prevalence of silent but substantial hepatic fibrosis with manifestations of leukopenia, lower albumin and higher aspartate aminotransferase (AST) levels in the serum [10]. Actually, hepatic injury can be induced by inflammatory responses and oxidative stress [11-13] and is usually accompanied by higher AST and alanine aminotransferase (ALT) serum levels [14,15].

Glutathione (GSH) is an important antioxidant protecting cells from damages of reactive oxygen forms. It exists in cells in two main forms: reduced GSH and oxidized GSH (GSSG). An increased ratio of GSSG to GSH is usually indicative of

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oxidative stress. The protective role of GSH has been uncovered in some diseases. For example, pretreatment of GSH in renal ischemia-reperfusion model rats relieves the damages in hepatocytes, which may be associated with decreased hepatic malondialdehyde (MDA) and serum ALT levels [16]. GSH also alleviates the oxidative hepatic injury induced by dengue virus via inhibiting proinflammatory cytokines [17]. However, the specific role of GSH in modulating hepatic injury during pSS has not been reported up until now.

This study aims to investigate whether GSH inhibits the hepatic injury during pSS. Non-obese diabetic (NOD) mice were used as the model of pSS, and GSH was administrated through tail intravenous injection, after which we examined hepatic histology by hematoxylin-eosin (HE) staining, and serum ALT and AST levels by enzyme-linked immunosorbent assay (ELISA). The levels of GSH, inflammatory factors, and oxidative stress-related factors in hepatocyte homogenates were also detected. By comparison, this study uncovers the protective role of GSH against hepatic injury in pSS mice and provides a potential therapeutic strategy for treating the hepatic injury in pSS.

MATERIALS AND METHODS

Animals

NOD mice of SPF grade (8 weeks old, Stock No. 001976, The Jackson Laboratory, Bar Harbor, ME) were used as models for pSS, based on previous studies [5,18], and C57BL/6J mice were used as normal control. The mice were randomly divided into three groups: The control group of 4 C57BL/6J mice, the pSS group of 4 NOD mice, and the pSS + GSH group, containing 4 NOD mice injected with GSH. The mice of each group were raised in sterile cages separately, with food and water *ad libitum* at 24°C. This study was conducted according to the guidelines of our institute and approved by the Local Animal Ethics Committee.

GSH administration and sampling

The mice of the pSS + GSH group were administrated by 5 mmol/kg body weight of reduced L-GSH (Sigma-Aldrich, Shanghai, China) via tail intravenous injection based on the references [17,19] and our pretests. At 24 hours after the administration, the mice of the three groups were anesthetized and sacrificed for sampling. Blood samples were collected from each individual and centrifuged at 4000 ×*g* at 4°C for 10 minutes to separate the serum for ELISA assay. One part of the hepatic tissues was washed in cold phosphate-buffered saline (PBS) for ELISA assay, and the other part was snap-frozen in liquid nitrogen for HE staining.

Histological analysis

The hepatic tissues were cut into slices of 10 μm by the frozen section method and applied to HE staining according to the standard procedure. Briefly, the sections were fixed in methanol for 2 minutes and stained with hematoxylin for 3 minutes after which the sections were immersed in 1% hydrochloric acid in ethanol for 5 seconds and flushed by water for 5 minutes. Then, the sections were stained in eosin for 1 minute, dehydrated in ethyl solution of gradient concentrations (75%, 95% and 100%), hyalinized in xylene, and mounted by neutral balsam. The samples of each individual were observed with an optical microscope (Leica Microsystems, Wetzlar, Germany).

ELISA

The concentration of AST and ALT in the serum and the concentration of GSH, tumor necrosis factor (TNF), interleukin 10 (IL10), integrin alpha M (ITGAM, alias CD11b), IL1B, MDA, nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4), and superoxide dismutases (SODs) in hepatic tissues were detected by ELISA with the corresponding commercial kits (the MDA kit of Blue Gene, Shanghai, China; the other kits of Cusabio, Wuhan, China). For hepatic tissues, homogenates were prepared from 100 mg tissues of each sample. The detection procedure was carried out in 96-well plates following the manufacturers' instructions.

Statistical analysis

All the experiments were performed in triplicate. Results were represented as the mean ± standard deviation. Data were analyzed using SPSS 20 (IBM, New York, USA), with *F*-test detecting the homogeneity of variance and then the *t*-test was used for determining if there is a significant difference between the groups. A probability value of $p < 0.05$ was considered statistically significant.

RESULTS

GSH attenuates hepatic injury in pSS

After the GSH injection, the serum concentrations of AST and ALT were detected by ELISA, and the results showed that the pSS group displayed significantly elevated AST and ALT serum levels ($p = 0.0021$ and 0.0048, Figure 1A and B) and that the pSS + GSH group had lower AST and ALT levels compared to the pSS group ($p = 0.0081$ and 0.0263). Thus, GSH was found to effectively control the AST and ALT serum levels in pSS.

Since pSS is sometimes accompanied by hepatic injury, hepatic histology was examined by HE staining. From the lower magnification, it could be observed that the hepatic plates in the control group were arranged radially around the

central veins (Figure 2), while those in the pSS group were restricted and less evident. In the view fields at higher magnification, clear hepatocyte boundaries were observed in the control group. However, in the pSS group, there was extensive hepatocyte steatosis or vacuolar degeneration, which led to the lighter stained hepatic cytoplasm by eosin. Less vacuolar degeneration and much neater hepatic plates were observed in the pSS + GSH groups, indicating the attenuated hepatic injury. The histological results suggested that pSS might lead to hepatic injury, which could be relieved by the GSH administration.

GSH regulates inflammatory factors and oxidative stress-related factors

The concentration of TNF, IL10, ITGAM, and IL1B was detected by ELISA in the hepatocyte homogenates, and the results showed that these inflammatory factors, especially TNF, IL10, and IL1B, were significantly elevated in the pSS group than the control (p = 0.0037, 0.0047, and 0.0161, Figure 3A). The administration of GSH markedly inhibited the TNF and IL10 levels (p = 0.0258 and 0.0203), but no significant down-regulation was found in the ITGAM or IL1B level (p = 0.1506 and 0.0774). According to our results, GSH could affect the production of inflammatory factors, which might be associated with its regulation of the hepatic injury during pSS.

The concentration of the GSH and some oxidative stress-related factors were also detected by ELISA in the hepatocyte homogenates. The GSH production in the hepatocytes was inhibited in the pSS group (p = 0.0034, Figure 3B) and elevated by the GSH injection (p = 0.0264). MDA and NOX4 were both promoted in the pSS group (p = 0.0108 and 0.0067), and the effects

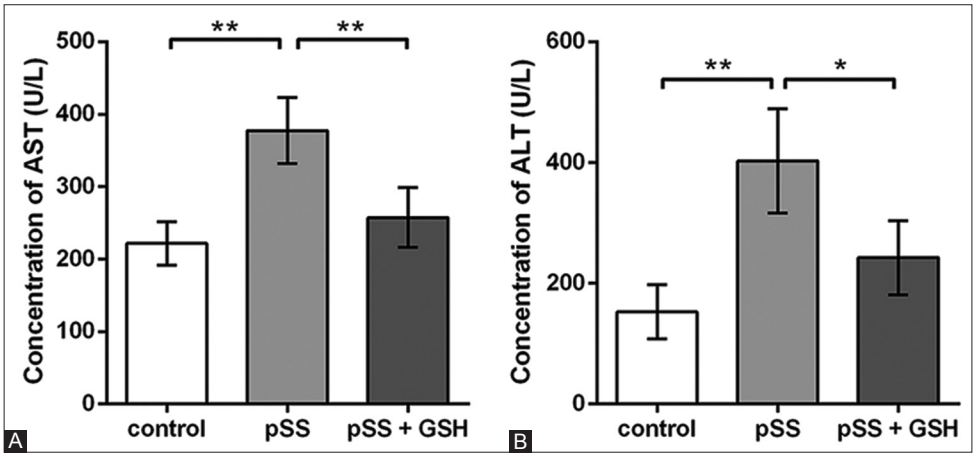

FIGURE 1. Concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum of the mice (n = 4). Concentration of AST (A) and ALT (B) is elevated in the pSS group and lowered after the GSH administration. Control: C57BL/6J mice as the control group. Primary Sjögren's syndrome (pSS): Non-obese diabetic (NOD) mice. pSS + reduced glutathione (GSH): NOD mice injected with GSH.

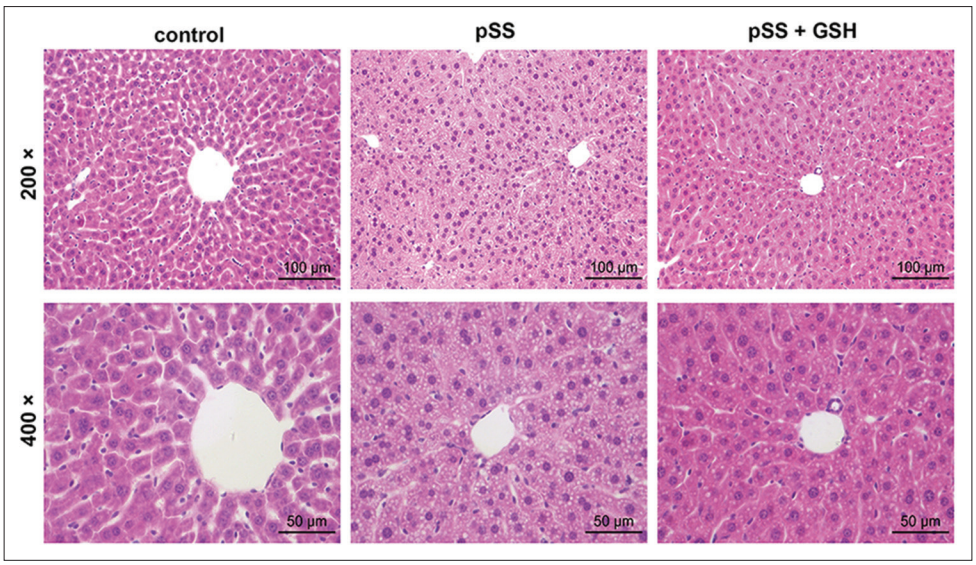

FIGURE 2. Histological examination by hematoxylin-eosin (HE) staining in the hepatic tissues (n = 4). Hepatic plates in the control group were arranged radially, with clear hepatocyte boundaries. Extensive hepatocyte steatosis or vacuolar degeneration was found in the pSS group, while GSH treatment decreased vacuolar degeneration in the pSS + GSH group. Control: C57BL/6J mice as the control group. Primary Sjögren's syndrome (pSS): Non-obese diabetic (NOD) mice. pSS + reduced glutathione (GSH): NOD mice injected with GSH.

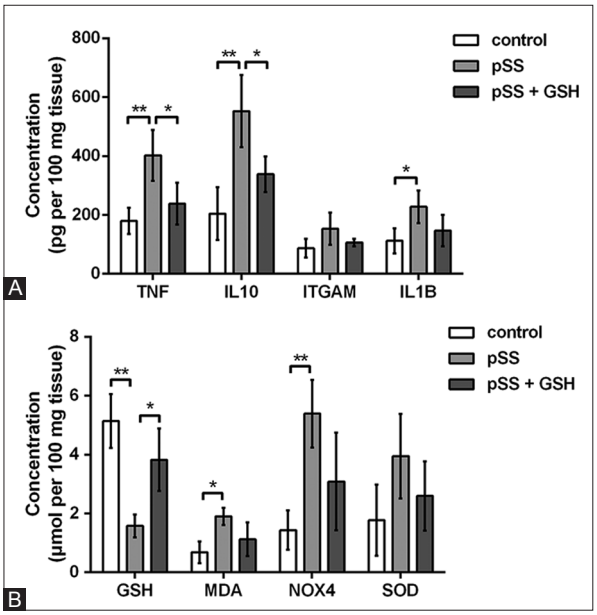

FIGURE 3. Concentration change in some inflammatory and oxidative stress-related factors in the hepatocyte homogenates (n = 4). Control: C57BL/6J mice as the control group. Primary Sjögren's syndrome (pSS): Non-obese diabetic (NOD) mice. pSS + reduced glutathione (GSH): NOD mice injected with GSH. (A) Concentration of tumor necrosis factor (TNF), interleukin 10 (IL10), integrin alpha M (ITGAM), and IL1B in the three groups, (B) Concentration of reduced GSH, malondialdehyde (MDA), nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) and superoxide dismutases (SODs) in the three groups.

of GSH failed to reach statistical significance (p = 0.1043 and 0.1189). The concentration of SODs exhibited the similar changing pattern to MDA and NOX4, but no significant change was detected (p = 0.1148 and 0.2766). These results implied that oxidative stress-related factors might be regulated by GSH during pSS.

DISCUSSION

In this study, we performed GSH administration in NOD mice to investigate the effect of GSH on a hepatic injury during pSS. The ELISA and histological examination results indicated that the up-regulated concentrations of AST and ALT in the NOD mice were inhibited by the GSH administration. GSH also showed protective effects against the hepatic injury in the NOD mice. The ELISA in the hepatocyte homogenates further indicated the regulation of some inflammatory and oxidative stress-related factors by GSH.

In the reported cases of hepatic injury induced by pSS, both a hepatocyte injury and cholangiolitic hepatitis were observed [20]. Some studies claimed that liver involvement in pSS patients is rare, and the histological features are mainly focused on primary biliary cirrhosis (PBC) [21]. However, PBC patients with pSS have a higher incidence of lymphoid non-suppurative cholangitis than those without pSS [22]. Moreover, it cannot be denied that PBC and pSS share common traits [23]. The involvement of hepatic injury in pSS was examined in this study. Higher serum AST and ALT levels were detected in the NOD mice than in the control group, indicating that pSS may be accompanied with hepatic dysfunction. Indeed, the histological examination suggested that the normal hepatic tissue structure was disturbed in the NOD mice. The GSH administration in the NOD mice decreased the serum levels of the two aminotransferases and benefited the hepatic tissue structure, implying the potential of GSH in protecting liver during pSS.

TNF, IL10, ITGAM, and IL1B are four inflammatory factors close related to hepatic injury and pSS. TNF, IL10, and IL1B are elevated in the peripheral blood of pSS patients, which may be generated from their gene polymorphisms and indicative of their crucial regulatory roles in inflammatory responses of pSS [24-26]. ITGAM, or CD11b, participates in liver protection, since the accumulation of Gr-1- and ITGAM-positive myeloid cells in the liver may help suppress T-cell activation and prevent hepatic injury [27]. The ELISA results of this study showed significant up-regulation of TNF, IL10, and IL1B in the hepatocyte homogenates of the NOD mice, which was then inhibited by the GSH administration. Inflammatory responses could be altered during pSS and GSH could influence the production of these inflammatory factors, which may be associated with the protective role of GSH during pSS. ITGAM did not show significant changes among the three groups, and we suspect that ITGAM is a further downstream effector which needs more accurate detection in future studies.

GSH in hepatocytes is crucial for the counteraction against oxidative stress and the induced hepatic injury [28]. Increased MDA production in Kupffer cells was observed concomitantly with oxidative stress-related features in hepatitis [29]. NOX4 is a major source of oxidative stress, and its inhibition reduced inflammation and fibrosis in the liver [30]. The ELISA in the hepatocyte homogenates indicated the inhibited GSH and promoted MDA and NOX4 levels in the NOD mice, which were consistent with their reported changing patterns of the oxidative stress. The GSH administration significantly promoted the GSH levels in the hepatocytes, possibly due to the elevated GSH re-synthesis, but the administration did not have marked influences on the MDA and NOX4 levels. As important antioxidant enzymes, SODs can easily respond to oxidative stress, thus being used to quantify oxidative stress in cells [31]. However, no significant changes in SODs were detected among the three groups, possibly due to the relatively short duration of our observation. More detailed information needs to be uncovered in further studies.

Taken together, NOD mice are prone to possess hepatic injury which is accompanied by pSS. GSH administration in NOD mice is capable of controlling hepatic injury, which may be associated with its regulation of some inflammatory and oxidative stress-related factors. Thus, GSH may be regarded as a potential therapeutic strategy for controlling hepatic injury during pSS, and this study provides valuable information for further research on pSS treatment.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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