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S3I-201 ameliorates tubulointerstitial lesion of the kidneys in MRL/lpr mice

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ABSTRACT

It is high incidence of tubulointerstitial lesion (TIL) in lupus nephritis (LN) and TIL can affect the prognosis of patients with LN. Signal transducer and activator of transcription (STAT) 3 was activated in LN and STAT3 inhibition could delay the onset of LN. Here, we evaluated the role of a well-known STAT3 inhibitor, S3I-201, on TIL in lupus nephritis. STAT3 was activated in MRL/lpr mice (a mouse model of lupus nephritis), and treatment with S3I-201 inhibited the activation of it. The level of 24-h urine protein and nitrogen urea increased in MRL/lpr mice and adminstration of S3I-201 reduced the level of urinary protein. In addition, S3I-201 attenuated the expression of α-smooth muscle actin (α-SMA), Fibronectin (FN) proteins, as well as the expression of monocyte chemotactic factor-1 (MCP-1) and intercellular adhesion molecule (ICAM-1). However, the expression of E-cadherin improved when treatment with S3I-201. These results revealed that the activation of STAT3 mediates tubulointerstitial lesion in mice with LN. S3I-201, by suppressing STAT3 activity, has therapeutic effect in lupus nephritis.

1. Introduction

Lupus erythematosus (SLE) is a systemic autoimmune disease that affects multiple organ systems. Involvement of the kidney, referred as to “lupus nephritis (LN)”, is a major cause of morbidity and mortality of SLE [1]. Kidney damage in patients with LN is often accompanied tubulointerstitial lesion (TIL), which portends poor long-term renal prognosis [2,3]. Yet, tubulointerstitial lesion of LN was not factored in the current therapy and its management remained to be elucidated.

Janus kinase-signal transducer and activator of transcription factor (JAK/STAT) pathway is known to play important role in the pathogenesis of LN. STAT proteins were first identified as DNA-binding proteins, which currently consists of seven members (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6). Dysfunction of STAT proteins has been implicated in numerous human immune-related disorders [4,5]. STAT3 is an important member of STAT family. It mediates intracellular signal transduction of many growth factors, cytokines and chemokines and has crucial functions in various tissues. Earlier report [6,7] has revealed that STAT3 is hyperactivated in bone marrow mononuclear cells, T cells from SLE patients and splenic B cells from lupus mice. Using small-molecule inhibitor Stattic to inhibit the activation of STAT3 in lupus-prone MRL/lpr mice, Edwards LJ [8] found that STAT3 inhibition delayed the onset of autoantibody production and proteinuria, reduced lymphadenopathy and the numbers of total T cells and Th. In addition, T cells, treated with Stattic, exhibited decreased proliferation and migration in vitro. So they proposed that STAT3 inhibition represents a therapeutic target in SLE, particularly lupus nephritis.

The above researches about STAT3 activity focused on immune cells in SLE. Arakawa T [9] reported that there was a marked increase in STAT3 activation in many forms of glomerulonephritis, including LN. In this study, we use S3I-201, which can selectively inhibit the DNA binding activity of STAT3, to reveal the effects of STAT3 inhibition on TIL in lupus nephritis.

2. Materials and methods

2.1. Animals and treatment

Female MRL/lpr and MRL/MpJ mice were provided by the Model Animal Research Center of Nanjing University. MRL/MpJ mice were used as control group (group N). And MRL/lpr mice (28 weeks)

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were randomly divided into LN group, S3I-201 treatment group, and vehicle (DMSO) control group. For treatment studies, S3I-201 (Selleck, Houston, HOU, USA) was intraperitoneally administered to MRL/lpr mice daily at a dose of 10 mg/kg for 14 days. All of the animals were housed in an environment with fixed light, temperature, and humidity as well as freely accessible food and water. At final harvest, serum, urine, and renal cortex samples were collected for further analysis. This study protocol was reviewed and approved by the Animal Care and Use Committee of Hebei Medical University.

2.2. Serum and urine analyses

Level of serum creatinine (Scr), blood urea nitrogen (BUN) and 24-h urinary protein (24 h Upro) were assessed using commercially available kits (BioSion, Beijing, China) according to the manufacturer's directions.

2.3. Immunohistochemistry

The kidney tissue sections were deparaffinized in xylene and rehydrated in graded alcohol series. Antigen recovery was performed by microwave. Then, the sections were incubated with primary antibodies against fibronectin (FN), respectively, overnight at 4 °C. The antibodies were purchased from Proteintech Group (Chicago, IL, USA). On the following day, after incubating sequentially with a biotinylated secondary antibody and horseradish peroxidase-conjugated streptavidin for 30 min at 37 °C, the sections were stained with 3,3-diaminobenzidine (DAB). As a negative control, the primary antibody was replaced by phosphate-buffered saline.

2.4. Western blot analysis

Tissue lysates were prepared according to our previous protocols [10]. The protein samples from renal cortex were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. Membranes were blocked with 5% non-fat milk for 2 h at 37 °C, followed by hybridization with anti-E-cadherin, α-SMA, MCP-1, ICAM-1, β-actin, phosphorylated signal transducer and activator of transcription (p-STAT)1, p-STAT3, total STAT1 and STAT3 antibodies overnight at 4 °C. The anti-β-actin antibody was purchased from ZSGB-BIO (Beijing, China), and the anti-p-STAT and total STAT antibodies were purchased from Cell Signaling (Boston, MA, USA). The other antibodies were purchased from Proteintech Group. Thereafter, the membranes were hybridized with a horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (ZSGB-BIO) and signals were detected by chemiluminescence techniques using Odyssey Infrared Imaging System (LI-COR, Lincoln, NE, USA).

2.5. Statistical analysis

The quantitative data are expressed as the mean ± standard deviation and analyzed by one-way analysis of variance (ANOVA) with the Student-Newman-Keuls test. $P < 0.05$ was considered to indicate a statistical significance.

3. Results

3.1. Effects of S3I-201 on the phosphorylation of STAT1 and STAT3 in kidney of MRL/lpr mice

S3I-201, known as a STAT3 inhibitor, is hypothesized to block STAT3 function by inhibiting the STAT3 DNA-binding activity. In order to investigate the mechanism of S3I-201 in LN, we examined the activation and expression of STAT1 and STAT3. As shown in Fig. 1, STAT1 and STAT3 were activated by tyrosine phosphorylation in LN mice. S3I-201 injection largely suppressed the level of p-STAT3. However the phosphorylation of STAT1 increased after S3I-201 treatment. These results suggest that both STAT1 and STAT3 were activated in kidney damage of SLE, whereas S3I-201 treatment inhibited mainly the activation of STAT3.

3.2. S3I-201 improved the renal function of MRL/lpr mice

Scr, BUN and 24 h Upro were detected (Fig. 2). The levels of BUN and Upro, instead of Scr, in MRL/lpr mice were higher than that in MRL/MpJ mice. Treatment with S3I-201 improved the level of Upro, but didn't affect the level of BUN.

3.3. S3I-201 ameliorated renal tubulointerstitial lesion of MRL/lpr mice

About half of lupus nephropathy patients were accompanied tubulointerstitial lesion (TIL), which included tubular epithelial cell injury, inflammation, and tubulointerstitial fibrosis. In LN mice, we found that tubular epithelial cells have the tendency to transdifferentiation, which was characterized by the down-regulation of E-cadherin (epithelial marker protein) and up-regulation of α-SMA (mesenchymal marker protein). S3I-201, instead of DMSO, treatment reversed their expression partially (Fig. 3 A and B).

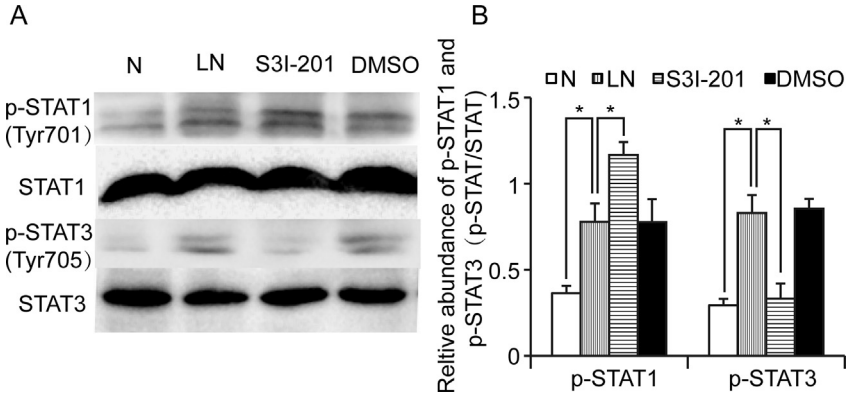

Fig. 1. The activation and expression of STAT in renal tissue. The expression of p-STAT1 and p-STAT3 was analyzed by Western blot (A) and quantified by densitometry (B) (mean ± SD). *p < 0.05.

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