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Beneficial effect of tripterine on systemic lupus erythematosus induced by active chromatin in BALB/c mice

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Abstract

The aim of this study was to determine whether tripterine, isolated from *Tripterygium wilfordii* Hoog f. in China, had beneficial effects on experimental systemic lupus erythematosus induced by active chromatin in BALB/c mice. BALB/c mice were immunized with active chromatin isolated from concanavalin A-activated syngenetic spleno-lymphocytes on day 0. Tripterine 6 or 12 mg kg^{-1} day^{-1}, or prednisone 5 mg kg^{-1} day^{-1} was given to BALB/c mice intragastrically from day 35 to day 50. Treatment with tripterine 12 mg kg^{-1} day^{-1} for 15 days protected renal from glomerular injury with a concomitant reduction of serum autoantibodies and total immunoglobulin G (IgG) also with a improvement of splenocyte proliferation stimulated with concanavalin A and lipopolysaccharide. The effects were associated with reduced interleukin-10 production and serum nitric oxide (NO) level but not interferon-γ compared with vehicle-treated control group. Tripterine 6 mg kg^{-1} day^{-1} had no significant protective effect against glomerular injury. It inhibited autoantibodies and interleukin-10 production but had no effect on splenocyte proliferation, serum NO level, and interferon-γ production. These findings suggested that tripterine had a beneficial effect on systemic lupus erythematosus induced by active chromatin in BALB/c mice.
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Keywords: Tripterine; Systemic lupus erythematosus; Chromatin; Autoantibody; Cytokine; Splenocyte proliferation

1. Introduction

Lupus nephritis is a prototype human immune complex disease. Nephritis and autoantibodies occur spontaneously in certain strains of mice, such as NZB/W (F1) and MRL/lpr. Lupus also can be induced using the hydrocarbon oil pristine (Satoh et al., 2000) or special antigen (Brosh et al., 2000) in non-autoimmune prone strains. All these murine lupus models facilitate studies on the relationship between autoantibodies formation and end organ damage, and help the screening for effective treatment. It has been found that active chromatin isolated from concanavalin A-activated syngenetic splenophocytes could induce systemic lupus erythematosus-like syndrome in BALB/c mice, a strain usually not considered to be genetically susceptible to lupus (Li et al., 2004a,b). The syndrome shares many of the characteristics of human lupus including both clinical features such as glomerular hypercellularity, glomerular immunoglobulin deposition, proteinuria, and the autoantibody profile such as anti-double stranded DNA (ds-DNA), anti-single stranded DNA (ss-DNA), and anti-histone, with complex immune abnormality.

Tripterine, one of the active components isolated from *Tripterygium wilfordii* Hoog f. in China, inhibited not only humoral and cellular immune responses but also some inflammatory reaction (Zhang et al., 1986, 1990). In vitro, tripterine inhibited interleukin-1 production from murine peritoneal macrophages induced by lipopolysaccharides, interleukin-2 production from concanavalin A-activated murine splenocytes, and prostaglandin E_2 released from synovial cells (Xu et al., 1991). Tripterine also shows therapeutic effect on collagen-induced arthritis, an animal model for rheumatoid arthritis (Li et al., 1997). The present work was to study the effect of tripterine on active

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chromatin induced systemic lupus erythematosus syndrome in mice.

2. Materials and methods

2.1. Induction of experimental systemic lupus erythematosus

Female BALB/c mice aged 6 weeks were purchased from Sino-British Sippr/BK Lab Animal Ltd. (SPF II Certificate; No 153) and bred and housed under a specific pathogen free condition with free access to food and water. Experiments followed the instructions for the care and use for animals provided by Fudan University (Shanghai, China).

Spleen cells from BALB/c mice were cultured in Rosewell Park Memorial Institute (RPMI)-1640 medium containing 10% fetal calf serum, 1 mM glutamine, 0.05 mM 2-mercaptoethanol, 100 μg/ml streptomycin, and 100 μg/ml penicillin. Some spleen cells were stimulated by concanavalin A (5.0 μg/ml), while others were kept in a mitogen-free medium for 48 h as non-activated spleen cells. Active and resting chromatin was extracted from activated and non-activated spleen cells, respectively according to Zhong methods (Zhong et al., 1994). Preparation of chromatin was performed at 4 °C. In brief, after washed with phosphate-buffered saline (PBS), spleen cells (1×10^7 cells) were homogenized in ice-cold buffer A (1:5, v/v) [0.25 M sucrose, 3.0 mM $MgCl_2$, 10 mM Tris–HCl pH 8.0, 0.6% Triton X-100, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF)] and centrifuged at $800 \times g$ for 15 min to obtain crude nuclear pellet. Purified nuclei were obtained by suspending crude nuclear pellet in 20 volumes (v/v) of ice-cold buffer B (2.2 M sucrose, 3 mM $MgCl_2$, 10 mM Tris–HCl buffer, and 0.1 mM PMSF) and centrifuging the mixture at $60,000 \times g$ for 90 min. Purified nuclei were re-homogenized in 20 volumes (v/v) of ice-cold buffer C (10 mM Tris–HCl buffer, pH 7.9, and 1 mM EDTA) and centrifuged at $800 \times g$ for 15 min. Chromatin sediment was suspended in 1% standard saline citrate (1:50, v/v; 150 mM NaCl, 15 mM Na citrate) and re-harvested by centrifuging at $800 \times g$ for 15 min. The pellet was vacuum dried and stored at −70 °C.

BALB/c mice were injected intradermally (i.d.) on the back and at the base of the tail (1–2 points) with 100 μg of chromatin emulsified in 200 μl cold Freund's complete adjuvant [containing bacillus Calmette-Guerin (BCG) 10 mg/ml] on day 0, then boosted with 200 μl (0.5 mg/ml) Freund's incomplete adjuvant on day 14. And on day 28 mice were re-boosted with an intraperitoneal injection of 200 μl chromatin suspension (0.5 mg/ml in 0.15 M NaCl). Mice were sacrificed on day 53 and serum was stored at −20 °C until measurement of antinuclear antibodies and NO levels.

2.2. Experimental protocol

Tripterine (red cubic crystal, mp 199–201 °C) was isolated by Prof De-ji Pan (Department of Pharmaceutical Chemistry, School of Pharmacy, Fudan University, Shanghai, China) according to a method described previously (Zhang et al., 1986). In brief, the alcohol extraction of the root of *T. wilfordii* was fractionated to give portions-petrol ether, ether, ethyl actate, acetone and methanol solution portions. A red crystal, tripterine (celastrol), was isolated from the ether soluble portion.

Tripterine and prednisone were ground and suspended in normal saline containing 0.5% sodium carboxymethyl cellulose (CMC) for administration, respectively. The placebo preparation was made of the same vehicle.

There were six groups in this experiment (6 mice in each group). Nil group was neither immunized nor treated. Negative group was immunized with resting chromatin. Active chromatin immunized mice were grouped randomly 35 days after immunization. One was given 0.5% CMC solution as vehicle-treated group, the other three groups were given tripterine 6 mg kg^{-1} day^{-1}, 12 mg kg^{-1} day^{-1}, or prednisone 5 mg kg^{-1} day^{-1} intragastrically from day 35 to day 50, respectively.

2.3. Immunoassay of antibodies

For the detection of specific antibodies in sera of immunized mice, enzyme-linked immunosorbent assay (ELISA) was carried out as described previously (Li et al., 2004a,b). Murine serum was diluted at 1:200 and horseradish peroxides (HRP)-conjugated goat anti-mouse IgG antibodies were diluted at 1:2000 (Sino-American Biotechnology Company, Shanghai, China). For the detection of anti-ds-DNA antibodies and anti-ss-DNA antibodies, 96-well plates (Costar, Corning, NY) were coated with calf thymus DNA (Sigma) or denatured calf thymus at 50 μg/ml. 10 μg/ml histone from calf thymus (Sigma) was used for detection of anti-histone antibodies. Optical density (O.D.) was monitored at 492 nm using a well scanner ELISA reader (Labsystems Dragon).

For the detection of total IgG, 96-well plates were coated with goat anti-mouse IgG 10 μg/ml, 100 μl/well. Sera were diluted at 1:160,000, 100 μl/well. HRP-conjugated goat anti-mouse IgG antibodies were added at a 1:40,000 dilution. The mice IgG standard (Sino-American Biotechnology Company, Shanghai, China) was used for standard curve fitting and immunoglobulin concentration calculating.

2.4. Splenolymphocytes proliferation assays

Isolated spleen cells were incubated (1×10^6–2×10^6 cells/well) in 96-well U-bottom plates with concanavalin A 6 μg/ml or lipopolysaccharide 10 μg/ml in RPMI-1640 medium supplemented with 10% fetal calf serum at 37 °C, 5% CO_2 for 48 h. After the removal of medium, 20 μl of 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT, Sigma; 5 mg/ml of medium) was added to each well, and the plate was incubated for 4 h at 37 °C. The plates were centrifuged at $800 \times g$ for 20 min and the supernatant was

carefully discarded, then 150 μl of dimethyl sulfoxide was added to each well. The color intensity was assessed with an ELISA plate reader at a wavelength of 570 nm (Labsystems Dragon). Proliferative responses were calculated as stimulation index (SI). SI = O.D. with mitogen/O.D. without mitogen (Denizot and Lang, 1986).

2.5. Nitrate measurement

Serum NO level was determined by nitrate reduction method with Griess reagents (Nanjing Jiancheng Biotechnology Co, China).

2.6. Interleukin-10 and interferon-γ measurement

For interleukin-10 detection, isolated peritoneal macrophages (1×10^6 cells/well) were added in 24-well plates (Costar, Corning, NY) and cultured in RPMI-1640 medium (1 ml) with 10% fetal calf serum for 2 h at 37 °C, 5% CO_2. After removal of non-adherent cells, lipopolysaccharide 10 μg/ml was added in each well and incubated for 24 h. For interferon-γ detection, splenolymphocytes (5×10^6 cells/well) were added in 24-well plates and cultured in RPMI-1640 medium (1 ml) with 10% fetal calf serum and concanavalin A 5 μg/ml for 24 h. The plates were centrifuged at $800\times g$ for 20 min to collect supernatant from macrophages and spleno-lymphocytes respectively.

Levels of interleukin-10 and interferon-γ in supernatants were determined respectively by using ELISA kits (Diaclone) according to the manufacturers' instructions.

2.7. Proteinuria assays and morphological examination

Proteinuria was measured by Coomassie brilliant blue test (Ma et al., 2000). Albumin (bovine serum) was used to produce standard curves. Murine urine was centrifuged at $6000\times g$ for 20 min. The supernatant was diluted at 1:10 with normal saline. The optical density was measured at 540 nm after addition of Coomassie brilliant blue solution.

Renal tissue was fixed in 3% formaldehyde and embedded in paraffin. Sections of 5-μm-thick were cut and stained with hematoxylin and eosin. Glomerular injury was blindly semi-quantified by a renal pathologist. Sections were graded as follows: 0, normal; 1, a small increase of cells in the glomerular mesangium; 2, a larger number of cells in the mesangium; 3, complex endocapillary hypercellularity sometimes with mild sclerosis or necrosis; 4, glomerular crescent formation, sclerosis, tubular atrophy and casts (Nicoletti et al., 2000). Usually, there were different grade lesions observed in a kidney, the most severe alteration was referred to as the grade of each mouse kidney and was taken into analysis.

2.8. Statistical analysis

Quantitative variables were expressed as means ± S.D. One-way analysis of variance (ANOVA) was used. If any significant change was found, *post hoc* comparisons were performed using Fisher's PLSD. Non-parametric data were analyzed by the Mann–Whitney U-test. P-values <0.05 was considered significant.

3. Results

3.1. Effect of tripterine on autoantibodies and total IgG production

Vehicle-treated group showed progressive increase in autoantibodies and IgG production compared with nil and resting chromatin-immunized group ($P<0.001$, $P<0.05$). Treatment with tripterine 6 and 12 mg kg^{-1} day^{-1} or prednisone 5 mg kg^{-1} day^{-1} for 15 days caused reduction of the three autoantibodies and IgG levels compared with vehicle-treated group ($P<0.01$, $P<0.001$). Tripterine 12 mg kg^{-1} day^{-1} had better effect in reducing anti-ss-DNA

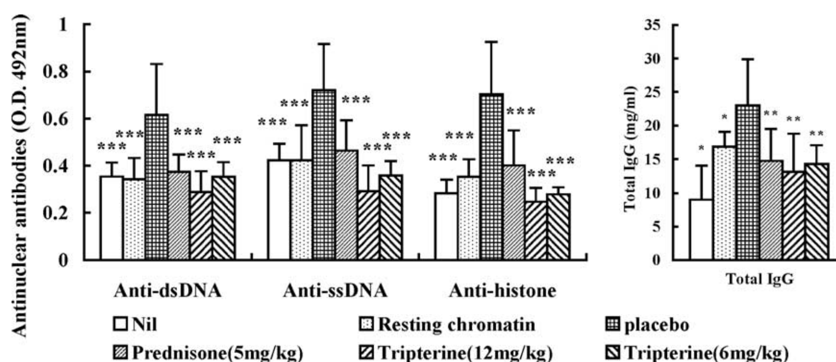

Fig. 1. The effect of tripterine on antinuclear antibodies and total IgG production in BALB/c mice. Mice were immunized with resting and active chromatin on day 0. Active chromatin immunized mice were grouped randomly and treated with tripterine 6 and 12 mg kg^{-1} day^{-1}, prednisone 5 mg kg^{-1} day^{-1} or placebo from day 35 to day 50; mice were sacrificed on day 53, serum was collected and diluted at 1:200 for antibodies detection; data were expressed as means ± S.D.; $n=6$ mice for each group. * Indicates $P<0.05$, **indicates $P<0.01$, ***indicates $P<0.001$ compared with placebo group.

($P=0.027$) and anti-histone ($P=0.033$) antibodies compared with prednisone 5 mg kg^{-1} day^{-1} (Fig. 1).

3.2. Effect of tripterine on spleno-lymphocyte proliferation

Spleno-lymphocyte proliferation in the absence of mitogen was higher in vehicle-treated group compared with that in nil and rest chromatin-treated group ($P<0.001$). But spleno-lymphocyte proliferation induced by concanavalin A or lipopolysaccharide was markedly lower in vehicle-treated group compared with nil and rest chromatin-treated group ($P<0.001$).

Treatment with tripterine 12 mg kg^{-1} day^{-1} for 15 days partly improved spleno-lymphocyte proliferation stimulated by concanavalin A and lipopolysaccharid compared with vehicle group ($P<0.05$), but had little effect on spleno-lymphocyte proliferation in the absence of mitogen. Treatment with tripterine 6 mg kg^{-1} day^{-1} for 15 days had no influence on spleno-lymphocyte proliferation in the presence or absence of mitogen compared with vehicle-treated group (Fig. 2).

Treatment with prednisone 5 mg kg^{-1} day^{-1} for 15 days inhibited the increased spleno-lymphocyte proliferation in the absence of mitogen and enhanced spleno-lymphocyte proliferation stimulated by concanavalin A ($P<0.001$) but had little effect on spleno-lymphocyte proliferation induced by lipopolysaccharid ($P=0.058$) compared with vehicle-treated group.

3.3. Effect of tripterine on interleukin-10 and interferon-γ production

Production of interleukin-10 from macrophages was elevated but production of IFN-γ from splenocytes was reduced in vehicle-treated group compared with nil- and rest chromatin-treated group ($P<0.001$, $P<0.05$).

Treatment with tripterine 6 and 12 mg kg^{-1} day^{-1} for 15 days inhibited IL-10 production ($P<0.001$) but had no influence on IFN-γ production compared with vehicle-treated group.

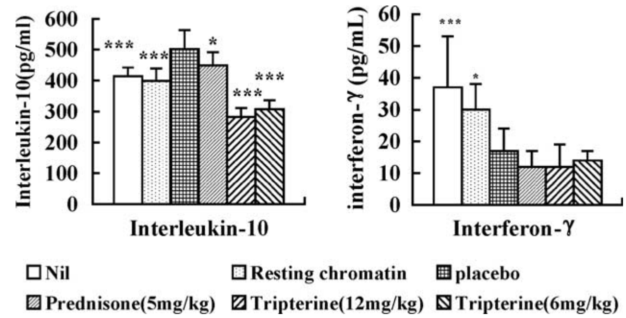

Fig. 3. The effect of tripterine on cytokine production in culture supernatants. Mice were immunized with resting and active chromatin on day 0. Active chromatin immunized mice were grouped randomly and treated with tripterine 6 and 12 mg kg^{-1} day^{-1}, prednisone 5 mg kg^{-1} day^{-1} or placebo from day 35 to day 50; mice were sacrificed on day 53. After 24 h of stimulation, interferon-γ was determined in supernatants of concanavalin A-stimulated splenocytes and interleukin-10 was measured in supernatants of lipopolysaccharide-stimulated peritoneal macrophages with ELISA kits based on standard curves. Data were expressed as means ± S.D.; $n=6$ mice for each group. *Indicates $P<0.05$, **indicates $P<0.01$, ***indicates $P<0.001$ compared with placebo group.

Treatment with prednisone 5 mg kg^{-1} day^{-1} for 15 days partly inhibited the increased IL-10 production ($P<0.05$) but had no effect on IFN-γ production (Fig. 3).

3.4. Effect of tripterine on proteinuria and serum NO level

Vehicle-treated group showed progressive increase in albuminuria compared with nil- and rest chromatin-treated group ($P<0.001$). Treatment with tripterine 12 mg kg^{-1} day^{-1} for 15 days caused a marked reduction of urinary albumin compared with vehicle-treated group ($P<0.05$). Treatment with tripterine 6 mg kg^{-1} day^{-1} or prednisone 5 mg kg^{-1} day^{-1} for 15 days caused a slight reduction of urinary albumin but the difference was not significant compared with vehicle-treated group (Table 1).

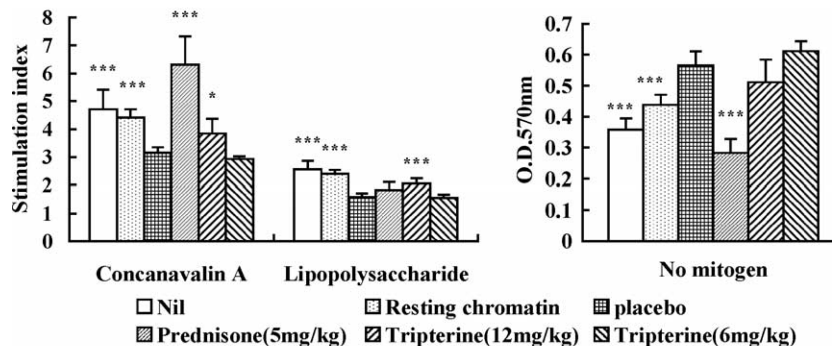

Fig. 2. The effect of tripterine on spleen lymphocyte proliferation (ex vivo) in BALB/c mice. Mice were immunized with resting and active chromatin on day 0. Active chromatin immunized mice were grouped randomly and treated with tripterine 6 and 12 mg kg^{-1} day^{-1}, prednisone 5 mg kg^{-1} day^{-1} or placebo from day 35 to day 50; mice were sacrificed on day 53, spleen lymphocytes were prepared from mice. Assay for proliferation in response to lipopolysaccharide and concanavalin A in duplicate as described in the Materials and methods and Results sections is presented as stimulation index. Cells cultured in the absence of mitogen are presented as O.D. value. Data were expressed as means ± S.D.; $n=6$ mice for each group. *Indicates $P<0.05$, **indicates $P<0.01$, ***indicates $P<0.001$ compared with placebo group.

Table 1
The effect of tripterine on kidney pathology, proteinuria and sera NO level

Group		Nephritic lesion score#	Proteinuria (mg/ml)	NO (μmol/l)
Nil	/	0 (0~0)	0.47 ± 0.15***	18.8 ± 4.2***
Resting chromatin	/	0 (0~0)	0.59 ± 0.10***	21.6 ± 8.3***
Active chromatin				
	Placebo	3 (3~3)	0.92 ± 0.15	43.6 ± 9.7
	Prednisone (5 mg/kg)	1.5 (1~3)**	0.80 ± 0.11	30.6 ± 5.3**
	Tripterine (12 mg/kg)	1.5 (1~3)**	0.75 ± 0.20*	25.6 ± 5.8***
	Tripterine (6 mg/kg)	2.5 (1~3)	0.86 ± 0.08	42.6 ± 10.7

Mice were immunized with resting chromatin and active chromatin on day 0. Active chromatin immunized mice were grouped randomly and treated with tripterine 6 and 12 mg kg^{-1} day^{-1}, prednisone 5 mg kg^{-1} day^{-1} or placebo from day 35 to day 50; mice were sacrificed on day 53 and samples were collected for detection; data expressed as means ± S.D.; $n=6$ mice for each group. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. placebo-treated group (Fisher's PLSD). #Median (minimum~maximum) tested by Mann–Whitney U-test.

Serum NO level was elevated in vehicle-treated group compared with nil- and rest chromatin-treated group ($P<0.001$). Treatment with tripterine 12 mg kg^{-1} day^{-1} for 15 days caused a marked reduction of NO level ($P<0.001$) but tripterine 6 mg kg^{-1} day^{-1} had no effect compared with vehicle-treated group. Treatment with prednisone 5 mg kg^{-1} day^{-1} for 15 days partly inhibited the elevated NO level compared with vehicle-treated group ($P<0.01$; Table 1).

3.5. Effect of tripterine on glomerular injury

Vehicle-treated group showed segmental or diffuse glomerular lesions ($n=6$). Glomerular lesions were characterized by apparent proliferation of mesangial cells in mesangium and mild influx of monocytes in the glomerular capillaries. These lesions were not apparent in nil- and rest chromatin-treated group.

Treatment with tripterine 12 mg kg^{-1} day^{-1} for 15 days markedly ameliorated the glomerular injury but tripterine 6 mg kg^{-1} day^{-1} had no significant effects (Table 1; Fig. 4).

Treatment with prednisone 5 mg kg^{-1} day^{-1} for 15 days ameliorated the glomerular injury (Table 1).

4. Discussion

Mammalian double stranded DNA has been proved to be poorly immunogenic. Immunization with resting chromatin could not induce lupus-like syndrome in BALB/c mice

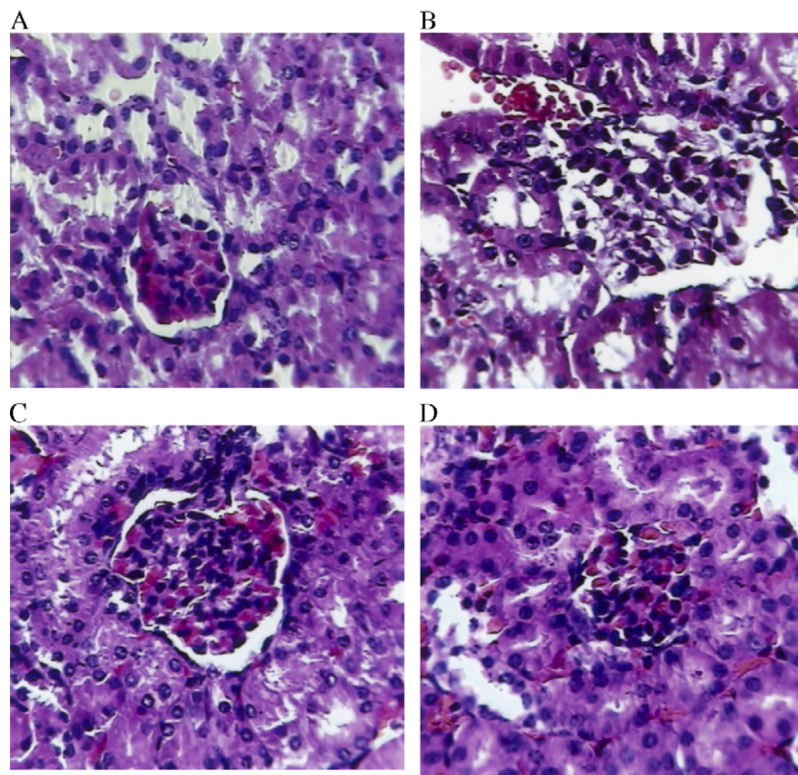

Fig. 4. The extent of renal pathological lesions were graded on a semiquantitative scale. (A–D) Light microscopy ×400. Renal tissue was fixed in 3% formaldehyde and 5-μm paraffin sections were stained with hematoxylin and eosin. (A) Grade 0: normal glomeruli, from resting chromatin immunized group. (B) Grade 3: extensive endocapillary hypercelluarity, thickening of the capillary wall, (note glomerular enlargement), from vehicle-treated group. (C) Grade 2: a larger number of cells in the mesangium, from tripterine 6 mg/kg treated group. (D) Grade 1: a small increase of cells in the glomerular mesangium, from tripterine 12 mg/kg treated group.

when compared with nil group. As the factors that might induce systemic lupus erythematosus could directly or indirectly activate lymphocytes, it is possible that the change of the antigenicity of chromatin form active lymphocytes is the common pathway to systemic lupus erythematosus pathogenesis (Burlingame and Rubin, 1996; Ma et al., 2000). Resting chromatin immunization group was served as the negative control in this experiment.

Anti-DNA autoantibodies are presented in human lupus nephritis. Active chromatin-treated mice had significantly elevated levels of IgG anti-ds-DNA at d18, anti-ss-DNA at d44 and anti-histone at d32 when compared with nil and resting chromatin group (Li et al., 2004a,b). Active chromatin immunized mice were grouped randomly and the treatment began from day 35 to day 50 according to the kinetics of antinuclear antibodies.

In untreated systemic lupus erythematosus patients, T- and B-lymphocytes as well as monocytes have immunoregulatory disturbance (Alcocer et al., 1984). Impaired responses to mitogen were also observed in MRL/lpr mice, a genetically determined mice model for systemic lupus erythematosus. These mice lack an important cell death receptor, Ras, and accumulate large amount of dysfunctional lymphocytes, most of them were $CD4^{-}CD8^{-}$ T lymphocyte (Katzav et al., 2001). In our active chromatin induced systemic lupus erythematosus model, when compared with nil and resting chromatin group, the higher spleno-lymphocyte proliferation in the absence of mitogen and the lower response to concanavalin A or lipopolysaccharide may also result from large numbers of the lymphocytes being non-functional. It shows systemic lupus erythematosus-like mice have abnormality in cellular immune response. The autoimmune pathology in these mice may be due to either impaired apoptosis of auto-reactive lymphocytes or to an autoimmune response to the lymphocytes that have inadequate apoptosis. Tripterine 12 mg kg^{-1} day^{-1} and prednisone administration partly recovered the proliferative response to concanavalin A or lipopolysaccharide stimulation.

Immunoglobulin deposition, renal histological changes and proteinuria in the active chromatin immunized mice indicate that the high levels of autoantibodies are pathogenic (Li et al., 2004a,b). Elevated plasma NO concentration is strongly associated with renal involvement in systemic lupus erythematosus patients (Ho et al., 2003). Mycophenolate mofetil, an immunosuppressive drug commonly used in organ transplantation, has its beneficial effect on lupus nephritis in MRL/lpr mice during the early period of the disease, which might be partly attributed to the inhibition of NO production (Lui et al., 2002). Tripterine 12 mg kg^{-1} day^{-1} and prednisone suppressed anti-nuclear antibodies, inhibited NO production in serum. All these could be helpful for reducing renal damage in mice. The therapy started when the symptom of the disease had appeared, this may account for the facts that tripterine 12 mg kg^{-1} day^{-1} and prednisone treated mice had less severe proteinuria and renal damage but the suppressive effect on immune response and NO production is much better.

The importance of T_{H1} and T_{H2} cytokines in lupus is controversial, perhaps reflected heterogeneity of the disease. Altered cytokine homeostasis is a feature common to human and experimental lupus. Several studies have shown that most cases of systemic lupus erythematosus appear to be mediated by T_{H2}-type cytokines. In systemic lupus erythematosus patients and in NZB/WF1 mice, there is an increase in T_{H2}-type cytokine production and a decrease in T_{H1}-type cytokine production or both (Beebe et al., 2002; Mocellin et al., 2004; Dayan et al., 2000). Tripterine and prednisone administration inhibit expression of T_{H2} cytokines interleukin-10, which may contribute to the pathogenesis of renal damage and lead to suppression of antinuclear antibodies (Appiah et al., 1999). The role of interferon-γ in systemic lupus erythematosus is complex (Nicoletti et al., 2000). There are several types of lupus nephritis, consistent with different pathogenic mechanisms. Experimental systemic lupus erythematosus can be induced by immunization with the human monoclonal anti-DNA antibody bearing the common idiotype 16/6 Id. The establishment of this experimental systemic lupus erythematosus in young mice is associated with a characteristic pattern of cytokine response, manifested by an initial increase in the pro-inflammatory cytokines, interleukin-1 and tumor necrosis factor-á followed by a peak in the production of the T_{H1}-type cytokines, interleukin-2 and interferon-γ. The T_{H2}-type cytokines, interleukin-4 and interleukin-10 peak later, at the time that clinical manifestations are observed. At this stage, the levels of the T_{H1}-type cytokines are lower than those in control mice (Segal et al., 2003). In our experiment, interferon-γ in supernatants of splenocytes was detected when the mice had lupus nephritis already, the level of interferon-γ was also decreased in our active chromatin immunized mice. Further studies will be needed to determine if experimental systemic lupus erythematosus induced by immunization with active chromatin is also associated with such a characteristic pattern of cytokine response. Tripterine and prednisone had little effect on interferon-γ production.

The treatment of systemic lupus erythematosus is mainly based on a number of traditional drugs such as corticosteroids, antimalarials, azathioprine and cyclophosphamide. As our knowledge on the mechanisms of immune response increases, new drugs that can interfere with T and B cell interaction and activation, production of anti-ds-DNA autoantibodies, immune-complexes deposition and cytokine activation have been developed and some of these are now under investigation in systemic lupus erythematosus. The use of these drugs might represent the keystone in the future management of systemic lupus erythematosus and other autoimmune diseases (Mosca et al., 2001). In the present study we explored the possible therapeutic potential of the tripterine in active chromatin induced systemic lupus erythematosus in BALB/c mice. The results show that

tripterine treatment (12 mg/kg/day) for a period of 15 days had a number of significant beneficial effects on the diseased BALB/c mice. The treatment resulted in a regained splenocyte proliferation to concanavalin A, lipopolysaccharide, and in a significant decreased antinuclear antibodies. The elevated levels of plasma NO and interleukin-10 were reduced. These immunological effects were associated with amelioration of proteinuria and histological changes. Studies have demonstrated that tripterine induced apoptosis in human T cell line CEM26T (Bao et al., 2003), decreased lipopolysaccharide-stimulated interleukin-1β and interleukin-1α production by human monocytes (He et al., 1998), which may also benefit in inhibiting further renal damage in the lupus-like mice.

Tripterine 12 mg/kg has a similar effect as prednisone 5 mg/kg in treating experimental systemic lupus erythematosus and has an even better effect on reducing antinuclear antibodies. Tripterine, an immunosuppressive agent, defines its effect mainly on the immune system without steroid-induced adverse reaction (Zhang et al., 1990). As chronic treatment often accompanies serious side effects, tripterine may have a less toxic effect when compared with prednisone.

In conclusion, our study demonstrated that tripterine could be a benefit for the improving of the abnormality in immune systems in mice, slowing down the renal damage. The result raises the possibility that tripterine could be a new agent for the treatment of autoimmune disease.

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