Effects of Shikonin Isolated from Zicao on Lupus Nephritis in NZB/W F1 Mice

Xin Chang WANG, Jian FENG, Feng HUANG, Yong Sheng FAN, Yan Yan WANG, Ling Yong CAO and Cheng Pin WEN

A Department of Rheumatology, Chinese PLA General Hospital; No. 28 Road Fuxing, Beijing 100089, China; B Department of Pharmaceutics, Zhejiang Chinese Medical University; and C School of Basic Medical Sciences, Zhejiang Chinese Medical University; No. 548 Road Binwen, District Binjiang, Hangzhou 310053, China.

Received May 12, 2009; accepted June 24, 2009; published online June 29, 2009

The present study was performed to evaluate the potential protective effects of Shikonin extracted from Zicao on lupus nephritis (LN) using NZB/W F1 mice. Oral administration of Shikonin (24 mg/kg body weight/d) or vehicle was applied to sixty female NZB/W F1 mice of 28-week-old with LN. Treatment with Shikonin for 14 weeks suppressed proteinuria dose-dependently with the mean proteinuria of 274.0 mg/dl and 160.3 mg/dl for low-dose and high-dose Shikonin groups, respectively, compared to 499.2 mg/dl for the vehicle. Also, Shikonin was observed to reduce circulating adhesion molecules significantly and down-regulate intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) mRNA expression in kidney. However, anti-double stranded (ds)DNA antibody in mice with low or high Shikonin dose administration both exhibited no significant elevation, differing from vehicle group. Kidney histological examination showed that renal glomerular lesions were alleviated after Shikonin application. These results suggest that Shikonin has therapeutic effects on LN in NZB/W F1 mice, to which inhibition of anti-dsDNA may be potential contribution, and its part mechanism is related to suppression of mRNA expression of cell adhesion molecules (CAMs) in the kidney.

Key words Shikonin; lupus nephritis; Zicao; NZB/W F1 mouse

Renal involvement in systemic lupus erythematosus (SLE) is a common manifestation and a strong predictor of poor outcome. Renal disease continues to cause major morbidity and some mortality for around 30—40% of patients with SLE. The recommended treatment for patients with lupus nephritis (LN) varies from patient to patient and conventional drugs are associated with marked toxicity. New drugs proposed for LN have to be at least as effective as and less toxic than existing therapies.1—4) Cell adhesion molecules (CAMs) including vascular cell adhesion molecule-1 (VCAM-1), E-selectin and intercellular adhesion molecule-1 (ICAM-1), are essential for cellular interactions and important in the activation and adhesion of cells.5,6) The up-regulation and enhanced expression of cell surface adhesion molecules on polymorphonuclear leukocytes and on normally non-adhesive vascular endothelium, lead to the adherence of inflammatory cells to the vessel wall and to their subsequent extravasation.7) Studies have shown that elevated levels of soluble VCAM-1 (sVCAM-1), soluble E-selectin (sE-selectin) and soluble ICAM-1 (sICAM-1) are related to disease activity in patients with various acute and chronic inflammatory diseases.8—10) The same relationships between LN activity and serum level of sVCAM-1 and sICAM-1 have been observed.11,12)

Zicao (purple gromwell), the dried root of Lithospermum erythrorhizon Sieb. et Zucc., Arnebia euchroma (Royle) Johnst., or Arnebia guttata Bunge, is a commonly used herbal medicine in China. Shikonin, a major active chemical component isolated from Zicao with a molecular weight of 288, has many pharmacological properties, including anti-inflammatory and anti-tumor properties. It has also been shown to be able to promote wound healing activity.13,14) Earlier studies have demonstrated that Zicao extract possesses multiple pharmacological activities. For example, the herbal extract can stimulate glucose uptake and potentiate insulin-stimulate glucose uptake in a concentration-dependent manner in 3T3-L adipocytes.15) It has been used to prevent or treat diabetic nephropathy and glomerulosclerosis,16) inhibit human mesangial cells proliferation, apoptosis, and extracellular matrix.17)

The (NZW X BXSB) F1 (W/BF1) mice is known as an autoimmune-prone strain which develops LN, thrombocytopenia due to platelet-specific autoantibodies, leukocytosis, and myocardial infarction.18) To estimate the potential therapeutic effect of Shikonin on LN in the present study, the NZB/W F1 mice of 28 weeks old with established nephritis were treated orally with vehicle only or the Shikonin for a total of 14 weeks. The change of each group’s proteinuria, anti-double stranded (ds)DNA, circulating adhesion molecule were observed in order to estimate the therapeutic effect of Shikonin on LN mice.

MATERIALS AND METHODS

Animals and Treatment Regimens Sixty eight-week-old female NZB/W F1/J mice were purchased from Jackson Laboratory (Bar Harbor, ME, U.S.A.) and maintained in a conventional animal housing facility throughout the experiment. At age 28 weeks, the animals were randomly divided into three groups (vehicle, Shikonin low dose and Shikonin high dose). Vehicle (2% dimethyl sulfoxide in water), the Shikonin at 24 mg/kg (equivalent to 1/20 of the LD50) and the Shikonin at 48 mg/kg (equivalent to 1/10 of the LD50) were orally administered respectively. Shikonin was dissolved in the vehicle solution to obtain an appropriate concentration for oral application (about 0.4 ml per animal per dose). Based on the information obtained from a pilot study that the half life of Shikonin was 7.2 h after oral administration, treatment was given daily, 5 d a week from Monday to Friday for a total of 14 weeks. The body weight was monitored weekly. If an...
animal lost more than 15% of body weight, treatment was terminated and the animal euthanized. Otherwise, mice were sacrificed after 14 weeks of treatment. Blood, kidneys, thymus and spleens were sampled from all experimental mice, including those dying before the completion of treatment and those completing the treatment protocol. All experiments were performed in accordance with the guidelines for the care and use of animals as established by Zhejiang University.

**Urine Collection and Proteinuria Assay** Urine from individual mouse was collected biweekly from the age of 24 weeks using metabolic cages. Proteinuria was tested by dipstrip (Chemstrip, Roche) and semiquantified as 0, ± (0 to 30 mg/dl), + (30 to 100 mg/dl), ++ (100 to 500 mg/dl) and +++ (+500 mg/dl). Proteinuria was also analyzed by spectrophotometer using a bichinchoninic acid based bichinchonic acid (BCA) protein assay kit (Pierce, Rockford, IL, U.S.A.) and standardized with bovine serum albumin (BSA). In the entire course of the study, proteinuria was measured first with the urinary analysis strips (Chemstick) followed by the spectrophotometer. It was found that results obtained from the Chemstick correlated with those obtained from the spectrophotometer. Because proteinuria was quantified more accurately, data generated using the spectrophotometer was used to plot the figures.

**Assay for Blood Urea Nitrogen** Serum blood urea nitrogen (BUN) was determined by the laboratory of the Zhejiang TCM hospital.

**Enzyme-Linked Immunosorbent Assay (ELISA) for Anti-dsDNA Antibodies** EIA/RIA plates (96-well; Corning Inc., Corning, NY, U.S.A.) were coated with 50 μl of 10 μg/ml type XV calf thymus DNA (Sigma, St. Louis, MO, U.S.A.) and left overnight at 4°C. After washing, the plates were blocked with 10% BSA/phosphate buffered saline (PBS). The plates were incubated with diluted serum (first dilution of 1:20 followed by serial 3-fold dilutions) for 2 h at room temperature. After washing with 0.5% Tween-20/PBS, the plates were incubated with alkaline phosphatase conjugated goat anti-mouse immunoglobulin G (IgG) (Fab) for 1 h followed by the alkaline phosphatase substrate p-nitrophenyl phosphate. The reaction was quenched by the addition of 25 μl of 5 N NaOH solution. The plates were assayed with a micro-plate spectrophotometer (Bio-Tek Instrument Inc., Winooski, VT, U.S.A.) at 405 nm. Sera from 10 C57BL6/J mice were employed as normal controls.

**Circulating Adhesion Molecule Assay** Serum levels of sVCAM-1 and sICAM-1 were measured with commercially available sandwich ELISA (sVCAM-1 was obtained from R&D Systems, Minneapolis, MN; sICAM-1 from Predicta, Cambridge, MA, U.S.A.). All measurements were made in duplicate. Microtiter plates were coated with a murine IgG class monoclonal antibody (MoAb) directed against one epitope on sVCAM-1 or sICAM-1. After samples were incubated in an appropriate dilution (VCAM-1, 1:50; ICAM-1, 1:100), a biotinylated murine IgG class MoAb directed against a second epitope on sVCAM-1 or sICAM-1 was added to the preparation. After the addition of streptavidin-conjugated horseradish peroxidase, a color reaction was obtained with tetramethylbenzidine and the plates were read by an automated multiscanner. Concentrations of circulating adhesion molecules were calculated using a standard curve.

**Pathological Study of Kidneys** Kidneys were harvested from the mice after spontaneous death or euthanasia. One of the freshly harvested kidneys was fixed in buffered 10% formalin and embedded in paraffin blocks. Seven micron thick sections were cut and stained with hematoxylin and eosin (Histoserv, Germantown, MD, U.S.A.). Sections were graded semi-quantitatively by two veterinary pathologists for glomerular lesions. Glomerular injury as defined by mesangial cellularity, capillary wall adhesions to Bowman’s capsule and inflammatory exudates, were scored blindly by a five-tier qualitative system (Table 1) from grade 0 to grade 4 (with 4 being the most severe) after examining 20 consecutive glomeruli.

**RNA Isolation and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)** Total RNA from 100 milligram kidney tissue was isolated by use of the TRIZOL reagent (Life Technologies, Grand Island, N.Y.). RT-PCR was performed with the GeneAmp RNA PCR kit (Roche Molecular Systems, Branchburg, NJ, U.S.A.). Thirty cycles were used for the amplification of ICAM-1, VCAM-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sequences. The primers specific for ICAM-1 were 5'ATCCCAAGGCTGACACCC3' and 5'ACATAAGAGCCTGCACTACG3', VCAM-1 were 5'CCTCAGACATTTACCGAGTT3' and 5'ACTCTGCTCTTGTGTTGGT3', GAPDH were 5'TGAAGGTCGAGTCAACGAGT3' and 5'GTGAGACGCCAGTGGACTC3'. PCR products were identified on 2% agarose gels after ethidium bromide staining and were documented photographically. To ensure the quality of the procedure, RT-PCR was also performed with primers specific for GAPDH. The relative levels of expression of ICAM-1 and VCAM-1 were quantified with the Image J program.

**Spleen and Thymus Weight** The animals were weighed at the time of sacrifice following which the spleen and thymus were excised and weighed.

**Statistical Analysis** For data from all NZB/W F1 mice that completed at least four weeks of the treatment, an intention-to-treat analysis was carried out. All statistical tests were two sided. Comparison of the mean values of individual variables measured at each time to the corresponding base-line values for mice of the same group was carried out using the Student’s t-test. The Kruskal–Wallis test was employed to compare each variable between groups before and after treatment. For proteinuria data, a last observation-carried-forward approach was used for the mice that died before the end of study.

### Table 1. Histological Grading of Renal Tissue

<table>
<thead>
<tr>
<th>Grade</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No glomerular lesions</td>
</tr>
<tr>
<td>1</td>
<td>Minimal thickening of the mesangium</td>
</tr>
<tr>
<td>2</td>
<td>Noticeable increase in both mesangial and glomerular cellularity</td>
</tr>
<tr>
<td>3</td>
<td>Above + superimposed inflammatory exudates and capsular</td>
</tr>
<tr>
<td>4</td>
<td>Adhesions</td>
</tr>
<tr>
<td></td>
<td>Obliteration of glomerular architecture in &gt;70% of glomeruli</td>
</tr>
</tbody>
</table>

**Outcome** Twenty mice were initially included in each group. One mouse from each group had severe proteinuria at...
the beginning of administration and was dead within four weeks of starting the treatment. Two mice from the vehicle group unexpectedly died of acute pulmonary edema caused by improper gavage. Data from these five mice were excluded from analysis of treatment efficacy. Two mice from the vehicle group and each mouse from two treatment groups completed more than four weeks of treatment, but died before the end of the treatment course after development of severe proteinuria and elevated BUN, suggesting disease-related death. These mice were not included in the analysis. Two mice from the Shikonin high dose group were euthanized at the eighth and ninth week of the Shikonin treatment because of weight loss reaching 15% of baseline. Despite this fact, there was no significant difference in the mean body weight between the three groups either before or after treatment (44.3 g, 41.5 g and 40.8 g before starting treatment and 40.1 g, 40.5 g and 39.2 g after treatment for vehicle, Shikonin low and Shikonin high dose groups, respectively). These mice were also included in the analysis.

**Therapeutic Effect of the Shikonin on Proteinuria and Renal Function**

All mice at age 28 weeks exhibited significant proteinuria (30 mg/dl or higher) before starting the treatment course. At this time, the mean proteinuria for the vehicle, Shikonin low and Shikonin high dose groups were 109.63 ± 10.27 mg/dl, 111.28 ± 14.63 mg/dl and 105.91 ± 12.48 mg/dl, respectively (Fig. 1). Notably, during the treatment course, proteinuria increased in the mice treated with vehicle, with a mean proteinuria of 300 mg/dl at the age of 36 weeks. Of 15 animals in the vehicle group, 13 (86.5%) developed severe proteinuria (>500 mg/dl) before the end of the study (age of 42 weeks). Only one mouse in the vehicle group continued to have proteinuria below 100 mg/dl at the end of study. In contrast, 12 mice (75%) from the Shikonin high dose group had 30 to 100 mg/dl of proteinuria at the end of treatment. Proteinuria worsened in only 4 animals. In 2 of the 4 mice, worsening of proteinuria was noted 2 and 4 weeks after starting treatment, respectively. Two mice developed severe proteinuria before starting treatment that was unchanged during the treatment course. Similar to the Shikonin high group, proteinuria was improved or maintained at a low level throughout the 14 week treatment in 8 out of 18 mice in the Shikonin low group. In 4 animals from the Shikonin low group, proteinuria was maintained at a low level and increased only at the very end of the treatment course. Four mice from the Shikonin low dose group had severe proteinuria at the beginning of treatment without improvement. Figure 1 shows the comparison of proteinuria between groups before and after treatment. There was no significant difference in mean proteinuria between the three groups at the beginning of treatment. At the end of the treatment course, however, mean proteinuria was 499.2 ± 33.61 mg/dl, 274.0 ± 22.36 mg/dl and 160.3 ± 17.58 mg/dl for the vehicle, Shikonin low and Shikonin high groups, respectively (p<0.005, vehicle versus Shikonin low; p<0.0001, vehicle versus Shikonin high).

The level of BUN was found to increase, which correlated with the severity of proteinuria in 3 groups. As shown in Fig. 2, 10 out of 15 tested animals from the vehicle group had increased BUN ranging from 47 to 152 mg/dl (normal range <27 mg/dl). In contrast, in 10 out of 18 mice from the Shikonin low dose group and 13 out of 16 mice from the Shikonin high dose group, BUN was within normal range. The mean BUN was 86.33 ± 7.72 mg/dl, 27.75 ± 4.31 mg/dl and 19.09 ± 2.90 mg/dl for the vehicle, Shikonin low and Shikonin high dose groups, respectively (p<0.001).

**Changes in Serum Levels of Anti-dsDNA Antibody**

Autoantibody against dsDNA was examined before and after treatment. Compared to those from normal C57BL6/J mice, sera from the NZB/WF1 mice of the three groups contained higher titers of anti-dsDNA antibody at 28 weeks of age. The relative titers of anti-dsDNA at this time for C57BL6/J mice ranged from 0.1 to 0.3, with an average of 0.25. All NZB/WF1 mice in the study exhibited elevated titers of this autoantibody, with averages of 1.11 ± 0.21, 1.27 ± 0.26 and 3.9 ± 0.15 for the vehicle, Shikonin low and Shikonin high dose groups, respectively. The levels of the anti-dsDNA autoantibody increased during the treatment course for all of the three groups. At the end of study, the mean anti-dsDNA titer was 2.07 ± 0.32, 1.56 ± 0.20 and 1.64 ± 0.12 for the vehicle, Shikonin low and Shikonin high dose groups, respectively (Fig. 3). As compared with that before treatment, anti-dsDNA antibody at the end of treatment increased markedly for the vehicle group (p<0.05). In contrast, there was no significant difference in the mean titers of this autoantibody before and after Shikonin treatment (p>0.05), either Shikonin low or Shikonin high group.

**Level of Circulating Adhesion Molecule**

The mean sICAM-1 was 7.6, 5.5 and 4.3 mg/l for the vehicle, Shikonin low and Shikonin high groups, respectively. The mean sVCAM-1 was 20.61, 18.746 and 15.93 mg/l for the vehicle, Shikonin low and Shikonin high groups, respectively (p<0.05). In contrast, there was no significant difference in the mean sICAM-1 and sVCAM-1 levels of each group.

![Fig. 1. Effect of Shikonin on Proteinuria in NZB/W F1 Mice](image1)

Proteinuria was determined by spectrophotometer as described in Materials and Methods. Data are the means±2-standard error of the mean of each group. Proteinuria was compared between groups before and after treatment. No significant difference was determined before treatment between the three groups. After treatment, proteinuria was significantly higher in the vehicle treated mice than in mice treated with the Shikonin (p<0.05, Shikonin low versus vehicle; p<0.01, Shikonin high versus vehicle).

![Fig. 2. Renal Function (BUN) of the NZB/W F1 Mice after Treatment](image2)

Numbers in parentheses indicate the animals examined. The horizontal bars indicate the median values of each group. **p<0.01, vehicle versus Shikonin low; ***p<0.001, vehicle versus Shikonin high.
The sVCAM-1 and sICAM-1 levels were significantly decreased in the Shikonin treatment groups compared to those in the vehicle group. The change of the Shikonin high group was more significant than those in the Shikonin low group. (Table 2).

### Spleen and Thymus Weight versus Body Weight Ratio (×100)

Our results showed that the mice’s spleen and thymus weight versus body weight ratio did not have a significant difference between the Shikonin treatment groups and vehicle group at the end of study (Table 3).

#### Shikonin Down-Regulates ICAM-1 and VCAM-1 mRNA Expression in Kidney

Our observation showed that in the mice treated with Shikonin, the expression of ICAM-1 and VCAM-1 mRNA became down-regulated compared to the vehicle group. The down regulation in the Shikonin high group was also more significant than in the Shikonin low group. (Table 3).

**DISCUSSION**

It is well recognized that renal involvement contributes substantively to the morbidity of patients with SLE. Early treatment of LN and the prevention of end stage renal disease are important objectives in management of patients with SLE.

In the present study, we selected NZB/W F1 mice to examine the potential therapeutic role of the Shikonin in SLE. The study was designed to start the therapy of the NZB/W F1 mice at the age of 28 weeks because all mice at this time had positive anti-dsDNA antibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive autoantibody titers and more than 90% of mice at the age of 28 weeks because all mice at this time had positive anti-dsDNA antibody titers and more than 90% of the mice had detectable proteinuria, indicating that autoimmune nephritis was established before the start of treatment in these mice.21) Our study showed that 93% of mice treated with vehicle had progressive glomerulonephritis, documented by severe proteinuria, elevated BUN and histological abnormalities at the end of the study. In contrast, serum BUN and proteinuria were significantly lower or maintained at mild levels of disease in most mice treated with the Shikonin, suggesting that the Shikonin exerted a therapeutic effect on established LN in NZB/W F1 mice. Similarly, anti-dsDNA titers increment occurred progressively throughout the experiment for all mice with vehicle treatment. Through Shikonin administration, however, the mean titers of the autoantibody in mice both low and high Shikonin groups exhibited no significant elevation, respectively. It indicated evidently that inhibition of anti-dsDNA may be potential contribution to ther-
apeutic effect of Shikonin on LN in NZB/W F1 mice, though its mechanism is still unknown now and also to be studied in our upcoming experiment. The finding that the Shikonin is effective as a therapy for established NZB/W F1 murine lupus suggests that its efficacy may be more easily translated to treatment of human lupus.

The data presented suggest that treatment of NZB/W F1 mice with Shikonin was effective in controlling the renal pathology in a dose-dependent manner and was not associated with the thymus and spleen index. Overall, the mice tolerated the treatment well without any gross and adverse effects by either agent. The treatment schedules were chosen with the intention to simulate some clinical protocols but because of the paucity of relevant data in the literature on murine models, a standard protocol was not available.

SLE is the prototypic systemic autoimmune disease. Adhesion molecules are of central importance in (auto)immune and inflammatory responses because they mediate the interactions of hematopoietic cells with endothelial cells during extravasation and homing. They increase T-cell–antigen-presenting-cell contact and deliver the necessary signals for effective B-cell activation and both T-helper and T-cytotoxic cell function.22) Aberrations in cell adhesive interactions in patients with autoimmune rheumatic diseases, including SLE and systemic sclerosis (SSc), have been reported. Overexpression of adhesion molecules at sites of inflammation and autoimmune injury in these patients has been shown at the mRNA level, as well as at the protein level, with specific monoclonal antibodies.23) CAMs regulate the interaction between leucocytes and renal parenchymal cells and are likely to play a major role in renal inflammation. CAMs expressed by the human kidney include the glycoproteins, ICAM-1 and VCAM-1, which belong to the immunoglobulin super gene family. The specific interaction of subsets of leucocytes with endothelium expressing VCAM-1, ICAM-1 and E-selectin is important in the adhesion of leucocytes to the endothelium and their transmigration across the endothelium to sites of inflammation.24,25) In addition, the interaction between lymphocytes and ICAM-1 and VCAM-1 provides an important co-stimulatory signal for T lymphocyte activation.26,27) Normal human glomerular endothelium expresses ICAM-1, and Bowman’s capsule cells express ICAM-1 and VCAM-1.28—30)

Immunohistochemical expression of E-selectin, VCAM-1, and ICAM-1 in skeletal muscle with perivascular infiltrates, in the renal glomerulus and on lesional, non-sun-exposed skin is increased evidently in patients with SLE versus healthy controls,23,29) especially during disease exacerbations.31) Furthermore, the elevated mean concentrations of both soluble ICAM-1 and VCAM-1 in serum were also observed significantly, and it is believed to correlate with both individual disease activity and erythrocyte sedimentation rate in patients with SLE, respectively.32—36,38) The sVCAM-1 level may be a useful marker of LN activity.38) Also, the same correlation between increased renal tubule expression of ICAM-1 and disease activity were proved.37) The increased expression of these adhesion molecules on activated endothelium is considered to represent a mechanism by which various leukocyte populations can be rapidly recruited into a site of inflammation or tissue injury. Actually, autoimmune MRL/lpr and NZB/W F1 mice show increased steady state levels of ICAM-1 transcripts in the kidney compared increased with normal or prenephritic mice.39) Elevated levels of VCAM-1 were expressed not only in the endothelium of MRL/lpr kidneys, but also in the cortical tubules and glomeruli.40) Accordingly, CAMs have an important role in the infiltration of LN with mononuclear cells and seem to play a part in the initiation and progression of the disease.
In the present study, the concentrations of sICAM-1 and sVCAM-1 were significantly raised in all NZB/NZW F1 mice, respectively. Also, the mRNA of ICAM-1 and VCAM-1 was highly expressed in the kidney of NZB/NZW F1 mice. However, the downregulated mRNA expression levels of ICAM-1 and VCAM-1 in the kidneys of the NZB/NZW F1 mice were found significantly in most mice treated with the Shikonin. It suggests that the mechanism by which Shikonin treats LN was to inhibit the CAMs produced from the kidney endothelial cell and stimulated by various cytokines.

CONCLUSION

In conclusion, the present study has shown that Shikonin, a major active chemical component extracted from Zicao, had a therapeutic effect for LN in NZB/W F1 mice. Its mechanisms may be associated with inhibition of the mRNA expression of CAMs in the kidney. Therefore, Shikonin may clinically provide a potential therapeutic approach for LN patients.

Acknowledgement This work was partly supported by Project No. 39870921 of the National Natural Science Foundation of China.

REFERENCES