Inflammation, Vol. 42, No. 3, June 2019 (© 2018)
DOI: 10.1007/s10753-018-0943-z

ORIGINAL ARTICLE

Schisandrin B Improves the Renal Function of IgA Nephropathy Rats Through Inhibition of the NF-κB Signalling Pathway

Jian-Hua Qin,[1] Jia-Ru Lin,[1] Wen-Fei Ding,[1] and Wei-Hua Wu[1,2]

Abstract—Schisandrin B (SchB) is an active compound extracted from the Chinese herb *Schisandra chinensis* and shows excellent anti-inflammatory activity. This study was performed to examine the effects of SchB in a rat model of IgA nephropathy (IgAN). IgAN was established in Sprague-Dawley rats by immunization with lipopolysaccharide (LPS), bovine serum albumin, and carbon tetrachloride. Renal function was evaluated by determining the levels of urinary red blood cells, proteinuria, blood urea nitrogen (BUN), and creatinine (Cr). Renal tissue and protein samples were collected for further analysis. Pre-treatment and treatment with SchB significantly ameliorated renal function of IgAN rats, which was evidenced by decreased levels of proteinuria, hematuria, BUN, and Cr. IgAN rats exhibited increased serum IgA, renal IgA deposition, mesangial cell proliferation, and inflammatory cell infiltration, which were significantly attenuated by intervention with SchB. Moreover, SchB inhibited infiltration of CD3+ and CD11b+ cells, decreased levels of tumour necrosis factor-alpha, interleukin-1β, and monocyte chemoattractant protein-1 in the kidney, and decreased the numbers of CD3+CD69+ cells in the spleen. Of note, SchB therapy significantly increased cytoplasmic p65 and IκB expression and decreased nuclear p65 levels both in the damaged renal tissue and LPS-stimulated HK-2 cells, indicating a direct inhibitory effect on the NF-κB pathway in IgAN rats. Taken together, our data provide insight into a new application of SchB for the treatment of IgAN and represent a novel mechanism behind these effects.

KEY WORDS: schisandrin B; IgA nephropathy; renal function; TNF-alpha; NF-κB.

INTRODUCTION

IgA nephropathy (IgAN) is the most common glomerulonephritis (GN) worldwide. It has been recently reported that the prevalence of IgAN is modest in the United States (10–20% of primary glomerulonephritis), higher in some European countries (20–30%), and highest in developed countries in Asia (40–50%) [1]. Development of IgAN causes progressive renal function impairment, finally resulting in end-stage renal disease (ESRD). Around 15–20% of biopsy-proven IgAN develops into ESRD in 10 years and 20–30% does so in 20 years [2]. For ESRD, the patients have no choice but to receive dialysis and kidney transplantation, which increases in cost yearly and becomes a heavy burden. On the other hand, no specific, safe, and effective therapy is available for IgAN presently. Glucocorticoid

[1] Department of Nephrology, Affiliated Hospital of Southwest Medical University, No. 25, Tai Ping Street, Jiangyang District, 646000, Luzhou, China
[2] To whom correspondence should be addressed at Department of Nephrology, Affiliated Hospital of Southwest Medical University, No. 25, Tai Ping Street, Jiangyang District, 646000, Luzhou, China. E-mail: younger1672@163.com

steroids have been widely used to treat IgAN patients, but their efficacy in preserving renal function and reducing proteinuria in IgAN remains unclear. Moreover, the adverse side effects are a concern because of the potential uncontrollable immunosuppressive effects caused by long-term use of glucocorticoids [3, 4]. Therefore, it is of great importance to find a novel and efficient way to inhibit IgAN progression.

Primary IgAN is well-known to be characterized by the deposition of the IgA antibody in the glomerulus, followed by the manifestation of mesangial cell proliferation, and extracellular matrix expansion. Although the mechanism of IgAN is complicated and not fully understood, it has been proven that renal inflammation and immune disorders are definitely involved in the development of IgAN. Briefly speaking, in IgAN progression to chronic renal failure, renal function closely correlates with high levels of mononuclear leukocytic infiltration into the kidney [5, 6]. The mononuclear leukocytes are often found around inflamed glomeruli and consist mainly of T cells, macrophages, and dendritic cells [6]. Moreover, Stangou et al. have stated that inflammatory cytokines were directly implicated in the renal pathology of IgAN and have a central role in inflammation and progression of kidney injury [7].

Schisandra chinensis (*S. chinensis*) is a famous traditional Chinese herb and possesses diverse biological activities. Schisandrin B (SchB) is the most abundant dibenzocyclooctadiene derivative found in *S. chinensis*. In previous reports [8–10], SchB shows anti-oxidative, anti-inflammatory, and hepatoprotective properties. In *in vivo* studies, SchB ameliorated myocardial ischemia/reperfusion injury, prevented chronic cardiotoxicity, and protected against tert-butylhydroperoxide-induced cerebral toxicity [11–13]. *In vitro* research has observed that SchB treatment regulated Th1/Th17 differentiation, inhibited inflammatory cytokine secretion, and blocked inflammation-related molecular pathways in several cell models [14–17]. Moreover, it has been recently demonstrated that SchB prevents cyclosporine A (CsA)- and gentamicin-induced nephrotoxicity in animal models both *in vivo* and *in vitro* [18, 19]. In agreement with these results, Stacchiotti and colleagues confirmed the protective effects of SchB on the mercury-induced renal damage *in vivo* and *in vitro* [20]. Therefore, in the present study, we tested the hypothesis that the application of SchB might prevent the progression of IgAN in a rat model by inhibiting renal inflammation.

MATERIALS AND METHODS

Cell Culture and Treatment

HK-2 cells, an adult human proximal tubule epithelial cell line, were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). HK-2 cells were cultured in Dulbecco's-modified Eagle's medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (Gibco, Gaithersburg, MD) in a humidified incubator with 5% CO_2 at 37 °C. HK-2 cells were stimulated by lipopolysaccharide (LPS, 5 μg/m, Sigma-Aldrich, St. Louis, MO, USA) for 12 h, and 10.0 μM of SchB (purity beyond 98%, Sigma-Aldrich, St. Louis, MO, USA) was added to the culture for 12 h.

IgAN and Treatment

A total of thirty-six healthy male Sprague-Dawley rats weighing 180–220 g were purchased from the experimental animal center of Southwest Medical University (Luzhou, China). All rats were maintained at controlled temperature (22–25 °C) under a 12 h light/12 h dark cycle, with free access to food and water. To induce IgAN [21], all rats except those in the control group received 400 mg/kg bovine serum albumin (by gavaging every other day) (Sigma) for 6 weeks, 0.05 mg LPS (intravenous injection at the end of week 6), and 0.1 ml CCl_4 dissolved in 0.5 ml castor oil (subcutaneous injection weekly) (Sinopharm Chemical Reagent Co. Ltd., China) for 6 weeks. In the present experiment, two schemes were included to analyze the effect of SchB on early-stage IgAN (Scheme 1) and on established IgAN (Scheme 2). In Scheme 1, the rats were randomly distributed into four groups with 6 rats in each group: control, IgAN, IgAN + SchB (50 mg/kg/d), and IgAN + losartan (Los, 25 mg/kg/d, Sigma, positive control). All groups received drug treatment or the same volume of menstruum (olive oil) for 12 weeks. In Scheme 2, all drugs were orally administered to the IgAN rats for 6 weeks (from the sixth to the twelfth weeks).

Clinical Evaluation

At each time point (0, 3, 6, 9, and 12 weeks), 24 h of urine was collected from each rat using metabolic cages for measuring urinary red blood cells (RBCs) and urinary protein. The 24 h urine protein was measured using a Hitachi 7080 automatic biochemical analyzer (Hitachi, Japan). The number of urine RBCs was observed and counted using a red blood cell counting plate, for which ≥ 3 per field of 10 with field vision at high magnification

was considered positive for microscopic hematuria. At 12 weeks, the animals were anesthetized by intraperitoneal injection of pentobarbital (30 mg/kg, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and blood samples were prepared. The renal function parameters of blood urea nitrogen (BUN) and blood creatinine (Cr) were determined by full automatic biochemical analyzers.

Determination of Renal and Plasma IgA

Kidney sections were prepared and analyzed for IgA deposition. The deposition of IgA was determined by direct immunofluorescence staining. Briefly, kidneys were frozen and sectioned to 5 μM and then stained with fluorescein-labelled goat anti-IgA (Sigma). The slides were viewed under a confocal microscopy microscope (Olympus, Tokyo, Japan) at 400× magnification. At least 20 area images per slide were captured. Serum IgA levels were measured using an enzyme-linked immunosorbent assay (ELISA) quantitation kit (Bethyl Laboratories, Montgomery, TX, USA).

Pathological Evaluation and Immunohistochemical Staining

Renal tissues were removed from all rats, and the specimens were fixed in a 10% formalin solution, dehydrated, and embedded in paraffin. The sections were stained with hematoxylin & eosin (H&E) and periodic acid-Schiff (PAS). Renal glomerular injury and mesangial hypercellularity were determined by examining 20 glomeruli in each sample. The pathologic evaluation was scored according to the previously published method [22]. Moreover, to assay inflammatory cells infiltrating the tissue, the specimens were deparaffinized in xylene, and antigen retrieval was performed by high-pressure repair for 2 min. Nonspecific staining was blocked with 1.5% blocking serum for 30 min. The sections were incubated with antibodies of CD3 and CD11b (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a 1:200 dilution overnight at 4 °C. The numbers of positive cells and total nuclear cells per glomerulus in 10 observation fields (magnification field 40×) were scored and averaged by two investigators, who were blinded to the groups of the samples.

Determination of Active T Lymphocytes

Splenocytes were prepared from the rats according to a previously published method [23]. The cells were stimulated by concanavalin A (ConA, 4 mg/l, Sigma) for 24 h and then were double-stained for activated T cell markers using FITC-conjugated anti-mouse CD3 and phycoerythrin-conjugated anti-mouse CD69 antibodies (BD Biosciences, San Jose, CA, USA). The cells were analyzed using flow cytometry (FACScan, Becton Dickinson, USA).

Determination of TNF-Alpha, IL-1β, and MCP-1

To determine the levels of renal pro-inflammatory cytokines, renal tissue was prepared and homogenized in RIPA lysates. The homogenate was centrifuged (10,000 ×*g*, 5 min) to separate the supernatant. The levels of tumour necrosis factor-alpha (TNF-alpha), interleukin-1β (IL-1β), and monocyte chemoattractant protein-1 (MCP-1) in the supernatant were assayed by commercial ELISA kits (R&D Systems, Minneapolis, MN, USA). The levels of TNF-alpha and IL-1β in the culture of LPS-stimulated HK-2 cells were detected in the same way.

Western Blot Analysis

Total protein was isolated from renal tissues using protein lysis buffer (Beyotime, Jiangsu, China). Cytoplasmic and nuclear proteins were extracted using a Nuclear/Cytosol fractionation kit (BioVision, Milpitas, CA, USA) according to the manufacturer's instructions. Equal amounts of sample protein (50 μg), determined by a BCA assay kit (Beyotime), were separated on 10% SDS-polyacrylamide gels and transferred to a nitrocellulose filter membrane (Millipore, Bedford, MA). The membranes were incubated overnight at 4 °C with primary antibodies of NF-κB p65, IκB, and p-IκB (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:1000 dilution and then incubated with a secondary antibody. Blots were visualized with enhanced chemiluminescence (Thermo, Rockford, IL, USA), and the relative density was quantitated using PRO plus software (Media Cybernetics, Rockville, MD, USA). The targeted proteins of HK-2 cells were measured in the same way.

Statistical Analysis

All data are expressed as the mean ± S.D. The data were analyzed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). Differences between groups were analyzed by one-way analysis of variances (ANOVA) followed by Student's *t* test. A difference was considered to be significant at $P < 0.05$.

RESULTS

SchB Improved Renal Function of IgAN Rats

As seen in Fig. 1, renal function was analyzed during the experimental period. IgAN rats showed the appearance of hematuria and increased proteinuria, BUN, and Cr compared with the normal group ($P < 0.01$). In contrast, both pre-treatment and treatment with SchB obviously decreased the levels of proteinuria, hematuria, BUN, and Cr ($P < 0.05$), which was similar to that observed in the positive control group (Los).

SchB Improved Pathologic Performance in IgAN Rats

As shown in Fig. 2a, b, h, and e, PAS staining demonstrated that IgAN rats developed significant mesangial cell proliferation and more inflammatory cell infiltration in interstitial areas, renal tubules, and areas around the glomerulus. Interestingly, the pathologic manifestation, such as mesangial cell proliferation and inflammatory hypercellular infiltration, was significantly ameliorated by pre-treatment or treatment with SchB and Los.

SchB Decreased Serum and Renal IgA Levels

As shown in Fig. 3a, serum IgA levels in IgAN rats at 12 weeks after induction were significantly higher than those in control rats ($P < 0.01$). Pre-treatment or treatment with SchB could reduce the IgA levels compared with those of the control ($P < 0.05$). Moreover, we have noted that Los showed similar results to those of SchB. As shown in Fig. 3b, immunofluorescent-marked IgA shows that normal rats had very little deposition of IgA in the glomeruli, while IgAN model rats exhibited increased fluorescence intensity of IgA. However, after treatment or pre-treatment with SchB or Los, IgA fluorescence intensity was reduced. These results indicated that SchB has direct inhibitory effects on the early-stage IgA deposition and on already deposited IgA.

SchB Inhibited Renal Inflammation of IgAN Rats

The renal inflammatory response has been implicated in the acceleration and progression of IgAN. To clarify the mechanism of the protective effect of SchB

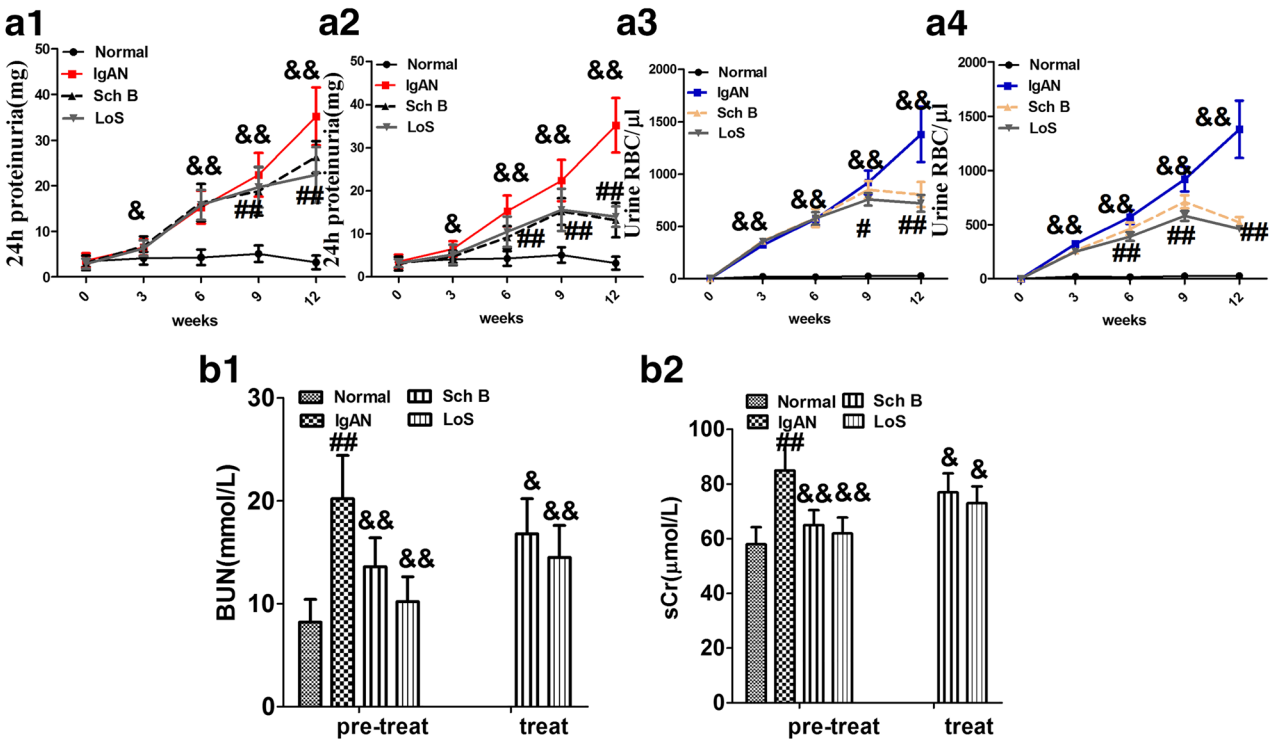

Fig. 1. SchB improved renal function of IgAN rats. **a1** Effects of SchB treatment on 24 h proteinuria. **a2** Effects of SchB pre-treatment on 24 h proteinuria. **a3** Effects of SchB treatment on urine RBC numbers. **a4** Effects of SchB pre-treatment on urine RBC numbers. **b1** BUN; **b2** Cr. Values are shown as the mean ± S.D. $N = 5–6$. $^{\#\#}P < 0.01$ *vs.* normal group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ *vs.* IgAN group.

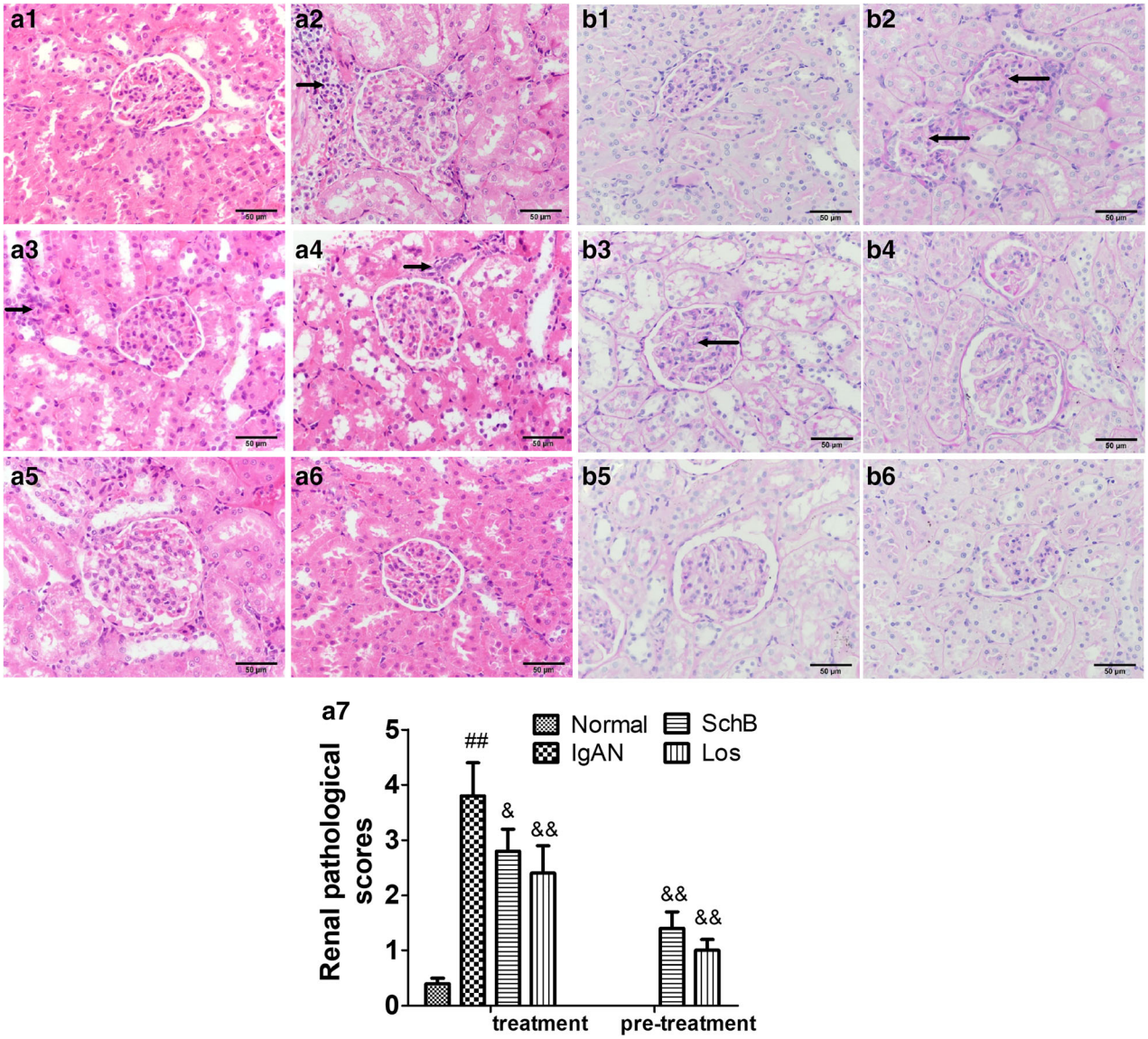

Fig. 2. HE and PAS staining of renal tissues. **a** HE staining with original magnification 400× (bar scale 50 μm). **a1** normal; **a2** IgAN; **a3** SchB (treatment); **a4** Los (treatment); **a5** SchB (pre-treatment); **a6** Los (pre-treatment); **a7** histopathological scores of renal tissues. **b** PAS staining with original magnification 400×. **b1** normal; **b2** IgAN; **b3** SchB (treatment); **b4** Los (treatment); **b5** SchB (pre-treatment); **b6** Los (pre-treatment). The arrows to the right indicate inflammatory infiltration, and the arrows to the left indicate inflammatory cell infiltration. Values are shown as the mean ± S.D. $N = 5–6$. $^{\#\#}P < 0.01$ *vs.* normal group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ *vs.* IgAN group.

on IgAN rats, we examined the effects of SchB on renal inflammation in IgAN rats. As shown in Fig. 4a, a significant increase in CD11b+ cell infiltration could be observed in the interstitial areas and the areas around the glomerulus of IgAN rats compared with that of control rats. Moreover, a significant increase in CD3+ cell infiltration could be observed in the interstitial areas, renal tubules, and around the glomerulus of IgAN rats compared with that of control rats ($P < 0.01$). However, treatment with SchB obviously reduced the number of these inflammatory cells in the IgAN rats compared with untreated IgAN rats ($P < 0.01$). Moreover, SchB reduced the levels of inflammatory cytokines (TNF-alpha and IL-1β) and

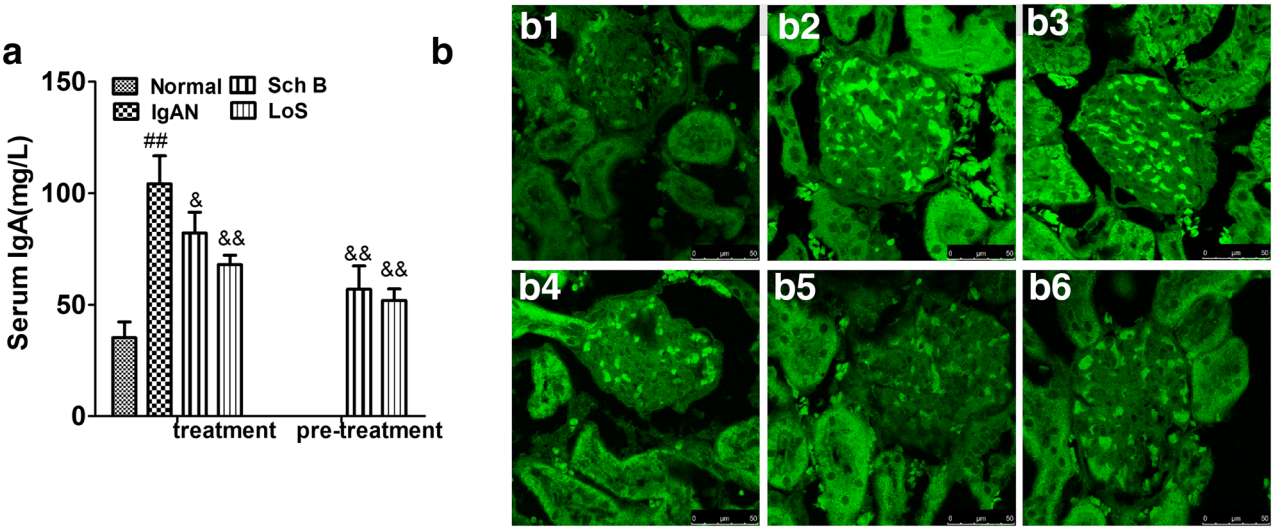

Fig. 3. Serum and renal IgA levels. **a** IgA levels in serum were measured by the commercialized quantitation kit. **b** IgA deposition in renal tissue was detected by fluorescent staining with original magnification 400×. **b1** normal; **b2** IgAN; **b3** SchB (treatment); **b4** Los (treatment); **b5** SchB (pre-treatment); **b6** Los (pre-treatment). Values are shown as the mean ± S.D. $N = 5–6$. $^{\#\#}P < 0.01$ *vs.* normal group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ *vs.* IgAN group.

MCP-1 in the IgAN damaged kidney (Fig. 5, $P < 0.01$). In addition, we further examined T cell activation in splenocytes by flow cytometry in IgAN rats. As shown in Fig. 5, an obvious increase in the percentage of CD3+CD69+ cells (activated T cells) was observed in the diseased rats compared to the normal rats ($P < 0.01$). As expected, SchB obviously reduced the number of activated T cells in the spleen of IgAN rats. Our data indicates that SchB therapy could inhibit renal inflammation of IgAN rats.

SchB Blocked Activation of the Renal NF-κB Pathway in IgAN Model Rats

Activation of the renal NF-κB pathway plays an important role in the drive of pro-inflammatory cytokine synthesis and amplification of the inflammatory response. We investigated the possible underlying mechanisms that might be involved in the renoprotective effect of SchB. As seen in Fig. 6, IgAN rats showed significantly higher levels of nuclear p65 and p-IκB in the kidney than those of the control group ($P < 0.01$), while the levels of cytoplasmic p65 and IκB showed opposite results ($P < 0.01$). However, in the rats treated with SchB, the levels of nuclear p65 and p-IκB were significantly reduced and the levels of cytoplasmic p65 and IκB were significantly increased ($P < 0.05$).

SchB Inhibited Activation of the NF-κB Pathway in LPS-Stimulated HK-2 Cells

To detect whether SchB directly inhibited NF-κB activation, we used LPS to activate the human renal tubular epithelial cell (HK-2) and observed the effects of SchB. As shown in Fig. 7, LPS stimulation for 12 h showed increased levels of TNF-alpha and IL-1β compared with the control group ($P < 0.01$). Moreover, LPS stimulation induced increased levels of nuclear p65 and cytoplasmic p-IκB and decreased levels of cytoplasmic p65 ($P < 0.01$), suggesting an enhanced p65 nuclear translocation in LPS-treated HK-2 cells. However, SchB treatment remarkably inhibited NF-κB activation and p65 translocation in HK-2 cells *in vitro* ($P < 0.01$).

DISCUSSION

This report is the first to identify beneficial effects of SchB against renal injury in IgAN rats. SchB has previously been reported to improve nephrotoxic drug- and compound-induced renal dysfunction in animal models. Chiu et al. [18] have demonstrated that long-term treatment with SchB caused significant decreases in the plasma Cr (10–30%) and BUN levels (14–32%) in gentamicin-induced nephrotoxicity rats. In other studies [19, 20], it has been reported that SchB significantly attenuated CsA- and Hgcl2-induced severe renal injury in rats as well. In

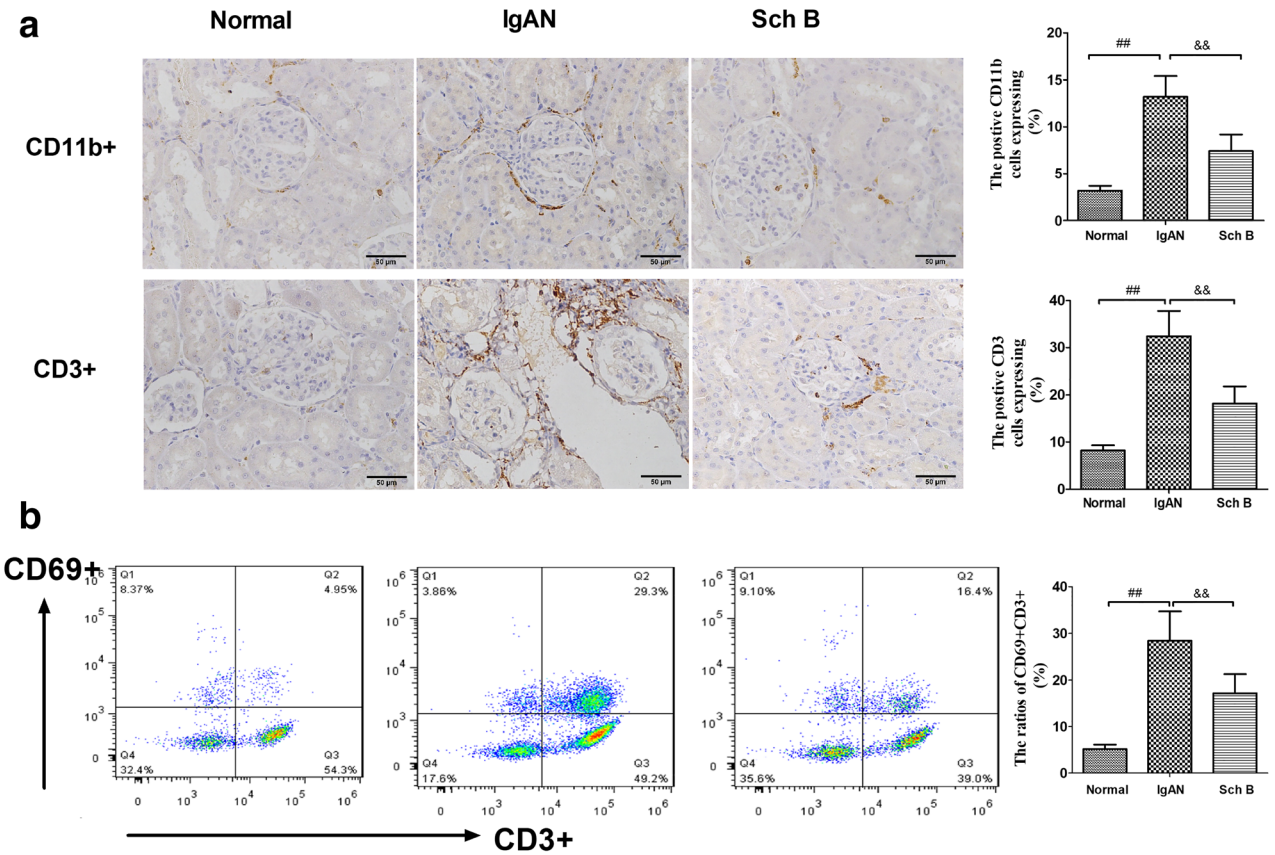

Fig. 4. SchB inhibited renal inflammatory responses in the IgAN rats. **a** Renal tissues stained with antibodies of CD11b and CD3, and the positive rates of stained cells are shown. Original magnification, 400× (bar scale 50 μm). **b** Percentage of CD3+CD69+ cells in splenocytes was detected by flow cytometry. Values are shown as the mean ± S.D. $N = 5–6$. $^{\#\#}P < 0.01$ *vs.* normal group; $^{\&\&}P < 0.01$ *vs.* IgAN group.

our study, we observed that short-term and long-term SchB administration was able to improve renal damage in IgAN rats, which was evidenced by a decrease in proteinuria, BUN, and Cr compared with the untreated group. Moreover, IgAN rats not only exhibited the increased IgA deposition in glomeruli but also developed significant mesangial cell proliferation and more inflammatory cell infiltration in interstitial areas, renal tubules, and areas around the glomerulus. However, these manifestations were significantly reduced and ameliorated by pretreatment or treatment with SchB and Los. Our results suggest that SchB inhibits the development of early-stage IgAN and established IgAN. Moreover, our data also indicated that SchB effectively prevents immunological factor-induced renal damage.

Although the pathogenesis of IgAN is complex and has not been clearly elucidated, many studies have suggested that inflammation plays a vital role in the evolution of IgAN. In patients with IgAN, there is good evidence for disturbances in immune cell populations. For example, it has been shown that the non-classical monocyte subset was significantly expanded in all IgAN patients [24]. Moreover, it has also been demonstrated that T cells appeared together with macrophages infiltrated in glomeruli, crescents, and tubulointerstitial lesions in IgAN patients [25]. Specifically, immune cells and immune complexes infiltrated in the kidney activate mesangial cells, resulting in mesangial cell proliferation and overproduction of the extracellular matrix, cytokines, and chemokines [1]. Some of these cytokines directly cause downstream podocyte injury and induce proteinuria. In a previous study, it was shown that urine levels of pro-inflammatory cytokines IL-1β and TNF-alpha were increased in IgAN patients [26]. Gene expression of TNF-alpha and IL-1β is associated with increased susceptibility to IgAN and related to the degree of mesangial matrix expansion in IgAN patients [27, 28]. Interestingly, some published reports demonstrated that for patients with renal amyloidosis and other forms of GN who

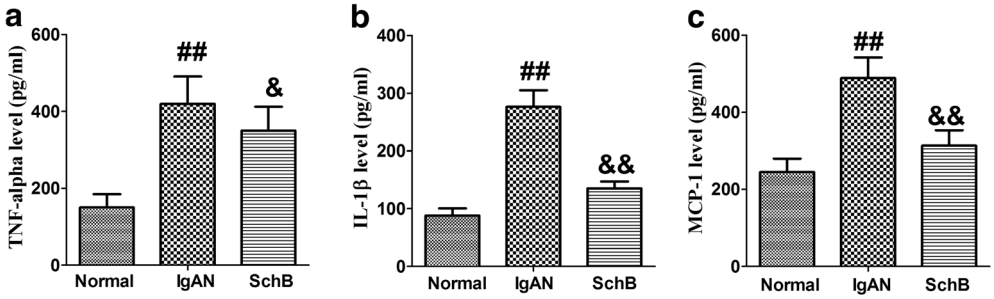

Fig. 5. SchB reduced renal inflammatory cytokine levels in IgAN rats. **a** TNF-alpha; **b** IL-1β; **c** MCP-1. TNF-alpha, IL-1β, and MCP-1 levels in renal tissue were measured by commercial ELISA kits. Values are shown as the mean ± S.D. $N = 5–6$. $^{\#\#}P < 0.01$ *vs.* normal group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ *vs.* IgAN group.

initially had normal creatinine levels, TNF-alpha blocker therapy resolved proteinuria, but this was not the case for patients with initial renal insufficiency [29]. Mechanically, mesangial-derived TNF-alpha is an important mediator involved in degrading the constituents of the glomerular basement membrane and glomerulotubular communication in the development of interstitial damage in IgAN. Infiltrating macrophages in nephropathy promote podocyte apoptosis *via* the TNF-α-mediated p38MAPK pathway [30–32]. Likewise, IL-1β is a mesangial cell growth factor in experimental mesangioproliferative nephritis [33]. Previous findings confirm that SchB possesses excellent anti-inflammatory activity, especially in the treatment of angiocardiopathy, liver disease, as well as neurodegenerative diseases. For instance, SchB exerts anti-neuroinflammatory activity by downregulating nitric oxide, TNF-alpha, prostaglandin E2, IL-1β, and IL-6 in LPS-stimulated microglia [34]. In an *in vivo* study, SchB oral administration attenuated LPS-induced interstitial edema, hemorrhage, and infiltration of neutrophils in lung tissue [35]. In agreement with these earlier results, we found that IgAN rats developed significantly increased inflammatory (CD11b+ and CD3+ cells) infiltration in the interstitial areas and the areas around the glomerulus compared with

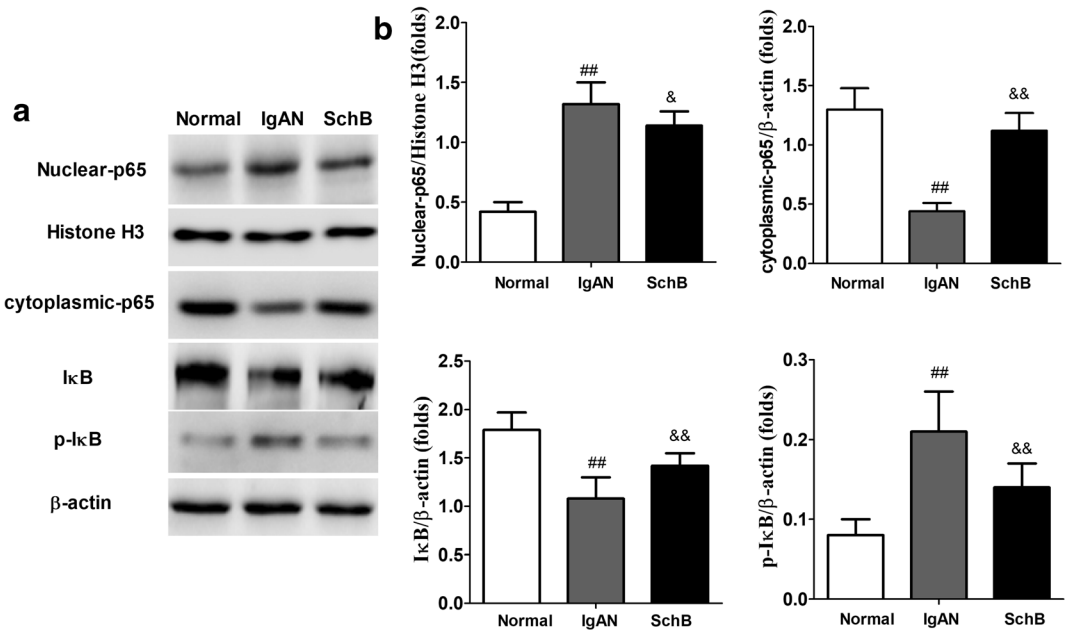

Fig. 6. SchB attenuated renal NF-κB p65 activation and nuclear translocation in IgAN rats. Nuclear and cytoplasmic fractions of NF-κB p65 were estimated by Western blot analysis. **a** Representative stripes of targeted protein. **b** Densitometric analysis. Values are expressed as the mean ± S.D. $N = 5–6$. $^{\#\#}P < 0.01$ *vs.* normal group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ *vs.* IgAN group.

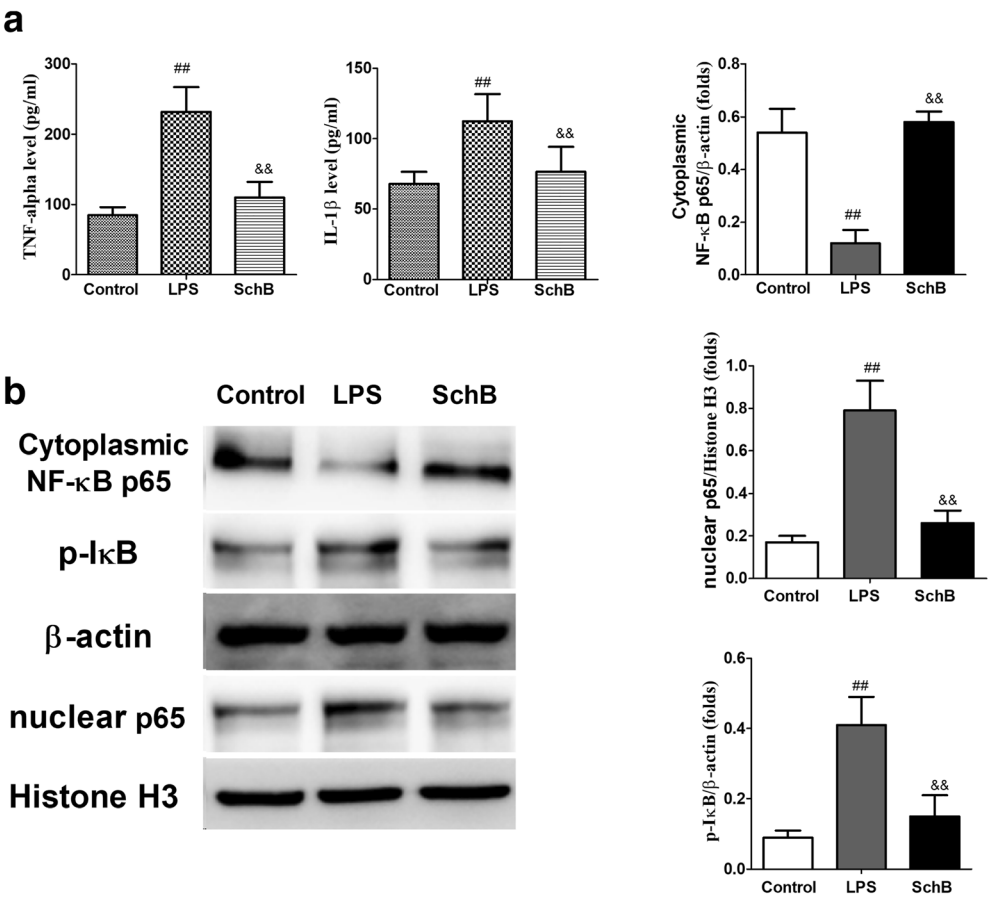

Fig. 7. SchB inhibited NF-κB p65 activation and nuclear translocation in LPS-simulated HK-2 cells. **a** Pro-inflammatory cytokine levels in the supernatant of LPS-treated HK-2 cells were measured by commercial ELISA kits. **b** Nuclear and cytoplasmic fractions of HK-2 cells were isolated, and p65 nuclear translocation was estimated by Western blot analysis. Representative bands and densitometric analysis are shown. Values are expressed as the mean ± S.D. $N = 3$. $^{\#\#}P < 0.01$ *vs.* control; $^{\&\&}P < 0.01$ *vs.* LPS group.

that of the control rats. However, treatment of IgAN rats with SchB significantly decreased inflammatory cell (CD3+ and CD11b+) infiltration in the damaged renal tissue and decreased the active T cell subpopulation in the spleen. Further experiments showed that SchB could reduce TNF-alpha, IL-1β, and MCP-1 in renal tissue. Based on these results, it is suggested that SchB protects the IgAN-damaged kidney, at least in part, possibly through inhibition of renal inflammation.

In previous reports, the renoprotective mechanism of SchB has been concluded as decreasing oxidative stress, cell death, and enhancing mitochondrial antioxidant status and structural integrity [18–20]. However, no more details are known. Chronic inflammation plays a pivotal role in the pathogenesis of IgAN, and SchB treatment significantly reduces renal inflammation. Therefore, we focused our attention on the inflammation-related cellular signalling pathway in IgAN animal models. The NF-κB pathway, known as a central signalling pathway in inflammation, is usually activated by a wide variety of extracellular stress stimuli, such as reactive oxygen species, TNF-alpha, IL-1β, LPS, and ionizing radiation [36]. Activation of NF-κB can induce the transcription of many cytokines, such as MCP-1, TNF-alpha, and the interleukin system. Normally, NF-κB complexes are inactive and mainly located in the cytoplasm in a complex with IκB proteins. When the cells receive stimulation, activation of NF-κB is initiated by the signal-induced degradation of IκB proteins, which is followed by the release and nuclear translocation of the NF-κB p65 subunit [36, 37]. In early studies, SchB has been shown to inhibit the activation of the NF-κB pathway in cisplatin-induced neurotoxic mice, inflammatory bowel mice *in vivo*, as well as in LPS-treated microglia *in vitro* [34, 35, 38, 39]. In agreement with these studies, we found

that SchB treatment could increase cytoplasmic p65 and IκB expression and meanwhile decrease nuclear p65 levels both in the damaged renal tissue and LPS-stimulated HK-2 cells, indicating direct prevention of the activation of the NF-κB signalling pathway in IgAN.

In conclusion, our results demonstrate that SchB shows protective effects on the renal function of early- and late-stage IgAN rats, which may be associated with its anti-inflammatory effects. Moreover, the inhibitory effects of SchB on renal inflammation in IgAN rats were associated with its inhibition of the NF-κB pathway. Therefore, our findings indicate that SchB may show beneficial effects on clinical IgAN patients.

ACKNOWLEDGEMENTS

This research was financially supported by the Medical Research Project of the Sichuan Medical Association (No. 201363).

COMPLIANCE AND ETHICAL STANDARDS

Conflicts of Interest. All contributing authors declare that they have no conflicts of interest.

Ethical Approval. The present study was approved by the Animal Care and Use Committee of Southwest Medical University (Luzhou, China), and all procedures performed in the studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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REFERENCES

1. Lai, K.N., S.C. Tang, F.P. Schena, J. Novak, Y. Tomino, A.B. Fogo, and R.J. Glassock. 2016. IgA nephropathy. *Nature Reviews Disease Primers* 2: 16001.
2. Xie, Y., and X. Chen. 2008. Epidemiology, major outcomes, risk factors, prevention and management of chronic kidney disease in China. *American Journal of Nephrology* 28: 1–7.
3. Katafuchi, R., K. Ikeda, T. Mizumasa, H. Tanaka, T. Ando, T. Yanase, K. Masutani, M. Kubo, and S. Fujimi. 2003. Controlled, prospective trial of steroid treatment in IgA nephropathy: A limitation of low-dose prednisolone therapy. *American Journal of Kidney Diseases* 41: 972–983.
4. Pozzi, C., P.G. Bolasco, G.B. Fogazzi, S. Andrulli, P. Altieri, C. Ponticelli, and F. Locatelli. 1999. Corticosteroids in IgA nephropathy: A randomised controlled trial. *Lancet* 353: 883–887.
5. Lan, H.Y., D.J. Paterson-Nikolic, W. Mu, and R.C. Atkins. 1997. Local macrophage proliferation in the pathogenesis of glomerular crescent formation in rat anti-glomerular basement membrane (GBM) glomerulonephritis. *Clinical and Experimental Immunology* 110: 233–240.
6. Ka, S.M., T.T. Hsieh, S.H. Lin, S.S. Yang, C.C. Wu, H.K. Sytwu, and A. Chen. 2011. Decoy receptor 3 inhibits renal mononuclear leukocyte infiltration and apoptosis and prevents progression of IgA nephropathy in mice. *American Journal of Physiology-Renal Physiology* 301: F1218–F1230.
7. Stangou, M., C. Bantis, M. Skoularopoulou, L. Korelidou, D. Kouloukouriotou, M. Scina, I.T. Labropoulou, N.M. Kouri, A. Papagianni, and G. Efstratiadis. 2016. Th1, Th2 and Treg/T17 cytokines in two types of proliferative glomerulonephritis. *Indian Journal of Nephrology* 26: 159–166.
8. Chen, Q., H. Zhang, Y. Cao, Y. Li, S. Sun, J. Zhang, and G. Zhang. 2017. Schisandrin B attenuates CCl4-induced liver fibrosis in rats by regulation of Nrf2-ARE and TGF-β/Smad signaling pathways. *Drug Design Development and Therapy* 11: 2179–2191.
9. Sun, R., R. Zhai, C. Ma, and M. Wei. 2017, 2017. The anti-growth and anti-metastasis effects of Schisandrin B on hepatocarcinoma cells in vitro and in vivo. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2017.06.022.
10. Lin, Q., X. Qin, M. Shi, Z. Qin, Y. Meng, Z. Qin, and S. Guo. 2017. Schisandrin B inhibits LPS-induced inflammatory response in human umbilical vein endothelial cells by activating Nrf2. *International Immunopharmacology* 49: 142–147.
11. Zhang, W., Z. Sun, and F. Meng. 2017. Schisandrin B ameliorates myocardial ischemia/reperfusion injury through attenuation of endoplasmic reticulum stress-induced apoptosis. *Inflammation* 40: 1903–1911.
12. Xu, Y., Z. Liu, J. Sun, Q. Pan, F. Sun, Z. Yan, and X. Hu. 2011. Schisandrin B prevents doxorubicin- induced chronic cardiotoxicity and enhances its anticancer activity in vivo. *PLoS One* 6: e28335.
13. Ko, K.M., and B.Y. Lam. 2002. Schisandrin B protects against tert-butylhydroperoxide induced cerebral toxicity by enhancing glutathione antioxidant status in mouse brain. *Molecular and Cellular Biochemistry* 238: 181–186.
14. Chen, Z., M. Guo, G. Song, J. Gao, Y. Zhang, Z. Jing, T. Liu, and C. Dong. 2016. Schisandrin B inhibits Th1/Th17 differentiation and promotes regulatory T cell expansion in mouse lymphocytes. *International Immunopharmacology* 35: 257–264.
15. Leong, P.K., and K.M. Ko. 2015. Schisandrin B induces an Nrf2-mediated thioredoxin expression and suppresses the activation of inflammasome in vitro and in vivo. *Biofactors* 41: 314–323.
16. Checker, R., R.S. Patwardhan, D. Sharma, J. Menon, M. Thoh, H.N. Bhilwade, T. Konishi, and S.K. Sandur. 2012. Schisandrin B exhibits anti-inflammatory activity through modulation of the redox-sensitive transcription factors Nrf2 and NF-κB. *Free Radical Biology and Medicine* 53: 1421–1430.
17. Park, E.J., J.N. Chun, S.H. Kim, C.Y. Kim, H.J. Lee, H.K. Kim, J.K. Park, S.W. Lee, I. So, and J.H. Jeon. 2012. Schisandrin B suppresses TGFβ1 signaling by inhibiting Smad2/3 and MAPK pathways. *Biochemical Pharmacology* 83: 378–384.
18. Chiu, P.Y., H.Y. Leung, and K.M. Ko. 2008. Schisandrin B enhances renal mitochondrial antioxidant status, functional and structural integrity, and protects against gentamicin-induced nephrotoxicity in rats. *Biological & Pharmaceutical Bulletin* 31: 602–605.

19. Zhu, S., Y. Wang, M. Chen, J. Jin, Y. Qiu, M. Huang, and Z. Huang. 2012. Protective effect of schisandrin B against cyclosporine A-induced nephrotoxicity in vitro and in vivo. *American Journal of Chinese Medicine* 40: 551–566.
20. Stacchiotti, A., Volti.G. Li, A. Lavazza, I. Schena, M.F. Aleo, L.F. Rodella, and R. Rezzani. 2011. Different role of Schisandrin B on mercury-induced renal damage in vivo and in vitro. *Toxicology* 286: 48–57.
21. Wei, L., Y. Du, L. Jia, X. Ma, Z. Chen, J. Lu, L. Tian, Z. Duan, F. Dong, Z. Lv, G. Yao, R. Fu, and L. Wang. 2017. Therapeutic effects of FK506 on IgA nephropathy rat. *Kidney & Blood Pressure Research* 42: 983–998.
22. Lai, K.N., L.Y. Chan, H. Guo, S.C. Tang, and J.C. Leung. 2011. Additive effect of PPAR-γ agonist and ARB in treatment of experimental IgA nephropathy. *Pediatric Nephrology* 26: 257–266.
23. Hu, Y., Z. Hu, S. Wang, X. Dong, C. Xiao, M. Jiang, A. Lv, W. Zhang, and R. Liu. 2013. Protective effects of Huang-Lian-Jie-Du-Tang and its component group on collagen-induced arthritis in rats. *Journal of Ethnopharmacology* 150: 1137–1144.
24. Cox, S.N., G. Serino, F. Sallustio, A. Blasi, M. Rossini, F. Pesce, and F.P. Schena. 2015. Altered monocyte expression and expansion of non-classical monocyte subset in IgA nephropathy patients. *Nephrology Dialysis Transplantation* 30: 1122–1232.
25. Sakatsume, M., Y. Xie, M. Ueno, H. Obayashi, S. Goto, I. Narita, N. Homma, K. Tasaki, Y. Suzuki, and F. Gejyo. 2001. Human glomerulonephritis accompanied by active cellular infiltrates shows effector T cells in urine. *Journal of the American Society of Nephrology* 12: 2636–2644.
26. Wu, T.H., S.C. Wu, T.P. Huang, C.L. Yu, and C.Y. Tsai. 1996. Increased excretion of tumor necrosis factor alpha and interleukin 1 beta in urine from patients with IgA nephropathy and Schönlein-Henoch purpura. *Nephron* 74: 79–88.
27. Lim, C.S., S. Zheng, Y.S. Kim, C. Ahn, J.S. Han, S. Kim, J.S. Lee, D.W. Chae, J.R. Koo, R.W. Chun, and J.W. Noh. 2001. Th1/Th2 predominance and proinflammatory cytokines determine the clinicopathological severity of IgA nephropathy. *Nephrology Dialysis Transplantation* 16: 269–275.
28. Syrjänen, J., M. Hurme, T. Lehtimäki, J. Mustonen, and A. Pasternack. 2002. Polymorphism of the cytokine genes and IgA nephropathy. *Kidney International* 61: 1079–1085.
29. Lee, S.H., E.J. Lee, S.W. Chung, R. Song, J.Y. Moon, S.H. Lee, S.J. Lim, Y.A. Lee, S.J. Hong, and H.I. Yang. 2013. Renal involvement in ankylosing spondylitis: Prevalence, pathology, response to TNF-a blocker. *Rheumatology International* 33: 1689–1692.
30. Lai, K.N., J.C. Leung, L.Y. Chan, M.A. Saleem, P.W. Mathieson, F.M. Lai, and S.C. Tang. 2008. Activation of podocytes by mesangial-derived TNF-alpha: Glomerulo-podocytic communication in IgA nephropathy. *American Journal of Physiology-Renal Physiology* 294: F945–F955.
31. Guo, Y., Z. Song, M. Zhou, Y. Yang, Y. Zhao, B. Liu, and X. Zhang. 2017. Infiltrating macrophages in diabetic nephropathy promote podocytes apoptosis via TNF-α-ROS-p38MAPK pathway. *Oncotarget* 8: 53276–53287.
32. Nee, L., N. Tuite, M.P. Ryan, and T. McMorrow. 2007. TNF-alpha and IL-1 beta-mediated regulation of MMP-9 and TIMP-1 in human glomerular mesangial cells. *Nephron Experimental Nephrology* 107: e73–e86.
33. Tesch, G.H., H.Y. Lan, R.C. Atkins, and D.J. Nikolic-Paterson. 1997. Role of interleukin-1 in mesangial cell proliferation and matrix deposition in experimental mesangioproliferative nephritis. *American Journal of Pathology* 151: 141–150.
34. Zeng, K.W., T. Zhang, H. Fu, G.X. Liu, and X.M. Wang. 2012. Schisandrin B exerts anti-neuroinflammatory activity by inhibiting the Toll-like receptor 4-dependent MyD88/IKK/NF-κB signaling pathway in lipopolysaccharide-induced microglia. *European Journal of Pharmacology* 692: 29–37.
35. Cai, Z., J. Liu, H. Bian, J. Cai, and G. Zhu. 2016. Suppression of P2X7/NF-κB pathways by Schisandrin B contributes to attenuation of lipopolysaccharide-induced inflammatory responses in acute lung injury. *Archives of Pharmacal Research* 39: 499–507.
36. Kopitar-Jerala, N. 2015. Innate immune response in brain, NF-kappa B signaling and cystatins. *Frontiers in Molecular Neuroscience* 8: 73.
37. Park, M.H., and J.T. Hong. 2016. Roles of NF-κB in cancer and inflammatory diseases and their therapeutic approaches. Cells 5(2). pii: E15.
38. Giridharan, V.V., R.A. Thandavarayan, H.N. Bhilwade, K.M. Ko, K. Watanabe, and T. Konishi. 2012. Schisandrin B, attenuates cisplatin-induced oxidative stress, genotoxicity and neurotoxicity through modulating NF-κB pathway in mice. *Free Radical Research* 46: 50–60.
39. Liu, W., Y. Liu, Z. Wang, T. Yu, Q. Lu, and H. Chen. 2015. Suppression of MAPK and NF-κ B pathways by schisandrin B contributes to attenuation of DSS-induced mice model of inflammatory bowel disease. *Pharmazie* 70: 598–603.