Protective effect of ganoderan on renal damage in rats with chronic glomerulonephritis

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Abstract

Purpose: To investigate the protective effect of ganoderan on renal damage in rat models with chronic glomerulonephritis induced by adriamycin.

Methods: 48 healthy Sprague-Dawley rats were randomly divided into three groups: control, nephritic model and ganoderan treatment groups. Changes of the following indices in the three groups were observed 6 weeks after treatment: 24-hour urine protein, albumen, serum creatinine, cholesterol. Histopathological observations of the renal cortex were made by light and electron microscopy.

Results: Compared with controls, levels of 24-hour urine protein ($9.60 \pm 0.57 \text{ mg/d}$ vs. $82.50 \pm 3.18 \text{ mg/d}$), serum creatinine ($35.25 \pm 2.63 \mu\text{mol/L}$ vs. $44.75 \pm 8.06 \mu\text{mol/L}$) and cholesterol ($1.15 \pm 0.10 \text{ mmol/L}$ vs. $4.02 \pm 0.25 \text{ mmol/L}$) of rats in the nephritic model group were increased ($P < 0.05$), and the concentration of albumen was decreased ($35.98 \pm 1.34 \text{ g/L}$ vs. $19.05 \pm 0.62 \text{ g/L}$, $P < 0.05$). Ganoderan administration decreased 24-hour urine protein ($82.50 \pm 3.18 \text{ mg/d}$ vs. $45.01 \pm 3.94 \text{ mg/d}$, $P < 0.05$). Following ganoderan, the

pathological changes in kidney tissue were improved compared with those in the nephritic model group.

Conclusion: Ganoderan exerts protective effects in rats with chronic glomerulonephritis induced by ADR. Ganoderan reduced 24-hour urine protein, serum creatinine, cholesterol, improving renal function and reducing the severity of renal injury.

Ganoderan contains the active components of *Ganoderma lucidum* (Ganoderma or lingzhi)¹, an Asian medicinal mushroom that has been used for two thousand years for the treatment of various diseases such as hypertension², hyperlipidemia³, diabetes⁴, hepatitis⁵, allergy⁶, anti-aging, anti-fibrosis⁷ and cancer⁸. Recent studies have indicated that the extract of *Ganoderma lucidum*-ganoderan, also has a wide range of pharmacological actions including suppressing inflammation and scavenging free radicals.⁹

Adriamycin (ADR) causes chronic nephropathy in rats, which corresponds clinically and histologically to chronic glomerulonephritis, focal and segmental glomerulosclerosis.^{7, 8} In the present study, we prepared rat models with chronic glomerulonephritis by the intravenous injection of ADR. Rats in the ganoderan treatment group were fed ganoderan for 7 days. The following indices were observed after 6 weeks treatment: urine protein quantitation (UPr) of 24h, serum cholesterol (Ch), serum albumin (Alb), serum creatinine (sCr). Histopathological examination of rat renal cortex was made by light and electron microscopy. Changes in these markers were compared among the control, nephritic model, and ganoderan treatment groups to investigate the protective effects of ganoderan on rats with chronic glomerulonephritis.

Materials and Methods

Animals

Forty-eight healthy Sprague-Dawley (SD) rats of sanitation grade, 5-6 weeks old and approximately 200±25g, were purchased from the *Department of Laboratory Animal Science, Guangzhou Medical College (Guangzhou, China)* respectively. The rats were housed and given a commercial diet and tap water for 1 week before experimentation at 24°±1°C and 50-70% humidity under a 12h light-dark cycle. Experiments were performed according to the "Guidelines for the Care and Use of Experimental Animals" of the Chinese Association for Laboratory Animals. After one-week adaptation, rats were randomly divided into three groups: control, nephritic model and ganoderan treatment groups with 16 rats in each group.

Materials

Adriamycin (ADR, #02D020-23214-92-8) was purchased from Asia Talent Chemical Supplier. Ganoderan, the active ingredient of the capsule containing mythic fungus spore powder, 0.3 g/capsule, was pur-

chased from the Fuzhou Institute of Green Valley Bio-Pharm Technology (Fuzhou, China). 10 mg ADR was added to physiological saline to make a solution with a concentration of 1g/L. The contents of the capsule containing ganoderan were dissolved in distilled water to provide 2ml/rat.

Preparation of Animal Models and Drug Treatment

In the nephritic model and the ganoderan treatment groups, chronic glomerulonephritis was induced with ADR, 3.5mg/kg, injected into the tail vein of conscious rats twice, 7 days apart. Rats in the control group received the same volume of physiological saline. Rats in the ganoderan group were given mythic fungus spore powder, 5g/kg, intragastrically for 7 days. Rats in the control and nephritic groups were given the same amount of distilled water. During the experiment, all rats were maintained in a standard environment with 12 h daytime, 12 h night, temperature 24°±1°C and humidity 50%-70%. Animals fed freely on whole-value particle animal feedstuff, made by our animal experimental centre, and tap water.

Urine Protein and Blood Biochemical Detection

24h UPr (expressed as urinary protein in mg/ml glomerular filtrate in 24h) was determined using spectrophotometry, after 3% sulfosalicylic acid precipitation of urine which was collected from rats individually housed in metabolic cages for 24h before ADR administration and 6 wk after drug treatment. Simultaneously, blood samples were drawn from rats in the three groups. Biochemical parameters (sCh, Alb, sCr) were measured by standard assays using diagnostic kits (Roche AG, Switzerland) and the biochemical analyzer Cobas Mira plus (Roche AG, Switzerland).

Histopathological Observation

Six weeks after drug treatment, rats were anaesthetized with 10% chloral hydrate, 0.5ml/100g, by i.p.injection. One kidney was removed from each rat

TABLE 1. Comparison of the mouse biochemical indicators in different groups, 49 days after drug treatment ($\bar{X} \pm s$)

Groups	Cases	24h UPr (mg/d)	Alb (g/L)	sCr (μ mol/L)	Ch (mmol/L)
Control	16	9.60 $\pm$ 0.57	35.98 $\pm$ 1.34	35.25 $\pm$ 2.63	1.15 $\pm$ 0.10
adriamycin group	16	82.50 $\pm$ 3.18*	19.05 $\pm$ 0.62*	44.75 $\pm$ 8.06*	4.02 $\pm$ 0.25*
Ganoderan group	16	45.01 $\pm$ 3.94* #	24.48 $\pm$ 0.91*	45.25 $\pm$ 5.68*	2.61 $\pm$ 0.29 * #

* $P < 0.05$, comparison with the control group;

$P < 0.05$, comparison with the adriamycin-induced nephritic model group.

and fixed in 10% formalin, embedded in paraffin and examined in multiple consequent sections. The histopathological study was carried out using HE staining (hematoxylin –eosin) and observed by light microscope. In addition, other partial kidney tissues were fixed with 3% glutaraldehyde and 1% osmic acid, and stained with uranyl acetate and citric acid. Histopathological changes in the rat renal cortex were also observed by electron microscopy.

Statistical Analysis

Data are presented as a mean $\pm$ standard deviation ($\bar{X} \pm s$) obtained from three independent experiments. Statistical analysis was determined by Dunnett t test (2-sided). P values < 0.05 were considered statistically significant.

Results

Effect of Ganoderan on the changes of biochemical indicator in rat models

The biochemical indicators in three groups are shown in Table 1.

Albumen levels in the nephritic group were lower than in the control ($P < 0.05$), but sCr and Ch were increased ($P < 0.05$), demonstrating the successful production of the nephritic model. Two weeks after ADR, hyperproteinuria had occurred in the nephritic model rats and continued to increase. Six weeks after ADR, it was higher than in the control group ($P < 0.05$). After ganoderan administration, urine protein and Ch levels were lower than those in the nephritic model group.

Effect of Ganoderan on renal pathology

Light microscopy

Renal tissues in the control group served as negative controls (Figure 1).

In the nephritic group, glomerular mesangium regions were expanded, intercapillary cells increased and basal material multiplied. The lumen of the blood vessels was narrowed and even disappeared. A few renal glomeruli and capsules were partly adherent. There were some casts in the renal interstitium with increased infiltration of inflammatory cell in the renal glomeruli and interstitium (Figure 2).

In the ganoderan treatment group, hyperplasia in the glomerular mesangium regions was reduced,

FIGURE 1. Renal pathological changes in rats of control group observed by light microscopy.

FIGURE 2. Renal pathological changes in rats of nephritic model group observed by light microscopy.

FIGURE 3. Renal pathological changes in rats of ganoderan treatment group observed by light microscopy.

FIGURE 5. Renal pathological changes in rats of nephritic model group observed by electron microscopy.

FIGURE 4. Renal pathological changes in rats of control group observed by electron microscopy.

FIGURE 6. Renal pathological changes in rats of ganoderan treatment group observed by electron microscopy.

pathological changes in the basal membrane were not obvious and there was no adherence in the renal capsules. Pathological changes in the renal tubules and interstitium were also improved with fewer casts. There was some infiltration of inflammatory cells in the renal glomeruli and interstitium, but less than in the nephritic model (Figure 3).

Electron microscopy

The normal renal tissues served as negative control for electron microscopy (Figure 4).

In the nephritic group, mesangial regions were expanded, intercapillary cells propagated, and the foot processes of the foot cells were mixed together and even disappeared with adherence in the renal capsules (Figure 5).

In the ganoderan group, foot processes were partly confluent compared with the nephritic group and, in some regions, the foot processes had recovered completely or were close to normal. Mesangial regions remained mildly expanded with relatively mild hyperplasia in the intercapillary cells (Figure 6).

Discussion

The fungus *Ganoderma lucidum*, also known as 'Lingzhi' in Chinese, 'Reishi' in Japanese, and 'Youngzhi' in Korean, is a member of the genus *Ganoderma* and has been used, traditionally, as a popular herbal medicine for the promotion of health in the Orient. In 1881, the genus *Ganoderma* was established in the west by the Finnish botanist, Karsten.¹⁰ Since then, more than 120 species have been reported world-wide. In the present study, the protective effect of ganoderan, the active ingredient of *Ganoderma lucidum*, on chronic glomerulonephritis was analyzed using the rat models induced by ADR. After ADR administration, 24UPr, sCh and sCr levels were increased. Proteinuria has a direct effect on cellular infiltration¹¹ and protein overload has been suggested to induce functional alterations of tubular cells, overexpressing proinflammatory mediators. Thus, tubulointerstitial inflammation in rat models with chronic glomerulonephritis corresponded in time with the overt proteinuria as our results. In the ganoderan treatment group, levels of these makers were decreased and renal function was improved. Moreover,

light microscopical and electron microscopical observation in the nephritic group demonstrated tubulointerstitial changes with cellular infiltration and vacuolar degeneration. Interstitial inflammation has been considered an important determinant of the outcome of glomerular inflammation. Several studies have suggested that myofibroblasts of the interstitium may play a crucial role in the pathogenesis of fibrosis in glomerular diseases.¹²⁻¹⁴ Thus, the tubulointerstitial cellular response and vacuolar degeneration in our rat models with chronic glomerulonephritis emphasize the severity of the illness. On the other hand, attenuation of interstitial inflammation and other renal injury may be secondary to inhibition of proteinuria by the ganoderan medication. These results confirm that ganoderan can postpone the progression of chronic glomerulonephritis.

Many biological effects of ganoderan have been reported. Renal interstitial fibrosis usually shows initial infiltration of lymph cells. Lymph cell culture fluid contains factors that may promote fibrocyte multiplication and collagen synthesis. Ganoderan may inhibit delayed anaphylaxis, restrain the primary power of antibody response and decrease the level of circulating antibody *in vivo* of rats.¹¹ Experiments *in vitro* have also shown that ganoderan may inhibit the proliferative response of the splenic lymphocytes of rats and the proliferative response of human tonsilla lymphocytes, which suggests that ganoderan may have a similar inhibiting effect on both human and rats' lymph cells. Some reports have indicated that, in mice, ganoderan decreases production of IL-2 by the splenic cells (IL-2 can promote the proliferation of T lymph cells¹²), and inhibit the mixed lymphocyte culture reaction of the allotype splenic cells. Ganoderan may also increase the peritoneal macrophage ACP, β -glucuronidase activity and the content of hydrogen dioxide, and antagonize the inhibiting effect of the splenic DNA synthesis by glucocorticoid.¹³ Thus, one may conclude that ganoderan inhibits both the body's cell immunity and humoral immune function.

The mechanism of the protective effects of ganoderan on ADR induced chronic glomerulonephritis is unclear. ADR exhibits its cytotoxic effect mainly by inhibiting topoisomerase II, which causes DNA strand breaks. ADR also may induce ROS production, causing oxidative stress.¹⁵ The effects of ganoderan on ADR may, at least in part, be based on: (1) attenuation of ADR-induced oxidative stress by ganoderan and (2) inhibition of cells to ADR-induced DNA strand breaks by ganoderan through simulation of the DNA excision repair system.

In conclusion, ganoderan exerts protective effects on ADR-induced chronic glomerulonephritis in rats. By reducing excretory quantity of proteinuria, 24UPr, and Ch, improving renal function and lessening the severity degree of glomerulosclerosis ganoderan retards development of the disease. Further research is needed to confirm the mechanism of the interaction between ganoderan and ADR.

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