Anti-Proteinuric Effect of Sulodexide in Adriamycin-Induced Nephropathy Rats

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SUMMARY. This study investigated the anti-proteinuric effect of sulodexide in rats with adriamycin (ADR) nephropathy. A total of 40 healthy male Sprague-Dawley (SD) rats were randomly assigned to four groups: normal control group (Control-group), ADR control group (ADR-group), sulodexide treatment group (SUL-group), and losartan treatment group (LOS-group). The ADR-induced rat models were established by injecting two different doses of ADR (4 and 3.5 mg/kg) into the caudal vein of rat for two consecutive weeks. After that, SUL-group and LOS-group were respectively treated with sulodexide (10 mg/kg/day) and losartan (10 mg/kg/day) for an additional 4 weeks period. Samples of 24-hour urine were harvested at 3, 4, 5, and 6 weeks after the model establishment. The pathological change in renal tissues was observed by light microscopy, the function of liver and kidney were assayed at week 6th. The results showed that the urinary excretion of protein progressively increased in ADR-group, and accompanied with severe nephrotic syndrome such as massive albuminuria, proteinuria, and hyperlipidemia. Sulodexide effectively reduced the 24-hour urinary protein excretion of ADR-induced nephropathy rats, preventing focal segmental glomerulosclerosis. There was no significant difference between LOS-group and SUL-group for reducing urinary protein excretion (P < 0.05). Sulodexide alleviated ADR-induced nephrotoxicity as good as losartan in a short period of treatment.

INTRODUCTION

Sulodexide is a purified glycosaminoglycan (GAG) obtained from porcine mucosa and composed of 80 % low-molecular mass heparin and 20 % dermatan sulfate. It has antithrombotic, profibrinolytic, and antilipemic effects. Clinical trials have demonstrated that sulodexide has beneficial effects in the treatment of deep vein thrombosis. Sulodexide probably binds to the surface of the endothelial cells, which additionally protects against formation of thrombus. For now, sulodexide is widely used in various cases of ischemia caused by atherosclerosis and/or thrombosis cause for its longer half-life and a reduced effect on systemic clotting and bleeding. Furthermore, it can reduced infarct size and inflammation during reperfusion in animals with myocardial ischaemia.

Sulodexide can also reduce proteinuria of diabetic nephropathy, both in animal models and in human subjects. For example, sulodexide reduced extracellular matrix deposition and transformed growth factor-β (TGF-β) over expression, restored anionic charges lost from the endothelial surface and reduced endothelial injury in a streptozocin-induced diabetic nephropathy rat model. In humans, several small scale clinical studies in humans with diabetic nephropathy have demonstrated a consistent trend for the reduction of urinary albumin excretion. And a 6-month treatment with sulodexide showed a tendency to increase the time-dependent anti-proteinuric effect in IgA nephropathy patients.

Adriamycin (ADR, doxorubicin hydrochloride) is a highly potent antineoplastic agent that...
induces cell killing. Its clinical efficacy is limited because of severe cytotoxic side effects. However, intravenous administration of ADR in rats can induce a nephrotic syndrome, which is characterized by proteinuria, albuminuria and hypoalbuninemia. The degree of renal toxicity is a function of the cumulative dose of ADR, the glomerular sclerosis and tubulointerstitial damage are similar to humans. Intensive proteinuria associated with focal loss of podocyte foot processes, swelling and vacuolization of epithelial and mesangial cells are landmarks of early stage of ADR nephrotoxicity. Therefore, ADR-induced nephropathy rat is widely used as a nephropathic model.

Angiotensin II (ANG II) has been proved that it plays an important role in the progression of chronic renal failure. Short-term losartan treatment can improve glomerular filtration rate and ameliorated glomerulosclerosis resulting in decreased proteinuria. Prolonged treatment with losartan showed further reduction of glomerulosclerosis associated with reduced progression of tubular atrophy and interstitial fibrosis. Although it has been demonstrated that sulodexide can reduce proteinuria of diabetic nephropathy, diabetes-induced nephropathy is different with ADR-induced nephropathy, for instance, the pancreatic-β cells are not damaged. Since there is seldom study of sulodexide special focusing on renal disease progression in rat with ADR-induced nephropathy, and no compare between sulodexide and angiotensin II in anti-proteinuric effect. Thus, the aim of the present study is to examine short-term (4 weeks) anti-proteinuric effects of sulodexide and losartan in rat with ADR nephropathy.

**MATERIAL AND METHODS**

**Animals and chemicals**

A total of 40 male Sprague-Dawley (SD) rats weighing 234 ± 19 g were obtained from Wenzhou Medical College Laboratory Animal Center (Wenzhou, China). All experimental procedures were conducted according to the Institutional Animal Care guidelines and approved ethically by the Administration Committee of Experimental Animals, Zhejiang Province, China.

Adriamycin, sulodexide and losartan were obtained from Hisun Pharmaceutical Co Ltd (Zhejiang, China), Alfa Wassermann SpA, Bologna, (Italy) and MSD Pharmaceutical Co Ltd (Hangzhou, China), respectively.

**Experimental protocol**

Forty healthy male rats were randomly divided into four groups: (1) the normal control group (Control-group, n = 10), intravenously injected an equal volume of normal saline through the caudal vein; (2) the ADR control group (ADR-group, n = 10), intravenously injected 4 mg/kg of ADR through the caudal vein at first week, 3.5 mg/kg of ADR at second week; animals of both control groups drank tap water throughout the experiment and they were killed after 6 weeks; (3) the sulodexide treatment group (SUL-group, n = 10), intravenously injected ADR as in the ADR-group and administered sulodexide 10 mg/kg per day orally for 4 weeks after the ADR-induced nephrotic model establishment; (4) the losartan treatment group (LOS-group, n = 10), intravenously injected ADR as in the ADR-group and administered losartan 10 mg/kg per day orally for 4 weeks after the ADR-induced nephrotic model establishment. During the treatment period, body weights of the rats were measured every week, and the doses of ADR, sulodexide and losartan were adjusted according to the change in body weight.

**Determination of 24-h Urinary Protein Excretion**

All the rats were placed in metabolism coops at week 3, 4, 5, and 6 after two weeks administration of ADR. The samples of 24-h urine were collected for 24 h. The concentration of total protein in urine was measured by Cobas Integra 400 Chemistry Analyzer (F. Hoffmann-La Roche, Basel, Switzerland).

**Determination of Biochemical Parameters**

The abdominal aorta was carefully isolated and 5 mL of arterial blood was collected into tubes containing heparin, after rats were anesthetized by 10 % chloral hydrate (3 mL/kg). The samples were immediately centrifuged at 3000 rpm within 10 min. The plasma was separated and stored at -80 °C until further analysis.

The biochemical parameters of hepatic and renal functions, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin (ALB), blood urea nitrogen (BUN), serum creatinine (Cr), total cholesterol (TC), total triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) were measured by Hitachi 7180 Automatic Biochemical Analyzer (Hitachi High-Tech Science Systems Corporation, Japan).
Histological Procedures

After the arterial blood was collected into tubes, the kidneys of rats were removed immediately and immersion-fixed in 4% paraformaldehyde phosphate-buffered saline (PBS) solution. Afterward, paraffin-embedded sections (thickness = 4 µm) of cortex tissues were dewaxed, stained with haematoxylin and eosin, then dehydrated, cleared and mounted for light microscopic examination.

Statistical analysis

Biochemical Parameters are expressed as means ± SD when a normal distribution was present and ANOVA with a post hoc test (differences between groups) were used. A one-way repeated measures analysis of variance was used to analyze the changes in the excretion of 24-h urinary protein. Statistical analyses were performed using SPSS statistical software, version 15.0. Statistical significance was assumed at the 5% level.

Results

Excretion of 24-h Urinary Protein

Excretion of 24-h urinary protein at week 3, 4, 5, and 6 was increased significantly in the ADR-group as compared with that in the Control-group, SUL-group and LOR-group (Fig. 1). All the data were met Spherically Symmetric Test (P=0.081), and repeated measures analysis of variance was used to analyze the relationship between four group. The results indicated that there was significant difference in the excretion of 24-hour urinary protein in four group. The excretion of 24-hour urinary protein at week 6 were: 6.86 ± 3.03 mg/24h (Control-group), 14.83 ± 4.71 mg/24h (SUL-group), 29.43 ± 8.77 (ADR-group), 16.17 ± 2.64 (LOR-group). The data were analyzed with one-way ANOVA (Table 2). The results showed there was significant difference between ADR-group and SUL-group (P < 0.05), however there was no difference between SUL-group and LOR-group (P >0.05).

Biochemical Parameters

Body weights and biochemical parameters are shown in Table 1. Body weight was comparable among the four groups at week 6. There were: 223.20 ± 43.72 g (Control-group), 193.80 ± 15.99 g (ADR-group), 195.75 ± 33.65 g (SUL-group), 200.00 ± 36.06 g (LOR-group). Data are expressed as mean ± SD. aP < 0.05 vs. ADR-group, bP < 0.01 vs. ADR-group. Control-group n =10; ADR-group n = 8; LOR-group n = 7; and SUL-group n = 8.

Table 1. Body weight and biochemical parameters in experimental groups. Control-group = with normal saline injection; ADR-group = adriamycin-induced rats; SUL-group = sulodexide-treated rats; LOR-group = losartan-treated rats. Data are expressed as mean ± SD. * p < 0.01, ADR-group vs. Control-group, SUL-group and LOR-group. ** p < 0.05, ADR-group vs. Control-group.
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was no difference in the body weight between experimental groups. Alanine aminotransferase, aspartate aminotransferase, high density lipoprotein, low density lipoprotein, creatinine and blood urea nitrogen have no significantly difference among the four groups. Although serum albumin was significantly decreased in ADR group compared with that in Control-group (P < 0.05), there was no difference when it compared to LOR-group and SUL-group (P > 0.05). Total Cholesterol and Glycerin were increased in ADR group, however there no statistically difference between ADR-group and SUL-group.

**Histological studies**

The histological studies of four groups are shown in Figure 2. There was no obvious pathological changes were found in the glomeruli of ADR-induced nephropathy rats under light microscopy when short-time treatment with the sulodexide (SUL-group) or AT-1 receptor antagonist losartan (LOR-group). And focal segmental glomerulosclerosis with capsular adhesion and tubular atrophy with protein casts were present in ADR-groups.

**DISCUSSION**

In the present study, the effect of sulodexide on ADR-induced nephropathy in rats which is regarded as an experimental model for human minimal change nephrotic disease has been investigated. After two different dose of ADR were injected through the caudal vein for two consecutive weeks, ADR successfully induced a severe nephrotic syndrome with massive albuminuria, proteinuria, hyperlipidemia. The parameters of serum albumin, total triglyceride and 24-h urinary protein excretion were dramatically changed in ADR-group. These characteristic features of ADR-induced nephropathy are similar to those previously reported by other investigators. The changes reflect many functional alterations such as a drop in glomerular filtration rate, glomerular capillary damage.

It has been demonstrated that ACEI and ARB has the renoprotective effects, either alone or in combination, they reduce both systemic and glomerular capillary pressure. Furthermore, they could prevent the changes of TGF-β1 expression and the development of proteinuria. TGF-β1 is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein that performs many cellular functions, including the control of cell growth, cell proliferation. In this study losartan effectively reduced the 24-h urinary protein excretion of ADR-induced nephropathy rats. There was no significant difference between LOR-group and SUL-group. Based on the above, we could draw a conclusion that sulodexide was effective in attenuating ADR-induced nephrotoxicity in rats, and the effect is same to losartan for short-term use.

The protective mechanisms of losartan relates to systemic hemodynamic changes and inhibition of angiotensin II-mediated synthesis of local growth factors such as TGF-β1. However, sulodexide is a GAG-based drugs that will not affect the systemic and glomerular capillary pressure. Its anti-proteinuric mechanism may be associated with maintaining the composition of glomerular basement membrane (GBM), improving the function of vascular endothelia, alleviating podocyte injury and preventing TGF-β1 over-expression in renal tissue.

In conclusion, our research showed sulodexide could alleviate ADR-induced nephrotoxicity as good as losartan. This study provided theoretical evidence for the use of sulodexide in the treatment of glomerular diseases, however the molecular mechanism of sulodexide need further investigation.

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**REFERENCES**


