Effect of GM6001 on the expression of syndecan-1 in rats with acute kidney injury and its protective effect on the kidneys

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Received November 7, 2019; Accepted December 3, 2019

DOI: 10.3892/etm.2020.8892

Abstract. Expression of syndecan-1 (SDC-1) in rats with acute kidney injury and the protective effect of GM6001 on the kidney were investigated. Fifty SD rats were selected and randomly divided into control group (CG) (n=15), treatment control group (TCG) (n=10), module group (MG) (n=15) and treatment group (TG) (n=10). In TG, the model of acute renal injury (AKI) in rats was established after pretreatment of intraperitoneal injection of GM6001 one day before modeling. In MG, the same amount of saline was injected intraperitoneally one day before modeling and the same treatment was done on the day of modeling. In CG, the same amount of saline was injected intraperitoneally one day before modeling but the model was not made. In TCG, rats were pretreated with intraperitoneal injection of GM6001 one day before modeling but the model was not made. The contents of blood urea nitrogen (BUN) in serum, serum creatinine (SCR), uric acid (UA) and blood β2-microglobulin (β2-MG) were detected by ELISA. The content of SDC-1 in renal tissues was detected by qRT-PCR and western blotting. Expression of SDC-1 in renal tissue of 24 rats after modeling was lower than that of MG (P<0.050). SDC-1 expression was the highest in TG (P<0.05). Compared with before modeling, the contents of BUN, SCR, UA and β2-MG in MG and TG increased (P<0.05). After modeling, the contents of serum BUN, SCR, UA and β2-MG in TG were significantly lower than those in MG (P<0.05). The levels of SDC-1 in renal tissue of rats with acute kidney injury increased. After GM6001 treatment, SDC-1 levels can be improved and has a certain protective effect on the kidneys.

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Key words: syndecan-1, acute kidney injury, rat model, GM6001

Introduction

Acute kidney injury (AKI) is a life-threatening disease syndrome characterized by rapid loss of renal excretion function (1). This serious complication often occurs in critically ill patients with devastating consequences (2). Dialysis is an important choice for the treatment of acute renal failure in clinical treatment, but maintenance of dialysis leads to a higher daily medicine burden and peritoneal dialysis patients are at great risk of infection (3). At present, many drugs used for preventing and treating AKI have shown benefits in clinical models, but there is no specific drug for a curative effect in treatment of humans (4). The drug treatment of acute kidney injury needs further investigation.

Syndecan-1 (SDC-1) is a ubiquitous and multifunctional extracellular matrix proteoglycan, which can mediate the binding and activity of basic fibroblast growth factor (bFGF) and plays an important role in cell adhesion and maintenance of epithelial integrity (5). Lu *et al* (6) confirmed that ischemic AKI can be effectively prevented by inhibiting shedding of SDC-. Junior *et al* (7) also found that SDC-1 is a new biomarker for renal injury and endothelial dysfunction in HIV patients. The abscisic enzymes of matrix metalloproteinase (MMP) 7 (8), MP9 (9) and other SDC-1 proteases participate in the proteolysis of SDC-1 extracellular domain. GM6001 is a broad-spectrum MMP inhibitor (10), which acts on MMP-1, MMP-2, MMP-3 and MMP-8. It was considered that the application of GM6001 can reduce the proteolysis of SDC-1 by inhibiting the expression of MMPs, thus achieving the purpose of inhibiting SDC-1 to treat or prevent the development of AKI and effectively protect renal tissues of patients.

In this study, the expression of SDC-1 in the kidney tissues of rats was detected after the application of GM6001 to explore the protective effect of GM6001 on SDC-1 and kidney by establishing the rat model of acute kidney injury.

Materials and methods

Animals. Fifty clean grade 2 week-old SD rats, weighing ~180-250 g, were purchased from Kay Biological Technology (Shanghai) Co., Ltd. The rats were raised at the temperature of 24.00±2.00˚C, humidity 50.00±5.00%, natural light, free access

to food and drink. The experiment was approved by the animal ethics committee of Weifang People's Hospital (Weifang, China).

Methods

Experimental methods. The rats after adaptive feeding were randomly assigned as CG (n=15), TCG (n=10), MG (n=15) and TG (n=10). Modeling method: Rats were anesthetized with phenobarbital. Bilateral renal arteries and renal veins were bluntly separated through the abdominal median incision. Bilateral renal arteries were clamped with non-invasive vascular clamps. The vascular clamps were loosened after 1 h. Renal artery blood flow was restored and abdomen was closed layer by layer. Then the other groups of rats were treated: In TG, after pretreatment of intraperitoneal injection of GM6001 one day before modeling, the kidney injury model of rats was established according to the above method on the day of modeling. In MG, the same amount of saline was injected intraperitoneally one day before modeling and the same treatment was done on the day of modeling. In CG, the same amount of saline was injected intraperitoneally one day before modeling but the model was not made on the day of modeling. In TCG, rats were pretreated with intraperitoneal injection of GM6001 one day before modeling and the model was not made on the day of modeling. The contents of blood urea nitrogen (BUN), serum creatinine (SCR), uric acid (UA) and blood β2-microglobulin (β2-MG) in the above four groups were detected, respectively, before modeling and 24 h after successful modeling. All rats were sacrificed and the kidney tissues of the rats were stripped off. One piece of tissue was taken from each part and placed in 10% neutral formaldehyde at 4˚C overnight for fixation to detect the contents of SDC-1 in kidney tissue.

Samples detection

QRT-PCR: Detection of the contents of renal serum SDC-1. The collected serum was accelerated to coagulate, left to stand for 30 min and centrifuged at 4˚C for 5 min at 400 x g. The serum was taken and stored in a 1.5 ml centrifuge tube in a refrigerator at-80˚C for testing. Total RNA was extracted. The concentration and purity of the extracted RNA were determined by using an ultraviolet spectrophotometer. The A260/A280 ratio should be between 1.8 and 2.1. The RNA concentration was calculated for further experiments. Then TransScript Green miRNA Two-Step qRT-PCR SuperMix was used for reverse transcription into cDNA. After the reaction, the cDNA sample was used as the template of qRT-PCR (Thermo Fisher Scientific - CN) reaction and put into the refrigerator for testing. The reaction conditions were cDNA 1 μl, forward primer 0.4 μl, Universal miRNA qPCR Primer 0.4 μl, 2X TransStart Tip Green qPCR SuperMix 10 μl, Passive Reference Dye (50X) (Optional) 0.4 μl and total volume 20 μl. ddH_2O was used to complete to 20 μl. The conditions were as follows: pre-denaturation at 95˚C for 5 min, then denaturation at 90˚C for 15 sec, annealing and extension at 60˚C for 30 sec and the cycle was repeated 40 times. The relative expression of SDC-1 was expressed by $2^{-\Delta Ct}$. All the tests were repeated 3 times and the results were averaged (Table I).

Western blotting: Detection of the contents of SDC-1 in kidney tissue. After the collected rat kidney tissues were fully ground, the total protein was extracted by RIPA lysis. The protein concentration was detected by BCA (NC-BIO, LCB004). The protein concentration was adjusted to 4 μg/μl. 12% SDS-PAGE electrophoresis separation was carried out. The membrane was transferred to PVDF membrane after ionization and soaked with PBST for 5 min for washing. 5% skim milk powder was used for sealing for 2 h and first antibody (1:1,000 Abcam, ab34164) was added for sealing overnight at 4˚C. The first antibody was removed by washing the film. Horseradish peroxidase-labeled sheep anti-mouse second antibody (1:5,000 LD, LD-BJ-101891) was added, incubated at 37˚C for 1 h and rinsed 3 times with PBS for 5 min each time, and then developed in the darkroom. The excess liquid on the film was dried with a filter paper. The ECL was illuminated and developed. The protein bands were scanned and the gray values were analyzed in the Quantity One. Relative expression level of its protein = the gray value of the target protein band / the gray value of the β-actin protein band.

ELISA: Detection of renal injury indexes in rat blood. Renal function indexes include BUN, SCR, UA and β2-MG. The detection was performed by using automatic biochemistry analyzer. Detection kit was BUN (Shanghai Kanglang Biological Technology Co., Ltd., KLJC0193), SCR (Shanghai Yuanye Biological Technology Co., Ltd., S83553), UA (Chundu Biological Technology Co., Ltd., CD-1161-LIN) and blood β2-MG (SenBeiJia, SBJ-R0215).

Observation indexes. Levels of BUN, SCR, UA and β2-MG of rats in four groups before and after injection. SDC-1 levels of rats in four groups after treatment.

Statistical methods. All the experimental results were statistically calculated by using SPSS 24.0 statistics (Shanghai Yuchuang Network Technology Co., Ltd.). Graphpad 8 (Shenzhen Tianruiqi Software Technology Co., Ltd.) was used to draw graphs and check the statistical calculation. The results of the experiments were all expressed in the form of (mean number ± standard deviation). Single factor analysis of variance was used for comparison among groups. t-test was used for comparison between two groups of data conforming to normal distribution. Nonparametric test was used for data of non-normal distribution. Paired t-test was used for comparison before and after modeling. The difference was statistically significant at $P<0.050$.

Results

Modeling results. Among the 50 rats, 25 were modeled and 1 died in MG. The success rate of modeling was 96%.

Effect of GM6001 on SDC-1 expression in TG and TCG. The expression of SDC-1 protein in TG was 5.68±0.74 and it was the highest among the three groups ($P<0.001$). Expression of SDC-1 protein in CG was 1.49±0.34, which was not significantly different from the expression of SDC-1 protein in TCG of 1.45±0.48 ($P>0.050$) and was significantly lower than that in the other two groups ($P>0.001$) (Fig. 1).

The levels of SDC-1 mRNA in TG were 54.39±5.34 and it was also the highest among the three groups ($P<0.001$). The

Table I. Primer sequences.

	Upstream sequence (5'-3')	Downstream sequence (5'-3')
SDC-1	CAGCAGCAACACCGAGAC	GATTGGCAGTTCCATCCTC
GAPDH	TGGCAAAGTGGAGATTGTT	CTTCTGGGTGGCAGTGAT

SDC-1, syndecan-1.

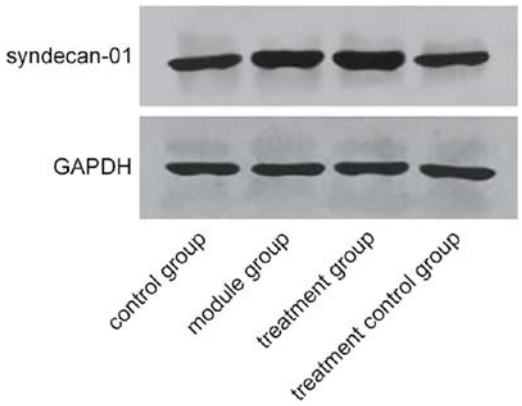

Figure 1. Detection results of western blotting.

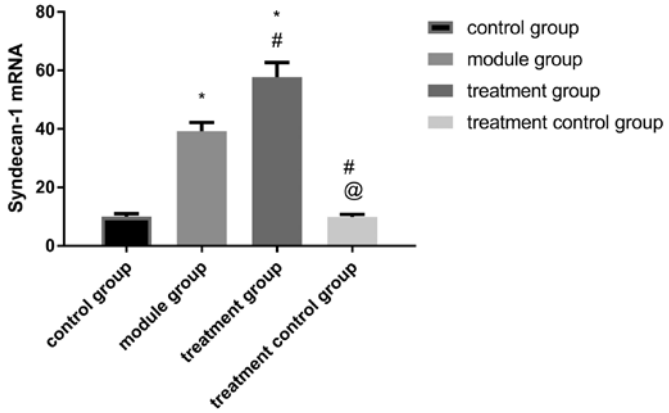

Figure 2. Detection of SDC-1 mRNA expression of rats in each group after treatment by qRT-PCR. *P<0.001, compared with CG; #P<0.001, compared with MG; @P<0.001, compared with TG, SDC-1, syndecan-1. CG, control group; MG, module group; TG, treatment group.

levels of SDC-1 mRNA in CG was 9.74±1.39, which was not significantly different from the levels of SDC-1 mRNA in TCG of 9.61±1.87 (P>0.050) and was lower than that in the other two groups (P>0.001) (Fig. 2).

Correlation between SDC-1 protein expression in tissues and SDC-1 mRNA levels in serum. Through Pearson correlation analysis of the relationship between SDC-1 protein expression in rat tissues and SDC-1 mRNA levels in serum of each group, it was found that SDC-1 protein expression in rat tissues was positively correlated with SDC-1 mRNA levels in serum (Fig. 3).

Changes of renal function indexes of rats in each group before and after treatment. There was no significant difference in BUN, SCR, UA and β2-MG contents among the groups before modeling (P>0.05). Compared with before modeling, there was no significant difference in the contents of BUN, SCR, UA and β2-MG between CG and TCG after modeling (P>0.05). Compared with before modeling, the contents of BUN, SCR, UA and β2-MG in MG and TG increased (P<0.05). After modeling, there was no significant difference between CG and TCG (P>0.050), while the contents of BUN, SCR, UA and β2-MG in TG were significantly lower than those in MG and higher than those in CG and the TCG (P<0.05) (Table II).

Discussion

AKI is an extremely painful and costly disease that affects more than 13 million people each year, 85% of whom live in developing countries (10). However, the prevalence of acute kidney injury is also increasing in developed countries. The incidence rate is estimated to be as high as 15% in hospital patients and it is more common in severe patients, with the prevalence rate estimated to be as high as 60% (11). How to treat acute kidney injury is currently a major medical problem in the world.

SDC-1 is a member of the transmembrane sulfate acid heparin proteoglycan family. Through its sulfate acid heparin chain, SDC-1 covalently binds to a variety of extracellular ligands (12,13). It mediates cell adhesion to several extracellular matrix molecules and promotes cell proliferation (14). Relevant reports have also proven that down-regulation of SDC-1 induces glomerular endothelial cell dysfunction by regulating the internalization of VEGFR-2, suggesting that SDC-1 may be a new target for the treatment of AKI (15). BUN and Cr are important indicators of the severity of renal function injury (16-18). The increase of β2-MG in urine can predict residual renal function (19), which is currently the most commonly used renal function detection indicator in clinical and animal research and can reflect renal state to some extent. In this study, AKI rats were treated with different treatment methods and the expression of SDC-1 and renal function levels in rat kidney tissue were measured to explore the protective effect of GM6001 on the kidneys.

The results of this experiment showed that compared with CG, the expression of SDC-1 protein and mRNA in MG were increased and the levels of renal function were decreased. The difference was statistically significant, indicating that SDC-1 participates in the development and progression of AKI. According to the study of de Melo Bezerra Cavalcante *et al* (20), the expression levels of SDC-1 in AKI

Table II. Changes of renal function indexes before and after treatment.

	CG	MG	TG	TCG	F	P-value
Before modeling						
BUN (mmol/l)	5.32±1.39	5.34±1.42	5.38±1.37	5.33±1.41	0.004	0.999
SCR (μmmol/l)	85.52±6.34	83.46±5.97	83.48±6.51	83.33±5.94	0.002	0.999
UA (μmol/l)	128.45±14.37	127.25±15.540	127.32±12.53	128.39±13.59	0.024	0.995
β2-MG (mg/l)	0.089±0.028	0.090±0.027	0.089±0.029	0.087±0.029	0.019	0.996
After modeling						
BUN (mmol/l)	5.32±1.41	12.53±2.52[a,d]	9.24±1.94[a,b,d]	5.38±1.32[b,c]	33.600	<0.001
SCR (μmmol/l)	83.63±6.39	139.43±13.45[a,d]	114.50±16.35[a,b,d]	83.47±5.45[b,c]	53.96	<0.001
UA (μmol/l)	129.73±15.74	198.35±31.53[a,d]	169.36±29.45[a,b,d]	127.46±13.94[b,c]	19.470	<0.001
β2-MG (mg/l)	0.089±0.027	0.213±0.059[a,d]	0.175±0.045[a,b,d]	0.088±0.028[b,c]	22.130	<0.001

[a]P<0.050, compared with the CG at the same time; [b]P<0.050, compared with the MG at the same time; [c]P<0.050, compared with the TG at the same time; [d]P<0.050, compared with before treatment at the same group. BUN, blood urea nitrogen; SCR, serum, serum creatinine; UA, uric acid; β2-MG, β2-microglobulin.

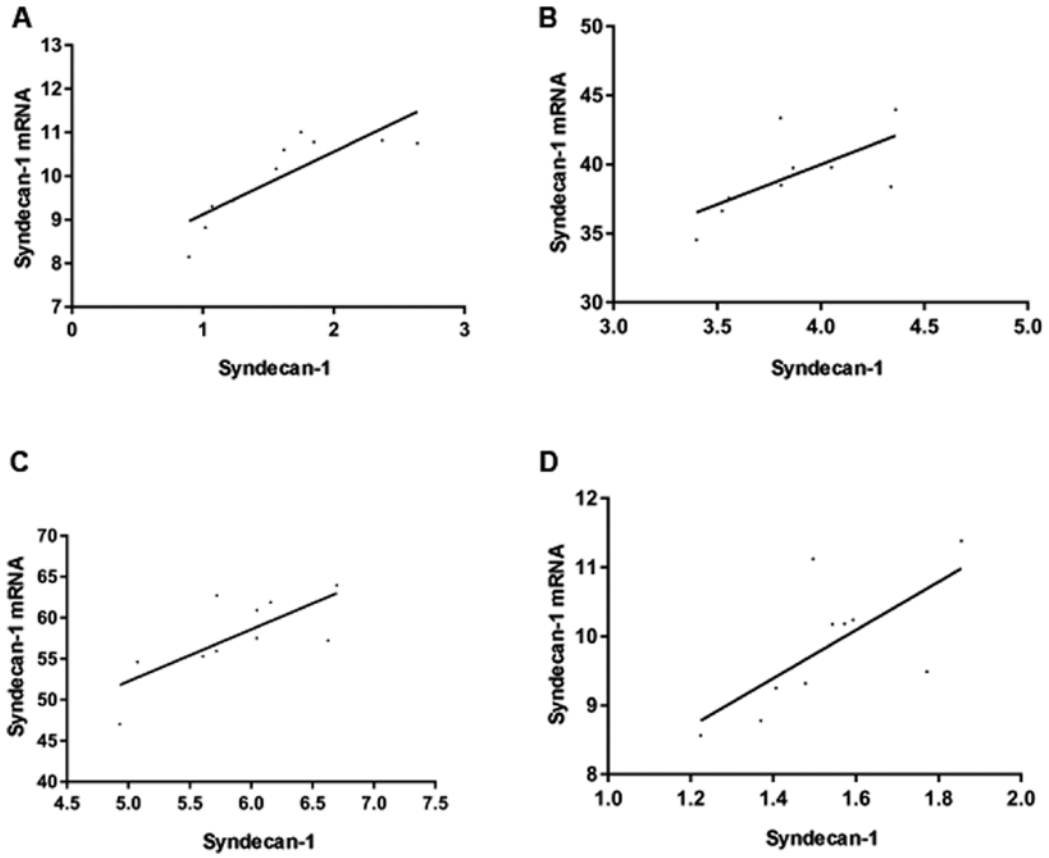

Figure 3. Correlation between SDC-1 protein expression in tissues and SDC-1 mRNA levels in serum. (A) SDC-1 protein expression in the control group (CG) was positively correlated with SDC-1 mRNA levels in serum (r=0.838, P=0.003). (B) SDC-1 protein expression in the model group was positively correlated with SDC-1 mRNA levels in serum (r=0.657, P=0.031). (C) SDC-1 protein expression in the treatment group (TG) was positively correlated with SDC-1 mRNA levels in serum (r=0.735, P=0.015). (D) SDC-1 protein expression in the treatment of CG was positively correlated with SDC-1 mRNA levels in serum (r=0.670, P=0.027). SDC-1, syndecan-1.

caused by pediatric cardiac surgery were increased, which is approximately consistent with the results of this study. In studies of Mosaad *et al* on adolescent systemic lupus erythematosus patients (21), it was found that the serum levels of SDC-1 in patients were higher than that in healthy control group (CG), which can further support the results of this study. Our research also showed that the expression of SDC-1 protein in rat tissues was positively correlated with the levels of SDC-1 mRNA in serum, which further confirms the relationship between the two. However, we found that the SDC-1 content

in TG was higher than that in MG and the renal function level in TG is better than that in MG, suggesting that the application of GM6001 before AKI can inhibit SDC-1 hydrolysis and has a protective effect on renal function level. However, there was no significant difference in renal function between TCG and CG, which also indicated that GM6001 has no negative effect on renal function of rats and can be used for treatment of AKI. The reason is that soluble SDC-1 can participate in the repair process of damaged tissues and promote the regeneration and repair of renal tubules when AKI occurs in the body (22). After the development of AKI, renal expression levels of MMP-2 and MMP-9 significantly increased (23), while matrix metalloproteinases such as MMP-7 (24) belong to hydrolase and participate in the down-regulation of SDC-1, thus reducing the levels of SDC-1 in the body and weakening the repair function of renal tissue. GM6001 has been proved to be able to inhibit MMP extensively (25). GM6001 changes the three-dimensional structure of the enzyme by binding with MMPs polypeptide to inhibit MMPs activity (26), reduce SDC-1 hydrolysis and to improve the renal protection role of SDC-1.

This study found that GM6001 can reduce SDC-1 hydrolysis by inhibiting MMP expression by establishing the AKI rat model. However, due to the short experimental period, the long-term effect of GM6001 on AKI were not observed. In this experiment, PCR was used for analysis. As the best quantitative detection method currently recognized in clinical practice, PCR is highly representative. In order to make the experimental data reliable, we used tissue testing, which can only be carried out when the rats were sacrificed. Therefore, no detailed multi-time point data was possible.

In conclusion, the increasing concentration of SDC-1 indicates that renal dysfunction is gradually aggravated. GM6001 can effectively improve renal function in rats after the treatment of AKI and is expected to become an effective drug for treating AKI.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KZ wrote the manuscript. KZ and RL conceived and designed the study. RL and GX were responsible for the collection and analysis of the experimental data. GX and HH interpreted the data and drafted the manuscript. LQ performed PCR, Western blot analysis and ELISA. KZ and LQ revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Weifang People's Hospital (Weifang, China).

Patient consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no competing interests.

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