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Smad signaling pathway in pathogenesis of kidney injury induced by calcium oxalate stone in rats

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ABSTRACT

Objective: To investigate the involvement of Smad signaling pathway in the pathogenesis of kidney injury induced by calcium oxalate stone in rats to provide a reference for clinical treatment. **Methods:** Clean SD rats were randomly divided into 3 group, namely the control group, model group and pirfenidone group. Ethylene glycol + hydroxy vitamin D3 was used as a stone-inducing agent to replicate the renal calcium oxalate stone model. Rats in the pirfenidone group were treated with pirfenidone intragastric administration. The serum Cr, BUN and 24-hour oxalate and calcium in renal tissues were assayed. The expressions of Bax/Bcl2 protein, Caspase3 protein, TGF β , Smad1, Smad2 and Smad3 proteins were detected by the fluorescent quantitation PCR method. **Results:** Compared with the rats of the control group, the results showed that the levels of serum BUN, Cr and 24-hour oxalate in rats of the model group were increased greatly, *Bax* and *Caspase3* mRNA also increased while the level of *Bcl2* decreased significantly, and the expressions of TGF β , Smad1, Smad2 and Smad3 proteins increased distinctly as well (P<0.01). These abnormal parameters could be normalized effectively by pirfenidone. **Conclusions:** Activated TGF β /Smad signaling pathway is involved in the pathogenesis of kidney injury induced by calcium oxalate stone in rats.

1. Introduction

Kidney injury is a common clinical consequence of a variety of pathological processes such as urolithiasis, diabetes, high blood pressure and so on, which has a strong impact on the life qualities and survival conditions of patients[1,2]. The pathological process of kidney injury is concerned with many factors, such as the oxidative status, high glucose, ischemia, *etc*. However, the exact pathogenesis of the disease still remains unknown[3,4]. TGF β /Smad signaling pathway is existing popularly in cells and participating in processes such as diabetes, high blood pressure, ischemia reperfusion injury and so on. Besides, it could be used as a possible target in the treatment of kidney injury[5,6]. This study aimed to investigate the involvement of Smad signaling pathway in the pathogenesis of kidney injury induced by calcium oxalate stone in rats so as to provide a reference for future clinical treatment.

2. Materials and methods

2.1. Animals and sources

Clean SD rats weighting from 220-250 g were purchased from Shanghai Slack Experimental Animal Center. Those rats were allowed to acclimate for 10 days. After that, they were randomly divided into the control group, model group and pirfenidone group (n=10). The renal calcium oxalate stone model was replicated in accordance with the previous reported method[7,8]. Rats in the pirfenidone group received drug intragastric administration.

2.2. Reagents

Pirfenidone was bought from Sigma Aldrich; Bax/Bcl2 and Caspase3 amplification primers were entrusted to Shanghai

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Biosynthesis; TGF β protein was acquired from Wuhan Boster Bio-engineering Co. Ltd; Smad1 and Smad2 detection kits were bought from Santa Cruz and Smad3 protein detection kit was from Abcam; detection kits of Cr, BUN and 24-hour oxalate were purchased from Nanjing Detection Bioengineering Institute; the total protein extraction kit was form Beijing Tiangen and the rest reagents were commercially available.

2.3. Fluorescent quantitation PCR method

The extraction and reverse transcription of mRNA were all carried out in accordance to the specifications. The reaction conditions of PCR included 94 ℃-3 min, 93℃-30 s, 55℃-30 s, 72℃90 s and 32 cycles. And the primer sequences included *Bax* forward primer 5'-TCCACCAAGAAGCTGAGCGAG-3', reverse primer 5'-GTCCAGCCCATGATGGTTCT-3'; *Bcl2* forward primer 5'-TTCTTTGAGTTCGGTGGGGTC-3', reverse primer 5'-TGCATATTTGTTTGGGGCAGG-3' and *Caspase3* forward primer 5'-TGTGGCATTGAGACA GAC-3'and its reverse primer 5'-CAC TTGCCATACAAACTA-3′.

2.4. Data statistics

SPSS19.0 was applied for data analysis. Measurement data were expressed by mean±sd and comparisons between groups were illustrated by One-way ANOVA. $P<0.05$ suggested that differences were statistically significant.

3. Results

3.1. Changes of serum Cr, BUN and 24–hour oxalate levels in the model of kidney injury induced by calcium oxalate stone in rats

It was found that the levels of the serum Cr, BUN and 24-hour oxalate and calcium in renal tissues increased significantly. Compared with the control group, the differences were statistically significant ($P<0.01$) (Table 1).

3.2. Change of protein expression of apoptosis in renal tissues in model of kidney injury induced by calcium oxalate stone in rats

It was shown in researches that apoptosis protein *Bax* and *Caspase3* mRNA increased significantlywhile the level of *Bcl2* mRNA decreased significantly. Compared with the control group, the differences showed evident statistical significances ($P<0.01$). The above indexes in rats of the pirfenidone group were recovered significantly ($P<0.01$) (Figure 1).

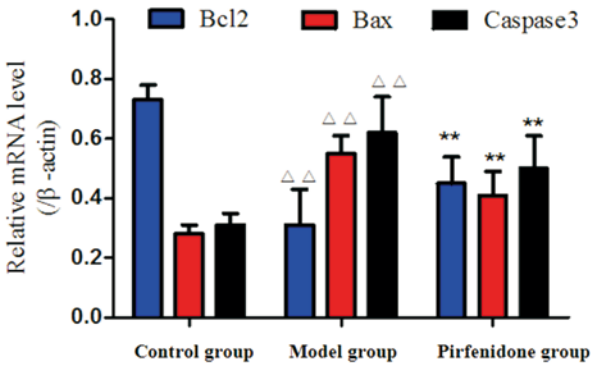

Figure 1. Change of protein expression of apoptosis in renal tissues in the model of kidney injury induced by calcium oxalate stone in rats.
Compared with the control group, $^{\triangle\triangle}P<0.01$; compared with the model group, $^{**}P<0.01$.

3.3. Changes of TGF β , Smad1, Smad2 and Smad3 protein expressions in renal tissues of model of kidney injury induced by calcium oxalate stone in rats

It was discovered that the expressions of TGF β , Smad1, Smad2 and Smad3 proteins in renal tissues in the model of kidney injury induced by calcium oxalate stone in rats increased distinctly. Also, compared with the control group, the differences had distinct statistical significances ($P<0.01$). The above parameters in rats of the pirfenidone group were recovered significantly ($P<0.01$) (Figure 2).

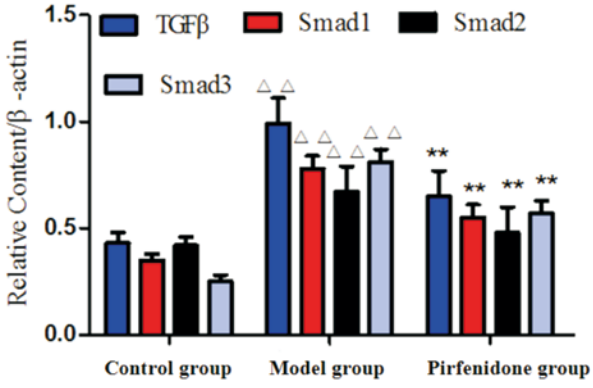

Figure 2. Changes of TGF β , Smad1, Smad2 and Smad3 protein expressions in renal tissues of model of kidney injury induced by calcium oxalate stone in rats.
Compared with the control group, $^{\triangle\triangle}P<0.01$; compared with the model group, $^{**}P<0.01$.

Table 1
Changes of serum Cr, BUN and 24-hour oxalate levels in the model of kidney injury induced by calcium oxalate stone in rats.

Term	Control group	Model group	Pirfenidone group	*P*
Cr (mmol/L)	7.12 ± 1.32	19.4 ± 2.98	14.4 ± 2.77	0.002
BUN (μmol/L)	28.8 ± 5.41	99.7 ± 12.6	76.2 ± 11.7	0.005
24-hour oxalate (μmol/24 h)	21.7 ± 3.09	80.9 ± 5.10	47.8 ± 5.89	0.004
Calcium in renal tissues (mg/g)	1.32 ± 0.09	111.5 ± 14.3	62.5 ± 10.3	0.007

4. Discussion

Urolithiasis is a common disease with a high postoperative reoccurrence rate affecting human health. There are many factors inducing urolithiasis. More than 80% of them are caused by calcium oxalate stones. At present, extracorporeal shock wave lithotripsy (ESWL) is commonly used in the treatment of the disease on clinic, but its pathogenesis is still unclear. In this study, the involvement of Smad signaling pathway in the pathogenesis of kidney injury induced by calcium oxalate stone in rats was explored. The results showed that the levels of serum BUN, Cr and 24-hour oxalate in rats of the model group were increased greatly, *Bax* and *Caspase3* mRNA also increased evidently while the level of Bcl2 decreased significantly, and the expressions of TGF β , Smad1, Smad2 and Smad3 proteins increased distinctly as compared with those in rats of the control group (P<0.01). These abnormal parameters could be normalized effectively by pirfenidone. Hence, it is concluded that activated TGF β /Smad signaling pathway participates in the pathogenesis of kidney injury induced by calcium oxalate stone in rats.

TGF β /Smad signaling pathway is a common pathway for multiple pathological processes of kidney injury. Moreover, it is a potential target for drug treatment. It is learned from the study of the effects of ginseng imperial sugar pills on the expressions of TGF- β 1 and Smad2/3 in the kidney of diabetic nephropathy rats that ginseng could decrease the fibrosis of the renal tissues of the diabetic rats and improve their pathologic structure by inhibiting the over-activation of the signaling pathway[9]. It was also approved in the research of the effect of formula for promoting blood circulation and removing phlegm on the expression of TGF- β and Smad7 in in the kidney of diabetic nephropathy rats that drugs delay the development of diabetic kidney fibrosis and improve kidney injury mainly by inhibiting the TGF β /Smad signaling pathway[10]. The study of the effect of Yishen capsule on the expression TGF- β 1 and Smad7 in rats with diabetic nephropathy also confirmed that drugs could improve kidney fibrosis and recover kidney injury by inhibiting the above parameters[11]. Ren *et al* also affirmed that the effects of activin A on renal tubulointerstitial fibrosis in diabetic rats were achieved mostly by restraining the over-activation of TGF β /Smad signaling pathway[12]. It was found in this study that the expression levels of protein TGF β , Smad1, Smad2 and Smad3 in renal tissues of the model of kidney injury induced by calcium oxalate stone in rats increased significantly and the differences showed significantlystatistical significances as compared with the control group. However, those indexes in rats of the pirfenidone group were recovered distinctly, which indicated that TGF β /Smad signaling pathway is involved in the pathogenesis of kidney injury induced by calcium oxalate stone in rats and pirfenidone can improve the kidney injury by restraining the over-activation of the signaling pathway.

Therefore, it can be concluded that activated TGF β /Smad signaling pathway participates in the pathogenesis of kidney injury induced by calcium oxalate stone in rats.

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