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

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Calcium oxalate stone
Smad signaling pathway
Apoptosis
Cr
Oxalate

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 groups, namely the control group, model group and pirfenidone group. Ethylene glycol + α-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, TGFβ, 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 TGFβ, Smad1, Smad2 and Smad3 proteins increased distinctly as well (P<0.01). These abnormal parameters could be normalized effectively by pirfenidone.

Conclusions: Activated TGFβ/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]. TGFβ/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
Biosynthesis; TGF-β protein was acquired from Wuhan Boster Biotechnology 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 from 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'-GTCCAGCCCATGATGGTCTC-3'; Bel2 forward primer 5'-TTCCTTTGAGTTCGGTGGGTG-3', reverse primer 5'-TGATATTGGGGGCAGG-3' and Caspase3 forward primer 5'-TGGTCATTTGGGGGCAGG-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 significantly while the level of Bel2 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).

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).

Table 1

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

Urolithiasis is a common disease with a high postoperative recurrence 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, showed that the levels of serum BUN, Cr and 24-hour oxalate in induced by calcium oxalate stone in rats was explored. The results of Smad signaling pathway in the pathogenesis of kidney injury but its pathogenesis is still unclear. In this study, the involvement (ESWL) is commonly used in the treatment of the disease on clinic, oxalate stones. At present, extracorporeal shock wave lithotripsy Urolithiasis is a common disease with a high postoperative.

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.

References

[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 significantly statistical 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.