Effect of Yishen Huayu Fang on kidney tissue E-cadherin expression in unilateral ureter ligation in rats

Zhang Lin-qi, Li Wei-ming, Sun Wei

Abstract

OBJECTIVE: To observe E-calcium sticky protein (E-cadherin) expression in kidney tissues in a rat model of unilateral ureter ligation and the effect of Yishen Huayu Fang (formula of tonifying the kidney and dissolving accumulated blood stasis) on the expression.

METHODS: A total of 150 clean grade male rats were randomly divided into a control group, model group, low-dose Yishen Huayu Fang group (low-dose group), high-dose Yishen Huayu Fang group (high-dose group), and Lotensin group. A renal fibrosis model was established with unilateral ureteral obstruction (UUO). Pathological changes of rat renal tissue were observed with light microscopy on days 3, 7, 14, 21, and 28 after UUO. Changes in kidney tissue E-cadherin expression were observed with immunohistochemistry.

RESULTS: Three days after modeling, kidney edema appeared followed by gradual inflammatory cell infiltration, and part of the small tubules disappeared while the renal cortex thinned. Meanwhile, the E-cadherin expression level dropped, which was negatively correlated with the obstruction time. After intervention, E-cadherin expression was increased in all treatment groups ($P<0.01$ or $P<0.05$), while there were no significant differences between the high-dose and Lotensin groups.

CONCLUSION: Yishen Huayu Fang delays the renal fibrosis process by promoting E-cadherin expression in renal tissues and reducing extracellular matrix deposition.

INTRODUCTION

Renal interstitial fibrosis (RIF) is the main basic pathology of various renal diseases and results in progression to end-stage renal disease. It features excessive accumulation of extracellular matrix and fibroblast proliferation in the renal interstitium. Epithelial-to-mesenchymal transition (EMT) is reportedly an important mechanism in the occurrence of RIF, and abnormal expression of E-calcium sticky protein (E-cadherin) plays a significant role in the process of EMT. How to effectively inhibit RIF progression and occurrence of EMT is a crucial issue in the prevention and cure of chronic renal failure. The formula for tonifying the kid-
ney and dissolving accumulated blood stasis is formed based on traditional Chinese medicine, which believes that deficiency of the kidney and blood stasis is the pathogenesis of chronic renal failure. This formula can improve renal function after long-term clinical verifications, and a positive treatment effect has been shown. However, its mechanism of action needs further discussion. In this study, a rat unilateral ureteral obstruction (UUO) model was adopted to dynamically view the influence of the formula of tonifying the kidney and dissolving accumulated stasis of blood on renal tissue pathology and E-cadherin expression. The possible mechanism of the formula in preventing and curing RIF is also discussed.

MATERIALS AND METHODS

Laboratory animals
A total of 150 clean grade male SD rats, 4 to 6 weeks old and weighing 180 to 220 g, were purchased from Henan Laboratory Animal Center (Zhengzhou, China; animal license No. SCXK (Yu) 2005-0001) and raised in the Laboratory Animal Center of Henan University of Traditional Chinese Medicine.

Medicines and reagents
Yishen Huayu Fang, or granular formula of tonifying the kidney and dissolving accumulated stasis of blood in English (Huang Qi/Radix Astragali 30 g, Tu Si Zi/Se-men Cuscutae 15 g, Zhi Da Huang/Radix et Rhizoma Rhei Praeparatus 15 g, Dan Shen/Radix Salviae Miltiorrhizae 30 g, and E Zhu/Rhizoma Curcumae 10 g; produced by Jiangsu Jiangyin Tianjiang Pharmaceuticals Co., Ltd., Jiangyin, Jiangsu, China), was purchased from TCM Pharmacy of the First Affiliated Hospital of Henan University of Traditional Chinese Medicine (batch No. 0608635). The medicine was dissolved and diluted by distilled water in specific proportions before use. Rabbit anti-rat E-cadherin monoclonal antibody was obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China), and goat anti-rabbit secondary antibody and DAB color-developing agent were obtained from Beijing Zhongshan Jingqiao Biotechnology Co., Ltd. (Beijing, China).

Molding and grouping
The rats were randomly divided into a sham operation group, UUO group, high-dose group of formula of tonifying the kidney and dissolving accumulated stasis (high-dose group), low-dose group of formula of tonifying the kidney and dissolving accumulated stasis (low-dose group), and Lotensin group (n=30 in each group). For performance of UUO, an intraperitoneal injection with 10% chloral hydrate was given to anesthetize the rats. A vertical incision of about 3.0 cm into the left abdomen was then made after shaving locally, conducting routine disinfection, and covering a hole towel. The skin and muscular layer were respectively cut to reach the abdominal cavity. The kidney and ureter were dissociated, and the central section of the left ureter was raised after the ureter was separated with tissue forceps. The section close to the renal pelvis of the left ureter was ligated twice with 4.0 sutures at both ends, the ureter was snipped, and the kidney was placed in its original position. The skin was then sutured layer by layer. The UUO model was thus completed. For the sham operation group, the left ureter was separated without ligation. Lavage was performed in animals of all groups on the day following molding; normal saline (20 mL/kg) was used in the sham operation and UUO groups, formula of tonifying the kidney and dissolving accumulated stasis (32 or 8 g/kg) was used in the high- and low-dose groups, and Lotensin (1.6 mg/kg) was used in the Lotensin group.

Observational indices
Semi-quantitative analysis of pathological changes in renal tissue: Routine hematoxylin and eosin staining and Masson staining were conducted on renal tissue slices to observe the pathomorphological changes in renal tissue with an optical microscope. E-cadherin expression in renal tissue was detected with immunohistochemistry (PV two-step approach): 1) paraffin sections were gradually dehydrated; 2) antigen was retrieved; 3) primary antibody was added; 4) second antibody was added; 5) hydrochloric acid was developed with DAB; 6) alcohol was differentiated; 7) ethanol was gradually dehydrated, transparent, and finalized; and the 8) E-cadherin result was determined as follows. Ten non-repeated renal tubule-mesenchymal views of each section were randomly selected under a 200× optical microscope. The area and total optical density of each staining view was analyzed with Image-Pro Plus 6.0 Image Analysis software (Media Cybernetics, MD, USA). The integral optical density (IOD) value was also determined, and the mean IOD value of each view was calculated.

Statistical analysis
A general linear model was created using SPSS 13.0 statistical software (Statistical Product and Service Solutions Inc., Chicago, IL, USA), comparison among groups was performed with two-way ANOVA, and the statistical significance level was P<0.05.

RESULTS

Pathomorphological change in renal tissue
Three days after the operation, slight expansion of kid-
neuropathy and focal aggregation of interstitial mono-
nuclear cells, lymphocytes, and plasma cells were
found in rats of the UUO group. With progression of
the disease, the kidney tubules gradually expanded, ep-
ithelial cells swelled with vacuolar degeneration, inter-
stitial infiltration of inflammatory cells increased, and
the renal cortex thinned. Finally, the tubules atrophied and
disappeared, and the degree of fibrosis gradually wors-
ened. Throughout the disease course, pathological
changes in renal corporuses were of slightly different de-
grees. Protein and cellular casts in rats of each of treat-
ment group were improved, kidney tubule and renal in-
terstitial inflammatory cell infiltration were reduced,
and kidney tubule expansion and focal RIF were ame-
liorated as well. The high-dose and Lotensin groups
were superior to the low-dose group in terms of the
above pathologic processes. No obvious abnormalities
were found in renal corporuses. Table 1 shows compari-
sions of the renal tubular injury index among the
groups of UUO rats at different time points.

Table 1 Comparisons of renal tubular injury index among groups of UUO rats at different time points (n=30, ±x 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>3d</th>
<th>7d</th>
<th>14d</th>
<th>21d</th>
<th>28d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation group</td>
<td>1.67±0.72</td>
<td>2.10±0.88</td>
<td>2.33±0.55</td>
<td>2.50±0.25</td>
<td>2.46±0.84</td>
</tr>
<tr>
<td>UUO group</td>
<td>3.50±0.84</td>
<td>4.33±0.32</td>
<td>5.31±0.54</td>
<td>6.77±0.82</td>
<td>8.67±0.52</td>
</tr>
<tr>
<td>High dose group</td>
<td>2.17±0.75</td>
<td>2.67±0.51</td>
<td>3.50±0.55</td>
<td>4.83±0.65</td>
<td>5.50±1.05</td>
</tr>
<tr>
<td>Low dose group</td>
<td>2.83±0.89</td>
<td>3.66±0.72</td>
<td>4.67±0.84</td>
<td>5.50±0.86</td>
<td>6.17±0.75</td>
</tr>
<tr>
<td>Benazepril group</td>
<td>2.00±0.89</td>
<td>3.05±0.84</td>
<td>4.01±0.63</td>
<td>4.83±0.90</td>
<td>5.67±0.82</td>
</tr>
</tbody>
</table>

Notes: *P<0.05, **P<0.01 at the same time point compared with the sham operation group; *P<0.05, **P<0.01 at the same time point compared with the UUO group; *P<0.05 at the same time point compared with the high-dose group.

Changes in E-cadherin expression in renal tissue

Three days after the operation, renal tubular epithelial
cell E-cadherin in the sham operation group was posi-
tively expressed; E-cadherin expression was obviously
reduced in the UUO group compared with that in the
sham operation group. The expression of E-cadherin
progressively decreased with extension of the obstruc-
tion time (P<0.01). Compared with the UUO group,
the high-dose, Lotensin, and low-dose groups showed
decreased expression (P<0.01 or P<0.05, Table 2).

Table 2 Comparison of e-cadherin expression (IOD Value) in renal tissue among groups of UUO rats at different time points (n=30, ±x 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>3d</th>
<th>7d</th>
<th>14d</th>
<th>21d</th>
<th>28d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation group</td>
<td>37.70±1.16</td>
<td>37.64±1.51</td>
<td>37.45±1.25</td>
<td>36.68±0.90</td>
<td>37.30±1.39</td>
</tr>
<tr>
<td>UUO group</td>
<td>31.32±1.52</td>
<td>24.95±2.06</td>
<td>20.87±1.79</td>
<td>17.05±1.38</td>
<td>15.02±0.96</td>
</tr>
<tr>
<td>High dose group</td>
<td>36.43±1.48</td>
<td>32.29±1.29</td>
<td>30.82±0.69</td>
<td>28.70±1.42</td>
<td>28.00±1.47</td>
</tr>
<tr>
<td>Low dose group</td>
<td>32.44±1.64</td>
<td>29.38±2.05</td>
<td>26.48±1.05</td>
<td>24.13±0.60</td>
<td>22.82±1.13</td>
</tr>
<tr>
<td>Benazepril group</td>
<td>37.80±1.20</td>
<td>30.16±1.19</td>
<td>28.82±1.56</td>
<td>26.73±0.99</td>
<td>25.14±1.17</td>
</tr>
</tbody>
</table>

Notes: *P<0.05, **P<0.01 at the same time point compared with the sham operation group; #P<0.05, ##P<0.01 at the same time point compared with the UUO group; *P<0.05 at the same time point compared with the high-dose group.

DISCUSSION

When chronic kidney disease occurs, progressive fibro-
sis is almost inevitable, which greatly threatens human
health. Related research shows that RIF plays a leading
role in the outcome of kidney disease. It is the most im-
portant index of the severity of kidney dysfunction and
prognosis[5]. A variety of cells and growth factors are in-
volved in the occurrence of RIF. They interact one an-
other and eventually lead to increased synthesis, re-
duced degradation, and excessive deposition of the ex-
tracellular matrix, resulting in RIF[8]. The clinical
course of RIF is long, and its pathogenesis is complex.
Although various clinical intervention methods have
been developed, approximately 25% of patients still ex-
perience continual advancement of their kidney dis-
ease. Currently available treatments cannot completely
treat chronic kidney disease or prevent its development
to end-stage renal failure[10]. Therefore, it is a priority in
nephrology to study the pathogenesis of RIF and ex-
ploring effective treatments to block the progression of
RIF. However, because of the limitation of obtaining
kidney tissues, it is difficult to study the dynamic
pathologic process of RIF. To further study the patho-
logical mechanism of tubulointerstitial fibrosis, it is
necessary to establish a typical and stable RIF animal
model. The UUO model is a currently accepted classi-
cal model that can be used to study pathological chang-
enes in RIF. It can represent the processes of obstructive
and fibrotic kidney diseases in humans. The effects of
drugs on RIF can also be effectively evaluated with this
model[8]. Many quantifiable pathophysiological chang-
es occur within 1 w after UUO occurs, which makes it an attractive model. Comparisons were conducted between studies using this model and observations in renal patients after UUO. Much evidence has shown that UUO rodent models can reflect human kidney disease processes[11,12]. In this experiment, the pathological changes in the kidney tissues of the UUO rats were observed at five time points (days 3, 7, 14, 21, and 28) to elucidate the disease process from damage to fibrosis. On the third day after obstruction, the renal tubules showed mild expansion, inflammatory cell infiltration was observed, the kidney size macroscopically increased and lightened in color, and the renal cortex thinned. With prolonged obstruction, a large amount of renal tubular structural damage occurred, renal tubular epithelial cells expanded or shrank and collapsed, and there were casts in parts of the renal tubules. The renal tubular basement membrane structure was incomplete, and the tubular and mesenchymal structure basically disappeared. There were large numbers of renal interstitial lymphocytes and mononuclear cells infiltrating with fibrosis, while the pathological changes of the glomerulus were mild to different degrees. These changes indicated a successful model. These results are consistent with those reported in the literature[19].

E-cadherin is a member of the cadherin family and is widely present in many types of epithelial cells. Its main role is to mediate the adhesion reaction between the same type of cells and to serve as a cytoskeleton.[14] In the kidney, it is mainly present in the renal tubular epithelial cells. Normally, renal tubular epithelial cells are closely connected to one another to form a complete epithelial layer. As the adhesion molecules of the epithelial cells, E-calcium and a complete tubular epithelial basement membrane play an important role in maintaining the integrity of renal tubular epithelial cell structure and cell polarity, as well as in participating in regulation of differentiation[19]. It has been thought that renal tubular epithelial cell activation is an important cytological event in the RIF induction phase. Deletion of E-cadherin expression and damage to epithelial integrity are early events in the process of renal tubular epithelial cell transformation[10]. Deletion of E-cadherin expression will eliminate cell polarity and cause them to separate from the surrounding cells. The present study shows that E-cadherin expression in renal tubular epithelial cells in model rats started to decrease on day 3 after modeling. As the time after modeling increased, the renal tubular structural damage continued, a large number of renal tubules atrophied, renal fibrosis became severe, and E-cadherin expression progressively declined. There was a significant difference (P<0.01) between the model group and the sham group at the same time point. E-cadherin expression was significantly different in renal tubular epithelial cells with different severities of interstitial damage, and it was negatively correlated with the severity of renal fibrosis. There is no terminology for renal fibrosis in traditional Chinese medicine, but the research on the disorder has been performed from the viewpoint of traditional Chinese medicine. Some traditional Chinese medicine scholars believe that the main pathogenetic characteristics of the renal fibrosis are asthenia of healthy qi and sthenia of pathogenic factors[17], and a consensus was reached that deficiency of the kidney is the cause of renal fibrosis[19]. From the perspective of traditional Chinese medicine, while the clinical syndromes of various chronic kidney diseases are not identical, they all share the common pathogenesis of "kidney deficiency" and "blood stasis"[19]. Our previous study found that the key pathogenesis of patients with chronic renal failure was "deficient kidney as the root cause and blood stasis as the main symptom"[19]. Comprising Huang Qi (RadixAstragal), Tu Si Zi (Semen Cuscutae), Dan Shen (Radix Salviae Miltiorrhizae), Z. Zhu (Rhizoma Curcumae), and Zhi Da Huang (Radix et Rhizoma Rhei Praeparatus), Yishen Huayu Fang (formula of tonifying the kidney and dissolving accumulated blood stasis) is the authors’ principle formula to treat chronic renal failure. The results of the present study show that after treatment with different dosages of the formula, infiltration of inflammatory cells in the kidney decreased, the expansive renal tubular and focal fibrosis improved, and E-cadherin expression in renal tissues improved. The therapeutic effect of the high dose of Yishen Huayu Fang was similar to that of Lotensin, and both were better than low doses of Yishen Huayu Fang (P<0.01 and P<0.05). This suggests that Yishen Huayu Fang might benefit the kidney by improving the E-cadherin expression in the kidney tissues to reduce inflammatory cell infiltration and extracellular matrix deposition, thus treating renal fibrosis and slowing the process of chronic renal failure. However, because Yishen Huayu Fang is a compound preparation, further in-depth research is needed to explore the effective acting targets and molecular mechanism of the formula in terms of its anti-renal interstitial fibrosis function.

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