Correlation of renal peroxidative damage with apoptosis and autophagy in rats with chronic fluorosis

Yi-Min Duan¹, Dan Pu¹, Chen-Chen Wang¹, Ling Zhang¹, Shu-Jie Zhang²

¹ Department of Endemic Disease, Centers for Disease Control and Prevention of Xinjiang Uygur Autonomous Region, Urumchi 830002, China
² Department of Urology, the Fourth Affiliated Hospital of Xinjiang Medical University, Urumchi 830000, China

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Objective: To study the correlation of renal peroxidative damage with apoptosis and autophagy in rats with chronic fluorosis. Methods: Male SD rats were selected as experimental animals and randomly divided into model group and control group. After chronic fluorosis model rats were established, renal injury marker contents in serum as well as oxidative stress injury marker contents, apoptosis gene expression and autophagy gene expression in kidney tissue were determined. Results: 1 month, 2 months and 3 months after model establishment, serum BUN and Scr contents of model group were significantly higher than those of control group, and ROS contents, calpain-1, caspase-3, Notch1, Hes1, mTOR and Beclin-1 mRNA expression as well as LC3-II/LC3-I ratio in kidney tissue of model group were significantly higher than those of control group. Pearson test showed that ROS content in kidney tissue was positively correlated with BUN and Scr contents in serum as well as calpain-1, caspase-3, Notch1, Hes1, mTOR and Beclin-1 mRNA expression and LC3-II/LC3-I ratio in kidney tissue. Conclusion: The renal injury induced by peroxidation in rats with chronic fluorosis is closely related to the excessive activation of apoptosis and autophagy.

1. Introduction

Fluorine is one of the essential micronutrients in the body, and fluorine is commonly found in nature. The human body has a low demand for fluorine, and the long-term high fluorine environment or excessive fluorine intake can cause the fluorine accumulation in the body and cause tissue organ damage. The kidney is the main organ of the body to excrete fluorine, and about 85% of the fluorine is filtered through the glomeruli and excreted in the urine. The accumulation of fluorine in the kidney can cause glomerulus and renal tubule damage, which can cause renal insufficiency[1,2]. The studies about the tissue viscera damage due to chronic fluorosis in recent years suggest that the oxidative stress damage mediated by excessive oxygen free radical generation is an important factor of excessive fluorine accumulation to cause tissue damage[3,4], but the regulating mechanism of downstream oxidative stress is not yet clear. Apoptosis and autophagy are important biological behaviors, and excessive apoptosis and autophagy are thought to be associated with damage to various tissue organs. In the following studies, the correlation of renal peroxidative damage with apoptosis and autophagy in rats with chronic fluorosis was analyzed.

2. Experimental materials and experimental methods

2.1 Experimental materials

The experimental animals were a total of 60 male SD rats with body mass of 180-200 g, which were supplied by the laboratory animal center of Fourth Military Medical University. Animal experiments passed the centre ethical review, and the animal experiments and animal processing after death were carried out according to regulation. The ROS test kits were purchased in Shanghai Beyotime Company, and the Trizol lysis buffer and fluorescence quantitative PCR kits were purchased from Invitrogen Company.
2.2 Experimental methods

2.2.1 Chronic fluorosis rat model establishment

The rats were randomly divided into model group and control group, 30 in each group. The following method was followed to establish model group of rats into chronic fluorosis rat models: high fluoride water containing 60 mg/L sodium fluoride was configured, and model group of rats were free to drink high fluoride water during model establishment; control group were free to drink regular drinking water during the experiment.

2.2.2 Detection of renal injury markers in serum

1 month, 2 months and 3 months after model establishment, 10 rats were randomly selected from each group and put to death, blood specimens were collected immediately and centrifuged to separate serum, and automatic biochemical analyzer was used for determining the contents of BUN and Scr.

2.2.3 Detection of oxidative damage, apoptosis and autophagy markers in kidney tissue

1 month, 2 months and 3 months after model establishment, the rats were put to death and anatomized to get kidney tissue and divide it into two parts. One was added in protein lysis buffer and homogenized to get kidney tissue suspension, and the ROS content was determined by the ROS testing kit; the other was added in Trizol lysis buffer and homogenized to separate and extract total RNA for reverse transcription into cDNA, and fluorescence quantitative PCR kit was used to determine calpain-1, caspase-3, Notch1, Hes1, mTOR, Beclin-1, LC3-I and LC3-II mRNA expression.

2.3 Statistical processing methods

SPSS 19.0 software was used for t test of serum detection indexes and kidney tissue detection indexes of two groups of rats, and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Oxidative damage marker contents in kidney tissue and serum

1 month, 2 months and 3 months after model establishment, analysis of the contents of oxidative damage marker ROS (U/L) in kidney tissue and renal injury markers BUN (μmol/L) and Scr (mmol/L) in serum between two groups of rats was as follows: ROS contents in kidney tissue as well as BUN and Scr contents in serum of model group were significantly higher than those of control group. Differences in ROS contents in kidney tissue as well as BUN and Scr contents in serum were statistically significant between two groups of rats 1 month, 2 months and 3 months after model establishment (P<0.05). Pearson correlation analysis showed that ROS content in kidney tissue was positively correlated with BUN and Scr contents in serum.

3.2 Apoptosis marker gene expression in kidney tissue

1 month, 2 months and 3 months after model establishment, analysis of apoptosis marker genes calpain-1, caspase-3, Notch1 and Hes1 expression in kidney tissue between two groups of rats was as follows: calpain-1, caspase-3, Notch1 and Hes1 mRNA expression in kidney tissue of model group were significantly higher than those of control group. Differences in calpain-1, caspase-3, Notch1 and Hes1 mRNA expression in kidney tissue were statistically significant between two groups of rats 1 month, 2 months and 3 months after model establishment (P<0.05). Pearson correlation analysis showed that ROS content in kidney tissue was positively correlated with calpain-1, caspase-3, Notch1 and Hes1 mRNA expression in kidney tissue.

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time points</th>
<th>n</th>
<th>ROS</th>
<th>BUN</th>
<th>Scr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model group</td>
<td>1 month after model estab</td>
<td>10</td>
<td>7.5±0.93</td>
<td>11.25±1.38</td>
<td>165.52±22.42</td>
</tr>
<tr>
<td></td>
<td>2 months after model estab</td>
<td>10</td>
<td>11.36±1.52</td>
<td>13.52±1.93</td>
<td>225.29±31.08</td>
</tr>
<tr>
<td></td>
<td>3 months after model estab</td>
<td>10</td>
<td>15.45±2.26</td>
<td>15.78±2.27</td>
<td>272.83±42.47</td>
</tr>
<tr>
<td>Control group</td>
<td>1 month after model estab</td>
<td>10</td>
<td>2.42±0.36</td>
<td>6.47±0.93</td>
<td>84.52±9.32</td>
</tr>
<tr>
<td></td>
<td>2 months after model estab</td>
<td>10</td>
<td>2.29±0.34</td>
<td>6.65±0.86</td>
<td>86.12±11.27</td>
</tr>
<tr>
<td></td>
<td>3 months after model estab</td>
<td>10</td>
<td>2.55±0.32</td>
<td>6.52±0.91</td>
<td>83.48±10.86</td>
</tr>
</tbody>
</table>

*: comparison between model group and control group, P<0.05.

Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time points</th>
<th>n</th>
<th>Calpain-1</th>
<th>Caspase-3</th>
<th>Notch1</th>
<th>Hes1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model group</td>
<td>1 month after model estab</td>
<td>10</td>
<td>1.84±0.25</td>
<td>1.62±0.19</td>
<td>1.77±0.22</td>
<td>1.58±0.18</td>
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<td></td>
<td>2 months after model estab</td>
<td>10</td>
<td>2.55±0.37</td>
<td>2.31±0.36</td>
<td>2.21±0.35</td>
<td>2.19±0.32</td>
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<td>3 months after model estab</td>
<td>10</td>
<td>3.41±0.55</td>
<td>2.98±0.42</td>
<td>2.89±0.46</td>
<td>3.05±0.56</td>
</tr>
<tr>
<td>Control group</td>
<td>1 month after model estab</td>
<td>10</td>
<td>1.02±0.17</td>
<td>1.04±0.15</td>
<td>1.03±0.15</td>
<td>0.97±0.12</td>
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<tr>
<td></td>
<td>2 months after model estab</td>
<td>10</td>
<td>0.98±0.11</td>
<td>0.97±0.10</td>
<td>0.96±0.11</td>
<td>1.04±0.11</td>
</tr>
<tr>
<td></td>
<td>3 months after model estab</td>
<td>10</td>
<td>0.99±0.10</td>
<td>0.98±0.11</td>
<td>0.98±0.12</td>
<td>1.01±0.15</td>
</tr>
</tbody>
</table>

*: comparison between model group and control group, P<0.05.
Kidney tissue of model group showed that ROS contents in kidney tissue of model group were significantly higher than those of control group 1 month, 2 months and 3 months after model establishment. Further analysis of the correlation between ROS generation and renal injury showed that ROS content in kidney tissue was positively correlated with BUN and Scr contents in serum. This shows that the over-generated in the kidney tissue of rats with chronic fluorosis can cause oxidative damage to renal function.

Peroxidative damage can not only cause kidney function damage through direct action of reactive oxygen species, but can also act on the downstream apoptosis, autophagy and other biological behaviors. Apoptosis will directly affect the normal biological function of the cells, and then affect the function of the corresponding tissues and organs. Calpain-1 is a kind of pro-apoptosis molecule regulated by calcium overload, excessive fluorine accumulation will cause calcium overload and activate calpain-1, and the activated calpain-1 can cause anti-apoptosis molecule XIAP cutting and remove the inhibitory effect of XIAP on a variety of downstream caspase so as to activate the cascade amplification reaction mediated by caspase and execute apoptosis process by caspase.[10,11]. Notch1 signaling pathway is also closely related to cell apoptosis, Notch-1 shifts into the nucleus after activation and induces the expression of target genes Hes1 so as to affect cell growth process.[12,13]. In the study, analysis of the expression of above apoptosis markers in kidney tissue of model group showed that calpain-1, caspase-3, Notch1 and Hes1 mRNA expression in kidney tissue of model group were significantly higher than those of control group 1 month, 2 months and 3 months after model establishment. This shows that chronic fluorosis can activate the apoptosis mediated by calpain-1/caspase-3 and Notch1/Hes1 in kidney tissue. Further analysis of the correlation between ROS generation and apoptosis showed that ROS content in kidney tissue was positively correlated with calpain-1, caspase-3, Notch1 and Hes1 mRNA expression in kidney tissue of model group showed that ROS content in kidney tissue of model group were significantly higher than those of control group 1 month, 2 months and 3 months after model establishment.

### 3.3 Autophagy marker gene expression in kidney tissue

1 month, 2 months and 3 months after model establishment, analysis of autophagy marker genes mTOR and Beclin-1 mRNA expression as well as LC3-II/LC3-I ratio in kidney tissue between two groups of rats was as follows: mTOR and Beclin-1 mRNA expression as well as LC3-II/LC3-I ratio in kidney tissue of model group were significantly higher than those of control group. Differences in mTOR and Beclin-1 mRNA expression as well as LC3-II/LC3-I ratio in kidney tissue were statistically significant between two groups of rats 1 month, 2 months and 3 months after model establishment (*P*<0.05). Pearson correlation analysis showed that ROS content in kidney tissue was positively correlated with mTOR and Beclin-1 mRNA expression and LC3-II/LC3-I ratio in kidney tissue.

### 4. Discussion

Chronic fluorosis caused by excessive fluorine intake can cause damage to bone, kidney, liver, and other tissues and organs.[5-7]. Kidney is the main organ of the body to excrete fluorine, and excessive fluorine intake can cause increased accumulation of fluorine in the kidney and lead to glomerular filtration function and renal tubular excretion function damage. In the study, high fluoride drinking water was provided to establish the chronic fluorosis rat model, and the analysis of serum kidney function marker content at different time points after model establishment showed that BUN and Scr contents in serum of model group were significantly higher than those of control group 1 month, 2 months and 3 months after model establishment. This shows that the intervention of high fluorine drinking water can establish the chronic fluorosis model and cause kidney damage in the model rats. Studies about the tissue viscera damage due to chronic fluorosis in recent years have shown that the excessive fluorine accumulation can cause excessive oxygen free radical generation, and thus result in peroxidation damage to tissue organs.[8,9]. In the study, analysis of oxygen free radical generation in kidney tissue of model group showed that ROS contents in kidney tissue of model group were significantly higher than those of control group 1 month, 2 months and 3 months after model establishment.
II programmed cell death. mTOR is the most important upstream signal molecule to regulate autophagy process, and the hypoxia, oxidative stress, poisoning and other external stimuli will activate the autophagy process mediated by mTOR signaling pathway, increase the expression of Beclin-1 and interact with a variety of Atg molecules to participate in the formation of autophagosome\cite{14,15}. LC3-I transforms to LC3-II when the autophagy is activated\cite{16}. During physiologic autophagy, the cells use their own lysosome to degrade the damaged organelles and macromolecules, which can not only remove the damaged macromolecules and organelles, but also provide energy for cells by degradation products\cite{17}. However, the persistence of external damaging factors will cause the continuous activation of autophagy process, and then cause cell damage. In the study, analysis of the expression of above autophagy markers in kidney tissue of model group showed that mTOR and Beclin-1 mRNA expression as well as LC3-II/LC3-I ratio in kidney tissue of model group were significantly higher than those of control group 1 month, 2 months and 3 months after model establishment. This suggests that chronic fluorosis will activate the autophagy mediated by the mTOR signaling pathway in the kidney tissue. Further analysis of the correlation between ROS generation and autophagy showed that ROS content in kidney tissue was positively correlated with mTOR and Beclin-1 mRNA expression as well as LC3-II/LC3-I ratio. This shows that the over-generated reactive oxygen species in the kidney tissue of rats with chronic fluorosis can cause excessive activation of autophagy.

To sum up, it is believed that chronic fluorosis can cause peroxidative damage to the kidney tissue; the excessive generation of reactive oxygen species in the kidney tissue can cause excessive activation of apoptosis and autophagy, and then cause kidney damage.

References