Short Communication

Preventive Effects of Taurine and Vitamin C on Renal DNA Damage of Mice Exposed to Arsenic

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Arsenic (As), as a pollutant, ubiquitously exists in well water and contaminated soil. There is also exposure to arsenic in some industries, such as non-ferrous smelting, wood preservation, and electronics. The risk of As-induced human diseases is high in some developing countries such as Bangladesh, India, and China1,2. Epidemiologic investigations and animal experiments have demonstrated that acute and chronic exposure to As can cause injury to the kidney and increase the risk of renal cancer3, so the kidney may be a major target of toxicities induced by As. It was reported that As depresses the functions of the antioxidant defense system and led to oxidative damage to cellular macromolecules4,5. The above results show that increase of oxidative stress and subsequent oxidative DNA damage of cells may be involved in the renal disorders induced by As. Taurine, an end product of l-cysteine metabolism, is the most abundant free amino acid in many tissues and protects against the adverse effects of reactive oxygen species (ROS) caused by various toxic substances6. Vitamin C (Vit C), as a kind of water-soluble antioxidant, also prevents oxidative damage to many important macromolecules7.

8-OHdG is formed from deoxyguanosine (dG) in DNA by hydroxy free radicals. Because of its stability, 8-OHdG is known as one of the most reliable markers of oxidative DNA damage8. In the present study, in order to investigate the protective effects of taurine and Vit C against As-induced nephrotoxicity, we examined the expression of 8-OHdG and the histopathological changes in the renal tissues of mice administered As or both As and taurine or Vit C.

Materials and Methods

Chemicals
Arsenic trioxide, taurine and Vit C were purchased from the Sigma Chemical Company (St. Louis, USA). Mouse monoclonal anti-8-OHdG antibody was obtained from the Japan Institute for the Control of Aging (Fukuroi, Japan). Diaminobenzidine (DAB) color reagent kit and UltrasensitiveTM S-P kit were purchased from MAIXIN-Bio (Fuzhou, China).

Animal and treatment
Forty Kunming mice (20 male and 20 female) weighing 20 ± 2 g were purchased from the Experimental Animal Center, Dalian Medical University. These mice were randomly divided into 4 groups of 5 mice in each gender. Group 1 received drinking water alone as controls. Group 2 received 4 mg/l arsenic trioxide. Group 3 and group 4 received 4 mg/l arsenic trioxide with 150 mg/kg taurine or 45 mg/kg Vit C (as combined treatment groups), respectively. Arsenic trioxide was given through drinking water for 60 days. Taurine and Vit C were administered by gavage twice a week. The animals were maintained on a standard diet and water ad libitum. They were caged under a 12-h dark-light cycle under standard conditions of temperature (18–22°C) and humidity (50%). After the last administration of arsenic trioxide, the thoraxes of the mice were opened under urethane (1 g/kg, ip) anesthesia, and tissue fixative (10% formalin) was injected via a needle inserted into the left ventricle of the heart; and then, the kidneys were removed and placed in fixative. The animal experiment was performed in accordance with the Animal Guideline of Dalian Medical University and in agreement with the Ethical Committee of Dalian Medical University.

Histopathological examination
The formalin-fixed kidney tissues were embedded in paraffin, sliced at 5 µm, mounted on glass slides coated with poly L-lysine, and subjected to hematoxylin and eosin staining according to routine histopathological methods. Histopathological changes were observed under a light microscope.

Immunohistochemistry for 8-OHdG formation
For immunohistochemical staining with the streptavidin-biotin-peroxidase method, 3-µm tissue sections were deparaffinized, dehydrated in graded alcohols and subjected to hematoxylin and eosin staining according to routine histopathological methods. Histopathological changes were observed under a light microscope.

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with diaminobenzidine (DAB) chromogen solution. Finally, sections were counterstained with hematoxylin and mounted in xylene-based mountant. Five fields selected randomly were observed for each section and the total optical density value of 8-OHdG was analyzed quantitatively using an image analyzer (Image-Pro Plus 4.5, Media Cybernetics).

Statistical analysis

Values were expressed as mean ± SD for the 10 mice of each group, and the significances of the differences between mean values were determined by one-way analysis of variance (ANOVA) followed by the Duncan test for multiple comparison using the Statistical Package for Social Sciences 11.5 (SPSS 11.5) computer package.

Table 1. Optical density value of 8-OHdG in kidney tissues of mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Chemicals</th>
<th>No. of Animals*</th>
<th>Optical density value (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (controls)</td>
<td>drinking water</td>
<td>10</td>
<td>4.94 ± 1.67</td>
</tr>
<tr>
<td>Group 2</td>
<td>4 mg/l As₂O₃</td>
<td>10</td>
<td>5,522.71 ± 686.34ab,c</td>
</tr>
<tr>
<td>Group 3</td>
<td>4 mg/l As₂O₃ + 150 mg/kg taurine</td>
<td>10</td>
<td>25.83 ± 5.90</td>
</tr>
<tr>
<td>Group 4</td>
<td>4 mg/l As₂O₃ + 45 mg/kg Vit C</td>
<td>10</td>
<td>23.32 ± 12.12</td>
</tr>
</tbody>
</table>

* Five mice of each gender. Because there was no significant difference between genders (data not shown) in each group, the optical density values of 8-OHdG of both genders were pooled into one group for calculation of the mean ± SD. a p<0.01, compared with controls; b p<0.01, compared with group 3; c p<0.01, compared with group 4.

Results

Expression of 8-OHdG in kidney tissue of mice

As shown in Fig. 1, intensive 8-OHdG expression was found in the kidney tissues of mice exposed to As. The 8-OHdG expression was mainly distributed in the regions of the glomerulus and renal tubules. Especially, high expression of 8-OHdG was show in the proximal convoluted tubule and Bowman’s capsule. In contrast, in the two combined treatment groups receiving taurine or Vit C, weak expression of 8-OHdG was observed in the kidney tissues. In controls, no 8-OHdG expression was seen. Optical density values of 8-OHdG were quantitatively evaluated using an image analyzer and the results are shown in Table 1. Because there was no significant difference between the genders (data not shown) in each group, the optical density values of 8-

Fig. 1. 8-OHdG immunoreactivity in the renal tissues of mice given various arsenic treatments. Mice of four groups were treated respectively with drinking water (a, as control), 4 mg/l arsenic trioxide (b), 4 mg/l arsenic trioxide and 150 mg/kg taurine (c), and 4 mg/l arsenic trioxide and 45 mg/kg vitamin C (d). PCT: proximal convoluted tubule, DCT: distal convoluted tubule. G: glomerulus. Each 8-OHdG-positive cell appears brown, and had stained nuclei. Original magnification ×200.
OHdG of both genders were pooled into one group for calculation of the mean ± SD. The optical density value of the As-exposed group was significantly higher than those of the other three groups (p<0.01).

**Histopathological changes in kidney tissues of mice**

Histopathological changes in kidney tissues of mice are shown in Fig. 2. Cellular swelling and tubular dilatation were observed in kidney tissues of mice exposed to As. Especially, more evident structural damage, such as loss of cell-cell contacts and loss of microvilli were seen in the epithelium of proximal convoluted tubule. In the glomerulus, pathological changes were mainly found in the area of Bowman’s capsule. Bowman’s capsules were diminished or disappeared in the group exposed to As. However, dilation and hyperemia of glomerular capillaries and mild cellular proliferation were also observed in the glomerulus. In contrast, the pathological changes in the two combined treatment groups were mild and showed only slight swelling of the cell body in the proximal convoluted tubules. Moreover, the cell boundary was clear and without any changes in the cytoplasm and nucleus. In controls, there were no histopathological changes in the proximal convoluted tubules and glomerulus. The regions where these histopathological changes presented in the kidneys of mice exposed to As were consistent with the distribution of 8-OHdG expression.

**Discussion**

Epidemiologic investigations and animal experiments have shown that acute and chronic exposure to As induces dysfunction of the renal system and renal diseases, indicating that As possesses nephrotoxicity. It has been reported that As generates ROS, inducing lipid peroxidation and the oxidative damage of proteins as well as DNA. As DNA is more sensitive to oxidative stress, 8-OHdG an oxidative product of DNA, is used for evaluating oxidative damage in vivo.

In the present study, obvious expression of 8-OHdG in kidney tissues was observed in the group which received As alone. The 8-OHdG expression was mainly concentrated in the regions of the glomerulus and renal tubules. Especially, high expression of 8-OHdG was seen in the proximal convoluted tubules and Bowman’s capsule. Histopathological changes such as cellular swelling, dilation and hyperemia of glomerular capillaries in kidney tissue were also found in the As-exposed group. In particular, the more evident pathological changes were seen in the proximal convoluted tubules and Bowman’s capsule. Moreover, the regions where these histopathological changes presented in the kidneys of the mice exposed to As were consistent with the distribution of 8-OHdG expression.

Fig. 2. Histopathological changes in the renal tissues given various arsenic treatments. The treatment and abbreviations are the same as in the legend to Fig.1. Histopathological changes were examined by H&E staining. Original magnification ×200.
of 8-OHdG expression. These results suggest that the kidney may be a major target of As toxicity, and that the epithelial cells of proximal convoluted tubules and Bowman’s capsule seem to be more sensitive to As-induced nephrotoxicity. Many recent studies have provided experimental evidence that As exposure can result in the generation of ROS and cause cell damage or death through the ROS signaling pathway. These studies and the result of our immunohistochemistry analysis indicate that the pathological changes in the kidney tissues of mice exposed to As may be related to the As-induced oxidative stress. The proximal convoluted tubules are particularly sensitive to their high reabsorptive activity and anatomical position as the first renal epithelial cell to be exposed to filtered toxicants. It was reported that chronic toxicity of As to kidney resulted in proteinuria. However, the mechanism is still unclear. Recent studies by many investigators have shown that the podocyte is important for maintaining permselectivity. Podocytes line the outer aspect of the glomerular basement membrane and serve as the final defense against urinary protein loss. Once the podocytes are damaged, loss of permselectivity follows. Therefore, we suspect that proteinuria resulting from chronic As poisoning may be associated with As-induced damage to podocytes.

In the two combined treatment groups receiving taurine or Vit C, weak expression of 8-OHdG in the kidney tissues was observed. Moreover, the optical density value of the combined treatment 8-OHdG expression of these two groups was significantly lower than that of the As-exposed group ($p<0.01$). In these two groups, the pathological changes showed slight swelling of the cell body in the proximal convoluted tubule. However, the cell boundary was clear. These results indicate that taurine and Vit C protect the kidney against arsenic-induced oxidative DNA damage and pathologic changes. It is well known that taurine is the major intracellular free $\beta$-amino acid and plays an important physiological role in the prevention of oxidant-induced injury to many tissues. The preventive effect of taurine as an antioxidant for As-induced damage may be attributed to its ability to stabilize biomembranes, scavenge ROS, and reduce the production of lipid peroxidation. In addition, many studies have demonstrated that Vit C, as a water-soluble antioxidant, can readily scavenge ROS, reactive nitrogen species (RNS), increase As excretion and prevent oxidative damage to many important biological macromolecules such as DNA, lipids, and proteins. Hence, the preventive effect of Vit C on renal damage induced by As may be associated with its antioxidant capacity. In summary, the above results show that taurine and Vit C protect against As-induced nephrotoxicity. Further studies are required to explaining the molecular mechanisms of their preventive action.

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References