**Panax notoginseng** saponins attenuated cisplatin-induced nephrotoxicity

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**KEY WORDS** cisplatin, ginseng, saponins, cultured cells, blood urea nitrogen, creatinine, cell survival, DNA, cross-link reagents, calcium

**ABSTRACT**

**AIM** To study protective effects of *Panax notoginseng* saponins (PNS) against cisplatin-nephrotoxicity.

**METHODS** Cisplatin-induced nephrotoxicity in mice in vivo and primary culture of rabbit proximal tubular cells (PTC) in vitro were established. Blood urea nitrogen, serum creatinine, cell viability, DNA interstrand cross-link, DNA-protein cross-link and cytosolic free Ca2+ were assayed with diacetyl monoxime, alkaline picrate, trypan blue, ethidium bromide binding and Fur 2-AM respectively.

**RESULTS** With pretreatment for 2 d in mice, PNS 100 and 200 mg kg⁻¹ d⁻¹ suppressed cisplatin-induced high blood urea nitrogen level to 83 % and 31 % respectively. Preincubated with PTC for 24 h, PNS 10 and 100 mg L⁻¹ inhibited cisplatin-induced decrease of cell viability from 78 % to 81 % respectively. P < 0.05 and 89 % respectively. P < 0.01. DNA interstrand cross-link to 47 % and 40 % respectively. DNA-protein interstrand cross-link to 77 % and 42 % respectively. Cytosolic free Ca2⁺ overload in PTC to 70 % and 63 % respectively, P < 0.01.

**CONCLUSION** PNS was a prophylactic for cisplatin-induced nephrotoxicity and mechanisms were relevant to the effects that PNS reduced cisplatin-induced cytosolic free Ca2⁺ overload and formations of DNA interstrand cross-link and DNA-protein cross-link.

**INTRODUCTION**

Cisplatin is an effective antitumor agent but its nephrotoxicity is serious characterized by high blood urea nitrogen, BUN and serum creatinine Cr levels. Cisplatin induced lipid peroxidation and oxygen free radical generation in kidney and these effects damaged the kidney. However, as genetic substance was a target for cisplatin-induced nephrotoxicity cisplatin-induced nephrotoxicity was more serious than carboplatin. In addition, cytosolic free Ca²⁺ overload was important for cisplatin-induced nephrotoxicity.

*Panax notoginseng* saponins PNS showed extensive biological activities, e.g. protection from damage of genetic substance and antagonizing cytosolic free Ca²⁺ overload. Therefore, it was examined if reduction of cisplatin-induced nephrotoxicity took place with PNS pretreatment in this study.

**MATERIALS AND METHODS**

Materials RPMI-1640 culture medium was obtained from Gibco USA. HEPES and ethidium bromide were purchased from Fluka Switzerland. β-Mercaptoethanol was obtained from Sigma USA. 125I was made in Chinese Atomic Energy Isotope Co. PNS 83.5 % pure contained ginsenoside Rb₁ 31.8 % Rg₁ 29.1 %, R₁ 10.7 % and R₂ 11.9 % was obtained from Mr. WANG Yao-De in Sichuan Institute of Chinese Materia Medica. Cisplatin was obtained from Shandong Qilu Pharmaceutical Factory. New Zealand white rabbits about one month old and Kunming mice Grade II Certificate No 99058 were obtained from Center of Laboratory Animal Third Military Medical University.

Primary culture of kidney proximal tubular cells PTC PTC were prepared with an established procedure. Alkaline phosphatase of brush border and epithelium keratin in PTC were stained with cytochemistry. Gomori Ca-Co and anti-keratins.

**Animals** Kunming mice were randomly divided into
5 groups. Control vs. Cisplatin group. Cisplatin 5 mg kg⁻¹ d⁻¹ ip for 4 d. Treatment groups after PnS 50 mg 100 mg 200 mg kg⁻¹ d⁻¹ ip for 2 d. then with PnS 50 100 200 mg kg⁻¹ d⁻¹ ip and cisplatin 5 mg kg⁻¹ d⁻¹ ip for another 4 d.

PTC groups. Control group PTC were incubated without cisplatin and PnS for 48 h. Cisplatin groups PTC were incubated without cisplatin and PnS for 24 h then with cisplatin 26 µmol L⁻¹ for another 24 h. Treatment group PTC after PTC were preincubated with PnS 100 mg L⁻¹ for 24 h cisplatin 26 µmol L⁻¹ was added into culture and incubated for another 24 h.

Assays of blood urea nitrogen and serum creatinine Urea nitrogen in blood was reacted with diacetyl monoxime to produce diazine with maximal absorbance at 540 nm. Creatinine in separated serum was combined with alkaline picate to produce creatinine-picate compound with maximal absorbance at 510 nm [10].

Cell viability Cell viability of PTC was counted with trypan blue.

Assay of DNA interstrand cross-link The DNA interstrand cross-link was measured with ethidium bromide binding assay [11]. The DNA interstrand cross-link was expressed as ISC.

Assay of DNA-protein cross-link The DNA-protein cross-link was measured with [12] postlabelling [12]. DNA-protein cross-link DPC was expressed as Bq g⁻¹ DNA.

Assay of cytosolic free Ca²⁺ Cytosolic free Ca²⁺ in PTC was measured with Fura 2-AM [13].

Statistical analysis Results were expressed as x ± s. Newman-Keuls test was used.

RESULTS

Effects of PnS on BUN and Cr in mice by cisplatin After mice were injected with cisplatin 5 mg kg⁻¹ d⁻¹ ip for 4 d BUN and Cr in cisplatin group were increased to 4 and 4.4 times respectively of those in control group P < 0.01. But BUN and Cr in PnS 100 mg kg⁻¹ d⁻¹ ip group were decreased to 83 % and 86 % respectively of those in cisplatin group P < 0.01. In PnS 200 mg kg⁻¹ d⁻¹ ip group BUN and Cr were 31 % and 42 % respectively of those in cisplatin group P < 0.01. However BUN and Cr in PnS 50 100 200 mg kg⁻¹ d⁻¹ ip were higher than those in control group P < 0.01 Tab 1.

Characterization of PTC Cells were stained black and brown with cytochemistry and anti-keratin respectively. These results showed that cultured cells were PTC.

Effects of PnS on cell viability of PTC by cisplatin Cisplatin decreased cell viability from 98 % in control to 78 % in cisplatin 26 µmol L⁻¹ P < 0.01. PnS increased cell viability from 78 % to 81 % in 10 mg L⁻¹ P < 0.05 and 89 % in 100 mg L⁻¹ P < 0.01 Tab 2.

Tab 1. Influence of PnS on blood urea nitrogen and serum creatinine in mice. n = 8. x ± s. aP < 0.01 vs single treatment with cisplatin 5 mg kg⁻¹ d⁻¹. bP < 0.01 vs control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PnS/ Cr/</th>
<th>Cisplatin/</th>
<th>BUN/</th>
<th>Cr/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
<td>mmol L⁻¹</td>
<td>µmol L⁻¹</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>8 ± 1</td>
<td>65 ± 9</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>0</td>
<td>5</td>
<td>36 ± 6f</td>
<td>284 ± 18f</td>
</tr>
<tr>
<td>Treatment</td>
<td>50</td>
<td>5</td>
<td>40 ± 2f</td>
<td>303 ± 49f</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5</td>
<td>30 ± 3f</td>
<td>246 ± 29f</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>5</td>
<td>11 ± 1f</td>
<td>120 ± 18f</td>
</tr>
</tbody>
</table>

Tab 2. Influence of PnS on cell viability cisplatin-induced formations of DNA interstrand cross-link and DNA-protein cross-link. x ± s. aP < 0.05 bP < 0.01 vs single treatment with cisplatin 26 µmol L⁻¹. cP < 0.01 vs control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PnS/ Cisplatin/</th>
<th>Cell viability</th>
<th>ISC</th>
<th>GBq g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg L⁻¹</td>
<td>µmol L⁻¹</td>
<td>%</td>
<td>n = 8</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>98 ± 2</td>
<td>0.02 ± 0.04</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>0</td>
<td>26</td>
<td>78 ± 3f</td>
<td>1.53 ± 0.09f</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>26</td>
<td>76 ± 4f</td>
<td>1.60 ± 0.12f</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>26</td>
<td>81 ± 4f</td>
<td>0.73 ± 0.06f</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>26</td>
<td>89 ± 4f</td>
<td>0.62 ± 0.06f</td>
</tr>
</tbody>
</table>

Effects of PnS on cisplatin-induced formations of DNA interstrand cross-link and DNA-protein interstrand cross-link in PTC ISC and Bq g⁻¹ DNA in 26 µmol L⁻¹ of cisplatin were increased to 77 and 11 times respectively of those in 0 µmol L⁻¹ P < 0.01 Tab 2. ISC in PnS 10 mg L⁻¹ groups was decreased to 47 % and 40 % respectively of that in cisplatin group P < 0.01 but was higher than that in control group P < 0.01. Bq g⁻¹ DNA in PnS 10 mg L⁻¹...
100 mg L⁻¹ groups was decreased to 77 % and 42 %, respectively, of that in cisplatin group but was higher than that in control group P < 0.01. Tab 2

Effects of PrnS on cytosolic free Ca²⁺ in PTC by cisplatin Cytosolic free Ca²⁺ in cisplatin 13 μmol L⁻¹ and 52 μmol L⁻¹ was increased to 160 % and 250 %, respectively, of that in control P < 0.01. Cytosolic free Ca²⁺ in PrnS 10 μmol L⁻¹ groups were decreased to 70 % and 63 %, respectively, of that in cisplatin 26 μmol L⁻¹ P < 0.01 but was higher than that in control P < 0.01. Tab 3

### DISCUSSION

Cisplatin-induced high BUN and Cr were decreased by PrnS 100 mg kg⁻¹ and 200 mg kg⁻¹ d⁻¹ ip in mice. The range of decrease in BUN and Cr was not great by PrnS 100 mg kg⁻¹ d⁻¹. However, PrnS 200 mg kg⁻¹ d⁻¹ could greatly decrease BUN and Cr. The protective effects of PrnS 100 mg kg⁻¹ d⁻¹ were not significant. The range of decrease in BUN and Cr was not great by PrnS 100 mg kg⁻¹ d⁻¹. However, PrnS 200 mg kg⁻¹ d⁻¹ could greatly decrease BUN and Cr. The protective effects of PrnS 100 mg kg⁻¹ d⁻¹ were not significant.

ISC and Bf g⁻¹ DNA in cisplatin 26 μmol L⁻¹ were increased to 77 and 11 times of those in 0 μmol L⁻¹ but cell viability was decreased from 98 % in control to 78 % in cisplatin 26 μmol L⁻¹. Therefore, the genetic toxicity of cisplatin was more serious than cellular toxicity. DNA interstrand cross-link and DNA-protein cross-link could suppress duplication of DNA expression of mRNA and synthesis of protein. Thus, formations of DNA interstrand cross-link and DNA-protein cross-link induced cisplatin nephrotoxicity. PrnS 10 μmol L⁻¹ inhibited cisplatin-induced formations of DNA interstrand cross-link and DNA-protein cross-link in PTC. Cisplatin directly induced formations of DNA interstrand cross-link and DNA-protein cross-link, therefore, mechanism of PrnS induced ion of attenuated cisplatin-induced nephrotoxicity is due to the fact that PrnS inhibited cisplatin-induced DNA interstrand cross-link and DNA-protein cross-link and not lipid peroxidation and oxygen free radical generation. PrnS 10 μmol L⁻¹ also antagonized cisplatin-induced cytosolic free Ca²⁺ overload in PTC. Cisplatin-induced cytosolic free Ca²⁺ overload seriously damaged PTC for example it inhibited the respiratory rate of mitochondrion. There can be two reasons relevant to the antagonistic effects of PrnS on cytosolic free Ca²⁺ overload: blocking of Ca²⁺ channels activation of Na⁺-K⁺ ATPase. Thus, the antagonistic effect of PrnS against cisplatin-induced cytosolic free Ca²⁺ overload in PTC led to the protective effects of PrnS against cisplatin-induced nephrotoxicity.

### REFERENCES


