Stimulation of p-Aminohippurate Transport in Renal Cortical Slices Prepared from Rats Treated with Ginsenosides

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The effect of treatment of rats with ginsenosides (extracted from Panax ginseng) on transport of p-aminohippurate (PAH) in renal cortical slices was studied for an in vitro reflection of PAH secretion in the proximal tubules in the kidney. The treatment of rats with ginsenosides stimulated PAH accumulation in the slices and tended to prevent the decrease in PAH accumulation in incubated slices from the rats with acute renal failure caused by cisplatin or ischemia followed by reperfusion. Ginsenoside treatment affected other biochemical responses in renal cortical slices, with a decrease in adenosine triphosphate level and an increase in potassium level. The latter may lead to the stimulation of PAH transport in the slices, but additional information on the cellular action of ginsenosides is needed for this conclusion. It may be that the stimulatory effect of ginsenosides on PAH accumulation in the slices counterbalances the decrease in PAH accumulation in the slices from rats with acute renal failure.

Keywords — acute renal failure; p-aminohippurate transport; cisplatin; ginsenoside; ischemic kidney; renal cortical slice

Introduction

Shibuya et al.1) have shown that the Chinese traditional medicine ginseng and Tang-kuei Ten Combination depressed the lethal toxicity and nephrotoxicity (assessed by the rise in the blood urea nitrogen level) caused by cisplatin in mice. Chen et al.2) reported that ginsenosides extracted from Panax ginseng helped to prevent myocardial damage in ischemia with reperfusion in rats. These findings suggest that ginsenosides affect cellular functions of kidney and heart, especially in acute failure of both organs which are usually induced by toxic substances or ischemia followed by reperfusion.

Necrosis of cells in a portion of the proximal tubules has been observed in acute renal failure induced by either cisplatin, an antineoplastic agent with nephrotoxicity,3,4) or ischemia, despite the different etiology.5) One function of the proximal tubules in the kidney is the secretion of p-aminohippurate (PAH), a prototype for organic anions transported in the tubules. PAH accumulation in renal slices, which can be used experimentally as an in vitro reflection of that function, is inhibited by cisplatin treatment or by ischemia followed by reperfusion.6,7)

This study was undertaken to examine the effect of treatment of rats with ginsenosides on PAH accumulation and other biochemical responses in kidney cortical slices and also on the decreased PAH accumulation in the slices prepared from rats after cisplatin treatment or ischemia with reperfusion.

Materials and Methods

Animals — Male Sprague-Dawley rats were housed in a room with controlled temperature (24 ± 1 °C), humidity (55 ± 10%), and light (12-h dark, 12-h light cycle) and given feed and water ad libitum. Rats weighing 215 ± 5 g were usually used and rats weighing 300 ± 10 g were used for the experiments with ischemia followed by reperfusion.

Chemicals — Ginsenosides, which were extracted from Panax ginseng C. A. Meyer by the method of Shibata et al.,8) were generously provided by Dr. Xiu Chen of Hunan Medical University (Changsha, P.R.C.). Cisplatin was pur-

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chased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of commercial reagent grade.

**Experiments with Ginsenoside and Cisplatin Treatments** — The rats were injected intraperitoneally (i.p.) with ginsenosides (50 mg/kg twice a day for two consecutive days). In the case of cisplatin treatment, they were given ginsenosides (50 mg/kg, i.p.) 26 h and again 2 h before the administration of cisplatin (7.5 mg/kg, i.p.). Ginsenosides and cisplatin were dissolved in 0.9% NaCl. Two days after the cisplatin treatment, the animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and the kidneys were rapidly removed, decapsulated, and placed in ice-cold 0.9% NaCl for preparation of slices.

**Experiments with Ischemia and Reperfusion** — Rats were treated i.p. with ginsenosides (50 mg/kg) for three consecutive days. The last dose was injected 30 min before the left renal artery was occluded surgically, as follows. Renal ischemia was induced in rats anesthetized with pentobarbital sodium (50 mg/kg, i.p.). The abdomen was opened and the renal arteries of both kidneys were occluded with silk thread. The right kidney was removed as a control immediately after the ligature of the right renal artery. After ischemia for 1 h and 3 h of reperfusion through the left renal artery, the left kidney was removed and placed in ice-cold 0.9% NaCl for the preparation of slices.

**Studies of Slices** — Slices were prepared from kidney cortex with a razor blade and immersed in chilled saline medium that contained 134 mM NaCl, 5.9 mM KCl, 1.5 mM CaCl₂, 1.2 mM MgCl₂, 11.5 mM glucose, and 5.8 mM HEPES (N-2-hydroxyethylpiperazine-N' -2-ethanesulfonic acid, adjusted to pH 7.4 with NaOH) as described elsewhere. In brief, the slices were transferred to 10 ml of an incubation medium that was identical to the above saline medium except that it also contained 0.074 mM PAH and 1% inulin. The inulin was added to estimate the extracellular space of the slices. The slices were incubated at 37 °C for 30 min with a gas phase of 100% oxygen to measure PAH accumulation, adenosine triphosphate (ATP) levels, potassium levels, water content, and extracellular (inulin) space in the slices. PAH and inulin were analyzed by the methods of Bratton and Marshall and Roe et al., respectively. PAH accumulation in the slices was expressed as the slice–medium concentration ratio (S/M) of PAH. ATP was measured by enzymatic spectrophotometry with a luciferase enzyme system as previously described. Potassium in the slices was assayed with the use of a flame photometer (Hitachi 205D) as reported before. The water content in the slices was the difference in their weights before and after desiccation at 100 °C overnight. The extracellular space of the slices was estimated from the ratio of the inulin concentrations in the slices and the medium.

**Statistics** — Values are expressed as means ± S.E. Statistical analysis was performed by using Student's t-test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAH accumulation (S/M)</th>
<th>ATP levela</th>
<th>Potassium levelb</th>
<th>Total water contentb</th>
<th>Extracellular spaceb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before incubation</td>
<td>0.92 ± 0.05</td>
<td>24.5 ± 0.77</td>
<td>82.6 ± 1.8</td>
<td>20.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>After incubation</td>
<td>12.5 ± 0.92</td>
<td>1.52 ± 0.07</td>
<td>59.4 ± 1.27</td>
<td>77.2 ± 0.9</td>
<td>32.1 ± 1.5</td>
</tr>
<tr>
<td>Ginsenosides</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Before incubation</td>
<td>0.71 ± 0.03</td>
<td>18.3 ± 0.55</td>
<td>84.5 ± 0.8</td>
<td>20.9 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>After incubation</td>
<td>15.1 ± 0.44</td>
<td>1.00 ± 0.05</td>
<td>67.1 ± 1.42</td>
<td>78.2 ± 1.3</td>
<td>33.3 ± 5.2</td>
</tr>
</tbody>
</table>

Slices were prepared from rats 2 d after the last intraperitoneal injection of ginsenosides (50 mg/kg twice a day for two consecutive days) and incubated at 37 °C for 30 min. Results are expressed as means ± S.E. of four experiments. a) μmol/g wet wt. b) % of wet wt. c) p < 0.05, d) p < 0.01, e) p < 0.005 vs. before incubation. f) p < 0.05, g) p < 0.01, h) p < 0.001 vs. saline (control) after incubation.
Results and Discussion

Table I presents that PAH was prominently accumulated by rat kidney cortical slices incubated for 30 min, by which PAH accumulation reached steady-state condition (data not shown) as previously reported. The administration of ginsenosides (50 mg/kg twice a day for two consecutive days) to rats significantly increased the ability of slices prepared from kidney cortex of the treated animals to accumulate PAH. This enhanced accumulation of PAH by ginsenosides may be mediated by their effect on intrinsic cellular responses in the slices. For instance, PAH is actively transported in the proximal tubular cells via the basolateral sodium/PAH cotransporter, which is driven by the sodium gradient. This gradient is maintained by the sodium/potassium ATPase in the basolateral membrane which consumes ATP as an energy source. To test this, experiments were designed to evaluate the effect of ginsenoside treatment on the biochemical responses of the incubated slices (Table I). The increase in the ATP level in the slices after incubation was significantly depressed by ginsenoside treatment. An increase in the potassium level in the slices after incubation was somewhat enhanced. Changes in the water content and extracellular (inulin) space in the slices after incubation was not affected by treatment with ginsenosides.

The injection of cisplatin to rats decreased the ability of the slices to accumulate PAH (Table II) as previously reported. Treatment of rats with ginsenosides before the injection of cisplatin prevented the depression by cisplatin of PAH accumulation in the slices. However, ginsenoside treatment itself increased PAH accumulation in the slices as shown in Table I, even when used in a lower dose (50 mg/kg per day for two consecutive days).

Acute renal failure caused by renal ischemia followed by reperfusion has been reported to increase the ability of renal cortical slices to accumulate PAH. We tried to examine the effect of ginsenosides on PAH accumulation in slices prepared from ischemic kidneys (Table III). Ginsenosides alone increased PAH accumulation in the slices as shown above. Their treatment had no significant effect but tended to prevent the decrease in PAH accumulation in the slices prepared from kidneys after 1 h of ischemia followed by 3 h of reflow.

It would seem from the above results that PAH accumulation in the slices was balanced to about the control level by both the inhibitory effect of cisplatin or renal ischemia and the stimulatory effect of ginsenosides, each with different mechanisms.

PAH transport is energy-dependent through being influenced by the sodium gradient maintained by the sodium pump coupled with potassium influx. Our results suggest that a decrease in the ATP level would depress PAH accumulation by reduced activity of the sodium pump. So, the stimulatory effect of ginsenosides on PAH transport cannot

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**Table II.** Effect of Treatment with Ginsenosides on PAH Accumulation in Slices Prepared from Rats 2 d after an Intraperitoneal Injection of Cisplatin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAH accumulation (S/M)</th>
<th>Effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>11.90 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>Cisplatin alone</td>
<td>8.59 ± 0.18</td>
<td>–28</td>
</tr>
<tr>
<td>Cisplatin + ginsenosides</td>
<td>11.05 ± 0.49</td>
<td>–7</td>
</tr>
<tr>
<td>Ginsenosides alone</td>
<td>15.38 ± 0.15</td>
<td>+29</td>
</tr>
</tbody>
</table>

Slices were prepared from rats treated with ginsenosides (50 mg/kg, i.p.) at 26 and 2 h before the injection of cisplatin (7.5 mg/kg, i.p.) and incubated at 37 °C for 30 min. Results are expressed as means ± S.E. of three experiments. 

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**Table III.** Effect of Treatment with Ginsenosides on PAH Accumulation in Slices Prepared from Ischemic Kidney of Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAH accumulation (S/M)</th>
<th>Before ischemia</th>
<th>After ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>11.38 ± 0.54</td>
<td>6.12 ± 0.86</td>
<td></td>
</tr>
<tr>
<td>Ginsenosides</td>
<td>14.86 ± 1.01</td>
<td>8.73 ± 1.00</td>
<td></td>
</tr>
</tbody>
</table>

Rats were treated intraperitoneally with ginsenosides at the dose of 50 mg/kg per day for three consecutive days. The last dose was injected 30 min before occlusion of the left renal artery. a) Slices were prepared from the right kidney just before occlusion of the left renal artery. b) Slices were prepared from the left kidney after 1 h of ischemia followed by 3 h of reperfusion. These slices were incubated at 37 °C for 30 min. Results are expressed as means ± S.E. c) p<0.001, d) p<0.05 vs. saline, before ischemia.
be explained by an effect on ATP metabolism. An increase in the potassium level resulted in a loss of the sodium level in the slices (data not shown), indicating an increase in the sodium gradient which leads to the stimulation of PAH transport. However, it is not conclusive from our findings whether there is correlation between the stimulation of PAH accumulation and the slight increase in the potassium level in the incubated slices by ginsenoside treatment.

Stimulation of PAH accumulation in slices has been reported in the presence of acetate, pyruvate, verapamil, dibucaine, or trifluoperazine in the incubation medium.\textsuperscript{12,18-21} Endogenous substances such as acetate and pyruvate can enhance energy metabolism in renal tissues,\textsuperscript{18} although the exact mechanism by which PAH transport is stimulated is not clear. This explanation does not apply with ginsenosides since they are not endogenous. Stimulation by verapamil, dibucaine, and trifluoperazine may be describable by their effects on membrane stabilization.\textsuperscript{12,20} Additional information on the ginsenoside action may be obtained by studying its effect on membrane stabilization. The stimulatory effect of ginsenosides on PAH accumulation probably is not due to water distribution, water content and extracellular space, because of no effect of ginsenosides on them in the incubated slices.

Cisplatin or ischemia followed by reperfusion can damage the epithelium of the proximal tubules where PAH transport occurs.\textsuperscript{5,6} Another factor in the acute renal failure induced by cisplatin or ischemia, besides leakage across the damaged tubular epithelium, is probably decreased renal blood flow caused by renal vasoconstriction and resulting in decreased renal function.\textsuperscript{22} It has been observed that ginsenosides slightly contract the renal vein of rabbits and also potentiate the contractile responses of the renal vein of dogs and rabbits to prostaglandin F\textsubscript{2a}.\textsuperscript{23} So, the possibility that the prevention by ginsenosides of the depression of PAH transport in acute renal failure induced by cisplatin or ischemia arises from a renal vascular effect of the ginsenosides can be ruled out.

Superoxide dismutase and antioxidants ameliorate the acute renal failure caused by cisplatin in rats.\textsuperscript{24-26} Paller \textit{et al.}\textsuperscript{27} suggested that reperfusion after ischemia of the kidney results in the production of oxygen free radicals, which then cause renal damage by lipid peroxidation. Chen \textit{et al.}\textsuperscript{28} reported that the components of ginsenosides counteract the increase in lipid peroxidation in ischemic myocardium. These results suggest that the prevention by ginsenosides of the depression of PAH accumulation in the slices in acute renal failure induced by cisplatin or ischemia followed by reperfusion could be explained by their being an antioxidant. However, the present results indicate that ginsenosides affect biochemical responses, including PAH transport, in renal cortical cells when given to rats and that one such response counterbalances the decrease in PAH transport in renal cortical slices prepared from rats in acute renal failure induced by cisplatin or ischemia followed by reperfusion. Further studies must be performed to clarify a mechanism by which ginsenosides stimulate PAH transport.

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References


Ginsenosides on PAH Transport


