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 （*Pn*S） 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 $[Ca^{2+}]_i$ were assayed with diacetyl monoxime，alkaline picrate，trypan blue，ethidium bromide binding，^{125}I-postlabelling，and Fur 2-AM，respectively. RESULTS：With pretreatment for 2 d in mice，*Pn*S 100 and 200 $mg \cdot kg^{-1} \cdot d^{-1}$ suppressed cisplatin-induced high blood urea nitrogen level to 83 % and 31 %，and serum creatinine level to 86 % and 42 %，respectively （$P < 0.01$）. Preincubated with PTC for 24 h，*Pn*S 10 and 100 $mg \cdot L^{-1}$ inhibited cisplatin-induced decrease of cell viability from 78 % to 81 % （$P < 0.05$） and 89 % （$P < 0.01$），respectively. *Pn*S 10 and 100 $mg \cdot L^{-1}$ suppressed formations of DNA interstrand cross-link to 47 % and 40 %，DNA-protein interstrand cross-link to 77 % and 42 %，and cytosolic free $[Ca^{2+}]_i$ overload in PTC to 70 % and 63 %，respectively. （$P < 0.01$）. CONCLUSION：*Pn*S was a prophylactic for cisplatin-induced nephrotoxicity，and mechanisms were relevant to the effects that *Pn*S reduced cisplatin-induced cytosolic free $[Ca^{2+}]_i$ 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[1]. Cisplatin induced lipid peroxidation and oxygen free radical generation in kidney，and these effects damaged the kidney[2]. However，as genetic substance was a target for cisplatin-induced nephrotoxicity cisplatin-induced nephrotoxicity was more serious than carboplatin[3]. In addition，cytosolic free $[Ca^{2+}]_i$ overload was important for cisplatin-induced nephrotoxicity[4].

Panax notoginseng saponins （*Pn*S） showed extensive biological activities，eg，protection from damage of genetic substance，and antagonizing cytosolic free $[Ca^{2+}]_i$ overload[5-7]. Therefore，it was examined if reduction of cisplatin-induced nephrotoxicity took place with *Pn*S 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）. ^{125}I was made in Chinese Atomic Energy Isotope Co. *Pn*S （83.5 % pure，contained ginsenoside R_{bl} 31.8 %，R_{gl} 29.1 %，R_e 10.7 %，and R_f 11.9 %） was obtained from Mr WAN 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，♀ or ♂） and Kunming mice （Grade Ⅱ，Certificate № 98058，♂） 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[8].

Alkaline phosphatase of brush border and epithelium keratin in PTC were stained with cytochemistry （Gomori Ca-Co） and anti-keratin[9].

Animals Kunming mice were randomly divided into

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5 groups：Control；Cisplatin group (cisplatin 5 mg·kg^{-1}·d^{-1} ip for 4 d)；Treatment groups (after *Pn*S 50，100，200 mg·kg^{-1}·d^{-1} ip for 2 d，then with *Pn*S 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 *Pn*S for 48 h)；Cisplatin groups (PTC were incubated without cisplatin and *Pn*S for 24 h，then with cisplatin 26 μmol·L^{-1} for another 24 h)；Treatment groups (after PTC were preincubated with *Pn*S 1，10，100 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 picrate to produce creatinine-picrate 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 ^{125}I-postlabelling[12]. DNA-protein cross-link (DPC) was expressed as Bq·g^{-1} DNA.

Assay of cytosolic free $[Ca^{2+}]_i$ Cytosolic free $[Ca^{2+}]_i$ in PTC was measured with Fura 2-AM[13].

Statistical analysis Results were expressed as $\bar{x} \pm s$. Newman-Keuls test was used.

RESULTS

Effects of *Pn*S 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 *Pn*S (100 mg·kg^{-1}·d^{-1}，ip) group were decreased to 83 % and 86 %，respectively，of those in cisplatin group ($P < 0.01$). In *Pn*S (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 *Pn*S (50，100，200 mg·kg^{-1}·d^{-1}，ip) were higher than those in control group ($P < 0.01$) (Tab 1).

Tab 1. Influence of *Pn*S on blood urea nitrogen and serum creatinine in mice. $n = 8$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* single treatment with cisplatin (5 mg·kg^{-1}·d^{-1}). $^fP < 0.01$ *vs* control.

Groups	*Pn*S/ mg·kg^{-1}·d^{-1}	Cisplatin/ mg·kg^{-1}·d^{-1}	BUN/ mmol·L^{-1}	Cr/ μmol·L^{-1}
Control	0	0	8 ± 1	65 ± 9
Cisplatin	0	5	36 ± 6^f	284 ± 18^f
Treatment	50	5	40 ± 2^f	303 ± 49^f
	100	5	30 ± 3^{cf}	246 ± 29^{cf}
	200	5	11 ± 1^{cf}	120 ± 18^{cf}

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 *Pn*S on cell viability of PTC by cisplatin Cisplatin decreased cell viability from 98 % in control to 78 % in cisplatin (26 μmol·L^{-1}) ($P < 0.01$). *Pn*S 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 2. Influence of *Pn*S on cell viability，cisplatin-induced formations of DNA interstrand cross-link and DNA -protein cross-link. $\bar{x} \pm s$. $^bP < 0.05$，$^cP < 0.01$ *vs* single treatment with cisplatin (26 μmol·L^{-1}). $^fP < 0.01$ *vs* control.

Groups	*Pn*S/ mg·L^{-1}	Cisplatin/ μmol·L^{-1}	Cell viability (%) ($n = 8$)	ISC ($n = 4$)	GBq·g^{-1} (DNA) ($n = 5$)
Control	0	0	98 ± 2	0.02 ± 0.04	2 ± 1
Cisplatin	0	26	78 ± 3^f	1.53 ± 0.09^f	22 ± 2^f
Treatment	1	26	76 ± 4^f	1.60 ± 0.12^f	19 ± 5^f
	10	26	81 ± 4^{bf}	0.73 ± 0.06^{cf}	17 ± 1^{cf}
	100	26	89 ± 4^{cf}	0.62 ± 0.06^{cf}	6 ± 1^{cf}

Effects of *Pn*S 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 *Pn*S (10，100 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 *Pn*S (10，

100 mg·L^{-1}) groups was decreased to 77 % ($P < 0.01$) and 42 % ($P < 0.01$), respectively, of that in cisplatin group, but was higher than that in control group ($P < 0.01$). (Tab 2)

Effects of *Pn*S on cytosolic free $[Ca^{2+}]_i$ in PTC by cisplatin Cytosolic free $[Ca^{2+}]_i$ in cisplatin 13, 26, and 52 μmol·L^{-1} was increased to 160 %, 250 %, and 258 %, respectively, of that in control ($P < 0.01$). Cytosolic free $[Ca^{2+}]_i$ in *Pn*S (10, 100 mg·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)

Tab 3. Influence of *Pn*S on cytosolic free $[Ca^{2+}]_i$ in PTC. $n = 4$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* single treatment with cisplatin (26 μmol·L^{-1}). $^fP < 0.01$ *vs* control.

Groups	*Pn*S/ mg·L^{-1}	Cisplatin/ μmol·L^{-1}	$[Ca^{2+}]_i$/ nmol·L^{-1}
Control	0	0	112 ± 5
Cisplatin	0	6.5	123 ± 8
	0	13	179 ± 8^f
	0	26	230 ± 6^f
	0	52	320 ± 17^f
Treatment	1	26	230 ± 12^f
	10	26	162 ± 19^{cf}
	100	26	146 ± 13^{cf}

DISCUSSION

Cisplatin-induced high BUN and Cr were decreased by *Pn*S (100, 200 mg·kg^{-1}·d^{-1}, ip) in mice. The range of decrease in BUN and Cr was not great by *Pn*S 100 mg·kg^{-1}·d^{-1}, thus, protective effects of *Pn*S 100 mg·kg^{-1}·d^{-1} were not significant. However, *Pn*S 200 mg·kg^{-1}·d^{-1} could greatly decrease BUN and Cr, thus, *Pn*S 200 mg·kg^{-1}·d^{-1} attenuated cisplatin-induced nephrotoxicity.

ISC and Bq·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, 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. *Pn*S (10, 100 mg·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 *Pn*S induced ion of attenuat cisplatin-induced nephrotoxicity is due to the fact that *Pn*S inhibited cisplatin-induced DNA interstrand cross-link and DNA-protein cross-link, and not lipid peroxidation and oxygen free radical[12] generation. *Pn*S 10, 100 mg·L^{-1} also antagonized cisplatin-induced cytosolic free $[Ca^{2+}]_i$ overload in PTC. Cisplatin-induced cytosolic free $[Ca^{2+}]_i$ overload seriously damaged PTC, for example, it inhibited the respiratory rate of mitochondrion[13]. There can be two reasons relevant to the antagonistic effects of *Pn*S on cytosolic free $[Ca^{2+}]_i$ overload (1) blocking of Ca^{2+} channels[14]; (2) activation of Na^+-K^+ ATPase[15]. Thus, the antagonistic effect of *Pn*S against cisplatin-induced cytosolic free $[Ca^{2+}]_i$ overload in PTC led to the protective effects of *Pn*S against cisplatin-induced nephrotoxicity.

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三七皂苷对抗顺铂导致的肾毒性

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关键词 顺铂；人参；皂苷类；培养的细胞；血尿素氮；肌酸酐；细胞存活；DNA；交联试剂；钙

目的：研究三七皂苷（*Pn*S）对顺铂肾毒性的防护作用. 方法：采用小鼠和原代兔肾近端小管细胞培养（PTC）建立体内外顺铂肾毒性模型. 用双乙酰、苦味酸、溴乙锭、台酚蓝、^{125}碘标记和 Fura 2-AM 方法分别测血尿素氮、血清肌酐、细胞存活率、DNA 链间交联、DNA-蛋白交联和细胞内游离钙离子. 结果：预先 2 d 给 *Pn*S（100，200 mg·kg^{-1}·d^{-1}）使顺铂导致的小鼠血尿素氮下降到 83 %和 31 %，血清肌酐下降到 86 %和 42 %（$P < 0.01$）. 提前 24 h *Pn*S（10，100 mg/L）与 PTC 孵育，细胞存活率从顺铂组的 78 %提高到 81 %和 89 %，DNA 链间交联下降到 47 %和 40 %，DNA-蛋白交联下降到 77 %和 42 %，细胞内游离钙下降到 70 %和 63 %（$P < 0.01$）. 结论：*Pn*S 可预防顺铂的肾毒性，其机制是降低顺铂导致的 DNA 链间交联、DNA-蛋白交联和钙离子超载.

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