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Effects of *Panax notoginseng* saponins on posthypoxic cell damage of neurons *in vitro*

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AIM: To study cerebral protective mechanism of *Panax notoginseng* saponins (PNS). **METHODS**: Cultured neurons of chick embryo cerebral hemisphere were used as an *in vitro* system for investigating the effects of PNS. The hypoxic cell damage of neurons cultured were induced by NaCN. The levels of adenosine triphosphate (ATP) were determined with HPLC. PNS was added 30 min before, beginning or after hypoxia. **RESULTS**: PNS 50 and 100 mg L^{-1} retarded the break down of ATP of cultured neurons after 2-h hypoxia for 11.3±1.5 ($P<0.05$) and 12.8±2.2 μmol/g protein ($P<0.01$), respectively and accelerated the restoration of ATP during 30-min reoxygenation for 21.0±2.0 ($P<0.05$) and 22.7±2.6 μmol/g protein ($P<0.01$), respectively. PNS also reduced the release of creatine kinase (CK) from 75±8 kU L^{-1}/g protein to 52±6 ($P<0.05$) and 41±3 kU L^{-1}/mg protein ($P<0.01$), respectively and promoted the restoration of ATP of neurons 20 h after hypoxia when administered in the beginning of hypoxia from 13.0±0.9 μmol/g protein to 18.1±1.4 and 20.5±2.1 μmol/g protein ($P<0.01$), respectively. PNS still promoted the restoration of ATP from 13.0±0.9 nmol/mg protein to 14.9±1.0 and 18.3±0.7 nmol/mg protein ($P<0.01$), respectively and reduced (PNS 100 mg L^{-1}) the CK release of neurons 20 h after hypoxia even when added in the recovery. **CONCLUSION**: The protection against hypoxic damage of PNS was related to improving energy metabolism, preserving the structural integrity of neurons.

KEY WORDS *Panax notoginseng*; ginseng; saponins; cultured cells; neurons; anoxia; adenosine triphosphate; creatine kinase

Panax notoginseng saponins (PNS) dilated blood vessel, depressed myocardial contratility, decreased oxygen consumption of myocardium[1], and had anti-arrhythmic effects[2]. PNS protected myocardium against ischemia/reperfusion injury in the anesthetized rat and in conscious rabbit[3,4]. PNS had some protective effects against cerebral ischemia/reperfusion injury[5,6]. The present study was to investigate the effects of PNS on posthypoxia cell damage of cultured neurons, to provide a further understanding of its cerebral protective mechanism.

MATERIALS AND METHODS

Reagents PNS, a yellowish brown powder, with 7 stains by thin layer chromatography, containing total saponins content more than 80 % was supplied by Guanxi Institute of Nature Materia Medica. ATP-Na_2 was from Sigma. Bovin serum albumin was from Shanghai Biochemical Reagent Factory. Dulbecco's phosphate buffered saline without Ca^{2+} and Mg^{2+}: NaCl 136.75, KCl 2.68, Na_2HPO_4 8.10, KH_2PO_4 1.47 mmol L^{-1}. The other reagents were all of AR.

Neuronal cultures Chick eggs (Leihorn) were purchased from Nanjing Agricultural Chemistry Machinery Factory.

Primary neuronal cultures were obtained from the

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cerebral hemispheres of 7-d old chick embryo[7]. Hemispheres were cleared from the meninges. Squeezed through a sterile nylon sieve (pore 40 μm), further dissociated by trituration, and seeded into polylysine-coated Falcon dish (60 mm diameter) containing culture medium 5 mL with a cell density of approximately 10^5 cells cm^{-2}. The culture medium consisted of 80 % Dulbecco's modified Eagle medium (DMEM, Gibco) with sodium pyruvate, 1 g L^{-1} glucose, 20 % heat-inactivated fetal calf serum (Division of Cranial Nerves, Suzhou Medical College affiliated First Hospital), gentamycine 40 kU L^{-1} and ampicillin 125 mg L^{-1}. Cultures were incubated grown at 37 ℃ in humidified 95 % air+5 % CO_2. The culture medium was changed after 3 and 6 d of cultivation.

Hypoxia and recovery Cytotoxic hypoxia was induced after 6—7 d in culture by adding NaCN to the culture medium to give a final concentration of 1 mmol L^{-1}. After 120 min, the medium was replaced by a NaCN-free medium when the cells should recover from hypoxia. Metabolism of the cells was stopped by rinsing the cells 3 times with ice-cold buffer solution (Dulbecco's phosphate buffered saline without Ca^{2+} and Mg^{2+}).

Extraction and determination of ATP and protein The cells were suspended in perchloric acid 0.3 mol L^{-1} containing edetic acid 1 mmol L^{-1}. After homogenization (teflon homogenizer, 4 C) the suspention was spinned at 12 000×g for 20 min. The pellet was used for determination of protein content of the culture. The supernatant was neutralized with a mixture of KOH, KCl, and imidazole (1.5, 0.3, and 0.4 mol L^{-1}, respectively), and the precipitate of $KClO_4$ was removed by centrifugation. The ATP in the extract was determined with HPLC (Waters) with a M510 pump, a U6K injection system coupled to a detector and Zorbax ODS column (250 mm × 4.6 mm ID 7 μm) with a precolumn. The mobile phase consisted of KH_2PO_4-Na_2HPO_4 0.15 mol L^{-1} buffer (pH 6.85). The flow-rate was 1 mL min^{-1}. This was a modification of the procedure[8]. ATP was detected by UV detector at 254 nm. The protein content of the culture was measured colorimetrically[9] with bovine serum albumin as a standard.

Determination of creatine kinase CK in the culture medium was determined with the Monarch Chemistry system.

Drug PNS was dissolved in redistilled water. This stock solution was added to each culture dish containing culture medium 5 mL, giving final PNS concentration of 50 or 100 mg L^{-1}. PNS was added at 30 min before, beginning or after cytotoxic hypoxia. Control cultures received an equal volume 0.2 mL of distilled water.

Statistics The results were represented by ANOVA, group t test was used.

RESULTS

Effects of PNS on ATP of cultured neurons Prehypoxic addition of PNS 50 and 100 mg L^{-1} retarded the breakdown of ATP, and accelerated the restoration of ATP during 30 min reoxygenation (Tab 1).

Tab 1. Effects of PNS on ATP levels and CK release of cultured neurons. $n=5-6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

PNS/ mg L^{-1}	ATP, μmol/ g protein	Protein, mg/dish	CK, kU L^{-1}/ g protein
H_{120}/R_0	PNS was added before hypoxia		
0	9.5±1.0	0.33±0.06	
50	11.3±1.5^b	0.29±0.04	
100	12.8±2.2^c	0.31±0.05	
H_{120}/R_{30}	PNS was added before hypoxia		
0	16.1±2.5	0.45±0.05	
50	21.0±2.0^b	0.47±0.03	
100	22.7±2.6^c	0.41±0.04	
H_{120}/R_{1200}			
PNS was added at the beginning of hypoxia			
0	13.0±0.9	0.39±0.04	75±8
50	18.1±1.4^c	0.42±0.05	52±6^b
100	20.5±2.1^c	0.47±0.03	41±3^c
H_{120}/R_{1200}			
PNS was added at the beginning of reoxygenation			
0	13.0±0.9	0.39±0.04	75±8
50	14.9±1.0^c	0.42±0.03	69±10^a
100	18.3±0.7^c	0.45±0.03	53±3^c

H_{120}/R_0, H_{120}/R_{30}, H_{120}/R_{1200}: 0, 30, and 1200 min reoxygenation after 120-min hypoxid.

When administered in the beginning of hypoxia or recovery, PNS raised the ATP level in the cultured neurons (Tab 1).

Effects of PNS on CK release in cultured neurons When given at the beginning of hypoxia, PNS (50 and 100 mg L^{-1}) markedly diminished the levels of CK in the culture medium 20 h after hypoxia. PNS 100 mg L^{-1} but not 50 mg L^{-1} also reduced the release of CK from neurons 20 h after hypoxia even when given after hypoxia (Tab 1).

DISSCUSION

The present study showed that PNS had beneficial effect on the energy metabolism after hypoxia/reoxygenation in the cultured neurons. The ATP enhancing effect seemed to the more pronounced with increase of PNS doses though we did not try to evaluate a concentration-response relationship. We assumed that these effects had something to do with Na^+-K^+-ATPase activation of PNS[10]. Because PNS activated Na^+-K^+-ATPase, leading to a reduced Na^+-Ca^{2+} exchange, meantime, keeping polarization of the neuronal membrane. Thus, energy consumptions of PNS-treated neurons are reduced.

The results indicated that the CK levels were raised significantly in the culture medium at 20-h recovery after 2 h hypoxia. It was almost twice as much as CK levels of the normal control (38.6±1.8 kU L^{-1}/g protein) which indicated the structural intergrity and membrane stability of neurons was severely damaged. PNS significantly inhibited the neurons CK release in a dose-dependent manner and the corresponding increase of ATP contents of neurons. It was confirmed that PNS had the protection against hypoxic damage. Unfortunately, there was no sufficient assessing the effects of ENS on morphological change of neurons in our study.

In conclusion, the results showed that the protection against hypoxic damage of PNS was related to improving energy metabolism, preserving the structural interity of neurons.

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三七总皂甙对神经细胞在体外缺氧性损伤的作用

R965

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主题词在下页

目的：探讨三七总皂甙(PNS)的脑保护作用机制. **方法**：体外培养鸡胚脑神经细胞，用氰化钠造成缺氧，作为试验PNS作用的模型，细胞内三磷酸腺苷(ATP)和培养液中肌酸激酶浓度作为观察指标，分别用HPLC-UV和全自动生化分析仪定量. **结果**：缺氧前30 min将PNS 50和100 mg L^{-1}加入到培养液中能明显延缓缺氧2 h细胞内ATP的耗竭(分别为11.3 ± 1.5和12.8 ± 2.2 μmol/g protein)，促进再给氧30 min时细胞内ATP的恢复(分别为21.0 ± 2.0和22.7 ± 2.6 μmol/g protein). PNS于缺氧开始或再给氧时给予，仍能促进再给氧期细胞内ATP的恢复，减少神经细胞内肌酸激酶的释放.
结论：PNS对培养神经细胞缺氧性损伤具有保护作用，其机制可能与改善能量代谢，保护细胞结构完整性有关.

关键词 三七；人参；皂苷类；培养的细胞；神经元；缺氧症；腺苷三磷酸；肌酸激酶

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Pharmacokinetics of ceftizoxime in renal failure patients without dialysis

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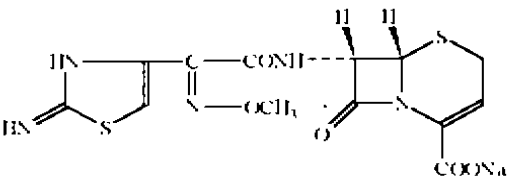
AIM: To investigate the pharmacokinetics of ceftizoxime (Cef) in renal failure patients without any dialysis and supply the basis for a suitable clinical regimen. **METHODS**: Cef in plasma and urine was assayed by HPLC. **RESULTS**: After injecting Cef 16.7 mg kg^{-1}, Cef concentration in blood was described as a 2-compartment open model. The main pharmacokinetic parameters were V_d 0.55 ± 0.17 L kg^{-1}; AUC 879 ± 460 mg L^{-1} h; Cl 27 ± 11 mL kg^{-1} h^{-1}. $T_{\frac{1}{2}\beta}$ was 15 ± 4 h. **CONCLUSION**: $T_{\frac{1}{2}\beta}$ in renal failure patients was about 10 times longer than that in normal volunteers. The clinical regimen should be adjusted in renal failure patients with infection, either prolonging the interval between Cef administration, or decreasing Cef dosage.

KEY WORDS ceftizoxime; kidney failure; pharmacokinetics

Ceftizoxime (Cef) is a potent, β-lactamase stable cephalosporin of the 3rd generation, against a wide spectrum of *Gram*-positive and -negative bacteria[1]. Since Cef is eliminated mainly through urinary route, renal function plays a major role in its elimination[2]. This study was to investigate the pharmacokinetics of Cef in renal failure patients without any dialysis and to design a



Ceftizoxime sodium

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