

Anti-inflammatory effects of total saponins of *Panax notoginseng*

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KEY WORDS ginseng; saponins; inflammation; non-steroidal anti-inflammatory agents; phospholipase A₂; dinoprostone; calcium; carrageenan; neutrophils; dexamethasone

ABSTRACT

AIM: To study the anti-inflammatory effects of total saponins of *Panax notoginseng* (*PnS*). **METHODS:** Rat air-pouch acute inflammatory model was established with sc carrageenan (Car, 25 mg · kg⁻¹). The protein content in exudate was measured. Microacid titration assay and radioimmunoassay (RIA) were applied respectively to investigate effects of *PnS* on phospholipase A₂ (PLA₂) activity and dinoprostone (Din) content in exudate. Fura-2 fluorescence technique was used to determine the intracellular free calcium concentration in neutrophils (Neu-[Ca²⁺]_i). **RESULTS:** At 12 h, *PnS* 60 - 240 mg · kg⁻¹ ip reduced Neu counts, protein content [(7.7 ± 1.3) to (4.4 ± 1.4) g · L⁻¹], and Din content [(1619 ± 391) to (883 ± 268) ng · L⁻¹]; inhibited the PLA₂ activity in exudate [(248 ± 42) to (157 ± 35) kU · L⁻¹] in a dose-dependent manner. *PnS* 60, 120, and 240 mg · kg⁻¹ lowered the level of Neu-[Ca²⁺]_i with the inhibitory rate of 9.1%, 33.2%, and 39.4%, respectively. **CONCLUSION:** *PnS* has an obvious anti-inflammatory effect and its mechanisms are related to the inhibition of the Neu-[Ca²⁺]_i level and PLA₂ activity, and reduction of Din content.

INTRODUCTION

Saponins of *Panax notoginseng* (*PnS*) is the

mainly effective constituent of *Panax notoginseng* (Burk) FH Chen. Its excellent anti-inflammatory effects on several inflammatory models were reported^[1]. It was supposed that *PnS* might augment adrenocortical function by indirect acting on the pituitary or other parts above pituitary^[2]. The fact that *PnS* reduced the dinoprostone (Din) content in exudate of inflamed feet^[3] indicates that one of the anti-inflammatory mechanisms of *PnS* may be related to its inhibitory effects on phospholipase A₂ (PLA₂). Ca²⁺ was one of the activating factors of PLA₂^[4] and *PnS* could inhibit extracellular calcium ion entry through blocking receptor-operated calcium channel (ROC) in the vascular strip experiments^[5], suggesting that the blocking effect on ROC may play an important role in the anti-inflammatory mechanisms of *PnS*. The present study was to explore the effects of *PnS* on the [Ca²⁺]_i level in neutrophils (Neu-[Ca²⁺]_i), PLA₂ activity, Din content, and their relationship with the anti-inflammatory effects of *PnS*.

MATERIALS AND METHODS

Rats and reagents Adult Wistar rats (♂, n = 48) weighing 185 g ± s 34 g were provided by Experimental Animal Center of The Third Military Medical University (Certificate No 24301050). *PnS* was extracted and purified by Kunming Institute of Botany, Chinese Academy of Sciences, purity >99%. Carrageenan (Car) was the product of Liaoning Institute of Materia Medica. Dexamethasone (Dex) was obtained from Institute of Drug Control, Ministry of Public Health. Fura 2-AM was the product of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Din RIA kit was the product of Department of Biochemistry, General Hospital of PLA. Phosphatidylcholine was purchased from Sigma. All other reagents were of AR.

Rat grouping The rats were randomly divided

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into 6 groups ($n = 8$, each group): normal group, Car group (12 h), Car with ip *PnS* 60, 120, and 240 $\text{mg} \cdot \text{kg}^{-1}$ groups, and Car with ip Dex 1 $\text{mg} \cdot \text{kg}^{-1}$ group. *PnS* or Dex was injected ip 30 min before and 6 h after sc Car. The normal group and Car group were given equal volume of solvent. Rats were killed 12 h after sc Car.

Preparation of rat air-pouch acute inflammatory model The model was established according to the method^[6] with some modification. Car 25 $\text{mg} \cdot \text{kg}^{-1}$ was injected sc into the air pouch to induce the inflammation. At 12 h rats were killed. The air-pouch was washed with ice-cold Ca^{2+} -free Hanks' solution (HBSS) 4 mL immediately and the exudates were harvested and spun (4 °C, 1800 $\times g$, 10 min). The supernatants were stored at -70 °C until analysis for PLA_2 and protein. The cells were washed twice with HBSS (1800 $\times g$, 5 min). An aliquot of cell suspension was taken for trypan-blue test and its results consistently showed 97 % cellular viability rate. Neutrophil (Neu) was demonstrated by staining classification occupied 95 %. Cells were suspended with HBSS for $[\text{Ca}^{2+}]_i$ measurement.

Protein measurement^[7]

Fura-2 loading and $[\text{Ca}^{2+}]_i$ measurement

Neu- $[\text{Ca}^{2+}]_i$ was determined according to the method^[8] with some modification. Cells ($1 \times 10^9 \cdot \text{L}^{-1}$) were washed once with HEPES-Krebs-Rins (HKR) solution, pH 7.4, containing NaCl 116, KCl 5.4, MgCl_2 1.0, HEPES 20, and glucose 10 $\text{mmol} \cdot \text{L}^{-1}$. The cells were resuspended with HKR and added Fura 2-AM 2 $\text{mmol} \cdot \text{L}^{-1}$ (in Me_2SO) to a final concentration of 4 $\mu\text{mol} \cdot \text{L}^{-1}$. The cell suspensions were incubated at 37 °C for 40 min and washed twice with HKR prior to measurement.

A spectrofluorophotometer (Hitachi F-3010) was used for fluorescence determination (λ_{ex} : 345 nm, λ_{em} : 500 nm). $[\text{Ca}^{2+}]_i$ was calculated with K_d of 224 $\text{nmol} \cdot \text{L}^{-1}$; $[\text{Ca}^{2+}]_i = K_d(F - F_{\text{min}})/(F_{\text{max}} - F)$

The maximal fluorescence (F_{max}) was determined with 0.09 % Triton X-100. The minimal fluorescence (F_{min}) was determined with egtazic acid 9 $\text{mmol} \cdot \text{L}^{-1}$ (pH > 8.5) to deplete calcium. Autofluorescence was determined on control cells without Fura 2-AM.

Assay of PLA_2 The samples (0.5 mL) were diluted 1:4 with tissue buffer (boric acid 3.57, sodium

deoxycholate 6.03, glycine 100 $\text{mmol} \cdot \text{L}^{-1}$, pH 7.4) and stored at -70 °C after incubation in water at 60 °C for 30 min. The PLA_2 activities were measured according to the method^[9].

Determination for Din Din was extracted by a modification of the method^[10]. Redistilled ethylacetate (5.0 mL $\times 2$) was added into 0.5 mL of sample and spun at 3000 $\times g$ for 15 min at 4 °C. The supernatants were lyophilized at -70 °C. The contents of Din were determined according to the instruction of Din RIA kit.

Statistical analysis The results were compared with ANOVA and PDA-3.

RESULTS

Neu count and protein content in exudate

The Neu count and protein content in exudate increased after sc Car ($P < 0.01$). *PnS* + Car reduced Neu count and protein content in exudate dose-dependently (Tab 1).

Din content in exudate *PnS* reduced Din content in a concentration-dependent manner. But Din content was also > that of the normal group after ip *PnS* 240 $\text{mg} \cdot \text{kg}^{-1}$ ($P < 0.01$) (Tab 1).

PLA_2 activity in exudate The Car-induced elevation of PLA_2 activity was inhibited by *PnS* in a dose-dependent manner, but the PLA_2 activity did not recover to the normal level after ip *PnS* 240 $\text{mg} \cdot \text{kg}^{-1}$. Dex also had the same effect (Tab 1).

Neu- $[\text{Ca}^{2+}]_i$ Neu- $[\text{Ca}^{2+}]_i$ increased rapidly at 12 h after sc Car (nearly 2.2 times that of normal groups, $P < 0.01$). *PnS* 60, 120, and 240 $\text{mg} \cdot \text{kg}^{-1}$ lowered the level of Neu- $[\text{Ca}^{2+}]_i$, with the inhibitory rate of 9.1 %, 33.2 %, and 39.4 %, respectively. However, the Neu- $[\text{Ca}^{2+}]_i$ did not reach normal level even for the 240 $\text{mg} \cdot \text{kg}^{-1}$ group. Dex 1 $\text{mg} \cdot \text{kg}^{-1}$ had no obvious effect (Tab 1).

Analysis of correlation The correlation coefficient (r) between protein content and PLA_2 activity was 0.8956 ($P < 0.01$). The r between $[\text{Ca}^{2+}]_i$ level and PLA_2 activity was 0.7287 ($P < 0.01$).

DISCUSSION

PnS 60, 120, and 240 $\text{mg} \cdot \text{kg}^{-1}$ ip obviously

Tab 1. Effects of total saponins of *Panax notoginseng* (*PnS*) and dexamethasone (*Dex*) on Neu count, protein content, PLA_2 activity, PGE_2 content in exudate, and the elevation of $Neu-[Ca^{2+}]_i$ in Car-induced subcutaneous air-pouch acute inflammation. $n = 8$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs normal group. $^dP < 0.05$, $^eP < 0.01$ vs Car group.

Group /mg·kg ⁻¹	10 ⁻⁹ × Neuro- phils·L ⁻¹	Protein content /g·L ⁻¹	PLA ₂ /kU·L ⁻¹	Din /ng·L ⁻¹	Neu-[Ca ²⁺] _i /nmol·L ⁻¹
Normal	3.0 ± 0.5	0.59 ± 0.20	38 ± 9	397 ± 41	216 ± 48
Car	48 ± 15 ^c	7.7 ± 1.3 ^c	248 ± 42 ^c	1619 ± 391 ^c	485 ± 126 ^c
Car + <i>PnS</i> (ip) 60	30 ± 10 ^{cd}	7.4 ± 1.6 ^c	202 ± 47 ^c	1396 ± 437 ^c	441 ± 131 ^c
120	21 ± 7 ^{cd}	5.7 ± 1.5 ^{cd}	195 ± 41 ^{cd}	1098 ± 318 ^{cd}	324 ± 115 ^{bc}
240	17 ± 6 ^{cd}	4.4 ± 1.4 ^{cd}	157 ± 35 ^{cd}	883 ± 268 ^{cd}	294 ± 86 ^{bf}
Car + <i>Dex</i> (ip) 1	17 ± 7 ^{cd}	5.4 ± 1.2 ^{cd}	159 ± 13 ^{cd}	703 ± 11 ^{cd}	480 ± 106 ^c

reduced protein content and Neu count in exudate, indicating that *PnS* had significant anti-inflammatory effects on acute inflammation. Din, which increased the vascular permeability and led to the exudation of acute inflammation^[11], was decreased dose-dependently following *PnS* treatment, showing that *PnS* may exert its anti-inflammatory effects by inhibiting the metabolism of arachidonic acid (AA). PLA_2 , a velocity-limit enzyme of AA metabolism, induced rat paw oedema by subplantar injection of 25 mg·L⁻¹ (*Naja naja*)^[12]. Similar to *Dex*, *PnS* inhibited the PLA_2 activity greatly. Furthermore, there is a positive correlation between the protein content and PLA_2 activity ($r = 0.8956$, $P < 0.01$), which leads to the suggestion that inhibition of PLA_2 activity and subsequent reduction of AA metabolism and decrement of Din was one of the important anti-inflammatory mechanisms of *PnS*.

Ca^{2+} was one of the active factors of PLA_2 and Neu activation^[13]. In our experiment, *PnS* 60, 120, and 240mg·kg⁻¹ ip prevented the $Neu-[Ca^{2+}]_i$ elevation induced by Car in a concentration-dependent manner, but *Dex* did not in any way. However, both *PnS* and *Dex* inhibited PLA_2 activity and decreased the content of Din, implying that their anti-inflammatory mechanisms were different in some way. There was a significant correlation between the reduction of PLA_2 and that of $Neu-[Ca^{2+}]_i$ ($r = 0.7287$, $P < 0.01$), indicating that blocking effect on $Neu-[Ca^{2+}]_i$ elevation was one primary action site of *PnS* on PLA_2 . In addition, the reduction of Neu counts by *PnS* was parallel to that of $Neu-[Ca^{2+}]_i$, suggesting that decrement of Neu migration resulted from the inhibition

of Neu activation following blocking effects of *PnS* on $Neu-[Ca^{2+}]_i$.

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磷脂酶 A 类; 地诺前列酮; 钙; 角叉菜胶; 中性白细胞; 地塞米松

目的: 研究三七总皂苷(PnS) 抗炎作用. 方法: 用角叉菜胶复制大鼠气囊滑膜炎模型. 蛋白含量、PLA₂ 活性、Din 含量及炎细胞内游离钙水平分别用 Lowry 法、微量酸滴定法、放射免疫分析法及 Fura 2-AM 荧光分析法进行测定. 结果: PnS (60-240 mg·kg⁻¹) 明显抑制角叉菜胶诱导的白细胞数升高和蛋白渗出; 抑制灌洗液 PLA₂ 活性, 降低 Din 含量; 阻止炎细胞内游离钙水平的升高. 结论: PnS 具有明显抗炎作用, 其作用机制与其阻止炎细胞内游离钙水平的升高及其抑制 PLA₂ 活性、减少 Din 释放有密切关系.

三七总皂苷的抗炎作用

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关键词 人参; 皂苷类; 炎症; 非甾醇抗炎剂;

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