

Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds¹

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ABSTRACT

AIM: To investigate the anti-inflammatory effect and mechanism of proanthocyanidins (PA) from grape seeds.

METHODS: Croton oil-induced ear swelling in mice and carrageenan-induced hind paw edema in rats were prepared. The nitric oxide synthase (NOS) activity was measured by NADPH-diphorase stain assay, nitric oxide (NO) content by Griess diazotization assay, *N*-acetyl- β -*D*-glucosaminidase (β -NAG) activity by spectrophotography, malondialdehyde (MDA) content by thiobarbituric acid (TBA) fluorescence technique, and IL-1 β , TNF α , and PGE₂ content by radioimmunoassay (RIA). **RESULTS:** PA 10-40 mg/kg ip inhibited carrageenan-induced paw edema in rats and croton oil-induced ear swelling in mice in a dose-dependent manner. PA 10 mg/kg reduced MDA content in inflamed paws, inhibited β -NAG and NOS activity, and lowered the content of NO, IL-1 β , TNF α , and PGE₂ in exudate from edema paws of rats induced by carrageenan. The inhibitory effect of PA on all above indices was more evident than that of dexamethasone 2 mg/kg.

CONCLUSION: PA has anti-inflammatory effect on experimental inflammation in rats and mice. Its mechanisms of anti-inflammatory action are relevant to oxygen free radical scavenging, anti-lipid peroxidation, and inhibition of the formation of inflammatory cytokines.

INTRODUCTION

Proanthocyanidins, naturally occurring antioxidants widely available in fruits, vegetables, nuts, seeds, flowers and bark, especially grape seeds, have been reported to possess a broad spectrum of biological, pharmacological, and therapeutic activities against free radicals and oxidative stress. A large number of reports demonstrated that proanthocyanidins were highly bioavailable and provide potent protection against free radical-induced diseases, such as ischemia and reperfusion injury of many organs, advancing age, tumor promotion, and carcinogenesis^[1-3], etc. However, fewer reports demonstrated their effects on arthritis. Proanthocyanidin from *Polypodium feei* roots, *Hamamelis virginiana* bark and avocado had been reported possess antiphlogistic, antiviral, and analgesic activity^[4-6]. In this study, we evaluated whether proanthocyanidins from grape seeds possessed anti-inflammatory activity and preliminarily explored its mechanisms of anti-inflammatory action.

MATERIALS AND METHODS

Chemicals and animals Proanthocyanidin (PA), brown-red powder (purity: 96%), extracted from grape seeds, provided by Laboratory of Applied Organic Chemistry, Lanzhou University. Dexamethasone (Dex) was purchased from Lanzhou Pharmaceutical Factory (Batch number 990903). All above reagents were diluted with normal saline (NS). Carrageenan was the product of Liaoning Institute of Pharmacy, and diluted to 10 g/L with NS. Nitric oxide (NO), nitric oxide synthase (NOS) and *N*-acetyl- β -*D*-glucosaminidase (β -NAG) detection kit were obtained from Jiancheng Institute of Biotechnology (Nanjing, China). IL-1 β RIA kit was the product of Beijing North Biotechnology Company. TNF α RIA kit was the product of Beijing Bangding Biotechnology Company. Prostaglandin E₂ (PGE₂) RIA kit was the product of Department of

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Thrombus, Suzhou Medical College, China. All other reagents were of analytical purity.

Kunming mice (weighing $22 \text{ g} \pm 2 \text{ g}$, $\hat{\sigma} \hat{\sigma}$) and Wistar rats (weighing $218 \text{ g} \pm 26 \text{ g}$, $\hat{\sigma} \hat{\sigma}$) were obtained from the Experimental Animal Center of Lanzhou Medical College (Grade II, Certificate No 14-005 and 14-006 respectively).

Croton oil-induced ear edema in mice⁽⁷⁾

Kunming mice ($n = 50$), were randomly divided into 5 groups (see Tab 1). PA and Dex were injected ip 30 min respectively before croton oil inducing ear edema. Croton oil mixture was prepared⁽⁸⁾ and applied (0.2 mL each mouse) topically on the right ear pinna of mice. The left pinna was used as control. The mice were killed 4 h later. Disks from the pinnae were taken with a punch 7.0 mm in diameter. The edema extent was expressed in terms of the weight difference between the inflamed and the control.

Carrageenan-induced arthritis in rats^(8,9)

Fifty Wistar rats were randomly divided into control (ip NS), PA 10, 20, 40 mg/kg and Dex 2 mg/kg groups to determine the anti-inflammatory effect of PA. NS, PA, or Dex were injected ip 30 min before carrageenan (1% carrageenan, 0.1 mL each rat) was given sc in right hind paw plantar to induce the inflammation. Paw thickness was measured prior to or 1, 2, 3, 4, 6, 10, and 12 h after challenge with carrageenan. The swelling degree was calculated according to the augment of paw thickness after inducing arthritis.

Another 30 rats were randomly divided into control, PA 10 mg/kg, and Dex 2 mg/kg groups (Tab 3) treated as above experiment to assay the effect on formation of inflammatory factors. The other 10 rats, 0.1 mL normal saline sc in right hind paw plantar and 5 mL/kg NS ip, were used as blank control. All the rats were killed 4 h later after inducing inflammation, the skin of inflamed paw was cut open and the entire inflamed paw was washed by extrusion with ice-cold PBS (50 mmol/L) 1 mL. Then the inflamed paw and exudate were harvested and stored at $-20 \text{ }^{\circ}\text{C}$ before use.

MDA determination in inflamed paws

Inflamed rat paw was taken off skin and soaked in 2 mL 15% trichloroacetic acid for 3 h ($4 \text{ }^{\circ}\text{C}$). The supernatant (1.5 mL) was mixed with 2 mL TBA reagent [1:1 (v:v) mixture of 0.57% TBA and acetic acid]. The reaction mixture was boiled for 25 min. Then *n*-butanol (2 mL \times 2 times) was added. The mixture was shaken vigorously for 1 min, and centrifuged at $3000 \times g$ for 10 min. The *n*-butanol layer was assayed by spectrofluoro-

meter with λ_{ex} 520 nm and λ_{em} 550 nm. MDA content was calculated by reference to standards prepared from 1, 1,3,3-tetraethoxypropane (Fluka).

Determination of inflammatory factors in exudate IL-1 β , IL-8, and TNF α were measured by RIA method, NOS activity by NADPH-diaphorase stain assay, nitric oxide (NO) content by Griess diazotization assay, and β -NAG activity by colorimetry. The detecting methods were according to the protocol enclosed in the detection kit.

Statistics Data were expressed as $\bar{x} \pm s$ and assessed by *t*-test.

RESULTS

Effect of PA on ear edema in mice PA 10–40 mg/kg reduced ear edema of mice induced by croton oil in a dose-dependent manner. The potency of PA 40 mg/kg was obviously higher than that of Dex 2 mg/kg (Tab 1).

Tab 1. Effect of PA on ear edema induced by croton oil in mice. $n = 10$. $\bar{x} \pm s$. ^b $P < 0.05$, ^c $P < 0.01$ vs control.

Drugs	Dose/ $\text{mg} \cdot \text{kg}^{-1}$	Edema degree /mg	Inhibition rate /%
Control	–	8.9 ± 2.0	
Dex	2	4.3 ± 1.7^c	51.7
PA	10	6.8 ± 2.3^b	23.6
	20	4.5 ± 1.1^c	49.4
	40	3.8 ± 1.4^c	57.3

Effect of PA on arthritis induced by carrageenan in rats PA 10–40 mg/kg obviously inhibited paw edema of rats induced by carrageenan in dose-dependent manner. The potency of PA 20 and 40 mg/kg arrived peak at 6 h after carrageenan and administration with inhibition rate (IR) of 71.3% and 83.6%, respectively, which were much higher than that of Dex. The lasting time of action was longer in groups treated with PA than that treated with Dex 2 mg/kg (Tab 2). The plateau of inhibitory rate of PA 10 mg/kg on inflammation was seen at 3–4 h with IR 48.1% and 39.3%, respectively; and then faded. So we adopted 4 h as final point and PA 10 mg/kg as the dose for the evaluation of the anti-inflammatory mechanism of PA in the following test.

Effects of PA on formation of MDA, NO, and

Tab 2. Effect of PA on paw edema degree (PED, mm) induced by carrageenan in rats. $n = 10$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs control. IR: inhibition rate (%).

Drugs mg·kg ⁻¹	1 h		2 h		3 h		4 h		6 h		10 h		12 h	
	PED	IR	PED	IR	PED	IR	PED	IR	PED	IR	PED	IR	PED	IR
Control	1.6±0.5	-	2.2±1.0	-	3.1±1.1	-	3.7±1.0	-	4.5±0.6	-	4.7±0.7	-	4.6±0.3	-
PA 10	1.1±0.3 ^b	30.1	1.4±0.4 ^b	36.9	1.6±0.6 ^c	48.1	2.2±0.6 ^c	39.3	3.4±0.9 ^c	24.2	4.1±0.6 ^b	12.1	4.4±0.5 ^a	3.9
20	0.8±0.4 ^c	54.0	0.9±0.4 ^c	59.6	1.0±0.6 ^c	66.6	1.0±0.6 ^c	72.4	1.3±1.0 ^c	71.3	2.0±0.8 ^c	56.5	2.2±0.7 ^c	51.4
40	0.6±0.4 ^c	66.2	0.6±0.3 ^c	74.2	0.8±0.4 ^c	75.3	0.8±0.4 ^c	79.2	0.7±0.5 ^c	83.6	1.6±0.6 ^c	66.7	1.3±0.6 ^c	70.7
Dex 2	1.19±0.25 ^b	27.0	1.1±0.5 ^c	49.3	1.7±0.4 ^c	44.8	2.11±0.29 ^c	42.3	3.1±0.3 ^c	20.6	4.2±0.3 ^b	11.7	4.4±0.4 ^a	3.3

inflammatory cytokines, NOS activity, and β -NAG release in carrageenan-induced rat arthritis

PA 10 mg/kg markedly suppressed the generation of MDA in inflamed paws, inhibited the NOS activity and β -NAG release, and reduced content of NO and cytokines such as IL-1 β , and TNF α , PGE₂ in exudates from rat paw stimulated by carrageenan. The inhibitory potency of PA on all above indices was higher than that of Dex 2 mg/kg (Tab 3, 4).

Tab 3. Effects of PA on formation of MDA and NO, NOS activity, and β -NAG release in carrageenan-induced rat arthritis. $n = 10$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs control.

Drugs mg·kg ⁻¹	MDA/ $\mu\text{mol}\cdot\text{L}^{-1}$	NO/ $\mu\text{mol}\cdot\text{L}^{-1}$	β -NAG/ $\text{U}\cdot\text{L}^{-1}$	NOS/ $\text{U}\cdot\text{L}^{-1}$
Blank	0.35±0.09 ^c	13±9 ^c	8±3 ^c	1001±530 ^c
Control	0.78±0.18	34±21	15±3	5815±804
PA 10	0.33±0.21 ^c	20±5 ^c	9±3 ^c	1992±899 ^c
Dex 2	0.35±0.09 ^b	34±16 ^b	11.3±2.7 ^b	4286±1502 ^b

DISCUSSION

Proanthocyanidin, contained in a large number of plants, is very important in human health protection and

diseases prevention. Our results showed that PA from grape seeds dose-dependently inhibited the plantar edema of rats induced by carrageenan and ear edema of mice induced by croton oil. The effective dosage of PA from grape seeds was 10 mg/kg, which was lower than that of PA from *Polypodium feei* roots, 200 mg/kg^[4]. The antiphlogistic mechanism of PA from grape seeds was partly due to the inhibition of prostaglandin biosynthesis same as PA from *Polypodium feei* roots.

β -NAG was released from lysosome, its change of activity in the inflammatory exudate reflected stability of lysosome membrane. PA marked lessened β -NAG activity demonstrating that PA had protective effect on lysosome membrane.

Free radicals-induced lipoperoxidation has been implicated in over a hundred diseases including arthritis. Recent studies showed that nitric oxide (NO), which showed free radical feature, was also a key inflammatory mediator^[9]. In our experiment, PA from grape seeds significantly suppressed the content of lipoperoxidation product MDA in carrageenan-induced inflamed paws of rats similar to that *in vitro*^[10], and markedly lessened the activity of NOS and the content of NO in exudate of carrageenan-induced paw edema in rats. These results demonstrated that inhibition of lipoperoxidation and NO formation was one of anti-inflammatory mechanisms of

Tab 4. Effect of PA on content of IL-1 β , TNF α , and PGE₂ in carrageenan-induced rat arthritis. $n = 10$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs control.

Drugs/ mg·kg ⁻¹	PGE ₂ / $\mu\text{g}\cdot\text{L}^{-1}$	Inhibition rate/%	TNF α / $\text{ng}\cdot\text{L}^{-1}$	Inhibition rate/%	IL-1 β / $\text{ng}\cdot\text{L}^{-1}$	Inhibition rate/%
Blank	0.12±0.07 ^c	-	10±7 ^c	-	0.5±0.3 ^c	-
Control	6.2±1.0	-	351±194	-	34±8	-
PA 10	2.8±1.0 ^c	55.2	126±90 ^b	64.0	6±4 ^c	82.2
Dex 2	3.2±1.1 ^c	48.9	133±94 ^b	62.3	20±17 ^b	40.8

PA.

It is well known that IL-1 β and TNF α were proinflammatory cytokines. They can interact with other inflammatory factors such as PGE₂, NO, and free radicals, and take part in the formation and development of rheumatoid arthritis^[11-13]. This experiment demonstrated that PA obviously inhibited formation of IL-1 β and TNF α , suggesting that the major anti-inflammatory mechanism of PA is related to its suppressive effect on the formation of inflammatory cytokines. Because it also inhibits the lipoperoxidation and the formation of PGE₂ and NO, so its action range is quite extensive. But what the pivotal role of anti-inflammation of PA is and at what point, PA affects inflammatory network remained to be clarified.

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葡萄籽中原花青素的抗炎作用和机制¹

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关键词 原花青素; 葡萄籽; 炎症; 白介素-1; 肿瘤坏死因子; 一氧化氮; 丙二醛; 前列腺素 E 类; 角叉菜胶

目的: 研究葡萄籽中原花青素(PA)的抗炎作用及机制。 **方法:** 用巴豆油诱导的小鼠耳肿和角叉菜胶诱导的大鼠足肿模型, 研究 PA 对炎症的影响。 NOS 活性用 NADPH 黄酶染色法, NO 含量用 Griess 重氮化反应法, β -NAG 用对硝基酚比色法, MDA 用 TBA 荧光法测定, IL-1 β 、TNF α 和 PGE₂ 含量用放免法测定。 **结果:** PA 10-40 mg/kg ip 剂量依赖性地抑制角叉菜胶诱导的大鼠足肿和巴豆油诱导的小鼠耳肿。 PA 10 mg/kg 减少大鼠致炎足 MDA 生成, 抑制炎症渗出液中 NOS 和 β -NAG 活性, 降低 IL-1 β 、TNF 和 PGE₂ 含量。 其作用强于地塞米松 2 mg/kg。 **结论:** PA 对大鼠和小鼠实验性炎症有明显的抗炎作用。 其抗炎机理和清除氧自由基、抗脂质过氧化和减少细胞因子的生成有关。

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