3 个剂量组均能抑制 Neu 释放 $O_2^{\cdot-}$, 且呈剂量依赖性, 其抑制率分别为 10.19%, 29.96%, 41.43%。

2.4 对灌洗液中 Neu 内 cAMP 含量的影响: 由表 2 可见, Car 致炎后, 灌洗液 Neu 内 cAMP 含量明显降低($P< 0.01$); PnS 能剂量依赖性地增加 Neu 内 cAMP 含量, 且其大剂量组能使 Neu 内 cAMP 含量恢复到正常水平。Dex 也具有类似作用。

3 讨论

自由基是广泛存在于生物体组织细胞内的非特异性损伤因素, 也是炎症发生发展的重要病理机制之一[2]。炎症时, 活化的白细胞呼吸暴发过程产生的 $O_2^{\cdot-}$ 等活性氧自由基, 不仅具有增加血管通透性的作用, 而且极易与生物膜的多不饱和脂肪酸发生脱氢反应, 从而诱发组织细胞的脂质过氧化损伤[10]。本实验结果发现, PnS 3 个剂量组均能剂量依赖性地抑制 Car 致炎大鼠 Neu 释放 $O_2^{\cdot-}$、降低灌洗液中脂质过氧化物 MDA 的含量, 且与其降低 Neu 数量及蛋白含量呈平行性变化。这与 Pns 对体外多形核白细胞呼吸暴发时产生的氧自由基及黄嘌呤氧化酶体系产生的 $O_2^{\cdot-}$ 均具有清除作用的结果相一致[11]。

细胞内的第二信使分子 cAMP 在调控炎症发生发展中具有重要作用, 胞内升高的 cAMP 可抑制炎细胞释放自由基而产生抗炎活性[3]。本实验观察到, Car 致炎后, 气囊灌洗液中 Neu 内 cAMP 含量明显下降 ($P< 0.01$), PnS 3 个剂量组均能剂量依赖性升高 Neu 内 cAMP 含量, 相关分析结果显示, PnS 升高 Neu 内 cAMP 含量与其抑制 Neu 释放 $O_2^{\cdot-}$ 呈显著负相关 ($r= -0.938\ 4$, $P< 0.01$)。上述结果表明, 升高 Neu 内 cAMP 从而抑制 Neu 释放 $O_2^{\cdot-}$、减轻脂质过氧化损伤是 PnS 发挥抗炎作用的重要分子机制之一。

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Studies on the Basic Principles for the Processing of *Rhizoma Cibotii*

Part Ⅰ Influence of *Rhizoma Cibotii* and Its Processed Samples on Thrombin Induced Rabbit Platelet Aggregation

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摘 要 比较研究了狗脊及其不同炮制品对凝血酶诱导的兔血小板聚集作用的影响, 各炮制品均有抑制血小板聚集作用, 抗血小板聚集作用砂烫品> 盐制品> 酒蒸品> 单蒸品> 生品。

关键词 狗脊 凝血酶 血小板聚集作用

Abstract The influence of *Cibotium barometz* (L.) J. Sm. and its processed samples on thrombin-induced platelet aggregation in rabbits was studied. The results showed that all differently processed samples tested could inhibit platelet aggregation, with activities in the decreasing order of *Rhizoma Cibotii* roasted in stirring sand> steamed after being salted> steamed after steeped in wine> simply steamed> the unprocessed crude *Rhizoma Cibotii*.

Key words *Rhizoma Cibotii* thrombin platelet aggregation

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Rhizoma Cibotii (RC) is the dried rhizome of *Cibotium barometz* (L.) J. Sm. It is a traditional Chinese herbal drug in common use to invigorate the liver and kidney, strengthen the bones and muscles and relieve rheumatism, by driving away the pathogenic factors of cold dampness and motivate the joint from its numbness and rigidity, which is claimed to be specially good for the elders. Since these therapeutic efficacies may be related to its action by promoting blood circulation and resolving blood stasis, it would be justifiable to delve into the influence of RC and its processed samples on platelet aggregation.

1 Materials and Instrument

1.1 Plant Materials: The raw RC was bought from Shenyang Madicinal Materials Company and authenticated by Professor Zheng Taikun at Liaoning College of TCM.

The processed samples, including RC roasted in stirring sand, steamed after being salted, steamed after steeped in wine, and simply steamed were processed as described in the methods recorded by Wang Xiaotao[1]. They were all grounded, including the crude RC, to pass 20-meshe sieve for extraction and preparation into sample solutions Ⅰ and Ⅱ.

1.2 Reagents: Thrombin was bought from Beijing Biological Product Inspection Institute. Aspirin, the positive control, was obtained from the Affiliated Hospital of Liaoning College of TCM.

1.3 Animals: Rabbits were supplied by the Experiment Animal Center of Liaoning College of TCM.

1.4 Instrument: Aggregometer, model BS-631, was purchased from Beijing Biopharmaceutical Factory.

2 Method and Result

2.1 Preparation of Sample Solutions: 10 g of the powdered crude and processed RC were weighed accurately and sparately extracted with 95% alcohol by reflux. Each individual alcoholic extract was filtered after cooling and the solvent recovered as completely as possible to give a residue which was treated with 4 times its amount of water and left overnight. They were filtrated and concentrated on the next day and the resultant residue was dissolved in distilled water in a 5 mL volumetric flask to give sample solutions with final concentrations equivalent to 10 g of crude or processed RC per 5 mL. This was designated as solution Ⅰ.

Another 10 g portions of crude or processed RC were likewise extracted with 95% alcohol, treated similarly as above, but the final water solutions were further subjected to extraction with ethyl acetate to remove the presence of any lipophilic contents. The water layer was concentrated, transferred to 5 mL volumetric flasks with distilled water to give solutions also with final concentrations equivalent to 10 g of crude or processed RC per 5 mL, which were designated as solutions Ⅱ.

2.2 Preparation of Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP): Rabbits of either sex (n = 8, weighing 2～3 kg) were anaesthetized by intraperitoneal (ip) injection of urethane. The citrated carotid blood was centrifuged for 5 min at 1 000 r/min to obtain PRP with platelet numbers about 2.0×10^6/mL, the rest blood was continually centrifuged for 10 min at 3 000 r/min to obtain PPP. 3.8 percent of sodium citrate solution was used as anticoagulant.

2.3 Mesurements and Results[2,3]: Turbidimetry was used. 0.5 mL of PRP was placed into each turbidimetric tube. 25, 50 μL of distilled water were added respectively into the blank control tubes. 15 μL of aspirin solution (3 g dissolved in 100 mL of normal saline solution) was added into the positive control tube. Solution Ⅰ and solution Ⅱ were added into the test drug tubes. All tubes were placed in the aggregometer and incubated for 2 min at 37 ℃. The thrombin (1.70 mg/100 units dissolved in 3 mL of normal saline solution) was added as platelet aggregation inducer to each tube. The curves of absorbance by thrombin-induced platelet aggregation were recorded.

The inhibitory percentage of platelet aggregation was calculated by the following formula:

$$I = \frac{A_B - A_S}{A_B}$$

Where: I= Platelet aggregation inhibition (%)

A_B = Curve of absorbance (mm) of blank control

A_S = Curve of absorbance (mm) of sample

The data were expressed as $\bar{x} \pm s$ and analyzed with analysis of variance. (See Tab. 1)

Tab. 1 Influence of Rhizoma *Cibotii* and its Processed Samples on Thrombin Induced Rabbit Platelet Aggregation (n= 8, $\bar{x} \pm s$)

Group	Dosage (μL)	Solution Ⅰ Absorbance (mm)	Solution Ⅰ Inhitition (%)	Solution Ⅱ Absorbance (mm)	Solution Ⅱ Inhibition Ⅱ (%)
1	25	143.4±11.8			
	50	144.1± 8.9			
2	15	65.5±11.5**	54.3		
3	25	122.2±22.2	14.8	131.0±34.8	8.6
	50	104.4±16.5*	27.6	103.3±36.6*	28.3
4	25	97.8±11.1**	31.8	90.7±25.7**	36.1
	50	71.8±19.6**	50.2	60.7±20.4**	57.8
5	25	122.2±33.0	14.8	102.8±20.5**	28.3
	50	94.7±27.4**	34.3	82.7±12.9**	42.6
6	25	123.6±22.1	13.8	116.4±15.8	18.8
	50	84.6±18.3**	41.3	77.8±15.2**	46.0
7	25	92.8±19.5**	35.3	109.2±30.6	23.8
	50	75.4±19.5**	47.7	68.1±29.6	52.7

Statistical significance vs distilled water: * $P< 0.01$ ** $P< 0.001$

1. distilled water 2. asiprin 3. crude RC 4. RC roasted in stirring sand 5. RC simply steamed 6. RC steamed after steeped in wine 7. RC steamed after being salted

Compared with t test, there is no significantdifference between the inhibition of solution Ⅰ and that of solution Ⅱ.

3 Discussion

3.1 Compared with the Blank-Control Group: RC and all of its processed samples showed significant inhibitory actions on platelet aggregation, with inhibitory intensity of platelet aggregation in the decreasing order of RC roasted in stirring sand, RC steamed after being salted, RC steamed after steeped in wine, RC simply steamed and the crude RC.

3.2 The Inhibitory Activity of Solution Ⅱ: the water part after ethyl acetate extraction, is slightly stronger than that of solution Ⅰ, not subjected to ethyl acetate extraction, though statistically non significant lead us to suppose that the active constituent for inhibition of platelet aggregation is water-soluble. Based on our previous study on the "Basic Principles for the Processing of RC", a research project granted by SPAC, we have detected the presence and estimated the contents of protocatechuic acid and protocatechualdehyde in various processed RC by HPLC (restricted publication data), it would be justifiable to speculate that the anti-platelet activity may be closely related with the presence of these two compounds, especially the decreasing order of their contents was found to be closely coinsident with that of the platelet inhibition activity. Whether such speculation can achieve a firm stand, or there are some other chemical constituents responsible for the diverse pharmacological activities of RC, need further study.

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