这些结果提示肿瘤发生使机体红细胞膜收缩蛋白、锚蛋白和带 3 蛋白发生交联变构而引起带 3 蛋白含量的下降, 进而导致红细胞膜脂流动性的降低[7,8]。红细胞膜脂流动性的降低又引起 GPA (血型糖蛋白 A, 富含 SA) 等多种重要膜蛋白构象改变, SA 含量大幅减少, 引起膜表面的电负性降低, 红细胞聚集性增加而无法正常识别和清除肿瘤细胞进而导致肿瘤细胞血行转移。而本实验中两种 AP 中、高剂量组均可显著降低膜蛋白聚集性而提高带 3 蛋白含量, 使 S_{180}小鼠膜脂流动性升高, 红细胞膜生理功能趋于正常, 这可能是 AP 促进红细胞免疫功能发挥抗肿瘤作用的主要机制之一。

此外, 实验中还发现 AP 在 100 mg/kg 剂量下作用最佳, 当剂量达到 200 mg/kg 时对实验中的各项指标的作用反而有所下降。这一现象表明 AP 存在作用的最佳剂量。超过这一数值, 剂量再增加, 作用反而下降, 这与以往对于其他多糖类成分活性研究的结论相一致。

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Antilipid peroxidation of polyamines from pilose antler

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Abstract: Object To investigate the antioxidant activity of polyamines isolated from pilose antler (PAIPA). **Methods** The effects of PAIPA on the lipid peroxidation (MDA formation) in microsomes of rat brain, liver, and kidney induced by NADPH-Vitamine C (Vc) and ferrous-cysteine systems *in vitro*, the superoxide anion radical production (reduced cytochrome C formation) in xanthine-xanthine oxidase system *in vitro*, and the CCl_4-and ethanol-induced MDA formation in mice liver *in vivo* were evaluated. **Results** PAIPA could significantly inhibit the lipid peroxidation (MDA formation) in microsomes of rat brain, liver, and kidney induced by NADPH-Vc and ferrous-cyctein, the superoxide anion radical production (formation of reduced cytochrome C) in xanthine-xanthine oxidase system *in vitro*, and the CCl_4- and ethanol-induced MDA formation in mice liver *in vivo*. **Conclusion** PAIPA exhibits an antioxidant activity.

Key words: pilose antloer; polyamines; lipid peroxidation; superoxide anion radical

鹿茸多胺的抗脂质过氧化作用

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摘 要: 目的 研究鹿茸多胺的抗氧化作用。方法 测定鹿茸多胺在体外对 NADPH-维生素 C 和 Fe^{2+}-半胱氨酸系统诱发的微粒体脂质过氧化反应 (MDA 形成) 的影响, 对黄嘌呤-黄嘌呤氧化酶系统超氧阴离子自由基 ($O_2^{\cdot-}$)

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产生 (还原型细胞色素 C 形成) 的影响, 在体内对 CCl_4 和乙醇诱发的小鼠肝脂质过氧化反应 (MDA 形成) 的影响。结果 鹿茸多胺在体外能明显抑制 NADPH-维生素 C 和 Fe^{2+}-半胱氨酸系统诱发的大鼠脑、肝、肾微粒体脂质过氧化反应 (MDA 形成), 及黄嘌呤-黄嘌呤氧化酶系统 $O_2^{\cdot-}$ 的产生 (还原型细胞色素 C 形成)。在体内能抑制 CCl_4 和乙醇诱发的小鼠肝脂质过氧化反应 (MDA 形成)。结论 鹿茸多胺具有抗氧化作用。

关键词: 鹿茸; 多胺; 脂质过氧化; 超氧阴离子自由基
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The unossified pilose antler of *Cervus nippon* Temminck var. *mantchuricus* Swinhoe is one of the most famous Chinese traditional medicines, and is used for the treatment of aging syndrome, anemia, neurosis, impotence, seminal emission and premature ejaculation. In the previous papers, that the extract of pilose antler significantly improved age-related biochemical factors in aged mice and showed obvious inhibition on MAO-B activity[1-3] was reported. The polyamines isolated from pilose antler (PAIPA) could increase the synthesies of protein and RNA, and the activity of RNA polymerase Ⅱ in mice liver cell[4,5]. However, the antioxidant properties of PAIPA have not yet been clarified. The present paper describes the antilipid peroxidation of PAIPA *in vitro* and *in vivo*.

1 Materials and methods

1.1 Animals: Male Kunming mice (20±2) g and male Wistar rats (220±20) g were obtained from writer's institute animal center and housed in free condition with food and water supply *adlibitum*. The number of animal eligibility was 980101018 and 980101017, respectively.

1.2 Medicine: The unossified pilose antler of *C. nippon* var. *mantchuricus* Swinhoe was suppled by Antu Pharmaceutical Factory of Jilin Province, identified by Prof. GUO Chai-yu from Laboratory of Traditional Chinese Materia Medica in writer's institute. The PAIPA was supplied by the Laboratory of Traditional Chinese Medicine Formula in writer's institute, composed of 70.9% putrescine, 26.3% spermine, and 2.8% spermidine after HPLC analysis.

1.3 Chemical reagents: Nicotinamide adenine dinucleotide phosphate (NADPH), superoxide dismutase (SOD, 3 000 U/mg), xanthine oxidase (XOD, 10 U/mL), xanthine (XAN), thiobarbituric acid (TBA), 1, 1, 3, 3-tetramethoxypropane and bovin serum albumin were the products of Sigma Chemical Co. All the chemicals used are of analytical grade.

1.4 Preparation of microsome: The tissues of Wistar rat (fasted for 24 hours before experiments) brain, liver, and kidney were homogenized with the four volume of TMS buffer (0.05 mol/L Tris-HCl, 0.2 mol/L sucrose, 3 mmol/L $MgCl_2$, pH 7.5) at 4 ℃, respectively. The homogenate was centrifuged at 10 000 r/min for 20 min, and the supernatant was further centrifuged at 105 000 r/min for 90 min. The pellet of microsome fraction of brain, liver, and kidney was resuspended with TMS buffer. The protein content was determined by the method of Lowry[6]. The proteins of microsomal suspension from rat brain (3 mg/mL), liver (15 mg/L), and kidney (5 mg/mL) were used for this experiment.

1.5 Measurement of lipid peroxidation of microsome induced by NADPH-Vc: The reaction system containing 0.1 mL of brain, liver, or kidney microsomal suspension, respectively, PAIPA 0.01 mL at different concentrations, NADPH 0.1 mL (1.8 mmol/L), Vc 0.005 mL (5 mmol/L) and PBS 0.8 mL (0.1 mol/L KH_2PO_4, 0.14 mol/L NaCl, pH 7.4) was incubated at 37 ℃ for 15 min. After addition of the TBA solution (0.67%) to the system, the content of malondialdehyde (MDA) from lipid peroxidation was measured by TBA method[7].

1.6 Measurement of lipid peroxidation of microsome induced by ferrous-cysteine: A reaction system of 1 mL containing 0.1 mL of brain, liver, or kidney microsomal suspension, respectively, PAIPA 0.01 mL at different concentrations, cysteine 0.02 mL (0.01 mol/L), ferrous sulfate 0.05 mL (1 mmol/L) and PBS[7] was incubated. The MDA formation was detected as described above.

1.7 Detection of superoxide anion radical production in xanthine-xanthine oxidase system: The superoxide anion production was detected by the method of cy-

tochrome C reduction[8] and the formation of the reduced cytochrome C was used to indicate the producing superoxide anion radical in xanthine-xanthine oxidase system indirectly.

1.8 Measurement of lipid peroxidation of mice liver induced by CCl_4 after PAIPA treatment *in vivo*: All the mice were randomly divided into four groups, each consisted of 10 mice, control, CCl_4, PAIPA 10 and 20 mg/kg. PAIPA was iv given to the two PAIPA groups at dose of 10 and 20 mg/kg, respectively, and the saline was iv given to the control and CCl_4 group at dose of 10 mL/kg, for three days. One hour after the last administation, 0.1% CCl_4 (dissolved with bean oil) was ip given to the CCl_4 and two PAIPA groups at dose of 10 mL/kg, the control group was only ip given with bean oil at the same dose. Two hours later, all mice were killed by decapitation and protions of the liver were rapidly sampled to measure the MDA content by the TBA method as described above.

1.9 Measurement of lipid peroxidation of mice liver induced by ethanol after PAIPA treatment *in vivo*: Mice were divided into four groups, control, ethanol, PAIPA 10 and 20 mg/kg. PAIPA was iv given to two PAIPA groups at doses of 10 and 20 mg/kg, respectively, and the saline was iv given to the control and ethanol group at dose of 10 mL/kg, for three days. After the last administration, all mice were fasted for eight hours, then 50% ethanol was ig given to the ethanol group, and two PAIPA groups at dose of 15 mL/kg. Twelve hours later, mice were decapitated and the liver was dissected out to measure the MDA content as described above.

1.10 Statistical analysis: Data presented were $\bar{x} \pm s$ and statistically evaluated by Student's *t*-test.

2 Results

2.1 Effect of PAIPA on lipid peroxidation of microsomes from rat brain, liver, and kidney induced by NADPH-Vc: As shown in Table 1, PAIPA could significantly inhibit the lipid peroxidation (MDA formation) in microsomes of the rat brain, liver, and kidney induced by NADPH-Vc at concentrations of 10—100 μg/L, and it showed the obvious concentration-effect relationship.

Table 1 Effect of PAIPA on NADPH-Vc induced MDA formation in microsomes of rat tissues ($\bar{x} \pm s$, $n = 4$)

Groups	Dose /(μg·mL^{-1})	MDA/(μmol·g^{-1}) Brain	Liver	Kidney
control	–	6.6±0.2	11.5±0.5	5.9±0.5
PAIPA	1	6.3±0.3	11.1±0.5	5.5±0.4
	10	5.4±0.5*	9.6±0.7*	4.6±0.4*
	100	4.9±0.6**	8.6±0.6**	4.0±0.3**

* $P < 0.05$ ** $P < 0.01$ *vs* control group

2.2 Effect of PAIPA on lipid peroxidation of microsomes from rat brain, liver, and kidney induced by ferrous-cysteine: As shown in Table 2, PAIPA could obviously inhibit the lipid peroxidation in microsomes of the rat brain, liver, and kidney induced by ferrous-cysteine at the concentration of 100 μg/L, and it also showed the obvious concentration-effect relationship.

Table 2 Effect of PAIPA on ferrous-cysteine induced MDA formation in microsomes of rat tissues ($\bar{x} \pm s$, $n = 4$)

Groups	Dose /(μg·mL^{-1})	MDA/(μmol·g^{-1}) Brain	Liver	Kidney
control	–	5.5±0.5	6.4±0.2	5.7±0.3
PAIPA	1	5.2±0.5	6.1±0.3	5.3±0.5
	10	4.9±0.6	5.7±0.4*	5.1±0.5
	100	4.6±0.5*	4.7±0.6**	4.5±0.3*

* $P < 0.05$ ** $P < 0.01$ *vs* control group

2.3 Effect of PAIPA on superoxide anion radical production in xanthine-xanthine oxidase system: As shown in Table 3, PAIPA could significantly inhibit the formation of reduced cytochrome C (superoxide anion radical production) in xanthinexanthine oxidase system at a concentration of 100 μg/L.

2.4 Effect of PAIPA on lipid peroxidation of mice liver induced by CCl_4 *in vivo:* As shown in Table 4, PAIPA could significantly inhibit the lipid peroxidation in mice liver induced by CCl_4 at doses of 10 and 20 mg/kg, and it showed the obvious dose-effect relationship.

2.5 Effect of PAIPA on lipid peroxidation of mice liver induced by ethanol *in vivo :* In Table 5 , the

Table 3 Effect of PAIPA on formation of reduced cytochrome C in xanthine-xanthine oxidase system ($\bar{x} \pm s$, $n = 4$)

Groups	Dose /(μg·mL^{-1})	Reduced cytochrome C /(nmol·mg^{-1}·min^{-1})
control	–	16.8±1.3
PAIPA	1	16.1±1.6
	10	15.9±2.4
	100	13.8±2.1*

* $P < 0.05$ *vs* control group

Table 4 Effect of PAIPA on CCl_4-induced MDA formation in mice liver *in vivo* ($\bar{x} \pm s$, $n = 4$)

Groups	Dose/(mg·kg^{-1})	MDA/(μmol·g^{-1})
control	–	42.3± 6.2**
CCl_4	–	132.2±30.1
CCl_4+ PAIPA	20	72.3±12.2***
	10	102.3±18.2**

** $P < 0.01$ *** $P < 0.001$ *vs* CCl_4 group

Table 5 Effect of PAIPA on ethanol-induced MDA formation in mice liver *in vivo* ($\bar{x} \pm s$, $n = 10$)

Groups	Dose/(mg·kg^{-1})	MDA/(nmol·g^{-1})
control	–	44.5±12.6***
ethanol	–	98.1±30.1
ethanol+ PAIPA	20	48.7±13.5***
	10	55.6±24.5**

** $P < 0.01$ *** $P < 0.001$ *vs* ethanol group

lipid peroxidation in mice liver induced by ethanol was significantly reduced by PAIPA at doses of 10 and 20 mg/kg, and it showed the obvious dose-effect relationship.

3 Discussion

Lipid peroxidation by free radicals are involved in many physiological and pathological processes, such as the toxic injury, reperfusion injury, aging and carcinogenesis. Superoxide anion radical ($O_2^{\cdot-}$) could be generated in the NADPH-Vc induced microsome lipid peroxidation systems, hydroxyl radical (·OH) and superoxide anion radical ($O_2^{\cdot-}$) could be generated in the ferrous-cysteine systems. In the experiments of NADPH-Vc and ferrous-cysteine initiated lipid peroxidation of microsomes from the rat brain, liver, and kidney, PAIPA showed an antilipid peroxidation action *in vitro*. It was known that CCl_4 could be metabolized to generate the radical ($\cdot CCl_3$) evoking lipid peroxidation after cytochrome activation in liver cell. Ethnol also could initiate liver lipid peroxidation. In this experiment, PAIPA could significantly inhibit the CCl_4- and ethanol-induced lipid peroxidation in mice liver *in vivo*. In order to elucidate mechanisms of the antioxidant action of PAIPA, the inhibition of $O_2^{\cdot-}$ radical production (formation of reduced cytochrome C) in xanthine-xanthine oxidase system was detected. The results showed that PAIPA could significantly inhibit the production of ($O_2^{\cdot-}$) radical. It was suggested that one of the mechanisms of antioxidant action of PAIPA possibly be related to PAIPA's inhibiting the production of $O_2^{\cdot-}$ radical. Furthermore, the molecular mechanism of PAIPA scavenging oxygen radicals directly merits further studies.

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