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## 人参皂苷 $Rg_1$ 对大鼠海马 c-*fos* 基因表达和 cAMP 含量的影响

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**关键词** 人参；皂苷类；*fos* 基因；原癌基因蛋白 c-*fos*；腺苷环一磷酸；RNA 印迹；蛋白质印迹；海马 cAMP 大鼠

**目的**：探讨 $Rg_1$ 对神经系统作用的机制。**方法**：采用 Northern 和 Western 印迹分析法，检测了 $Rg_1$ 处理前后大鼠海马组织的 c-*fos* 基因和蛋白的表达。**结果**：老年鼠 c-*fos* 基因和蛋白的表达明显低于青年鼠，但给 $Rg_1$ 后老年鼠和青年鼠均呈现显著性增强效应。此外，$Rg_1$ 还明显增加青年鼠和老年鼠海马组织的 cAMP 含量。**结论**：$Rg_1$ 升高 cAMP 水平及促进 c-*fos* 基因和蛋白的表达有助于阐明其促智和抗衰老作用的机制。

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# Effects of *Coriolus versicolor* polysaccharides on superoxide dismutase activities in mice

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**KEY WORDS** *Coriolus versicolor*; polysaccharides; superoxide dismutase; delayed hypersensitivity; Ehrlich tumor carcinoma; radiation

**AIM**: To study if *Coriolus versicolor* polysaccharides (CVP) influence the superoxide dismutase (SOD) activities in mice. **METHODS**: Normal, tumor-bearing, and radiated ICR mice were injected ip with CVP daily for 3 - 15 d. The SOD activity was assayed by epinephrine autoxidation test. **RESULTS**: The SOD activities in lymphocytes and thymus were increased by CVP in both the normal mice with or without delayed hypersensitivity (DH). In tumor-bearing mice, CVP exerted not only inhibitory effects on tumor growth and SOD activity in tumor tissue but also complete or partial restorative effects on the suppressed DH and on the declined SOD activities in lymphocytes, spleen, and thymus. The total SOD and manganese-containing SOD (MnSOD) activities in lymphocytes and thymus were dose-dependently enhanced by CVP (5 - 20 mg · kg$^{-1}$) on d 3 after the tumor transplantation. In the mice exposed to $^{60}$Co (3 or 6 Gy),

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DH and SOD activities were dose-dependently decreased. These changes were completely or partly prevented by CVP. **CONCLUSION**: CVP exerted the favorable effects on SOD activities in mice.

*Coriolus versicolor* polysaccharides (CVP) exert inhibitory effects on experimental and clinical tumors. These effects are presumed to be mediated mainly by host-defence mechanism, especially immunological responeses. Superoxide dismutase (SOD) plays an important role in protecting cells against superoxide radical ($O_2^-$) damages and overproduction of $O_2^-$ or SOD abnormities exist in many diseases[1-4]. The present study was to investigate if the CVP could exert some favorable effects on SOD activities *in vivo*.

## MATERIALS AND METHODS

ICR mice, ♂ & ♀ (22.5 ± *s* 2.3 g) were purchased from the Experimental Animal Center of Second Military Medical University (SMMU). CVP was isolated and analyzed by the Department of Photochemistry of SMMU[5]. CVP was dissolved in normal saline (NS) just before use. The stock solution of *l*-epinephrine (Fluka) 50 mmol·L$^{-1}$ was made in HCl 0.1 mol·L$^{-1}$, which was diluted each time with redistilled water to make fresh 1 mmol·L$^{-1}$ solution for assay. The buffers and solutions were prepared with deionized glass-redistilled water and all the reagents were of AR.

**Experimental protocol** The experiment was made on normal, tumor-bearing, and radiated ICR mice. Solid tumor was transplanted by sc implantation of $5 \times 10^6$ Ehrlich ascites carcinoma cells (EAC) into mice[2]. DH was induced by dinitrofluorobenzen[6] in some mice 9 d after implantation or 1 d after radiation. Briefly, CVP was injected ip at 1 h after implantation in tumor-bearing mice for 3 - 15 d and on d 3 before DH induced (eg, 2 d before radiation) in radiated mice for 9 d. Finally, the mice were decapitated and blood samples were collected for separating lymphocytes. Organs and tumors were excised, rinsed, and frozen at - 40 ℃ until assay.

**Preparation of SOD crude extracts**[2,4] The lymphocytes separated from 1 mL of blood or 0.1 g of the samples (spleen, thymus or tumor tissue) were homogenized in 2 mL of ice-cold potassium phosphate buffer 50 mmol·L$^{-1}$ (pH 7.4) for 2 min. After extraction at 4 ℃ for 30 min, the homogenates were centrifuged at 17 000 × *g* for 40 min (4 ℃). The supernatants were the SOD crude extracts.

**Measurement of SOD activity** The SOD activity was assayed by the epinephrine autoxidation test of Sun and Zigman[7]. One unit (U) of SOD was defined as the amount of enzyme that induced a 50 % of inhibition of the epinephrine autoxidation in 1 mL of the assay system. The protein content of the SOD crude extracts was measured using Coomassie brilliant blue G-250 (Fluka)[8]. To assay total SOD activity, 25 μL of the SOD crude extract or potassium phosphate buffer (as matched blank control) and epinephrine (1 mmol·L$^{-1}$) 0.3 mL were added to 2.675 mL of sodium carbonate buffer (50 mmol·L$^{-1}$, pH 9.9), respectively. After shaken, the final total 3 mL of reaction mixtures were incubated in water at 30 ℃ for 3 min, and then the *A* value of the mixtures was measured immediately at 320 nm. The MnSOD activity was assayed by the same procedure at 20 min after adding NaCN to the crude extracts at 2 mmol·L$^{-1}$. CuZnSOD activity was obtained by subtraction of the MnSOD from the total SOD and the errors were calculated by propagation-of-error theory.

**Statistical analysis** All data are expressed as $\bar{x} \pm s$. Significance of the differences was determined by *t*-test (between two groups) or ANOVA and Newman-Keuls' test (multiple comparisons) at the $\alpha = 0.05$ level.

## RESULTS

CVP inhibited the growth of the tumor and suppressed the SOD activity in the tumor. The depressed DH was enhanced by CVP on d 15 after sc EAC (Tab 1).

**Tab 1. Effects of ip CVP (10 mg·kg$^{-1}$·d$^{-1}$) on DH, Ehrlich solid tumor, and SOD activity of tumor in mice after sc $5 \times 10^6$ Ehrlich cells. $^cP < 0.01$ *vs* NS (9 d). $^fP < 0.01$ *vs* NS (15 d).**

| | *n* | Days after sc EAC | DH/mg | Wet wt of tumor/mg | SOD activity, U/mg protein |
|---|---|---|---|---|---|
| NS | 7 | 9 | - | 320 ± 34 | 48 ± 5 |
| CVP | 7 | 9 | - | 235 ± 27$^c$ | 27 ± 7$^c$ |
| NS | 6 | 15 | 3.4 ± 2.5 | 558 ± 29 | 39 ± 13 |
| CVP | 7 | 15 | 12 ± 5$^f$ | 287 ± 23$^f$ | 14 ± 4$^f$ |

MnSOD activity was increased in lymphocytes ($P < 0.05$) and thymus ($P < 0.01$) after mouse DH was induced, and total SOD and copper- and zinc-containing SOD (CuZnSOD) in lymphocytes and MnSOD activity in lymphocytes and thymus were increased by CVP in both normal mice with or without DH (Tab 2).

SOD activities were decreased except total SOD (remained unchanged) and CuZnSOD (increased) in lymphocytes on d 3 after implantation of EAC in

**Tab 2. Effect of ip CVP on SOD activities in lymphocytes, spleen, and thymus of normal mice. A) Control, $n = 10$. B) + DH, $n = 8$. C) + CVP, $n = 7$. D) + DH + CVP, $n = 7$. $^bP < 0.05$, $^cP < 0.01$ *vs* A. $^eP < 0.05$, $^fP < 0.01$ *vs* B.**

| | Group | SOD activity, U/mg protein MnSOD | CuZnSOD | Total SOD |
|---|---|---|---|---|
| Lymphocytes | | | | |
| | A | 50 ± 9 | 84 ± 19 | 134 ± 22 |
| | B | 61 ± 8 | 89 ± 12 | 151 ± 14 |
| | C | 63 ± 11$^b$ | 101 ± 9$^b$ | 164 ± 19$^b$ |
| | D | 75 ± 15$^e$ | 97 ± 7$^e$ | 172 ± 18$^e$ |
| Spleen | | | | |
| | A | 67 ± 14 | 66 ± 23 | 133 ± 20 |
| | B | 76 ± 14 | 74 ± 24 | 150 ± 21 |
| | C | 80 ± 10 | 65 ± 20 | 145 ± 28 |
| | D | 91 ± 13 | 75 ± 20 | 167 ± 16 |
| Thymus | | | | |
| | A | 23 ± 6 | 67 ± 14 | 89 ± 16 |
| | B | 32 ± 6 | 65 ± 23 | 97 ± 23 |
| | C | 40 ± 11$^c$ | 62 ± 12 | 101 ± 18 |
| | D | 41 ± 4$^f$ | 69 ± 19 | 110 ± 21 |

tumor-bearing mice. CVP (once daily for 3 d) enhanced the total SOD and MnSOD activities in a dose-dependent manner, while the increase in CuZnSOD activity was not much ($P > 0.05$) (Fig 1).

The SOD activities in lymphocytes, spleen, and thymus, especially the MnSOD were declined 3, 9, and 15 d after implantation of EAC. These changes were completely or partially restored by CVP (Fig 2).

In mice exposed to $^{60}$Co-γ rays (1.0522 Gy/min), DH and SOD activities were decreased in a radiation dose-dependent way. The changes were completely or partially prevented by CVP (Tab 3).

## DISCUSSION

The effects of CVP on tumor and DH are consistent with those reports by most of other investigators up to now. Our present study showed that tumor burden or radiation depressed the SOD activities in lymphocytes, spleen, and thymus and that these changes were completely or partially restored by CVP. SOD may be a major intracellular enzyme against $O_2^-$ toxicity through catalysis of the removal of $O_2^-$[3]. Therefore, our present study may partly account for the antitumor and immunomodulator

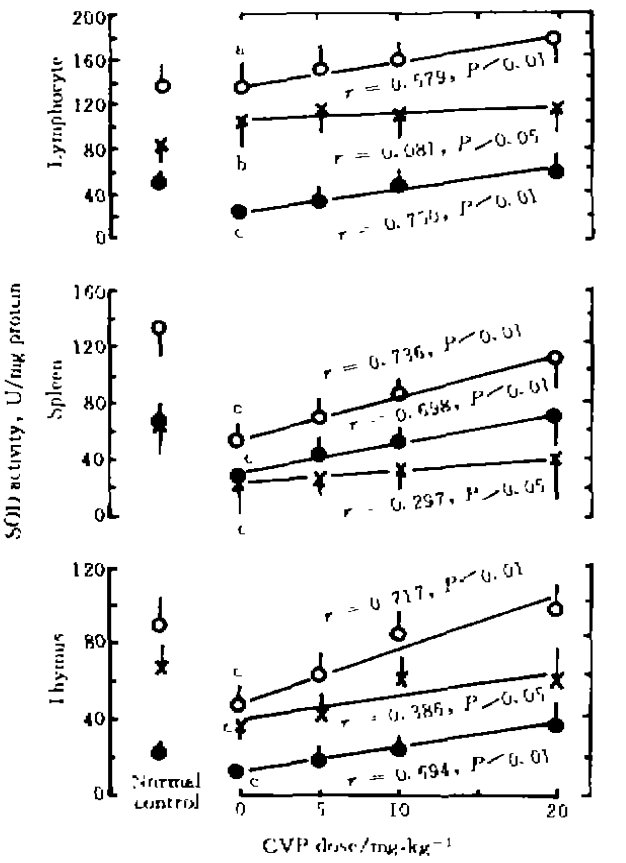


**Fig 1. Effects of CVP on SOD activities in tumor-bearing mice 3 d after sc EAC. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. ○ = Total SOD. ● = MnSOD. × = CuZnSOD. Control, $n = 10$; the other, $n = 7$.**

properties of CVP.

Oxy-radicals such as $O_2^-$ are by-products of many biological oxidation and their formation appears to be greatly increased during stress condition[9]. Radiation also has sufficient intrinsic energy to produce $O_2^-$. Although $O_2^-$ at physiological concentrations seems particularly harmless, its toxicity *in vivo* arises by a metal ion-dependent conversion into hydroxyl radicals (the Haber-Weiss reaction) which can cause damage to the body. SOD serves a protective role in all aerobic organisms by converting $O_2^-$ to $H_2O_2$, so, the abnormal pattern of SOD activities occured in tumor-bearing and radiated mice might result in a variety of $O_2^-$-mediated damages to the immune system. Of particular importance is that the decrease in SOD activities can lead to $O_2^-$-mediated lipid peroxidation and cellular injury. Under these conditions, increase in SOD activities by CVP may prevent immune system from

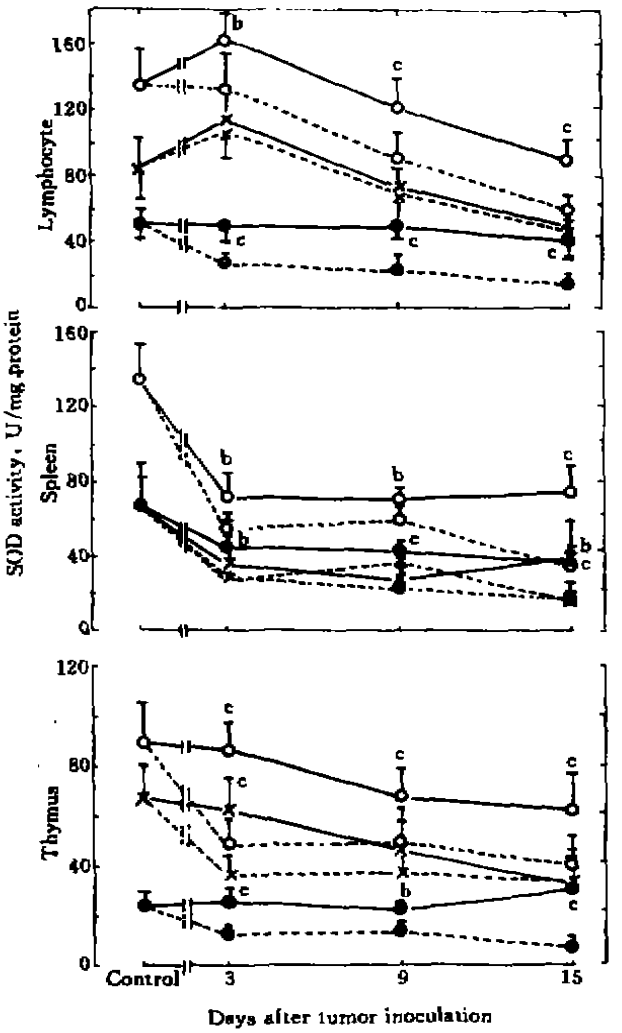


**Fig 2. Effect of CVP (10 mg·kg$^{-1}$, ip) on SOD activities in mice after tumor inoculation. $n = 7$ except control ($n = 10$) and 15 d NS ($n = 6$). ○ = Total SOD. ● = MnSOD. × = CuZnSOD. CVP (——). NS (……). $^{b}P < 0.05$, $^{c}P < 0.01$ vs NS.**

$O_2^-$-mediated toxicity and restore the immune function, which can partly explain why CVP could enhance the DH. As the SOD activities in both normal and tumor-bearing mice were increased by CVP, it is proposed that CVP enhanced induction of SOD.

In eukaryotic cells, 2 forms of SOD, CuZnSOD, and MnSOD, are present. SOD, especially MnSOD activity was lower in tumor cells than in their normal cell counterparts[2,4]. In this study no MnSOD activity was measured and the CuZnSOD activity was very lowered in the tumor. The tumor-bearing mice treated with CVP had lower SOD activity in tumor tissue than with NS. The mechanism for such change is unknown at present. The cytotoxicity of tumor necrosis factor (TNF) might be partly mediated through induction of toxic $O_2^-$, and cellular sensitivity to TNF might be correlated with antioxidant enzymes such as SOD[10]. Apparently, a decrease in SOD activity in tumor tissue may present with an increase in tumor cellular sensitivity to TNF. It is possible that cytocidal activity of TNF against tumor may be potentiated by CVP.

In conclusion, the antitumor and immunorestorative properties of CVP were partially related to its effects on SOD activities.

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**Tab 3. *Effects of* CVP (10 mg·kg$^{-1}$·d$^{-1}$ *ip for* 9 d) on DH and SOD activities in radiated mice.** $n = 8$ except the group of 6 Gy + NS ($n = 7$). $^{b}P<0.05$, $^{c}P<0.01$ *vs* 3 Gy + NS. $^{e}P<0.05$, $^{f}P<0.01$ *vs* 6 Gy + NS.

| *Group* | *DH/mg* | *Tissue* | SOD activity, U/mg protein | | |
|---|---|---|---|---|---|
| | | | Total SOD | MnSOD | CuZnSOD |
| Normal mice + NS | 13 ± 3 | Lymphocytes | 151 ± 14 | 61 ± 8 | 89 ± 12 |
| | | Spleen | 150 ± 21 | 76 ± 14 | 74 ± 24 |
| | | Thymus | 97 ± 22 | 32 ± 6 | 66 ± 23 |
| Radiated mice | | | | | |
| 3 Gy + NS | 7.4 ± 2.5 | Lymphocytes | 119 ± 18 | 42 ± 9 | 77 ± 17 |
| | | Spleen | 110 ± 11 | 54 ± 6 | 56 ± 15 |
| | | Thymus | 78 ± 9 | 21 ± 7 | 57 ± 7 |
| 3 Gy + CVP | 13 ± 3$^{c}$ | Lymphocytes | 146 ± 20$^{b}$ | 54 ± 10$^{b}$ | 92 ± 21 |
| | | Spleen | 128 ± 19 | 67 ± 12 | 58 ± 18 |
| | | Thymus | 92 ± 10$^{b}$ | 26 ± 9 | 66 ± 14 |
| 6 Gy + NS | 3.7 ± 1.4$^{b}$ | Lymphocytes | 92 ± 11$^{c}$ | 39 ± 6 | 52 ± 13$^{c}$ |
| | | Spleen | 88 ± 10$^{b}$ | 43 ± 10 | 45 ± 13 |
| | | Thymus | 59 ± 19$^{b}$ | 15 ± 6 | 43 ± 17 |
| 6 Gy + CVP | 10.8 ± 2.4$^{f}$ | Lymphocytes | 127 ± 21$^{f}$ | 49 ± 8$^{e}$ | 79 ± 17$^{e}$ |
| | | Spleen | 116 ± 20$^{e}$ | 62 ± 10$^{f}$ | 54 ± 17 |
| | | Thymus | 87 ± 17$^{f}$ | 25 ± 8$^{e}$ | 62 ± 14 |

and its TNF-resistant variant.
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## 云芝多糖对小鼠超氧化物歧化酶活力的影响

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**关键词** 云芝; 多糖类; 超氧化物歧化酶; 迟发型超敏感性; Ehrlich 瘤癌; 辐射 抗肿瘤 免疫力

**目的**: 研究云芝多糖(CVP, ip)对小鼠有关组织中超氧化物歧化酶(SOD)活力的作用. **方法**: 实验在正常, 荷瘤和$^{60}$Co-γ 线辐射损伤小鼠中同步进行. SOD 活力测定采用肾上腺素自氧化法. **结果**: CVP 既能不同程度地增强正常小鼠和正常迟发型超敏感性(DH)小鼠淋巴细胞, 脾及胸腺中 SOD 的活力, 又能明显恢复或防止肿瘤或辐射对小鼠 DH 和淋巴细胞, 脾及胸腺中 SOD 的抑制效应. CVP 对肿瘤生长和肿瘤组织中 SOD 活力均有明显抑制作用. **结论**: CVP 对小鼠 SOD 活性有促进作用, CVP 的抗肿瘤和免疫恢复作用与其对 SOD 的作用有一定的联系.

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## Information for authors

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