

Original Research

Restorative effect of *Coriolus versicolor* polysaccharides against γ -irradiation-induced spleen injury in mice

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KEY WORDS *Coriolus versicolor*; polysaccharides; experimental radiation injuries; spleen; DNA

AIM: To study the restorative effect of *Coriolus versicolor* polysaccharides (CVP) on spleen injury induced by γ -ray irradiation in mice. **METHODS:** ICR δ mice, 6-8 wk old, were divided at random into 3 groups: A) normal control; B) irradiated with 1 Gy; and C) after 1 Gy irradiation, given CVP 60 mg·kg⁻¹(ig) daily for 10 d continuously. Body weight (BW), spleen weight (SW), relative SW (RSW), DNA synthesis of splenocytes (DNA-SS), and relative DNA-SS were measured on d 5, 12, 19, 26, and 33 after irradiation. **RESULTS:** SW, RSW, DNA-SS, and relative DNA-SS decreased after irradiation. CVP enhanced the recovery of SW, RSW, DNA-SS, and relative DNA-SS inhibited by irradiation. **CONCLUSION:** CVP has the restorative effect against spleen injury induced by γ -ray irradiation in mice.

Coriolus versicolor polysaccharides (CVP), Krestin, containing 18% - 38% protein were extracted from the mycelia^[1]. It has the positive effects on tumor control^[2]. CVP enhanced the proliferation of T-cells and B-cells^[3], inhibited tumor growth but restored NK cell activity impaired by cisplatin in nude mice bearing human ovarian carcinoma^[4] and promoted macrophage activity^[5]. Spleen is an important organ relative to immunity. Ionizing radiation inhibited both humoral and cellular immunities while the radiosensitivity of T-cells was less than that of B-cells^[6]. In this study, we studied the restorative effect of CVP against radiation-induced spleen injury in mice.

MATERIALS AND METHODS

1 CVP Made by Sankyo Co, Japan, lot number is K200A10. CVP is protein-bound polysaccharides containing about 18% - 38% of protein, with mean molecular weight of about 100 000. Crude polysaccharides extracted with hot water from mycelia of *Coriolus versicolor* (Fr) Quel (Kawaratake) was fractionated with barium hydroxide and respective fractions obtained were examined. As the result monosaccharide comprising sugar part for the major fractions was identified to be D-glucose, which was assumed to be beta-D-glucan by the tests for specific rotation, zymolysis with cellulase, etc. CVP is a brown or brownish powder with slight specific odor, tasteless hardly soluble in methanol, pyridine, chloroform, benzene and hexane. The pH of water solution (1→100) is 6.6 - 7.2.

2 Mice ICR strain δ mice ($n = 180$), 6-8 wk old, were fed rod lab chow (bought from Fu-Show Co in Taiwan) and water *ad lib*.

3 Irradiation The machine was ceiling mounted ¹³⁷Cs γ -ray irradiation (Picker T55-266). The mice were exposed in whole body irradiation with a single dose of 1 Gy (exposure dose in air) at a rate of 0.6 Gy·min⁻¹. Each group of 5 mice was placed in a round wooden box (20 cm in diameter) on a turning table under the irradiation source. The distance between the source and the back of mice was 33 cm.

4 Grouping The mice were divided into 3 groups: A) normal control; B) 1 Gy whole-body γ -ray irradiation; and C) after irradiation received CVP 60 mg·kg⁻¹·d⁻¹(ig) for 10 d continuously.

5 Parameters

5.1 Body weight Eight mice from each group were weighed on d 5, 12, 19, 26, and 33 after irradiation.

5.2 Spleen weight Eight spleens of mice from each group were weighed on d 5, 12, 19, 26, and 33 after irradiation.

5.3 Relative spleen weight (RSW) = [spleen weight (mg)/body weight (g)] × 100 %

5.4 DNA synthesis of splenocytes (DNA-SS) According to the method of Michell and Dutton^[7]. The suspension of $1 \times 10^3 \cdot L^{-1}$ splenocytes in the RPMI-1640 medium (pH

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7.2 - 7.4), was supplemented with 5 % fetal calf serum, benzylpenicillin 1×10^8 IU·L⁻¹, and streptomycin 1×10^5 mg·L⁻¹. Suspension (0.1 mL) in a well of microplate was incubated at 37 °C in 5 % CO₂ for 48 h. [³H]thymidine (37 kBq) was added and incubated for another 18 h. The cells were harvested on filter by cell harvester (Flow Science Lab) and the radioactivity (counts per minute, CPM) were counted with an automatic liquid scintillator.

5.5 Relative DNA-SS Relative

DNA-SS = [(cpm of the experimental group)/(cpm of normal control)] × 100 %

6 Statistics Indices were expressed as mean ± standard deviation and analyzed by *t*-test.

RESULTS

1 **Body and spleen weights** In groups B and C, the body weights did not change much after γ -ray irradiation, but the splenic weights decreased on d 5 and recovered gradually. The spleen weights in group C were higher than those of group B on d 19 and 26 after irradiation. The spleen weights of groups B and C elevated to the normal range since d 26 (Tab 1).

2 **RSW** RSW in groups B and C decreased

on d 5 and recovered gradually. The RSW of group C was higher than that of group B on d 12 and d 26 after irradiation. The RSW in groups B and C recovered to nearly the normal level on d 33 (Tab 1).

3 **DNA-SS** The DNA-SS of groups B and C treated with irradiation decreased on d 5, and then recovered gradually. The DNA-SS of group C was higher than that of group B on d 5, 12, 19, 26, and 33 after γ -ray irradiation. The DNA-SS in groups B and C elevated to the normal range on d 33 (Tab 1).

3.1 **Relative DNA-SS** The outcome of the relative DNA-SS was similar to that of DNA-SS. The relative DNA-SS in group C was 83.0 % and in group B was 52.1 % on d 12, in group C was 93.9 %, and in group B was 69.2 % on d 26 (Tab 1).

DISCUSSION

The previous study in our laboratory indicated that the total number of splenocytes and the number of splenocytes per unit weight in spleen was

Tab 1. Changes in body weight (g), spleen weight (mg), relative spleen weight (mg/g), DNA synthetic rate ($10^{-3} \times$ cpm), and relative DNA synthetic rate (%) of mice after treatment. $n=8$, $\bar{x} \pm s$. ^a $P > 0.05$, ^b $P < 0.05$, ^c $P < 0.01$ vs group B. NC: Normal control. RT: Radiation treatment (1 Gy γ -ray irradiation). CVP: *Coriolus versicolor* polysaccharides.

Item tested	Group	Treated condition		Days after γ -ray irradiation				
		Mode	Fraction	5	12	19	26	33
Body weight	A	NC		30.7 ± 1.7 ^a	24 ± 3 ^a	29 ± 3 ^a	29.3 ± 1.3 ^c	28 ± 5 ^a
	B	RT	1	27.3 ± 1.9	28 ± 3	27.3 ± 1.3	23.0 ± 2.2	31.0 ± 2.2
	C	RT+	1	28 ± 3 ^a	27 ± 3 ^a	25.7 ± 1.4 ^b	26 ± 4 ^a	28 ± 3 ^a
		CVP	10					
Spleen weight	A	NC		130 ± 5 ^a	119 ± 8 ^a	149 ± 52 ^a	145 ± 7 ^a	146 ± 27 ^a
	B	RT	1	99 ± 34	107 ± 12	98 ± 5	155 ± 65	185 ± 76
	C	RT+	1	91 ± 24 ^a	99 ± 29 ^a	175 ± 33 ^c	224 ± 12 ^a	158 ± 57 ^a
		CVP	10					
Relative spleen weight	A	NC		4 ± 3 ^a	5.0 ± 0.3 ^c	5.3 ± 2.2 ^a	4.96 ± 0.20 ^a	5.3 ± 1.0 ^a
	B	RT	1	3.6 ± 1.0	3.8 ± 0.5	3.6 ± 0.3	7 ± 4	5.8 ± 2.0
	C	RT+	1	3.3 ± 0.7 ^a	3.8 ± 1.3 ^a	6.8 ± 1.2 ^a	8 ± 4 ^a	6 ± 3 ^a
		CVP	10					
DNA-SS	A	NC		11 ± 4 ^c	11 ± 3 ^c	12 ± 3 ^c	12 ± 3 ^c	11 ± 4 ^a
	B	RT	1	4.0 ± 1.2	6.0 ± 1.1	6.7 ± 2.1	8.2 ± 1.5	11 ± 3
	C	RT+	1	8 ± 3 ^c	9.5 ± 2.3 ^c	10 ± 3 ^c	11 ± 3 ^c	13 ± 4 ^a
		CVP	10					
Relative DNA-SS (%)	A	NC		100	100	100	100	100
	B	RT	1	36.5	52.1	58.4	69.2	93.5
	C	RT+	1	75.0	83.0	88.7	93.9	111.1
		CVP	10					

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analyzed by a flow cytometer, and both of them markedly decreased on d 7 after 4 Gy γ -ray irradiation; and similar effect was shown on the spleen weight^[8]. The study also indicated that 4 Gy γ -ray irradiation could inhibit the relative splenocyte percentage significantly, and that the increase in spleen weight appeared to be due to the increased number of splenocytes^[8]. The present results showed that 1 Gy γ -ray irradiation decreased SW, RSW, DNA-SS, and relative DNA-SS in mice. The reason was that 1 Gy γ -ray irradiation might induce mitotic delay^[9], decreased the synthetic rates of DNA, RNA, and protein in splenocytes of mice^[10]; and decreased the number of the splenocytes and spleen weight^[8].

Hirai *et al* stated that CVP could enhance the proliferation of T-cells and B-cells^[3]. Our previous study reported that CVP could promoted the total number splenocytes in spleen, the number of splenocytes per unit weight, the splenocyte proliferation and immunocompetence T-cells inhibited by γ -ray irradiation^[9]. The present result showed that CVP was effective in enhancing the BW, SW, RSW, DNA-SS, and relative DNA-SS in γ -ray irradiated mice. The mechanism of action could be considered as that CVP could promote the synthetic rate of DNA, then promoted the splenocyte proliferation, the number of splenocytes and spleen weight. And spleen function was enhanced by CVP on the proliferation of T-cells and B-cells inhibited by γ -ray irradiation^[3].

In conclusion, CVP has the restorative effect against spleen injury induced by γ -ray irradiation in mice.

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REFERENCES

- 1 Taguchi T. Experimental and clinical studies on Krestin. Osaka, Japan, Osaka University 1981: 1 - 2.
- 2 Dennert G, Tucker D. Antitumor polysaccharide lentinan. AT-cell adjuvant. J Natl Cancer Inst 1973; 51: 1727 - 9.
- 3 Hirai R, Oguchi Y, Sugita N, Matsunaga K, Nomoto K. Enhancement of T-cell proliferation by PSK. Int J Immunopharmacol 1993; 15: 745 - 50.
- 4 Ishii K, Kikuchi Y, Hirate J, Tode T, Kita T, Nagata I, *et al*. Antitumor effect of PSK in nude mice with human ovarian carci-

- noma cells and its combined effect with cisdiammine-dichloroplatinum (II). Cancer 1993; 6: 87 - 90.
- 5 Kobayashi H, Matsunaga K, Fujli M. PSK as a chemopreventive agent. Cancer Epidemiol Biomar Prevent 1993; 2: 271 - 6.
- 6 Kataoka Y, Sado T. The radiosensitivity of T and B lymphocytes in mice. Immunology 1975; 29: 121 - 30.
- 7 Michell RI, Dutton RW. Immunization of dissociated spleen cell cultures from normal mice. J Exp Med 1967; 126: 423 - 8.
- 8 Chen WC, Hau DM, Wang CC, Lin IH, Lee SS. Effects of *Ganoderma lucidum* and Krestin on subset T-cell in spleen of γ -irradiated mice. Am J Chin Med 1995; 23: 289 - 98.
- 9 Yu CK, Sinclair WK. Mitotic delay and chromosomal aberrations induced by X-rays in synchronized Chinese hamster cells *in vitro*. Cancer Inst 1967; 39: 610 - 32.
- 10 Goud SN. Effect of irradiation on lymphocyte-proliferation and differentiation-potential of IL-6 in augmenting antibody-responses in cultures of murine spleen-cells. Int J Radiat Biol 1995; 67: 461 - 8.

瓦茸多糖体对照射过 γ 射线小白鼠脾脏损伤之修复作用

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关键词 瓦茸; 多糖类; 实验性辐射性损伤; 脾; 脱氧核糖核酸

目的: 评估瓦茸多糖体对于照射过 γ -射线小白鼠脾脏损伤之修复作用. **方法:** 本研究所使用之药品为瓦茸多糖体 (*Coriolus versicolor* polysaccharides, CVP, PSK). 瓦茸(又名黑云茸)多糖体是一种与蛋白质结合的多糖体, CVP 是从培养的担子菌类胡孙眼科瓦茸之菌丝体精制而成. 本研究选取 6-8 周大之 ICR 种系小白鼠 180 只, 分为 3 组: A 组为正常对照组; B 组为辐射对照组, 仅接受 1 Gy γ 射线的全身辐射; C 组为实验组, 于接受 1 Gy γ -射线之次日起 10 d, 每日用胃管喂以 CVP 60 mg·kg⁻¹. 于辐射后的第 5, 12, 19, 26 及 33 日, 分 5 批处理, 每批每组各取小鼠 8 只测量体重后, 取出脾脏称重, 制成脾脏细胞的悬浮液. 用 [³H]TdR 追踪法测定脾脏细胞之 DNA 合成速率. **结果:** 小鼠在接受 γ -射线后, 其脾脏重量及脾脏细胞之 DNA 合成速率皆有下降. 在照射后接受 CVP 处理之 C 组, CVP 能促进提早恢复因 γ -射线所导致的抑制效果. **结论:** CVP 对于照射过 γ -射线小白鼠之脾脏损伤具有显著修复作用.