RESEARCH COMMUNICATION

Polysaccharide Extracted from Rheum Tanguticum Prevents Irradiation-induced Immune Damage in Mice

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Abstract

Aim: To investigate the protective effect of purified fraction 1 polysaccharide extracted from *Rheum tanguticum* RTP1 on irradiation-induced immune damage in mice. Methods: Kunming mice were randomly divided into five groups: normal group (NC), irradiation control group (IC), RTP1 low dose (200 mg/kg), middle dose (400 mg/kg) and high dose (800 mg/kg) groups. RTP1 was adminstered by the gastric route for 14 d, mice in the NC and IC groups being given by 0.9% sodium chloride solution in the same way. The mice in all groups except NC group were irradiated with 2.0 Gy ^{60}Co γ-ray on the fourteenth day. Immune indives of non-specific immune function, cellular immunity and humoral immunity were assessed at the 24th hour after radiation. Results: Compared with the IC group, the spleen index, thymus index, rate of carbon clearance, phagocytic function of macrophages, lymphocyte proliferation, hemolysin value of blood serum and NK activity were increased markedly ($P < 0.05$ or $P < 0.05$). Conclusion: RTP1 has an obvious protective effects on damage in γ-ray radiated mice.

Keywords: Polysaccharide of *Rheum tanguticum* - immune function - radiating damage - ^{60}Coγ Ray

Asian Pacific J Cancer Prev, **13**, 1401-1405

Introduction

Radiation therapy is one of the most important strategies in cancer treatment with irreplaceable advantages. However, it should not be overlooked that the immune damage caused by radiation therapy, which means that radiation therapy could lead to the immune function lessening and immune organ damage (Hu et al., 1988; Uh et al., 1994). The immune system damage could further weaken the immune function and leave the tumor out of control. At the same time, patients experienced physical deterioration, ruining their quality of life. Therefore, the research on radioprotector has been the hot spot in radiation therapy.

In recent years, scholars in many countries have extracted a variety of polysaccharide from plants which could strengthen the immune system, such as ginseng polysaccharides (Lim et al., 2004), the polysaccharide from mulberry leaves (Hwan et al., 2000), polysaccharide from mugwort leaves (Lan et al., 2010), ganoderma lucidum polysaccharides (Gao et al., 2003; Zhua et al., 2007), wolfberry polysaccharide (Tang et al., 2011), *Achyranthes bidentata* polysaccharides (Chen et al., 2003), *Grifola frondosa* polysaccharide (Yin et al., 2007), lentinan (Yin et al., 2007), polyporus polysaccharide (Wong et al., 2011), angelica polysaccharide (Yang et al., 2006). All of them have been verified that they could enhance immunity. In addition, because of its wide material sources, natural, non-toxic and low cost, plant polysaccharides is a promising radioprotector (Schepetkin and Quinn, 2006).

Rheum tanguticum is Chinese traditional and commonly used herbs, and has a long history of medicinal application. We isolated and extracted five kind of RTP (Rheum tanguticum polysaccharide, RTP) from Rheum tanguticum with different molecular weight, and we verified that component 1 (RTP1, molecular weight $6\times10^5 \sim 8\times10^5$) could promote the proliferation of mouse splenocytes, and coordinate to enhance the concanavalin (ConA)-induced T cell proliferation and bacterial lipopolysaccharide (LPS)-induced B cell proliferation (Jia et al., 2002; Zhang et al., 2006; Liu et al., 2009). We exposed mice to ^{60}Co γ ray and studied its effect on mice immune system. The studies will provide the basis for the discovery of new anti-radiation drugs.

Materials and Methods

Animals

Clean level Kunming mice are provided by Laboratory Animal Center of The Fourth Military Medical University. License number: SCXK (Army) 2002-2005. The number of male and female mice is equal, weighing 18 ~ 22 g.

Instruments and reagents

CO_2 incubator (U.S. Nuaice); enzyme-linked immunoassay instrument (East China Electron Tube Factory); DU800 UV-visible spectrophotometer (U.S. Beckman); Rheum tanguticum polysaccharide component 1 (RTP1, molecular weight $6 \times 10^5 \sim 8 \times 10^5$), provided

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by the Department of Pharmacology, The Fourth Military Medical University; concanavalin (ConA, Sigma); Methylthiazolyldiphenyl-tetrazolium bromide(MTT, Sigma); dimethyl sulfoxide (DMSO, Tianjin Chemical Reagent Factory); RPMI1640 medium (Gibco). 100 U/ml penicillin, 100 μg/ml streptomycin and 10% of the ultrafiltration of fetal bovine serum (Hangzhou Evergreen biological Engineering Company) should be added when RPMI1640 medium is used.

Animal grouping and treatment

In each experiment, 50 Kunming mice were randomly divided into 5 groups (n = 10), respectively, the normal control group (Normal Control, NC), the radiation control group (Irradiation Control, IC), RTP1 low-dose group (RTP1-L, 200 mg/kg), RTP1 medium-dose group (RTP1-M, 400 mg/kg) and the RTP1 high-dose group (RTP1-H, 800 mg/kg). RTP1 was given to group RTP1-L, RTP1-M, and RTP1-H by gastric gavage (0.1 ml/10g) for 14 d, while the NC and IC groups were given the same amount of saline. On the 14th day, all the mice except that in the NC group were addition to the NC group, the mice received ^{60}Co γ-ray total body irriadiation, with the irradiation distance of 80 cm and with a dose of 2.0 Gy per mouse. Various immunity functions were detected 24 hours after the irradiation.

Weighting of mouse immune organs

The mice were killed in orbital bleeding way 24 hours after ^{60}Coγ ray irradiation. Then we weight these mice with electronic balance before and after exteriorizing thymus and spleen. The thymus and spleen index was calculated used the following formula:

Thymus (spleen) index = weight of thymus (spleen)/ weight of mice × 100%.

Carbon clearance test

30 min after the last gavage in the 14th day, India ink 0.01 ml/g (ink was firstly diluted 10 times with saline) was injected via tail vein. 20 μl blood samples were collected from the eyepit at 1 and 5 min respectively after injection. Then the samples were dissolved in 0.1% $NaCO_2$ and shaked up in 2 ml tube. Measure the absorbance (OD) value at 600 nm, and calculate carbon clearance index K (Chen, 1993):

Clearance index K = log OD1- log OD2/t2-t1

Detect the phagocytic function of macrophages from mouse enterocoelia

The mice were treated by intraperitoneal injection of 0.5 ml 5% (V/V) chicken erythrocytes (CRBC, prepared suspension (Chen, 1993). The mice were sacrificed by cervical dislocation after 1h. Cut the abdominal skin and inject 2ml saline with gentle massage. Draw the abdominal wash fluid, drop on the slides and keep 37 °C incubation for 30 min. Then wash cells with saline rinse and remove the CRBC which has not been swallowed. Dry and fix the cells for 5min in 1:1 acetone methanol. Stain the slides for 3 min 4% Giemsa-weight. Observe these slides with oil immersion lens after air dry. Calculate phagocytic index α according to following fomula (Chen, 1993):

Phagocytic index α= swallowed CRBC / 100 macrophages

ConA-induced lymphocyte proliferation reaction assay / MTT assay

After the mice were sacrificed by cervical dislocation, we remove the spleen under sterile condition and set them in RPMI 1640. After grinding the sample with frosted glass, we get the single cells with 200 mesh stainless filter and wash the cells twice with Hanks solution (1000 r/min.5min). The cell density was adjusted to 3x10^6/ml in two holes of 24-well culture plate with RPMI 1640, 1ml per hole. One hole was added with 75 μl ConA solution, and another hole was set as control. The plate was placed in 5% CO_2, 37 °C incubator for 72 h. At 4h before the end of incubation, we remove 0.7 ml supernatant from each well and add 50 μl MTT and 0.7 ml RPMI 1640 medium without fetal calf serum in each well. Then the cells were cultured for 4 hours. Each hole was added 1ml dimethyl sulfoxide after the end of culture. Make sure that the blue-violet crystals were dissolved completely. The OD value (absorbance) was detected at 570 nm wavelength. The value of Plus ConA hole minus the one without ConA represents the ability of lymphocyte proliferation.

Genesis assay of serum hemolysin

50 mice were grouped and treated as previously. They were sensitized at 1w before irradiation with intraperitoneal injection of 5% chicken erythrocyte suspension, 0.2 ml per mouse (Chen, 1993), once a day for one week. All mice except for the NC group were exposed at ^{60}Coγ ray after 7 d. The eye blood was collected, centrifuged, and diluted with saline to 100 times. Mixture of 1 ml diluted serum, 0.5 ml 5% chicken erythrocyte suspension and 0.5 ml 10% complement (fresh guinea pig serum with saline dilution to 10 times) was placed at 37 °C incubator for 30 min, then to 0 °C refrigerator for 5 min. The OD values of supernatant were detected at 540 nm wavelength by UV spectrophotometer after centrifugation (1500 r/min). Set the value of supernatant from tube without serum as zero. 1.9 NK cells activity assay YAC-1 cells (target cells) were subcultured at 24h before experiment, and the cell density was adjusted to 3 x 10^5/ml with RPMI 1640. Remove the spleen under sterile condition and prepare spleen cells suspension. Wash the cells twice with Hanks and discard the supernatant. Then add 3 ml NH4Cl lysis solution to crack erythrocyte. 10 min later, add 7 ml Hanks solution. Centrifuge the cells with 1000 r/min for 10 min. Adjust the cell density with RPMI 1640 to adjust 2 x 10^7/ml. Take the target cells and spleen cells respectively 100 μl (50:1) into the 96-well culture plate. 100 μl of cell suspension and medium were added into the naturally released well. 100 μl of cell suspension and 1% NP40 were added into the maximum released well. The plate was placed in 37 °C, 5% CO_2 incubator for 4 h and centrifuged. The 100 μl supernatant was placed in 96-well plate. Add 100μl LDH matrix liquid for 6 min. terminate the reaction with 1mol/L HCl. The OD values were measured at 490 nm with ELISA Reader, which represent the activity of NK cells (Chen, 1993).

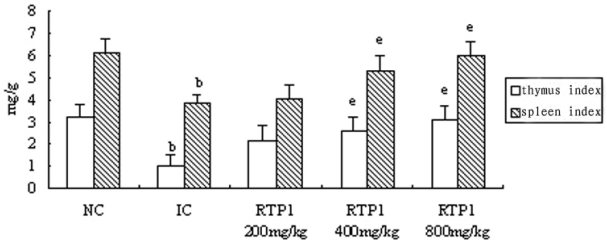

Figure 1. The Effect of RTP1 on the Weight of Immune Organs in Irradiated Mice ($\chi \pm s$, n = 10). $^{b}P < 0.05$ compared with NC group; $^{e}P < 0.05$ compared with the IC group

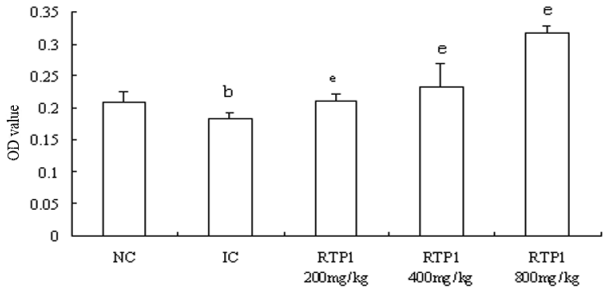

Figure 2. The Effect of RTP1 on ConA-induced Lymphocyte Proliferation In Mice ($\chi \pm s$, n = 10). $^{b}P < 0.05$ compared with NC group; $^{e}P < 0.05$ compared with the IC group

Results

The effect of RTP1 on the weight of immune organs in irradiated mice

The index of thymus and spleen in IC group is lower than NC group ($P < 0.05$). Compared with the IC group, moderate and high dose of RTP1 group can significant increase the index of thymus and spleen in mouse ($P < 0.05$). The results are shown in Figure 1.

The effect of RTP1 on carbon clearance index K and phagocytosis index α

The clearance index K and the phagocytic index α in IC group significantly reduced compared with NC group ($P < 0.05$ or $P < 0.01$). The K value and α value in RTP1 group significantly increase with the increased dose, in which the moderate and high dose groups can completely antagonize the radiation-induced reducing K value ($P < 0.01$). These results in Table 1 show that the RTP1 can promote the non-specific immune function.

The effect of RTP1 on ConA-induced lymphocyte proliferation

The spleen lymphocyte proliferation in IC group was significantly lower than the NC group ($P < 0.05$). Compared with the IC group, the spleen cell proliferation in RTP1 groups was significantly higher than the IC group ($P < 0.05$). The results are shown in Figure 2.

The effect of RTP1 on the hemolysin genesis

The value of hemolysin genesis in IC group is significantly lower than the NC group ($P < 0.01$). The value in small dose of RTP1 group showed no significant difference with IC group. The hemolysin level of the moderate and high dose groups significantly increased compared with IC group ($P < 0.01$). The results are shown in Table 2.

Table 1. The Effect of RTP1 on Carbon Clearance Index K and Phagocytosis Index α in Irradiated Mice (n = 10)

groups	carbon clearance index (K)	phagocytosis index(α)
NC	0.036 ± 0.0107	4.783 ± 0.528
IC	0.023 ± 0.014^{c}	4.302 ± 0.567^{b}
RTP1 200mg/kg	0.028 ± 0.017^{e}	6.731 ± 0.623^{f}
RTP1 400mg/kg	0.046 ± 0.015^{f}	5.724 ± 0.594^{f}
RTP1 800mg/kg	0.059 ± 0.023^{f}	5.119 ± 0.578^{f}

$^{b}P < 0.05$; $^{c}P < 0.01$ compared with NC group; $^{e}P < 0.05$; $^{f}P < 0.01$ compared with the IC group

Table 2. The Effect of RTP1 on the Hemolysin Genesis (n = 10)

grouops	OD value
NC	0.102 ± 0.012
IC	0.051 ± 0.015^{c}
RTP1 200mg/kg	0.063 ± 0.010
RTP1 400mg/kg	0.089 ± 0.007^{f}
RTP1 800mg/kg	0.092 ± 0.013^{f}

$^{b}P < 0.05$; $^{c}P < 0.01$ compared with NC group; $^{e}P < 0.05$; $^{f}P < 0.01$ compared with the IC group

Table 3. The Effect of RTP1 on the NK Cell Activity of Irradiated Mice (n = 10)

groups	NK cell activity(%)
NC	65.37 ±5.52
IC	12.51 ± 2.25^{d}
RTP1 200mg/kg	21.34 ±2.84^{f}
RTP1 400mg/kg	48.19 ±3.70^{f}
RTP1 800mg/kg	68.67 ±4.39^{f}

$^{b}P < 0.05$; $^{c}P < 0.01$ compared with NC group; $^{e}P < 0.05$; $^{f}P < 0.01$ compared with the IC group

The effect of RTP1on the NK cell activity

As shown in Table 3, the radiation can significantly reduce the activity of NK cell, compared with NC group ($P < 0.01$). The RTP1 can promote the activity of mouse NK cells and enhance the lethality of the mouse spleen cells to target cells with the doses from 200 to 800 mg/kg.

Statistical analysis

Experimental data are presented as mean ± standard deviation (s). Comparisons of group means were assessed with t-test of SPSS 10.0.

Discussion

Currently, the radioactive treatment is being increasingly and widely applied, especially in cancer treatment. However, at the same time of killing cancer cells, both the humoral and cellular immune are inhibited when body is exposed to more than moderate doses irradiation. Also because of the impact of immunity, systemic or local toxic reaction will occur (Hu et al., 1988; Lee et al., 1994). Chemical protection (drugs protection) from radiation damage usually refers to the body or a biological system is given a chemical substance before ionizing radiation in order to reduce the radiation damage and promote recovery, but the majority of current anti- radiation drugs have reduced clinical value due to

strong toxicity. So looking for non-toxic or low toxic anti-radiation drugs from natural products become a new hotspot in recent years (Jagetia, 2007; Hosseinimehr, 2007).

A large number of studie shave shown that the polysaccharide is the major immune active substance in a plenty of Chinese herbal medicine, which can be as immune promoters and regulators. A variety of active polysaccharide extracted from plants can influence the humoral immunity, innate and adaptive immunity. Now, the lentinan and polysaccharides of ganoderma lucidum, bidentata, polyporus, huangqi, yunzhi and etc are commonly used in clinical (Tzianabos, 2000; He et al., 2012). The immunologic enhancement of Chinese herbal medicine is realized via the following pathway: (1) activation of the reticuloendothelial and addiment system; (2) activation of macrophages, T and B lymphocytes, NK cells; (3) regulating the activity of immune promoters and inhibitors to enhance immunity of erythrocyte; (4) regulating the neuroendocrine network to play an immunomodulatory role; (5) induced various cytokines (CKs), including interferon (IFN-a, β, γ), interleukin (IL), tumor necrosis factor (TNF-a, β), colony-stimulating factor (CSF) and dozens of species from four series so far (Vincent et al., 2000; Lin, 2005; Xie et al., 2006; Jane et al., 2010; Jiang et al., 2010).

Our experiments show that radiation impairs the innate immune function in mice. The appearances include that carbon clearance index K and the phagocytic index α was significantly reduced compared with the normal control group, the immune organs (thymus and spleen) lose weight, and the response of spleen cells to the ConA reduced. The carbon clearance index and phagocytic coefficient can be a indicator of hagocytosis by the reticuloendothelial system. The results show that the component1 of Rheum tanguticum polysaccharide can increase the radiation-induced carbon clearance index and phagocytic coefficient, meanwhile increase the index of mouse thymus and spleen, and the response of spleen cells to ConA. These results indicate that the RTP1 can promote the innate immune function.

The levels of serum hemolysin and NK cell activity are two important indicators of humoral and cellular immunity, respectively. RTP1 can significantly increase the level of serum hemolysin and the activity of NK cells from mice spleen, suggesting it can antagonize the radiation-induced deficiency in humoral and cellular immunity, in line withthe literature (Vincent et al., 2000; Xie et al., 2006; Jiang et al., 2010), but it is still to be further studied that whether the immune enhancement is due to the presentation of MHC II molecules and direct or indirect identify of αβ-TCR, which leads to the activation of the adaptive cellular immune response or the generation of specific antibodies through the effective activation of B lymphocytes.

Acknowledgements

This study is supported by National Natural Science Foundation-funded project (81073135).

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