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Aloe Polysaccharides Mediated Radioprotective Effect through the Inhibition of Apoptosis

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Aloe polysaccharides/Radioprotective effects/Apoptosis.

Polysaccharides from aloe are always considered an effective radioprotector on irradiation-induced skin damage. The aim of this study was to determine if aloe polysaccharides (AP) have radioprotective effects on normal human cells in vitro and mouse survival in vivo and to explore the mechanism. Pretreatment with 50 μg/ml AP could improve the surviving fraction at 2 Gy (SF_2) of three normal cell lines 293, ECV304, and C. liver from 41.5%, 46.5%, and 40.9% to 49.4%, 72.1%, and 89.1%, respectively. AP could also reduce the apoptotic rate of C. liver cells from 9.5% and 43.0% to 2.2% and 10.9% 48 h and 72 h after 2 Gy irradiation, respectively. Western blot analysis showed that pretreatment with AP could block the upregulation of pro-apoptotic p53, Bax, and Bad and the downregulation of Bcl-2 by irradiation. AP could lower thymocyte apoptosis of mice in vivo after 6 Gy irradiation and abrogate the cell cycle perturbation. Fifty mg/kg of AP treatment for 30 min before 7.5 Gy irradiation provided the best radioprotective effect and improved the 30-day survival rate of mice to 86.0%, from 10.0%. AP exerted radioprotective effects in vitro and in vivo through an inhibition of apoptosis.

INTRODUCTION

Radiation is increasingly used for medical and occupational purposes and is an established weapon in the diagnosis and therapy of cancer. The amounts of ionizing radiation that can be given to treat malignant tumors are often limited by the toxicity to surrounding normal tissues and organs. Therefore the availability of agents that can preferentially protect normal tissue from the damaging effects of radiation will allow to increase doses and may improve the outcome of radiation therapy[1)]. At present, most chemicals that are tested for radioprotective activity are sulphydryl compounds. Among them, WR-2721, s-2-(3-aminopropylamino) ethylphosphoro- thioic acid, is the only one that has undergone phase I and II clinical trials[2–4)].

In traditional Chinese medicine, aloe is commonly used to treat constipation, irritability, epilepsy, convulsion, twitch, indigestion, and verminosis. At present, it is also a popular supplement in health foods for treating obesity, hyperlipaemia, and acne[1)]. Furthermore, aloe has been traditionally used worldwide as a folk remedy for various diseases because of its multiple biological activities in wound healing, antigastric ulcers, and antiinflammation[5)].

Some plant polysaccharides are well known to protect against deleterious effects of radiation. Irradiated animals treated with polysaccharides display improved haematopoietic recovery with increased bone-marrow cellularity, circulating granulocytes, lymphocytes and platelets similar to that seen in animals receiving syngeneic bone marrow transplantation[6)]. Aloe is a rich source of polysaccharides. A wound dressing gel that contains acemannan extracted from aloe leaves can reduce acute radiation-induced skin reactions in C3H mice[7)], though it was not thought that aloe vera gel protects against radiation therapy-induced dermatitis[8)]. Aloe polysaccharides (AP) was also found to have immunoregulatory activity, which may play an important role in radioprotection[9)]. Our previous studies demonstrated that AP could evidently inhibit the tumor growing in mouse[4)]. In the present study, we report that it exhibited radioprotective activity in vivo and in vitro. A mechanistic study showed that AP significantly inhibited the apoptosis of normal cell lines in vitro and thymocytes in vivo by blocking the upregulation of pro-apoptotic p53, Bax, and Bad and the downregulation of anti-apoptotic Bcl-2 after irradiation. Our findings present the first evidence that AP mediated

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radioprotection through an inhibition of apoptosis.

MATERIALS AND METHODS

Preparation of AP

AP was prepared from the gel of aloe vera L. var. *chinensis* (Haw.), according to the method that we had described previously[1]. In brief, the fresh, colorless gel fillet was separated from the green rind, then incubated with cellulase for 1 h and terminated when the mixture was heated. The mixture was then dealt with charcoal absorbing, extensive water dialysis, and 80% ethnol precipitating successively. The precipitate was dissolved in distilled water, and the solution was superfiltrated with molecular filters 10 kD and 50 kD in diameter. So three extract sections were obtained. Considering the pre-experimental results, in the present study we used a test drug of 10 kD–50 kD molecular weight. The stock solution that contained 2 mg AP in 1 ml medium was filtered by a microdiameter filter to keep it sterilized, then stored at 4°C.

Cell cultures and reagents

The normal human cell line Chang liver (C. liver) was purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). Two other normal human cell lines, 293 (embryo kidney) and ECV304 (umbilicus vein endothelium), were purchased from Type Culture Center at Wuhan University (Wuhan, China). All cells were cultured by the use of RPMI-1640 medium (Life Technologies, Inc.) supplemented with fetal bovine serum (10%), penicillin (100 units/ml), and streptomycin (100 μg/ml). The cells were routinely incubated at 37°C in a humidified atmosphere containing 5% CO2. All chemicals used in this study were obtained from Sigma Co., and all the antibodies were purchased from Santa Cruz.

Animals

Specific pathogen-free 4-week-old NIH mice (body weight 18–22 g) were purchased from the Medical Animal Center of Guangdong Province, China. They were kept in a clean conventional environment at 24 ± 1°C and 60 ± 10% relative humidity and given nutritional chow and water adlibitum. The mice were maintained on a 7 a.m. to 7 p.m. light-dark cycle and acclimatized to laboratory conditions for 1 week before use. The study was conducted according to the Guidelines for Animal Welfare and Experimentation issued by Sun Yat-sen University.

MTT assay

The logarithmically growing cells were plated at a density of 2×10^4 cells/well in a 96-well plate. The stock of AP was diluted with medium and added to the wells for the desired final concentrations. After 72 h, the cell viability in each well was measured by MTT assay as described previously[2,3]. Briefly, 10 μl of 5 mg/ml MTT was added into each well and incubated for an additional 4 h followed by 200 μl of DMSO being added to dissolve the formazan. The cell growth inhibition was obtained from the absorbance in the microplate reader 550 model with 570 nm wavelength. The IC_{50} values were determined by the use of POMS software.

Radiation exposure

For X-ray in vitro exposures, we delivered irradiation at room temperature with a 210 kV X-ray generator (Dongfanghong F34-1, Beijing, China) that had a total filtration of 0.25 mm Cu. The X-ray dose-rate measured at the same position was about 108.75 cGy/min. Field size (10×15 cm^2) and source-surface distance (40 cm) were constant for all experiments. The cells were seeded into six-well dishes at an initial density of 1.5×10^3 –2×10^3 cells/well. After treatment in the presence or absence of AP for 3 h, the cells were irradiated with X-ray. AP was released, and instead of fresh culture medium after exposure to AP for 24h. After 7–10 days, colonies larger than 50 cells were coded and scored by two people working independently. The data presented were the averages derived from a minimum of three independent experiments, each performed in triplicate.

For in vivo treatments, the whole-body animals were irradiated with a ^{60}Co γ-ray teletherapy unit (PHOENEX) at a dose rate of 1.02–1.04 Gy/min. All groups were consistent with the same field size (25×25 cm^2) and source-surface distance (80 cm). After irradiation, all mice were assessed daily for survival for 30 days.

All the above equipment was at the Department of Radiotherapy, Cancer Center, Sun Yat-sen University, Guangzhou, China.

Western blot analysis

Lysates were prepared from 1×10^6 cells by dissolving cell pellets in 100 μl of lysis buffer (20 mM Na_2PO_4 (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% aprotinin, 1 mM phenymethysulfonyl fluoride, 10 mg/ml leupeptin, 100 mM NaF, and 2 mM Na_3VO_4). Lysates were centrifuged at 12,000 rpm for 10 min. The supernatant was collected. The protein content was determined by use of the Bio-Rad protein assay (Bio-Rad laboratories, Hercules CA). SDS-PAGE sample buffer (10 mM Tris-HCl, pH 6.8, 2% SDS, 10% Glycerol, 0.2 M DTT) was added to the lysates, which were heated to 100°C for 5 min, and 40 μg of protein was loaded into each well of a 10% SDS-PAGE gel. The resolved proteins were electrophoretically transferred to nitrocellulose and incubated sequentially with primary antibody and secondary antibody. The immunoreactive bands were revealed by ECL chemiluminescence detecting reagent and exposure of X-ray films.

Flow cytometry analysis

The method of flow cytometry analysis was according to the previous description and modified[2,3]. After AP and/or irradiation treatment and cultivation for the indicated time, the cells were harvested by trypsinization, washed twice with ice-cold PBS, resuspended in cold PBS, and fixed with 70% ethnol. After refrigeration at –20°C overnight and subsequent rehydration in PBS for 30 min at 4°C, the cell nuclei were stained for 30 min in darkness with 50 μg/ml propidium iodide (Sigma) containing 125 units/ml protease-free Rnase. The cells were filtered trough 95 μ pore nylon mesh, and a total of 12,000 stained nuclei were analyzed with a FACScan flow cytometer (Becton Dickinson, San Jose, CA) in combination with BD Lysis II software.

As for the in vivo thymocytes test, single mouse thymocytes were harvested according to the reference[4]. Briefly, thymus was separated from mouse after it was sacrificed, then washed in PBS. Single cells were obtained by thymus ground and filtered with 200-pore filtration. The concentration of cells was adjusted to 1×10^7/ml and washed twice with ice-cold PBS, then resuspended in cold PBS and fixed with 70% ethnol for flow cytometry analysis. The survival rate was determined by trypan blue.

Statistical analysis

The data were expressed as mean ± S.D. The mean and SD of mice or cell survival fractions and cell cycle distributions were calculated with reference to untreated controls defined as 1.0 or in a percentage scale, respectively. The tatistical difference was assessed by analysis of Student's t-test. A comparison of ANOVA was used to determine significant differences in multiple comparison. The *x*-square test was employed to assess the statistical significance of the survival rate of mice. The values of $p<0.05$ were considered significant.

RESULTS

Effect of AP on growth of normal cell lines

After 72 h of AP addition alone, the result of MTT analysis demonstrated that the concentrations of AP inhibiting 50% of cell growth (IC_{50}) on 293, ECV304, and C. liver cell lines were 1231.6, 1782.4, and 891.8 μg/ml, respectively (Fig. 1). In the next radioprotective experiments, AP was

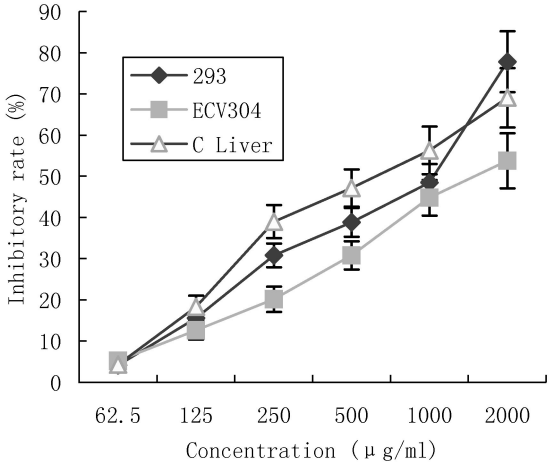

Fig. 1. The cytotoxity of AP on normal cells lines, 293, ECV304, and C. liver. Cells were treated with various concentrations of AP as indicated for 3 days. Cell viability was determined with MTT assay and shown as inhibitory rate.

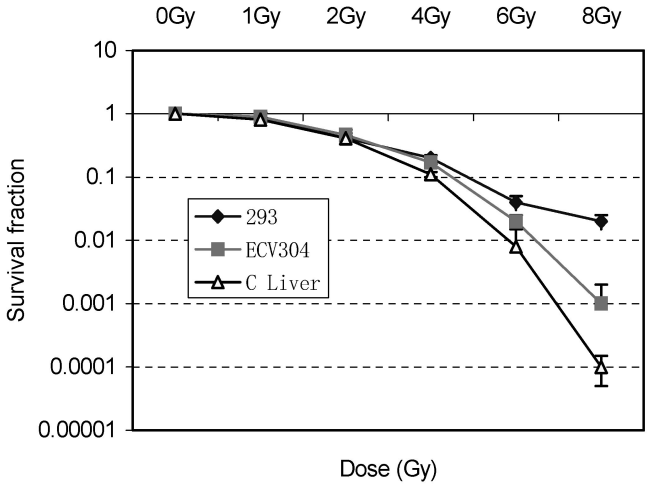

Fig. 2. Cell survival of three normal cell lines, 293, ECV304, and C. liver as determined by the clonogenic assay after different dosage of X-ray irradiation.

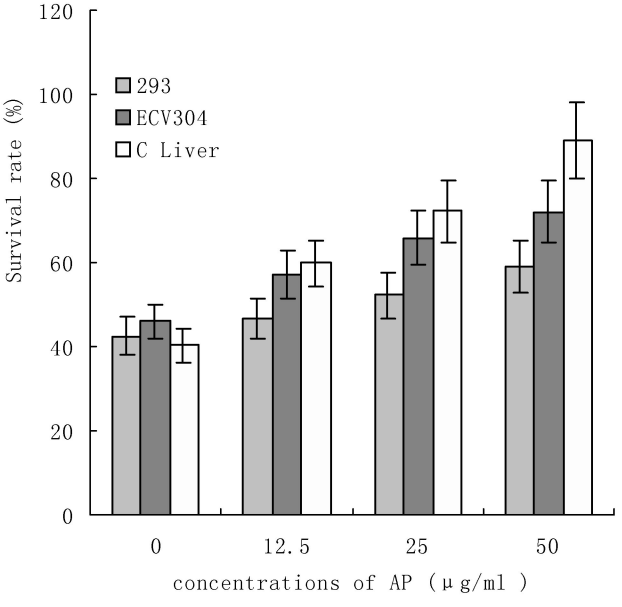

Fig. 3. Comparison of the dose-effct curves for AP plus 2 Gy irradiation in three normal cell lines, 293, ECV304, and C. liver. All three lines showed a radioprotective effect upon AP exposure. Data points show the mean ± SD of three independent experiments.

used at concentrations of 10–50 μg/ml. It indicated that the inhibitory effect of AP on normal cell growth was weak.

Radioprotective effect of AP on the survival of normal cell lines

The intrinsic radiosensitivity of the three normal cell lines was determined by the colony-forming assay. As shown in Fig. 2, C. liver cells were the most sensitive line with a survival fraction at 2 Gy (SF_2) of 40.9%. Meanwhile, 293 cells were the lowest in sensitivity to radiation in three cell lines.

To investigate whether AP in a concentration that does not affect cell survival to a significant degree can modulate the radiation response of these normal cell lines after single-dose exposure, we pretreated the cells with 12.5, 25, and 50 μg/ml of AP 3 h before 2 Gy X-ray irradiation. As Fig. 3 shows, 7–10 days after irradiation pretreatment with AP led to a shift of the dose-response curves, which can be interpreted as radioprotection. At the concentration of 50 μg/ml, SF_2 of cell lines 293, ECV304, and C. liver increased to 49.4%, 72.1%, and 89.1%, from 41.5%, 46.5%, and 40.9%, respectively.

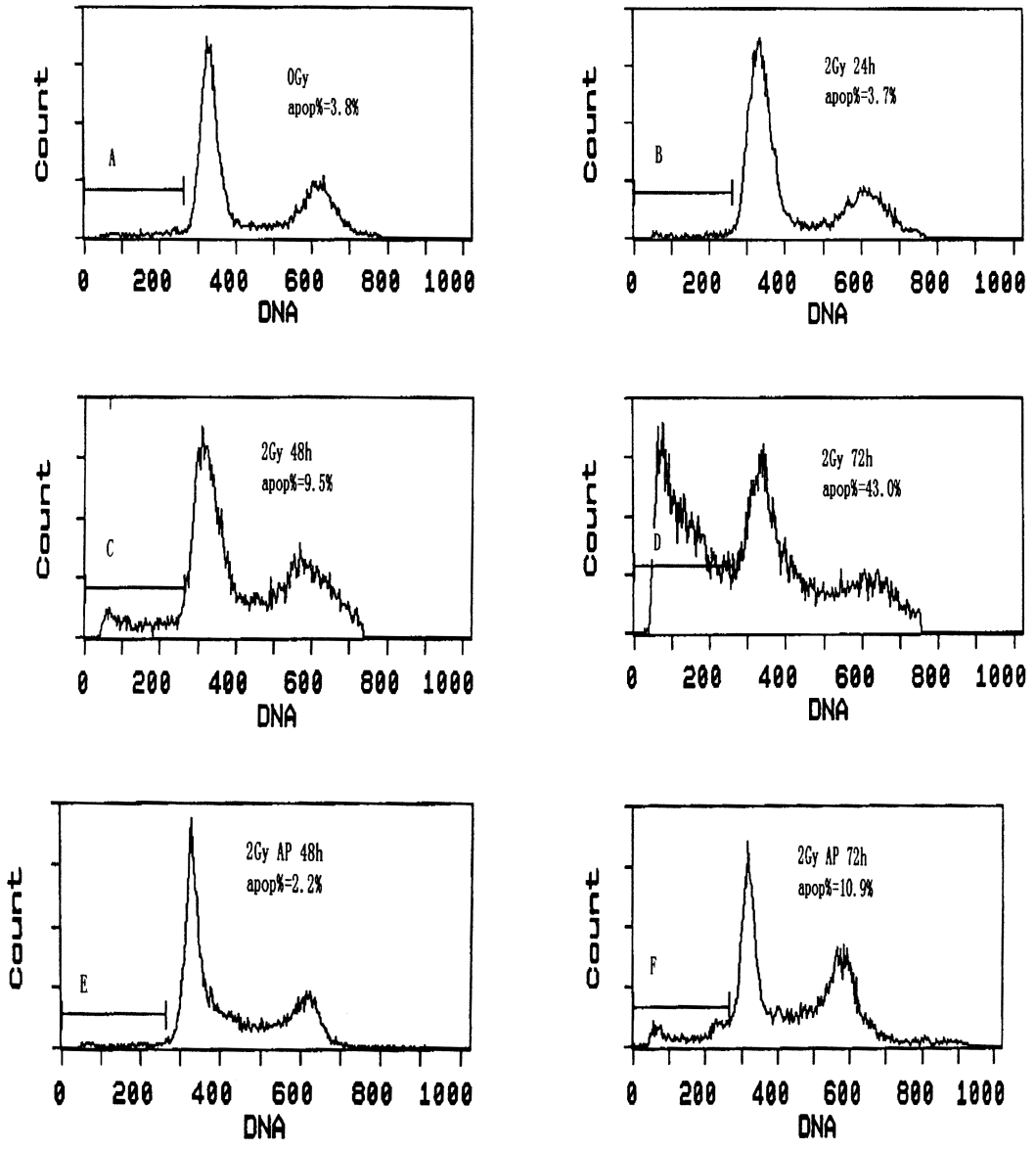

Fig. 4. Flow-cytometric analysis of C. liver cells following X-irradiation and pre-treatment with AP (50 μg/ml) before irradiation. Cellular DNA was stained with propidium iodide. The first peak represents the fraction of cells in G_1, and the second represents G_2. Pretreatment with AP (50 μg/ml) results a decrease in the pre-G1 apoptotic peak increased by X-irradiation. Similar results were found for two other independent experiments we investigated. A: normal control; B: 24 h after irradiation alone; C: 48 h after irradiation alone; D: 72 h after irradiation alone; E: 48 h treatment with irradiation and AP; F: 72 h treatment with irradiation and AP.

The effect of AP on the apoptosis of C. liver cell line and thymocytes

To assess the effect of AP on the extent of irradiation-induced apoptosis, we analyzed the appearance of the sub-G1 population by use of flow cytometry. As indicated in Fig. 4, irradiation resulted in a significant apoptotic peak in C. liver cells 48 h and 72 h after X-ray irradiation. Pretreatment of AP could significantly reverse this situation and significantly reduce the apoptotic rate from 9.5% and 43.0% to 2.2% and 10.9%, 48 h and 72 h after 2 Gy irradiation, respectively (Fig. 4).

First, mice were treated with 6 Gy ^{60}Co γ-ray irradiation alone or pretreated with 50 μg/ml AP (*i.p*) 30 min before irradiation. Then at 2 h, 4 h and 8 h after irradiation, the single thymocytes were harvested from animals. It is amazing that the result is very similar to that of the cell lines mentioned above. As shown in Fig. 5, from 2 h to 8 h after 6 Gy irradiation the apoptosis rate of thymocytes abruptly ascended to 37.8%, from 20.5%, and reached peak value (49.6%). Afterward, it gradually descended and arrived to the normal level of 48 h after irradiation (data not shown). On the other hand, pretreatment with AP could evidently lower the apoptotic rate to 17.7%, 32.8%, and 31.7% 2 h, 4 h, and 8 h after 6 Gy irradiation, respectively.

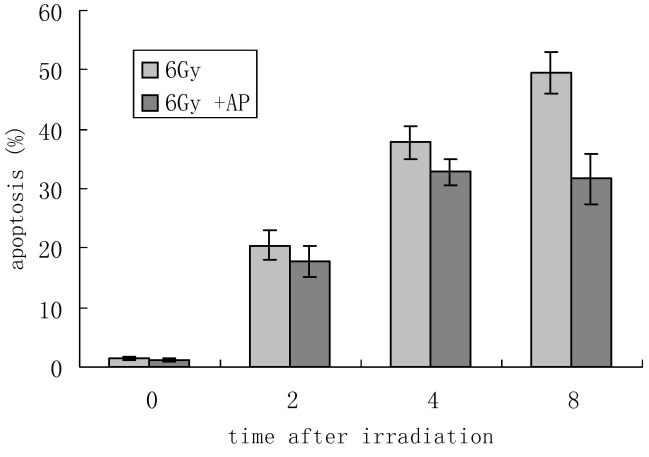

Fig. 5. Effect of AP on the apoptosis in mouse thymocytes. Animals were treated with 6 Gy ^{60}Co γ-ray irradiation alone or pretreated with 50 μg/ml AP (i.p.) 30 min before irradiation. At 2 h, 4 h, 8 h, 12 h, and 24 h after irradiation the single mouse thymocytes were harvested. The cell concentration was adjusted to 1×10^7/ml and washed twice with ice-cold PBS, then resuspended in cold PBS and fixed with 70% ethnol for a flow cytometry assay. The data show the mean ± SD of five independent animals.

^{60}Co γ-ray irradiation in 6 Gy dose also resulted in the cell cycle turbulence for in vivo mouse thymocytes that mainly manifested as the G2/M phase and S phase population accumulation, but the G0/G1 phase population decreased especially at 4 h and 8 h after irradiation. Pretreatment with AP could abrogate the cell cycle turbulence to a certain extent (Table 1).

The effect of irradiation and pretreatment of AP on the expression of the pro- and anti-apoptotic proteins

To examine the role of AP in inhibiting in vivo and in vitro apoptosis after irradiation, we investigated whether it would alter the expression of the pro- and anti-apoptotic proteins in C. liver cells. The cells were exposed in 2 Gy X-ray irradiation or pretreatment with 50 μg/ml of AP. At 24 h and 48 h postirradiation, the cells were harvested and the levels of protein expression were analyzed by Western blot. As shown in Fig. 6, we observed that the basal level of Bcl-2, an anti-apoptotic protein, was high and irradiation evidently could not change it, not only within 24 h, but also within 48 h after irradiation. However, pretreatment with AP could abruptly induce upregulation of Bcl-2 48 h after irradiation. For pro-apoptotic p53, Bax and Bad, only very low or even zero basal levels were found in control cells. Irradiation induced their remarkable overexpression. Pretreatment with

Table 1. Cell cycle distribution in mouse thymocytes after exposure to irradiation alone or pretreatment with AP[a].

Group	Cell cycle								
	2 h after irradiation			4 h after irradiation			8 h after irradiation		
	G_0/G_1(%)	S(%)	G_2/M(%)	G_0/G_1(%)	S(%)	G_2/M(%)	G_0/G_1(%)	S(%)	G_2/M(%)
0Gy	92.5±6.9	4.1±0.4	3.4±0.4	83.2±6.8	6.3±0.7	10.5±2.3	85.1±12.5	12.1±2.4	2.8±0.3
4Gy	77.2±4.6	15.9±2.7	7.0±1.3$^{\#}$	69.4±4.5	12.7±1.2	17.8±1.5$^{\#}$	77.0±8.7	6.7±1.1	16.3±0.8$^{\#}$
4Gy+AP	79.2±3.5	14.8±1.9	5.9±0.6	82.7±7.5*	7.0±1.3*	10.3±1.8*	82.3±6.9	15.9±1.4**	1.8±0.4**
6Gy	69.0±3.6	25.1±4.2	5.8±0.3	56.8±4.6	31.2±5.4	12.0±0.2	66.8±6.2	29.7±2.3	3.5±0.3
6Gy+AP	75.7±3.9	19.0±3.0	5.2±0.9	70.7±7.2*	20.3±2.4*	9.0±1.1*	72.1±7.4	26.1±1.7	1.8±0.3*

[a]Animals were treated with 4 or 6 Gy ^{60}Co γ-ray irradiation alone or pretreated with 50 μg/ml AP (*i.p*) 30 min before irradiation. Then at 2 h, 4 h, and 8 h after irradiation, the single mouse thymocytes were harvested. The cell concentration was adjusted to 1×10^7/ml and washed twice with ice-cold PBS, then resuspended in cold PBS and fixed with 70% ethnol. The data show the mean ± SD of five independent animals. Compared with normal control group, $^{\#}P < 0.05$; compared with group that irradiated alone, $P < 0.05$,$^{**}P < 0.01$

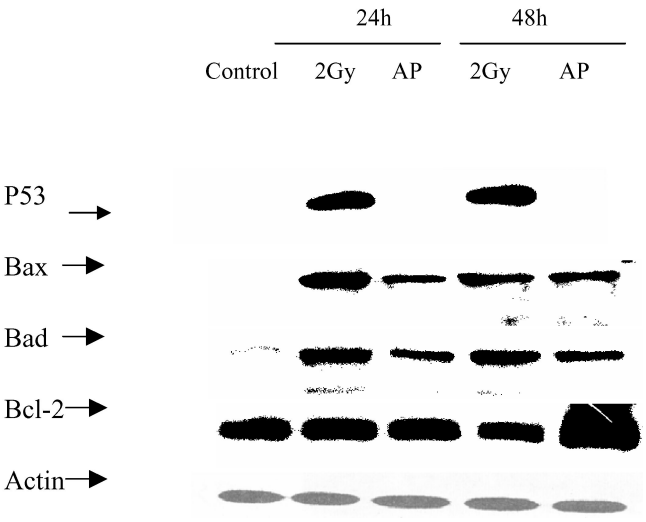

Fig. 6. Western blot analysis of pro- and anti- apoptotic proteins in C. liver cell lines after X-ray irradiation alone or pretreatment with 50 μg/ml AP. Protein extracts of irradiated cells were isolated 24 h and 48 h postradiation exposure. Eighty micrograms of total cellular proteins were analyzed by Western blot analysis. Actin was used as an internal control to ensure an equal loading of each lane. This figure is representative of three independent experiments.

AP could sharply block the upregulation of p53, Bax and Bad, by irradiation. All loading was normalized by β-actin. Taken together, these data suggest that AP causes a reduction in the cellular levels of the pro-apoptotic proteins and an increase in anti-apoptotic proteins (seen in Fig. 6).

The radioprotective effect of AP on mouse survival after irradiation

The studies above have shown that AP has a radioprotective effect on normal cell lines in vitro and thymocytes from irradiation-treated mice. Therefore it is interesting to investigate its action on mice survival after exposure to γ-ray irradiation at a dose of 7.5 Gy. As shown in Tables 2 and 3, treatment with various doses of AP (12.5, 25, 50, and 100 mg/kg body weight, i.p. 30 min before irradiation) could substantially increase the 30-day survival rate of mice from 10.0% in animals irradiated alone to 30.0%, 60.0%, 86.0% and 72.0%, respectively. It is obvious that 50 mg/kg AP provided the best protection. To demonstrate the time-effect relation, the mice were treated i.p. with AP at 30, 60, and 90 min before irradiation, respectively. It could be shown that at the doses of 25 and 50 mg/kg body weight, 30 min before irradiation was the optimum time for the radioprotective effect of AP (Table 3).

Table 2. The effect of AP on the mouse survival 30-day postirradiation[a].

Group	Drug dosage (mg/kg BW)	Survival (%)
Control	–	100
Irradiation only	–	10
AP treated+Irradiation	12.5	30.0*
AP treated+Irradiation	25	60.0*
AP treated+Irradiation	50	86.0**
AP treated+Irradiation	100	72**

[a] Forty mice were used in each group, and the dose of γ-ray irradiation was 7.5 Gy. The data presented are averages derived from independent triplicate experiment. One asterisk and two asterisks indicate $P < 0.01$ and $P < 0.001$, respectively, as compared with the control group (*x*-square test).

Table 3. Pretreated time required for radioprotective effect of AP[a].

Group	Drug dosage (mg/kg BW)	Drug exposure time (min)	Survival (%)
Control	–		100
Irradiation only	–		16.6
AP treated + Irradiation	25	30 min before irradiation	65.0**
AP treated + Irradiation	25	60 min before irradiation	50.0*
AP treated + Irradiation	25	90 min before irradiation	60.0**
AP treated + Irradiation	50	30 min before irradiation	87.5**
AP treated + Irradiation	50	60 min before irradiation	80.0**
AP treated + Irradiation	50	90 min before irradiation	82.3**

[a] Forty mice were used in each group, and the dose of γ-ray irradiation was 7.5 Gy. The data presented are averages derived from an independent triplicate experiment. One asterisk and two asterisks indicate $P < 0.01$ and $P < 0.001$, respectively, as compared with the control group (*x*-square test).

DISCUSSION

Several studies have discovered that aloe vera gel components could prevent UV or γ-ray radiation-induced skin reactions not only in mice, but also in humans. Acemannan and other modified polysaccharides are mainly responsible for this action by preventing the suppression of contact hypersensitivity or immune suppression induced by irradiation[7,8]. In our laboratory, we extracted and purified Aloe polysaccharides (AP) from a variety of aloe vera and aloe vera L. var. *chinensis*, which grows mainly in China. Although pretreatment with AP caused a transient increase in WBC and platelet counts compared with that irradiated alone[1], these results seem insufficient to explain the significant radioprotective effect of AP. To elucidate its mechanism, we designed and developed the present study.

Apoptosis is critical in many physiological contexts, including embryogenesis, immune cell maturation and response, and tissue homeostasis, and in the celluar response to injury[9]. In pathological states, excessive apoptosis may contribute to organ injury[10,11]. Thus a selective preservation of cells through a modulation of the apoptotic process promises to influence the treatment of many diseases. Studies have indicated that apoptosis plays an important role in the process of radiation-induced mouse thymus lymphocytes and bone marrow cell damage and repair[12,13]. In this study, irradiation induced observable apoptosis in normal cell lines, 293, ECV304, and C. liver in vitro and in mouse thymocytes in vivo. Pretreatment with AP evidently reduced the apoptosis and halted the cell cycle. Simutaneously, not only the surviving fraction in C. liver, but also the 30-day survival rate significantly improved following the pretreatment of AP. On the other hand, from 7 days after irradiation we have clearly observed by the naked eye that mouse thymus condensed abruptly (data not shown). The irradiated animal death may be mainly attributed to immune organic pathological changing and immune suppression. Taken together, it can be concluded that, at least partly, the radioprotective effect of AP was attributed to inhibiting the apoptosis following the alleviating of a thymus organ injury. This function of AP is similar with such immunomodulators as S-TDCM and AM5[14–16]. It needs advanced experiments to verify its further mechanism and whether other organs, especially in the immune system, such as the spleen, have similar alterations.

Cell fate is regulated by a complex balance in a signal transduction pathway between such apoptotic factors as p53, Bax, and Bad, such antiapoptotic factors as the Bcl-2 family, and the inhibitors of apoptosis protein (IAP)[17]. The accumulation of p53 and Bax while Bcl-2 decreases following high-dose whole-body irradiation of mice has been shown to be associated with apoptosis in the spleen[13,18]. Our present study results showed that irradiation caused p53 overexpression in C. liver. Irradiation- induced DNA damage activates p53 and leads to the regulation of the downstream pathway that controls cell cycle progression and apoptosis[19]. It has been known that C. liver is a p53-proficient cell line[2]. Pretreatment with AP obviously suppressed the p53 upregulation by irradiation. Meanwhile, AP reversed irradiation-induced G_2/M block and increased G_0/G_1 phase cell proportion not only in C. liver, but also in mouse thymocytes.

From these results it can be concluded that AP-induced cytoprotection is mediated via the pathway that is dependent on p53 protein and with subsequent cell cycle changing. This effect is consistent with two other radioprotectors, amifostine and nitric oxide[20,21].

The Bcl-2 family is the key regulator of apoptosis. Wild-type p53 is known to be an upstream regulator of the Bax and Bad gene that contain p53 binding sites and they can be directly activated by wild-type p53[22]. Another member of the Bcl-2 family, Bcl-2 protein, is an antagonist of apoptosis[23,24]. AP blocked the p53 upregulation and activation of Bax and Bad. Simutaneously, AP induced Bcl-2 overexpression 48 h after irradiation, the right time AP elaborated its strongest effect in an inhibition of apoptosis. As shown in Figs. 2 and 3, AP exhibited its best radioprotective effect on C. liver cells, the most radiosensitive line. This may be why the activation of the p53 pathway obviously plays a role in radiation-mediated apoptosis in C. liver cells, but the pretreatment of AP can block p53 upregulation by radiation; thus AP elicited the best radioprotective effect on C. liver cells.

We have demonstrated that the naturally occurring plant polysaccharides, AP, exerted radioprotective effect in vivo and in vitro. This function has contributed to its inhibition of pro-apoptotic protein expression and overexpression in anti-apoptotic proteins with a subsequent inhibition of apoptosis and cell cycle perturbation. Our other processing research showed that AP exerted a protective effect from toxicity on normal cells induced by radiotherapy to a much greater extent than on tumor cells, and this effect is connected with the p53 status of the cells. Not only from a mechanistic point, but also from a clinical point we find it very interesting that AP acts as a radioprotector. Therefore our results suggest that the adjuvant therapy of cancer patients with AP may improve patients suffering from toxic therapeutic regimens and provide an alleviation of the symptoms resulting from radiation-induced normal organ injuries.

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