

Radioprotective Effects of *Cordyceps sinensis* Extracts on γ -Irradiated Mice

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- Abstract -

Effect of single intraperitoneal administration of *Cordyceps sinensis* (Cs) extract at 24 hour before whole-body γ -irradiation on the survival ratio, body weight, organ weight changes and serum metabolites in the irradiated mice were investigated. The single pre-administration of Cs extract increased the 40-day survival ratio of irradiated mice from 66.7% to 83.4%. The administration of Cs extract completely prevented weight reductions of spleen and thymus produced by γ -irradiation ($P < 0.05$, $P < 0.01$).

Similar but somewhat less radioprotective effect was also found in the testis of the Cs treated mice. The administration of Cs inhibited the serum hyperglycemia produced by irradiation on the day 7th ($P < 0.01$). However, it did not influence the serum cholesterol and protein levels on the days examined. The present study is the first report regarding Cs which was tested and found to be radioprotective.

Introduction

Cordyceps sinensis (Cs) is a parasitic fungus whose host is a larva of Lepidoptera. The fruit bodies of *Cordyceps sinensis* have been used as the traditional medicine in China¹⁾. Various effects of *Cordyceps sinensis* on animals *in vitro* and *in vivo* have been reported. These include inotropic effects on the left atrium²⁾ and a cure of tuberculosis and a restorative action after diseases³⁾. Recent studies have revealed that *Cordyceps sinensis* has the antitumor activity^{4,5)} and the erythropoietic action⁶⁾. However, effects of mycelial extracts of cultured *Cordyceps sinensis* remain unknown.

Ionizing radiation exerts the bulk of its toxic effects through the generation of oxygen-derived free radicals, in particular the hydroxyl radical^{7,8)}. Such free radicals also occur during aerobic metabolism as a byproduct⁹⁾. Therefore, the development of effective free radical scavenging compounds is important issue from the standpoint of radiobiology and gerontology.

A number of studies on the radioprotective compounds such as thiol complex, interleukin - 1, tumor necrosis factor and granulocyte colony-stimulating factor were carried

out¹⁰⁻¹³⁾. However, they are not actually useful because of their additional toxic effect^{12,14)}. Recently, study on the protective effects of natural products such as herbs against irradiation has become of general interest^{15,16)}.

Since little or no information is available regarding the effect of *Cordyceps sinensis* extract on mice exposed to γ -irradiation we attempted to look at the effect of *Cordyceps sinensis* extract on the survival ratio, body weight and organ weight change and serum metabolites after whole-body γ -irradiation.

Materials and Methods

Chemicals

All chemicals used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.)

Cordyceps sinensis Extract

The cultured mycelium of *Cordyceps sinensis* were provided by Dr. Wang, Institute of Insect mycology,

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Table 1. Effect of *Cordyceps sinensis* extract on 40-day survival ratio of mice after irradiation.

Group	Number of survived mice	40-day survival ratio (%)	significance against control
Control	10 of 10	100	----
Rad	20 of 30	66.7	P<0.01
Rad + Cs	25 of 30	83.4	P<0.05

Rad, irradiated group; Rad + Cs, *Cordyceps sinensis* treated group before γ -irradiation.

Anhui Agricultural University, China. The mycellium of *Cordyceps sinensis* was extracted with water at 95°C for 8 hrs. The extract was filtrated through a membrane filter (0.22 μ m) and lyophilized.

Treatment of Animals

ICR mice, 2 - weeks old male, were obtained from Green Cross Co, Ltd (Korea). They were housed in cages in a room at 23 \pm 2°C and a humidity of 60~70%. They were maintained on a 12 hr - light/12 hr - dark cycle, with the light phase beginning at 09:00 hr.

They had free access to a commercial mouse diet and water until they reached approx. 28 g body weight. The mice were then divided into three groups: group 1 received intraperitoneally 0.5 ml saline solution (0.9 % NaCl) only; group 2 received 0.5ml saline solution at 24 hr before whole - body irradiation with a ⁶⁰Co γ - ray source to 5.0 Gy in a homogeneous field of rays (dose rate : 2.5 Gy/min); group 3 received 0.5ml saline solutions containing *Cordyceps sinensis* extract (100mg/kg body weight) at 24 hr before irradiation under same condition to group 2. Animals were killed by decapitation and the blood was drawn into plastic tube containing EDTA acting as an anticoagulant.

The collected serum which was obtained by centrifuging the blood at 800 \times g for 10min was immediately analyzed.

Determination of Metabolites

The concentration of glucose was determined by Falis¹⁷⁾, cholesterol by Abell et al.¹⁸⁾ and protein by Lowry et al.¹⁹⁾.

Statistical Analysis

Analysis of variance was conducted according to

Snedecor and Cochran²⁰⁾ and treatment differences were subjected to the Student's Newman - Keuls multiple range test as outlined by Kirk²¹⁾.

Results and Discussion

The effect of the single administration of *Cordyceps sinensis* (Cs) extract prior to γ -irradiation on body weight change in mice was shown in Fig. 1. The growth of 3 weeks old male mice in the irradiated group was slightly retarded as compared to those of control mice and to mice treated with Cs extract prior to γ - irradiation.

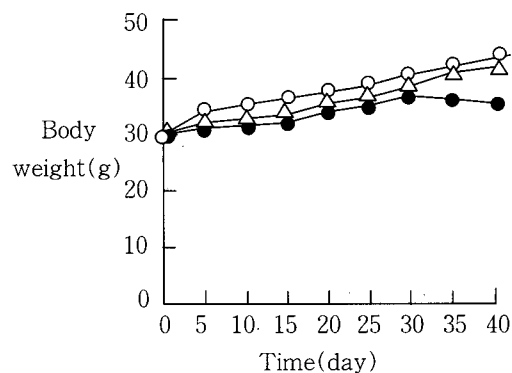


Fig. 1. Effect of the intraperitoneal administration of *Cordyceps sinensis* extract prior to γ -irradiation on body weight change in mice.

Administration of saline and *Cordyceps sinensis* extract and γ -irradiation were described in Materials and Methods.

○, control group ; ●, irradiated group
 △, *Cordyceps sinensis* extract treated group before γ - irradiation.

The effect of Cs extract on 40-day survival ratio of mice after irradiation is shown in Table 1. The single pre-administration of Cs extract increased the 40 - day

Table 2. Effect of *Cordyceps sinensis* extract on organ weight of mice after irradiation *

Tissues	Control	Rad	Rad + Cs
	g		
Heart	0.17±0.02 ^a	0.16±0.02 ^a	0.16±0.02 ^a
Kidney	0.71±0.03 ^a	0.65±0.04 ^a	0.63±0.03 ^a
Liver	1.62±0.16 ^a	1.55±0.17 ^a	1.62±0.18 ^a
Spleen	0.13±0.01 ^a	0.09±0.01 ^b	0.14±0.01 ^a
Testis	0.35±0.02 ^A	0.09±0.01 ^C	0.15±0.01 ^B
Thymus	0.06±0.01 ^A	0.03±0.00 ^B	0.07±0.01 ^A

*Values are means ± S.D. for 10 animals sacrificed on day 40th.

Means not sharing a common superscript letter within a row are significantly different (^{A,B,C} P<0.01 or ^{a,b,c} P<0.05).

Rad, irradiated group; Rad + Cs, *Cordyceps sinensis* treated group before γ - irradiation.

survival ratio of irradiated mice from 66.7% to 83.4%.

The average spleen and thymus weights of the irradiated mice were lower than those of control mice. The decreases of both organ weights in irradiated mice may be due to the marked reduction of lymphocytes which are mainly produced in these organs²²). In contrast, the spleen and thymus weights of the Cs extract treated mice were almost same as those of control mice. The result is a good agreement with the previous report on the hematopoietic action of Cs⁶) and indicates that Cs extract may accelerate regeneration of both organs.

The weight of testis in the irradiated mice was significantly reduced (P<0.01) as compared to that of control mice. However, the weight reduction of testis in the Cs extract treated mice was relatively slight as compared to that in the irradiated mice. This indicates that Cs extract exerted relatively a little protective action on testis as compared that on the spleen and thymus. Unlike these organs there were no significant differences in the weights of heart, kidney and liver among three groups (Table 2). Since the spleen, thymus and testis have been well known as radiosensitive organs²³), the protective action of Cs extract on irradiated mice may be responsible for its enhancing recovery of these organs.

As shown in Fig. 2 the glucose level in the irradiated group was dramatically increased on the first day irradiation (P<0.01). This kind of hyperglycemic effect was very well demonstrated on previous studies with

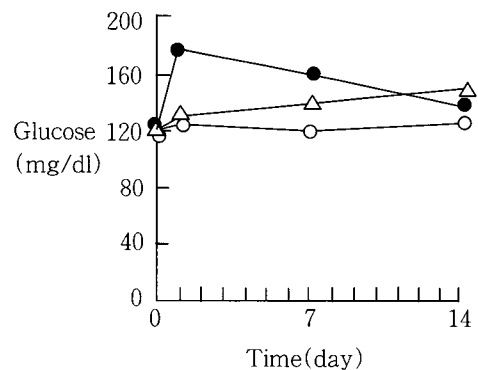


Fig. 2. Effect of the intraperitoneal administration of *Cordyceps sinensis* extract prior to γ - irradiation on the concentration of glucose in the serum of mice.

○, control group ; ●, irradiated group ; △, *Cordyceps sinensis* extract treated group before γ - irradiation.

whole body X-ray irradiated rats²⁴).

Similar studies regarding the effect of irradiation on the carbohydrate metabolism in mammals elucidated that the breakdown of glycogen into the glucose was inhibited resulting in the increases of glucose level in the liver, muscle and serum²⁵). On the 7th and 14th day, the glucose levels in the irradiated group were lower than that on the first day but were still higher than that in the control group. However, the glucose levels of the Cs extract treated group were not virtually unchanged on the

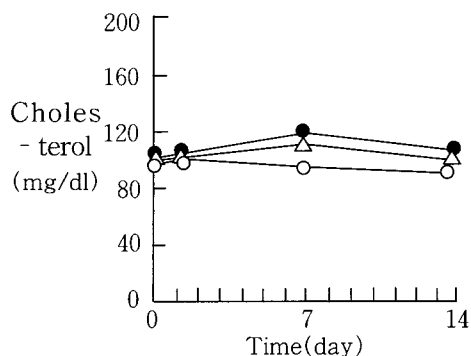


Fig. 3. Effect of the intraperitoneal administration of *Cordyceps sinensis* extract prior to γ - irradiation on the concentration of cholesterol in the serum of mice. ○, control group ; ●, irradiated group ; △, *Cordyceps sinensis* extract treated group before γ - irradiation.

days examined ($P > 0.05$).

It appears that *Cs* by an unknown mechanism protected the carbohydrate metabolism system against irradiation.

Fig. 3 shows that levels of the serum cholesterol were not changed among all groups on the first day. However, the level of cholesterol in the irradiated group was higher than that of control group on the 7th day ($P < 0.05$). Similar observations were also made with X - ray irradiated mice²⁶⁾ and γ - irradiated rats²⁴⁾. The increases of cholesterol level in these studies may be due to the interference with a circulating blood²⁶⁾. The level of cholesterol in the *Cs* extract treated group was also higher than that in the control group on the 7th day. The result indicates that *Cs* did not exert any radioprotective action on the change of cholesterol level produced by irradiation. On the 14th day those in the irradiated and *Cs* extract treated group were lowered to the level in the control group.

In the case of serum total protein levels there were no any differences among all groups (Fig. 4). The result demonstrates that total protein status in the serum under irradiation was the most stable among all metabolites examined.

Although the exact mechanism in protective effect of *Cs* extract on irradiated mice is not clear yet, the present study is the first report regarding the *Cs* which was tested and found to be a potential radioprotective agent. Further investigation with fractionation and subsequent examination of *Cs* extract is required for elucidating major active component of *Cs* in its radioprotective action.

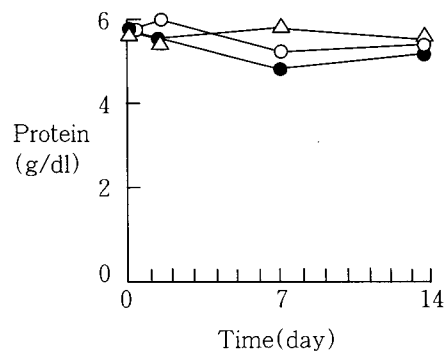


Fig. 4. Effect of the intraperitoneal administration of *Cordyceps sinensis* extract prior to γ - irradiation on the concentration of total protein in the serum of mice.

○, control group ; ●, irradiated group ; △, *Cordyceps sinensis* extract treated group before γ - irradiation.

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<국문 초록>

동충하초 추출물이 감마선에 조사된 생쥐에 미치는 방사선방호효과

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전신 γ - 선 조사 24 시간 전에 *Cordyceps sinensis* (*Cs*) 추출물을 복강에 단일 투여하였을 때 γ - 선 조사된 생쥐의 생존율, 체중, 기관 무게 및 혈청 대사물에 미치는 효과를 조사하였다. *Cs* 추출물의 투여는 γ - 선을 조사한 생쥐의 40 - day 생존율을 66.7%에서 83.4%로 증가시켰다. 또한 *Cs* 추출물의 투여는 γ - 선 조사에 의한 비장과 흉선의 무게 감소를 완전히 막아 주었다 ($P < 0.05$, $P < 0.01$).

한편 이와 유사하나 다소 적은 방사선 방호효과가 *Cs* 투여군의 고환에서도 관찰되었다. *Cs* 추출물의 투여는 γ - 선 조사시 나타나는 조사 7 일째의 고혈당 효과를 억제하였다 ($P < 0.01$). 그러나 조사된 기간 동안 혈청 콜레스테롤과 단백질의 수준에는 아무런 영향을 주지 않았다. 본 연구는 *Cs*가 방사선 방호효과를 보여주는 최초의 보고이다.