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≪Original≫ Protective Effect of Acetylbenzoylaconine Against Gamma-radiation

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Abstract

The protective effects of acetylbenzoylaconine, 2-aminoethylisothiouronium bromide hydrobromide, β-mercaptoethylamine HCl, and L-thiazolidine-4-carboxylic acid were studied on the white male mice, aged 5-6 weeks. The toxicity test of acetylbenzoylaconine revealed that the LD_{50} was 2.5 mg/kg of body weight. After the administration of test substances, mice were irradiated with whole body dose of 800 rad by the Co-60 source. Observing the number of surviving mice for 30 days, the survival coefficients for the test groups were calculated and with these the protective coefficients against radiation injury, PCR, were also calculated. The PCR values are 2.24, 2.95, 2.78, and 1.23 for acetylbenzoylaconine, 2-aminoethylisothiouronium bromide hydrobromiide, β-mercaptoethylamine HCl, and L-thiazolidine-4-carboxylic acid respectively. These values reveal that the acetylbenzoylaconine has protective potency against radiation injury on white male mice.

요 약

Acetylbenzoylaconine, 2-aminoethylisothiouronium bromide hydrobromide (AET), β-mercaptoethylamine HCl (MEA), L-thiazolidine-4-carboxylic acid (thioproline)의 Co-60 감마선에 대한 방어효과를 5~6주된 백색마우스를 이용하여 연구하였다. 백색마우스에 대한 *acetylbenzoylaconine*의 독성실험 결과 LD_{50} 은 2.5mg/kg 이었다. 위의 4종 실험약제를 백색마우스 복강내에 주사한 후 Co-60 감마선 800 rad를 전신조사하고 30일간 계속 관찰하여 실험군들에 대한 생존계수를 계산하였다. 이 생존계수치로 방사선장해에 대한 방어계수를 계산한 결과 acetylbenzoylaconine, AET, MEA, thioproline에 대해 각각 2.24, 2.95, 2.78, 1.23 으로, acetylbenzoylaconine은 백색마우스를 Co-60 감마선장해로 부터 방어할 수 있는 능력이 있음을 알았다.

1. Introduction

The protective effects against radiation of 2-aminoethylisothiouronium bromide hydrobromide (AET), β-mercaptoethylamine HCl (MEA), and L-thiazolidine-4-carboxylic acid (thioproline) were reported by several investigators [1-3], but the acetylbenzoylaconine was not reported. Through a series of preliminary tests of several substances, the acetylbenzoylaconine showed a protective effect against gamma radiation. However, the protective

effects of acetylbenzoylaconine, AET, MEA, and thioproline are evaluated by the same experimental method for the white male mice in order to determine the protective potency of acetylbenzoylaconine.

2. Materials and Methods

Test substances; The acetylbenzoylaconine ($C_{34}H_{47}NO_{11}$) used was purely crystalized material from E. Merck AG, and AET, MEA and thioproline were obtained through SIGMA Chemical Company. Solutions were prepared by weight and all reagents employed were of analytical grade.

Animal; The white male mice, aged 5-6 weeks and weighed 20±2gm were used for the experiments.

Toxicity; The toxicitv test was done only for acetylbenzoylaconine. In order to prepare the solution, 10mg acetylbenzoylaconine was dissolved in 0.6ml ethylalcohol and diluted by 9.4ml distilled water, and made the concentration 1 mg/ml. The white male mice were given 1.5 to 3.0mg/kg of acetylbenzoylaconine solution by intraperitoneal injection. The toxicity data for AET, MEA, and thioproline were relied on the published data[3].

Irradiation; Co-60 gamma-ray irradiation was done to the white male mice with 800 rad of whole body dose, which is equivalent to $LD_{99/30}$ for the white male mouse used. The dose was administered in a single irradiation with dose rate 112 R/min at a distance of 50 cm from Co-60 source.

Experiment; The solution of the test substance was administered intraperitoneally about 15 minutes before gamma-ray irradiation. In most cases, each test group was composed of 10 animals of the same sex, age, and weight. In each experiment, four such groups were used; Group-c received saline solution only (control); Group-s received the test substance only; Group-r received saline solution and 800 rad radiation; Group-t received the test substance and 800 rad radiation. Three such experiments were carried out. The dosage of the test substance was, in all cases, insufficient to cause any deaths. Each day, the number of surviving mice was counted for 30 days and the survival coefficient, S, for the four groups was calculated from the equation

$$S=(n_1+n_2+\cdots+n_i+\cdots+n_{a-1}+n_a)/a.n_1$$

Where n_i is the number of mice at the i-th day and a is the observation period of 30 days.

The protective coefficient against radiation injury (PCR) is then defined by the following combination of the four S values which are independent each other, in the four groups as defined above[3]

$$PCR=S_t/(S_c.S_s.S_r)$$

The standard deviation for PCR value was calculated with the results of three experiments.

3. Results

Fig. 1 illustrates the results of toxicity test for the acetylbenzoylaconine to white male mice. From this survival curve, the LD_{50} was 2.5mg/kg (or 0.0039mM/kg) of white male mouse weight. Therefore, the dose used in the experiment was taken as 1.5mg/kg(or 0.0023 mM/kg).

Fig. 2 illustrates the survival hystograms of white male mice in five different experiments. Fig. 2 (a) shows the survival hystogram for saline injection into the peritoneum of white male mouse about 15 minutes before Co-60 gamma-ray irradiation with whole body dose of 800 rad. Fig. 2 (b) shows the survival hystogram for acetylbenzoylaconine injection into the peritoneum of white male mouse about 15 minutes before Co-60 gamma-ray irradiation with whole body dose of 800 rad. fig. 2

Table 1. Biological properties of chemical protectors

Protective substance	LD_{50}		Dose		Survival after 30 days	PCR (mean value)
	(mg/kg)	(mM/kg)	(mg/kg)	(mM/kg)		
Acetylbenzoylaconine	2.5	0.0039	1.5	0.0023	0.58±0.06	2.24±0.61
AET	635	2.26	281	1.0	0.78±0.06	2.95±0.92
MEA	507	4.46	284	2.5	0.87±0.08	2.78±0.72
Thioproline	140	1.05	100	0.75	0.33±0.11	1.23±1.03

(c) shows the survival hystogram for AET injection into the peritoneum of white male mouse about 15 minutes before Co-60 gamma-ray irradiation with whole body dose of 800 rad. Fig. 2 (d) shows the survival hystogram for thioproline injection into the peritoneum of white male mouse about 15 minutes before Co-60 gamm-ray irradiation with whole body dose of 800 rad. Fig. 2 (e) shows the survival hystogram for MEA injection into the peritoneum of white male mouse about 15 minutes before Co-60 gamma-ray irradiation with whole body dose of 800 rad.

In Table 1, the toxicity values, the protective dose, the survival coefficients and the protective coefficients against radiation injury (PCR) are summarized for four chemical substances.

Table 2 lists all the characteristics of four chemical substances used. The survival coefficients with standard deviation are listed in the fourth column of Table 1, as 0.58±0.06, 0.78±0.06, 0.87±0.08, and 0.33±0.11 for acetylbenzoylaconine, AET, MEA, and thioproline respectively. The protective coefficients against radiation injurey (PCR) are listed with standard deviation in the fifth column of Table 1 as 2.24±0.61, 2.95±0.92, 2.78±0.72, and 1.23±1.03 for acetylbenzoylaconine, AET, MEA, and thioproline respectively.

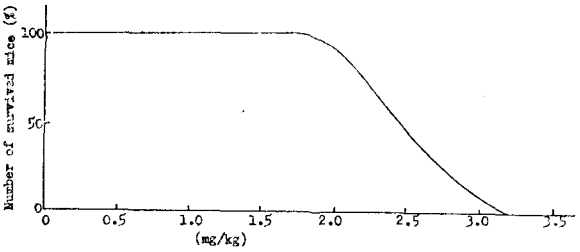

Fig. 1. Survival curve of the white male mice by intraperitoneal injection of the acetylbenzoylaconine solution in different concentration.

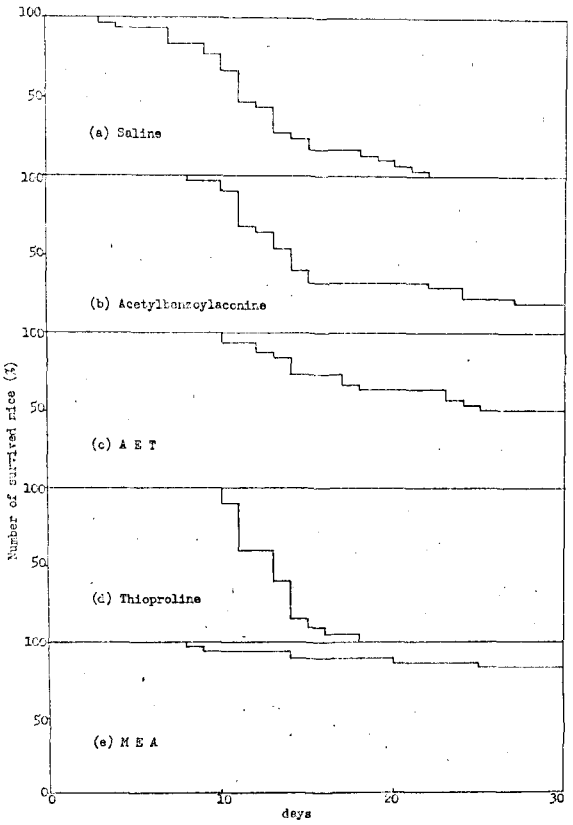

Fig. 2. Survival curves after 800 rad gamma-ray irradiation.

4. Discussion

Among the four chemical substances, AET, MEA and thioproline are well known chemical

protectors in different degrees, but the acetylbenzoylaconine was never been reported about its protective potency against gamma radiation. Acetylbenzoylaconine is rather unstable alkaloid, which is known as a peripheral and central stimulant and depressant. The peculiar stimulation and anesthesia of sensory nerves, without inflammatory phenomena, was used against neuralgic and rheumatic pains. Larger doses than 1.5mg/kg are highly toxic, producing great general depressions, auricular fibrillation, extreme irregularity of the heart, and death by arrest of the respiration or heart.

In this investigation, exactly same experimental method was applied to all four chemical substances in order to compare the protective effectiveness of acetylbenzoylaconine with other known chemical protectors. The highest survival coefficient is 1 in this experiments, which is a perfect protection, but the survival coefficient of acetylbenzoylaconine was 0.58 ± 0.06 and it reveals a little bit of protective potency. On the other hand, the lowest PCR value should not be less than 1 if there are any protective potency. The PCR value of acetylbenzoylaconine was 2.24 ± 0.61 and it certainly has a protective potency in 95% confidence level. The acetylbenzoylaconine is a little less protective than MEA and AET, but it is slightly more protective than thioproline.

Since the toxicity of acetylbenzoylaconine is higher than that of three other chemical substances, the practical applicability to the therapy is doubtful, because a slightly higher dose of acetylbenzoylaconine will cause toxic effect and also the slowing down of heart beat has nothing to contribute to the therapy itself.

5. Conclusion

The toxicity test of acetylbenzoylaconine revealed that the LD_{50} was 2.5mg/kg of body weight.

The acetylbenzoylaconine solution was injected intraperitoneally to the white male mouse of about 20 ± 2 gm weight with the dose of 1.5mg/kg (or 0.0023mM/kg). About 15 minutes after the injection, the white male mouse was irradiated by Co-60 gamma radiation with the whole body dose of 800 rad. Observing the number of white male mice daily, it was found that the survival coefficient of acetylbenzoylaconine injected group was 0.58 ± 0.06 and the protective coefficient against radiation injury was 2.24 ± 0.61. These results reveal that the acetylbenzoylaconine has protective potency against Co-60 gamma radiation in 95% confidence level.

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