Effect of Adrenaline Against Total Body Gamma-irradiation on Serum Protein

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γ-線 全身照射時 血淸蛋白質에 미치는 Adrenaline의 影響

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摘 要

가물치 (*Ophicephalus argus* CANTOR) 성우를 材料로 하여 두 實驗群으로 나누어서 對照群에 副致死量인 250γ의 ^{137}Cs-γ 線을 照射하고 實驗群에는 250γ의 ^{137}Cs-γ 線을 照射 即後 adrenaline (0.5 cc/80 g±18%)을 每日 投與하여 約 23°C. 에서 飼育하면서 血淸蛋白質의 電氣泳動像과 總血淸蛋白質量의 變化를 1, 4, 7, 10, 13 및 16 日區間으로 分離 即 時日的 經過로 調査하였다. 한편 가물치에서의 γ 線 致死量은 310γ이다.

1) 兩群에서 一般的으로 1, 4, 7, 10 및 13日區에 顯著한 albumin 分屑의 減少가 나타났다. 即 對照群인 adrenaline 非投與 照射群에서 α_1 및 α_2 globulin 分屑의 增加와 實驗群인 adrenaline 投與 照射群에서 α_1 및 β-globulin 分屑의 增加에 따른 albumin 分屑의 減少가 나타났다. 따라서 A/G 比도 亦是 時日的 經過에 따라 減少된 値를 나타내었다. 그러나 特히 adrenabine 投與 照射群의 1日區에서 A/G 比가 顯著하게 減少의 遲滯性을 나타내었다.

2) 總血淸蛋白質量은 兩群에서 一般的으로 1, 4 및 7日區에서 顯著하게 減少되나 13 및 16日區에서 가물치의 正常値로 近接된 値를 나타내었다. 그러나 特히 adrenaline 投與 照射群에서 4 및 7日區에서 顯著하게 減少의 遲滯性을 나타내었다.

3) 上記의 結果로 미루어보아 明白하게 血淸蛋白質에 미치는 adrenaline 處理의 防禦效果가 γ線 照射後 特히 初期狀態에서 顯著하게 招來된다고 생각된다.

INTRODUCTION

Gray et al. (1952 a) have demonstrated a protective effect of pitressin and epinephrine against lethal X-irradiation injury of the survival rate of rats. Gray et al. (1952 a) and Gray et al. (1952 b) have reported that pitressin, epinephrine and serotonin are believed to protect via a vasoconstrictor action that produces a temporary tissue hypoxia. Stearner et al. (1954) have reported that epinephrine, as well as low oxygen tension, markedly reduced radiation mortality in young chicks, while combined treatment had a synergistic, protective effect. On the other hands, Ellinger (1952) pointed out that saline injection reduced mortality produced by X-ray on mice.

Electrophoretic studies have revealed that the blood protein pattern of a variety of animals may be changed by altering the physiological and environmental condition. Shin and Nam (1964) found that globulin increased in the gamma-irradiated snake head, while Nam et al. (1964), using snake head, observed that the albumin-globulin ratio decreased by ultraviolet-light irradiation.

However, there have been only a few report on the effect of adenaline against total body gamma-irradiation on serum protein. This paper is concerned with the effect of adrenaline against total body gamm-irradiation on the electrophoretic fraction, the albumin-globulin ratio and total protein of serum in snake head.

MATERIALS and METHODS

Snake heads, *Ophicephalus argus* CANTOR of both sex, 80 g±18% of body weight, were obtained from a dealer in

Seoul. Irradiation was carried out with gamma-ray emitted from ^{137}Cs. Conditions of irradiation are as follow; dose rate was 28 r/min., fishes were irradiated in a wood cylindrical vessel, 15 cm in diameter and with water measuring 10 cm in depth. Dose of gamma-irradiation was 250 r.

A water-bath of approximately 50 liters capacity, equipped was maintained by means of the thermostat at 23±1°C. Fishes fed under the same conditions were divided into two groups (each group 81 fishes). One group which was irradiated with 250 r of gamma-ray (whole body) was used as control. Other group was given daily injection of adrenaline. Adrenaline (Bosmin, Liq. Epirenam. Hydrochlor., Daiichi Seiyaku Co., Ltd., Tokyo, Japan) was administered daily in amounts of 0.5 cc intramusculary, starting immediately after exposure, for an over-all period of 16 days. Fishes irradiated by gamma-ray on the both group were sacrificed at 1, 4, 7, 10, 13 and 16 days after gamma-irradiation.

When the exposure of irradiation was over, at different day period the fishes of the both group were starved 15 hours and then a blood sample was obtained by a cardiac puncture. Blood was allowed to clot before centrifugation and serum used for electrophoretic separation. Paper electrophoresis runs were made using Toyo No. 51 filter paper strips, 3 cm wide and extending 38 cm between baths of barvital buffer of pH 8.6, and ionic strength 0.05. The strips were dipped into the buffer, blotted and laid on a flat plastic support, on the upper surface of which narrow ribs, running longitudinally 2 cm apart, supported the paper above the flat surface. The ends were dipped into the buffer, and the paper was allowed to equilibrate with the buffer and atmosphere in a closed cabinet at 18—19°C for 30 minutes. Samples of 0.03 ml of sera were pipetted to line on the filter paper sideway between the buffer vessel and two adjacent longitudinal ribs. A tube connecting the buffer vessel for liquid level equilibration was clamped shut, and a constant voltage and ampere was applied for 13 hours at 2.7 volts/cm and 0.2 mA/cm.

At the end of the run, the paper was removed and dried for 20 minutes in a forced-draft oven at 60°C. Protein was stained with bromophenol blue. Optical density was determined by Toyo densitometer with wave length of 580 mμ. Diagrams of optical density were constructed and serum protein of snake head identified by comparing the electrophoretic mobility to human serum. Total protein of serum was determined by Kjieldahl's and Biuret's methods.

For graphic presentation of results the albumin-globulin ratio and total protein of serum of adrenaline-injected animals are compared with that of the non-administered irradiation control group.

RESULTS

Lethal dose was 310 r in snake head. Although the general condition seemed to have deteriorated during the

Table 1. Effect of adrenaline on percent fraction of serum proteins of gamma-irradiated on snake head.

Adrenaline treatment	Gamma-ray treatment	Days after irradiation	No. of fishes	Globulin %				Albumin %	A/G ratio
				α_1	α_2	β	γ		
None	250r 28r/min.	0	15	*28.47±2.71	28.79±4.12	4.67±1.20	5.44±1.39	32.62±2.07	0.484
		1	11	51.98±8.24	16.31±3.25	6.60±2.81	10.81±2.92	13.67±2.56	0.163
		4	11	36.86±4.76	31.21±4.15	7.79±2.90	5.35±0.33	16.26±2.70	0.209
		7	11	39.18±6.74	33.87±3.24	4.67±2.81	7.61±2.12	15.39±1.13	0.180
		10	11	35.19±6.49	39.51±4.72	5.79±2.41	7.26±1.04	12.57±4.12	0.143
		13	11	37.54±3.23	31.21±0.91	5.42±1.01	7.47±1.82	18.39±3.43	0.123
		16	11	43.70±7.11	38.40±10.52	6.95±5.61	11.46±2.21	11.53±2.33	0.123
0.5cc/80g± 18%, after Gamma-ray	250r, 28r/min.	0	15	28.47±2.71	28.78±4.12	4.67±1.20	5.44±1.39	32.62±2.07	0.484
		1	11	28.60±5.32	19.57±5.42	12.70±3.61	11.68±4.57	19.69±3.72	0.239
		4	11	42.84±8.42	27.85±6.10	8.58±2.43	8.75±5.14	18.86±4.63	0.215
		7	11	35.05±4.12	35.32±7.05	5.72±1.62	5.18±1.30	15.55±3.54	0.188
		10	11	48.93±6.23	22.41±3.87	13.32±6.12	6.11±1.65	11.77±1.85	0.167
		13	11	37.89±8.76	24.54±1.64	19.49±9.08	4.67±0.81	13.40±4.44	0.155
		16	11	36.85±3.49	29.66±9.32	9.34±2.12	5.65±0.64	14.17±2.54	0.163

*Mean±S.D.

Table 2. Effect of adrenaline on total protein of gamma-irradiated snake head.

Adrenaline treatment	Gamma-ray treatment	Experimental, days after irradiation						
		0	1	4	7	10	13	16
None	250r, 28r/min.※	2.82±0.32	1.48±0.21	1.32±0.15	1.40±0.37	2.17±0.22	2.27±0.15	2.50±0.23
0.5cc/80g±18% after Gamma-ray	250r, 28r/min.	2.82±0.32	1.55±0.27	1.55±0.37	1.67±0.35	2.37±0.27	2.30±0.27	2.60±0.28

※ g/dl, Mean±S.D.

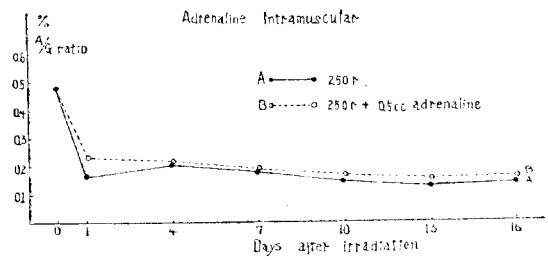

Fig. 1. Influence of intramuscular adrenaline injection on radiation-induced albumin-globulin ratio changes. 15—11 fishes were used for each measurement.

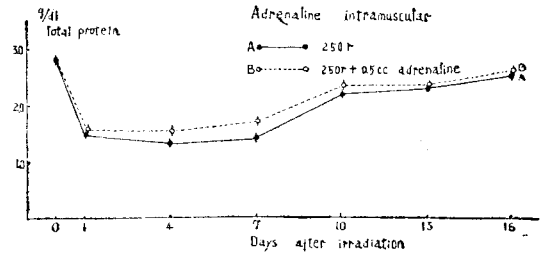

Fig 2. Influence of intramuscular adrenaline injection on radiation-induced total protein changes. Vertical lines represent 2 standard deviations of the mean. 15—11 fishes were used for each measurement.

experimental period, all animals survived without any mortality.

Diagrams of optical density revealed no significant difference between the two sexes under the same experimental condition. Hence, the relative proportions of the serum protein components under experimental condition are shown in Table 1 and Fig. 1, without refencence to the sex of the animal. The relative amounts of total protein of serum under experimental condition are shown in Table 2 and Fig. 2.

Serum electrophoretic pattern showed a marked decrease in albumin fraction at 1, 4, 7, 10 and 13 days on the adrenaline-injected irradiation group and the non-administered irradiation control group. Percentage increase in alpha-1 and alpha-2 fraction on the non-administered irradiation control group and alpha-1 and beta fraction on the adrenaline-injected irradiation group occured at different time period generally but interpreted as only apparent changes accompanying in the greater albumin fall. Hence, the albumin-globulin ratio decreased at 1, 4, 7, 10 and 13 days remarkedly on the both group but the adrenaline-injected irradiation group on the albumin-globulin ratio caused a delay in the development of decrease produced by gamma-irradiation for period of 1 day than control group.

Total protein of serum changed according to suggestive changes in electrophoretic pattern of serum. Total protein of serum decreased remarkably at 1, 4 and 7 days and increased approching normal values of snake head at 13 and 16 days on the both group but total protein of serum on the adrenaline-injected irradiation group for period of 4 and 7 days produced a slight but statistically not significant delay of decrease induced by gamma-irradiation than control group.

DISCUSSION

Attension is called to the fact that adrenaline administration subsequent to sublethal gamma-ray dose may produce a delay of decrease on the albumin-globulin ratio and total protein of serum.

As indicated in Fig. 1 and Fig. 2, the albumin-globulin ratio and total protein of serum in snake head after adrenaline administration were more delayed than control group at 1 day and 4 days respectively.

It is known that an immediate but short lived increase in the oxygen consumption follows the administration of

adrenaline. The mechanism of this action is not known. The increase in the oxygen consumption following adrenaline administration and the vasoconstrictive action of adrenaline, decreasing the tissue supply of oxygen, may lead to a temporary anoxia. Another possibility is that the vasoconstrictive action of adrenline may produce a more profound hypoxia in certain critical tissues than can be accomplished by reduction in the ambient oxygen concentration. In mammals it is established that large dose of adrenaline constrict arterioles of skin, skeletal muscle and splanchnic bed, and blood is shunted into brain, heart and lungs (Salter 1952).

It is generally assumed that many of the biological effects of X-irradiation can be attributed to the action of the decomposition products of water which result from the irradiation. It is also becoming increasingly evident that the degree of X-radiation effects is, to an important extent, dependent on the oxygen content in the cellular fluids.

Gray et al. (1952 a) have reported that the protective effect of epinephrin against total body X-irradiation may be due to their property of producing a temporary tissue anoxia. It is postulated that the decreased supply of oxygen to the tissue produced by exposure at low oxygen tension against X-irradiation is argumented by epinephrin so that a more severe hypoxia of the visceral organ is produced (Stearner et al., 1954).

A reduction in the albumin-globulin ratio from the normal to a subnormal level could be due to many types of disorders in which the albumin and/or globulin metabolism are modified. Abdel-Wahab et al. (1950) have shown that a fall in the albumin concentration may be due to an impaired synthesis or an increased breakdown by the liver or from a selective elimination of this fraction by the kidney. Also according to them, a rise in the globulin concentration may be due to a stimulation of reticulo-endothelial system or an accumulation of abnormal protein. Little is known about the control of blood protein concentration or of the origin and function of the various protein fraction. Since the synthesis of albumin and most globulin take place in the liver (Madden and Whipple, 1948), any change in the liver function is likely to be accompanied by a shift in the serum protein pattern. In other words, the variation of the albumin-globulin ratio and total protein of serum under this condition are a result of many complex changes and thus hard to elucidate a single mechanism. From these facts, apparently the protective effect of adrenaline administration against total body gamma-irradiation on the albumin-globulin ratio and total protein of serum is upon the initial phase of the chain of events initiated by irradiation.

SUMMARY

Snake heads, *Ophicephalus argus* CANTOR fed under the same conditions were divided into two gropus (each group 81 fishes). One group was used as control which was irradiated with sublethal dose, gamma-ray (250 r) emitted from ^{137}Cs. The lethal dose was 310 r in snake head. Other group was given daily injection of 0.5 cc of adrenaline into fish of 80 g±18% body weight subsequent to the exposure to gamma-ray dose (250 r). Each group determined serum protein fraction, the albumin-globulin ratio and the contents of total serum protein after 1, 4, 7, 10, 13 and 16 days.

1) At the experimental days, each serum sample was analyzed by paper electrophoresis. Serum electrophoretic pattern showed a marked decrease in albumin fraction at 1, 4, 7, 10 and 13 days on the group. Percentage increase in alpha-1 and alpha-2 fraction on the control group, and alpha-1 and beta fraction on the group of daily injection of adrenaline occured at different time period generally but interpreted as only apparent changes accompanying in the greater albumin fall. Hence, the albumin-globulin ratio decreased at 1, 4, 7, 10 and 13 days remarkably on the both group but the group of daily injection of adrenaline on the albumin-globulin ratio caused a delay in the development of decrease produced by gamma-irradiation for period of 1 day than control group.

2) Total protein of serum changed according to suggestive changes in elctrophoretic pattern of serum. Total protein of serum decreased remarkably at 1, 4 and 7 days and increased approching normal values of snake fish at 13 and 16 days on the both group but total protein of serum on the group of daily injection of adrenaline for period of 4 and 7 days produced a slight but statistically not significant delay of decrease induced by gamma-irradiation.

3) Apparently, the protective effect of administration of adrenaline is upon the initial phase of the chain of events itiniated by gamma-ray irradiation.

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