CHANGES OF CATHEPSIN D AND α $_2$– MACROGLOBULIN IN RATS AFTER ACUTE TOTAL BODY IRRADIATION*

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ABSTRACT

α $_2$– macroglobulin (α $_2$M) could stimulate the regeneration of thymic and bone marrow cells in rats received γ – irradiation, but there was very few reports concerning its mechanism. Wistar rats were irradiated by ^{16}Co at 7 Gy, 8.5 Gy, 15 Gy total body doses. Blood plasma and some tissue's extracts were collected α $_2$M level. α M activity and cathepsin D activity, malonaldehyde level were determined by radioimmunoassay, modified Schidlow's method, Barrett's method and Ohkawa's method respectively.

Key words: Alpha– 2– macroglobulin Cathepsin D Malonaldehyde Radiation Rat

I . INTRODUCTION

There was increase of plasma α $_2$M levels as well as plasma α M activities after 7 Gy, 8.5 Gy, 15 Gy irradiation. Both of them were in parallel with the increase of radiation doses. The changes of plasma malonaldehyde also correlated with the elevation of plasma α $_2$M concentration. There was marked elevation of cathepsin D activities in splenic tissue but no increase of α $_2$M level in spleen in the first 24 h, after 8.5 Gy exposure. It seems that the ability of α $_2$M synthesis by splenic lymphatic cells was reduced during severe radiation injuries.

Burger et al. found that sheep splenic extracts and human serum α $_2$M could stimulate the regeneration of thymic and bone marrow cells in rats received γ – irradiation[1]. In 1974 I. Berenblum et al. further proved that α $_2$M, just like splenic extracts, could reduce the incidence of lymphatic leukemia in irradiated rats[2], but there was very few reports concerning its mechanism.

α $_2$M is a protease inhibitor of wide spectrum. It regulates the level of intracellular and extracellular proteolytic enzymes[3]. Recently it was reported that combined with protease α $_2$M was a free radical scavenger as well[4].

During irradiation there is an increase of free radical flux in the organism. Free

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radicals accelerated proteolysis of tissue proteins[5]. And proteases were involved in the production of superoxide radical in polymorphonuclear neutrophiles too[6], so it was enhanced the radiation injury, and was speculated that the beneficial effects of α ₂M might be both binding with proteases and scavenging of free radicals.

Cathepsin D, being one of the important proteolytic enzymes in lysosomes, presents widely in mammalian cells with tissue proteolytic effects, for which there are very few natural inhibitors[7]. Normally, cathepsin D could be trapped by α ₂M in pH≥6[8]. In this study changes of cathepsin D, α ₂M and malonaldehyde (MDA) (one kind of lipid peroxidation products in free radical chain reaction) were investigated in irradiated rats.

Ⅱ. MATERIALS AND METHODS

240 male Wistar rats (Shanghai laboratory animal permission. No. 32* —34*) with body weight 211±31 g ($\bar{X}$±SD) were devided into several groups with 5—8 rats each. The total body irradiated groups received 7 Gy, 8.5 Gy and 15 Gy separately from ^{60}Co source with dose rates 0.03483—0.04257 C/(kg.min). The distance between ^{60}Co source and rats was 80cm. Blood and tissue samples from each group were collected at 1/4, 1, 3, 7 days after irradiation, some even longer, Plasma was obtained from heparinize blood after centrifugation. Tissue samples were obtained after bleeding and irrigating with 0.9% NaCl solution through abdominal vessels. Then it was homogenized and centrifugalized, and supernatants were used for examination.

By using bovine hemoglobin as the substrate the activities of cathepsin D were determined by Barrett's method[9].

Rat's acute phase plasma α ₂M was purified through Sephacryl S– 300 gel filtration and affinity chromatography coupled with anti– normal rat's plasma. Immunopure α ₂M showed only one band in poly– acrylamide gel electrophoresis was obtained[10]. Anti– α ₂M antiserum was collected by immunizing rabbits as usual.

Plasma concentrations of α ₂M were determined by rocket electrophoresis[11]. Estimation of α ₂M contents in liver, spleen, kidney, parotid gland and skin tissues was done by radioimmunoassay[12]. Changes of α ₂M concentration in rats after irradiation varied remarkablely individually, so statistic analysis on logarithm was performed.

Plasma α M activities were tested by modified Schidlow's method using BAPNA as its substrate[13].

Plasma concentration of MDA was measured according Leu Shizhong's method by using thiobarbituric acid as chief reagent[14]. MDA in tissues was estimated by modified Ohkawa's method[15] and protein concentration in tissue's extracts by Lowry's method[16].

Ⅲ. RESULTS

Total body irradiated rats with 7 Gy were all alive at the 28th day (8/8). 12/16 irradiated rats with 8.5 Gy were still living 7 days after exposure. 4/8 15 Gy irradiated rats were alive at the 3rd day.

There was no significant changes of plasma cathepsin D activities in the first few days after 7 Gy, 8.5 Gy and 15 Gy doses of ^{60}Co irradiation. But a tendensy of increase in cathepsin D activities was observed in spleen 7 days after 7 Gy exposure (irradiated: control = 40.8 ± 10.2* : 34.5 ± 5.8), and a pronounced rise was observed 6 h after 8.5 Gy irradiation and persisted even more than 7 days in spleen (Tab.1). An increase of cathepsin D activities was also detected in the liver at 7th day after 8.5 Gy irradiation (Tab.1).

Table 1

Cathepsin D activities in plasma (mu/ml) and tissue extracts (mu/mg protein) of rats after 8.5 Gy ^{60}Co total body irradiation (TBI) ($X \pm SD$) ($n = 8$)

Groups		Plasma	Spleen	Kidney	Liver	Skin
Control		22.5 ± 3.0	31.7 ± 3.6	15.5 ± 8.4	5.7 ± 1.4	27.4 ± 7.8
Postirrad.	1/4d	23.4 ± 2.8	53.7 ± 9.6* * *	9.5 ± 2.4	4.9 ± 1.9	21.9 ± 3.8
	1d	21.8 ± 0.9	48.7 ± 3.9* * *	17.2 ± 3.8	3.6 ± 0.7	27.6 ± 4.7
	3d	19.7 ± 3.0	49.4 ± 11.2* * *	18.9 ± 4.6	6.4 ± 1.8	35.9 ± 7.6
	7d	21.6 ± 6.9	53.0 ± 7.4* * *	13.8 ± 2.9	11.5 ± 3.6* * *	31.6 ± 5.5

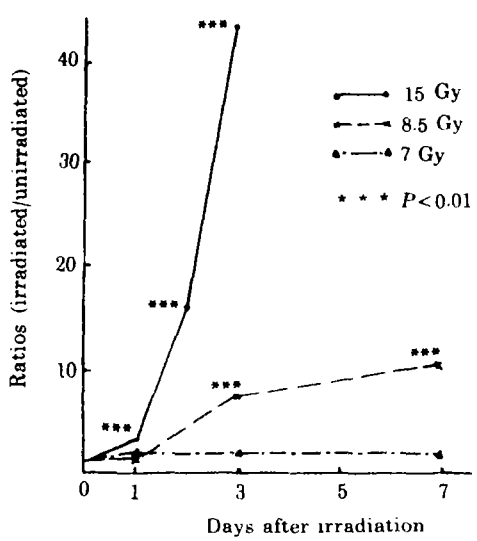

Fig. 1 Changes of plasma α_2 M levels in rats after irradiation with different doses

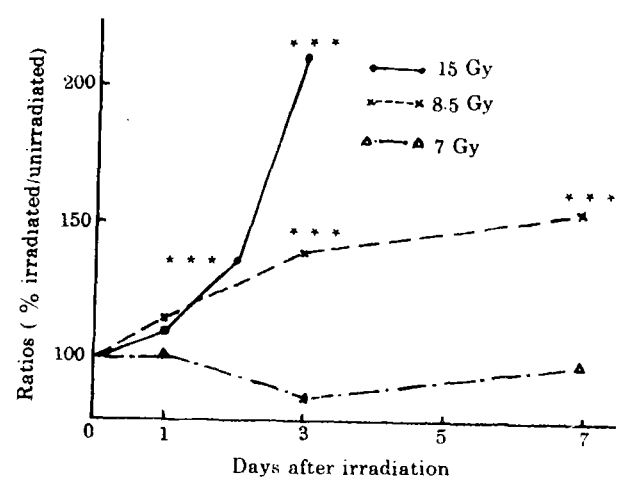

Fig. 2 Changes of plasma α M activities in rats after irradiation with different doses

Plasma α ₂M levels increased 28 days after 7 Gy irradiation (irradiated: control = 66.5±2.8* * * :25.0±1.3), but without much changes in α M activities. Both plasma α ₂M levels and activities were increased evidently 3 days after 8.5 Gy exposure and 1 day after 15 Gy irradiation (Fig.1, 2). 3 days after exposure both plasma α ₂M contents and activities increased in parallel with increases of radiation doses ($r=0.6739$, $P=0.003$ & $r=0.8566$, $P=0$ respectively). And there was correlation between plasma α ₂M levels and α M activities too ($r=0.7535$, $P=0.0005$).

In 8.5 Gy irradiated rats, α ₂M levels in liver, kidney, parotid gland, skin and spleen rose and correlated with plasma α ₂M levels ($P=0$, $P=0$, $P=0$, $P=0$ & $P=0.0019$ respectively). But there was no increase of α ₂M in the spleen in the first 24h and less increase of α ₂M in spleen than other tissues in 7 days after irradiation (Tab. 2).

Table 2

α ₂M levels in tissue extracts (ng/mg protein) in rats after 8.5 Gy ^{60}Co TBI

[geometric mean $\sqrt{(\log X)\pm(\overline{\log X})}$]

Sample	Control	Post-irradiation			
		1/4d	1d	3d	7d
	($n=8$)	($n=7$)	($n=8$)	($n=7$)	($n=7$)
Spleen	12.59	11.75	10.96	52.48	81.28
	1.1±0.06	1.07±0.08	1.04±0.08	1.72±0.20* * *	1.91±0.13* * *
Skin	33.88 +	41.69	53.70 +	104.71	380.19
	1.53±0.05	1.62±0.05	1.73±0.03	2.02±0.21	2.58±0.19* * *
Parotid	9.12	13.80	13.49	40.74	102.33
gland	0.96±0.06	1.14±0.06	1.13±0.03	1.61±0.19* *	2.01±0.25* * *
Kidney	5.25	10.23 #	8.71	56.23 +	77.62
	0.72±0.06	1.01±0.11	0.94±0.07	1.75±0.06* * *	1.89±0.26* * *
Liver	1.32	4.90	1.70	15.14	33.88
	0.12±0.13	0.69±0.16	0.23±0.11	1.18±0.25* * *	1.53±0.29* * *

* * $P<0.05$ * * * $P<0.01$ $^{+}n=7$ $^{\#}n=8$ $^{+}n=6$

The increase of plasma α M activities was positively correlated with increase of cathepsin D activities in spleen ($r=0.4285$, $P=0.0065$).

Plasma concentration of MDA increased on the 3rd and 28th day after 7 Gy radiation, they were 7.71±0.23* * * and 7.18±1.71* * nmol/ml respectively with 5.77 ± 1.06 nmol/ml in control groups. MDA concentration in plasma, liver, kidney and testis of 8.5 Gy irradiated rats all increased one day after exposure, and still higher than control in plasma, kidney and testis 7 days after exposure (Tab.3). There was correlation between plasma MDA and plasma α ₂M level with $r=0.707$ and $P=0$. In 15 Gy group, plasma levels of MDA increased 1,2 and 3 days after irradiation (9.2± 0.7* * *, 9.3± 1.4* *, 26.8 respectively with control 7.8± 0.8 nmol/ml), and it correlated with the increase of α ₂M contents in plasma too ($r=0.7676$, $P=0$).

Table 3

MDA in plasma (nmol/ml) and tissue extracts (n mol/mg protein) of rats after 8.5 Gy ^{60}Co TBI (X±SE) (n=8)

Sample	Control	Post- irradiation		
		1d	3d	7d
Liver	339.6±33.8	457.3±38.8* *	370.3±19.5	306.0±37.4
Kidney	97.1±7.2	130.5±9.0* * *	217.5±21.1* * *	146.2±10.8* * *
Testis	127.4±7.3	210.3±31.9* *	152.9±28.6	282.6±12.3* * *
Submax. gland	65.0±6.2	67.6±4.3	89.5±19.4	51.0±6.2
Plasma	6.81±0.17	7.98±0.18* * *	8.62±0.65	7.73±0.37* *

IV. DISCUSSION

It is well known that there is an increase of free radical flux in organism after high dose ionizing radiation. Free radicals induces lipid peroxidation with increase levels of MDA in bone marrow, spleen, kidney, plasma etc[17].

Hectepehk reported that in rats 3 days after 12 Gy ^{60}Co TBI the cathepsin D activities increased 3—5 times in small intestinal extracts than control group[18]. Our observation revealed similar findings as the MDA increased in plasma, liver, kidney, testis and cathepsin D activities also increased in spleen in 8.5 Gy ^{60}Co irradiated rats.

Weimer et al.[19] found that 7 days after 0.0516,0.1032,0.1548 and 0.2064 C/kg ^{60}Co irradiation α ₂M appeared in rat's plasma, and was even detected 24h after 800 r irradiation. Rat's α ₂M is one kind of acute phase proteins. Konnova et al.[20] considered that rat plasma α ₂M activities reduced to almost 58% in the first 3 days after 6 Gy exposure. Our results showed there were increases both of α ₂M levels and α M activities with increase of radiation dosage. It seemed that rat's plasma α ₂M was a kind of acute phase proteins, it increased significantly after radiation stimulation to compensate the requirement in microenvironment for binding of proteases, such as cathepsin D etc, and scavenging free radicals.

α ₂M is synthesized by liver parenchymal cells, Kupffer's cells and other reticulo- endothelial cell system as well as fibroblasts[21]. In acute phase there is increase of α ₂M synthesis by lymphatic cells too[22]. In our study after 8.5 Gy exposure the α ₂M level in spleen increased gradually and less than other tissues. It seemed that the ability of α ₂M synthesis by splenic lymphatic cells was reduced with severe radiation injuries.

V. SUMMARY

There was marked elevation of cathepsin D activities in splenic tissues and no

increase of α_2M levels in spleen in the first 24h after 8.5 Gy TBI by ^{60}Co in male Wistar rats. The increase of splenic cathepsin D activities persisted even 7 days after exposure, while splenic α_2M levels increased less than other tissues (liver, kidney, skin etc).

There was increase of plasma α_2M levels as well as α M activities after 7 Gy, 8.5 Gy and 15 Gy irradiation. Both of them were in parallel with the increase of radiation doses.

There was increase of MDA levels in plasma, liver, kidney, testis etc. The changes of plasma MDA also correlated with the elevation of plasma α_2M concentration.

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