THE EFFECT OF X-RAY ON THE ENERGY METABOLISM OF MOUSE EHRLICH ASCITES TUMOR CELLS

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エールリッヒ腹水癌細胞の力源代謝に及ぼすX線の作用

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X線全身照射後担癌マウスより分離したエールリッヒ腹水癌細胞においてはその酸化的リン酸化反応は顕著に阻害され，一方好気的乳酸形成は顕著な増大を示す．かゝる現象は前に報告した癌細胞に高級不飽和脂肪酸を添加した場合に観察された結果と酷似している．

X線全身照射後癌細胞で観察されるかゝる障害は本文に詳述せる如く間接的作用による結果であり，同様の阻害は照射担癌マウス腹水液中の脂質成分により誘発されることを明らかにした．

Contents

I. Introduction

To study the effect of the X-irradiation on the energy metabolism of cancer cells is an important problem in connection with the synthesis of nucleic acid and protein.

Van Bekkum[1)2)] reported that by the whole body irradiation of a rat lesion appears in oxidative phosphorylation, but no explanation is given to its mechanism.

The author[3)] reported previously the effect of higher unsaturated fatty acid fraction extracted from irradiated rabbit liver on the energy metabolism of cancer cells. In connection with the above study a work has been made on perceiving the disturbance of lipid metabolism when tumor bearing mouse is exposed to whole body X-irradiation.

II. Materials and Methods

Ehrlich ascites tumor cells (6,000, 000 cells) was transplanted into the peritoneal cavity of Strong A mouse and after 9 days this tumor bearing mouse was used for the experiment.

The mice were irradiated in a Petri dish under condition of maximum backscatter. The physical factors were as follows: 200 KV, 25 mA, filter; 0.5 mm Cu+0.5mm Al, H.V.L.; 1.37 mm Cu, dose rate; 116 r/min., whole body dose was 100, 500 and 1000 r.

After irradiation Ehrlich ascites tumor cells were removed from the mice and then immediately oxygen uptake, lactate formation and P^{32} incorporation into Δ 10 P fraction of the cells were measured with the method[3] reported previously.

In order to study the direct action of X-ray on the oxidative phosphorylation of tumor cells, the tumor cells were removed from the tumor bearing mice. Suspending the tumor cells in yeast extract-lactoalbumin hydrolyzate-Earle salt (YLE) solution, they were exposed to 1000 r irradiation. After irradiation, the tumor cell suspension was incubated for 6 hours at 37°C and then oxygen uptake and P^{32} incorporation into Δ 10 P fraction were measured.

Ascites fluid was obtained from the tumor bearing mice 4 hours after 1000 r whole body irradiation. The effect of this ascites fluid on the oxidative phosphorylation of the tumor cells was observed as follows: taking 2 ml of ascites fluid in a test tube containining heparin 5 units and adding 2 ml of tumor suspension (20,000,000 cells/ml of Krebs-Ringer (K-R) solution), and the tumor cells were preincubated for 2 hours at 37°C. The tumor cells were washed twice with 0.9% sodium chloride solution by centrifugation and once with K-R solution. Lastly, oxygen uptake and P^{32} incorporation into Δ 10 P fraction of the tumor cells were measured.

With the object to assay the compound which uncouples the oxidative phosphorylation in the ascites fluid of the irradiated tumor bearing mice, Wojtczak and Lehninger method[4] was applied. 4 ml of ascites fluid was taken in a test tube, boiled for 10 minutes at 100°C, and separated its supernatant and residue by centrifugation at 13,000 r p m for 15 minutes in 4°C. After the residue was washed with distilled water, it was extracted repeatedly three times by 5 ml of ethanol-ether (3 : 1) in order to extract lipid fraction. This lipid fraction was evaporated in vaccuo and dried. Then it was solved with 0.2 ml of ethanol and mixed with 4.3 ml of 0.125 *M* KCl-0.02 *M* Tris buffer solution (pH 7.4). Suspension of rat liver mitochondria (0.5 ml) was added to the lipid solution and the change of the absorbancy at 520 mμ was measured for 40 minutes at 38°C. Mitochondria were prepared from rat liver with Hogeboom method[5]. After preparation it was immediatly used for the experiment.

III. Results

Fig. 1 shows the changes of oxygen uptake and aerobic lactate formation of Ehrlich ascites tumor cells removed 12 hours after whole body irradiation on the tumor bearing mice.

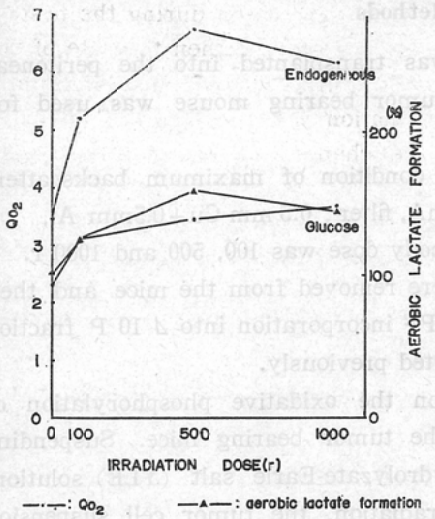

Fig. 1. Effect of irradiation on the oxygen uptake and aerobic lactate formation of Ehrlich ascites tumor cells

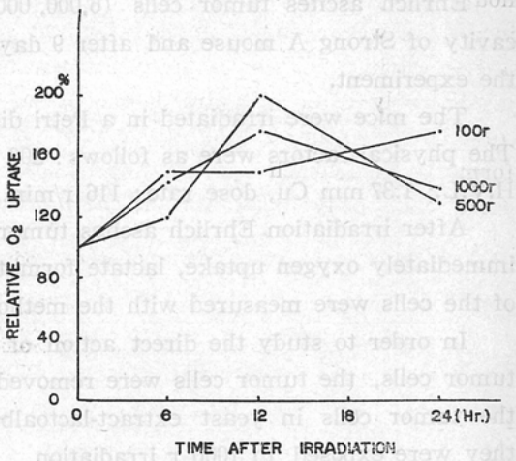

Fig. 2. Effect of irradiation on the oxygen uptake of Ehrlich ascites tumor cells.

Endogenous oxygen uptake extremely increased by whole body irradiation in each dose. In the presence of glucose both oxygen uptake and lactate formation were considerably enhanced by whole body irradiation.

Fig. 2 and Fig. 3 show the changes of endogenous oxygen uptake and the incorporation of P^{32} into Δ 10 P fraction of Ehrlich ascites tumor cells by whole body irradiation. Irradiating the whole body of tumor bearing mice with 100, 500 and 1000 r dose, the tumor cells were removed after 6, 12 and 24 hours.

During the experiment oxygen uptake increased by 100 r dose, and by 500 and 1000 r dose the oxygen uptake increased continously during the first 12 hours after whole body irradiation, but the rate of the increased-oxygen uptake weakened after 24 hours later.

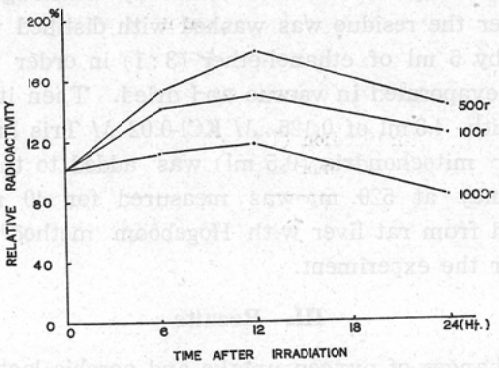

Fig. 3. Effect of irradiation on the incorporation of P^{32} into labile phosphate of Ehrlich ascites tumor cells

The incorporation of P^{32} into Δ 10 P fraction of the cells increased during the first 12 hours after whole body irradiation with 100 and 500 r dose, but then the rate of the increment weakened. The incorporation of P^{32} into Δ 10 P fraction was not significantly altered during the first 12 hours after whole body irradiation with 1000 r, but inhibited considerably after 24 hours later. Table 1 shows the changes of endogenous oxygen uptake, P^{32} incorporation into Δ 10 P faction, relative P/0 ration and aerobic lactate formation of Ehrlich ascites tumor cells, which were removed 1/4, 6, 12 and 24 hours after whole body irradiation on the tumor bearing mice with 1000 r.

Table 1. Effect of whole body irradiation (1000 r) on the energy metabolism of Ehrlich ascites tumor cells

Time after irradiation hr.	Relative O_2 uptake (%)	Relative radioactivity of Δ 10 P (%)	Relative P/O ratio	Relative lactate formation (%)
0	100	100	1.00	100
1/4	104	106	1.02	99
6	143	112	0.79	143
12	180	125	0.70	146
24	140	90	0.64	215

Oxygen uptake increased during the first 12 hours after X-irradiation but decreased after 24 hours. In this case relative P/O ratio (relative radioactivity of Δ 10 P/relative oxygen uptake) decreased 79% in 6 hours after whole body irradiation and 64% in 24 hours. In the presence of glucose the aerobic lactate formation of the tumor cells increased about 1.5 and 2.2 times in 12 and 24 hours, respectively, after X-irradiation.

To find out whether the lesion of oxidative phosphorylation in Ehrlich ascites tumor cells by whole body irradiation depend upon the direct action of X-ray on the tumor cells, the tumor cell suspension was exposed to X-irradiation with dose of 1000 r.

At six hours after direct irradiation on tumor cells, endogenous oxygen uptake and P^{32} incorporation into Δ 10 P fraction were measured. As shown in Table 2, oxygen uptake and P^{32} incorporation into Δ 10 P fraction have little difference compared with the control. Irradiating the whole body of tumor bearing mice with dose of 1000 r, separating the ascites fluid after 4 hours later and then non-irradiated Ehrlich ascites tumor cells were preincubated with the ascites fluid for 2 hours at 37°C. The oxygen uptake of the cells increased 17% but the incorporation of P^{32} into Δ 10 P fraction decreased 74%.

Table 2. Effect of X-irradiation (1000 r) on the oxidative phosphorylation of Ehrlich ascites tumor cells

	Control (%)	Tumor cell irradiation	Whole body irradiation	Incubation with ascites fluid from irradiated mice
Relative O_2 uptake	100	106	146	117
Relative radioactivity of Δ 10 P	100	108	112	74
Relative P/O ratio	1.00	1.02	0.79	0.64

Consequently, in this case, it was clear that the uncoupling oxidative phosphorylation of tumor cells was induced by the ascites fluid from the X-irradiated mice.

The swelling action of water soluble and lipid fractions from the ascites fluid of X-irradiated mice on rat liver mitochondria were measured to obta in some clue in what fraction the uncoupler of oxidative phosphorylation exists. By measuring the swelling action it was found that remarkable swelling action was observed in the lipid fraction from the ascites fluid of tumor bearing mice 6 hours after whole body irradiation with dose of 1000 r. As shown in Fig. 4, the swelling activity of the lipid fraction was proportional with the duration of the X-irradiation to the separation of the lipid fraction from the ascites fluid of the X-irradiated mice.

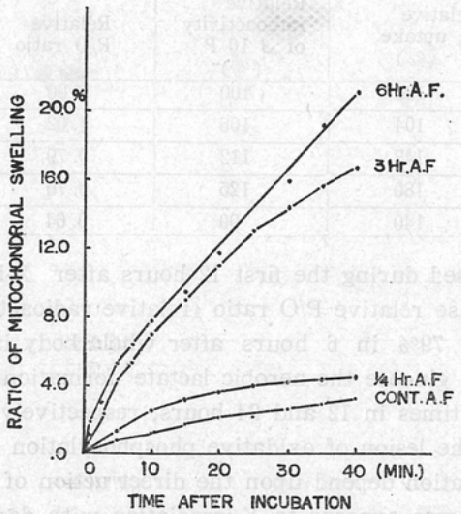

Fig. 4. The swelling action on rat liver mitochondria of ascites lipid fraction from X-irradiated mice

IV. Discussion

It is well known that some fatty acids act as an uncoupler of oxidative phosphorylation of rat liver mitochondria[6] and tumor cells[7,8]. Van Bekkum[1] reported that uncoupling oxidative phosphorylation is inducible in radiosensitive tissues with X-irradiation, but the mechanism was not clear. Uncoupling oxidative phosphorylation was also observed in the case of mouse Ehrlich ascites tumor cells after the whole body irradiation and the same degree of the uncoupling was also in the non-irradiated tumor cells incubated with the ascites fluid or ascites lipid fraction from X-irradiated tumor bearing mice[9]. Therefore it is clear that the uncoupling oxidative phosphorylation of the tumor cells after the whole body irradiation was caused with indirect action of X-ray. Di Luzio et al.[10] suggested that a post-irradiation hyper-lipemia was induced by the abnormal lipoproteins of chylomicra which may be formed in X-irradiated rabbits and could not efficiently be metabolized. The mechanism by which X-ray disturbs the lipid metabolism is not clear, however, it is likely that by the whole body irradiation the hormonal imbalance would occur and as a result some abnormal lipids would be released

into blood and ascites fluid as discussed by Spitzer and McElroy[11] on the hormonal controlling on the lipids of dog plasma.

To clarify the chemical properties of the lipid fraction acting as an uncoupler of oxidative phosphorylation, further analytical investigations are necessary.

The mechanism by which X-ray stimulates indirectly the aerobic lactate formation in the tumor cells is not clear, but, it appears possible that the stimulated-lactate formation may be induced by some lipids of the ascites fluid as observed in the case of the tumor cells incubated with higher unsaturated fatty acids[3]. In this case the membrane-permeability would be stimulated and the mitochondrial function would be damaged.

V. Summary

1. Irradiating the whole body of tumor bearing mice with 100, 500 and 1000 r dose of X-ray and at various periods the Ehrlich ascites tumor cells were removed, and oxygen uptake and P^{32} incorporation into Δ 10 P fraction of the tumor cells were determined. Oxygen uptake of the tumor cells was enhanced but the relative P/0 ratio decreased during 24 hours after whole body irradiation with dose of 1000 r.

2. The tumor cells removed from the irradiated tumor bearing mice displayed remarkable increment in the aerobic lactate formation in the presence of glucose.

3. The lesion of oxidative phosphorylation in the tumor cells with whole body irradiation is a secondary action of X-ray. Because it has became clear that the same lesion occurs in non-irradiated tumor cells by incubation with the ascites fluid obtained from irradiated tumor bearing mice, and the swelling action on rat liver mitochondria was especially displayed in the lipid fraction from the ascites fluid of the X-irradiated tumor bearing mice.

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