THE INFLUENCE OF HYPOTHERMIC CONDITIONS ON THE RADIOSENSITIVITY OF YOSHIDA ASCITES SARCOMA CELLS IN VITRO

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吉田肉腫細胞の放射線感受性（生体外照射）に及ぼす低温の影響

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放射線感受性に及ぼす酸素効果に関連して，照射時の組織の低温状態がその組織の放射線障害を減少せしめるという報告があり，これが，臨床的にも応用されつつある．

我々は，生体外に取り出された吉田肉腫細胞の放射線感受性が照射時の環境温度を低温にする事によつて受ける影響を実験した．

1） 150g雄ラットに吉田肉腫細胞移殖後5日目の腹水を採り，室温，0°C及び−10°C，の3つの温度条件でいづれも1500r，一回照射を行ない，照射直後に新らしいラット腹腔内に移植して，そのラットの死亡率によつて肉腫細胞の放射線感受性を判定した．

2） 生体外1500r は，肉腫細胞にかなりの障害を与え延命効果をもたらす．

3） −10°Cに数分間保つても肉腫細胞に影響はみられない（対照群Ⅰ及びⅡ）．

4） 照射時の環境温度が低い程，移植動物群の平均余命の短縮に有意の差が認められ，低温に伴う放射線感受性の減少が推定される．

5） −10°Cの場合は，低温の影響の他に腫瘍細胞浮遊液の固相化による放射線の関接作用の影響が検討されなければならないであろう．

The dependence of radiosensitivity of mammalian cells on oxygen tension at the time of irradiation were reported by many investigators (Gray, 1953; Conger, 1956; Adachi, 1961). Their experiments have shown that radiosensitivity of cells paralleled with oxygen tension of cellular environment. For preparations of various conditions of oxygen tension at the time of irradiation, the partial pressure of oxygen in breathing gas of animals or the composition of gas which was used in a form of jet were changed. Recently, irradiation of cells of animals under hypoxic conditions became possible by cooling animals. Weiss (1960) has shown the protective effect of hypothermia for the

radiation damage of irradiated rat tissues when animals were kept at low temperature (1°±1°C) throughout irradiation.

Gafford (1958) reported the lower radiosensitivity to gamma radiation under lyophilized condition comparing with the effect for the same dose in the aerated aqueous suspension. We present here a report of an effect of hypothermia on radiosensitivity of Yoshida sarcoma cells in ascites form.

MATERIAL AND METHOD

Male adult rats of Saitama colony approximately 150 g in body weight were used throughout the experiments. All animals were fed on Oriental solid diet and water ad libitum and were housed two per cage. One thousand to one hundred thousand sarcoma cells in ascites fluid were inoculated in their peritoneal cavities on every sixth day by means of a glass capillary pipette. Irradiation of X-rays was made in vitro on the fifth day after inoculation.

Conditions of irradiation were as follows; 200 kVp, HVL 1.0 mm Cu. Distance between focus and glass tube in which sarcoma cells were suspended was 30 cm and dose rate at the centre of the tube was 250 r per minute. Dosimetry was carried out by Victoreen 250 r condenser chamber (model 570) at the position of glass tube embeded in a rice phahtom. Sarcoma cells were irradiated with a dose of 1500 r by single exposure. Irradiation of sarcoma cells were made under three different environmental temperatures. The first was at room temperature (18°C), the second was at O°C and the last was at -10°C. The second condition was prepared by ice and water and the last one was by ice and sodium chloride. Glass tube containing sarcoma cells was immersed in cooling medium for about five minutes before irradiation and then irradiated under the same condition for six minutes. For the first two groups, air was insufflate into the ascites fluid by a small air pump but the procedure was not adapted for irradiation at —10°C, because the suspending medium became frozen. Controls were treated in the same way except for irradiation.

Table 1 Number of rats for each type of treatment.

Group	Types of treatment	Number of animals
I	Control I (only transplantation)	42
II	1500 r (under room temperature)	18
III	1500 r (under 0°C)	15
IV	Control II (—10°C, no irradiation)	13
V	1500 r (under —10°C)	14

About ten thousand of treated cells were transplanted immediately after exposure into the peritoneal cavities of new healthy rats. Number of rats for each group and types of treatment were listed in Table 1. Number of survivors were recorded every

day and a series of survival curves was drawn. Median survival time for each group was estimated graphically from the curves.

RESULTS

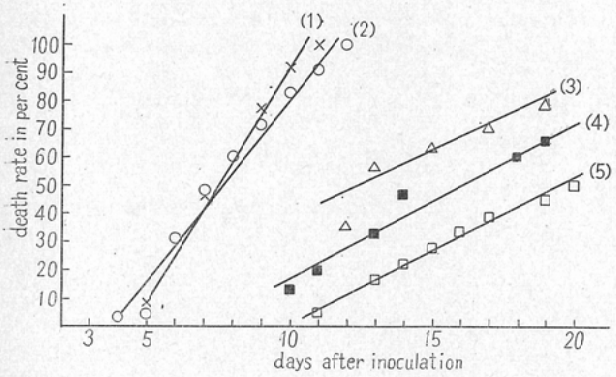
No difference was observed between median survival times for two control groups which were treated under conditions of hypothermia (-10°C) and roome temperature. The tumorcidal effect of irradiation was evidenced by the longer survival time of animals which were transplanted with sarcoma cells received 1500 r of radiation than those inoculated with non-treated sarcoma cells. Rediosensitivity of sarcoma cells was remarkably influenced by low temperature of environment of sarcoma cells at the time of irradiation. Number of dead animals were recorded every day after inocultation and were listed in Table 2. Median survival times of animals were shown at the bottom of the table and the relation between accumulated death rate and the day after inoculation were given in Figure 1.

Table 2.

Days after inoculation	Number of animals deceased				
	Group				
	I	II	III	IV	V
0					
1					
2					
3					
4	1				
5	1			1	
6	11			0	
7	7			5	
8	5			0	
9	5			4	
10	5		2	2	
11	3	1	1	1	
12	4	0	0		5
13		2	2		3
14		1	2		0
15		1	0		1
16		1	0		0
17		1	0		1
18		0	2		0
19		1	1		1
20		1 (+9)*	0 (+5)*		0 (+3)*
Number of animals treated	42	18	15	13	14
Median survival time (days)	7.6	19.5	15.9	7.5	13.0

(* No. of animals survived)

Fig. 1



(1) Control II (group IV)
(2) Control I (group I)
(3) —10°C, 1500 r (group V)
(4) 0°C, 1500 r (group III)
(5) 18°C, 1500 r (group II)

DISCUSSION AND CONCLUSION

In vitro radiosensitivity of Yoshida sarcoma cells was influenced remarkably by the environmental temperature at the time of irradiation. It is suggested that the oxygen concentration in tumour cells may be affected by thermal conditions during irradiation and biochemical reactions which induce radiation damage of sarcoma cells may be depressed by low temperature.

Under the condition, in which ascites fluid was frozen (-10°C), further decrease in radiosensitivity of sarcoma cell was observed. It will be assumed from these results that this decrease in radiosensitivity was not only caused by the lower temperature than 0°C but by the depression of the indirect action of ionizing radiation as reported by Gafford (1958).
