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ABDIOSENSITIZATION BY HALOGENATED PYRIMIDINE ANALOGUES

BY

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ピリミジン誘導体による放射線増感

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最近来ハロゲン元素 I,Br,F, を５－の位置に持つたピリミジン誘導体が放射線感受性を高めることが知られるようになつた．之等のものの増感剤としての効果を in vivo にて検討し，次の結果を得た．

1） 臓器重量から見て，放射線感受性の高い臓器程 5-Bromodeoxyuridine (BUDR) による放射線増感作用が著明であつた．

2） エールリツヒ腹水癌の分裂係数では，BUDRとX線の併用群に於て，X線照射群に比べ，照射６～10時間後での分裂係数の回復が遅れるのが見られた．

3） 皮下腫瘍の場合は，BUDRを腹腔内注射を行つた場合は，対照群との差がなかつたが，腫瘍内に直接注入を行つた場合には，照射後８～10日に於て対照群との間に比較的著明な差を認めた．

4） BUDRのみでは，分裂係数，腫瘍の発育率の低下は見られなかつた．

之等の作用機序としては Brom 原子がメチール基と Special Configuration が，にているため，Brom が Thymine の５－の位置と置換されて，之がDNAの中に incorporate され，之が放射線に対するもろさを増すものと思われる．生体使用の場合は，遺伝的な問題や，分割照射の時の効果等種々検討すべきと考えられる．

INTRODUCTION

The possibility of selective modification of cellular radiosensitivity by pretreatment with various chemical agents has received increasing attention in recent years.[1)2)]. Among those studies, there have been occuring an attempt to enhance the radiosensitivity of malignant tumor by chemical and physical agents[3)]. Such substances are called as potentiator or radiosensitizer.

The roles of oxygen in the biological effects of ionizing radiations has been clearly described by Dr. L.H. Gray[4], and was found oxygen had a remarkable radiosensitizing effects in vivo as well as in vitro.

Some authors reported that clinical applications of oxygen especially at high oxygen tension had a good results at the time of irradiation of cancer patients[5)].

Mitchel et al. reported Synkavi i.e. vitamin K derivatives had also radiosensitizing effects[6],

In addition, the intermediate metabolism of the synthesis of DNA and its pecursors has clarified. Taking advantage of the suggestion that uracil was utilized for nucleic acid synthesis in tumors, Heiderbeger et al[7]. created a new class of cytotoxic agents, the fluorinated pyrimidines. It was found that 5-Fluorouracil was effective clinically at very wide range in malignant disease particularly in solid tumors[8][15].

It has been reporting in recent years that the nucleosides and pyrimidine bases having I, Br, F, at 5 position of pyrimidine ring had the effects to enhance the radiosensitivities[9].

In the present paper, we examined the effects as the radiosensitizer of these pyrimidine derivatives and the mechanism of action against malignant tumor in vivo system.

EXPERIMENTAL METHODS AND RESULTS

The halogenated pyrimidine analogues which we used are as follows;

a) 5-Bromodeoxyuridine (BUDR)

b) 5-Bromodeoxycytidine (BCDR)

c) 5-Iododeoxyuridine (IUDR)

d) 5-Bromouracil (BU)

These constitutional formula are shown Fig.1 and Fig.2. The Chemicals were dissolved into distilled water and used.

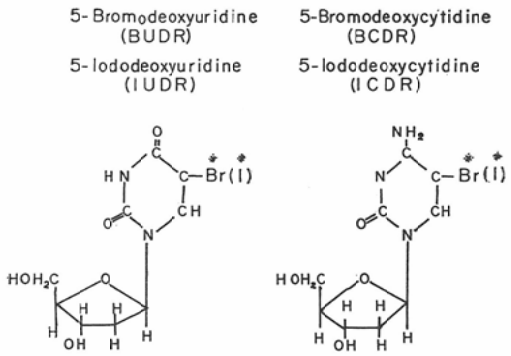

Fig. 1. Halogenated pyrimidine analogues

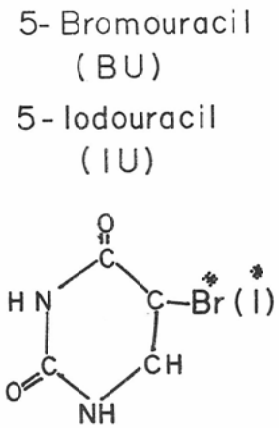

Fig. 2. Halogenated pyrimidine analogues

The physical factors of the X-irradiation were: 200kvp, 20mA, filtration 1.5 mm of Cu, HVL 1.8 mm of Cu, distance to target 50 cm, dose rate 42 r/min.

1) As the first experiment of BUDR etc in vivo, the influence against the organ weights were examined. CFI-mice, weighing 15 to 20 gm, were used. BUDR 10γ/g was injected intraperitoneally and 24 hours after injection, mice were given whole-body irradiation with 500r, and measured the weights of spleen, liver, thymus, ovarium, testis and kidney at 3 and 5 days after irradiation.

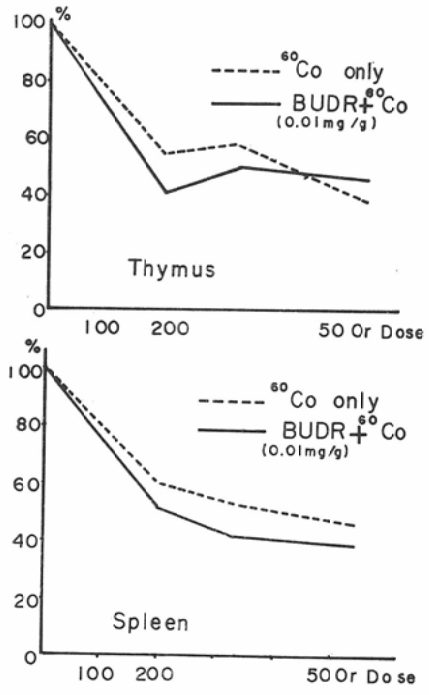

Fig. 3. Effects of ^{60}Co-ray on organ weights (thymus, spleen)

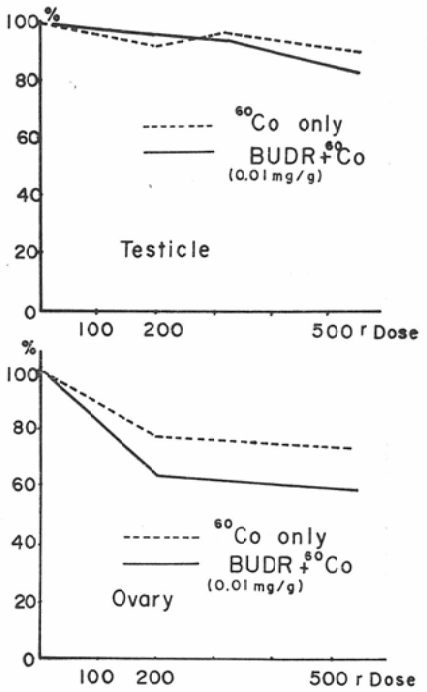

Fig. 4. Effects yf ^{60}Co-ray on organ weights (testicle, ovary)

The changes of organ weights of spleen and thymus were shown in Fig. 3. It was statistically different in spleen and thymus between group of ^{60}Co-irradiation only and group of ^{60}Co-irradiation combined with BUDR at the dosis of 200—330 r i.e. the combined group was much decreased its weight.

There were no difference in testis, but in ovarium the combined group was much decreased at the all test dosis and there was significant difference between them as shown Fig.4.5 We could not find difference, however, between both groups in liver and kidney. Fig. 5 represents these results. In view of these results, it shows that BUDR is more effective in the radiosensitive organs.

2) Males of d-d strain mice, weighing from 15—20 g, were used. Ehrlich ascites tumor were intraperitoneally tranplanted, and then BUDR (0.05 mg/g, body weight) intraperitoneally injected at 7 days after transplantation. Then the animals were given whole-body irradiation with 100, 200, and 300 r, three hours after injection of BUDR.

Mitotic index were examined 3, 6, 10, and 24 hours after irradiation.

The stain of tumor cells were performed with Dahlia violet and the numbers of mitosis among 1,000—2,000 tumor cells were examined. Fig. 6-a shows the experiment injected with BUDR (0.05 mg/g) and irradiated with 100 r.

Mitotic index decreased immediately after irradiation and reached maximum decrease between 2-3 hours after irradiation, and then gradually rose to normal or higer values by 24 hours. The decrease of mitotic index after irradiation was much greater in combined

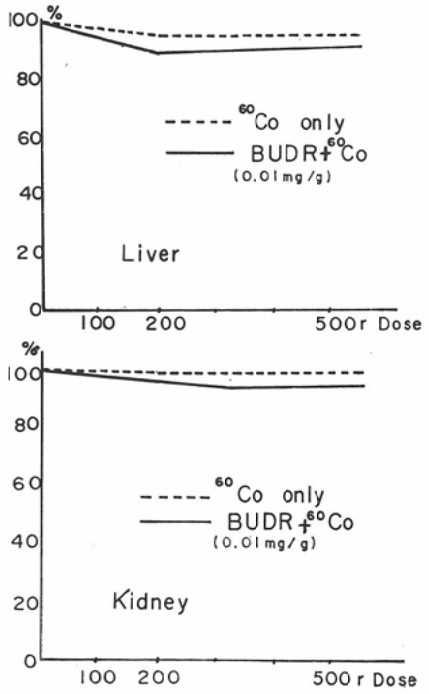

Fig. 5. Effects of ^{60}Co-ray on organ weights(liver, kidney)

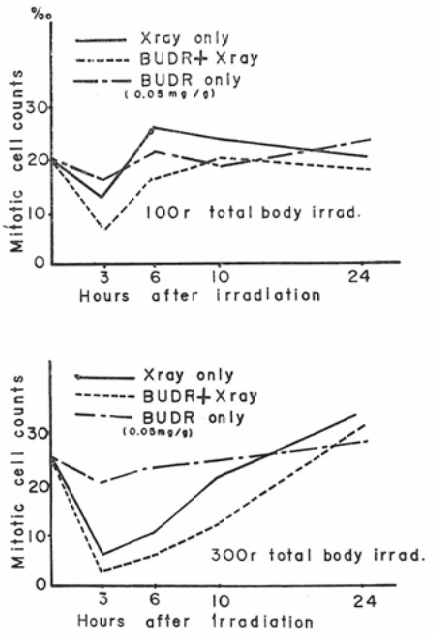

Fig. 6. Mitotic index in Ehrlich ascites tumor
a) 100 r total body irrad.
b) 300 r total body irrad.

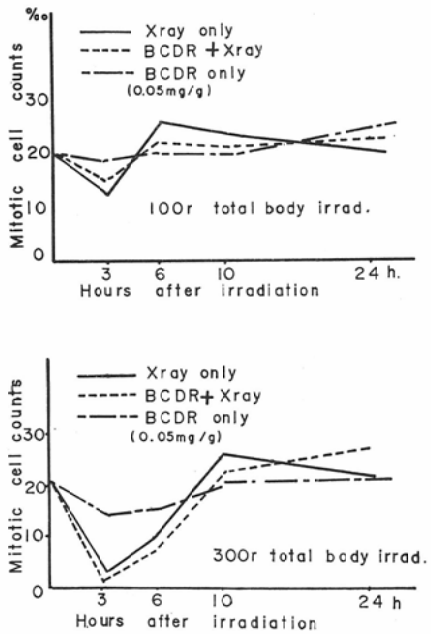

Fig. 7. Mitotic index in Ehrlich ascites tumor (BCDR)

Table 1. Recovery times of mitotic index to the values before irradiation (200 r total body irrad.)

Xray only	12.0h.	BUDR Xray	18.0h.	1.50
		IUDR Xray	16.4	1.36
		BU Xray	14.5	1.21
		BCDR Xray	11.5	0.95

(0.05mg/g)

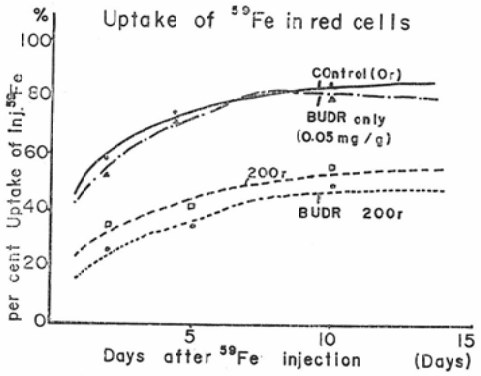

Fig. 8. Uptake of ^{59}Fe in rad cells

groups as shown Fig. 6-a, and statistically significant in 3,6, and 10 hours after irradiation. From these data, it is evident BUDR has a sensitizing effect. Fig. 6-b showed the case of 300 r total body irradiation and the combined groups also much decreased than X-ray only groups in the same manner Fig. 6-a. But mitotic index in the groups of BUDR only injected showed no significant changes. This indicated that BUDR had no antitumor properties in the dosage which we used.

As far as BCDR was concerned, there was no significant difference between groups of BCDR combined with X-irradiation and those of X-irrdiation only as shown Fig. 7.

Recovery times of mitotic index to the values before irradiation were measured by total body irradiation with 22 r. As shown Table 1, that of X-irradiation only group was 12.0 hours, but that of BUDR plus X-irrad. was prolonged to 18.0 hours, and IUDR, BU, and BCDR were 16.4, 14.5, and 11.5 respectively. In a standpoint of mitotic index, BUDR was most effective, next IUDR. BCDR was most ineffective as a sensitizer.

3) Twenty-four hours after irradiation with 200 r, 1 μc of ^{59}Fe was injected into the femoral vein of the rat. The uptake of ^{59}Fe in the red cells of the rat was followed over a period of 15 days using the method given by Hennessy and Huff[10]. After about 5 to 7 days in the control animal, the uptake of ^{59}Fe in red cells reached its maximum valus of around 80% of the injected amount.

In the combined group (BUDR+200 r) the uptake was decreased very markedly initially

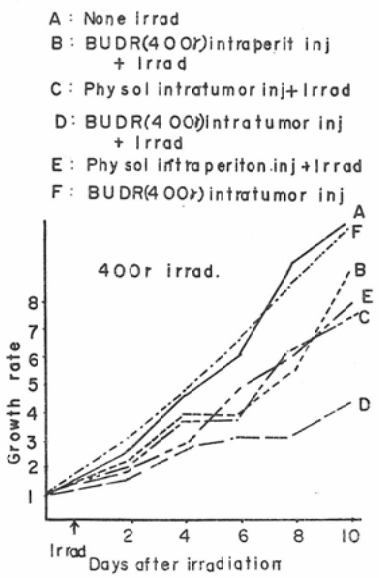

Fig. 9. The growth rate on the transplanted tumors

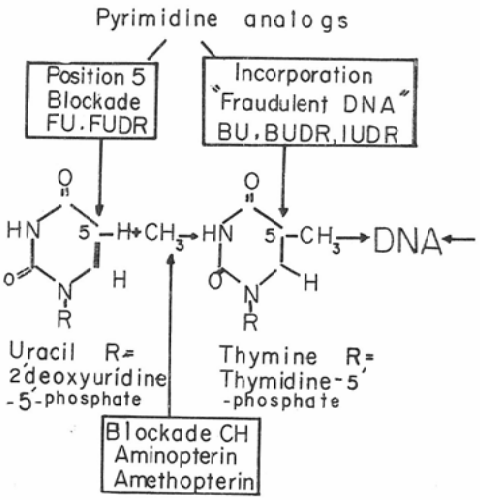

Fig. 10

and then rose to a constant maximum value at a lower than that of a group of 200 r irradiation only as shown Fig 8.

4) Solid tumors (AIM/4) were transplanted in the left groin region subcutaneously and examined the effects against the growth rate on transplanted tumors. The tumors grew gradually about 10×10×10 mm – 20×20×20mm eight days after transplantation and the tumors of these size were examined. Tumor growth rate was shown as (Major axis)×(minor axis) cm /initial tumor growth.

Transplanted mice were arranged into six groups:

A) none irradiation B) BUDR (0.4 mg/anim) intraperitoneally injection plus irradiation.

C) physiologic solution intratumor injection plus irrad.

D) BUDR intratumor injection plus irrad.

E) physiologic solution intraperitoneally injection plus irrad.

F) BUDR intratumor injection only

Local irradiation at the site of tumor with 400 r were performed 24 hours after injection. None irradiated tumors (A) was rapidely increased its size as shown Fig 9.

There were no significant difference between the groups of intraperitoneal injection of BUDR in conjunction with X-irradiation and those of X-irradiation only. Intratumor injected groups regressed more rapidly than other groups (B,E,C) by 10 days after irradiation. By this, it was found that intratumor injected group (D) showed the radiosensitizing effects. BUDR only injected groups(F) was no difference with none irradited groups (A), i.e. BUDR only had no antitumor properties in dosage which we used.

DISCUSSION

Many authors have reported radiosensitizing effects of halogenated pyrimidine analogues such as BUDR at a cellular level. Greer[11] has reported a striking increase of ultraviolet sensitivity in E. coli after incorporation of the thymine analogue, BU.

Djordjevic and Szybalski[9] have reported a similar response to ultraviolet in mammlian cells cultivated in vitro in the presence of BUDR, as well as the corresponding iodinated compound (IUDR). H.S.Kaplan[12] also demonstrated that both ultra-violet X-ray sensitivity of E. coli are enhanced when appreciable proportions of the natural bases of DNA are substituted by any of several purine and pyrimidine analogues. But little work has been done to study in vivo system.

The mechanims of BUDK is considered as follows[2]. Because the special configuration of the bromine atom closely resembles that of the methyl group, bromine may be substituted in the 5 position and actually incorporated into the DNA molecule as 5-bromouracil, replacing thymine, thus it is possible to trick the cell into synthesizing fradulent DNA. It has been suggested that the structural integrity of the DNA molecule is thereby compromised and that this might lead to an increased friability during roentgen irradation. Some of the biosynthetic pathways of DNA metabolism which may be interrupted by blockade with antimetabolities are diagrammatically summarized in Fig 10. Metabolic products of FU or FUDR (5-fluorouracil or 5-fluorodeoxyuridine) Prevent the utilization of uracil in the synthesis of thimine, a necessary constituent of DNA.

When BUDR was injected 24 hours before irradiation, the weights of radiosensitive organ such as spleen, ovarium and thymus were much regressed than those of radioresistant organ as shown Fig 3,4 and 5. It is clear from these data that BUDR is bocalifed in radiosensitive organs by means of preferential incorporation into proliferenting cells.

These results suggest that the radiosensitizing effect of BUDR opens new possibilities for radiotherapy of localized tumors for the selectivity of the radiosensitizing action for dividing cells i.e. primarily for neoplastic tissues. It is found that BUDR has very low toxicity for mice. That raised a quetion, however, as to whether BUDR might be incorporated into normal radioresistant tissues as well as neoplastic tissue later when BUDR would be delivered daily.

It is obvious that studies of the distribution of BUDR in normal and tumor tissue in the body are needed.

The influence of BUDR upon the genetics also remain obscure. It is an important problem how BUDR is given in vivo system. Kriss and Revesz[13] demonstrated in the rat that ^{82}Br-BUDR was rapidly debrominated by liver. Their detailed studies on the metabolic pathways of BUDR and BCDR also demonstrated that in the rat ^{82}Br-BUDR injected intravenously disappeared from the circulating blood. It was found in our experiments that intratumor injected groups had a radiosensitizing effect but intraperitoneally injected groups had not as shown Fig 9.

Cramer et. al. have found that ICDR and IUDR appeared to be equally effective in vivo

inhibiting the growth of lymphomas[14]. We could not find, however, BUDR only had an inhibiting effect, perhaps since the dosis were Small.

SUMMARY

We examined the radiosensitizing effects of halogenated pyrimidine analogues such as BUDR and BCDR etc in vivo system. The results were as follows:

1) It was found that the weights of radiosensitive organ were much decreased than that of radioresistant organ when BUDR was injected 24 hours before irradiation.

2) When the mitotic index of Ehrlich ascites tumors was used as a indicator, it was demonstrated that BUDR also had the effect as a potentiator.

3) So far as the transplanted subcutaneous tumor was concerned, it was more radiosensitive when BUDR was injected intratumor than intraperitoneally.

4) Iron uptake was also more strongly inhibited when BUDR was used before irradiation.

5) It was found that BUDR only had no effect as a inhibitor in the dosis which we used.

The mechanism of halogenated pyrimidine analogues which act as a radiosensitizer and its possibilities for radiotherapy have been briefly discussed.

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