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RADIOSENSITIZING EFFECT OF MISONIDAZOLE ON MAMMARY CARCINOMA OF C3H MICE

Kouji ONO, Toshifumi NAKAJIMA, Masahiro HIRAOKA, Akira MATSUMIYA and Yasuto ONOYAMA

Division of Radiation Therapy, Department of Radiology
Kawasaki Medical School, Kurashiki 701-01, Japan

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Abstract

Local tumor control rates and histopathological changes of spontaneous mammary carcinoma of C3H mice were investigated to assess the radiosensitizing effect of misonidazole (2-nitroimidazole). The dose of radiation to control a half of tumors was reduced to about 1/2 after i.p. administration of the drug. Histopathological study also elucidated a marked radiosensitizing effect of the drug to hypoxic cells in the tumor.

INTRODUCTION

Hypoxic cells in tumors are thought to be responsible for the failure of local control by irradiation in some instance, because these are more resistant to conventional radiation, such as X or γ rays, by a dose factor of 2.5-3.0[1~4]. One method to overcome the relative radioresistance of hypoxic cells is the use of high LET radiation, which kills cells in a way that is less dependent on the oxygen concentration than when X rays are used. Hypoxic cells are only 1.6 times more resistant to neutrons, and no more resistant to very heavy accelerated nuclei, in contrast to X ray. Fast neutrons produced by cyclotron or even more expensive negative pions produced by high energy proton synchrotron or linear accelerator are prime candidates at present[5~7].

Another method is the breathing high pressure oxygen before and during irradiation to increase the oxygen concentration in the blood, and hence in the tumor cells[8]. In theory, hyperbaric oxygen should raise the radiosensitivity of the hypoxic tumor cells by a factor of 2.5-3.0, but the clinical results reported from most institutes are rather disappointing[9]. The cause of its failure to raise the local control rates in human tumors is mainly attributed to the fact

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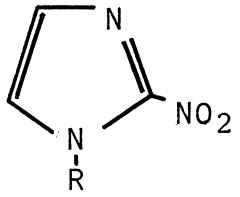
that oxygen is metabolized rapidly as it diffuses through the cells surrounding a capillary in the tumor and unable to sensitize the hypoxic tumor cells at a distance from the capillary. An alternative method to overcome this problem is the use of electron affinic drugs that can mimic the sensitizing effect of oxygen but are not so metabolized[10~12,18]. The most promising compounds of this kind are nitro-imidazoles, especially misonidazole (Fig. 1), which have been shown to sensitize hypoxic cells in vitro and in vivo[13~17,19].

The purpose of the present paper is to assess the effect of misonidazole as a hypoxic sensitizer, on the basis of the assay of 50% tumor control dose (TCD_{50}) for animal tumors of various sizes and the histological study of the irradiated tumor in the presence of this drug. This paper is an interim report on studies in progress, but the results obtained so far permit some tentative conclusions.



$R=CH_2CH(OH)CH_2OCH_3$

Fig. 1. Chemical structure of misonidazole (Ro-07-0582).

MATERIALS AND METHODS

Mice and tumors :

Eight-ten weeks old C3H/He mice of both sexes bred at the animal center of Kawasaki Medical School were used for this experiment. Spontaneous mammary carcinoma of the mice was stored in a refrigerator and the second generation isotransplant grown in subcutaneous tissue of the mice was removed and minced. The mince was diluted with Eagles' MEM containing 5% fetal calf serum, and the supernatant was filtered through a swinny filter to make a single cell suspension. Ten μl of the suspension, containing 10^5–10^6 viable cells, was injected subcutaneously into the right hind leg of the mice. The tumor reached a mean diameter of 8 mm 3.5 weeks after the injection, and 13 mm 4.5 weeks.

Irradiation :

Tumors were irradiated when they reached a given diameter. To irradiate the tumor, the mice were anesthetized with the intraperitoneal injection of

sodium pentobarbitone (Nembutal) at a dosage of 50 mg/kg body weight, or slightly less (ca. 25%) when the animals received the sensitizing drug. The mice were mounted 5-6 at a time on the 15 mm thick Acryl plate which provided a build up of the secondary electrons of 6MV X ray. Tumor bearing legs were irradiated through the plate with a single dose ranging from 2500 to 8000 rad. The target skin distance was 60 cm and the dose rate was about 500 rad/min. During irradiation, the mice were breathing air at atmospheric pressure and at room temperature, and no attempts were made to obstruct the blood flow to the tumor.

Drug :

Mice receiving the drug, misonidazole were injected intraperitoneally 30 minutes before the start of irradiation, with a dose of 1 mg of misonidazole disolved in sterile saline solution per gram body weight.

TCD_{50} assay :

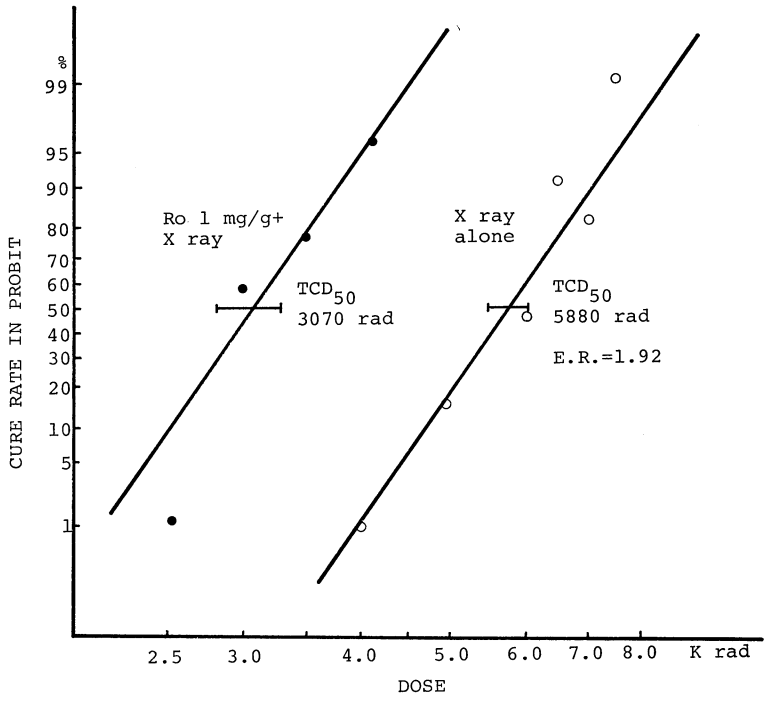
After irradiation, animals were inspected for the presence of the tumor and for the tumor size at intervals of one or two weeks. At 80 days, all palpable tumors were scored as uncontrolled. Questionable tumors were examined histologically. Based on tumor control frequency at each dose level of radiation on day 80, the dose required to yield 50% local tumor control (TCD_{50}) was determined by means of logit analysis.

Histological study :

Tumors of mean diameter of 15 mm were used for this study, because the tumor cords were not clear in the smaller ones. Six hours after the single dose irradiation of 1250 rad, about 2 ml of Indian ink was injected through the tail vein for microangiography. The tumor was extirpated en bloc, and fixed with 10% formaldehyde solution. H. E. stained thin sections (3μ thick) were prepared for histological study. The frequency of the degenerated nuclei in relation to the distance from capillary was recorded at intervals of 10 μ. Nuclei showing aggregation of chromatin granules, pycnosis and fragmentation were scored as degenerated.

RESULTS

Fig. 2 shows the radiation dose-local tumor control response curves for smaller (under 10 mm in diameter) and larger tumors (between 10 and 15 mm) treated by single irradiation with or without sensitizing drug. TCD_{50} for the smaller tumors were 5880 rad (5360-6440 rad) in the irradiated controls and 3070 rad (2830-3330 rad) in the sensitized ones. For the larger tumors, the values were 7000 rad (6770-7230 rad) and 3360 rad (3180-3550 rad) respectively. Figures in parenthesis mean the 95% confidence limits. The enhance-


CURE RATE IN PROBIT
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Ro 1 mg/g+
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3070 rad
X ray
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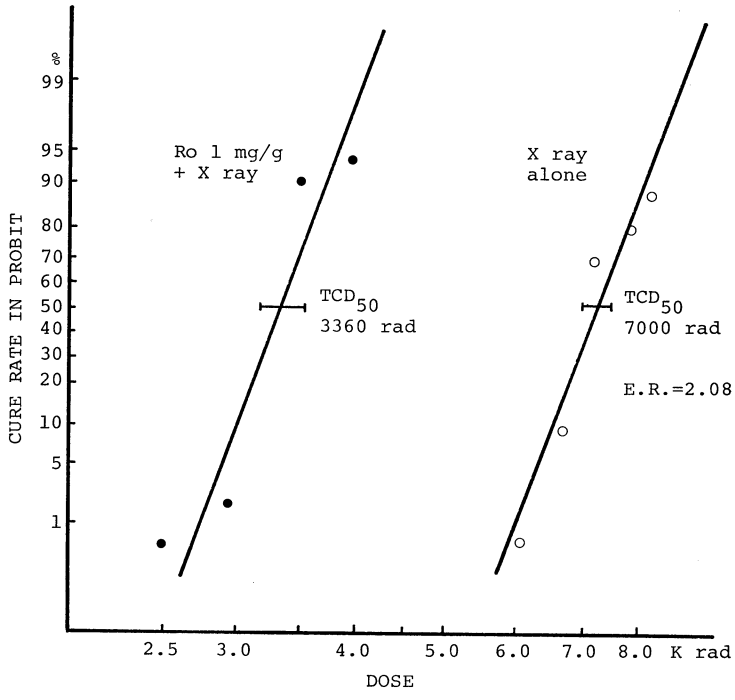
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Fig. 2. Proportion of isotransplants of C3H mouse mammary carcinoma controlled at 80 days vs single radiation doses. Open circles, radiation alone. Closed circles, radiation delivered 30 min. after i.p. injection of 1 mg/g body weight of misonidazole. a. for smaller tumors (under 10 mm in diameter), and b. for larger ones (between 10 and 15 mm).

ment ratios, TCD_{50} drug $(-)/TCD_{50}$ drug $(+)$ were 1.92 for the smaller tumors and 2.08 for the larger.

The relative frequency of the degenerated nuclei for unirradiated tumors is plotted in Fig. 3 as a function of distance from capillary. At 70–80μ from a central vascular core, the incidence of abnormal nuclei begins to increase gradually, and most tumor cells show necrosis beyond 100μ. This means the limit of diffusion of oxygen and other nutrients from the capillary. Fig. 4 shows the microphotographs of the tumor irradiated in the presence of misonidazole. The two curves in Fig. 5, solid line for drug (+) group and dotted for X ray alone, show the same degrees of the relative incidence of degenerated nuclei in the vicinity of the capillary. The frequency of degenerated nuclei in the tumor irradiated without the drug drops markedly at a distance ranging from 40 to 60μ from the capillary, however no such decrease is

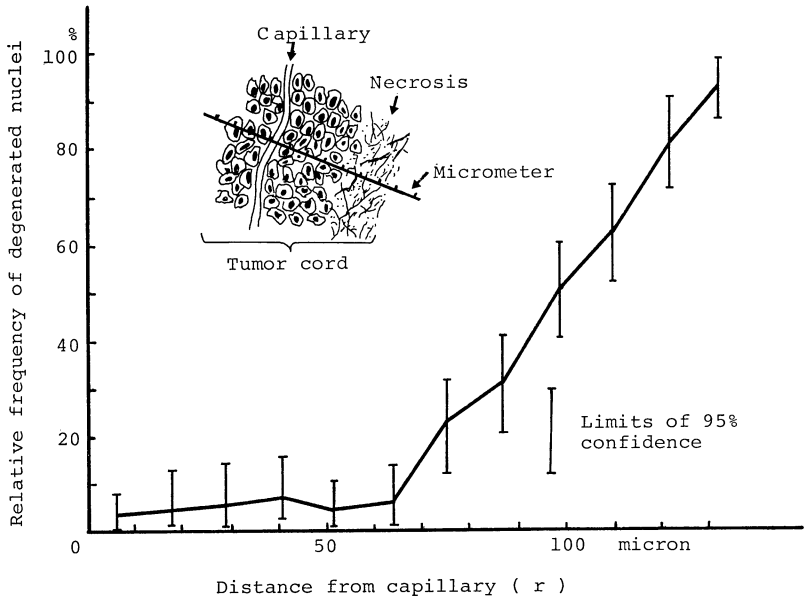

Fig. 3. Plot of the relative frequency of degenerated nuclei as a function of distance from capillary for the unirradiated tumor.

observed in the sensitized tumor. The differences in these region are statistically significant. Beyond 80μ, the frequency increases again equally in both groups to the same level in the spontaneously necrotic region of the unirradiated tumor.

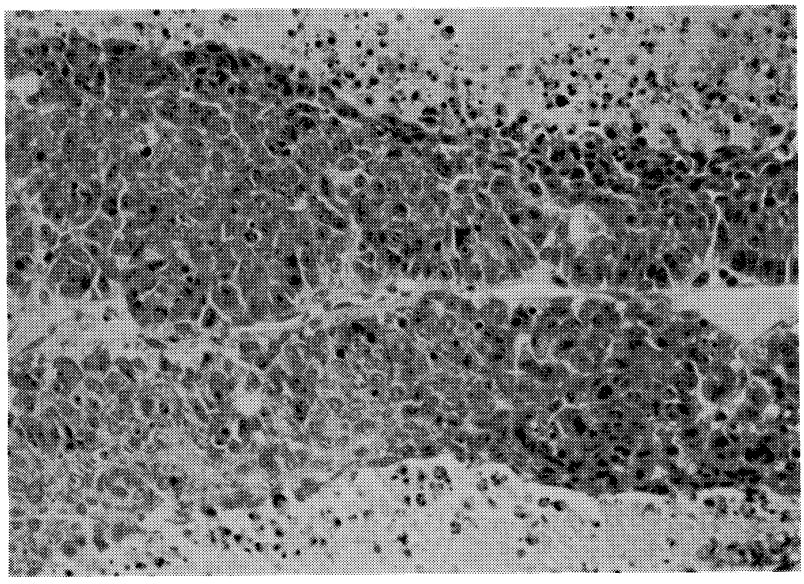

Fig. 4. Microphotograph of the tumor 6 hours after a single dose irradiation of 1250 rad in the presence of misonidazole (H. E. stain). ×200 Tumor cord and core capillary are clearly visible and numerous tumor cells show nuclear degeneration due to irradiation.

DISCUSSION

To assess the effect of misonidazole as a hypoxic sensitizer, we determined first the degree of sensitization of the drug as a function of tumor volumes. In tumor radiotherapy, it is well known that the larger the tumor is, the more difficult to obtain local control, if the other conditions are the same. The dose of radiation to control the tumor correlates in a complex way with the number of tumor cells, the size of clonogenic cell fraction and so on, however a part of low radiocurability of the larger tumor is attributable to higher proportion of hypoxic but viable cells in the larger as compared with the smaller[20]. In our present experiment, the dose response curve for larger tumors irradiated without the drug not only shows shift to the higher dose side, but also shows more steep slope than for the smaller (Fig. 2). This indicates that there is a prominent variation in the proportion of hypoxic cells among smal-

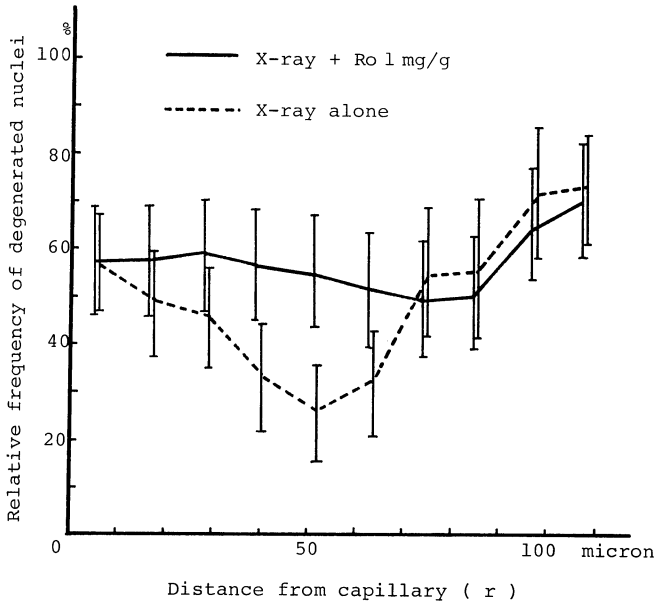

Fig. 5. Plot of the relative frequency of degenerated nuclei as a function of distance from capillary for the tumors 6 hours after a single dose irradiation of 1250 rad. Solid line, radiation alone. Dotted line, irradiated 30 min. after i. p. injection of misonidazole at 1 mg/g body weight.

ler tumors, whereas most tumor cells of the larger are hypoxic even in air-breathing condition. Therefore, we intended to assess the effect of misonidazole as a hypoxic sensitizer by means of determinating the relationship between tumor size and TCD_{50} after irradiation with or without the drug.

The dose required to control a half of tumors by X ray alone was significantly higher for the larger tumors as compared with that for the smaller, but much less difference was observed in the presence of misonidazole (Fig. 2). Enhancement ratios in our present experiment using misonidazole at 1 mg/g body weight, 1.92 for smaller tumors and 2.08 for larger ones, were similar those reported by other authors.[14,15,17] There is no statistically significant difference between the enhancement ratios for both groups, however these figures suggest that misonidazole can reach to the hypoxic cells at a distance from capillary, and sensitize them even in the larger tumors, which would contain more hypoxic cells than the smaller. Furthermore, the ability of misonidazole reaching to all the hypoxic cells is clear from the parallel shift of the dose response curve to the left in the presence of the drug, which is in no case for the hyperbaric oxygen therapy[20].

Histopathological study of the irradiated tumor also proved the diffusability of misonidazole to the hypoxic cells at a distance from capillary. As expected from the gradient of oxygen tension along the radii of tumor cord, the relative frequency of damaged cells tended to decrease in accordance with the increase of the distance from capillary for the tumor treated by X ray alone. On the contrary, no such decrease was observed for the tumor irradiated in the presence of misonidazole (Fig. 5). The same degree of nuclear degeneration continued to occur until reaching to the region of spontaneous necrosis. This fact strongly suggests that misonidazole, unlike oxygen, can sensitize all the hypoxic cells regardless of the distance from capillary.

Our experimental results indicate that misonidazole is markedly effective to sensitize radioresistant hypoxic cells of animal tumor, from the view points of both tumor control rate and histological changes of the irradiated tumors. For clinical use, further studies are necessary on the effect at a lower dosage of the drug applicable in radiation therapy for human cancers, maximum tolerance dose of the drug, and the optimal schedule of radiation therapy in the presence of this drug.

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