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# MISONIDAZOLE WITH SMALL DOSE FRACTIONS IN AN EXPERIMENTAL OSTEOSARCOMA

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**Summary.—The mouse osteosarcoma C22LR was irradiated with a single X-ray dose of 1000 or 1500 rad (to prevent rapid growth) and then with fractionated doses of 300 or 600 rad each. Half the mice were given 0·8 mg/g of misonidazole 1 h before irradiation. Tumour growth delay was determined. The radiosensitizer did enhance the effect of small (300 rad) doses per fraction, as well as that of 600 rad doses.**

IN an earlier publication (van Putten and Smink, 1976) we have shown that in our slowly reoxygenating mouse osteosarcoma misonidazole does not cause sensitization if applied with dose fractions of 300 rad or less. Although this tumour is slow in reoxygenation and may be shown to contain a large fraction of hypoxic cells in studies in which the surviving clonogenic cells are analysed by transplantation studies, it is possible that the hypoxic cells, if left *in situ*, are not relevant for the regrowth. It has been suggested (van Putten, 1968, 1977; MacNally, 1973) that *in situ* many of the hypoxic cells die, especially if pushed away from nutrient capillaries by continuing proliferation of well oxygenated cells. For this reason further tests were performed with this system.

## MATERIALS AND METHODS

Mouse osteosarcoma C22LR is a non-immunizing tumour, induced by strontium-90 in 1957 in a hybrid of CBA/Rij and C57BL/Ry mice. A large number of ampoules of passages 75 and 76 are stored in liquid nitrogen and all experiments are performed with passages 76 to 83 derived from this source. Flank tumours are obtained by the s.c. inoculation of $10^6$ cells, the tumours are measured twice weekly by caliper in 3 dimensions at right angles. Relative tumour volumes are obtained by multiplication of the 3 diameters and treatment is usually started when this parameter is between 50 and 150 $mm^3$. Groups of 6 to 10 mice were used to compare tumour growth without treatment or after irradiation with and without sensitizer. Results are expressed as relative tumour volume compared to the volume at the start of treatment and averaged for all tumours per group.

Misonidazole, a gift of Roche Products Ltd., Welwyn Garden City, was administered i.p. as a suspension in 2% carboxymethylcellulose. Local tumour irradiation under nembutal anaesthesia was performed with a Philips-Muller X-ray machine operated at 300 kVp, 10 mA giving a dose rate of about 350 rad/min.

## EXPERIMENTAL PROGRAMME AND RESULTS

In previous studies we encountered the difficulty of measuring growth delay in a tumour that continues to grow under radiotherapy and in which the initially hypoxic cells may have died and thus failed to contribute to tumour regrowth. To avoid this, each treatment week was preceded by a high radiation dose before the fractionated therapy combined with the sensitizer was started. In the first experiment 1000 rad was used initially, followed by 3 doses per week of 300 or 600 rad, preceded by a sensitizer 1 h before irradiation 800 mg/kg or not. The results presented in Fig. 1 show that a clear effect of the sensitizer given before each 600 rad dose is evident but the effect with the 300 rad doses are less clear. In the second experiment of this type the initial weekly

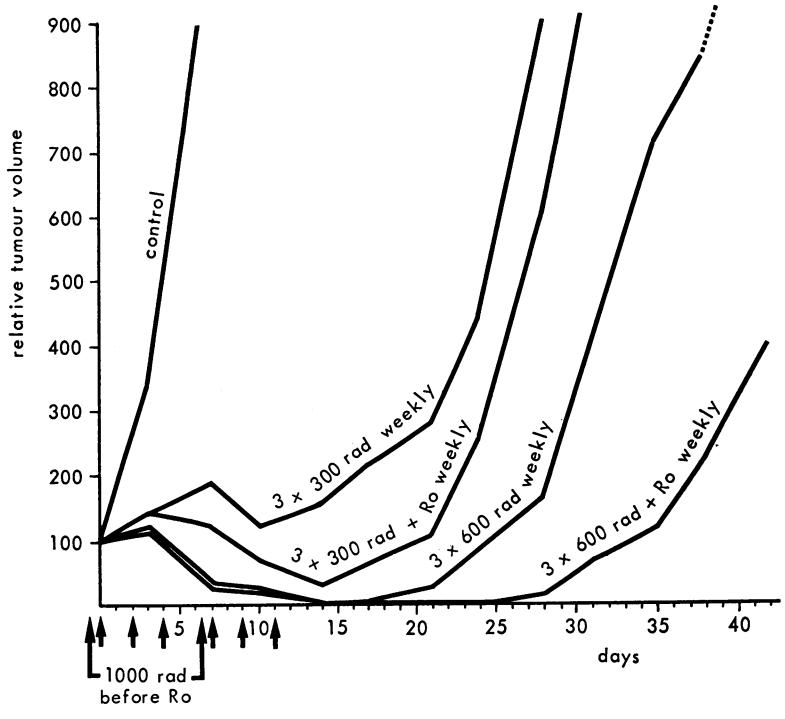


FIG. 1.—Effect of misonidazole applied with small fractions of radiotherapy to a mouse osteosarcoma. In order to enhance the hypoxia and the survival of hypoxic cells all treated groups received a weekly additional radiation dose of 1000 rad before injection of the sensitizer. Misonidazole 800 mg/kg i.p. was given 1 h before the small dose irradiation.

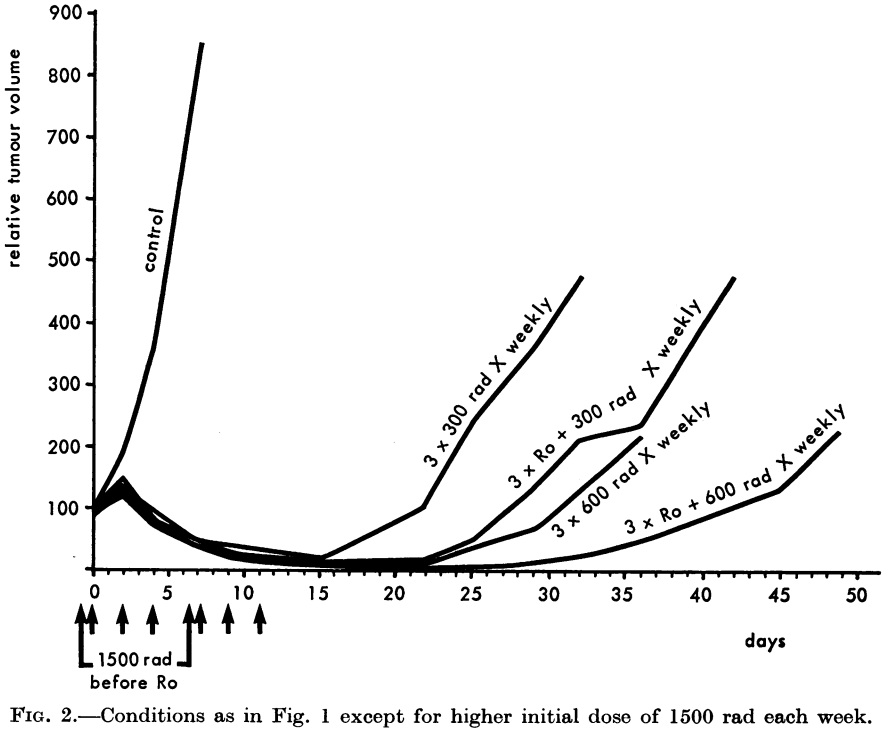


FIG. 2.—Conditions as in Fig. 1 except for higher initial dose of 1500 rad each week.

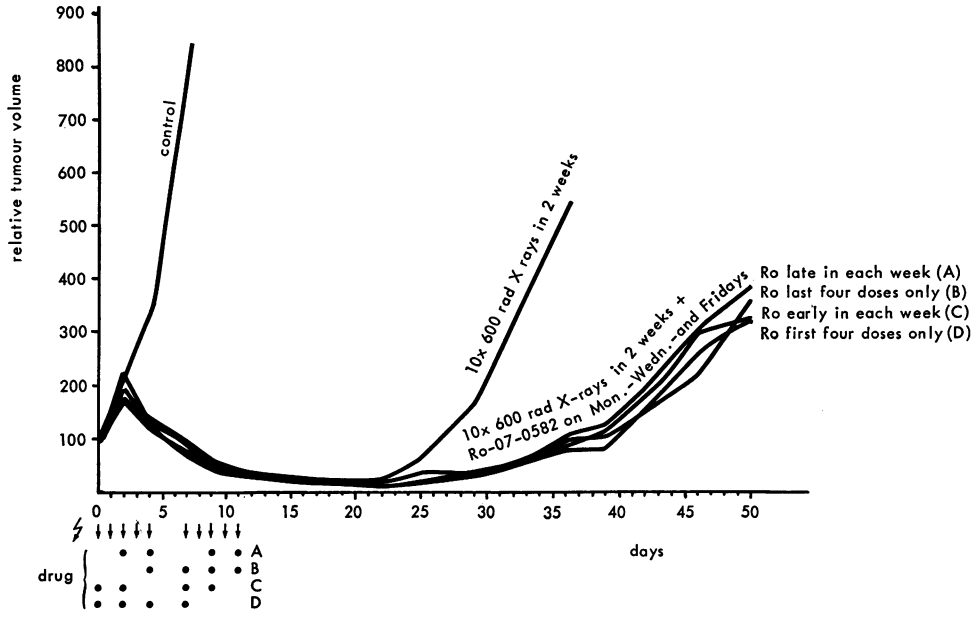


FIG. 3.—Optimal timing of sensitization. Tumour growth delay was measured after exposure to X-rays: weekly 5 × 600 rad for 2 weeks with or without misonidazole 800 mg/kg 1 h before X-rays 4 times in different schedules.

dose was raised to 1500 rad and in this case the results (Fig. 2) clearly indicate a marked effect of the sensitizer if combined with 300 rad fractions. Evidently, the increased hypoxia and rapid shrinkage induced by the initial large radiation dose facilitated the observation of the sensitization with small doses.

Since toxicity will not permit the administration of the sensitizer with each of a large number of small dose fractions, additional studies were performed to see whether an optimal timing of sensitization in the course of fractionated treatment could be identified. Treatment was given with 600 rad 5 times weekly for 2 weeks. Since alternate day treatment with the sensitizer would avoid acute toxicity, it was administered only on Mondays, Wednesdays or Fridays. Of the total of 6 possible days of treatment, 4 were selected: either the first 4 days, the last 4 days, the first 2 treatments each week or the last 2 each week. Results are presented in Fig. 3. This indicates that there is no difference in effectiveness depending upon the selection of sensitizer timing.

## CONCLUSIONS

These studies using tumour growth delay by radiation as an endpoint, have conclusively indicated that potentially the hypoxic sensitizer misonidazole can enhance the effect of small doses (300 rad per fraction) of X-rays although the reoxygenation occurring during fractionated radiotherapy will usually hide this capacity of the drug. If applied with larger doses of radiation (600 rad per fraction) the sensitizer could be shown to be effective but no preference among 4 possible dosage schedules could be detected.

After presentation of these data it was found that due to a conversion calculation error all radiation doses were actually 11·5% lower than given in this paper.

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## REFERENCES

MACNALLY, N. J. (1973) A Comparison of the Effect of Radiation on Tumour Growth Delay and Cell Survival. The Effect of Oxygen. *Br. J. Radiol.*, **46**, 450.

VAN PUTTEN, L. M. (1968) Oxygenation and Cell Kinetics after Irradiation in a Transplantable Osteosarcoma. In *Effects of Radiation on Cellular Proliferation and Differentiation*. Ed. Vienna: I.A.E.A., p. 493.

VAN PUTTEN, L. M. (1977) Reoxygenation of Hypoxic Tumour Cells. *Strahlentherapie*, **153**, 380.

VAN PUTTEN, L. M. & SMINK, T. (1976) Effect of Ro 07-0582 and Radiation on a Poorly Reoxygenating Mouse Osteosarcoma. In *Modification of Radiosensitivity of Biological Systems*. Ed. Vienna: I.A.E.A., p. 179.