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INCREASED ENHANCEMENT OF TUMOR RESPONSE TO X RAYS AND HIGH-LET NEON IONS BY DESMETHYLMISONIDAZOLE RELATIVE TO MISONIDAZOLE

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T.S. Tenforde, S.B. Curtis, S.S. Parr,  
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INCREASED ENHANCEMENT OF TUMOR RESPONSE TO X RAYS AND HIGH-LET  
NEON IONS BY DESMETHYLMISONIDAZOLE RELATIVE TO MISONIDAZOLE

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Short communication:

Increased enhancement of tumor response to X rays and high-LET  
neon ions by desmethylmisonidazole relative to misonidazole

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1. Introduction

The electron affinic compound misonidazole and its O-demethylated derivative, desmethylmisonidazole, have both been demonstrated to exert a significant sensitization of hypoxic cells to killing by low linear-energy-transfer (LET) radiation from X rays (Adams et al. 1976, Flockhart et al. 1978, Rauth et al. 1978). Recent studies have demonstrated that misonidazole is effective as an in vivo sensitizer for tumors exposed to high-LET radiation from cyclotron neutrons (Denekamp et al. 1977, Porschen et al. 1978, Guichard et al. 1980) and accelerated beams of carbon and neon ions (Tenforde et al. 1981a, Guichard et al. 1982). The experiments reported here were designed to test the relative effectiveness of misonidazole and desmethylmisonidazole for the enhancement of tumor growth delay when used in combination with high-LET radiation from neon ions in the extended-peak ionization region.

## 2. Materials and methods

### 2.1. Rhabdomyosarcoma tumors and volume response measurements

Measurements of radiation-induced growth delay were made with rhabdomyosarcoma R-1 tumors transplanted subcutaneously in the thoracic region of syngeneic WAG/Rij rats (Barendsen and Broerse 1969, 1970). The tumor subline used in these experiments, designated R1/LBL, was previously used for measurements of growth delay following irradiation with accelerated beams of helium, carbon, neon and argon ions (Curtis et al. 1978, Tenforde et al. 1981b), and with carbon and neon ions administered in combination with misonidazole (Tenforde et al. 1981a).

The regression and regrowth of R1/LBL tumors following irradiation were monitored by caliper measurements of tumor volume using methods described previously (Curtis et al. 1978). Radiation-induced growth delay was calculated from the difference in time for irradiated and nonirradiated tumors to grow to twice their volumes measured on the day of irradiation. The average initial volume of the tumors used in these experiments was  $0.53 \pm 0.03$  (S.E.)  $\text{cm}^3$ , and the volume doubling time for nonirradiated tumors of this size was 4.5 days.

### 2.2. Sensitizers: toxicity and drug kinetics

Misonidazole (Ro 07-0582; NSC 261037) and desmethylmisonidazole (Ro 05-9963; NSC 261036) were kindly supplied by Dr. C.E. Smithen of Roche Products Ltd. (Welwyn Garden City, Herts, England) and by the Drug Liaison and Distribution Section of the National Institutes of Health (Bethesda, Maryland). The chemical identity of the two drugs was confirmed by mass spectrographic analysis.

The toxicity and in vivo uptake and clearance kinetics of misonidazole in WAG/Rij rats have previously been described in detail (Tenforde et al. 1981a).

In single-dose experiments involving the combined administration of misonidazole and either X rays or charged-particle radiation, the sensitizer was injected intraperitoneally at the maximum tolerated level of 500 mg/kg. At this dose level, no lethality resulted from the administration of misonidazole either with or without tumor irradiation (Tenforde et al. 1981a).

As a preliminary to radiation response studies with desmethylmisonidazole, the gross toxicity resulting from the injection of a single dose of this compound was assessed in WAG/Rij rats. The drug was dissolved in 37°C physiological saline solution at a concentration of 75 mg/ml, and injected into groups of five rats as an intraperitoneal bolus at doses of 1000, 1500, 1750, 2000, 2500 and 3000 mg/kg. No mortality occurred with doses of 1000 or 1500 mg/kg. At dose levels of 1750, 2000 or 2500 mg/kg, 40 percent of the animals expired within two days following the drug injection. With a 3000 mg/kg dose, 60 percent of the rats died within two days post-injection. The WAG/Rij rats receiving a 1500 mg/kg dose were observed for a period of one year following drug administration, and were found to exhibit no signs of paralysis or other neuropathic symptoms during this interval. Studies of tumor radiation response with combined administration of desmethylmisonidazole were therefore conducted using the maximum tolerated drug level of 1500 mg/kg.

Because a high concentration of misonidazole has been reported to be directly cytotoxic to tumor cells (Brown 1977), experiments were carried out to determine whether the intraperitoneal dose levels of misonidazole and desmethylmisonidazole used in our studies produced tumor growth delay in the absence of irradiation. As described previously (Tenforde et al. 1981a), a 500 mg/kg intraperitoneal dose of misonidazole was not found to be cytotoxic to R1/LBL tumors. Following an intraperitoneal 1500 mg/kg dose of desmethylmisonidazole, the average doubling time for a group of 7 tumors was observed to

be  $4.3 \pm 1.1$  (S.D.) days; a parallel set of 7 tumor-bearing rats were sham-injected with physiological saline solution, and their average tumor doubling time was  $3.8 \pm 0.7$  days. These data clearly indicate that desmethylmisonidazole is also noncytotoxic at the dose level used in our experiments.

The uptake and clearance of desmethylmisonidazole in the circulation and in R1/LBL tumors were assayed spectrophotometrically by the procedures used previously in measurements of misonidazole kinetics (Tenforde et al. 1981a). Desmethylmisonidazole was extracted from blood plasma and homogenized tumor tissue with 95 percent ethanol, and the concentration of sensitizer was determined from the optical density of the extract at 317 nm in comparison with a desmethylmisonidazole standard curve (extinction coefficient at 317 nm in 95 percent ethanol:  $7.21 \text{ cm}^2/\text{micromole}$ ). The results of these kinetic studies are shown in figure 1. The concentration of desmethylmisonidazole achieves a peak level in blood plasma of 5.5–6.0 micromoles/ml at 15–60 min following an intraperitoneal 1500 mg/kg dose. A peak level of 4 micromoles/g is reached in tumor tissue at 30–60 min post-injection. Desmethylmisonidazole was therefore administered at 30–60 min prior to tumor irradiation in order to obtain a maximum sensitizing effect of the drug. After a peak concentration of desmethylmisonidazole was reached, the drug was cleared exponentially from plasma and R1/LBL tumors with a  $t_{1/2}$  of 1.3 hr.

The in vivo uptake and clearance pattern for desmethylmisonidazole shown in figure 1 is significantly different from that described previously for misonidazole (Tenforde et al. 1981a). First, when administered to WAG/Rij rats at the maximum tolerated intraperitoneal dose level, desmethylmisonidazole achieves a concentration in the blood plasma that is twice as high as the peak concentration reached by misonidazole (6mM versus 3mM). The maximum tumor level of desmethylmisonidazole is also elevated by one third above that reached



by misonidazole (4 micromoles/g versus 3 micromoles/g). Second, after reaching a peak level in plasma and tumor tissue, the concentration of desmethylmisonidazole decreases significantly faster than misonidazole, which has a clearance  $t_{1/2}$  of 3 hr for plasma and 4 hr for tumor tissue (Tenforde et al. 1981a). Third, at 30-60 min following injection of desmethylmisonidazole, approximately 20 percent (3/14) of the injected animals exhibited a drug concentration in blood plasma and tumor tissue that was more than one standard deviation below the mean value observed for the other rats in the same group. A similar heterogeneity in sensitizer concentration was not observed with misonidazole. It was determined that the animals exhibiting low levels of desmethylmisonidazole in the blood plasma and tumor tissue at 30-60 min post-injection also had low levels of the drug in lung tissue and in the abdominal cavity, i.e., at the site of injection. From these observations, it was concluded that a small fraction of the injected animals rapidly metabolized and/or excreted the desmethylmisonidazole. However, despite the heterogeneity in the maximum drug concentration reached in tumor tissue, an evaluation of the radiation-induced growth delay data presented in this report did not reveal a well-defined sub-population of tumors exhibiting an atypically small response to combined treatment with the sensitizer and radiation.

### 2.3. Radiation procedures

Tumors were exposed to high-energy beams of fully-stripped neon ions produced at the Berkeley Bevalac facility (Grunder et al. 1971). The tumors were positioned in the distal 1.5 cm portion of a 4 cm extended-peak ionization region, as shown in figure 2. In experiments with both misonidazole and desmethylmisonidazole, the neon-ion beam was accelerated at an initial energy of 557 MeV/u. In our irradiation procedure, the residual range in water of this beam was 22 cm. The dose-averaged LET values within the tumor treatment

zone ranged from 115-240 keV/ $\mu$ m.

Details of beam collimation techniques, dosimetry and tumor irradiation procedures have been described previously (Curtis *et al.* 1978). Dosimetry was performed with parallel plate ionization chambers, and the dose calibration was made by placing a tissue-equivalent EGG chamber (EGG Corporation, Goleta, California) at the tumor location. The absorbed dose rate in the tumor mass ranged from 2-10 Gy/min. Homogeneity of the radiation field across the breadth of the tumors was confirmed by exposing therapy verification film (Kodak V/RP, Eastman-Kodak, Rochester, New York) at the tumor irradiation position. From densitometer scans of the developed film, it was determined that the dose delivered across the width of the tumors was uniform to within  $\pm$  5 percent.

The reference source of low-LET radiation was 225-kVp X rays from a Philips RT200/250 unit, with a total filtration of 0.35 mm Cu (mean photon energy = 75 kV; HVL = 1.08 mm Cu). Procedures for collimation of the X-ray beam have been described previously (Curtis *et al.* 1978). The X-ray dose rate measured with a 250 R Victoreen ionization chamber was 6 Gy/min at the tumor location.

Animals were anesthetized with Metofane (Pitman-Moore, Inc., Washington Crossing, New Jersey) during tumor irradiation with both X rays and neon ions. Misonidazole and desmethylmisonidazole were administered intraperitoneally at 30-60 min prior to irradiation without the use of anesthesia.

### 3. Results

Figure 3 presents the growth delay versus dose curves obtained for R1/LBL tumors administered single doses of X rays and extended-peak neon-ion radiation, either alone or in combination with a 500 mg/kg intraperitoneal dose

of misonidazole or a 1500 mg/kg intraperitoneal dose of desmethylmisonidazole. It is apparent for both radiation modalities that a sensitizing action resulted from the administration of these drugs, with the enhancement of tumor radiation response by desmethylmisonidazole exceeding that achieved by misonidazole. In Table 1, the dose enhancement ratios are listed at the 20-day and 50-day growth delay levels for misonidazole and desmethylmisonidazole administered in combination with X rays and extended-peak neon ions. When combined with 225-kVp X rays, the enhancement ratios for desmethylmisonidazole (2.1-2.4) were approximately 15 percent higher than the enhancement ratios for misonidazole (1.8-2.1). A similar percentage difference between the enhancement ratios for these two compounds was observed with neon ions, namely, 1.4-1.5 for desmethylmisonidazole as compared to 1.2 for misonidazole.

Values of the relative biological effectiveness (r.b.e.) at the 20-day and 50-day growth delay levels are listed in table 2 for extended-peak neon ions administered alone or in combination with misonidazole or desmethylmisonidazole. It is evident that the r.b.e. decreases by 35 to 40 percent when the tumor irradiation is combined with administration of either of the hypoxic cell sensitizers. This reduction in r.b.e. results from the fact that the enhancement of tumor radiation response by both sensitizers is much greater with low-LET X rays than with high-LET neon ions. It should also be noted that the r.b.e. for extended-peak neon ions without administration of a radiosensitizing drug is greater than the enhancement ratios observed for X rays in combination with either misonidazole or desmethylmisonidazole. From this observation it can be concluded that the neon-ion dose required to produce a given level of tumor growth delay is less than the X-ray dose that must be given to produce the same growth delay even when the X-irradiation is administered in combination with either of the two hypoxic cell sensitizers.

#### 4. Discussion

The radiation-induced tumor growth delay studies reported here demonstrate that desmethylmisonidazole exerts a significantly greater radiosensitizing effect than misonidazole when these compounds are administered at their maximum tolerated intraperitoneal dose levels. It has been further demonstrated that the increase in dose enhancement ratios for desmethylmisonidazole relative to misonidazole is similar with low-LET X rays and high-LET neon ions in the extended-peak ionization region. Because the one-electron reduction potentials of misonidazole and desmethylmisonidazole are identical (Wardman and Clark 1976), it would be expected that their radiosensitizing activities would also be similar if both compounds were present in tumor tissue at identical concentrations. The most probable explanation for the greater enhancement of tumor radiation response by desmethylmisonidazole is therefore the higher concentration in tumor tissue that was achieved with this sensitizer (4 micromoles/g) relative to misonidazole (3 micromoles/g). It was possible to obtain this differential in sensitizer concentrations within R1/LBL tumors because of the threefold greater tolerance of WAG/Rij rats for desmethylmisonidazole relative to its less hydrophilic parent compound misonidazole.

In summary, when misonidazole and desmethylmisonidazole are administered under conditions that achieve maximum levels of these two sensitizers in tumor tissue, the single-dose enhancement ratios with extended-peak neon ions are 1.4-1.5 for desmethylmisonidazole as compared to 1.2 for misonidazole. The results of these studies therefore indicate the significant potential of desmethylmisonidazole for the enhancement of tumor response to high-LET radiation from charged-particle beams.

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Table 1. Enhancement ratios for radiation-induced growth delay of R-1 tumors following combined treatment with accelerated neon ions and misonidazole or desmethylmisonidazole

Radiation Modality <sup>+</sup>	Sensitizer <sup>‡</sup>	ER <sub>20</sub> <sup>§</sup>	ER <sub>50</sub> <sup>§</sup>
X rays	Misonidazole	1.8	2.1
	Desmethylmisonidazole	2.1	2.4
Neon ions	Misonidazole	1.2	1.2
	Desmethylmisonidazole	1.4	1.5

<sup>+</sup> The X-ray energy was 225 kVp. For the neon-ion irradiations, tumors were positioned in the distal 1.5 cm portion of a 4 cm extended-peak ionization region.

<sup>‡</sup> Misonidazole and desmethylmisonidazole were administered intraperitoneally 30-60 min prior to irradiation at dose levels of 500 and 1500 mg/kg, respectively.

<sup>§</sup> The ER<sub>20</sub> and ER<sub>50</sub> values are dose enhancement ratios at the 20-day and 50-day growth delay levels, respectively. Each enhancement ratio was calculated as the ratio of radiation doses required to produce a given level of growth delay without and with administration of the sensitizer.

Table 2. RBE values for radiation-induced growth delay of R-1 tumors following combined treatment with accelerated neon ions and misonidazole or desmethylmisonidazole

Treatment scheme <sup>+</sup>	RBE <sub>20</sub> <sup>‡</sup>	RBE <sub>50</sub> <sup>‡</sup>
Neon ions	2.9	2.6
Neon ions + misonidazole	1.9	1.6
Neon + desmethylmisonidazole	2.0	1.7

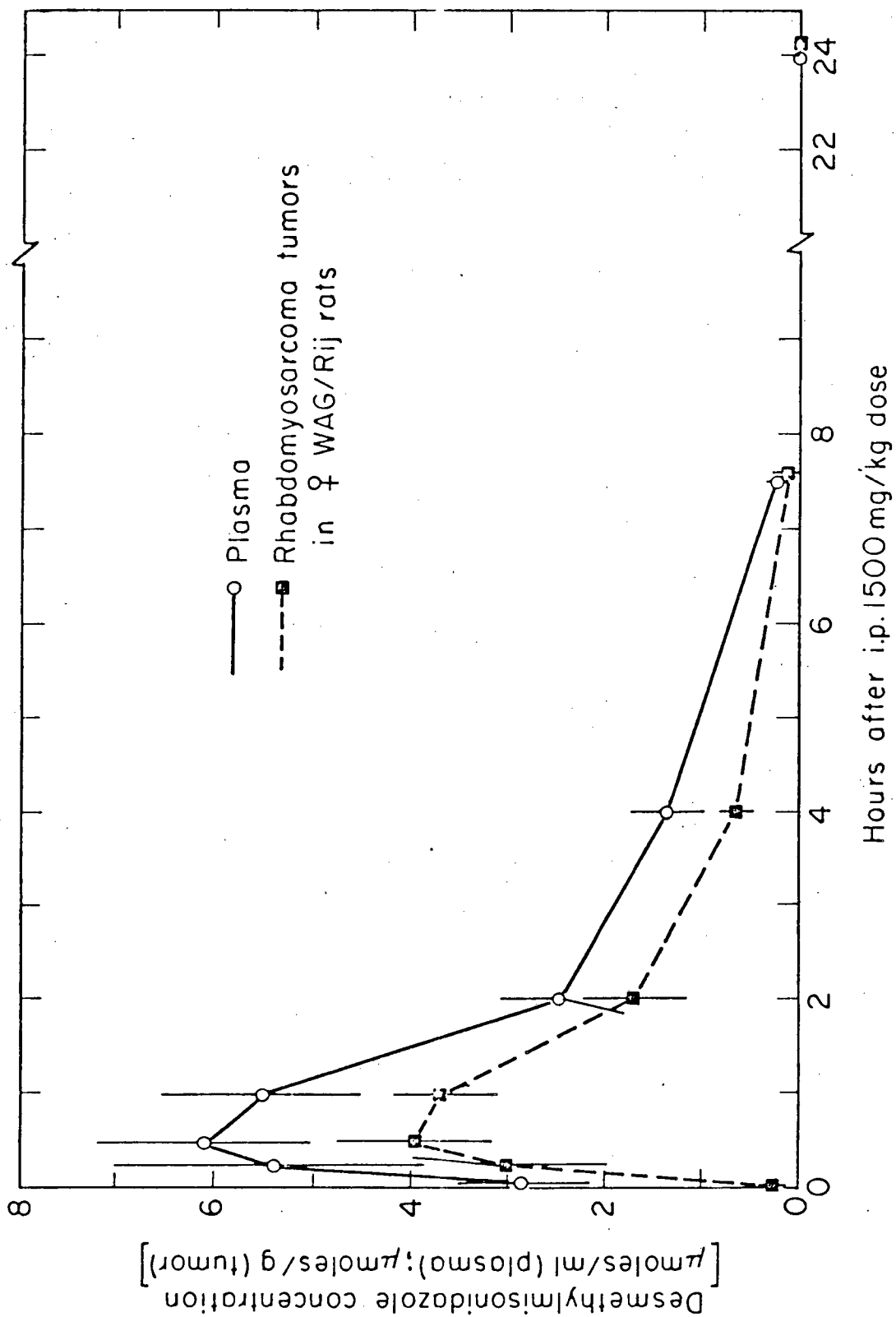
<sup>+</sup> The irradiation conditions and sensitizer dose levels were the same as those described in table 1.

<sup>‡</sup> The reference radiation for r.b.e. calculations was 225-kVp X rays. The r.b.e.<sub>20</sub> and r.b.e.<sub>50</sub> values were calculated from the ratio of the X-ray to the charged-particle dose required to produce growth delays of 20 days and 50 days, respectively.



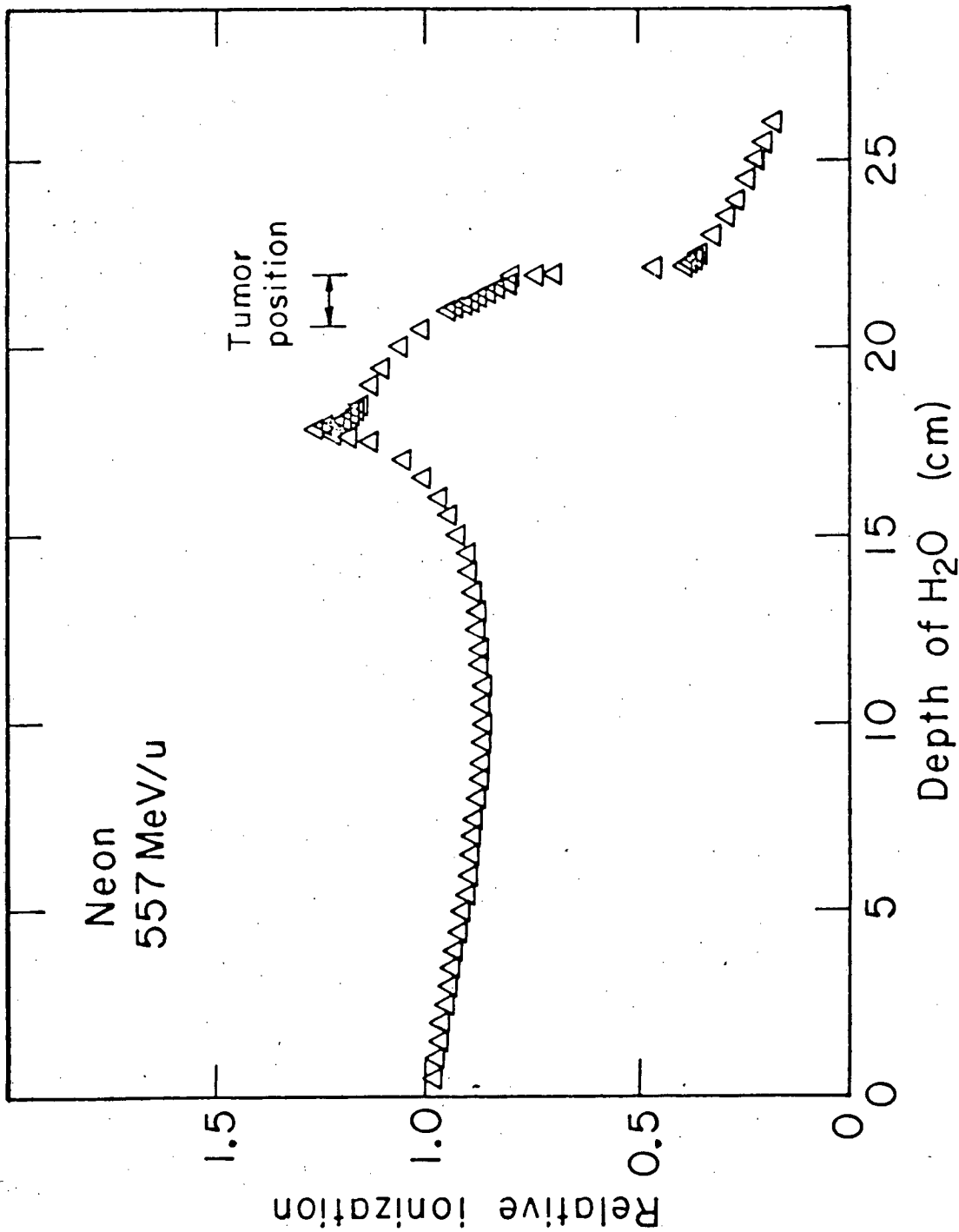
Figure legends

- Figure 1. Uptake and clearance kinetics in the blood plasma and in R1/LBL tumors in WAG/Rij rats are shown for the hypoxic cell sensitizer desmethylmisonidazole. The sensitizer was injected intraperitoneally as a single dose at the subtoxic level 1500 mg/kg body weight. Error bars represent one standard error of the mean. The desmethylmisonidazole concentration was assayed in the blood plasma and tumors of 6-9 rats at each of the time points between 0 and 1 hr. Assays were performed on an average of 3 animals at each of the later time points.
- Figure 2. The depth-dose curve measured in water is shown for the neon-ion beam with an initial energy of 557 MeV/u. The peak ionization region was spread to a width of 4 cm by means of a variable-thickness absorber (ridge filter). Brackets in the distal portion of the extended-peak region denote the tumor treatment zone.
- Figure 3. Radiation-induced growth delay curves are shown for R1/LBL tumors receiving single doses of misonidazole (500 mg/kg i.p.) or desmethylmisonidazole (1500 mg/kg i.p.) in combination with X rays (panel a) or extended-peak neon ions (panel b). The sensitizers were injected 30-60 min prior to irradiation in order to achieve a maximum drug concentration in the tumors at the time of radiation. Error bars on each data point represent one standard error of the mean growth delay measured for the number of tumors shown in parentheses.



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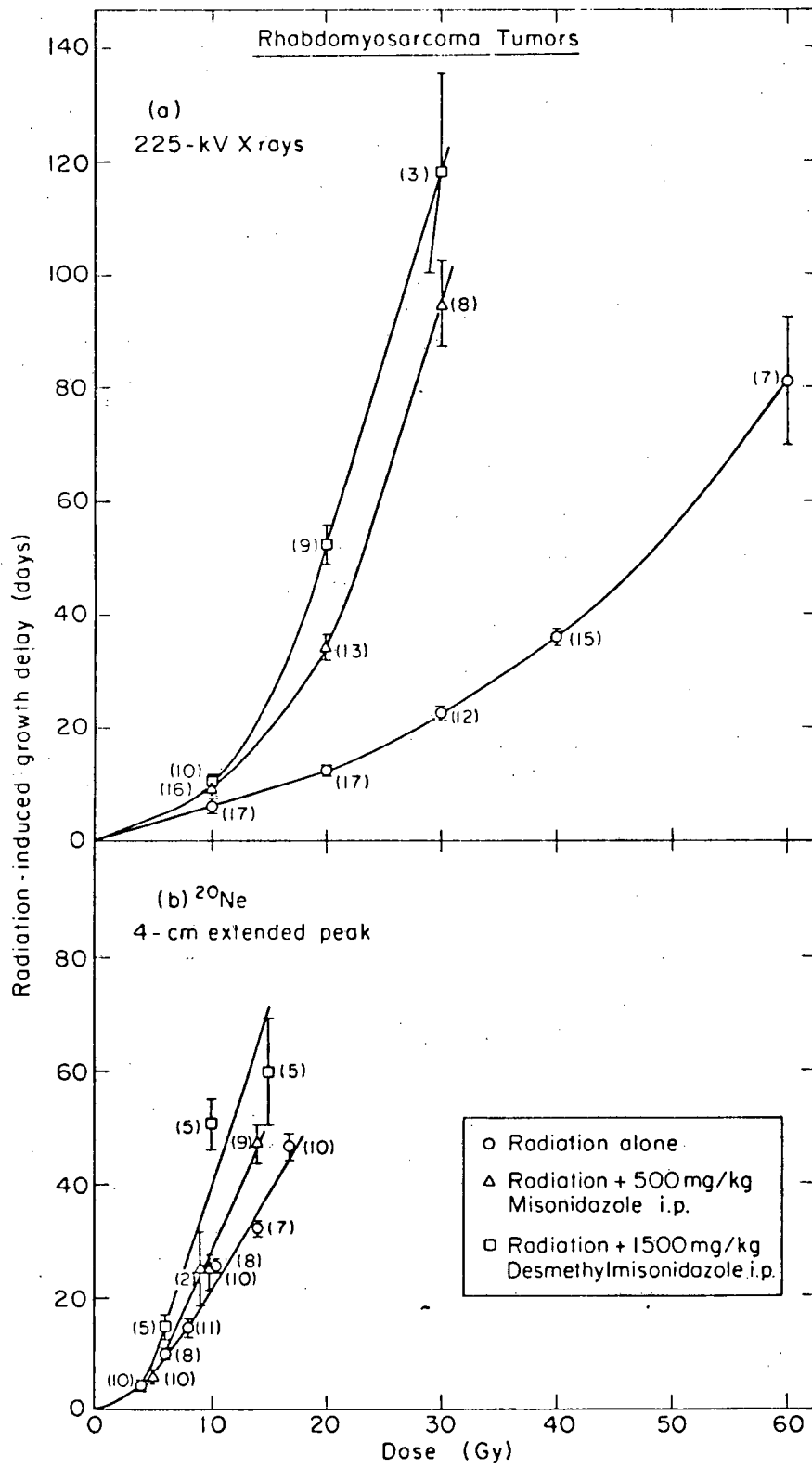
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FIGURE 1



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FIGURE 2



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FIGURE 3

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