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HYPOXIC CELL SENSITIZERS AND HEAVY CHARGED-PARTICLE RADIATIONS

J. D. CHAPMAN,*[1] R. C. URTASUN,*[2] E. A. BLAKELY,†
K. C. SMITH† AND C. A. TOBIAS†

*From the *Department of Radiation Oncology, Dr W. W. Cross Cancer Institute and the Department of Radiology, University of Alberta, Edmonton, Alberta T6G 1Z2, and the †Biomedical Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA. 94720*

Summary.—Stationary-phase populations of Chinese hamster V-79 cells were irradiated with 250 kV X-rays and the Bragg peaks (spread to a width of 4 cm) of energetic He-, C-, Ne-, and A-ion beams produced at the 184-inch cyclotron and BEVALAC at Lawrence Berkeley Laboratory. Survival curves were generated with each radiation for cells suspended in air-saturated and nitrogen-saturated medium with and without sensitizer present. The oxygen enhancement ratios (OERs) measured for X-rays with 1 mM metronidazole and 0·5 mM misonidazole were 2·0 and 1·6 respectively. The OERs without sensitizer for He-, C-, Ne-, and A-ion Bragg peaks were 2·4, 1·7, 1·6 and 1·4 respectively. For each type of radiation tested the presence of hypoxic-cell sensitizers resulted in an additional reduction in the measured OERs, indicating that these drugs should be of benefit in the radiotherapy planned with these and other high LET radiations.

THERE is evidence to suggest that hypoxic cells occur in several human tumours which are currently treated with radiation therapy and that their relative radioresistance may result in a poor rate of local control. Two techniques under current development which can reduce the differential radiosensitivity between hypoxic and aerated tissues are (1) the use of hypoxic cell sensitizers with conventional radiation (Adams, 1973) and (2) the use of high LET radiations, in particular the use of heavy charged-particle beams (Tobias, 1973). During the course of preclinical characterizations of the various high LET beams produced at Lawrence Berkeley Laboratory, the effectiveness of various hypoxic cell sensitizers in combination with the charged particles was measured. The studies reported in this paper indicate that whereas each of the individual new modalities goes a long way to reduce the radiobiological oxygen effect observed with mammalian cells, the lowest oxygen enhancement ratios (OERs) were measured when hypoxic cell sensitizers were used in combination with the high LET radiations.

MATERIALS AND METHODS

Populations of Chinese hamster cells (line V79-379A) which had been grown to stationary phase in suspension culture were used in this study. Five-ml aliquots of cells at a concentration of 10^5 cells/ml were transferred to cylindrical glass irradiation chambers which were positioned in the various heavy-ion beams so that their cylindrical axes coincided with the beam axis. Cells were made hypoxic by passing ultra-pure N_2 plus 5% CO_2 through the chamber for at least 40 min before the beginning of irradiation. The cells were stirred in suspension to facilitate the removal of O_2 from the sample and to average the biological effectiveness of a 1·5 cm segment of the spread Bragg peaks. A variable absorbing "ridge-filter" was used

[1] Experiments performed while on attachment to Lawrence Berkeley Laboratory from Atomic Energy of Cannda Ltd, Pinawa, Manitoba.

[2] On sabbatical at Lawrence Berkeley Laboratory.

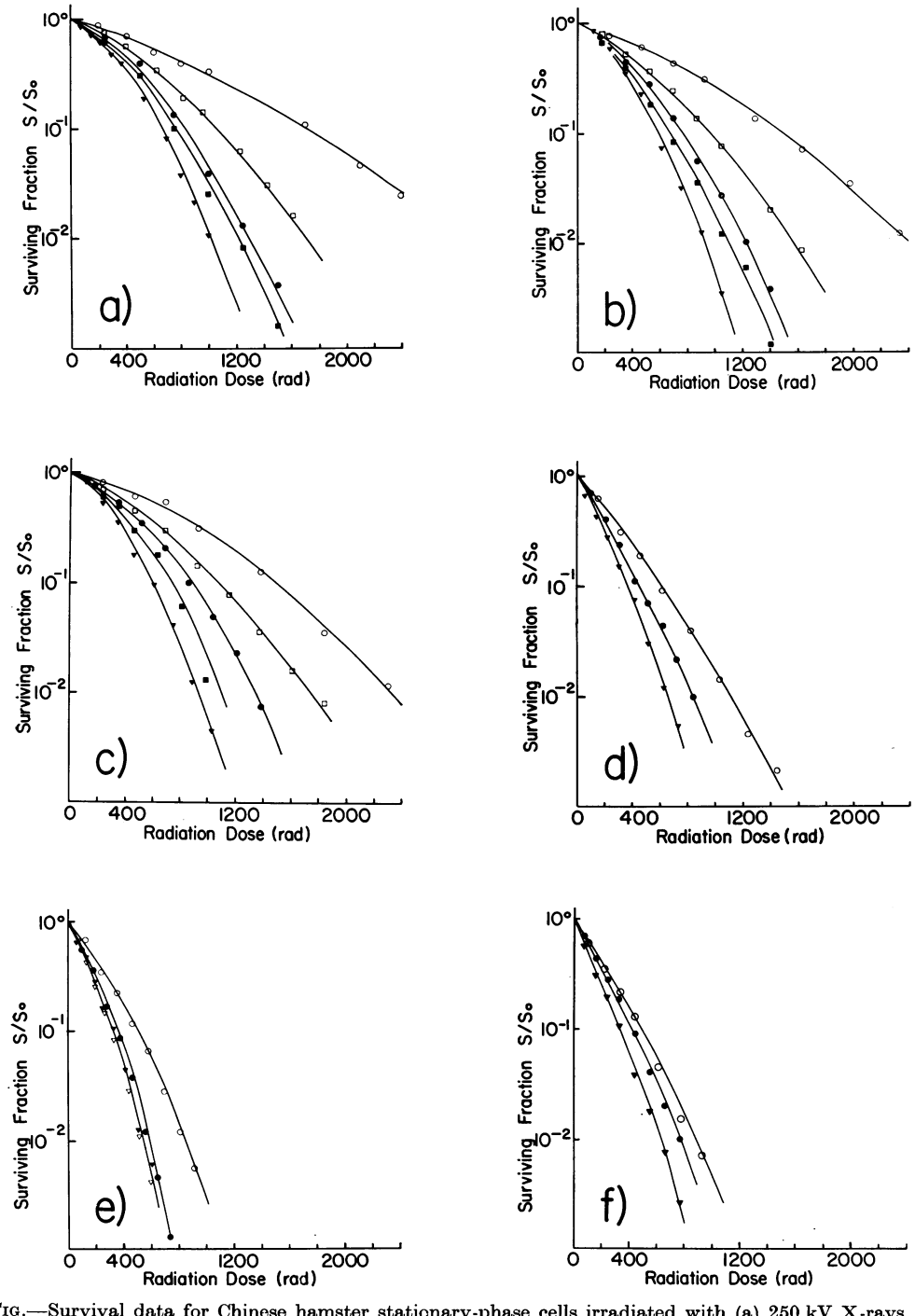

FIG.—Survival data for Chinese hamster stationary-phase cells irradiated with (a) 250 kV X-rays, (b) Bragg-peak He ions, (c) plateau C ions, (d) Bragg-peak C ions, (e) Bragg-peak Ne ions, and (f) Bragg-peak A ions. ▼ air; ▽ air + 5 mM misonidazole; ○ nitrogen; ● nitrogen + 5 mM misonidazole; □ nitrogen + 5 mM metronidazole; ■ nitrogen + 5 mM Ro-07-0741.

to spread the Bragg peak over 4 cm in the case of the carbon-, neon- and argon-ion beams and over 8 cm for the helium-ion beam. The LET distribution of particles which deposited energy in this irradiation chamber would be broad, since both energetic particles which traverse the chamber, and particles which stop in the chamber, would contribute to the absorbed dose. Spread Bragg peaks are considered necessary for many applications of high LET radiation in radiotherapy and it was felt that this experimental system could directly measure the average radiobiological response along a significant segment of a "therapeutic" beam setup. Additional details of the irradiation chamber, the gassing techniques, and the depth-dose distributions of the various particles studied have been described (Chapman *et al.*, 1977).

Cells were irradiated in aerated or hypoxic suspensions, with or without hypoxic cell sensitizers at the time of irradiation. Samples were removed after appropriate doses, diluted, and plated in complete monolayer medium for colony assay after 6 days of incubation at 37°C.

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) was kindly supplied by Poulenc Ltd., Montreal, Canada. Misonidazole (1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol) Ro-07-0741 (1-(2-nitro-1-imidazolyl)-3-floro-2-propanol) and Ro-05-9963 (1-(2-nitro-1-imidazolyl)-3-hydroxy-2-propanol) were kindly supplied by Hoffman–La Roche Inc., Nutley, New Jersey. These compounds were known to be effective radiosensitizers of hypoxic mammalian cells (Chapman and Urtasun, 1977).

RESULTS

Doses of each type of radiation were chosen so that cell inactivation could be established for at least two decades of kill for each condition studied. In each heavy-ion experiment, different aliquots of the same cell population were used to establish the effectiveness of the different drugs. In this way radiobiological differences between different cell populations were minimized. Fig. 1a shows survival data for stationary-phase Chinese hamster cells irradiated with 250 kV X-rays in the presence of 5 mM metronidazole, 5 mM misonidazole and 5 mM Ro-07-0741. Oxygen enhancement ratios were determined at the 10% survival level and are shown in Table I. The radiosensitizing effect of each of these compounds was tested at concentrations of 0·5 mM and 1·0 mM and the OERs obtained in these experiments are given in Table I. The OERs observed with the compound Ro-05-9963 at concentrations of 1 mM and 5 mM are also shown in this table.

TABLE I.—*Oxygen Enhancement Ratios* for Chinese Hamster Cells Irradiated with 250 kV X-rays in the Presence of Hypoxic Cell Sensitizers*

	0 mM	0·5 mM	1·0 mM	5·0 mM
Metronidazole	2·8	2·16	1·95	1·60
Misonidazole	2·8	1·63	1·40	1·27
Ro-07-0741	2·8	1·52	1·30	1·17
Ro-05-9963	2·8	—	1·50	1·34

* At 10% survival level.

Figs. b, c, d, e, and f show the survival data for stationary-phase Chinese hamster cells irradiated with Bragg-peak helium-ions, plateau carbon-ions, Bragg-peak carbon-ions, Bragg-peak neon-ions and Bragg-peak argon-ions, respectively. Only high concentrations (5 mM) of metronidazole, misonidazole and Ro-07-0741 were used with the heavy charged-particle beams. The OERs at the 10% survival level are shown in Table II. For every case

TABLE II.—*Oxygen Enhancement Ratios* for Chinese Hamster Cells Irradiated with 250 kV X-rays and Various Heavy Charged-particle Beams in the Presence of Various Hypoxic Cell Sensitizers*

	No Drug	5 mM metronidazole	5 mM misonidazole	5 mM Ro-07-0741
250 kV X-rays	2·8	1·60	1·27	1·17
Helium ions (8 cm spread peak)	2·40	1·62	1·30	1·13
Carbon ions (plateau)	2·55	1·78	1·46	1·23
Carbon ions (4 cm spread peak)	1·65	1·40	1·23	1·15
Neon ions (4 cm spread peak)	1·57	—	1·10	—
Argon ions (4 cm spread peak)	1·43	—	1·28	—

* At 10% survival level.

studied, the presence of a radiosensitizer at the time of radiation produced a significant reduction in the OER measured for each particular radiation. It is apparent that 5 mM of misonidazole can reduce the high OERs of 250 kV X-ray, Bragg-peak helium-ions and plateau carbon-ions (which can be considered as low LET irradiations) to values of 1·3 to 1·4. For those radiations of higher LET (Bragg-peak carbon-ions, Bragg-peak neon-ions and Bragg-peak argon-ions) the resultant OER in the presence of 5 mM misonidazole is ~1·2.

DISCUSSION AND CONCLUSIONS

The studies described in this paper constitute the first look at the effectiveness of hypoxic cell sensitizers with heavy charged-particle radiations. They have been useful in establishing some general principles on the interaction of hypoxic cell sensitizers with these types of high LET radiations. These studies have also established the feasibility of asking several specific questions in future experiments using sensitizers and heavy charged-particle beams.

The data of Figs. b, c, d, e and f establish very clearly that high concentrations of hypoxic cell sensitizers are effective with heavy charged-particle radiations resulting in OERs which are considerably smaller than those which can be obtained by the high LET radiation alone. These results, along with the previous studies by Hall *et al.* (1975) and McNally (1976) using neutrons, and Raju *et al.* (1977) using a mixture of alpha-particles and X-rays, have established the generality of the radiosensitizing effectiveness of hypoxic cell sensitizers against both low LET and high LET radiations.

Table I indicates that hypoxic cell sensitizers in combination with conventional radiations can produce OERs equal to or lower than those obtained by high LET radiation alone. For 1 mM metronidazole and 0·5 mM misonidazole the resultant OERs with conventional radiation are 2·0 and 1·6, respectively. These concentrations of each drug are near to the maximum concentrations one can achieve in the serum and tissues of humans who have received the drug orally every second day 3 times a week for 3 weeks with a minimum of toxicity (Urtasun *et al.*, 1975, 1977). If more effective and less toxic compounds can be discovered, the very real prospect exists for using such compounds with conventional radiation to produce OERs lower than those obtained with high LET radiations. The cost/benefit of such sensitizing drugs is readily apparent. The potential benefits to radiotherapy of a "low OER radiation" resulting from drugs plus conventional radiation could be immediately available to all radiotherapy centres.

The low OER observed with high LET radiation is only one of several potential benefits of heavy charged-particle beams in radiation therapy. Other benefits have been described (Chapman *et al.*, 1978) and their relative importance to radiotherapy is a matter of current discussion. It is safe to say that an OER of near 1·0 over a significant tumour treatment volume will not be achieved by the use of high LET radiations alone. In this regard, it is encouraging to know that hypoxic cell sensitizers can reduce the low OERs obtained with high LET radiations to produce much lower values. The studies described in this paper indicate that the most effective means of dealing with the radioresistance of hypoxic cells to date is the combination of high LET radiations plus hypoxic cell sensitizers.

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