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TESTING OF HYPOXIC CELL RADIOSENSITIZERS *IN VIVO*

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Summary.—Use has been made of the transplantable KHT sarcoma in C3H mice to test the *in vivo* effectiveness of some 2-, 4-, and 5-nitroimidazoles as hypoxic cell radiosensitizers. A comparison of the *in vivo* versus the *in vitro* sensitizing ability of misonidazole and metronidazole indicates some differences, probably due to drug delivery problems *in vivo*. The relative sensitizing abilities of eight 2-nitroimidazoles, two 4-nitroimidazoles and two 5-nitroimidazoles are compared on the basis of the amount of drug injected and the plasma levels obtained.

MOST screening of potential hypoxic cell radiosensitizers has been carried out *in vitro* in bacterial and/or mammalian cell systems (Adams *et al.*, 1976). Positive results at this level have led to *in vivo* testing of these compounds. The step to an *in vivo* system introduces a number of problems which are not present in most *in vitro* systems: a choice of mode of drug administration (oral, intraperitoneal (i.p.), intravenous, or local injection); acceptable toxicity to the whole animal; and an ability of the drug to reach the cells of interest. In passing from the site of application to the tissue of interest the drug may be subject to solubility problems, metabolism, and diffusion barriers. Once present at the target site the drug must be effective at the high cell densities encountered in most *in vivo* situations. The ability of some *in vitro* sensitizers to work *in vivo* has been limited by the above demands (Rauth and Kaufman, 1975; Rauth, Kaufman and Thomson, 1975).

Metronidazole and misonidazole are two drugs which have been extensively screened for activity in a variety of *in vivo* test systems (Fowler, Adams and Denekamp, 1976; Rauth and Paciga, 1977). In addition, the toxicity and radiosensitizing ability of these two drugs has been assessed in a number of normal tissue systems including skin and gut (Stone and Withers, 1975; Brown, 1975). In the present work, Metronidazole and misonidazole have been studied using the KHT transplantable murine tumour in C3H mice and assayed by an *in vivo* lung colony assay (Rauth and Kaufman, 1975). The KHT tumour has a 10–30% viable hypoxic cell fraction which can be demonstrated to be a limiting factor in single dose cell survival curves. The radiosensitizing ability of different doses of these drugs given i.p. to tumour-bearing mice has

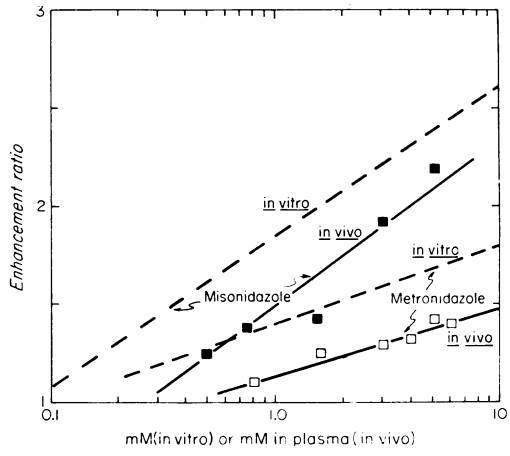


FIG. 1.—Enhancement ratios for metronidazole and misonidazole radiosensitization of KHT tumour cells *in vivo* as a function of the plasma drug concentration, measured polarographically at the time of irradiation. Dotted lines are the *in vitro* results of Whitmore and Gulyas (1977) for Chinese hamster ovary cells.

been determined by measuring single-dose survival curves for the KHT tumour cells. The final slopes of these survival curves can be used to determine relative enhancement ratios for both metronidazole and misonidazole. In Fig. 1 these enhancement ratios are plotted versus the plasma level of the drug at the time of irradiation. These data are compared to enhancement ratios observed with Chinese hamster ovary cells *in vitro* by Whitmore and Gulyas (1977), dotted lines. These drugs appear to be less effective *in vivo* than *in vitro*. The explanation for this difference appears to be one of drug supply to hypoxic cells. When misonidazole was given i.p., the amount of drug in the plasma was greater than gross tumour levels or the level in the necrotic fluid in the centre of this tumour up to 2 h after injection, Fig. 2. Thus, in mouse screening systems, where drug elimination is relatively rapid, times of tumour equilibration may be slow compared to peak plasma level times. This should be less of a problem in the clinical situation where drug half-lives are approximately 10 times longer (Fowler *et al.*, 1976).

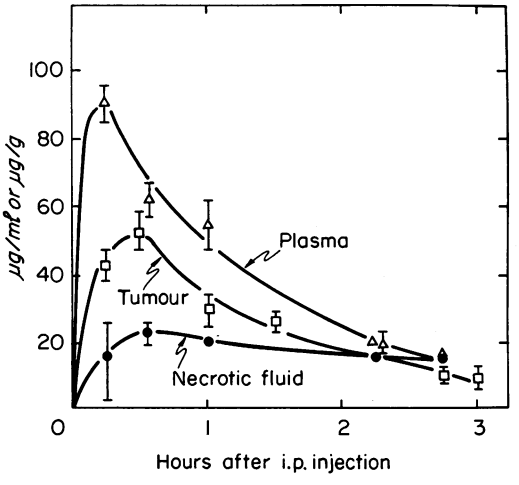
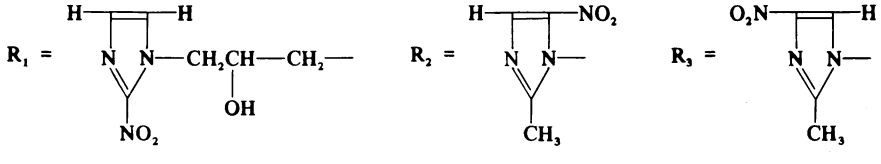


FIG. 2.—Levels of misonidazole measured polarographically in the plasma, whole tumour extract or necrotic fluid of KHT tumours as a function of time after i.p. injection 0·08 mg/g of the drug. Bars indicate the range of measured values.

TABLE.—*Summary of* In vivo *Screening Results*

Compounds	Structure	Partition coefficient	Maximum concentration tested (mg/g)	Plasma levels mM (60-100 min after injection)	*In vivo*† sensitizing ability
Ro 07-0269 (a)	$R_1 \cdot Cl$	1·4	0·125*	0·24	++
Ro 07-0582 (a)	$R_1—OCH_3$	0·48	1·5	5	++++
Ro 07-0741 (a)	$R_1 \cdot F$	0·33	0·75	3·1	++++
Ro 07-0913 (a)	$R_1—OCH_2CH_3$	0·86	2·5	5·8	+++
Ro 07-1127 (a)	$R_1—OC_6H_5$	25	0·5*	0·16	+++
Ro 07-1902 (a)	$R_1—OCH{=}CH—CH_3$	1·92	2·5	5·9	+++
Ro 07-2044 (a)	$R_1—OCH_2CF_3$	3·35	0·75	0·73	+++
Ro 05-9963 (a)	$R_1—OH$	0·14	2·5	3·5	++++
Metronidazole (b)	$R_2—CH_2—CH_2—OH$	0·9	1·5	4	+++
Ro 07-0207 (a)	$R_2—CH_2CHOHCH_2Cl$	4	1·0	2·5	+
R.P. 8532 (b)	$R_3—H$	1·1	0·6	2·2	+++
R.P. 8979 (b)	$R_3—(CH_2)_2OH$	0·26	1·5	2·1	++



(a) supplied by Hoffman-LaRoche, Nutley, New Jersey, U.S.A.
(b) supplied by Poulenc Ltd., Montreal, Canada

* Dissolved in DMSO (Rauth and Paciga, 1977).
† ++++ = E.R. 1·7 – 2·0, +++ = E.R. 1·35 – 1·70, ++ = E.R. 1·15 – 1·35, + = E.R. 1·0 – 1·15.

Using the KHT tumour system a number of 2-, 4- and 5-nitroimidazoles have been screened for their hypoxic cell radiosensitizing ability. Results for some of these compounds are listed in the Table. The general procedure was to inject i.p. different amounts of the drugs to be tested into KHT tumour-bearing mice, wait 30–60 min and irradiate with a large enough single dose of radiation that survival was controlled by the hypoxic cell fraction (Rauth and Kaufman, 1975). Immediately after irradiation animals were sacrificed and tumour-cell survival measured by a lung colony assay. Blood levels of drug were also measured at the time of animal sacrifice. Typical results for four 2-nitroimidazoles are shown in Fig. 3. On the basis of such data, each of the drugs listed in the Table have been assigned an *in vivo* sensitizing ability based on the minimum survival obtained, assuming the drugs are acting as dose-modifying agents. By this criterion all 2-nitroimidazoles were positive but some were better hypoxic cell sensitizers than others.

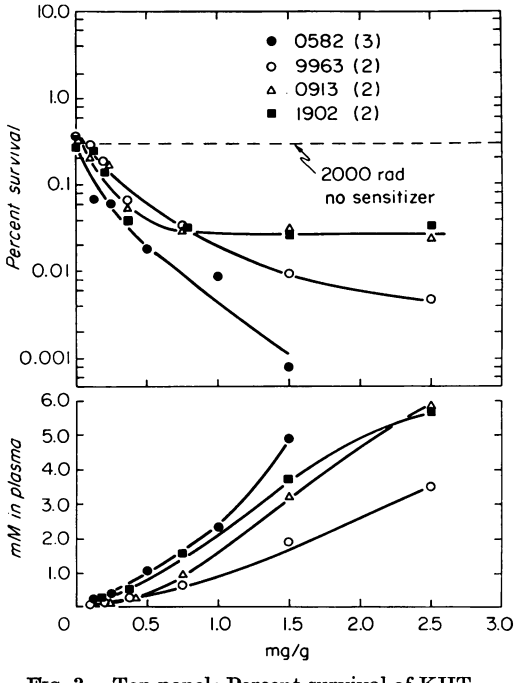


FIG. 3.—Top panel: Percent survival of KHT tumour cells in C3H mice injected i.p. with the indicated mg/g of drug and irradiated with 2000 rad, 30-60 min later. Numbers in brackets indicate the number of experiments averaged for the points plotted. Bottom panel: Drug concentration measured by polarography in mouse plasma 60-90 min after i.p. injection of the indicated mg/g of drug.

Another way of analysing data in the form shown in Fig. 3 is to plot survival as a function of the drug concentration in the plasma. When this is done for plasma concentrations of 1 mM or less, the results shown in Fig. 4 are obtained. That is, for the 2-nitroimidazoles little difference between drugs is seen in this concentration range. This indicates that, for these drugs at least, once a given plasma level is obtained the drugs behave similarly as radiosensitizers. Also it can be noted from the Table that this sort of similarity occurs for drugs whose partition coefficients measured in octanol-water differ by 100-fold. Thus, a major factor in selecting a "best sensitizer" from this group will have to do with the ease of obtaining good plasma levels and what

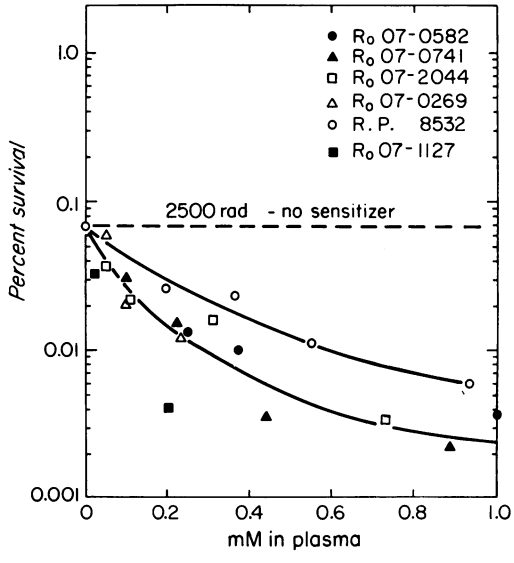


FIG. 4.—Percent survival of KHT tumour cells in C3H mice injected i.p. with drug and irradiated 30-60 min later with 2500 rad versus plasma drug concentration measured at the end of irradiation.

toxic side effects are present at these levels.

The mere attainment of sufficiently high plasma levels does not guarantee *in vivo* effectiveness as can be seen in the Table for Ro-07-0207, a 5-nitroimidazole. Levels of this drug were obtained which would have been expected to sensitize based on *in vitro* data but no hypoxic cell sensitization was seen *in vivo*. Also shown in Fig. 4 and the Table are data for R.P. 8532. This 4-nitroimidazole is a surprisingly good sensitizer *in vivo* and has been shown to primarily reduce the shoulder on the hypoxic cell survival curve *in vitro* (Whitmore and Gulyas, 1977). Thus, R.P. 8532 is not a dose modifying agent. For comparison R.P. 8979, a 4-nitroimidazole which is dose modifying *in vitro* (Whitmore and Gulyas, 1977) is less effective *in vivo*. The fact that R.P. 8532 is a relatively good sensitizer *in vivo* at low plasma levels and exerts its effect primarily on the shoulder of the *in vitro* mammalian cell survival curve suggests that this class of drug deserves further study.

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