Br. J. Cancer (1978) **37**, Suppl. III, 276

THE HYPOXIC CELL SENSITIZER PROGRAMME IN THE UNITED STATES

T. L. PHILLIPS,* T. H. WASSERMAN,* R. J. JOHNSON,† C. J. GOMER,† G. A. LAWRENCE,† M. L. LEVINE,* W. SADEE,‡ J. S. PENTA,§ AND D. J. RUBIN§

*From the *Division of Radiation Oncology, University of California, San Francisco, CA* 94143, *the †Department of Radiation Medicine, Roswell Park Memorial Institute, Buffalo, NY* 14263, *the ‡Department of Pharmaceutical Chemistry, University of California, San Francisco and the §Division of Cancer Treatment, National Cancer Institute, Bethesda, MD* 20014, *U.S.A.*

Summary.—The initial results of a Phase I evaluation of misonidazole in the U.S.A. are described, as well as the U.S. National Cancer Institute programme for radiosensitizer development. A total of 12 patients have been given 1–6 doses of 1–2 g/m^2. Serum levels ranged from 25–87 μg/ml at 4–6 h. One patient has developed mild peripheral neuropathy. Urinary excretion was chiefly of a demethylated metabolite as measured by HPLC assay.

THE National Cancer Institute, Division of Cancer Treatment (NCI-DCT), in conjunction with the Radiation Therapy Oncology Group (RTOG), has embarked upon a programme to develop and apply hypoxic cell sensitizers in the United States according to the C.R.O.S. Research Plan (Anonymous, 1976). This programme involves an NCI-DCT supported Hypoxic Cell Sensitizer Coordinating and Review Committee, direct NCI-DCT supported contracts in drug synthesis, *in vitro* and *in vivo* tumour model evaluation, small and large animal toxicology, dose formulation, and clinical evaluation in Phase I–III clinical trials. This entire mechanism is set up to work in conjunction with the existing chemotherapy drug development programme.

As a first clinical step in this programme, misonidazole was obtained by the NCI–DCT and an application for initial clinical trial was filed with the U.S. Food and Drug Administration (FDA) and approved for activation in July 1977, via the RTOG. This paper will describe the early clinical results of this initial Phase I study, which is still in progress.

The objectives of this trial are to determine the maximum tolerated dose of misonidazole, administered orally, on a once per week dose schedule for up to 6 weeks, and then a twice per week schedule for up to 6 weeks; to determine the qualitative and quantitative toxicities of the drug; to determine the pharmacologic properties of the drug as to serum peak levels, half-life, renal excretion, metabolism, and tumour tissue levels.

The misonidazole has been prepared as a 0·5 g enteric coated tablet. To date, 12 patients have entered the study, with all patients having far-advanced cancer, but normal renal and hepatic function. The dose schedule has been single oral weekly doses for either 3 or 6 weeks alternately in separate groups of 3 patients each. The initial dose was 1·0 g/m^2 with dose escalation by 1·0 g/m^2 in successive groups of 3 patients for each dose schedule. Radiation therapy is given at a dose of 600–800 rad, at an interval of 4–6 h after each drug dose once per week, to allow for maximum tumour tissue drug concentration. Where possible, objective parameters of tumour effect of the combined drug and radiation are studied, although this is not a primary objective of this trial.

Complete pharmacological evaluation has been done on most patients using

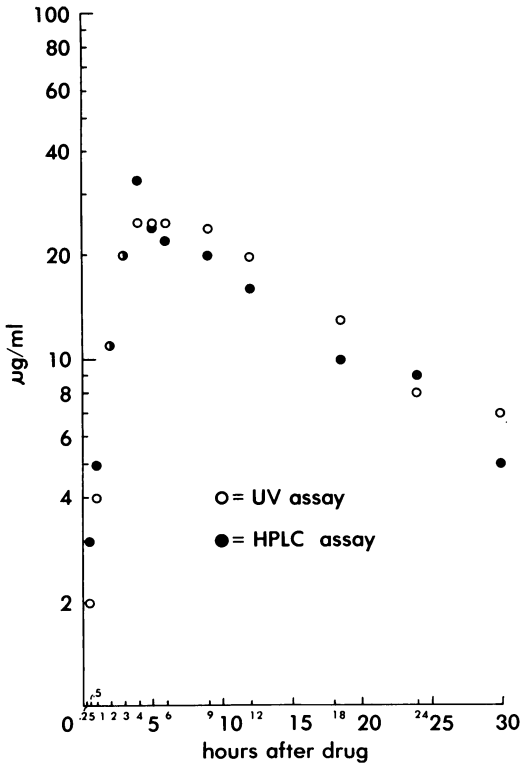

FIG. 1.—Serum concentration of misonidazole *vs* time as measured by HPLC and UV assays in Patient No. 2, given 1 g/m^2 = 1·5 g.

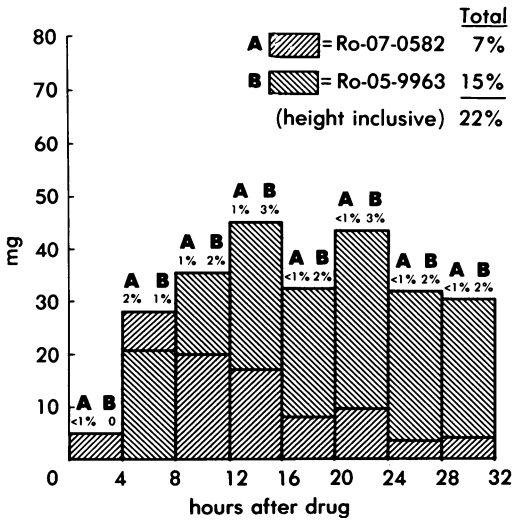

FIG. 2.—Urinary excretion of misonidazole and its metabolite as measured by HPLC assay for Patient No. 2 with 4 hourly urine collections. (Dose 1 g/m^2 = 1·5 g.)

measurement by UV spectrophotometry and a high pressure liquid chromatography method (HPLC) developed in the clinical pharmacology laboratory at the University of California, San Francisco, by one of the authors (W.S.). The HPLC method offers the advantage of being more sensitive at low levels, as well as measuring levels of each nitroimidazole specifically, rather than the group as a whole. This allowed for measurement of serum and urine levels of misonidazole and its principle metabolite, the demethylated Ro-05-9963. The absorbence of misonidazole by UV assay was established as linearly proportional to the drug concentration (μg/ml). The peak height in millimetres of the HPLC assay was established as linearly proportional to each of the component concentrations, misonidazole and Ro-05-9963, in μg/ml. Fig. 1 shows a serum concentration curve *vs* time in the second patient who received 1·0 g/m^2 (1·5 g total) of misonidazole. The levels for the UV assay and the HPLC assay parallel each other. The peak serum level at this dose was 33 μg/ml occurring at 4 h and the half-life was 12 h, consistent with the data of the British studies (Dische *et al.*, 1977; Foster *et al.*, 1975; Gray *et al.*, 1976).

Fig. 2 shows the urine concentrations in mg of misonidazole and Ro-05-9963 *vs* time for the same patient after the same dose of misonidazole. The data show that beyond the first 8 h of urinary excretion, more Ro-05-9963 was excreted than the parent compound misonidazole. The total urinary excretion of Ro-05-9963 was twice that of the misonidazole, with the 2 compounds accounting for 22% excretion of administered drug in 32 h. The metabolic conversion of misonidazole to Ro-05-9963 may be an important factor in the urinary excretion. It is known that Ro-05-9963 is an active radiosensitizer *in vitro* and *in vivo*. It is unknown which of these 2 compounds or which other, as yet unknown, metabolite of misonidazole is the neurotoxic species in man.

TABLE.—*Misonidazole* (*Ro*-07-0582) *Phase I Study Results*

Patient no.	Age	Sex	M^2	No. of doses	g/m^2	mg/kg	Mean 4–6 h sera level μg/ml	Total dose (g)	Neuro-pathy	Status
1	54	F	1·5	3	1	26	28	4·5	No	Expired
2	59	F	1·5	3	1	29	25	4·5	No	Expired
3	50	F	1·3	3	1	30	26	3·9	No	Expired
4	63	M	2·0	2	1	24	31 (1 only)	4·0	No	Did not complete dose schedule—refused.
5	84	F	1·25	1	1	22	38	1·25	No	Expired before completion of dose schedule.
6	60	F	1·8	6	1	24	29	10·5	No	
7	65	M	1·7	3	2	60	82	10·5	No	
8	69	M	2·1	3	2	42	63	12·0	No	
9	59	F	1·4	3	2	67	82	9·0	No	Expired
10	54	F	1·6	5	2	50	87 (1 only)	15·0	No	Did not complete dose schedule—lack of drug.
11	75	F	1·7	3	2	52	78	10·5	No	Expired before completion of dose schedule
12	76	M	1·6	5	2	46	77 (4 only)	15·0	Yes (mild)	Did not complete dose schedule—lack of drug.

Toxicity, so far, has consisted of mild to moderate nausea and vomiting. Neurotoxicity has been observed in the twelfth patient who was given 5×2 g/m^2 for a total of 15 g in 5 weeks. This toxicity is mild and under observation. Other patients have been observed to have clinically detectable (on careful neurological exam). but not symptomatic peripheral neuropathy *prior* to receiving any drug, presumably secondary to their advanced cancer. As the dose escalation continues, we will be carefully monitoring for neurotoxicity and decreasing the rate of dose escalation.

Following the completion of this Phase I study, a number of pilot Phase II studies will be instituted through the RTOG. The goal of such studies will be to test in groups of 20–30 patients of several different tumour types the clinical feasibility of administering misonidazole and radiation therapy together on a once or twice per week dose schedule.

At this time the NCI-DCT, in conjunction with its Hypoxic Cell Sensitizer Coordinating and Review Committee, is establishing programmes to derive data on other compounds which may ultimately offer a better therapeutic ratio as a clinical radiosensitizer. The initial step in this process is the selection and acquisition of compounds for evaluation. Prior to further *in vitro* and *in vivo* testing, some basic criteria for each compound will be reviewed by the Committee. These criteria include: (1) the compound has electron affinity; (2) the lipid water coefficient is measured and the compound is somewhat soluble in both; (3) the compound sensitizes hypoxic mammalian cells *in vitro* to radiation; (4) it does not significantly sensitize aerated cells *in vitro* to radiation; (5) the toxicity (cell kill) is limited at active levels *in vitro* for acute times of sensitization; (6) the structure is identified as one which is reasonably stable in solution; (7) the drug LD_{50} has been determined in the animal to be used for *in vivo* testing; and (8) activity of the compound is demonstrated in at least one *in vivo*-model tumour at doses below the drug LD_{50}.

Additionally, the compounds acquired for screening should be individually profiled as to quantity needed for further testing, physical constants, chemical structure, solubility, stability, purity and biological activity. After a compound is rationally selected, it will be tested in a variety of *in vivo* solid tumour models. These include only tumours with hypoxic cells. The exact spectrum of tumour models used may be varied in the future as new radiobiological data are obtained, under the knowledge that no single tumour model is known to predict perfectly for the clinical utility of a new radiosensitizing compound. Current tumours in the panel include the C_3H mammary cancer, Lewis lung, B16 melanoma, EMT6 and KHT sarcoma. Evaluation will be made in at least 3 different tumour models in the panel, with a total of 3 end-points including: (1) *in vivo* tumour cell survival modification as measured *in vitro*, by TCD_{50} or other assay; (2) tumour growth delay curve modification; (3) tumour cure dose (TCD_{50}) modification. In addition, drug LD_{50} and other toxicity should be determined for each animal strain used. All of the above experiments should have concurrent controls without drug and with the standard radiosensitizer (now misonidazole) tested under the same experimental conditions at equimolar or equitoxic doses.

Compounds which meet the above criteria, with acceptable toxicity and equal or superior efficacy to the standard radiosensitizer (now misonidazole), will undergo more detailed drug evaluation after large scale drug production and formulation. After such feasibility, as well as cost factors, are established as satisfactory, the compound will be entered into a toxicology programme in large animals, looking for general toxic effects as well as specific animal models which are to be developed to study the neurotoxicity. If such toxicological evaluation is deemed favourable, then the drug will be submitted for clinical evaluation.

It is beyond the scope of this paper to go into further details about the other

specifics of this development programme. They are more completely described in an available NCI-DCT brochure (1977).

It is the goal of the hypoxic cell sensitizer programme in the U.S.A. under the NCI-DCT to continue with the phased clinical evaluation of misonidazole, while continuing a major preclinical effort to seek an hypoxic cell sensitizer with a predicted better clinical therapeutic ratio.

Sponsored directly by the National Cancer Institute, Division of Cancer Treatment through the Radiation Therapy Oncology Group (Grant No. CA21439). Misonidazole supplied by the Drug Development Program, DCT-NCI. Some patients were admitted to the General Clinical Research Center, University of California, San Francisco.

REFERENCES

ANONYMOUS (1976) Radiation Sensitizers: Committee on Radiation Oncology Studies Research Plan, *Cancer*, **37**, 2062.

DISCHE, S., SAUNDERS, M. I., LEE, M. E., ADAMS, G. E. & FLOCKHART, I. R. (1977) Clinical Testing of the Radiosensitizer Ro-07-0582: Experience with Multiple Doses. *Br. J. Cancer*, **35**, 567.

FOSTER, J. L., FLOCKHART, I. R., DISCHE, S., GRAY, A., LENOX-SMITH, I. & SMITHEN, C. E. (1975) Serum Concentration Measurements in Man of the Radiosensitizer Ro-07-0582: Some Preliminary Results. *Br. J. Cancer*, **31**, 679.

GRAY, A. J., DISCHE, S., ADAMS, G. E., FLOCKHART, I. R. & FOSTER, J. L. (1976) Clinical Testing of the Radiosensitizer Ro-07-0582: I. Dose Tolerance, Serum and Tumor Concentrations. *Clin. Radiol.*, **27**, 151.

NCI-DCT, Program Linear Array—New Drug Development Flow-Radiosensitizers. July 1977.