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CLINICAL STUDIES WITH MISONIDAZOLE

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Summary.—The preliminary details of a randomized clinical trial of misonidazole for the radiotherapy of Grades 3 and 4 cerebral astrocytoma are described. Plasma concentrations of misonidazole and its O-demethylated metabolite, determined by high-pressure liquid chromatography analysis, are reported for 8 patients with astrocytoma, 1 with carcinoma of the bronchus and 1 with carcinoma of the breast. In the latter case, tumour concentrations are also presented.

THE TREATMENT of cerebral gliomas is far from satisfactory (reviewed by Sheline, 1975). Since these tumours rarely metastasize, failure of local control is the major problem. In the case of Grades 3 and 4 astrocytoma surgery alone yields extremely poor results.

Adjuvant radiotherapy improves the 5-year survival for Grade 3 but not Grade 4 tumours (see Sheline, 1975). The radiation dose is limited by the comparative radiosensitivity of the normal brain tissue. Moreover, the occurrence of significant regions of necrosis in cerebral gliomas suggests that hypoxia may be an important factor in their radioresistance.

A report by Urtasun *et al.* (1976) has suggested that the hypoxic cell sensitizer metronidazole may prolong survival in glioma patients receiving post-operative radiotherapy. Most experimental studies (see review by Fowler, Adams and Denekamp, 1976) have indicated that misonidazole (1-(2-nitroimidazol-1-yl)-3-methoxypropan-2-ol; Ro 07-0582, Roche Products) is likely to be the most effective of the hypoxic cell sensitizers currently available. Preliminary clinical trials have demonstrated radiosensitization with misonidazole in man (Thomlinson *et al.*, 1976).

The present study was designed to assess the value of combining misonidazole with post-operative radiotherapy in the treatment of Grades 3 and 4 glioma. During the early stages of the study we have taken the opportunity to investigate the pharmacokinetics of misonidazole in these and certain other patients with neoplastic disease.

PATIENTS AND METHODS

Treatment regimes.—Patients over the age of 18 years having histologically proven Grade 3 or 4 cerebral glioma are separately randomized according to one of 3 treatment schedules. These are:

(1) 5656 rad in 28 fractions of 202 rad/fraction over $5\frac{1}{2}$ weeks (1702 ret).
(2) 4352 rad in 12 unequal fractions over 4 weeks: Monday, 294 rad; Wednesday, 294 rad; Friday, 500 rad (1702 ret).
(3) Same as (2) with addition of misonidazole, 3 g/m^2 orally (tablets or capsules), 4 h before the 500 rad dose. (Total dose of misonidazole 12 g/m^2.)

Patients are maintained on dexamethasone throughout the treatment. Other medications are noted. Paracetamol and barbiturates are avoided.

Assessment.—Assessment is made on the basis of skin reaction, neurological function, computerized axial tomography, quality of life and survival. Full blood counts, liver function tests and urea and electrolyte estimations are carried out.

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Pharmacokinetics.—Heparinized blood samples were collected at ½, 1, 2, 3, 4, 5, 6 and 24 h after drug administration. Control samples were taken prior to treatment. In one patient with breast carcinoma serial tumour biopsy specimens were also obtained.

Concentrations of misonidazole and its O-demethylated metabolite (1-(2-nitroimidazol-1-yl)-2,3-propandiol; Ro 05-9963) in plasma and tumour were determined by reverse phase high pressure liquid chromatography (HPLC). Aliquots of plasma and tissue homogenate (20% w/v) were treated with 9 vol methanol containing the internal standard 1-(2-nitroimidazol-1-yl)-3-chloropropan-2-ol (Ro 07-0269, 111 μg/ml). After centrifugation 10 μl samples of supernatant were chromatographed using a Waters Model ALC/GPC-244 liquid chromatograph equipped with a U6K sample loop injector and a μBondapak C_{18} column (30 cm × 3·9 mm I.D.) (Waters Assoc., Milford, Mass.). The mobile phase, consisting of 18% methanol/water, was delivered at a constant flow rate of 2 ml/min (pressure = 2000 psi). The extinction of the column effluent was monitored at 313 nm using a Waters Model 440 absorbance detector. Concentrations of misonidazole and Ro 05-9963 were calculated on the basis of peak height or by peak area using an on-line Varian CDS 111 computer with reference to appropriate calibration curves.

RESULTS

Clinical

The study began in October 1976 and 29 patients have been entered of whom 11 have received misonidazole. Apart from occasional nausea and vomiting during the 24 h following drug administration, no toxicity has been encountered. In particular, no neuropathy has been observed.

In view of the small number of patients admitted and the limited follow-up time, no conclusions may be made as to the relative efficacy of the various treatment regimes.

Pharmacokinetics

Following oral administration of misonidazole both the drug and its O-demethylated metabolite were detected in the blood plasma of all the patients studied (*e.g.* see Fig. 1). A typical plasma time-course for these compounds is shown in Fig. 2. Good absorption of the drug was observed in all instances. Peak plasma concentrations occurred 1–4 h after drug administration. No differences in pharmacokinetics between capsule and tablet formulations were observed. Similarly, no changes were observed with repeated dosage. Plasma half-lives of misonidazole were in the range 4·3–12·5 h.

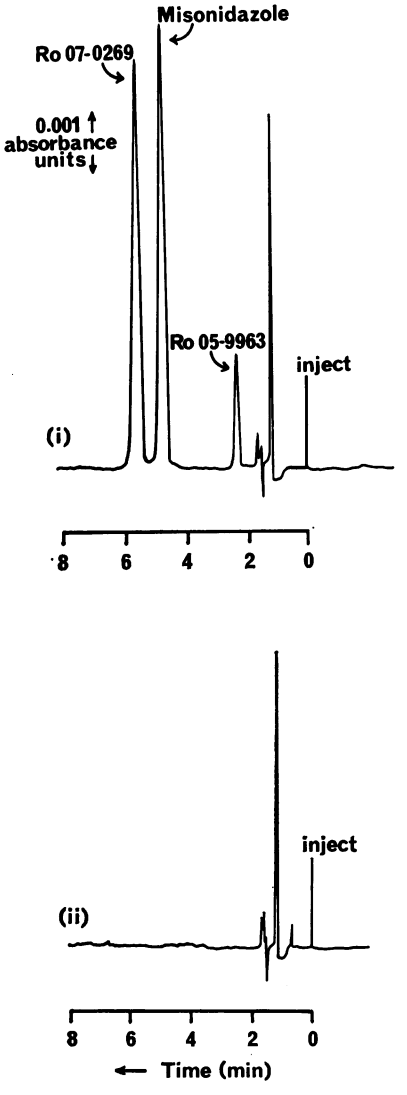

FIG. 1.—HPLC of a methanol extract of plasma from a glioma patient (No. 14). (i) 2 h after 3 g/m² misonidazole; (ii) immediately before receiving the drug.

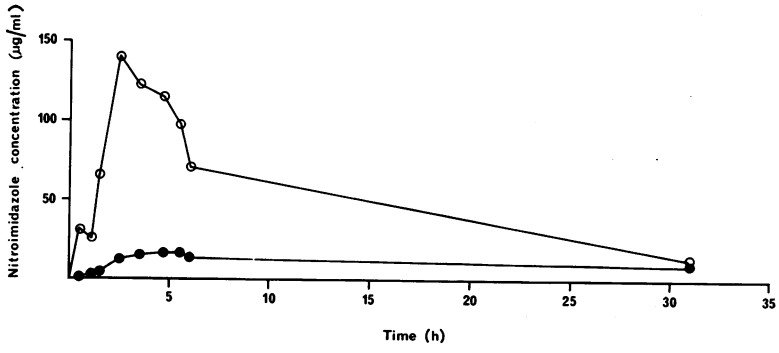

FIG. 2.—Typical plasma time-course of misonidazole (○) and Ro 05-9963 (●) in a glioma patient (No. 11) after 3 g/m² misonidazole.

Since radiotherapy is administered 4 h after drug administration, the nitroimidazole concentration at this time is particularly pertinent. These data are summarized in the Table.

TABLE.—*Plasma Nitroimidazole Concentrations 4 h after 3 g/m² Misonidazole (mean ± one standard error)*

Patients	Number	Misonidazole (μg/ml)	Ro 05-9963 (μg/ml)
Glioma	8	94·1 ± 12·8 (Range 55–168)	11·9 ± 0·5 (Range 9·3–14·8)
Total*	10	96·3 ± 7·3 (Range 55–168)	11·5 ± 0·5 (Range 9·3–14·8)

* Comprised: 8 glioma, one carcinoma of the breast and one carcinoma of the bronchus.

Plasma and tumour levels were determined in one patient with carcinoma of the breast (Fig. 3). It may be seen that over the period 1–24 h after administration tumour misonidazole concentrations were in the range 50–70% of the corresponding plasma level; tumour Ro 05-9963 concentrations were in the range 65–100% of the corresponding plasma level. There was good agreement between replicate tumour specimens (Fig. 3).

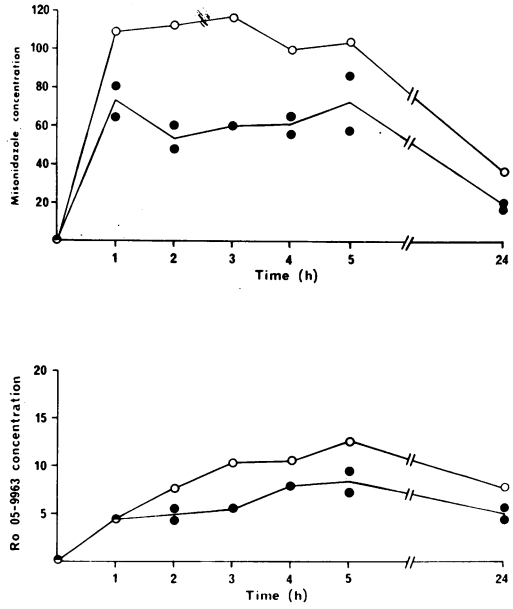

FIG. 3.—Concentrations of misonidazole (○) and Ro-05-9963 (●) in plasma and tumour of a patient (No. 10) with carcinoma of the breast after 3 g/m² misonidazole. Tumour and plasma concentrations are expressed as μg/g and μg/ml respectively.

DISCUSSION

In view of the presence of significant regions of necrotic tissue in cerebral gliomas and their inability to metastasize, these tumours may be considered particularly suited to the use of hypoxic cell radiosensitizers.

Urtasun *et al.* (1976) have demonstrated a beneficial effect for adjuvant metronidazole in the post-operative radiotherapy of cerebral gliomas at a rather low radiation dose. Studies on experimental tumour systems have suggested that misonidazole

is a more efficient radiosensitizer (see Fowler *et al.*, 1976).

In previous trials with misonidazole in man, neurotoxicity has occasionally been a side effect (Dische *et al.*, 1977). In an attempt to avoid this problem we limited the misonidazole to 12 g/m^2 total divided into weekly doses. It was for this reason that the rather unorthodox radiation fractionation schedule was selected. Using this regime, no serious toxicity has been encountered. However, no conclusions may yet be made as to the value of adjuvant misonidazole in the treatment of cerebral glioma.

Pharmacokinetics studies using HPLC analysis have demonstrated good absorption of misonidazole with peak plasma levels occurring 1–4 h after treatment. Typical plasma concentrations of misonidazole at the time of radiotherapy were around 100 μg/ml. Some variation in plasma nitroimidazole concentrations between patients has been noted. The levels observed are in good agreement with those obtained in previous studies in which polarography was used as the analytical technique (Foster *et al.*, 1975; Dische *et al.*, 1977). In addition, we have also observed significant plasma levels of the misonidazole O-demethylated metabolite Ro 05-9963.

Levels of nitroimidazoles in human glioma tissue have not been measured in the present study. However, we have observed concentrations of misonidazole and Ro 05-9963 in breast carcinoma tissue in the range 50–100% of the corresponding plasma levels. Similar results have been reported for misonidazole in various human neoplasms (Gray *et al.*, 1976; Dische *et al.*, 1977) and Urtasun *et al.* (1975) observed good penetration of metronidazole into human brain tumour tissue. Furthermore, unpublished data from this laboratory have revealed a similar distribution of misonidazole between plasma and central nervous tissue in the rat.

In view of the data summarized above, it appears reasonable to predict that misonidazole levels in cerebral glioma may be at least 50% of the corresponding plasma concentration. Reference to previous experimental data for mammalian cells *in vitro* (Asquith *et al.*, 1974) and *in vivo* (Denekamp, Michael and Harris, 1974) would suggest that significant hypoxic cell radiosensitization may be achieved at such concentrations.

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