Treatment of Radioresistant Cancers

M. Abe, K. Sakamoto & T.L. Phillips
editors

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Editors:
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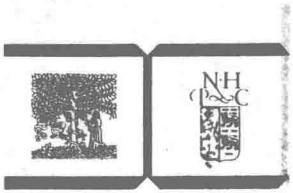

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PREFACE

With the advent of megavoltage sources, significant improvements in the cure rate of malignant disease have been achieved in radiotherapy. However, there are two limiting factors in current radiotherapy which have remained unresolved. One is that there exists highly radioresistant tumors such as osteosarcoma or hypoxic tumor cells which can hardly be eliminated by so called low LET radiation. The other is that if the tumor located near the radiosensitive critical structures, cancerocidal doses cannot be delivered. We must also admit that local failure is unfortunately still common even with the aid of megavoltage beams.

In this sense, we must say that radiotherapy has come to a turning point. The time has come for us to seek other radiation sources beyond photons or find biological methods to overcome these problems.

The purpose of this symposium is to explore ways to approach the resolution of these problems by physical and biological means. In the physical approach to these problems, enthusiasm has arisen for the study of high LET particles in clinical radiotherapy based on potential physical and biological advantages over the use of photons and electrons. The trial use of high LET radiations such as neutrons, heavy ions, pions as an alternative or additive to photon radiotherapy is actively underway in a number of centers. The results reported from these centers are most attractive. In the biological approach, chemical agents have been examined which will overcome the radioresistant hypoxic cells. We can, now, accept that chemicals exist which have the advantage of specifically sensitizing hypoxic tumor cells, while not affecting normal tissues. We also know that the combination of hyperthermia and radiation has the potential to become a powerful tool in the treatment of cancers.

It is for this reason, I think, that this symposium may be said to come at a most appropriate time. It is the purpose of this volume to review the current status of hyperthermia, hypoxic cell sensitizers and high LET radiations in radiotherapy and to suggest new approaches to the treatment of radioresistant cancers.

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RADIATION SENSITIZERS FOR HYPOXIC CELLS: PROBLEMS AND PROSPECTS

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INTRODUCTION

Despite the advanced treatment methods now available in radiotherapy, failure of local tumour control is a significant problem - in some sites particularly so. Although there are several reasons for this, a major cause of local failure is the problem of hypoxic cell radiation resistance. Hypoxic cells occur in a high proportion of human tumours. They arise as a result of tumour growth outstripping its blood supply and occur usually in, and around, areas of tumour necrosis[1].

Hypoxic cells are dangerous because of their relative radiation-resistance. These cells are in a resting state and would eventually die of oxygen starvation. However, subsequent to, or during, radiation treatment, tumour regression occurs due to the removal of oxic cells sterilized by the radiation. This permits some of the hypoxic cells to be re-oxygenated. They enter cycle, divide and provide a focus for the regrowth of the tumour.

Approaches to the hypoxic cell problem include treatment in hyperbaric oxygen chambers, unconventional fractionation regimes aimed at optimizing re-oxygenation processes during treatment, radiotherapy with high energy neutrons and finally radiosensitiz-drugs. Chemical agents which specifically increase the radiation sensitivity of hypoxic cells in tumours without increasing radiation damage to well-oxygenated normal tissue cells should be the preferred method of overcoming the hypoxic cell resistance. They would be cheap, and if free of complications, could be used

routinely in radiotherapy without the need to invest in expensive hardware or treatment machines.

DEVELOPMENT OF HYPOXIC CELL SENSITIZERS

Many chemical compounds exist that are able to sensitize hypoxic mammalian cells to radiation although not all of these display sensitizing activity in vivo. The largest class of hypoxic cell sensitizers is the "Electron-affinic" group, so called because their relative efficiencies are a direct function of their electron affinities or reduction potentials.

The electron affinity proposal[2] led to the examination of different types of chemical structures for evidence of radiation-sensitizing properties. Compounds shown to be active in irradiated hypoxic cultures of bacterial of mammalian cells included various conjugate diketones and diesters, quinones, aromatic ketones and numerous nitroaromatic or heterocyclic compounds. Some of the structures of the early compounds are shown in Figure 1.

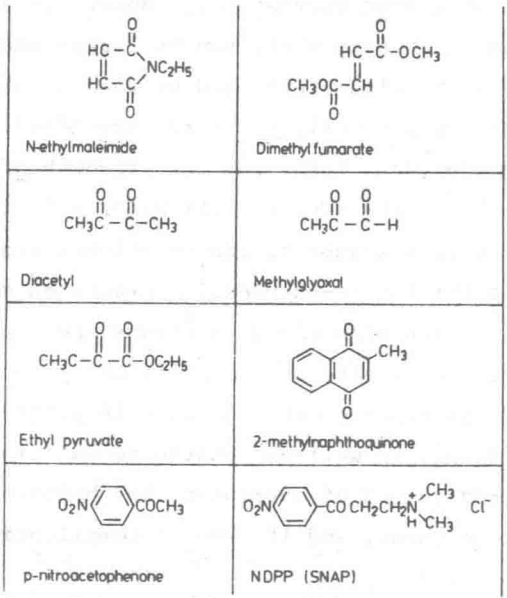


Fig. 1. Structures of some early sensitizers.

Bacterial systems were first used for assessing sensitization and many active compounds were found[3]. However, in general, little activity was found in mammalian cell systems in vitro. In 1971 the compound p.nitroacetophenone was found to be a potent sensitizer for hypoxic Chinese hamster cells irradiated in vitro[4,5]. This compound does not sensitize aerobic cells nor does the enhancement ratio for hypoxic cells vary very much with the position of the cell in the mitotic cycle. Evidence for sensitization by other nitrocompounds, the nitrofurans, soon followed[6]. This was interesting since several such compounds were in use clinically as antibiotics. However, sensitization in vivo is usually poor with these compounds since the large concentrations required for significant sensitization are often too toxic.

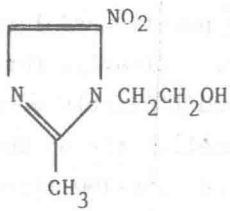
Although several hundred compounds of diverse chemical structure have demonstrated considerable sensitization of hypoxic cells in vitro, relatively few show pronounced activity in solid tumours in experimental animals. This is due mainly to the difficulties in penetration into the poorly-vascularized regions of tumours where hypoxic cells occur. Clearly, for a sensitizer to be effective in vivo, it must be sufficiently metabolically stable to enable it to diffuse intracellularly to the hypoxic cells which are situated probably about 150-100 microns distant from the nearest capillary.

METRONIDAZOLE AND MISONIDAZOLE

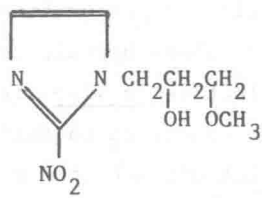
A considerable step forward occurred in 1973 with the discovery of the sensitizing action of metronidazole (Flagyl) in hypoxic bacterial and mammalian cells in culture[6,7]. Evidence soon followed of sensitization in various solid tumour systems, although the enhancement ratios observed were not large and large drug doses were generally required (for a review see references 8 and 9). Nevertheless, these results were import-

ant since they demonstrated the feasibility of the approach. Although metronidazole is relatively inefficient on a concentration basis, its half-life *in vivo* is fairly long thus apparently enabling the drug to diffuse into poorly-vascularized regions of tumours. Further encouragement followed with evidence from Urtasun and colleagues of apparent radiation sensitization of human gliomas by metronidazole[10].

The results with metronidazole led to the search for more active compounds in the nitroimidazole series. Electron affinity considerations indicated that the substituted 2-nitroimidazoles would be better sensitizers. This was based on the expectation that a nitro group substituted in the 2-position of the imidazole ring would interact to a greater degree with the π-electron system, of the heterocyclic ring than would a nitro group substituted in the 5-position. On examination of a range of 2-nitroimidazoles originally synthesised by Roche Products, one such compound Ro-07-0582, or misonidazole, was found to be a very efficient sensitizer both *in vitro* and *in vivo*[8,9].



Metronidazole



Misonidazole

Figure 2 illustrates the sensitizing ability of misonidazole in X-irradiated hypoxic Chinese Hamster cells[11]. Clearly, in hypoxia, appreciable sensitization at 1 mM is observed and at 10 mM the sensitization is comparable to that shown by oxygen itself.

Figure 3 shows data[12] on the sensitizing efficiency of misonidazole *in vitro* collected from several published studies. Both for metronidazole and misonidazole the data points do not

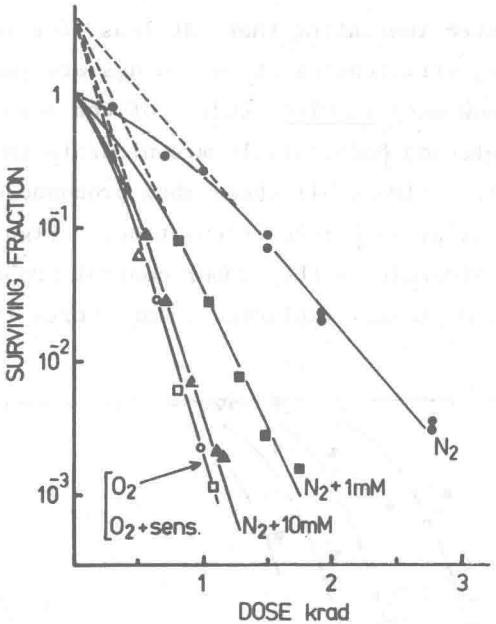


Fig. 2. Sensitization of hypoxic Chinese Hamster cells by misonidazole (ref. 11).

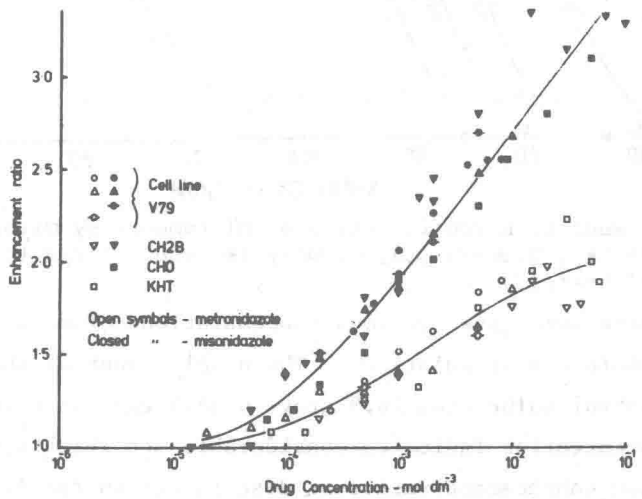


Fig. 3. Sensitization of a variety of cell lines by misonidazole and metronidazole (ref. 12).

show much scatter indicating that, at least for these cell lines, the sensitizing efficiencies of both drugs are fairly constant.

There are now many in vivo studies of the sensitizing ability of metronidazole and particularly misonidazole in experimental tumour systems. Almost all these show pronounced sensitization when the drug is given before irradiation. Figure 4 shows the effect of misonidazole on the tumour control probabilities for the anaplastic MT tumour implanted in an inbred strain of WHT/Ht mice[13].

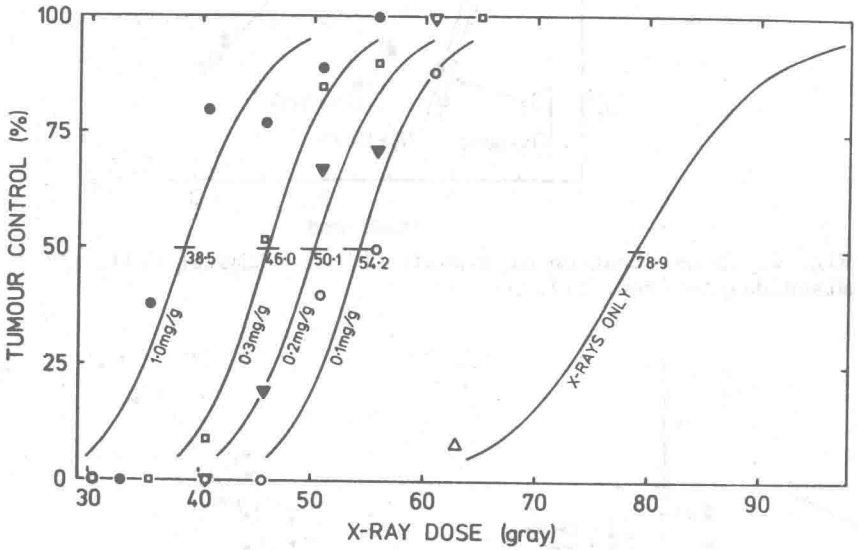


Fig. 4. Radiation sensitization of MT tumours by misonidazole at 1 mg/g (●), 0.3mg/g (□), 0.2mg/g (▼) and 0.1mg/g (○). X-rays alone (x) (ref.13).

The mice were given various concentrations of misonidazole 30 minutes before X-irradiation. The displacement of the 80 day TCD_{50} control value from 78.9 gray to 38.5 gray with increasing drug concentration indicates considerable sensitization. The calculated enhancement ratios or dose reduction factors are 1.46 (0.1 mg/g), 1.57 (0.2 mg/g), 1.17 (0.3 mg/g) and 2.05 (1.0 mg/g).

The large enhancement ratios found for single dose treatments

with X-rays and misonidazole would be expected to be smaller when the drug is given with multiple fractions of radiation spaced over a period, due to re-oxygenation processes reducing the hypoxic cell fraction. Numerous fractionation studies of this type have been carried out with misonidazole (collected papers in ref.14). An interesting result[15] showing that sensitization can occur in the MT tumour even with 20 fraction treatments is shown in Figure 5.

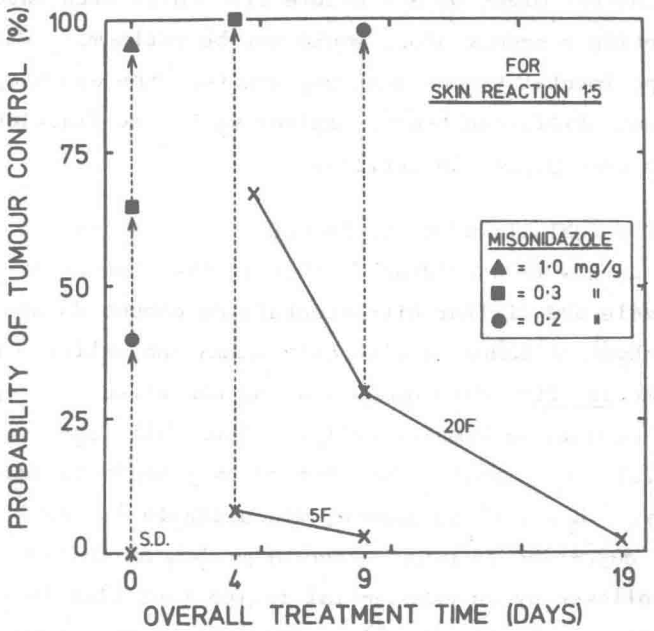


Fig. 5. Dependence of control probability for MT tumours X-irradiated after administration of misonidazole (see text) (ref. 15).

The Figure shows the tumour control probability of MT tumours treated with either single doses of radiation, 5 fractions in 4 or 9 days and 20 fractions in 4, 9 and 19 days. The mice were given 0.2, 0.3 of 1.0 mg/g with single radiation doses, 0.3 mg/g with each fraction of the 5 fraction treatments and 0.2 mg/g with each fraction of the 20 fraction treatments. Control probabil-

ities for each schedule were measured for the X-ray dose that would produce a constant level of skin damage (for details see reference). For X-rays alone, 20 fractions are better than 5 fractions for this tumour provided overall treatment time did not exceed 19 days. However, misonidazole improved both schedules to a uniform high level. This result suggests that by taking the criticality out of the choice of fractionation schedule, optimum therapy might be achievable clinically even though the fractionation schedule alone would not be optimum. With some other experimental tumour systems, however, the advantage afforded by misonidazole can become smaller with some fractionation schedules (see papers in ref.14).

HYPOXIC CYTOTOXICITY OF MISONIDAZOLE

There is now much evidence[14] that in the absence of radiation, misonidazole and similar nitro-containing compounds are much more toxic to hypoxic compared with oxic mammalian cells. Figure 6 shows some in vitro data demonstrating the effect in hypoxic cultures of Chinese Hamster cells. Oxic cells survive 5 mM misonidazole for almost 3 days before they begin to lose viability. However, in anoxia, misonidazole is highly toxic. The drug dose-time responses generally show an initial shoulder region followed by an exponential region (not clearly evident for the time scale in Fig. 6). Increase of drug dose causes both a reduction in the shoulder and an increase in the slope. The mechanism of this toxicity is believed to be associated with the production of a toxic substance following anaerobic metabolism of the drug[14]. There is now good evidence that this hypoxic cytotoxicity is due to processes other than those involved in hypoxic cell radiosensitization. For example, efficiency of sensitization in vitro shows little dependence on temperature yet the hypoxic toxicity in cells treated with misonidazole in the absence of radiation is very temperature-

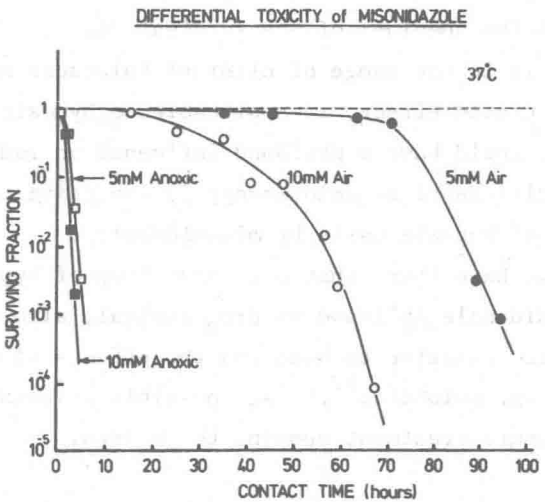


Fig. 6. The cytotoxic effect of misonidazole towards hypoxic and aerobic mammalian cells (ref. 12).

dependent. The question whether this cytotoxic effect can occur in human tumours has an important bearing on the possible future role of these drugs in combination with other cytotoxic drugs for the treatment of metastatic disease, in addition to their use in radiotherapy.

Pre-incubation effects of misonidazole

A phenomenon possibly related to the hypoxic cytotoxic properties of misonidazole is the potentiation of cellular damage by pre-incubation of hypoxic cells with the drug prior to exposure to radiation, heat or cytotoxic drugs.

Whitmore and colleagues[16] reported that if hypoxic cells are exposed to misonidazole for three hours and the cells are then irradiated after the drug has been removed by successive washing, the hypoxic survival curve shows a complete loss of the shoulder. In our laboratory[17] we have found that overnight incubation of hypoxic V79 cells with only 60μg/ml (0.3 mM) misonidazole followed