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METABOLISM AND RADIOSENSITIZATION OF 4,5-DIMETHYLMISONIDAZOLE, A RING-SUBSTITUTED ANALOG OF MISONIDAZOLE

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Abstract—4,5-Dimethylmisonidazole (DMM) is a ring-substituted derivative of the 2-nitroimidazole, misonidazole. 2-Nitroimidazoles are able to sensitize radioresistant hypoxic cells, and to kill them outright through bioreductive metabolism. The toxic process is believed to reflect the consequences of reductively activated drugs forming adducts with cellular (macro)molecules. Both this process and the radiosensitizing activity are thought to correlate with the electron affinity of radiosensitizing agents. In the present study, methyl groups were added to the imidazole ring of misonidazole in order to hinder adduct formation with cellular molecules after reductive-activation of the compound. It was anticipated that this would substantially decrease the hypoxic-cell toxicity of the parent drug. The presence of the two methyl groups reduced the half-wave reduction potential of DMM by about 70 mV, so we expected that its radiosensitizing ability would also decrease. In direct comparison with misonidazole, DMM, at equimolar concentrations, showed dramatically reduced binding to cellular macromolecules under bioreductive conditions, both *in vivo*, using a liver perfusion system, and *in vitro*, using tissue culture cells incubated under extreme hypoxia. However, DMM was only moderately less toxic than the parent compound, and showed greatly diminished radiation sensitization capacity. Since the decrease in toxicity was much less than expected, and the decrease in radiosensitization was much more than expected, this compound may be an important drug for continuing studies on the mechanisms of radiation sensitization, binding and cytotoxicity caused by electron affinic drugs.

A great deal of experimental and clinical evidence supports the importance of treatment-resistant, hypoxic cells in limiting tumor response to therapy [1–4]. Nitroheterocyclic drugs can be used to radiosensitize such cells, but prototypes of such drugs [e.g. misonidazole (MISO§), 3-methoxy-1-[2-nitroimidazole-1-yl]-2-propanol] have been too neurotoxic for such use in human patients at optimal concentrations [5–7]. Although it would be of great value to reduce the *neuro* toxicity of these drugs, their specific *hypoxic-cell* toxicity [8] and their ability to potentate the toxicity of chemotherapy agents [9, 10] are potentially useful properties, again for the elimination of hypoxic cells. Another important clinical use of nitroheterocyclic drugs at low, completely nontoxic concentrations is in the identification of hypoxic cells through metabolism-induced adducts to cellular (macro)molecules [11–13], this process being very sensitive to the ambient oxygen concentration [14, 15]. A reliable, biochemical monitor of oxygen concentration could be used in virtually all aspects of tumor treatments and management, and would be equally valuable in studies of the treatment and causation of many other types of disease and disease processes.

Development of better drugs requires a thorough understanding of their effects on cellular physiology/biochemistry, particularly if it were found possible to vary the properties of radiosensitization and toxicity independently. Although the precise mechanisms have not yet been worked out, radiosensitization is thought to be closely related to the electron affinity of the drugs [16, 17], as is their toxicity. The latter is thought to result from bioreductive activation, possibly to reactive radical intermediates followed by binding and/or inactivation of cellular target molecules [18, 19].

Extensive studies have been made for all of the above phenomena, *in vitro*, and *in vivo*, only for MISO. Most work on the chemistry and metabolism of MISO has focused on the requirement for (bio)reductive activation. The four-electron chemical reduction of MISO results in an activated compound that covalently binds to cellular macromolecules [11, 12, 18, 20] forms glutathione (GSH) conjugates [21], and undergoes imidazole ring fragmentation to glyoxal and 3-amino-1-methoxy-2-propanol [22]. The reactive metabolite of MISO responsible for covalent adduct formation does not alkylate extracellular protein, suggesting that its reactivity precludes diffusion across cell membranes [23, 24]. MISO forms a GSH conjugate in oxygen-deficient cultured cells [25] and the GSH conjugate is a major

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§ Abbreviations: MISO, misonidazole; MISO-amine, 3-methoxy-1-[2-aminoimidazol-1-yl]-2-propanol; DMM, 4,5-dimethylmisonidazole (3-methoxy-1-[2-nitro-4,5-dimethylimidazol-1-yl]-2-propanol); DMM-amine, 4,5-dimethylmisonidazole amine (3-methoxy-1-[2-amino-4,5-dimethylimidazol-1-yl]-2-propanol); GSH, glutathione; and EIMS, electron impact mass spectrometry.

metabolite in hypoxic isolated perfused rat livers [26]. Although radiosensitization of hypoxic cells by MISO is seen at low temperatures, where reductive metabolism is minimized, the mechanism of *enhanced* radiosensitization by MISO after incubation at 37° under hypoxia [27, 28] may depend in part on the depletion of GSH and other nonprotein sulfhydryl groups, and/or the inactivation of sulfhydryl-containing proteins via the formation of thiol conjugates [29, 30]. Synthetically prepared MISO–GSH conjugates consist of approximately equal amounts of 4- and 5-substitution on the imidazole ring [21, 31], whereas the GSH conjugate formed by isolated perfused rat livers is predominantly attached to the imidazole ring of MISO via position 5 (Smith BR and Born JL, unpublished results). McClelland *et al.* [32] have shown that the formation of the GSH conjugate of MISO occurs via an initial nucleophilic attack by protein thiols on MISO hydroxylamine, a bioreductive metabolite of MISO. Covalent binding of MISO to cellular proteins may occur via a similar nucleophilic attack by protein thiols on MISO hydroxylamine. Raleigh and Koch [33] have shown that nitroheterocycles irradiated under reducing conditions form covalent adducts with proteins at dramatically increased rates when the proteins contain cysteine moieties, and that under such favourable conditions, protein adducts can account for more than 20% of the reduced drug.

We have prepared a derivative of MISO, 4,5-dimethylmisonidazole (DMM; 3-methoxy-1-[2-nitro-4,5-dimethylimidazol-1-yl]-2-propanol), as a probe to investigate the role of covalent binding in the biological actions of MISO. DMM was designed with methyl groups at positions 4 and 5 of the imidazole ring to reduce the potential for covalent binding and GSH depletion via reductively activated metabolites. We anticipated that this derivative might be substantially less toxic than MISO but that the methyl groups would not greatly diminish the electron affinity of the drug and hence its radiosensitizing ability.

MATERIALS AND METHODS

Chemistry. DMM was prepared via the condensation of 4,5-dimethyl-2-nitroimidazole in ethanol with glycidyl methyl ether in the presence of K_2CO_3 to produce yellow crystals in 76% yield, m.p. (ethanol) 112°; ^{1}H-NMR ($CDCl_3$) 2.12 (s, 3H) (imidazole methyl), 2.26 (s, 3H) (imidazole methyl), 3.4 (s, 3H) (methoxy), 3.54 (m, 3H), 4.16 (m, 2H), and 4.56 (m, 1H); EIMS m/z = 229, 185 (M-NO_2), base peak 109 (M − 120).

DMM was converted to 1-[2-nitro-4,5-dimethylimidazol-1-yl]-3-methoxy-2-propanone (DMM-ketone) via oxidation with CrO_3 in acetone, yellow crystals; EIMS m/z = 227, 181 (M-NO_2). [2-^{3}H]4,5-Dimethylmisonidazole (8 mCi/mmol) was prepared via the reduction of DMM-ketone with $NaB[^3H]_4$ as reported for the synthesis of [^{3}H]MISO [34].

DMM-amine (3-methoxy-1-[2-amino-4,5-dimethylimidazol-1-yl]-2-propanol) was prepared by reduction of 4,5-dimethylmisonidazole with platinum dioxide and hydrogen; EIMS m/z = 199, 184 (M-CH_3), base peak 109 (M − 120).

The reduction potential, $E_{1/2}^{RED}$, of both MISO and DMM, was evaluated relative to Ag/AgCl (saturated) electrode by means of cyclic voltametry in an argon-purified solution of anhydrous acetonitrile with tetra-*n*-butylammonium perchlorate as the supporting electrolyte. Platinum wire was utilized as the working and auxiliary electrode.

Chromatography. An Alltech column (150 mm length, 4.5 mm i.d., 5 μm Ultrasphere-ODS) was used in the separation of DMM and its metabolites. Separations were obtained at a flow rate of 1 mL/min using a 0.05 M phosphate/methanol gradient (pH 2.3) which was 0% methanol at 5 min, 5% at 23 min and 25% at 43 min. In this system DMM had a retention time of 43 min and DMM-amine, the terminal reduction product of DMM, had a retention time of 37 min. Column eluate was collected at 1-min intervals, and the tritium content was quantitated by liquid scintillation counting.

In vivo *studies: Liver perfusion.* Male Sprague–Dawley rats (180–200 g) were anesthetized with methoxyfluorane and the livers (14.7 ± 1.2 g) were surgically prepared and perfused in a recirculating system using the procedures and apparatus reported earlier [26, 35, 36]. Oxygen concentration in the perfusion medium was monitored using a YSI model 53 oxygen monitor and the oxygen partial pressure was maintained at 10% or less in all experiments. DMM was dissolved in the perfusion medium (100 mL) by sonication to provide an initial drug concentration of DMM. The solubility of DMM in perfusion medium was greater than 5 mM. Perfusion medium and bile samples were removed at timed intervals for HPLC analysis of DMM and metabolites. DMM and its metabolites were recovered from the liver at the end of the experiment by homogenizing and extracting a liquid-nitrogen freeze-clamped tissue section with methanol. Protein was separated from the homogenate by centrifugation; then the supernatant was recovered and evaporated under reduced pressure prior to analysis by HPLC. Bile and perfusion medium samples were chromatographed directly. Concentrations of tissue non-protein sulfhydryl (NPSH, chiefly glutathione) were determined by the method of Ellman [37] using an extinction coefficient for reduced 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) of 13,600 M^{-1} cm^{-1}. Covalent binding of DMM-derived radioactivity was determined utilizing the phenol extraction method as described for the quantitation of carcinogens bound to macromolecules [38, 39].

In vitro *studies: Adduct formation and toxicity.* The cells utilized in these studies were derived from V79 Chinese hamster fibroblasts. The WNRE subline, selected for its ability to grow both in suspension and in monolayer culture, was obtained from Dr. J. D. Chapman. The cells were thawed from frozen stock on a roughly semi-annual basis, and tests were made routinely to ensure that the cultures were free from mycoplasma and other contaminations. The cells were cultured (37°, 95% air/5% carbon dioxide, 100% relative humidity) in the exponential phase of growth by twice weekly transfers using Eagle's Minimal Essential Medium containing 12.5% (v/v) fetal bovine serum and antibiotics (all culture solutions from GIBCO). On

the day before an experiment, cells were trypsinized and plated onto glass Petri dishes—approximately 200,000 cells confined to the central area of the dish as described previously [40] followed by overnight incubation at 37°. The dishes were then removed from the incubator and cooled to 0°, and their medium was replaced with a drug-containing medium, first as a rinse (1 mL) which was simply aspirated and then as the actual medium used for the experiment (also 1 mL). Dishes were placed in leak-proof aluminum chambers which were connected to a manifold allowing them to be deoxygenated with a series of gas exchanges taking approximately 30 min [41]. The confinement of cells to the central area of the dish and the use of a small volume of medium allow very rapid equilibrium of the gas and liquid phase to improve the control of oxygen concentration [41]. After deoxygenation, the chambers were incubated at 37°. To prevent minor gradients of oxygen or potentially larger gradients of nutrients/metabolites, the chambers were also shaken gently (1 Hz, 2.5 cm stroke).

To assay binding of radioactive nitroheterocycles after incubation under defined experimental conditions [14, 42], the chambers were removed from the incubator and opened (allowing immediate reoxygenation), and the dishes were cooled on ice. The radioactive medium was also removed. Two rinses with HEPES-buffered Earle's balanced salt solution (EBSS) were followed by the addition of fresh medium and the dishes were incubated at 37° for 15 min. Another rinse in EBSS was followed by cell removal with 0.05% trypsin (200:1 dilution of SIGMA "40×") in calcium and magnesium-free EBSS with citrate buffer. An equal volume of serum-containing medium was added to stop the trypsin and a portion of the cell suspension was counted. The cells were concentrated by centrifugation at 1000 rpm for 10 min followed by resuspension in 1 mL of EBSS, on ice, and then the cells were disrupted by the addition of 100 μL of 1 M ice-cold trichloroacetic (TCA). The TCA precipitate was concentrated by centrifugation at 2500 rpm for 20 min. The TCA supernatant was saved and the pellet was dissolved in 0.2 mL of 1 N NaOH, followed by neutralization with acetic acid. The clear TCA supernatant and the dissolved pellet were added with scintillation fluid to vials and counted in a Beckman liquid scintillation counter (LSC), resulting in a separation of acid-soluble and acid-precipitable counts. The above procedure results in the background levels of incorporated radioactivity when radioactivity sensitizers are incubated at low temperatures (4°) in hypoxia, or at 37° in air [14]. Since the cell number, drug concentration, specific activity, and LSC efficiency were all known, the absolute incorporation of adducts could be calculated as picomoles per cell per hour of incubation at 37°. For V-79 WNRE cells, which have a volume of about 0.75 pL [43], this unit is about 30% smaller than molar.

Cytotoxicity of the nitroheterocycles was assessed using similar techniques but at much higher concentrations of non-radioactive drug. After incubation with or without drug-containing medium, cells were trypsinized and plated for colony formation as described previously [42, 44].

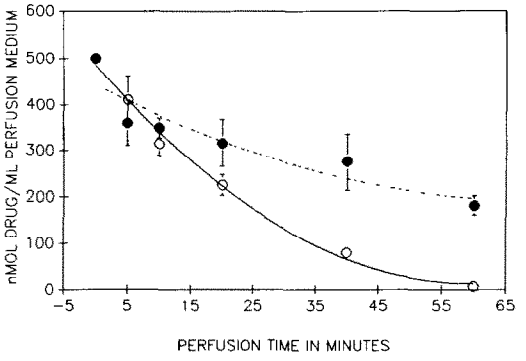

Fig. 1. Comparative clearance of 0.5 mM MISO (○) and 0.5 mM DMM (●) from the perfusion medium in an isolated, perfused hypoxic rat liver preparation. Values are means ± SD, N = 3.

Radiation sensitivity. Cells were treated as with the metabolism experiments except that gas equilibration and irradiation were performed at 0–4°. Survival was assessed by colony formation as described for the cytotoxicity experiments. Complete survival curves were performed and enhancement ratios (sensitivity under nitrogen/sensitivity with sensitizer) were calculated as described previously [41]. The radiation source used was a Gammacell 220 (Atomic Energy of Canada) which provided ^{60}Co radiation at a dose rate of approximately 20 Gy/min, or a Shepherd irradiator, which delivered ^{137}Cs radiation at a dose rate of approximately 12 Gy/min.

RESULTS

$E_{1/2}^{RED}$ values for MISO and DMM were 1.002 and 1.071 V, respectively. This result is consistent with the addition of two electron donating methyl groups on the imidazole ring which would serve to increase the relative electron density of the imidazole ring.

Metabolism and binding in vivo. The rate of disappearance of DMM from the perfusion medium in the hypoxic perfused rat liver preparation was much slower than that observed for MISO (Fig. 1), reflecting a slower rate of metabolism for the former. This comparative difference in clearance rates was observed in perfusions with both 0.5 and 2.5 mM DMM (not shown).

The profile of DMM-derived metabolites in the perfusion medium from hypoxic isolated perfused rat livers, following a 60-min perfusion, is shown in Fig. 2. The major biotransformation product in the perfusion medium was the amine metabolite of DMM (DMM-amine), which was identified by co-chromatography with an authentic standard. Both 0.5 and 2.5 mM perfusions of DMM resulted in a linear increase in the concentration of DMM-amine in the perfusion medium (Fig. 3). Although small quantities of DMM-amine were secreted into the bile, the major component in the bile was unchanged

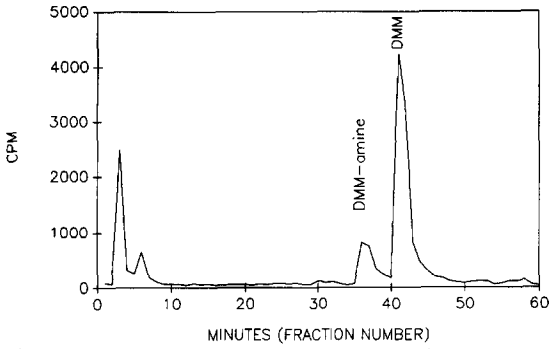

Fig. 2. Radiochromatogram of the perfusion medium resulting from a 60-min perfusion of DMM in an isolated hypoxic rat liver preparation.

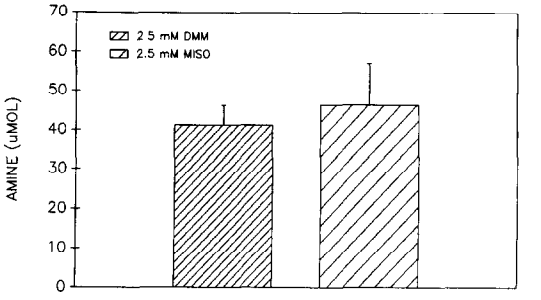

Fig. 4. Comparison of the total concentration of amine metabolites produced after 60-min perfusions of either 2.5 mM MISO or DMM in a hypoxic isolated rat liver preparation. These values were obtained by determining the concentration of amine metabolite, via HPLC analysis, in the perfusion medium, the bile, and in the tissue following perfusion with radiolabeled drug. Values are means ± SD, N = 3.

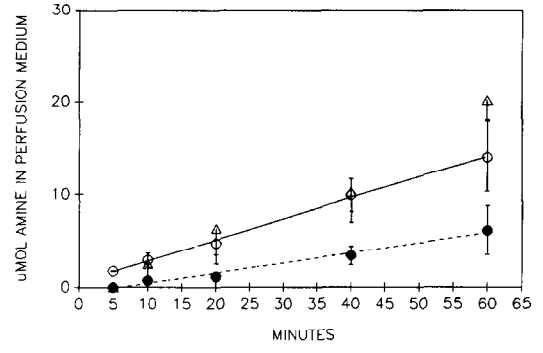

Fig. 3. Comparison of the concentration of amine metabolite of DMM and MISO in the perfusion medium from hypoxic isolated rat liver preparations: (●) 0.5 mM DMM; (△) 2.5 mM DMM; and (○) 0.5 mM MISO. Values are means ± SD, N = 3.

DMM (not shown). The total amount of amine metabolite in perfusion medium, bile and tissue was 8.52 ± 2 μmol for 0.5 mM perfusions (data not shown) and 41.16 ± 5.1 μmol for 2.5 mM perfusions (N = 3 preparations) as shown in Fig. 4. There was no significant difference in the quantity of amine produced from the reduction of either DMM or MISO in hypoxic perfused livers.

Hepatic, DTNB reactive, low molecular weight thiols, predominantly GSH, were not altered by perfusion with 2.5 mM DMM. In contrast, similar concentrations of MISO significantly decrease the concentration of hepatic thiols [26].

For 60-min perfusions, the covalent binding of DMM to tissue protein was 0.27 ± 0.11 nmol/mg for 0.5 mM drug and 0.68 ± 0.1 nmol/mg for 2.5 mM drug. The covalent binding of DMM to RNA and DNA was so limited that it could not be determined accurately.

Toxicity in vitro. Toxicity studies showed that DMM was somewhat less toxic than MISO. Data from several experiments showed consistently greater toxicity by 4 mM MISO than by 6 mM DMM (Fig. 5). The high degree of variability in the cytotoxic response, as yet poorly understood but described previously for MISO [44], is not likely caused by variability in GSH depletion. GSH analysis (as measured by the Tietze assay [45]) of cells treated in representative experiments to give approximately 10% survival showed extensive depletion by 4 mM MISO (50–75%) but none by DMM. At similar drug concentrations, no toxicity or thiol depletion was observed under aerobic conditions. At much higher concentrations (5 mM—approaching the solubility limit for DMM) and for longer times, aerobic exposure to DMM was *more* cytotoxic than MISO, and further investigations of this interesting finding are in progress.

Covalent binding in vitro. Adduct formation was measured using 20 μM drug (a completely non-toxic concentration) under conditions of extreme hypoxia. Acid-soluble products retained by cells incubated under hypoxia were produced by DMM at a rate which was about half that by MISO. In contrast, acid-precipitable products were produced by DMM at a rate which was only 1/20th that by MISO (Fig. 6).

Radiosensitization in vitro. Radiation sensitization by DMM is shown in Fig. 7. The radiosensitization resulting from irradiation by either 2 or 10 mM DMM was substantially less than by similar concentrations of MISO [46]. A measure of radiosensitization is the sensitizer enhancement ratio (SER) defined as the dose of nitrogen divided by the dose in air to give the same radiosensitizing effect. The SER values for 2 and 10 mM DMM were 1.5 and 1.7, respectively (Fig. 7), whereas the values for the same concentrations of MISO were 2.1 and 2.3, respectively [46].

DISCUSSION

The reductive metabolism of DMM is quantitatively and qualitatively different from that of MISO. In perfused rat liver, DMM was less extensively metabolized, and the major metabolite was the six-electron reduction product DMM-amine.

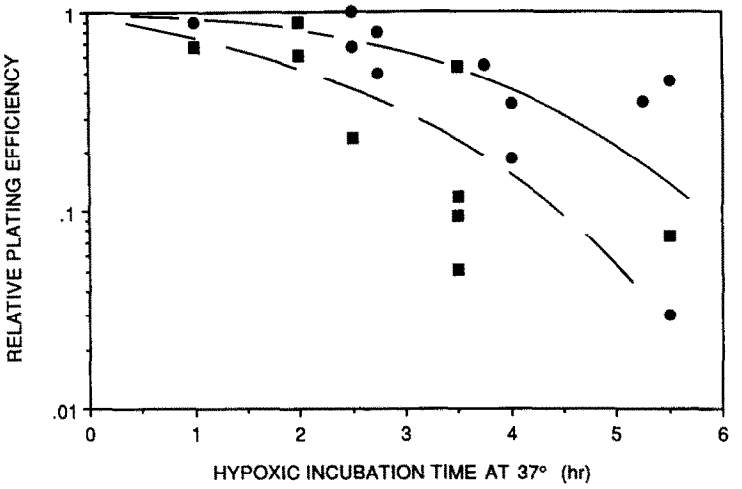

Fig. 5. Cytotoxicity towards V79-WNRE cells incubated *in vitro* at 37° under hypoxic conditions with DMM at 6 mM (●) vs MISO at 4 mM (■). Each point represents the average survival of several dishes of cells, corrected for plating efficiency. Individual points at the same or closely spaced times are from separate experiments.

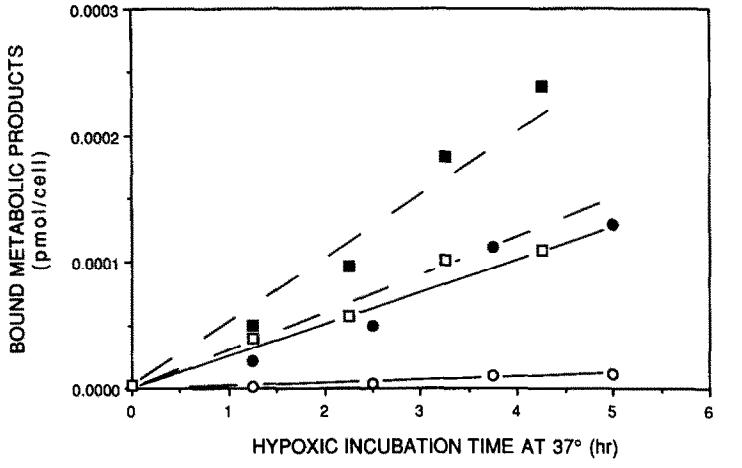

Fig. 6. Production of retained products of the metabolism of DMM and MISO *in vitro*; V79-WNRE cells were incubated at 37° under hypoxic conditions with 20 μM drug, and analyzed for acid-soluble (■, ●) vs -insoluble (□, ○) products. Key: MISO (■, □); and DMM (●, ○).

The extensive formation of GSH conjugates that occurred with perfusions of MISO was not observed in perfusions of DMM. The clearance of DMM from the perfusion medium, as shown in Fig. 1, was markedly slower than the clearance of MISO [35]. Consistent with the reduced clearance for DMM was the finding that the concentration of DMM-amine in the perfusion medium was lower than the concentration of the terminal reduction product of MISO, MISO-amine, in comparable experiments. In contrast, the fraction of total amine metabolite retained in the liver following the completion of the perfusions was proportionately greater for DMM than for MISO. Thus, when the retained amine was considered in liver perfusions with either MISO or DMM, the total amount of amine reduction production was similar for both compounds.

The reductive metabolism of MISO in the hypoxic isolated perfused rat liver preparation was accompanied by depletion of hepatic GSH and the formation of a MISO–GSH conjugate as the major biliary metabolite [26, 36]. In marked contrast, the bile from livers perfused with DMM contained DMM as the primary component although there were, in addition, a number of undefined minor peaks. The lack of GSH conjugates in the bile or perfusion medium from these preparations suggests that the placement of methyl groups on positions 4 and 5 of the imidazole ring of MISO inhibits the nucleophilic attack of GSH on bioactivated DMM intermediates.

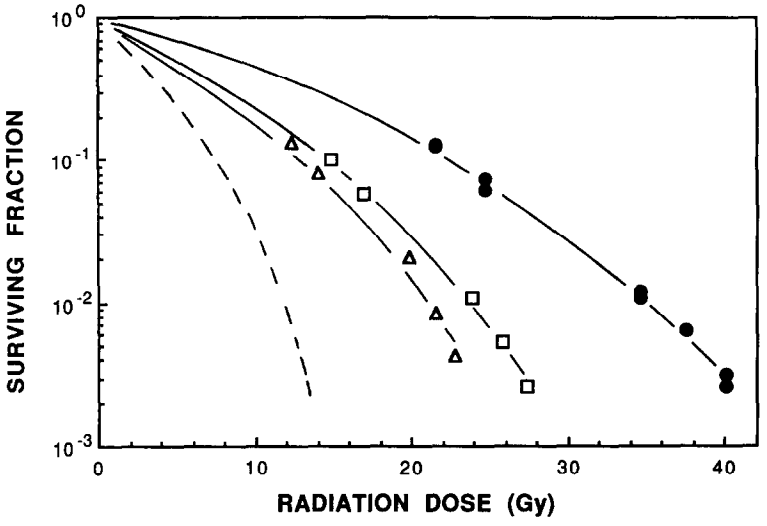

Fig. 7. Radiosensitization of hypoxic V79-WNRE cells by DMM at 0°. Drug concentrations were 0 (control cells, ●), 2 mM (□), and 10 mM (△). Each point represents the average survival of several dishes of cells, corrected for plating efficiency. All points are from the same experiments. A typical aerobic radiation response curve (i.e. 3-fold more sensitive than the hypoxic response curve) is shown by the dashed line without points.

These data suggest that the covalent bonding between DMM and cellular proteins should be significantly less than that observed with MISO.

This observation was confirmed by a comparison of the covalent binding of MISO and DMM *in vivo*. The decrease in covalent binding of DMM as compared to MISO occurred even though similar quantities of both MISO and DMM were converted to the respective amino terminal reduction products. The elimination of GSH depletion and the significant reduction of covalent bond formation observed in these experiments suggest that nucleophilic attack on an activated metabolite is precluded by the methyl groups on DMM.

We have shown that DMM undergoes a six-electron reductive biotransformation to produce DMM-amine. The concentration of DMM-amine is similar to the concentration of MISO-amine produced in similar experiments. However, the clearance of DMM was much slower than the clearance observed for MISO, suggesting a substantial difference in the pathways or in the reactive characteristics of reduced intermediates for these two compounds. In comparison to MISO, the reductive metabolism of DMM resulted in dramatically less covalent binding and no depletion of hepatic GSH. These results have a number of implications for the design of radiosensitizers and hypoxic cell markers. The failure of DMM to undergo extensive covalent binding suggests that hypoxic tissue markers may require an unsubstituted imidazole ring for maximum sensitivity.

The results *in vitro* add another interesting aspect to the bioreductive adduct-forming properties of DMM. The rate of adduct formation to acid-insoluble molecules was almost eliminated by the methyl-group additions. However, the low molecular weight or acid-soluble products of metabolism of DMM were reduced only 2-fold from those of MISO. Since the *in vitro* data do not indicate any GSH adducts, the identification of the acid-soluble products *in vitro* should prove interesting (work in progress). We know that these presumably low molecular weight compounds are not unmetabolized drug because this would be eliminated by the extensive rinses and incubations in air after the hypoxic incubation period (see Materials and Methods). It is well recognized that uncharged nitroheterocycles are freely diffused through cellular membranes [43, 47], and no low molecular weight material is retained after incubation in air at 37° or in nitrogen at 4°. This anomalous cellular retention of presumably non-GSH adduct metabolites has also been seen using MISO under conditions where the endogenous GSH had been almost completely removed [43].

Similarly, DMM was a much less efficient hypoxic-cell radiation-sensitizing agent than MISO. Although DMM did not have any of the properties which can lead to drug exclusion from the intracellular space (very high polarity, large molecular mass, or charge) [43, 47], the above results suggested the importance of confirming that DMM and MISO achieved similar intracellular drug concentration, compared with the drug concentration in the medium. In an experiment using the same batch of cells and techniques described previously [43], both sensitizers achieved approximately 65% equilibrium of intra- versus extracellular concentration (not shown).

The reduced efficiency of radiosensitization of DMM may involve at least two factors (not necessarily independent): the concentration of drug for half-maximal effect, and the magnitude of the maximal effect [48]. Investigations are underway to compare the radiosensitization properties of DMM

with both 2-nitroimidazoles like MISO as well as 5-nitroimidazoles such as metronidazole. The latter comparison will be made because a partial explanation for our findings rests with the decreased reduction potential of DMM (approximately 70 mV less positive than MISO). By way of comparison, metronidazole has a reduction potential of about 100 mV less positive than MISO [17] and metronidazole is certainly less toxic and is a less efficient radiosensitizer and adduct-forming drug [24]. Further work is planned to attempt to differentiate between the effects of ring methylation and electron affinity.

The small reduction in cytotoxicity of DMM versus MISO, even though the former did not deplete intracellular GSH, may indicate that adduct formation is *not* a primary cause of toxicity for the nitroheterocycles. In addition, since high (cytotoxic) concentrations of MISO are required to produce GSH depletion in hypoxic cells and GSH depletion enhances the cytotoxicity of MISO [30], the small reduction in toxicity seen for DMM versus MISO may simply reflect the fact that DMM does not deplete cellular GSH. DMM thus becomes an important model for the cytotoxic process, since it may have more specific effects on cellular metabolism due to the near elimination of adduct formation with high and low molecular weight thiols.

In summary, although we had originally hoped that DMM would be a good radiation sensitizer with reduced toxicity, our results are more consistent with the opposite effects. However, DMM appears to be a very useful molecule to investigate mechanisms of radiation sensitization, binding, and cytotoxicity by nitroheterocyclic drugs.

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