Pharmacokinetics of the Hypoxic Radiosensitizers Misonidazole and Demethylmisonidazole after Intraperitoneal Administration in Humans

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ABSTRACT

The hypoxic radiosensitizers misonidazole or demethylmisonidazole were administered i.p. in a 2-liter volume to 6 patients affected by advanced ovarian carcinoma, and the pharmacokinetic course of the two drugs was studied. The clearance of misonidazole and demethylmisonidazole from the peritoneal fluid was 19.1 and 12.4 ml/min, respectively. At 3 hr after drug administration, both radiosensitizers had peritoneal fluid concentrations more than 8 times larger than in the plasma.

The concentration x time exposure in the peritoneal fluid was 3.2 times larger than in plasma for misonidazole and 7.6 times for demethylmisonidazole. The advantage of i.p. delivery compared with systemic delivery decreases with distance from the peritoneal surface, but the advantage may be maintained for up to 1 mm or 100 cell layers. These differences between the two routes of administration provide a rational basis for the expectation that a substantial increase of the therapeutic benefits of misonidazole and demethylmisonidazole in potentiating radiation therapy or chemotherapy can be expected in treating tumors confined to the i.p. space.

INTRODUCTION

The 2-nitroimidazole derivative MIS2 and its analogue DMIS are the most widely investigated hypoxic radiosensitizers. However, both drugs cause cumulative, dose-dependent neurotoxicity (1, 10, 11) that reduces their therapeutic ratio and limits their administration to suboptimal and possibly ineffective doses during a typical fractionated course of radiation therapy.

A reasonable goal would thus be to increase the therapeutic ratio of the 2 hypoxic radiosensitizers by increasing the tumor tissue exposure to the drugs while maintaining the total plasma AUC within safe limits.

The i.p. route of administration provides a potentially useful alternative for the delivery of anticancer drugs to selected tumors involving i.p. structures. For drugs which have slower clearance from the peritoneal fluid than from plasma, theoretical analyses (8) and Phase I clinical trials (17) have suggested that large concentration differences can be maintained between peritoneal fluid and plasma when the i.p. route is used. However, a drug diffusing from the peritoneal fluid into surrounding tissues crosses capillary membranes and is removed by blood flow, with a rapid decrease in concentration within a few mm or less of the peritoneal surface. Thus, the ideal target for i.p. delivery is a tumor that diffusely seeds the peritoneum with small nodules (8).

In order to evaluate the i.p. route of delivering such radiation sensitizers, we began a study of i.p. administration of MIS or DMIS plus radiation in patients with advanced ovarian cancer. The pharmacokinetic analysis of the patients entered in this study is the subject of this report.

MATERIALS AND METHODS

Six patients, 4 treated with MIS and 2 with DMIS, were studied. They had histological proof of advanced ovarian carcinoma confined to the intra-abdominal space and had already failed conventional treatment with combination chemotherapy. Informed consent was obtained from all patients.

Peritoneal catheters were surgically implanted into the intra-abdominal cavity according to the described procedure (14). Patients were treated with MIS (NCS 261037) or DMIS (NCS 261036) in 2 liters of Inpersol (Abbott Laboratories, North Chicago, Ill.) containing 1.5% dextrose. The peritoneal fluid was drained after 3 to 4 hr of dwelling. All patients received concomitant whole abdominal irradiation in a variety of dose schedules dependent on their bulk of tumor and their symptomatology. The treatment was discontinued at first appearance of toxicity or clear evidence of progression.

MIS and DMIS Assay. MIS and DMIS in peripheral venous plasma and peritoneal fluid were assayed by HPLC. The samples were prepared for analysis by quantitatively diluting 0.4 ml of the sample with 1 ml of trichloroacetic acid for protein precipitation. Samples were then vortexed and spun for 10 min at 2000 × g in a refrigerated centrifuge. Injection of 0.02 ml of the peritoneal fluid supernatant and 0.1 ml of the plasma supernatant was automatically performed by a WISP injector (Waters Instruments, Milford, Mass.) into an HPLC system equipped with a Waters Model 440 UV detector with a 340-nm filter and a Waters Data Module integrator-recorder. A Waters HPLC system using a RCM-100 C18 reverse-phase column and a mobile phase of 200 mM sodium acetate buffer and acetonitrile (95:5 v/v; pH 3.4) was used isocratically at 2 ml/min for separation.

The retention time for DMIS and MIS was 2.5 and 7 min, respectively. The solvent front did not interfere with either peak for both plasma and peritoneal fluid samples. The integrated area under the peak was used for quantification. Two external standard curves of peak area versus concentration were constructed each day of analysis upon addition of known amounts of MIS and DMIS to either water for the peritoneal fluid samples (range of concentrations, 0.1 to 2 mg/ml) or pooled normal plasma for the plasma samples (range, 5 to 100 µg/ml). For both standard curves and for both drugs, the correlation coefficient was consistently greater than 0.98.

Pharmacokinetic Calculations. For all calculations, MIS and DMIS pharmacokinetics were assumed to be linear within the concentration range relevant to this study. The fraction (F) of the instilled dose which...
disappeared from the peritoneal fluid during the exchange was calculated as the ratio of the difference between the amount instilled and the residual amount of drug drawn at the end of the exchange divided by the initial amount instilled. Since no appreciable first-pass clearance through the liver takes place for the 2 drugs (20), F was considered equal to the amount of drug that entered the vascular compartment.

Total body clearance (\(C_{\text{TB}}\)) is a measure of the overall disappearance of the drug from the body after it reaches the central compartment. This value was calculated according to the equation:

\[
C_{\text{TB}} = \frac{F \times \text{(Dose)}}{\text{AUC}_p} \tag{A}
\]

The plasma area under the curve (\(\text{AUC}_p\)) is an expression of the concentration \(\times\) time exposure to the drugs in plasma, and it was calculated by the trapezoidal rule from Time 0 to 24 hr and then extrapolated to infinity according to the individual terminal half-time in plasma. For MIS, the extrapolation accounted for 20 to 25% of the total area; for DMIS, less than 5%. The AUC in the peritoneal space (\(\text{AUC}_i\)) was calculated according to the same rule from Time 0 to the end of the exchange but not extrapolated to infinity since there was complete removal of the drug from the i.p. space at the end of the exchange. Instead, the \(\text{AUC}_i\) from the end of the dwell to infinity was added to correct for the persistence of the drugs in the i.p. structures due to the plasma drug levels.

The disappearance of MIS and DMIS from the peritoneal fluid could not be considered a first-order kinetic process, as for other drugs (17), since the slow rate of drug disappearance from the body allows a back diffusion of MIS and DMIS from the plasma compartment to the peritoneal fluid. For this reason, the permeability-area product (PA) or drug clearance from the peritoneal fluid expressed in ml/min was calculated according to the equation:

\[
\text{AUC}_i = \frac{(\text{Cl}_{\text{TB}}/\text{PA}) + 1}{\text{F}\times(\text{AUC}_p - \text{AUC}_i)}
\]

rearranged to

\[
\text{PA} = \frac{(\text{Cl}_{\text{TB}}\times\text{AUC}_i)}{(\text{AUC}_p - \text{AUC}_i)} \tag{B}
\]

RESULTS

The i.p. administration of MIS or DMIS in combination with radiation therapy was well tolerated. While all patients experienced various degrees of nausea, vomiting, and diarrhea, the symptoms were not unusually pronounced and were mostly attributed to the abdominal irradiation. Patient J. D. (Table 1) developed acute peritonitis 2 days after the first course of MIS. This patient had been treated previously with i.p. 5-FU and had malignant ascites. No bacterial growth was evidenced by multiple analyses of the ascitic fluid, but the peritonitis promptly resolved with aggressive antibiotic treatment. This episode was considered a complication of catheter placement, although MIS-induced chemical peritonitis could not be ruled out. This patient had a second course of i.p. MIS without unusual side effects, and then treatment was discontinued because of progression of her malignant disease. All patients experienced mild back pain and temporary discomfort during the peritoneal exchanges, as expected with this modality of drug administration (14). One patient (W. B.) had severe pain and was unable to tolerate the dialysis after the first course of treatment. In the 2 patients treated with DMIS, mild and reversible paresthesias of the extremities developed after the seventh and eighth courses, respectively. Treatment was then discontinued.

Pharmacological study. The main pharmacokinetic constants for MIS and DMIS after i.p. administration are shown in Table 2. After a 3-hr dwell period, the i.p. drug concentrations declined to 27 ± 5.7% (S.E.) of the instilled concentration for MIS and to 36 ± 4.4% for DMIS.

The i.p. concentration measured after a 3-hr dwell period was consistently higher than that in the plasma at the same time (Table 3), with a larger difference for DMIS than for MIS, as might be expected from their respective \(C_{\text{TB}}\) (Table 2). Also, the ratio between the \(\text{AUC}_i\) and \(\text{AUC}_p\) was different for the 2 drugs and larger for DMIS than for MIS (Table 3).

Two typical overall kinetic courses of MIS and DMIS after i.p. administration of 4 g are illustrated in Chart 1. The plasma
kinetic profile is similar to that reported after p.o. administration of the drugs (1, 10). Early plasma appearance of DMIS after administration of MIS was observed. In Chart 1, the solid lines represent the kinetic course of the 2 drugs if the same dose was administered i.v. The values in the chart for i.v. administration were linearly scaled from published data (5, 20). As shown, the i.v. administration produces larger systemic AUC than does i.p. administration of the same dose, since part of the i.p. dose is withdrawn before absorption.

If peritoneal fluid were present during i.v. administration, the AUCp would be similar to AUCp. On the other hand, i.p. administration produces substantially higher AUCp compared with AUCp. Thus, the i.p. route produces lower systemic exposure, which is correlated with toxicity, and higher local concentrations, which is correlated with radiation sensitization.

**DISCUSSION**

The clinical value of MIS and DMIS has been overshadowed by their dose-dependent neurotoxicity (10, 11), which requires discontinuation of the drugs at a total p.o. dose of 12 g/sq m and prevents the achievement of adequate sensitizing concentrations at the tumor site during a conventional fractionated course of radiotherapy (1). The risk of developing peripheral neuropathy correlates better to the overall drug exposure expressed as AUCp than to the total dose per sq m (10, 16). For MIS, the risk increases sharply at an overall AUCp of 11,000 μg/ml-hr (16).

The goal of i.p. delivery is to increase the therapeutic index of MIS and DMIS by the achievement of higher tumor concentrations than can be achieved by systemic administration, while plasma concentrations are maintained in a safe range with respect to development of major neurotoxicity. As shown in Chart 1, the peritoneal fluid concentrations of drug following i.p. delivery were substantially greater than plasma concentrations after systemic delivery of the same dose. Since peritoneal fluid is expected to equilibrate with plasma following systemic delivery, it is clear that much higher peak peritoneal fluid concentrations can be achieved by i.p. delivery than by systemic delivery. The advantage of the local route of drug administration was also expressed as an increase in the AUCp and as a decrease in the AUCp per unit of delivered dose (Table 3). For MIS, p.o. doses in excess of 5 g/sq m (11) are required to obtain the same peritoneal fluid concentration which was achieved by i.p. delivery of an absorbed dose of 1.5 g/sq m.

A more practical approach to the comparison of the i.p. and i.v. routes is to evaluate differences in drug concentrations that could be achieved in a fractionated course of radiation. In the "Appendix," we used a rearrangement of Equation A ("Materials and Methods") to calculate the MIS dose per fraction that can be delivered during a 20-fraction course of abdominal irradiation before the cumulative AUCp limit for toxicity is reached. From the known dose-response curve for MIS (9), the peritoneal fluid concentration at 3 hr corresponds to an increase in hypoxic cell sensitivity to radiation of about 40% for the i.v. route and greater than 90% for the i.p. route.

Although impressive advantages for local delivery have been documented for peritoneal fluid or for cells at the peritoneal surface, most target tumor cells are located more than one or 2 cell layers from the surface. This is especially true for drugs like MIS and DMIS that are targeted to a hypoxic tumor cell population which is likely to be present more than 10 cell layers deep into the tumor mass (3). Since drug concentration will decrease with distance from the peritoneal surface, information about the concentration of drug in tissue is needed. The determination of this concentration profile in tissue is a formidable task. A diffusion-perfusion model has been published for i.p. 5-FUra (7) which also incorporates tissue metabolism. On the basis of molecular size, peritoneal disappearance, and pharmacokinetics, it appears that MIS and 5-FUra have similar physical and pharmacokinetic properties of tissue diffusivity and distribution space, while MIS has greater capillary permeability.

The 5-FUra model can be adapted for MIS assuming that tissue metabolism is negligible and capillary permeability is 50% higher than for 5-FUra. For exposure times of either 30 min or 3 hr, the model predicts (Chart 2) that tissue concentrations of MIS will be greater than 100 μg/ml for about 1 mm (100 cell layers) from the peritoneal surface. As seen in Chart 1, plasma concentrations of MIS are always less than 100 μg/ml beyond the early distribution phase.

Direct experimental data to support the calculations are not currently available. The measurement of tissue profiles requires techniques with a resolution of 100 μm or less. While several laboratories are working to obtain more direct information regarding this key aspect of i.p. delivery, the only available data at the moment are for model compounds in the rat (12).

The optimal choice for time of tissue exposure to drug before irradiation cannot be clearly established at the present time. For systemic delivery, a 2- or 3-hr interval between drug administration and radiation delivery has been used (2). In our study, radiation was delivered up to 3 hr after i.p. drug administration to allow sufficient time for tissue exposure, using the systemic experiments as a basis. Since the tissue concentration profile is fully established by 30 min, this would be the

**Pharmacokinetics of MIS and DMIS after i.p. Administration**

**Chart 1.** Kinetic course of MIS (top) or DMIS (bottom) after i.p. administration of 4 g of drug in 2 liters of dialysate for 3-hr dwelling. ▲, peritoneal fluid MIS concentrations; ◇, plasma MIS concentrations; ○, i.p. DMIS; ●, plasma DMIS. Values for i.v. administration of 4 g of both drugs (——) were derived from the literature (5, 20).
logical time to irradiate if peak concentrations were the only consideration (1, 15). However, our model calculations suggest that there is only a small decrease in concentrations between 30 min and 3 hr, especially for distances between 0.5 and 1 mm.

As a radiosensitizer of hypoxic tumor cells, DMIS is as effective as MIS (13). Because the advantage in concentration and AUC difference between peritoneal fluid and plasma is more pronounced for this analogue of MIS, even greater advantage in radiosensitizing hypoxic tumor cells at the peritoneal surface or in small nodules might be expected by use of the i.p. route of DMIS administration. The 2 patients treated with DMIS (Table 1) developed mild and reversible neurotoxicity at an overall dose which corresponds to an AUCp of about 4000 μg/ml-hr for the entire treatment. The appearance of toxicity at the latter value of overall drug exposure is consistent with what might be expected from published reports on the relationship between AUCp and risk of neurotoxicity for DMIS (10, 16).

The same considerations made for the radiosensitizing properties of the 2 nitroimidazoles also apply to the potentiation of other anticancer agents by MIS. The synergism with several alkylating agents and cis-platinum has been well characterized in vitro both in aerobic and hypoxic conditions (4, 18, 19). These reports indicate that the potentiation of other drugs by MIS is a function not only of dose, but also of time of drug exposure, suggesting a role for the total drug exposure of the latter value of overall drug exposure is consistent with what might be expected from published reports on the relationship between AUCp and risk of neurotoxicity for DMIS (10, 16).

Although the need for developing new radiosensitizers with better therapeutic ratios remains unchanged, it appears that the i.p. administration of MIS and DMIS may represent a clinical setting in which these drugs are more likely to show significant clinical activity.

APPENDIX

According to the data published by Schwade et al. (16), the risk of MIS-induced neurotoxicity significantly increases when a total AUCp of 11,000 μg/ml-hr has been reached during the overall treatment. In a 20-fraction course of abdominal irradiation, the overall limit corresponds to a plasma AUCp of 550 μg/ml-hr per fraction.

One can tailor a safe schedule to the individual patient by calculation of the i.p. dose per fraction according to the rearrangement of Equation 4 ("Materials and Methods"):

Dose i.p. = (Cliv)(AUCp)/F

(A.1)

Solving the equation for an AUCp of 550 μg/ml-hr and using the mean values of F (0.73) and Cliv (37 ml/min) from Table 2, the dose will be 1.67 g in a 2-liter volume (1 g/sq m). Such a dose corresponds to a 3-hr i.p. concentration of 225 μg/ml and to a plasma concentration of 27 μg/ml.

The i.v. dose which must be administered in order to keep the same safety limit of AUCp can be calculated by simply eliminating the absorption factor F from Equation A.1:

Dose i.v. = (AUCp)(Cliv)

(A.2)

Solving the equation for AUCp = 550 μg/ml-hr, the dose will be 1.22 g or 0.75 g/sq m. For such a dose, a 3-hr plasma concentration of 41 μg/ml can be calculated from Chart 1 as well as a dose ratio of 1.22 g or 0.75 g/sq m/2.45 g/sq m.

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