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SHORT COMMUNICATION

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Continuous intratumoral microdialysis during high-dose methotrexate therapy in a patient with malignant fibrous histiocytoma of the femur: a case report

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Abstract We used a microdialysis technique to assay intratumoral methotrexate (MTX) levels during high-dose (12 g/m^2 given as a 4-h infusion) therapy in a 43-year-old man with a malignant fibrous histiocytoma in the medial femoral condyle. Additional microdialysis probes were implanted in muscle tissue contralateral to the tumor and in an antecubital vein. Microdialysis was attempted during the initial two high-dose courses, but the two latter probes were removed at the start of the second treatment cycle due to leakage. No attempt to correct for microdialysis recovery was made. The intratumorally localized probe gave reproducible data on tumor MTX exposure of 9.3–14% of unbound systemic MTX. There was a close correlation between tumor and systemic levels for both MTX and its major extracellular metabolite 7-hydroxymethotrexate. Although limited to the study of MTX pharmacokinetics in a single subject, the experiment demonstrates that intratumoral microdialysis may provide data on tumor drug exposure, although of an indirect nature and dependent on the probe characteristics, the flow rate, and, possibly, the time after probe implantation. We propose that the application of microdialysis may prove useful for elucidation of the relationship between local drug exposure and the therapeutic response in normally inaccessible compartments during cancer pharmacotherapy.

Key words Microdialysis · Methotrexate · Tumor · 7-Hydroxymethotrexate

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Introduction

Drug therapy in oncology is crucially dependent on the maintenance of adequate dose intensity in target tissues. When local factors peculiar to tumor tissue are taken into account, a steady state for the in vivo penetration of anticancer agents into solid tumors may at best be subjected to considerable variation. This can in part explain the substantial interindividual differences observed in the treatment outcome after chemotherapy in patients with solid tumors.

Techniques for the direct monitoring of drug concentrations in tissue have not been generally available, and most studies have relied on the determination of drug levels in specimens obtained through biopsy or surgery. Although this approach offers the advantage of monitoring intracellular drug concentrations, it rarely allows serial sampling. This is reflected by the paucity of data on drug pharmacokinetics in solid tissues.

The antifolate methotrexate (MTX) is one of the most widely used cytotoxic drugs. High doses of this compound have been successfully employed in the treatment of leukemias and various solid tumors such as osteosarcomas, and its pharmacokinetics has been extensively studied. Measurements of serum/plasma drug levels are routinely employed during high-dose MTX treatment [1, 11, 12]. This has mainly been undertaken to facilitate the identification of individuals with impaired drug elimination who are prone to develop serious side effects. However, this practice also rests on an assumed relationship between the tumor response and the circulating levels of drug in these

patients. Although studies in patients with solid tumors have shown correlations between MTX blood levels and tumor response or survival [6, 7, 12], no consensus as to the importance of these findings has been reached.

In situ microdialysis has made possible the on-line monitoring of xenobiotics and endogenous substances in extracellular spaces, including tumor tissue. The method has thus far mostly been employed to measure endogenous substances in humans. With the exception of a single report on the monitoring of valproic acid by subcutaneous microdialysis in a small cohort of epileptic patients [17], we are not aware of any implementation of the technique for the clinical assay of drug levels during pharmacotherapy. In particular, no attempt at intratumoral monitoring during anticancer drug therapy appears to have been undertaken.

We have previously investigated the feasibility of obtaining reproducible pharmacokinetic data on MTX tissue exposure by employing the microdialysis technique in experimental animals given high doses of the drug by the intravenous route [3–5]. This series of experiments suggests that such an approach, which facilitates serial measurements in the extracellular part of the biophase, could be useful in attempts to elucidate the relationship between pharmacokinetic variables and outcome in the therapy of solid tumors. A logical extension of this work would be application of the microdialysis technique in cancer patients.

Herein we report our experiences with the technique during two high-dose MTX courses in a patient with malignant fibrous histiocytoma of the femur. To our knowledge, this is the first report of microdialysis of a drug in tumor tissue under clinical conditions.

Patient and methods

Patient

The patient was a 43-year-old farmer, previously in good health, who presented with a high-grade malignant fibrous histiocytoma in the right medial femoral condyle (see Fig. 1). He was treated with a neoadjuvant chemotherapy regimen consisting of three courses of high-dose MTX (12 g/m^2 given as 4-h infusions), cisplatin, and doxorubicin; reconstructive surgery with a knee-joint prosthesis; and four cycles of postoperative chemotherapy with etoposide, ifosfamide, mesna, and granulocyte colony-stimulating factor. Concomitant with an open biopsy of the tumor prior to therapy, flexible microdialysis probes were inserted into the center of the tumor, contralaterally in the muscle tissue of the left thigh, and into an antecubital vein. The probes (10-mm membranes with a diameter of 0.50 mm, 20-kDa cutoff size) were obtained from CMA/Microdialysis, Stockholm, Sweden. They were sterilized by gamma irradiation (32 kGy) prior to use, and subsequent in vitro experiments confirmed that the irradiated probes behaved identically to native probes from the supplier.

Until the removal of the microdialysis probes the patient was treated prophylactically with clindamycin at 150 mg q.i.d. The first MTX course was given on the day after the probes were inserted, and the second course was given 1 week subsequently. Microdialysis by the indwelling probes was attempted during these two initial MTX courses. The experimental procedures were approved by the Regional Ethics Committee, and prior informed consent was obtained from the patient.

Microdialysis

The microdialysis perfusion solution (Ringer acetate; Kabi Pharmacia AS, Halden, Norway) was delivered at ambient temperature by a CMA/100 syringe pump, and the perfusate was collected by a CMA/140 fraction collector. Before the start of the experiments the microdialysis probes were flushed with Ringer acetate at 10 μl/min to purge the membranes and tubing of air bubbles. At 30 min before drug administration the perfusate flow was reduced to 4 μl/min, and microdialysis was performed at this flow rate during the subsequent experiments.

Samples and sample treatment

Whole-blood samples were obtained by venipuncture on Vacutainer (Becton-Dickinson, Rutherford, N.J., USA) tubes with potassium-ethylenediaminetetraacetic acid (EDTA) as the anticoagulant prior to MTX infusion and at 4, 6, 8, 12, 24, 48, 72, and 96 h after the start of the 4-h infusions. Plasma was separated, and aliquots of each sample were immediately ultrafiltered by centrifugation at 3,600 *g* for 30 min through a 30-kDa-cutoff membrane (Ultrafree MC, Millipore, Bedford, Mass., USA) in a Microfuge 13 (Heraeus Sepatec, Osterode, Germany) for determination of unbound drug. Prior to analysis, all samples were stored under protection from light at −70 °C.

Samples of 30-min (120-μl) dialysis fractions were obtained continuously during the initial 12 h after the start of MTX infusion and, subsequently, at 24, 48, 72, and 96 h. Sample preparation prior to chromatography consisted of the addition of a 1/5 vol. of 2 *M* perchloric acid, vigorous mixing, centrifugation at 15 000 *g* for 5 min, and transfer of the sample to borosilicate glass autosampler vials (Chromacol Ltd., London, UK).

Drugs and chemicals

Formulated MTX was obtained from Lederle Laboratories (Pearl River, N.Y., USA). 7-OH-MTX was a kind gift from Dr. F.M. Sirotnak, Memorial Sloan-Kettering Cancer Center (New York, N.Y., USA). High-performance liquid chromatography (HPLC)-grade methanol was obtained from Rathburn Chemicals Ltd. (Walkerburn, UK). All other reagents were of analytical grade.

Analytical procedure

MTX and 7-OH-MTX levels were quantified by a modified isocratic HPLC assay described in detail elsewhere [13]. Chromatographic equipment was produced by Shimadzu Corporation (Tokyo, Japan). The solvent-delivery system consisted of a DGU-3A on-line degasser coupled to an LC-9A quaternary gradient pump. A column temperature of 40 °C was maintained using a CT0-6A column oven and on-line solvent preheater. Samples were injected by an SIL-9A autoinjector maintained at ambient temperature and detection was done by an SPD-6AV variable-wavelength UV detector. Peak area integrations were performed by a Chromatopac C-R6A integrator.

Chromatography was performed on a Supelcosil C18 octadecyldimethylsilyl (ODS) column (4.6 × 150 mm, particle size 3 μm; Supelco, Bellefonte, Pa., USA) equipped with a Supelguard ODS precolumn (4.6 × 20 mm, particle size 5 μm). The mobile phase consisted of a TRIS-sodium dihydrogen phosphate (both 0.1 *M*, pH

6.7): methanol mixture (80 : 20, v/v). The mobile phase was delivered at a rate of 1 ml/min. The UV detector was operated at 370 nm. Either 50 or 100 µl of sample was injected. Between analyses the autoinjector line was washed by flushing with a 60% aqueous methanol solution. MTX and 7-OH-MTX had retention times of 6.9 and 7.9 min, respectively. The within-run and between-run coefficients of variation (CVs) were $\leq 2\%$ and $< 10\%$, respectively, for both compounds. The recovery after protein precipitation was approximately 70%.

Calculations

Area under the concentration-time curve (AUC) calculations were performed by the computer program SIPHAR (Simed, Créteil, France).

Results

The microdialysis system showed no sign of malfunction during the first course. However, at the start of the second course 1 week later the intravenous and muscle probes leaked and had to be removed. Thus, only tumor microdialysis data were obtained during the second course. An X-ray of the distal femur that visualizes the tumor after the injection of contrast medium through the microdialysis probe at the end of the second high-dose MTX course is shown in Fig. 1 (right panel).

Elimination curves generated for total and unbound MTX and 7-OH-MTX as well as for unbound fractions of these two compounds in plasma are depicted in Fig. 2. The plasma elimination curves were similar for the two courses but slightly higher levels of both MTX and the 7-hydroxylated metabolite were noted during the second course. Plasma AUC values are given in Table 1. Protein binding in plasma was found to be variable in the 29–81% range for MTX and in the 90–96% range for 7-OH-MTX, with a tendency toward higher free fractions of both compounds being observed at early time points during the courses (Fig. 2).

Figure 3 presents data on MTX and 7-OH-MTX levels measured in microdialysis effluents during the two courses. During the first course, neither the drug nor the metabolite was detected in samples obtained beyond 24 h, whereas low levels of both MTX and 7-OH-MTX were measured at these time points during the second therapy cycle (data not shown). There was no difference in the patient's albumin and total protein serum concentrations at the commencement of the first and second courses of high-dose MTX, with albumin levels being 41 and 40 mg/l and total protein values being 72 and 71 mg/l, respectively. The AUC values determined for the microdialysis effluents are given in Table 1.

The degree of correlation between unbound drug concentrations in plasma and tumor microdialysis data was investigated. As shown in Fig. 4, unbound MTX in plasma and MTX in tumor microdialysis perfusate correlated well, with coefficients of correlation (r^2 values) exceeding 0.96. For the 7-hydroxylated metabolite this correlation was weaker, with r^2 values being 0.85 and 0.91. A high degree of correlation between both total and unbound plasma levels of both MTX and 7-OH-MTX and the concentrations of these compounds measured in microdialysis effluents from the intravenously situated probe was apparent during the first MTX course (mean r^2 0.97, range 0.94–0.99), whereas the degree of correlation between plasma and muscle microdialysis data was slightly less pronounced (mean r^2 0.88, range 0.83–0.93).

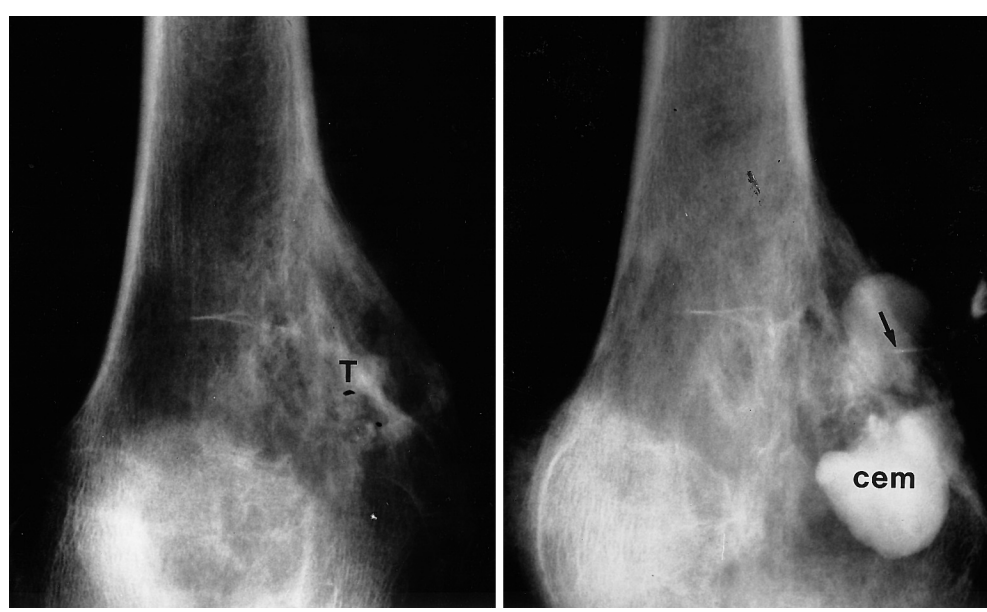

Fig. 1 X-rays of the tumor (T) in the right femur as obtained prior to biopsy (*left*) and at the end of the second course of high-dose MTX therapy (*right*) in a 43-year-old man with a malignant fibrous histiocytoma of the femur. In the *right image*, contrast medium has been injected for visualization of the microdialysis probe (*arrow*). (*cem* Bone cement used to close the lesion after open biopsy)

Table 1 AUC values determined in plasma and microdialysis effluents from probes in three different compartments for MTX and its metabolite 7-OH-MTX during two courses of high-dose MTX in a patient with a malignant histiocytoma of the femur. Data are given as $\mu mol \times h \times l^{-1}$. The unbound MTX and 7-OH-MTX percentages were calculated from the total drug levels measured in plasma (*1*), and dialysis-fluid percentages were calculated from the unbound drug concentrations measured in plasma (*2*). (*ND* Not determined because of probe failure)

Course number	Total in plasma (1)	Unbound in plasma (2)	Microdialysis, probe in		
			Tumor	Muscle	Vein
MTX					
I	6,800	4,300 (63%)	400 (9.3%)	230 (5.4%)	210 (4.9%)
II	7,200	3,900 (54%)	540 (14%)	ND	ND
7-OH-MTX					
I	1,600	110 (6.9%)	12 (11%)	7 (6.4%)	5 (4.6%)
II	1,700	86 (5.1%)	17 (20%)	ND	ND

Discussion

Our experience with the microdialysis technique during the initial two high-dose MTX courses in this single patient demonstrates that this approach is feasible and capable of supplying relevant pharmacokinetic data. We hope that the technique will contribute to current knowledge by shedding light on crucial yet mainly unanswered questions regarding anticancer drug distribution in solid tumors.

At 38 months after the initiation of anticancer chemotherapy with high-dose MTX the patient remains in complete remission. Although he has not resumed his work as a farmer, he has few complaints and is in good health. The patient encountered no specific problem during the 2 weeks of microdialysis. In particular, there was no sign of infection at the implantation sites, probably due to our strict attention to sterility during the probe implantation as well as to prophylactic treatment with a broad-spectrum antibiotic. The availability of factory-sterilized probes may

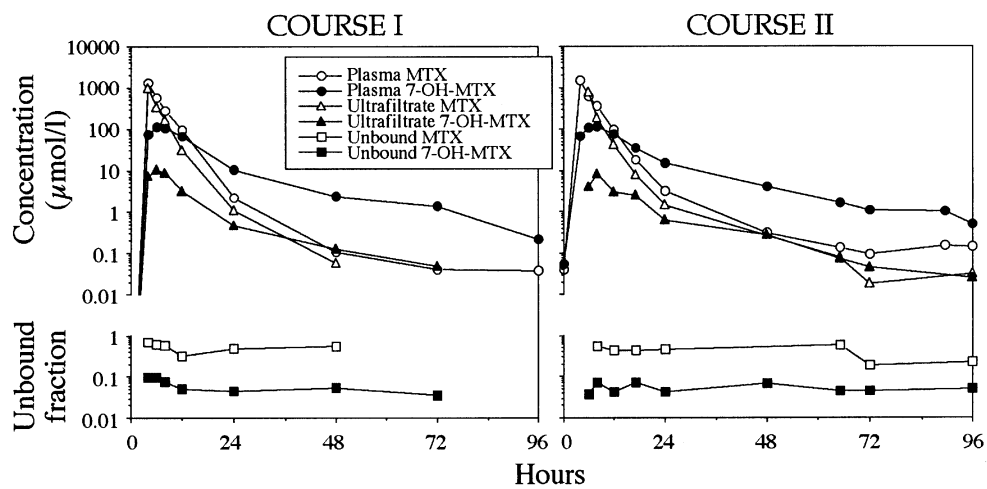

Fig. 2 Plasma pharmacokinetics of total, ultrafiltered, and unbound fractions of MTX and its extracellular metabolite 7-OH-MTX as determined during two courses of high-dose MTX in a 43-year-old man with a malignant fibrous histiocytoma of the femur. The two treatment courses were separated by a 1-week interval

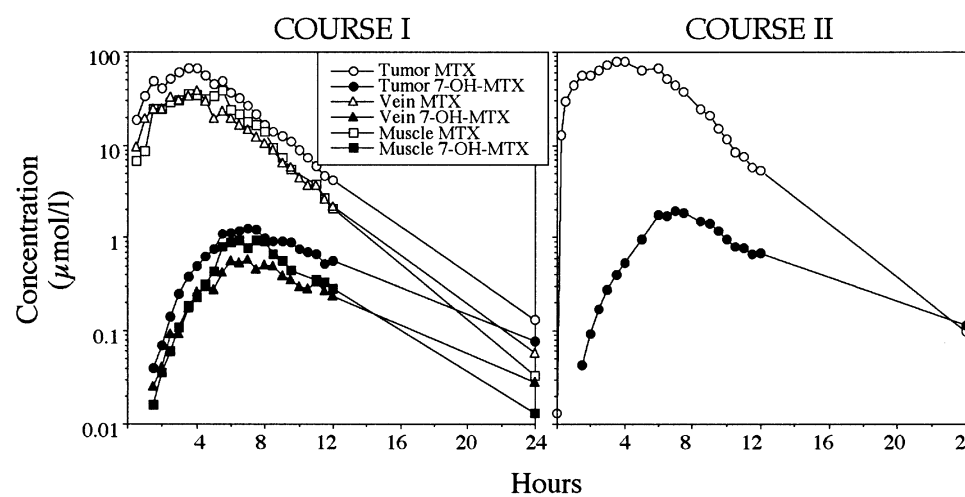

Fig. 3 Contents of MTX and its extracellular metabolite 7-OH-MTX measured in microdialysis effluents from probes placed intratumorally, contralaterally in muscle, and intravenously in a 43-year-old man with a malignant fibrous histiocytoma of the femur during the initial two high-dose cycles of neoadjuvant MTX. The two courses were separated by a 1-week interval, and by the start of the second course (*right*) the intravenous and intramuscular microdialysis probes had failed

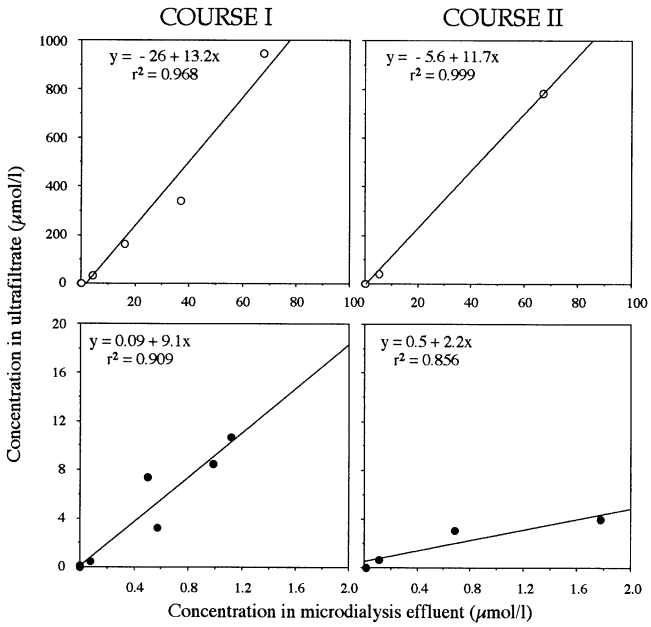

Fig. 4 Correlations between MTX (*white symbols*, *upper panels*) and 7-OH-MTX (*black symbols*, *lower panels*) concentrations measured in effluents from intratumorally located microdialysis probes and plasma ultrafiltrates during the first (*left panels*) and second (*right panels*) cycles of neoadjuvant high-dose MTX therapy in a patient with a malignant fibrous histiocytoma of the femur. Each plot is based on 6–9 observations, some of which overlap in the low concentration range

allow these procedures to be undertaken without the use of prophylactic antibacterial medication.

A noteworthy result of this single experiment lies in the demonstrated functional lifespan of the implanted microdialysis probes. Other investigators [2] have suggested that factors such as local reactions in the adjacent tissues and possible clogging of probe membranes may limit the functioning of microdialysis probes to a couple of days. In this experiment, neither of the three probes showed any sign of malfunction during the initial 5 days after implantation, and the probe localized in the tumor appeared to be functional for a total of 11 days. Since the patient moved about between the MTX cycles, it is likely that the leakage in the venous and muscular probes that became evident at the start of the second course were the result of mechanical stress rather than local perturbations at the dialysis site.

Plasma MTX pharmacokinetic profiles (Fig. 2) were found to be as expected. The observed tendency toward dose-dependent binding of MTX in plasma is in agreement with previous observations from in vitro binding studies with human plasma [18] as well as investigations similar to the present study but carried out in a rodent model [4]. The much higher degree of plasma 7-OH-MTX binding as compared with that of the parent compound also agrees with previously published results from equilibrium dialysis experiments [15, 16]. The AUC estimations (Table 1) suggest that 37–46% of MTX and 93–95% of 7-OH-MTX are bound to proteins in human plasma.

Of the three tissue compartments investigated by the microdialysis technique during the first MTX course, the highest concentrations of both the drug and the metabolite were measured in the tumor (Fig. 3), where the microdialysis AUC for MTX exceeded the values determined in the contralateral muscular tissue and venous blood by a factor of more than 1.7 (Table 1). These relatively high levels of MTX were reproduced during the second MTX course (Fig. 3), whereas comparative data were not obtained from venous and muscle microdialysis effluents due to probe failure. The reasons for the higher intratumoral MTX levels as compared with muscle and venous blood values remain to be determined. Systematic investigations of drug penetration into tumor tissue have generally demonstrated elevated intratumoral pressure, which should result in diminished convexion and a reduced degree of drug exposure [9, 10]. However, tumors like the present one are highly vascularized, and it is not unlikely that during therapy, extensive necrosis, reduced drug binding, and diminished drug efflux may contribute to a relative excess of MTX, as was seen in the present case.

Since MTX has a volume of distribution that approximates 1 l/kg [14], an even distribution of unbound drug between the bloodstream and the extracellular spaces in well-perfused organs without defined diffusion barriers for small molecules would be expected. In previous short-term in vivo experiments in rats we demonstrated that microdialysis effluents from intravenous probes contained considerably higher drug levels than did those from solid matrices such as the liver and kidney [3–5]. In these experiments we also showed that estimated extracellular muscle and liver MTX levels were similar to the unbound drug levels measured in the blood and that microdialysis recoveries varied from approximately 18% of the unbound drug in plasma to close to 5% of that in solid tissues [5]. Surprisingly, the venous drug levels achieved by microdialysis were lower than the comparable concentrations observed in both the tumor and the contralateral muscle tissue in the present case (Table 1). The reason for this is obscure, but it is not unlikely that fibrin deposits on the dialysis membrane of the intravenous probe may have made the venous compartment behave like a solid matrix. On the other hand, there is a high degree of similarity between previous animal data [5] and the fraction of systemic unbound drug recovered by microdialysis of muscle in the present experiment (Table 1).

There were good correlations between the microdialysis effluent concentrations of both MTX and its 7-hydroxylated metabolite achieved in the tumor as compared with unbound plasma levels, with r^2 values being in the 0.85–0.99 range (Fig. 4). In this respect the MTX data were reproduced from the first course to the

second, whereas the ratio of dialysate 7-OH-MTX in tumor to unbound metabolite in plasma was much higher during the second treatment. Whether this reflects intratumoral precipitation of the relatively poorly soluble 7-OH-MTX [8], altered microdialysis recovery, or other mechanisms is not known.

The experiment presented herein was limited to the study of MTX pharmacokinetics in a single subject by employment of the microdialysis technique. Despite its shortcomings, it demonstrates that intratumoral microdialysis may provide data on tumor drug exposure, albeit of an indirect nature and dependent on the probe characteristics, the flow rate, and, possibly, the time after probe implantation. Thus, the use of flexible, sterile microdialysis probes may allow serial sampling of intratumoral MTX during high-dose therapy. Logical extensions of the present work should include investigations into intra- and intertumoral variability in drug penetration as well as the relationship between the measured extracellular drug concentrations and the intracellularly sequestered drug levels. We propose that the application of the microdialysis technique may prove useful for elucidation of the relationship between drug exposure and the therapeutic response in the treatment of superficially located solid tumors.

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