The Use of Intracerebral Microdialysis for the Determination of Pharmacokinetic Profiles of Anticancer Drugs in Tumor-Bearing Rat Brain

E. C. M. de Lange,[1] J. D. de Vries,[1] C. Zurcher,[2] M. Danhof,[1] A. G. de Boer,[1] and Douwe D. Breimer[1]

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Purpose. The use of intracerebral microdialysis as a tool to measure the penetration of anticancer agents in brain tumor was investigated.
Methods. Following intravenous (iv) administration of 75 mg/kg. concentration-time profiles of methotrexate (MTX) were determined in brain cortical dialysate and in plasma. The individual ratio of the area under the curve of MTX in brain dialysate over that in plasma (MTX penetration) was determined in normal brain, in tumor-bearing brain and in brain after sham tumor implantation. Individual brains were examined histologically on the presence of tumor, as well as for other factors that might influence local MTX penetration. Histological scores were related to the individual data on penetration of MTX.
Results. MTX penetration values were higher in cortical brain at the site of the tumor, as compared to the levels measured in normal or sham implanted brain (mean increase to 250 %). In the cortical brain contralateral to the tumor, MTX penetration values were found to be lower than in normal brain (mean reduction of 65 %). Furthermore, it appeared that in the absence of tumor tissue, the presence of exudate around the probe was independently associated with increased penetration of MTX into the brain.
Conclusions. Tumor tissue appeared to be the most important parameter in changing local MTX penetration in brain after tumor implantation. In general, it is anticipated that intracerebral microdialysis combined with histological examination can be used to investigate effects of brain tumor presence on regional (periprobe) penetration of anticancer drugs into the brain.

KEY WORDS: Microdialysis: experimental brain tumor; pharmacokinetic; anticancer drugs; methotrexate; histology.

INTRODUCTION

Primary and metastatic brain tumors which cannot be controlled by either radiation or surgery are targets for chemotherapy. However, inadequate drug delivery to the brain, in part caused by the blood-brain barrier (BBB) or blood-brain tumor barrier (BBTB), probably accounts for poor therapeutic responsiveness to anticancer drugs by malignant metastatic and primary brain tumors (1,2,3,4).

For the effective treatment of brain tumors, a clear understanding of the targeting and disposition of chemotherapeutic agents into the tumor is necessary. Because it is likely that drug distribution is variable and will depend on factors like localization, size and type of the tumor (5,6), a suitable in vivo technique should be used to investigate the effects of such factors on concentrations of anticancer drugs reached in the tumor.

Intracerebral microdialysis can be used to monitor concentrations of compounds in the brain. In previous studies it was demonstrated that with this technique BBB transport characteristics of drugs can be studied (7,8,9), as well as changes thereof (8,9). Also local concentration differences within the brain can be demonstrated (10). Therefore it was considered to be an appropriate method for the determination of local pharmacokinetic profiles of anticancer agents in brain tumors. Such studies should allow to study the pharmacokinetics of anticancer agents in brain and brain tumor, in relation to their antitumor effects.

In the current investigation intracerebral microdialysis was used to monitor concentrations of methotrexate (MTX), hydrophilic anticancer drug, in normal and in tumor-bearing brain. As a tumor model a rhabdomyosarcoma was used. After implantation into the cortical brain this tumor infiltrates into the surrounding brain tissue. The transport of MTX in the cortex ipsi- and contralateral to the brain tumor implant was determined at different post tumor implantation intervals. MTX was administered intravenously and the extent of drug transport into the brain (MTX penetration) was expressed as the ratio of $AUC_{brain\ ECF}$ over AUC_{plasma}. The brains were studied histologically for the presence of tumor tissue as well as for a number of other parameters, and the outcomes were related to the pharmacokinetic data.

MATERIALS AND METHODS

Animals. Male SPF WAG/Rij rats were obtained from Harlan B.V. (Zeist, The Netherlands) and maintained under standard housing conditions on an ad libitum standard laboratory diet (RMH-TH, Hope Farms, Woerden, The Netherlands) with free access to water.

Tumor. The WAG/Rij rat derived solid R-6 rhabdomyosarcoma (11) was used. The tumors were maintained by subcutaneous passage to the flank of male WAG/Rij rats every 3-4 weeks, for at least three times. Tumors of about 1 cm^3 were aseptically removed from the flank and surrounding connective tissue and necrotic parts were separated from the vital parts. These vital parts were washed three times in saline and cut into 5 mm^3 pieces for subcutaneous implantation in the flank. For brain implantation 1 mm^3 tumor fragments were kept on Dulbecco's Modified Eagle's Medium (DMEM, Sigma, The Netherlands) at 4°C until implantation, maximally for 1 hour.

Tumor Implantation. Male WAG/Rij rats (body weight 160-200 g) were anaesthetized with an intramuscular injec-

[1] Leiden/Amsterdam Center for Drug Research, Division of Pharmacology, University of Leiden, Leiden, The Netherlands.
[2] TNO Institute for Aging and Vascular Research, Leiden, The Netherlands.
[3] To whom all correspondence should be addressed at: LACDR/Pharmacology Sylvius Laboratory, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

ABBREVIATIONS: AUC, area under the concentration-time curve; C_{plasma}, concentration-time profile in plasma; $C_{brain\ ECF}$, concentration-time profile in brain dialysate after correction for in vitro recovery; AUC_{plasma}, AUC in plasma; $AUC_{brain\ ECF}$, AUC in brain dialysate after correction for in vitro recovery; HPS, hematoxylin phloxine saffron; iv, intravenous; PTII, post tumor implantation interval; MTX, methotrexate.

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tion of 0.1 ml/kg Hypnorm® (Janssen Pharmaceutica, Goirle, The Netherlands). Incisions were made to expose the skull, that was subsequently anaesthetized locally with 0.6 % (w/v) lidocaine solution. Then, 2.2 mm left from the bregma, a hole was drilled into the skull using a 1mm dental burr. A guide cannula (Microlance needle, 21 G2, Becton Dickenson B.V., Etten-Leur, The Netherlands) and an inner cannula (a closed Microlance needle, 22 G¼) were used to place the tumor fragment on the left side into the cortex. The guide cannula together with the tumor fragment and the inner cannula were lowered into the brain parenchyma, to a depth of 1.9 mm with respect to the bregma. The guide cannula was slowly pulled out of the cortex, followed by the inner cannula, leaving the tumor fragment behind. The skull was blotted dry and covered with dental cement, whereafter the skin was sutured.

Microdialysis Probe Implantation. The microdialysis probe was implanted into the cerebral cortex approximately 24 hours prior to the microdialysis experiment. The rats were anaesthetized with an intramuscular injection of 0.1 ml/kg Hypnorm® (Janssen Pharmaceutica, Goirle, The Netherlands) and placed in a stereotactic frame. Incisions were made to expose the skull which was thereafter locally anaesthetized with a 0.6% (w/v) solution of lidocaine. Holes of 1.5 mm were drilled in the lateral plane of the skull, allowing the transversal introduction of a dialysis probe through the cortex, 2 mm below the bregma, using a tungsten wire (TW5-3, Clark Electro Medical Instruments, England). The dialysis fibre (O.D 0.29 mm, C-DAK artificial kidney 201-800 D 135 SCE, CD Medical B.V., Rotterdam, The Netherlands) was covered with silicon glue (Rhodosil CAF 3, Rhone-Poulenc, Amstelveen, The Netherlands) except for 3 mm on the left (ipsilateral) or right (contralateral) side. Stainless steel needles, glued to both ends of the dialysis fibre, were secured with dental cement on the top of the skull. A subcutaneous cannula (polyethylene tube, I.D. 0.58 mm, length ± 20 cm) was implanted at the back of the rat to allow the perfusate fluid to equilibrate at rat temperature before entering the microdialysis probe (8).

Effects of Covariates. All experimental conditions have been excluded from covariate effects by randomizing the tumor donor rat, the time of day of implantation of the tumor, the post tumor implantation interval and the time of day of performing the experiments.

Drug Administration. For intravenous (iv) drug administration and serial blood sampling polyethylene cannulas (OD 0.96 mm, ID 0.58 mm) were implanted into the femoral vein and femoral artery respectively under ether anaesthesia. The animals were allowed to recover from the ether anaesthesia for at least two hours before the start of the experiment.

Experimental Design

Study I. In this study four groups of 6 rats were used: A) normal = no tumor; B) sham tumor implantation with ipsilateral measurement; C) tumor implantation with ipsilateral measurement; and D) tumor implantation with contralateral measurement. Microdialysis measurements were performed 11 days after tumor or sham implantation.

Study II. This study was conducted at different post tumor implantation intervals (6, 11, 18, 25 days). For each post tumor implantation interval the microdialysis measurements were performed in the ipsilateral (n = 6) or contralateral cortex (n = 3).

Experimental Procedure

For the microdialysis experiments the stainless steel needles at both sites of the microdialysis probe were connected by means of polyethylene tubing (O.D. 0.61 mm, I.D. 0.28 mm) to a perfusion pump (Gilson, Medical Instruments Electronics Inc., Middleton, USA) and to the sample loop connected to HPLC ("on line"). The dialysis probe was perfused at 7 µl/min with 2 mM phosphate buffer containing 145 mM sodium, 2.7 mM potassium, 1.2 mM calcium, 1.0 mM magnesium, 150 mM chloride (all as ions) and 0.2 mM ascorbate, pH = 7.4 (12), at rat body temperature. Before administration of the drug the rats were dialyzed for at least 30 minutes in order to equilibrate and to obtain blank chromatograms. Then the animals received a single iv dose of 75 mg/kg MTX (Emthexate®, Pharmachemie B.V., Haarlem, The Netherlands) within 1 minute. Thereafter concentrations of MTX were determined in the dialysate samples every 10 minutes for 150 minutes.

Arterial blood samples of approximately 200 µl were collected into heparinized tubes at fixed time intervals, namely at 5, 10, 25, 40, 60, 90 and 120 minutes after the drug administration over a period of 120 minutes. The blood samples were kept at room temperature until the last blood sample was taken. The blood samples were centrifuged (room temperature, 10 minutes, 9000 rpm) and the plasma was removed. The collected plasma samples were stored at −80°C until analyzed for MTX concentrations.

At the end of the experiment, the animals were deeply anaesthetized with ether. All blood from the circulation was removed by perfusing the heart with saline and severing the inferior vena cava. Subsequently, an in situ fixation was performed by perfusion with a 0.1 M phosphate-buffered 4 % formaldehyde solution (pH = 7.4). Then the brains were removed from the cranial cavity and stored in 4 % buffered formaldehyde solution at 4°C.

Histology. After fixation in 4 % formaldehyde solution the right and left hemispheres were cut sagitally starting from and parallel to the midline plane at 3 and 5 mm. In this way 3 fronto-occipital brain slices were produced from right and left hemispheres which were numbered from the midline: R1 (3 mm), R2 (2 mm), R3 (<2 mm), L1 (3 mm), L2 (2 mm), and L3 (< 2 mm). After routine processing including dehydration in a graded alcohol series and paraplast embedding the blocks L1, L2, R1 and R2 were serially cut, starting from the 3 mm sagital plane. Every 40th 3 µm section was routinely stained with hematoxylin phloxine saffron (HPS) and used for microscopic examination. In this way the whole traject of the semipermeable part of the microdialysis membrane and the tumor implantation site could be screened for morphological changes.

All stained slides were semi-quantitatively examined for the severity and extent of: tumor growth; vacuolar change of white matter; necrosis and individual cell death; infiltration of granulocytes; cellular infiltration and glial proliferation (hypercellularity); fibrosis; hyaline proteinaceous exudate;

and hemorrhage. Severity and extent were determined for four concentric zones around the microdialysis probe with the following inner, respectively outer radius (mm): 0.15-0.2 (zone 1); 0.2-0.4 (zone 2); 0.4-0.7 (zone 3); and 0.7-1.0 (zone 4).

A. The severity was estimated by using the following grading system:

* Tumor growth (Fig 1)
  0 = absent
  1 = minimal (focal small tumor cell aggregates)
  2 = mild (focal small aggregates of tumor cells around vessels or in white matter)
  3 = moderate (multifocal moderately sized areas of tumor growth)
  4 = severe (large fields of massive tumor growth)
* For the other parameters
  0 = absent
  1 = minimal (focal spots)
  2 = mild (multifocal spots)
  3 = moderate (moderately sized areas)
  4 = severe (large fields)

B. The extent of each severity grade was expressed as a percentage of the total surface of a particular zone. A weighed value was calculated as follows to give the "tissue score." For example, in a particular zone for parameter "x" 50 % of severity 0; 25 % of severity 1; 12 % of severity 2; and 13 % of severity 4 was found. The tissue score for parameter x in that zone was calculated as [50*0 + 25*1 + 12*2 + 13*4]/ 100 = 1.0. Tissue score values thus ranges from 0 to 4.0. The tissue scores values were subsequently averaged for the total number of sections covering the semipermeable part of the dialysis probe, to provide the mean tissue score value.

Drug Analysis. The analysis of MTX was developed in our laboratory. The HPLC system consisted of a reversed phase column (Spherisorb, 10 cm* 2.0 mm I.D., S3 ODS 2, Phase Separations, Waddinxveen, The Netherlands) a precolumn (pellicular reversed phase, Chrompack, The Netherlands) and an electrochemical detector (glassy carbon electrode with an oxidation potential of 1000 mV versus an Ag/AgCl electrode, Antec Leyden B.V., Leiden, The Netherlands). The mobile phase consisted of 0.1 M Tris and

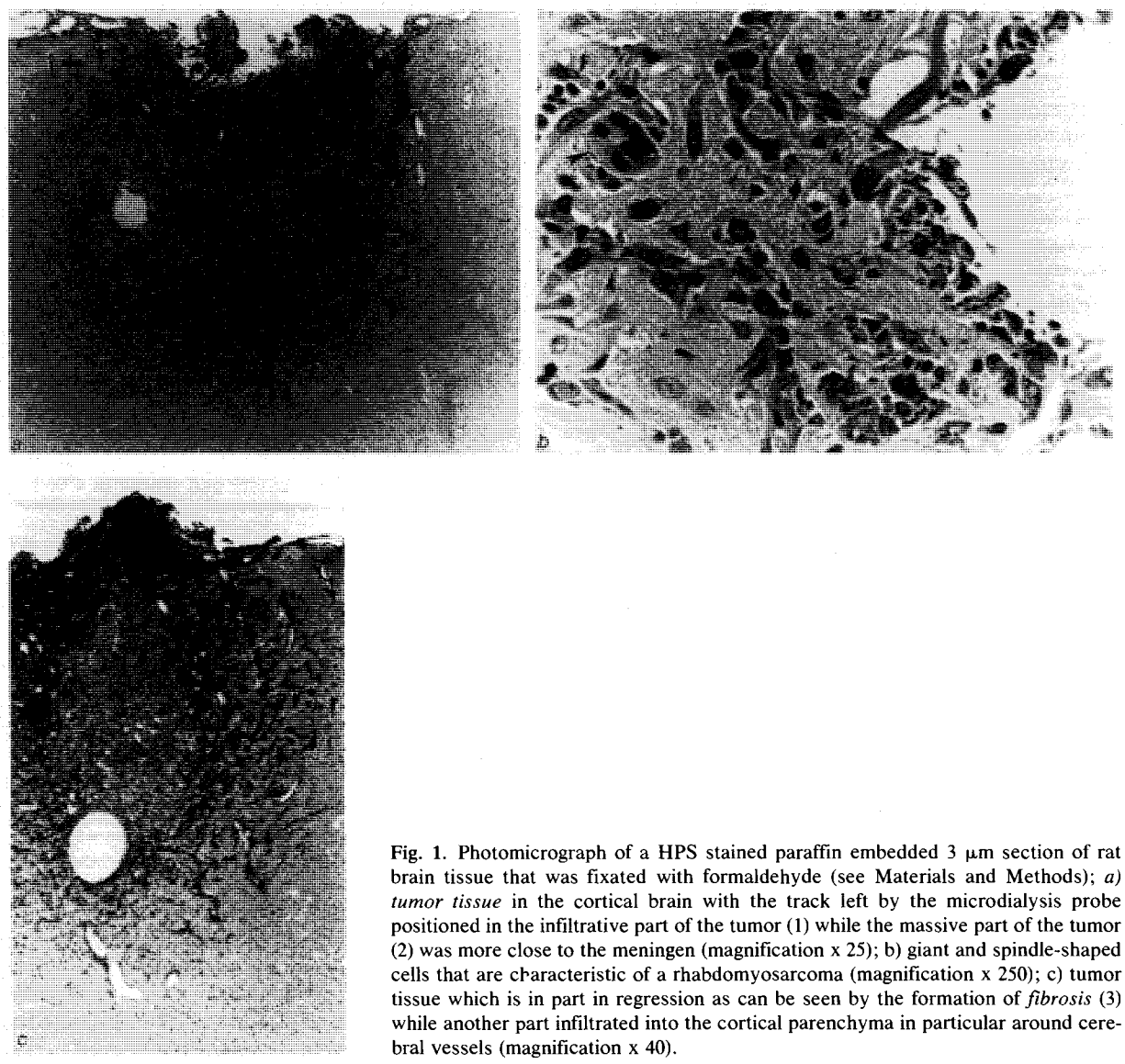

Fig. 1. Photomicrograph of a HPS stained paraffin embedded 3 μm section of rat brain tissue that was fixated with formaldehyde (see Materials and Methods); *a) tumor tissue* in the cortical brain with the track left by the microdialysis probe positioned in the infiltrative part of the tumor (1) while the massive part of the tumor (2) was more close to the meningen (magnification x 25); b) giant and spindle-shaped cells that are characteristic of a rhabdomyosarcoma (magnification x 250); c) tumor tissue which is in part in regression as can be seen by the formation of *fibrosis* (3) while another part infiltrated into the cortical parenchyma in particular around cerebral vessels (magnification x 40).

0.1 M phosphate at pH = 5.0, containing 20 % (v/v) MeOH and a small amount of EDTA. The flow was 0.2 ml/min. The dialysate that was collected in a 50 µl loop of an HPLC system was injected by hand every 10 minutes, until 150 minutes after administration of the drug. The plasma samples were diluted with water (Milli-Q, Water Purification System), ratio 1:30 and 1:60, and 10 or 20 µl samples were directly injected onto the column.

The coefficient of variation between-days for MTX over the whole concentration-range was 3.4 % (n = 9). The detection limit corresponded to less than 1 ng/ml (110 fmol) in the dialysate and 3 ng/ml (330 fmol) in plasma. The correlation coefficient for all calibration curves was greater than 0.9984.

In Vitro Recovery. The in vitro recovery of MTX was determined by placing a microdialysis cannula with a 9 mm dialysis zone in a glass vial with perfusion solution which had been spiked with MTX (10 and 100 ng/ml) at 37°C. The solution was not stirred. The perfusion solution was led through the dialysis cannula with a flow of 7 µl/min. The concentration of MTX inside the microdialysis cannula was determined and the ratio of the concentration of MTX inside over the concentration of MTX outside the microdialysis cannula was calculated. This ratio is equal to the in vitro recovery, which was 14.4 %. Assuming that the in vitro recovery is linearly related to length of the dialysis zone, the in vitro recovery of the microdialysis cannula with a dialysis zone of 3 mm is then equal to 4.8 %.

Data Analysis

The concentration-time profiles of MTX in the dialysate were corrected for the in vitro recovery to yield *estimations* of the profiles in brain ECF ($C_{brain\ ECF}$). $C_{brain\ ECF}$ and C_{plasma} were used to calculate the AUC(0→120 min) by means of the trapeozoidal rule (Siphar, modelling package, SIMED, Creteil, France). Thereafter the ratio of $AUC_{brain\ ECF}$ over AUC_{plasma} was calculated for each individual rat. Statistical evaluation was performed with the Kruskall Wallis test ($P < 0.05$).

RESULTS

Pharmacokinetics of MTX

In study I the penetration of MTX ($AUC_{brain\ ECF}$/AUC_{plasma}) was measured in normal brain cortex and compared to measurements, 11 days after tumor (sham) implantation, in the ipsilateral or contralateral cortex. Individual profiles of MTX in plasma and brain ECF (estimated by correction of dialysate concentrations for in vitro recovery) are shown in figure 2 for the rats with the semipermeable part of the dialysis membrane at the site of tumor implantation. The penetration of MTX into the brain was calculated for individual rats and the mean total values of group A (normal), B (sham, ipsilateral), C (tumor, ipsilateral) and D (tumor, contralateral) are presented in table I. The mean total value for A, B and D were similar, whereas the mean total value for group C was 2.2 fold higher (n.s.).

In study II the penetration of MTX in the ipsi- or contralateral cortex was measured at different periods after tumor implantation. Mean MTX penetration values are presented in table II. No statistically significant differences in the mean total values were found as a function of the post tumor implantation interval. Besides, no overall difference between mean total values of the ipsilateral and contralateral groups was found.

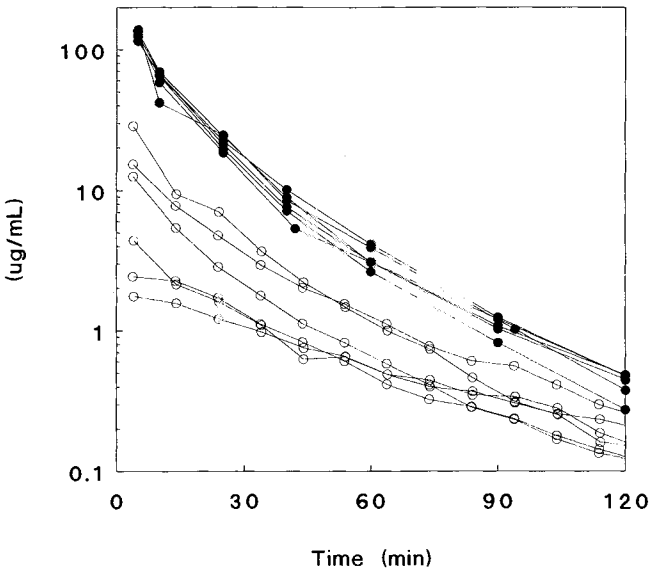

Fig. 2. Individual concentration-time profiles of methotrexate in plasma (●) and brain ECF (○) after iv bolus injection of 75 mg/kg, as measured for group C of study I (tumor implantation and microdialysis measurements 11 days post tumor implantation (PTII) in the ipsilateral cortical brain).

Histological Data

In order to relate MTX penetration with the presence of tumor, individual brains were examined by semiquantitative histology (Materials and Methods). A number of other parameters that might influence local MTX penetration were scored as well. These were vacuolisation of white matter; cell death; infiltration of granulocytes; hypercellularity; fibrosis; hyaline proteinaceous exudate; and hemorrhage. It appeared that after tumor implantation, 46 % of the animals (11/24) showed the presence of tumor tissue at the time of the experiment ("tumor take", fig. 1). In some other cases (day 6) reactive changes such as fibrosis were observed as a remains of the implantation (fig. 1c).

Relation between Histological and Pharmacokinetic Data

For the microdialysis measurements ipsilateral to the site of tumor implantation it was found that in case of "tumor take" or the presence of exudate, the MTX penetration values were higher than for measurements in the absence of these two factors (Table I and II). For measurements at the cortex contralateral to the tumor implantation site the opposite was found: tumor presence was associated with lower MTX penetration values (Table I and II). In figures 3 and 4 the individual MTX penetration values are presented together with the indication of tumor or exudate if present. The relation between MTX penetration and tumor size is presented in figure 5. Using a polynomal function (y = a0 + al. log x), the relation between tumor size and MTX penetration

Table I. Mean MTX Penetration Values ($AUC_{brain\ ECF}/AUC_{plasma}$) for the Different Experimental Groups of Study I (see Materials and Methods); Measurements of the Sham, Ipsilateral and Contralateral Group were Performed 11 Days after (Sham) Tumor Implantation in the Left Cortical Brain. Values are Presented as Mean ± SEM if Possible. (Kruskall Wallice Rank Sum Test, n.s. for $P > 0.05$)

Experiment	Total	No tumor or exudate "control"	Tumor	Exudate
A (normal)	0.053 ± 0.009 (n = 5)	0.053 ± 0.009 (n = 5)		
B (sham)	0.051 ± 0.013 (n = 6)	0.051 ± 0.013 (n = 6)		
C (tumor, ipsilateral)	0.131 ± 0.046 (n = 6)	0.050 (n = 2) 100%	0.112 (n = 2) 224%	0.232 (n = 2) 232%
D (tumor, contralateral)	0.051 ± 0.007 (n = 6)	0.068 (n = 2) 100%	0.043 ± 0.008 (n = 4) 63%	

can be described for ipsilateral (n = 20) and contralateral (n = 9) measurements, with a0 values of 0.063 and 0.064, a1 values of 0.03 and -0.01, and correlation coefficients of 0.53 and 0.68, respectively.

In an attempt to reveal a possible relation between the quantity of the presence of a parameter around the microdialysis probe and changes in MTX penetration, all parameters were scored in a semiquantitative way (data not shown). It was found that the mean tissue score values (of an individual parameter in each zone around the microdialysis probe) appeared not to be systematically correlated with changes in MTX penetration.

DISCUSSION

For the effective chemotherapeutic treatment of brain tumors a clear understanding of the targeting and disposition of anticancer agents into the tumor is necessary. In the present study intracerebral microdialysis was used to determine the disposition of the anticancer drug methotrexate (MTX) in an intracerebrally growing rhabdosarcoma. A difference in penetration of MTX into control cortical brain versus tumor-bearing cortical brain of WAG/Rij rats was demonstrated. This illustrates the potential use of the microdialysis technique in such studies.

Table II. Mean MTX Penetration Values ($AUC_{brain\ ECF}/AUC_{plasma}$) at Different Post Tumor Implantation Intervals (PTII) For Ipsilateral and Contralateral Measurements (Study II). Values are Presented as Mean ± SEM if Possible. *Significantly Different from Mean Control Value (Kruskall Wallice Test, $P < 0.05$)

PTII (days)	Total	No tumor or exudate "control"	Tumor	Exudate
ipsi 6	0.118 ± 0.032 (n = 6)	0.033 (n = 1)	0.105 ± 0.020 (n = 4)	0.235 (n = 1)
ipsi 11	0.088 ± 0.015 (n = 6)	0.037 (n = 1)	0.110 ± 0.007 (n = 5)	
ipsi 18	0.103 ± 0.013 (n = 6)	0.041 (n = 1)	0.116 ± 0.004 (n = 5)	
ipsi 25	0.156 ± 0.038 (n = 6)	0.070 (n = 2)	0.217 ± 0.042 (n = 4)	
Total ipsi	0.118 ± 0.013 (n = 24)	0.050 ± 0.009 (n = 5) 100%	0.131 ± 0.014 (n = 18) 254%*	0.235 (n = 1) 235%
contra 6	0.069 ± 0.008 (n = 3)	0.084 (n = 1)	0.061 (n = 2)	
contra 11	0.083 ± 0.011 (n = 3)	0.094 (n = 2)	0.061 (n = 1)	
contra 18	0.086 ± 0.012 (n = 3)	0.086 ± 0.012 (n = 3)		
contra 25	0.064 ± 0.014 (n = 3)	0.090 (n = 1)	0.051 (n = 2)	
Total contra	0.075 ± 0.006 (n = 12)	0.088 ± 0.005 (n = 7) 100%	0.057 ± 0.004 (n = 5) 65%*	

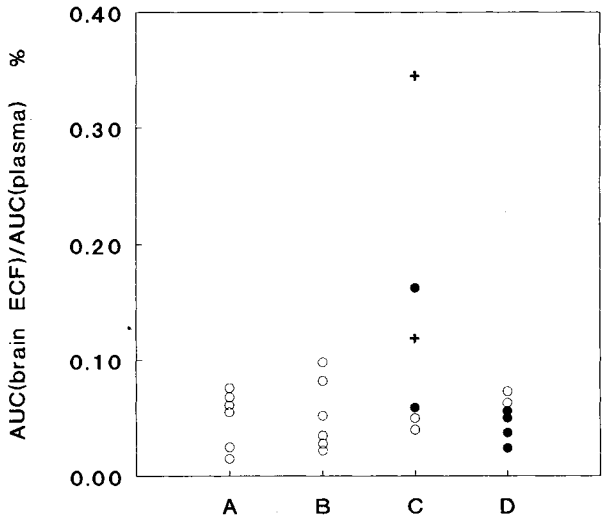

Fig. 3. Individual MTX penetration values ($AUC_{brain\ ECF}/AUC_{plasma}$) after iv bolus administration of 75 mg/kg methotrexate for the different groups of study I (see Materials and Methods); the post (sham) tumor implantation interval (PTII) was 11 days. A = normal, B = sham surgery with ipsilateral measurements, C = tumor implantation and ipsilateral measurement, and D = tumor implantation and contralateral measurement; (●) = presence of tumor, (+) = presence of exudate, and (○) = no tumor or exudate present.

The fact that the integrity of the BBB often becomes reduced within both metastatic and primary brain tumors has been known for a considerable period of time. However, this is not an all-or-nothing phenomenon (3,13,14,15). It is currently believed that the BBB or BBTB is a variable integrity within and between brain tumors (13). Obviously this is of importance for the delivery of (hydrophilic) anticancer agents to the brain tumor. In order to study local drug disposition into a brain tumor or tumor-bearing brain, autoradiography can be used. Although this technique offers the possibility to determine spatial distribution of the radiolabel, it does not discriminate between parent drug and metabolites. A more selective in vivo technique is intracerebral microdialysis, which can provide information about the local concentration of drug of interest in time. This technique has been shown to be a useful tool in the study of BBB transport of drugs (8,9,10), which it may also be in the study of disposition of anticancer drugs in brain tumors.

In the present studies, the penetration of MTX into cortical brain ECF was determined by estimation of the ratio of $AUC_{brain\ ECF}/AUC_{plasma}$. The effect of the supposedly tumor-bearing state was measured in the ipsilateral as well as contralateral cortical brain at different post tumor implantation intervals. Because it is known that tumor growth is highly variable it was necessary to examine the individual brains histologically, immediately after completion of the pharmacokinetic experiments. The presence of tumor tissue, but also of other parameters that might influence BBB permeability, was evaluated this way.

It was expected that an increase in MTX penetration would be found in the intracerebral tumor as compared to normal brain tissue, due to changes at the level the BBB. Indeed, it was found that the presence of tumor (rhabdomyosarcoma) was significantly correlated with an increase in ipsilateral MTX penetration values (to 250 %), while contralateral values were found to be lower (n.s., 65 %), as compared to the respective control values. In some previous studies it was found that drug penetration into brain tumors increased with the size of the tumor (3, 13), while others did not find such a relationship. Although the data showed substantial variability, in this study a positive relation between the tumor size and MTX penetration values was found for the ipsilateral measurements, while for the contralateral measurements the relation was negative. Apart from tumor,

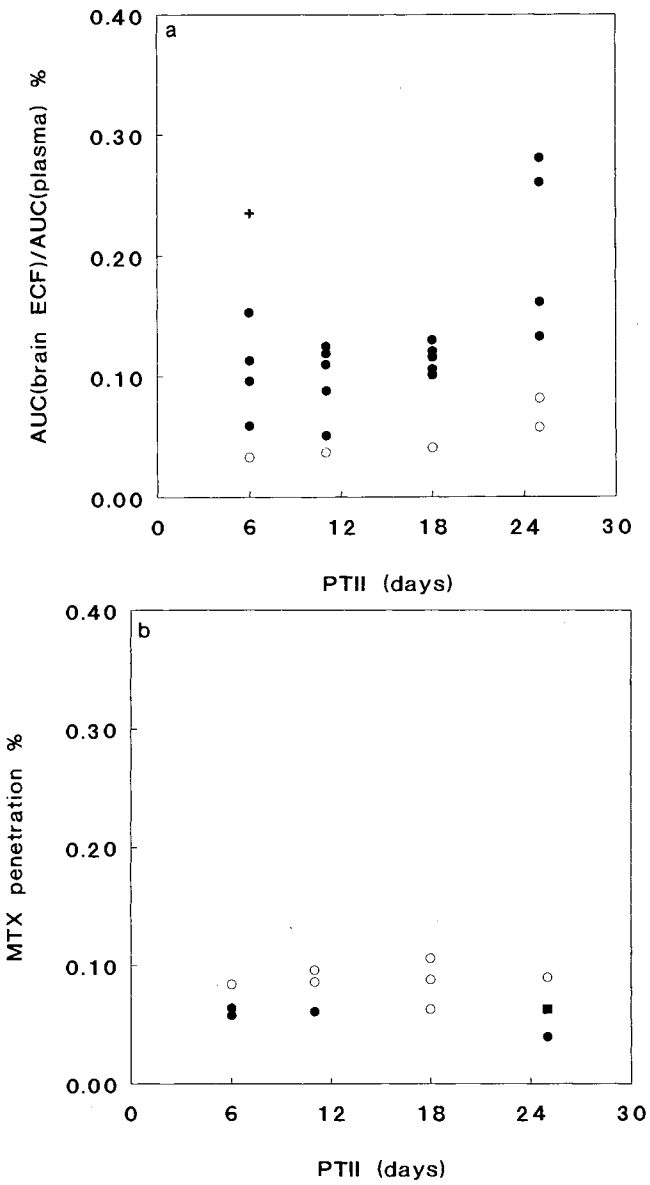

Fig. 4. Individual MTX penetration values ($AUC_{brain\ ECF}/AUC_{plasma}$) after iv bolus administration of 75 mg/kg methotrexate for the different groups of study II (see Materials and Methods); a) ipsilateral measurements; and b) contralateral measurements at different post tumor implantation intervals (days); (●) = presence of tumor at ipsilateral site or (■) ipsi- and contralateral site, (+) = presence of hyaline proteinaceous exudate, and (○) = no tumor or exudate present.

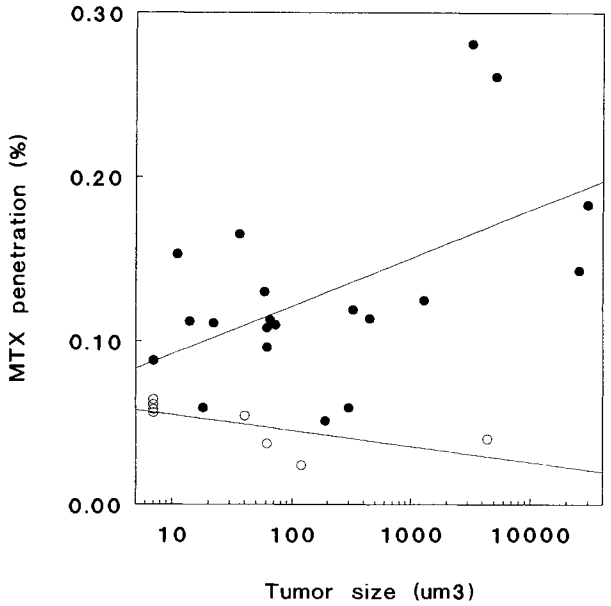

Fig. 5. Relationship between tumor size and individual MTX penetration values at ipsilateral (●) and contralateral (○) site. The data are fitted according to y = a0 + al * log x. (ipsilateral y = 0.063 + 0.029 * log x, r = 0.54; contralateral y = 0.064 - 0.009 * log x, r = 0.68)

the only other histologically determined parameter that was found to be associated with clearly increased MTX penetration values, was hyaline proteinaceous exudate (Table I and II). This is in line with the fact that hyaline proteinaceous exudate is indicative for a high vascular permeability.

Changes in MTX penetration found for ipsilateral measurements in tumor-bearing brain may arise from a number of factors like: changes in elimination of MTX; changes in protein binding of MTX in the diseased rat; changes in the recovery of the dialysis probe; and changes in the permeability of the BBB either by the tumor implantation procedure or by the tumor itself. First, the P-glycoprotein may have an important role in the elimination of certain anticancer drugs from the brain (16, 17). The functionality of this protein may be changed in tumor compared to normal tissue, which can in principle lead to different (net) penetration levels. However, MTX is no substrate for this elimination pump (18). To our knowledge no other active elimination mechanisms for MTX exist in brain. Then, the protein binding of (high-dose) MTX is about 50 %, and small changes in protein binding would probably not have led to any significant changes in the transport. Besides, these changes should have had identical effects on the transport in both the ipsilateral and the contralateral hemisphere, in contradiction to what is found in the present studies.

Furthermore, it is known that in tumor tissue the interstitial space is increased (19). This may lead to an increased diffusivity of solutes through the interstitial space and, therefore, to an increase of the recovery by the dialysis probe. However, the transport across the BBB is the limiting step in the flux of molecules to the microdialysis probe, for it is known that the micro-vascular of the brain allows for instantaneous solute equilibrium throughout the interstitial space once the solute traverses the BBB (20). This is endorsed by a microdialysis study in which dialysate levels of atenolol were substantially increased after osmotic BBB opening (9). This means that higher dialysate concentrations will be the reflection of an increase in transport across the BBB. Of course in this study the estimation of MTX levels in brain ECF is semiquantitative, for the correction of the dialysate levels have been based on vitro recovery instead of the in vivo recovery value. However, comparison of the different levels seems to be valid under the given experimental conditions.

The last reason mentioned for differences in MTX penetration may be by changes in BBB permeability due to the inevitably traumatic tumor implantation procedure, or by the presence of the tumor itself. In study I the effects of the implantation procedure was investigated by a sham treatment of the cortical brain. No differences were found in MTX penetration in the sham group as compared to the normal group. This means that differences in BBB permeability did not result from the implantation procedure itself. Moreover, the values obtained from rats showing no tumor take after tumor implantation were used to refer to the increase of MTX penetration to. This was considered to be a more appropriate control than the normal values. So, it seems that the increase (ipsilateral) or decrease (contralateral) in penetration of MTX into the brain was due to tumor associated factors.

The presence of tumor tissue resulted in lower MTX penetration values in the contralateral side (65 %). In principle, a decreased capillary blood flow could accompany the presence of tumor. In this case such a decrease is not supposed to be an appropriate explanation for our findings because MTX is a hydrophilic drug, and its delivery to the brain is in essence restricted by the limited transport across the BBB and not by blood flow (if not too low). Then, a diminished effective capillary surface (by increased intracranial pressure) could have resulted in a decreased extent of diffusion. No measurements on intracranial pressure have been performed. Although contralateral MTX penetration values tended to be lower with increased tumor size, which may be accompanied by higher intracranial pressure, no conclusions can be drawn from this study with respect to the effects of intracranial pressure.

The changes in MTX penetration have been compared to MTX uptake values in earlier studies. With Avian Sarcoma Glioma (21) and 471D SCLC tumor (22) respectively, the ratio of MTX concentrations in [tumor: brain around tumor: brain distant to tumor: contralateral brain] was [1.75: 4.1: 1: 0.79] and [5.4: 1: 0.75]. Shapiro et al. (23) found the ratio of [C6 glioma tumor: brain around tumor: normal cortex] of [2.1: 1.5: 1]. The data of the present study are similar, with the ratio [rhabdomyosarcoma: normal cortex: contralateral brain] of [2.5: 1: 0.65].

From this initial study it may be concluded that intracerebral microdialysis can be used to study the pharmacokinetics of anticancer drugs in a brain tumor. In combination with histological evaluation, factors that influence the pharmacokinetics can be revealed, which may provide important insights to be used for the treatment of brain tumors.

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