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Effect of X-Irradiation on the Pharmacokinetics of Methotrexate in Rats: Alteration of the Blood-Brain Barrier*

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Abstract—*This study was designed to evaluate the effects of brain irradiation on the permeability of the blood-brain barrier for methotrexate (MTX). Female WAG/Rij rats were cranially irradiated with a single dose of* 20 Gy *of* 300 kV *X-rays. At different times* (1–15 days) *after the exposure the rats were injected intravenously with MTX* (25 mg/kg *body wt). Irradiation had hardly any effect on the MTX concentrations in the plasma, heart and kidneys as determined by high-performance liquid chromatography. However, irradiation resulted in a significant increase of MTX (determined by* ^{125}I*-radioimmunoassay) in brain tissue per gram wet weight* (187.6 ± 17.9 pmol/g *vs* 46.4 ± 29.3 pmol/g *in unirradiated brain). This change in permeability of the blood-brain barrier lasted for about* 9 days. *The MTX elimination from the irradiated brain was the same as that from the non-irradiated brain. This indicates that only the MTX uptake and not the elimination by the brain was affected by the irradiation treatment.*

INTRODUCTION

FUNCTIONALLY, the blood-brain barrier can be represented as a membrane which selectively permits the passage of substances from plasma to the brain [1]. Most likely, the structural basis of this barrier is the presence of tight junctions between the endothelial cell membranes of the cerebral capillaries [2]. It has been postulated that the function of the blood-brain barrier is to maintain a specific internal environment essential for proper functioning of the brain. External factors that can influence the function of the blood-brain barrier are, among others, meningitis [1], microvascular injury [1], osmotic changes [3], microwaves [4, 5] and ionizing radiation [6–9]. These latter observations [6–9] are of special interest, since irradiation of the brain is common practice in the treatment and prophylaxis of intracranial metastases in cancer patients.

Most chemotherapeutic regimens for the treatment of childhood acute lymphocytic leukemia (ALL) include methotrexate (MTX), a cytostatic drug which can pass the blood-brain barrier to only a limited extent. Therefore, in order to prevent or treat central nervous system (CNS) relapse in childhood ALL [10], MTX is administered either intrathecally (i.t.) or systematically at a high dose. The MTX administration is often used in combination with cranial irradiation. However, this combination therapy can be associated with chronic neurotoxicity not only when MTX is given i.t. [11] but also after systemic administration of the drug [12–14]. The purpose of the investigation reported here was to determine the effects of cranial X-irradiation on the pharmacokinetics of MTX administered i.v. as a bolus injection, with special emphasis on the permeability of the blood-brain barrier.

MATERIALS AND METHODS

Experimental procedure

The animals used were 12 to 20-week-old female WAG/Rij rats bred in our own colony. They were cranially irradiated under sodium pentobarbital anesthesia (Nembutal; 60 mg/kg body wt) with a single dose of 20 Gy of 300 kV X-

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rays. Two opposed Philips-Müller X-ray generators were used (300 kV, 10 mA; half-value layer, 3.3 mm Cu), resulting in a homogeneous dose distribution over the brain. The dose rate was 2.0 Gy/min at a focus-to-midline distance of 30 cm. A large part of the oral epithelium was shielded to prevent acute radiation damage. During the experiment the rats showed a mean weight loss of 5 ± 2% after the first day; the weights gradually returned to normal within a few days. The weight loss during the first days after exposure cannot be explained by possible damage to the oral epithelium because this starts at about 5 days after irradiation and peaks at about 10 days, followed by a rapid recovery [15, 16]. Most likely, the weight loss was a result of the nembutal anesthesia, since sham-irradiated controls showed a comparable loss of weight. From 1 to 15 days after irradiation, rats were injected i.v. with MTX (Lederle) at 25 mg/kg body wt. At the indicated time intervals after drug administration they were bled under light ether anesthesia and, after dislocation, the brain, heart and kidneys were removed. Heparinized blood samples were centrifuged and the plasma was stored at -20°C. The organs were washed twice with 0.9% sodium chloride and also stored at -20°C.

MTX determinations

The concentration of MTX in the plasma and kidney and heart tissues was determined by high-performance liquid chromatography (HPLC) using a reversed-phase Lichrosorb 5RP18 column (Chrompack, Middelburg, The Netherlands) and an M440 UV detector (Waters) fitted with a 313 interferential filter. Separation was done with a mobile phase of 67 mM NaAc, 5% acetic acid/acetonitrile (88:12), pH 2.56, at a flow rate of 0.8 ml/min. The retention times for MTX and one of its metabolites are: MTX, 12.5 min; and 7-OH-MTX, 16 min. The limit of detection with this HPLC assay is about 0.2 nmol/ml biological substance (plasma or tissue homogenate).

Aliquots (500 μl) of plasma were deproteinized with 500 μl 10% TCA in 0.1 N HCl. After centrifugation (2 min at 8200 *g*), 100 μl of the clear supernatant were injected directly onto the column. Tissues were extracted as 10% homogenates in 0.9% sodium chloride with 10% TCA in 0.1 N HCl and, after centrifugation (2 min at 8200 *g*), 100 μl supernatant were injected onto the column.

In brain tissues the MTX was quantified by an MTX ^{125}I-radio-immunoassay (RIA) (Diagnostic Biochemistry Inc., San Diego, CA, U.S.A.). Brain tissue was extracted as a 20% homogenate with 10% TCA in 0.1 N HCl. After centrifugation the assay was performed on the supernatant. The lower limit of detection of the ^{125}I-RIA used was about 1 pmol/g wet wt brain tissue. The standards used for the quantification were made in 20% brain tissue homogenates.

The results presented in Figs 1 and 2 are expressed as the mean ± S.D. of 3 animals for the irradiated rats and for the non-irradiated ones as the mean ± S.D. of 6 animals. In Fig. 3 four separate experiments with one animal per time point are shown. Differences in the MTX concentrations between irradiated rats and control (unirradiated) rats were statistically analyzed by means of the Wilcoxon test.

The pharmacokinetic parameters of MTX were calculated using an open three-compartment model with drug excretion from the central compartment only [17].

RESULTS

MTX concentrations in plasma, heart and kidneys of irradiated rats

The MTX concentrations in the plasma, heart and kidneys on different days (1-15) after irradiation of the brain are shown in Fig. 1. The biological samples were taken at 1 hr after MTX administration. A striking difference in MTX concentration is seen between the heart and kidney tissues. In the kidneys, levels of up to 30 nmol/g wet wt were found, while the levels in the heart did not exceed 2 nmol/g wet wt. Great

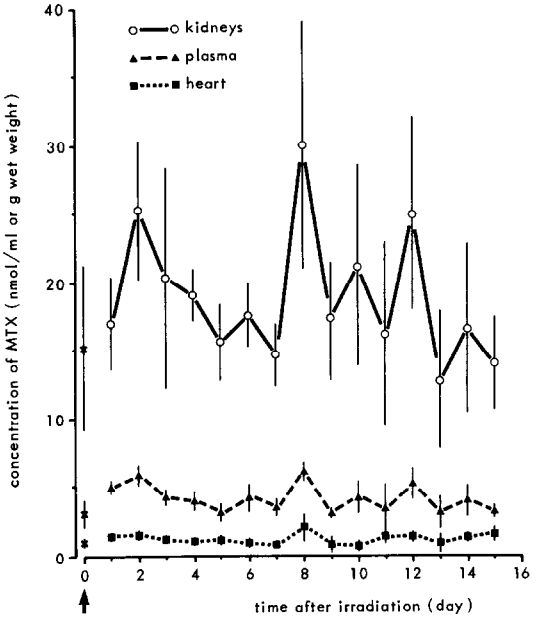

Fig. 1. Concentration of MTX in plasma, heart and kidneys at 1 hr after an MTX (25 mg/kg) i.v. bolus injection. The drug was administered at different time intervals (1-15 days) after irradiation (20 Gy) of the head. For the irradiated rats the results are expressed as mean ± S.D. of three rats. The arrow indicates time of irradiation. Control values (mean ± S.D. of six rats) for MTX found in unirradiated rats are indicated by an asterisk.

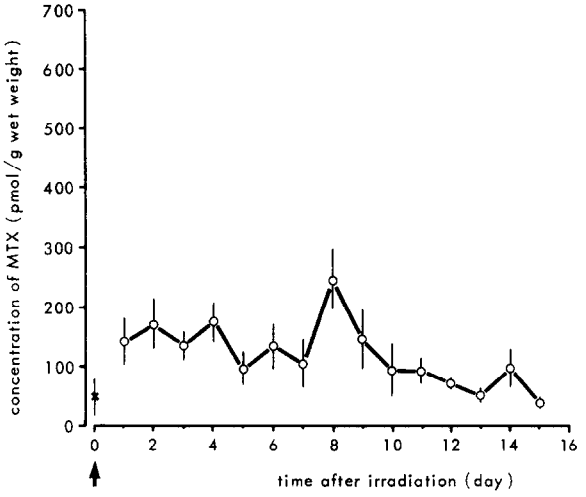

Fig. 2. Concentration of MTX in brain tissue (mean ± S.D. of three rats) at different time intervals after 20 Gy *whole-head irradiation. The arrow indicates time of irradiation.*

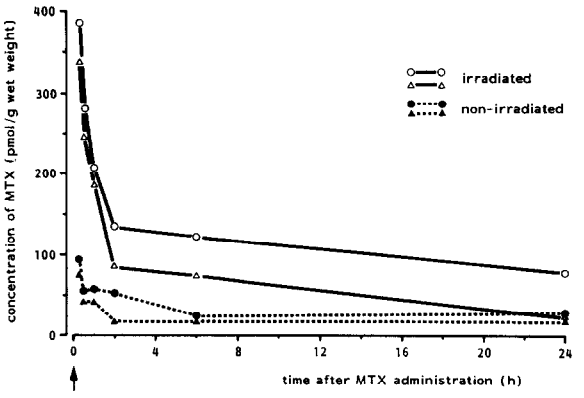

Fig. 3. Concentration-time course of MTX in brain tissue from non-irradiated and irradiated rats. The rats were injected i.v. with MTX (25 mg/kg) *at* 4 days *after irradiation. The results of four separate experiments are shown. The arrow indicates time of drug injection.*

variations are seen in MTX concentrations in the kidneys of irradiated rats. Although the kidney levels in the irradiated animals are somewhat higher as compared with the control value (in Fig. 1 indicated by an asterisk), these differences were not statistically significant. Also, the heart and plasma values did not change significantly in the irradiated animals as compared with the controls (in Fig. 1 indicated by an asterisk). In the plasma no metabolites of MTX were found and, among the tissues examined, only in the kidneys was 7-OH-MTX found in small amounts (maximally, 1.0 nmol/g wet wt tissue).

An MTX concentration-time course was followed in the plasma of non-irradiated rats and in rats which had undergone an irradiation treatment 4 days earlier. The plasma concentration-time data could be fitted very well by an equation describing drug distribution and elimination in an open three-compartment model with drug excretion from the central compartment only [17], with correlation coefficients of 0.9975 and 0.9998 for non-irradiated and irradiated rats respectively. In Table 1 are listed some relevant pharmacokinetic parameters of MTX in irradiated and non-irradiated rats. Only minor differences were found between non-irradiated and irradiated rats in $t_{1/2}\alpha$ and γ and AUC and, consequently, also in Vd and Clp.

MTX concentrations in brain tissue

Because the amount of MTX found in the brain was too small for detection by HPLC under the experimental conditions established, its concentrations in brain tissues were determined by RIA. In Fig. 2 are shown the concentrations of MTX in the brain at 1–15 days after irradiation of the brain of adult rats. The brain samples were

Table 1. Pharmacokinetic parameters of MTX (25 mg/kg) *administered as an i.v. bolus injection in non-irradiated and irradiated rats**

Pharmacokinetic parameters	Non-irradiated rats	Irradiated rats
$t_{1/2}\alpha$†	13.8 min	9.3 min
$t_{1/2}\beta$†	1.4 hr	1.4 hr
$t_{1/2}\gamma$†	77.0 hr	54.7 hr
$AUC_0 \rightarrow \infty$‡	46.5 nmol/ml × hr	51.8 nmol/ml × hr
Vd§	378 ml	301 ml
Clp‖	3.15 ml/min	2.47 ml/min

*The rats were injected with MTX at 4 days after irradiation.
†Half-time of plasma drug concentration during the α-distribution, β-intermediate and γ-elimination phases.
‡Area under the plasma drug concentration-time curve ($\int_0^\infty C(t)\,dt$).
§Volume of distribution(dose/β.AUC).
‖Drug plasma clearance (dose/AUC).

taken at 1 hr after MTX injection. As compared with the control values (46.4 ± 29.3 pmol/g wet wt), the amount of MTX in the brain of irradiated rats was significantly ($P < 0.05$) higher during the first 9 days after irradiation.

An MTX concentration-time course was also followed in the brain of non-irradiated rats and in some which had undergone an irradiation treatment 4 days earlier. After an i.v. MTX (25 mg/kg) injection the drug was taken up rapidly by the brain in both non-irradiated and irradiated rats (Fig. 3). The four concentration-time curves have nearly identical shapes. However, the curves representing the MTX concentrations in the brains of irradiated rats differ by a factor of 3-4 from the ones of the non-irradiated rats.

Since it has been reported [18] that the blood-brain barrier in younger rats (before the age of 7-8 weeks) is more permeable to drugs than in older rats, we also included in our study a comparison between young and adult rats in the blood-brain barrier permeability to MTX. Rats of 4 weeks of age were injected i.v. with MTX (25 mg/kg body wt) and 1 hr later assayed for MTX in the brain. The results are shown in Table 2. About twice as much MTX was found in the brains of young rats (119.2 ± 39.6 pmol/g wet wt)

Table 2. MTX concentration in rat brain tissue after an i.v. bolus injection (25 mg/kg *body wt*)

Age of rats	Concentration in brain* (pmol/g)
Adult† unirradiated	46.4 ± 29.3
Adult irradiated	187.6 ± 17.9
Young‡ unirradiated	119.2 ± 39.6
Young irradiated§	109.8 ± 16.1

Mean ± S.D. of 3-5 rats.
*1 hr after MTX administration.
†The adult rats were 13 weeks of age.
‡The young rats were 4 weeks of age.
§The rats were injected at 4 days after exposure.

than in those of adults (46.2 ± 29.3 pmol/g wet wt). However, brain irradiation in young animals did not result in elevated MTX levels as compared with the non-irradiated young rats. The differences in MTX brain concentrations between irradiated adults rats and young unirradiated and irradiated rats were not statistically significant.

DISCUSSION

When MTX is administered i.t. the brains are exposed to relatively high levels of drug and it has been shown [19, 20] that excessively high cerebrospinal fluid concentrations of MTX are directly responsible for the neurotoxicity observed with i.t. MTX therapy. Although it has been shown in man that MTX passes the blood-brain barrier poorly under normal conditions [21], neurotoxicity is also observed when MTX is injected i.v. in combination with cranial irradiation [12-14]. Indeed, the results of our experiments showed that in unirradiated adult rats MTX penetrates the blood-brain barrier poorly. However, irradiation resulted in a significant increase of MTX in the brain per g wet wt. In other organs, e.g. kidneys and heart, no significant increase in MTX concentration was found after irradiation. The kidneys of irradiated rats showed a large fluctuation in drug content. The reason for this fluctuation is obscure.

The effects of ionizing radiation on the blood-brain barrier permeability for MTX have been previously investigated by Griffin and co-workers [7]. They demonstrated that, after irradiation of the brain in mice with a single dose of 2000 rad of 250 kV X-rays, the blood-brain barrier permeability for MTX was altered so that injected MTX (100 mg/kg body wt) was able to pass into the brain. Methotrexate was detectable in the brain whether administered simultaneously with or up to 6 days after irradiation. We were able to repeat and extend the study of Griffin *et al.* [7]. Besides differences in species (mouse vs rat), route of administration (i.p. vs i.v.), drug assay (DHFR inhibition assay vs HPLC and RIA) and maximal drug concentration in the irradiated brain (28 pmol vs 230 pmol/g wet wt tissue), we have accumulated more quantitative data concerning the specificity and duration of the irradiation-induced permeability changes and the kinetics of the increased brain concentrations of MTX. Griffin *et al.* [7] mentioned that after 6 days the mice began dying as a consequence of high-dose oral irradiation. Obviously, they were studying MTX concentrations in plasma and brain tissues of severely toxified animals. In our study the rats in the experiment survived the irradiation treatment for at least 3 months. We experimentally followed the treated rats for up to 15 days and found that the permeability change was reversible and lasted for about 9 days. The experiments in which the kinetics of the irradiation-induced increased MTX concentrations in brain tissue was followed for 24 hr suggest that the drug uptake phase and not the drug elimination was affected by the irradiation treatment.

The cellular changes underlying the increased permeability of the blood-brain barrier for MTX are unknown at the moment. The possibility of increased permeability via the opening of the endothelial tight junctions, as has been found in the case of hypertonic shock [22], is not very likely.

Irradiation may directly damage endothelial cells of the blood vessels by a direct effect on, for example, membrane components or indirectly by, for example, the formation of free-radical species that cause the peroxidation of membrane lipids.

Our experiments with young rats show that the permeability of the blood-brain barrier for MTX is apparently not fully developed in rats of 4 weeks of age. A two-fold increase of MTX per g wet wt brain tissue was found in young unirradiated rats as compared with adult control rats. However, irradiation treatment in the young rats did not result in a further increase in MTX concentration.

An important question is whether the elevated drug levels in irradiated adult rats can be effective in terms of malignant cell kill in the case of CNS metastasis. Data found in the literature [23–25] indicate that, for effective treatment of, for example, overt CNS leukemia in man, MTX concentrations in cerebrospinal fluid have to be in the order of 100 pmol/ml. Although it is difficult to assess to what extent cerebrospinal fluid MTX concentrations can be compared with drug concentrations in brain tissue, our results suggest that, when doses equivalent to those in our rat studies are used in man, the findings of an increased MTX level in the brain after irradiation could be of therapeutic significance.

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