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Pharmacokinetics of high-dose methotrexate in dogs

An experimental model with diffusion chambers

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Summary. *The usefulness of a diffusion chamber method for determination of concentrations of cytostatic drugs in the interstitial fluid of tissues was tested. Chambers with a permeable membrane (pore size: 0.45 μm) were implanted in the liver, kidney, bladder wall, and prostate of dogs. After administration of high doses of methotrexate (100 mg/kg body wt) the concentrations in the chamber fluid and in serum were measured simultaneously and repeatedly for 72 h.*

The method proved to be effective for collecting data on the distribution of drugs in different organs. The results show that knowledge of the serum concentration does not permit predictions of the drug concentration in the interstitial fluid of various tissues to be made.

Introduction

Therapy with high doses of methotrexate (MTX) followed by leucovorin (citrovorum factor) rescue has been in use since 1966 for a number of neoplastic diseases [2, 7, 8, 10, 24]. A high dose of MTX, by definition, is more than 20 mg/kg body wt [3]. The counteractive effect of folinic acid when administered after MTX makes it possible to use doses of MTX that would otherwise be lethal. Use of higher doses of MTX with their inherent toxicological risks requires especially thorough knowledge of the pharmacokinetics.

Although isolated reports on the clinical use of high doses of MTX for treatment of kidney and bladder carcinomas exist [6, 12, 17, 24], we were unable to find any published data on drug concentrations in these or other organs. Due to the different intra- and extravascular distribution, the concentrations in serum do not allow any conclusion as to the concentrations in the target organ [4]. Therefore, the availability of MTX in the liver, kidney, bladder wall, and prostate of dogs following administration of a high dose followed by leucovorin rescue was investigated.

Organ homogenates did not appear to be suitable for determining the drug concentrations. The values thus obtained represent a conglomerate of concentrations of various compartments (interstitial fluid, serum, lymph, cellular cytoplasm, urine). Particularly in the case of substances eliminated via the kidney or gallbladder, the concentrations measured in tissue homogenates cannot be considered representative of the interstitial fluid [1, 5].

Thus, a modified diffusion chamber model was used, which has proved itself effective in the determination of antibacterial drug concentrations in interstitial fluid [9, 14, 19].

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Material and methods

Test animals. The investigations were carried out in four male beagle dogs, 2–4 years old. During the experiment they were kept in metabolism cages.

Diffusion chambers. The diffusion chambers used consist of a plastic ring (14 mm outer, 10 mm inner diameter, 4 mm high) with a millipore membrane (0.45 μm pore size) glued on each side. The capacity of each chamber is 0.2 ml. The plastic ring contains two adjacent holes through which two polyvinyl tubes (1 mm outer, 0.6 mm inner diameter, 60 mm long) were inserted and glued in place (MF cement). The free ends of both these catheters are constructed so that they can be punctured percutaneously with an injection needle after SC implantation.

Implantation and operation. The implantation of the diffusion chambers was performed during general anesthesia (thiopental and halothane-oxygen) and in aseptic conditions. The chambers were implanted in the liver and kidney after a median upper abdominal laparatomy. An incision was made in the convex side of the kidney, the chamber inserted, and the incision closed with sutures. The edges of the incision were also closed at the surface with Histoacryl tissue adhesive.

For implantation in the liver, an incision was made in the left lobe. It was closed in the same manner. The bladder and prostate were exposed by means of a paramedial lower abdominal laparatomy. An incision was made in a lateral lobe of the prostate. Following insertion of the chamber, the capsule was sutured and the incision also fastened with adhesive. Since direct implantation into the bladder wall itself was technically impossible, the chamber was placed in a duplicature of the bladder wall: the serosal layer of the bladder wall was removed, any bleeding being avoided. A pouch was formed and the diffusion chamber positioned therein, so that the chamber was completely surrounded by the muscular layer of the bladder. The pouch was closed with sutures. All aspiration tubes were passed through the abdominal wall and positioned SC.

The experiments were commenced at the earliest 2 weeks after implantation, and ended at the latest 8 weeks after implantation.

Membrane permeability. The permeability of millipore membranes has already been investigated previously in vitro with radiolabeled water, and also with amino acids, antibiotics, and albumin according to a standard procedure. The permeability was expressed in terms of the half-life periods required by the individual substances to achieve equilibrium by permeating into the chamber [19]. To exclude sources of error caused by delayed permeation, this value was also determined for MTX.

Pharmacokinetic investigations. A dose of 100 mg MTX/kg was dissolved in 250 ml physiological saline and infused IV for 1.5 h (MTX parenteral, sodium salt of 4-amino-N_{10}-methyl pteroylglutamic acid; Lederle, New York). At 8 h post-infusion, each dog received a short-term IV infusion of 60 mg calcium-leucovorin. The day after this administration, they received two doses of 6 mg each IM; and on the following day, two doses of 3 mg each IM (calcium-leucovorin; calcium salt of formyltetrahydropteroylglutamic acid; Cyanamid, Munich). The animals received a daily infusion of 500 ml physiological saline containing bicarbonate until day 3.

Blood samples of 1 ml each were taken from the veins of the forelegs (vena cephalica antebracchii). Samples were taken prior to the experiment, at the end of the infusion period, and at 15, 30, 45, and 60 min and 2, 3, 4, 8, 24, 48, and 72 h after infusion. For determination of cumulative urinary excretion, the urine volumes for the infusion period (90 min), the 1st h following application, and the periods 1–2, 2–4, 4–8, 8–24, 24–48, and 48–72 h were collected. Chamber fluid was withdrawn prior to infusion and then at 30 and 60 min and 2, 4, 8, 24, 48, and 72 h after the end of the infusion period.

The blood samples were centrifuged immediately and the serum removed. Chamber fluid was obtained by percutaneous puncture of the catheter conus. For each sample, about 0.02 ml, or $^1/_{10}$th of the chamber content, was withdrawn. The serum, chamber fluids, and urine samples were immediately frozen in liquid nitrogen and stored at −70° C.

Assay methods. MTX levels were determined with the homogenous enzyme immunoassay Syva Emit and MTX (E. Merck). All assays were performed in duplicate on a Syva Emit measuring unit consisting of a Gilford Stasar III photometer, a Syva Emit CP 5000 clinical processor, and a Syva pipettor dilutor. Measurements were taken at 30° C and 340 nm wavelength, and the measurement time was 30 s. The limit of detectability: 0.2 µmol/l.

Statistical evaluation. The mean and the standard deviation were calculated for the same time point for all animals. The pharmacokinetic parameters, in contrast, were determined for each animal separately, and then the mean values, standard deviation, and the area below the curve were calculated for all animals with the standard formulas [11, 20]. The AUC was calculated according to the observed concentration levels, while the $t_{1/2}$ was calculated from the adjusted model. The correlation coefficient *(r)* indicates the quality of approximation between the measured values and the calculated curve of the adjusted model. The coefficient of the exponential function and the standard deviation were calculated according to the 'exponential stripping method'. The cumulative urinary excretion was analyzed by the sigma minus and the 'rate' method [11].

Results

The histological examinations showed the diffusion chambers to be well tolerated. The demarcation zone was investigated 5–8 weeks after implantation. In the different organs the demarcation wall was of fairly uniform appearance. The device was regularly surrounded by a thin layer of fibroblasts showing some orientation parallel to the surface of the cellulose acetate membrane. This layer contains only a few collagen fibers, as could be shown by means of van Gieson stain, Foot's silver impregnation, and Lillie's allochrome connective tissue method. The zone is of almost uniform thickness and extremely poor in blood vessels. The demarcation wall was almost free of infiltrating cells. The mesenchymal wall gave way immediately to the adjacent interstitium with no cellular disarrangement of the parenchyma or secondary interstitial reaction. These findings gave no evidence of functional disturbance of the parenchyma due to interruption of the vascular supply.

The half-life of MTX until establishment of concentration equilibrium in vitro was 10 min. This implies that the membrane of the diffusion chambers caused a slight delay in diffusion, which was not taken into account in the calculation of the pharmacokinetic parameters.

The concentration-time curve of MTX in serum following IV administration of 100 mg/kg is shown in Fig. 1. At the end of the infusion a maximum concentration of 324 µmol/l was measured. The concentration dropped to 18 µmol/l after 4 h. At 8 h 5.7 µmol/l was still traceable; after 24 h, only traces could still be detected (Table 1). The simultaneously and repeatedly measured concentrations in the interstitial fluids of the organs studied can be seen in Fig. 2a–d. The maximum mean concentration values measured in the liver, bladder wall, and prostate were roughly the same (liver, 67.5; bladder wall, 73.8; and prostate, 55.3 µmol/l). The concentration curves had similar courses. After 24 h only slight concentrations could still be detected in the liver and bladder wall; those in the prostate were somewhat higher. In the kidney, the maximum concentration was 375.7 µmol/l after 2 h; 257.3 µmol/l was still detecable after 8 h; and 47.7 µmol/l even after 24 h (Table 1).

The cumulative urinary excretion shows that the drug is rapidly eliminated, 31% having been excreted by 8 h and 34% by 24 h, by which time renal elimination is practically complete (see Table 2 and Fig. 3).

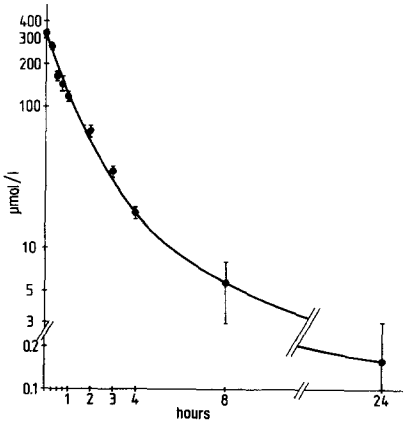

Fig. 1. Serum concentration of MTX after IV infusion of 100 mg/kg. Each *point* and *bar* represents the mean ± standard deviation in four dogs. The *solid line* represents the computer-fitted concentration-time curve

Table 1. Concentration of MTX in serum and tissue fluids after IV infusion of 100 mg/kg (μmol/l; mean ± standard deviation)

	Time after infusion										
	Immediately	15 min	30 min	45 min	1 h	2 h	3 h	4 h	8 h	24 h	48 h
Serum	324.02 ± 12.38	261.30 ± 14.22	166.67 ± 12.02	145.20 ± 16.66	118.26 ± 8.70	67.05 ± 10.90	35.46 ± 2.92	18.05 ± 1.78	5.68 ± 2.83	0.16 ± 0.14	0.16 – –
Liver	ND	ND	33.40 ± 7.98	ND	67.52 ± 30.68	66.99 ± 21.49	ND	62.59 ± 19.38	21.09 ± 1.25	5.96 ± 1.48	0.96 ±0.22
Kidney	ND	ND	216.68 ±120.25	ND	194.82 ±172.22	375.66 ±198.33	ND	306.45 ±168.53	257.32 ±148.87	47.66 ±35.37	5.15 ±2.93
Bladder wall	ND	ND	62.97 ± 20.85	ND	73.80 ± 9.24	72.80 ± 7.65	ND	56.33 ± 2.50	28.20 ± 10.81	3.93 ± 1.56	0.84 ±0.29
Prostate	ND	ND	33.73 ± 8.86	ND	41.07 ± 11.26	55.33 ± 10.32	ND	48.47 ± 8.69	34.35 ± 6.68	18.04 ± 6.37	2.48 ±1.34

ND, not done; – – –, incalculable

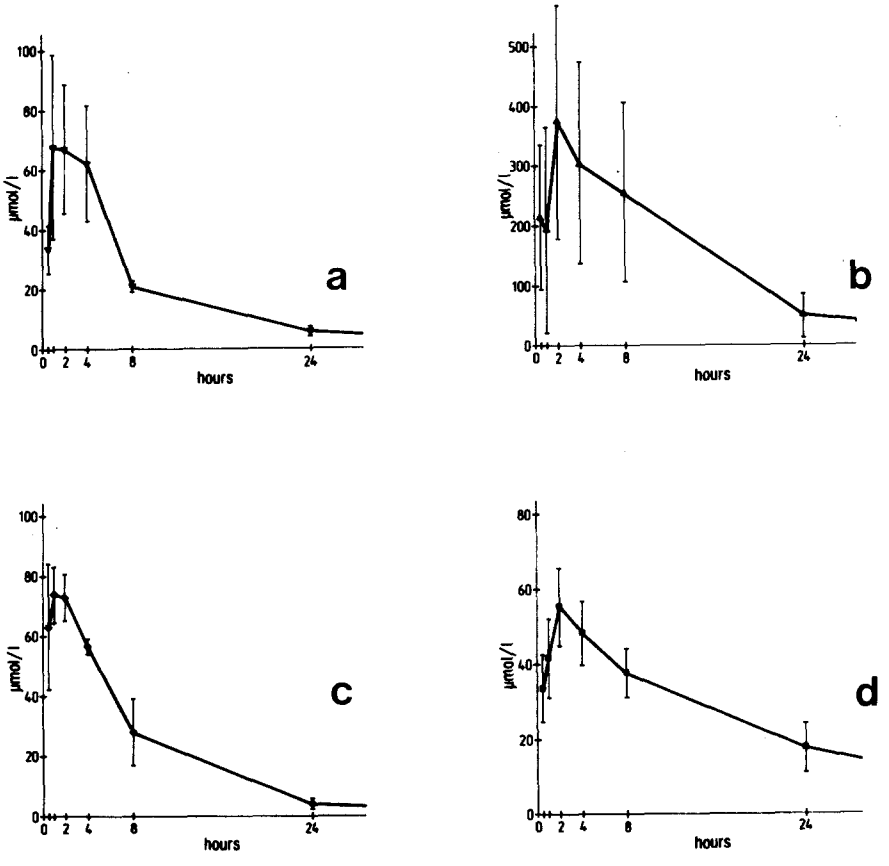

Fig. 2a–d. MTX concentrations in the liver **(a)**, kidney **(b)**, bladder wall **(c)**, and prostate **(d)** after IV infusion of 100 mg/kg. Each *point* and *bar* represents the mean ± standard deviation in four animals

Table 2. Cumulative urinary excretion of MTX as percentage of administered dose (100 mg/kg) (mean ± standard deviation)

1 h	2 h	4 h	8 h	1 day	2 days	3 days
14.892 ± 6.314	20.588 ± 7.626	26.645 ± 8.040	31.195 ± 7.260	34.855 ± 8.404	35.278 ± 8.303	35.308 ± 8.310

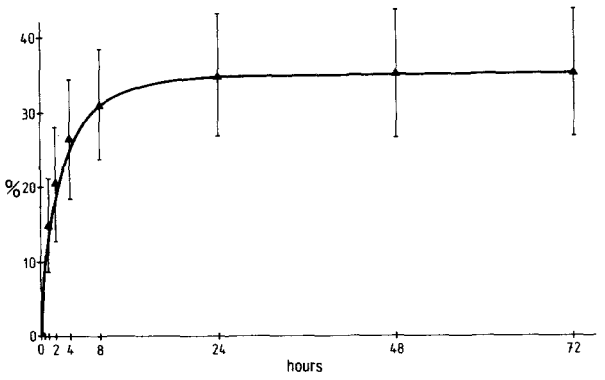

Fig. 3. Cumulative urinary excretion of MTX after IV infusion of 100 mg/kg. *Points* and *bars*, observed values (mean ± standard deviation); *solid line*, fitted curve

Table 3. Pharmakokinetic parameters in serum (MTX 100 mg/kg) (mean ± standard deviation)

$t_{1/2\,\alpha}$	$t_{1/2\,\beta}$	AUC	
0.20 ± 0.16	1.50 ± 0.34	495.73 ± 45.19	0.9936 ± 0.0004

$t_{1/2\,\alpha}$, distribution half-life time (h); $t_{1/2\,\beta}$, elimination half-life time (h); AUC, area under the curve (μmol/liter × h); *r*, correlation coefficient

Table 4. Pharmacokinetic parameters: MTX in interstitial fluids after IV infusion of 100 mg/kg (mean ± standard deviation)

	$t_{1/2}$	AUC	T_{max}	C_{max}	*r*
Liver	6.85 ± 1.65	612.98 ± 150.82	3.23 ± 0.26	44.74 ± 13.82	0.8925 ± 0.0537
Kidney	5.94 ± 1.14	5,262.99 ± 3,046.22	4.27 ± 0.91	377.31 ± 194.53	0.9795 ± 0.0114
Bladder wall	5.96 ± 1.03	707.04 ± 145.92	3.23 − − −	67.70 − − −	0.9590 ± 0.0021
Prostate	10.86 ± 3.64	937.40 ± 151.45	2.47 ± 0.44	56.19 ± 7.83	0.9772 ± 0.0074

$t_{1/2}$, elimination half-life time (h); AUC, area under the curve (μmol/l × h); T_{max}, time point of maximum concentration (h); C_{max}, maximum concentration (μmol/l); *r*, correlation coefficient; − − −, incalculable

The pharmacokinetic parameters are presented in Tables 3 and 4. In serum, the $t_{1/2\alpha}$ was 12 min and $t_{1/2\beta}$ 90 min. The half-life times in the liver, kidney, and bladder wall were all 6 h, and that in the prostate, 11 h. The AUC for the kidney was by far the highest (5,262.99 μmol/l × h).

Discussion

Following administration of 100 mg MTX/kg, the maximum serum concentration we measured (324 μmol/l) lies within the variation spectrum found in humans after the same or similar doses (100–1,000 μmol/l) [3, 18, 21]. The elimination half-life period in serum of 90 min also closely approaches that of 115–157 min in man [14, 18, 21, 23].

We were unable to find any published data on concentrations in organs or tissular interstitial fluid following the administration of high doses of MTX. Jain et al. [13] studied MTX concentrations in tumor interstitial fluid of transplanted tumors in rats (Walker 256 mammary carcinoma and hepatoma 5123), using micropore diffusion chambers. After IV administration of a single dose of 3 mg/kg they observed a rapid disappearance from plasma, followed by a steady decline of about tenfold the initial concentration between 5 and 120 min after the injection. The concentration of MTX in the tumor increased rapidly within a few minutes after the injection, then disappeared at a rate proportional to that in plasma. In another experiment, these authors measured MTX concentrations after various doses (0.03–30 mg/kg) in afferent and efferent tumor blood, and calculated the fraction of the administered dose retained by the tumor. They reported that the relative uptake by the tumor was eightfold more efficient with low than with high doses.

In our test series, we did not observe the wide variations in individual serum concentrations reported by many authors. However, considerable variations were found in the liver and kidney. Therefore, particularly the very high concentrations in the renal interstitial fluid must be assessed with due regard for their widely varying spectrum.

Data published on the cumulative urinary excretion vary widely. We found 31% of the administered dose in urine at 8 h, and 35% at 24 h. Thereafter, there was no notable excretion. Pratt et al. on the other hand, in man, found 41% after 6 h, and as much as 95% after 30 h, while Stoller et al. determined 50% of the administered dose in urine after 12 h [18, 21]. These differences may be due to the use of different analytical methods (dihydrofolate reductase method). Using a radioimmunoassay, Van Den Berg et al. found only 26% of the dose in urine within 48 h, and concluded that extrarenal excretion or metabolism had taken place [22]. Using the same method, Lenzhofer et al. found most of the administered dose had been excreted within 12 h [15]. These data in man coincide to a large extent with our findings.

On the whole it is difficult to compare results, because investigators have used a wide variety of doses (50–200 mg/kg), various durations of administration (bolus injection and infusion durations of up to 24 h), and different assay methods (enzymatic, fluorometric, and radioimmunoassays).

In conclusion, our results have shown that knowledge of the serum concentration does not permit prediction of the drug concentration in the various organs. Selection of drug dosage solely on the basis of the concentration in serum would denote inaccurate estimation of the cytotoxic effect on the tumor or the toxicity to healthy tissue [4]. The diffusion chamber

method appears to be a relatively simple and economical method, suitable for continuous measurement of cytostatic concentrations in tissular interstitial fluid.

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