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Evaluation of Cytostatic Drug Concentrations in the Kidney, Bladder Wall, and Prostate by Means of the Diffusion Chamber Technique in Dogs*

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Summary. The usefulness of a diffusion chamber model for measurement of concentrations of cytostatics in the interstitial fluid of tissues was tested. Chambers (pore size of the membranes: 0.45 mcm) were implanted in the kidney, bladder wall, and prostate of dogs. The concentrations after application of Doxorubicin-hydrochloride (2 mg/kg body wt.) and high doses of Methotrexate (100 mg/kg body wt.) were measured simultaneously and continuously in serum and in the above organs. The investigations showed that knowledge of the plasma level alone does not permit a prediction of the concentrations in the organs to be made. The diffusion chamber technique proved itself to be relatively simple to perform, economical, and it provides reproducible values.

Key words: Cytostatic drugs, Pharmacokinetics, Tissue concentrations, Diffusion chambers, Doxorubicin-hydrochloride, Methotrexate.

Introduction

The ultimate goal of pharmacokinetic studies of anticancer drugs is to offer a framework for the design of optimal therapeutic dosage regimens for individual patients. To optimize tumour kill while minimizing toxic effects of a given dosage regimen and route of administration, the drug concentration in different organs must be predictable at any point in time during therapy.

Predictive assays and techniques may in the future enable prediction of individual sensitivity to cytostatics and determination of the minimum cytotoxic concentration required. Serum levels of cytotoxic drugs give no indication what concentration will be present in tumour-containing or healthy organs [6].

The concentrations of chemotherapeutic drugs are usually measured in tissue or organ homogenates containing interstitial fluid, serum, lymph, cellular cytoplasm, and, in renal homogenates, urine. Thus, the measured concentration is a conglomerate of concentrations in various compartments. Particularly, in the case of renally eliminated substances with high urine levels, the concentrations measured in kidney homogenates cannot be considered representative of the interstitial fluid. An additional factor which could influence the drug concentration is the release of drug-inactivating enzymes [3, 7]. Disadvantages of organ homogenates are that the experiment cannot be repeated using the same animal, crossover tests are not possible, and considerably more animals are required.

These difficulties have led to the use of tissue chambers (tissue cages) for measuring antibacterial drug concentrations in interstitial fluid [2, 7, 11]. Chambers consisting of various inactive materials (e.g. PVC, silastic, polypropylene, metal) in various forms (cylindrical or round) with perforated surfaces have been implanted subcutaneously, in body cavities, and in organs. For this investigation, a modified diffusion chamber developed for antibiotic studies was used [13, 14, 20].

The suitability of this diffusion chamber model for continuous measurement of the concentration of cytostatics in the kidney, bladder wall, and prostate was tested. Two cytostatics were examined: *Doxorubicin-hydrochloride* (DXR), and *Methotrexate* (MTX) at a high dosage with Leucovorin-Rescue.

Material and Methods

Test Animals. All experiments were carried out on male Beagle dogs, 2–4 years-old, weighing 7.85–15 kg. They were kept in steel cages at room temperature, received a standard diet, and had free access to water. During the experiments, they were placed in metabolism cages.

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Diffusion Chambers. The diffusion chambers consisted of a plastic ring (14 mm outer, 10 mm inner diameter, 4 mm high) having a millipore membrane (0.45 micrometer pore size) glued on each side (MF-cement). The ring contained 2 adjacent holes through which two polyvinyl tubes (1 mm outer, 0.6 mm inner diameter, 60 mm long) were inserted and glued into place. The free ends were constructed so that they could be punctured with an injection needle. The chambers' capacity was about 0.2 ml. Before use, the chambers were sealed in plastic foil and sterilized for 6 h at 37 °C in ethylenoxide.

Operation Method. The implantation of the diffusion chambers was performed under general anesthesia and aseptic conditions. An upper abdominal incision about 10 cm long was made to gain access to the kidney. A 15 mm long and deep incision was made on the convex side of the kidney, and a diffusion chamber was placed in the opening. The incision was closed with sutures (Vicryl® 4-0, non-traumatic) and, in addition, the edges were attached with a tissue adhesive (Histoacryl®). The ends of the aspiration tubes were passed through the abdominal wall and positioned subcutaneously. The bladder and prostate were exposed by means of a 10 cm long paramedial incision. A chamber was placed in an incision made in one of the lateral lobes of the prostate. The incision was sutured and the edges also attached with an adhesive.

Since implantation of a chamber in the thin muscularis of the bladder wall or between the mucosa and muscularis was not possible, it was placed in a fold made in the bladder wall. The catheter ends were placed subcutaneously. The experiments were carried out 2–8 weeks after the chambers had been implanted.

Membrane Permeability. To exclude the possibility of inaccurate results due to delayed permeation of cytostatics through the membranes, DXR and MTX permeabilities were tested in vitro with a standard technique [26].

Drug Dosage and Application. Doses usually administered to humans were used. Each substance was tested on four dogs.

Doxorubicin-hydrochloride (DXR, syn.: Adriamycin-hydrochloride, Adriblastin®; Farmitalia, Carlo Erba GmbH, Milan) given at a dose of 2 mg/kg body wt. was dissolved in 100 ml physiological saline and administered i.v. for a 10 min period. Immediately afterwards, an additional 100 ml physiological NaCl was infused.

Methotrexate (MTX, sodium salt of 4 Amino-N_{10}-Methyl-pteroylglutamic acid, Methotrexate parenteral®; Lederle, New York), 100 mg/kg body wt. was dissolved in 250 ml physiological NaCl and infused for 1.5 h. 8 h post-infusion, each dog received 60 mg Calcium-Leucovorin i.v. (Citrovorum factor, calcium salt of Formyl-Tetrahydropteroylglutamic acid, Calcium-Leucovorin®; Cyanamid, Munich). The day after commencement of the experiment, they received two doses of 6 mg i.m. each; on the following day, two doses of 3 mg each i.m.

Sampling Procedures. Samples of diffusion chamber fluid were taken immediately before application of the drug, and 0.5, 1, 2, 4, 8, and 24 h after application; then every 24 h. Blood samples were taken before and after application, and at 5, 10, 15, 30, 45, 60 min; 2, 3, 4, 8, and 24 h; then every 24 h. The blood samples were immediately centrifuged and the serum removed.

Urine was collected for the periods 0–1, 1–2, 2–4, 4–8, 8–24, 24–48 h etc. in containers placed in dry ice. The experiments with DXR lasted 7 days; with MTX 3 days. The serum, chamber fluids, and urine samples were immediately frozen in liquid nitrogen and stored at minus 70 °C until assay.

Assay Methods. The quantitative assay for DXR was made using a radioimmunoassay kit from Diagnostic Biochemistry Inc., 10457-H Roselle St., San Diego, California 92121, USA, which was slightly modified. The samples were measured using a gamma counter of the type Multi Prias 1 from United Technologies Packard. The intra-assay variation was 8.25%, and the interassay variation 12.7%. Limit of detectability: 0.06 ng/ml.

MTX levels were determined with the homogenous enzyme-immunoassay Syva EMIT®- and Methotrexate; E. Merck. All assays were performed in duplicate on a Syva EMIT® measuring unit consisting of a Gilford Stasar III photometer, a Syva EMIT® clinical processor CP 5000, and a Syva pipettor dilutor. Measurements were made at 30 °C, at 340 nm wavelength, and measurement time was 30 s. Limit of detectability: 0.2 mcmol/l.

Statistical Evaluation. The calculation of the mean and the standard deviation was made for the same point in time from 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 [16, 27]. 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 calculation of the coefficient of the exponential function and the standard deviation was made with the "exponential stripping method". The cumulative urinary excretion was analyzed with the "sigma minus" as well as with the "rate"-method [16]

Results

The half-life times until establishment of concentration equalisation between the chamber content (aqua dest.) and the surrounding fluid having a defined substance concentration (20 mcg/ml) were 4 (DXR) and 10 (MTX) min in vitro.

In the histological preparation, the membranes of the diffusion chambers were covered by tissue layers mainly composed of fibroblasts and fibrocytes. This demarcation wall was poor in fibre, since no collagenic fibres were to be found. Moreover, there was no cellular infiltration within these cell layers in most cases.

Doxorubicin-hydrochloride. The mean values of the drug concentration measured after i.v. application of DXR are given in Table 1. Following application, a peak serum concentration value of 737.5 ng/ml was measured. The concentration-time curve then dropped relatively sharply until the 8th hour (20.6 ng/ml). After 168 h, a concentration of 3.6 ng/ml could still be measured.

The simultaneously and continuously measured concentrations in interstitial fluid from the kidney and prostate were similar (approx. 2–6 ng/ml). In the bladder wall, there was a slow but continuous rise up to 48 h after application (128.6 ng/ml). Only on day 3 did the levels drop to approximately those values measured in the interstitial fluid of the other organs (10.4 ng/ml) (Fig. 1–3). The cumulative amount excreted in the urine was 17.7% up to the 3rd day (Table 2).

In serum, the half-life time of the alpha-phase (distribution or invasion phase) was 5.8 min (0.097 h) and that of

Table 1. Concentration of DXR in serum and tissue fluids after i.v. application of 2 mg/kg (ng/ml, mean ± standard deviation)

	5 min	15 min	30 min	45 min	1 h	2 h	4 h	8 h	1 day	2 days	3 days	7 days
serum	737.50 ±131.05	440.00 ±76.84	341.00 ±52.82	268.00 ±43.91	209.25 ±39.42	129.33 ±26.26	66.50 ±14.31	20.67 ±9.68	6.73 ±2.14	6.40 ±1.45	4.40 ---	3.60 ±1.22
kidney	n.d.	n.d.	1.95 ±0.45	n.d.	2.15 ±0.43	2.38 ±0.19	2.98 ±0.40	3.71 ±0.52	4.00 ±0.68	4.03 ±0.55	4.93 ±0.64	u.l.d.
bladder wall	n.d.	n.d.	8.55 ±2.24	n.d.	15.88 ±2.35	24.55 ±2.13	32.80 ±2.21	62.83 ±8.49	97.08 ±26.37	128.60 ±31.52	10.45 ---	u.l.d.
prostate	n.d.	n.d.	5.43 ±2.11	n.d.	5.63 ±2.44	5.60 ±2.25	5.07 ±1.72	4.80 ±1.51	5.55 ---	4.00 ±0.95	3.33 ±0.38	u.l.d.

n.d. - not done
--- - incalculable
u.l.d. - under limit of detectability (0.06 ng/ml)

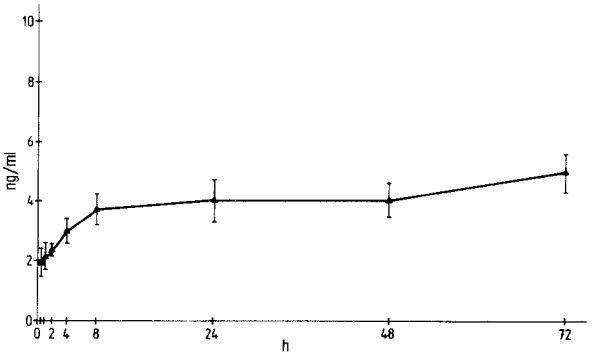

Fig. 1. Concentration of DXR in the kidney after intravenous application of 2 mg/kg

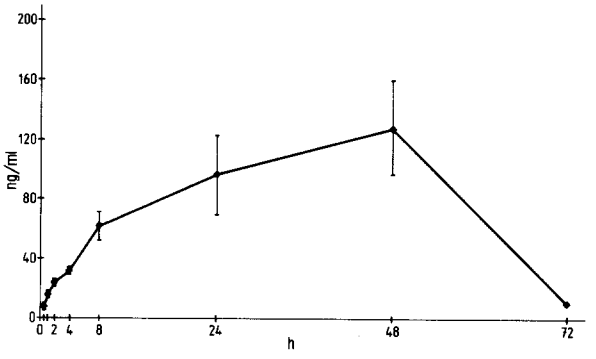

Fig. 2. Concentration of DXR in the bladder wall after intravenous application of 2 mg/kg

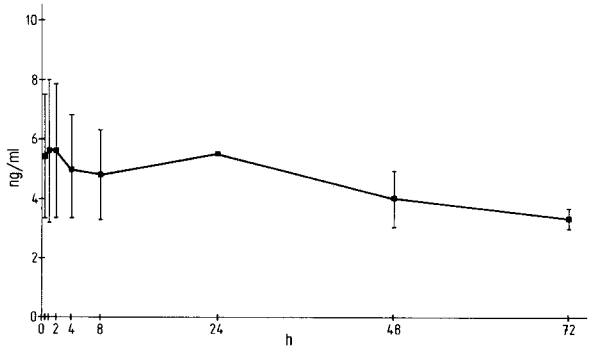

Fig. 3. Concentration of DXR in the prostate after intravenous application of 2 mg/kg

the beta-phase (elimination-phase) 98.4 min. (1.64 h). The AUC was 1760.93 ng/ml x h (Table 3).

The plateau shape of the kidney and prostate concentration-time curves made determination of elemination half-life periods and T_{max} and C_{max} impossible. These values could be formally calculated for the bladder wall fluid, however, the actual curve makes their clinical relevance doubtful (Table 4 and Fig. 2).

Since the AUC was calculated using the values actually measured, interpretation of it is allowable. The AUC for

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

	1 h	2 h	4 h	8 h	24 h	48 h	72 h
DXR	6.28 ±0.91	7.28 ±0.95	8.95 ±0.87	10.83 ±1.01	14.10 ±1.45	16.52 ±2.50	17.71 ±2.45
MTX	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

Table 3. Pharmacokinetic parameters in serum (DXR 2 mg/kg, MTX 100 mg/kg) (mean ± standard deviation)

	$t\,1/2_{(\alpha)}$	$t\,1/2_{(\beta)}$	AUC	R
DXR	0.097 ±0.023	1.64 ±0.37	1,760.93[a] ±314.21	0.997000 ±0.001414
MTX	0.20 ±0.16	1.50 ±0.34	495.73[b] ±45.19	0.993667 ±0.000437

$t\,1/2_{(\alpha)}$ – distribution half-life time (h)
$t\,1/2_{(\beta)}$ – elimination half-life time (h)
AUC – area under the curve – [a] ng/ml x h; [b] mcmol/l x h)
R – correlation coefficient

the bladder wall fluid was highest (4432.92) while those determined for the other organs were significantly lower (kidney: 256.87; prostate: 307.19) (Table 4).

Methotrexate. The concentration of MTX in serum and chamber fluids after i.v. application of 100 mg/kg bw. are found in Table 5. At the end of application, a maximum serum concentration of 324 mcmol/l was measured. The concentration drops relatively rapidly to 18 mcmol/l at 4 h. 5.7 mcmol/ml were evident at 8 h. Traces were still present after 24 h.

The concentrations in the bladder wall and prostate were similar (maximum values: 73.8 and 55.3 mcmol/l

Table 4. Pharmacokinetic parameters: DXR in interstitial fluids after i.v. application of 2 mg/kg (mean ± standard deviation)

	t 1/2	AUC	T_{max}	C_{max}	R
kidney	---	256.87 ±50.53	---	---	---
bladder wall	22.23 ±3.53	4,432.92 ±1,025.06	26.37 ±8.13	114.74 ±27.33	0.949250 ±0.024807
prostate	---	307.19 ±80.57	---	---	---

t 1/2 – elimination half-life time (h)
AUC – area under the curve (ng/ml x h)
T_{max} – time point of maximum concentration (h)
C_{max} – maximum concentration (ng/ml)
R – correlation coefficient
--- – incalculable

Table 6. Pharmacokinetic parameters: MTX in interstitial fluids after i.v. application of 100 mg/kg (mean ± standard deviation)

	t 1/2	AUC	T_{max}	C_{max}	R
kidney	5.94 ±1.14	5,262.99 ±3,046.22	4.27 ±0.91	377.31 ±194.53	0.979500 ±0.011405
bladder wall	5.96 ±1.03	707.04 ±145.92	3.23 ---	67.70 ---	0.959000 ±0.002161
prostate	10.86 ±3.64	937.40 ±151.45	2.47 ±0.44	56.19 ±7.83	0.97725 ±0.00740

t 1/2 – elimination half-life time (h)
AUC – area under the curve (mcmol/l x h)
T_{max} – time point of maximum concentration (h)
C_{max} – maximum concentration (mcmol/l)
R – correlation coefficient
--- – incalculable

Table 5. Concentration of MTX in serum and interstitial fluids after i.v. application of 100 mg/kg (mcmol/l, mean ± standard deviation)

	end of application	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 ---
kidney	n.d.	n.d.	216.68 ±120.25	n.d.	194.82 ±172.22	375.66 ±198.33	n.d.	306.45 ±168.53	257.32 ±148.87	47.66 ±35.37	5.15 ±2.93
bladder wall	n.d.	n.d.	62.97 ±20.85	n.d.	73.80 ±9.24	72.80 ±7.65	n.d.	56.33 ±2.50	28.20 ±10.81	3.93 ±1.56	0.84 ±0.29
prostate	n.d.	n.d.	33.73 ±8.86	n.d.	41.07 ±11.26	55.33 ±10.32	n.d.	48.47 ±8.69	34.35 ±6.68	18.04 ±6.37	2.48 ±1.34

n.d. – not done
--- – incalculable

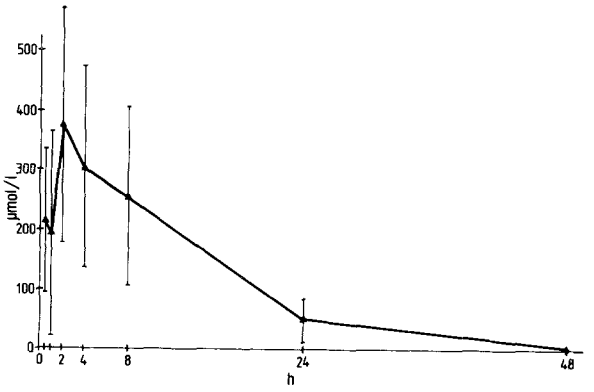

Fig. 4. Concentration of MTX in the kidney after intravenous infusion of 100 mg/kg

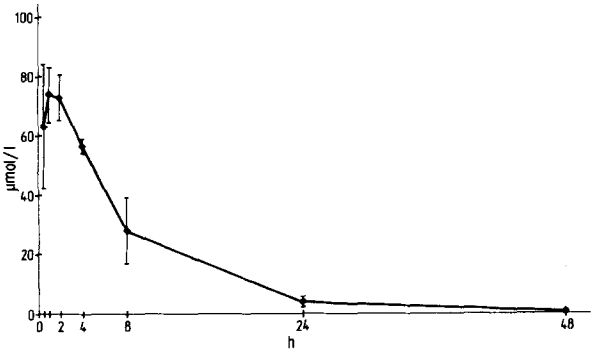

Fig. 5. Concentration of MTX in the bladder wall after intravenous infusion of 100 mg/kg

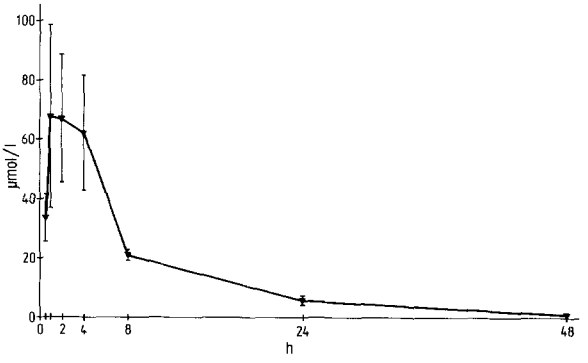

Fig. 6. Concentration of MTX in the prostate after intravenous infusion of 100 mg/kg

respectively). The concentration-time curves were similar, with only slight concentration in the bladder wall and somewhat higher concentration in the prostate still traceable after 24 h (Fig. 5 and 6). In the kidney, the maximum concentration of 375.7 mcmol/l was measured at 2 h. At 8 h, 257.3 mcmol/l, and at 24 h, 47.7 mcmol/l could still be measured (Fig. 4). However, due to the large variation range of the individually measured concentrations, these high values must be evaluated critically.

The substance was rapidly eliminated via urine, with 31% being excreted by 8 h and 34% of the dose by 24 h, renal elimination being therewith practically completed (Table 2).

In serum, t $1/2_{\alpha}$ was 12 min (0.2 h) and t $1/2_{\beta}$ 90 min (1.5 h) (Table 3). The half-life periods of the kidney and bladder were about 6 h; that of the prostate 11 h. The AUC for the kidney chamber fluid was by far the highest (5262.99) (Table 6).

Discussion

The membranes of the diffusion chambers cause only a slight delay in diffusion of the drugs under investigation. The equilibration half-life times in vitro were 4–10 min. This delay was not taken into account when calculating the pharmacokinetic parameters.

The histological findings indicate neither the occurrence of granulomatous tissues, nor is there any fibrous connective tissue to be found. Consequently, the entrance of interstitial fluid into the diffusion chamber does not seem to be altered to any significant degree.

Difficulties arise when the results are compared with those of other authors, mainly due to the varying techniques used. The most serious discrepancies are: the use of different animal species, other (mainly very high) doses, use of organ homogenates, and other methods to determine drug concentration. The main difference lies in the use of organ homogenates, as the concentrations thus obtained represent a mixture of interstitial fluid, cellular cytoplasm, lymph, plasma and possibly urine. Thus, the values, particularly those of the distribution phase, are considerably higher than those of interstitial fluids of the organs investigated. The concentration kinetics in serum and excretion in urine can be better compared.

Doxorubicin-hydrochloride. The maximum average serum-concentration we measured (728 ng/ml) after i.v. application of 2 mg/kg body wt. coincides well with that demonstrated by other authors who used a comparable dose in animal experiments [1, 5, 6, 17, 31] or on humans [5, 21]. The half-life periods (alpha-phase 5.8 min; beta-phase 98.4 min) we found also correspond with those reported for humans [10, 23].

Using the same assay method (RIA) and a comparable dose, Bachur reported finding about 0.8% in urine after 1 h and less than 2% after 10 h [1, 2]. In contrast, we found 6.28% of the administered dose after 1 h and 10.23% after 8 h in urine. Using rats, Kapelansky et al. [19] found 15.6 ± 2.4% of the applied dose (5 mg/kg body wt.) in urine after 48 h. These values come closest to our findings (16.52 ± 2.5%).

The DXR concentration in the kidney and prostate were similar (5–6 ng/ml) however, in the bladder wall, more than 20 times as much was measured (128 ng/ml). We have no experimental explanation for this phenomenon, and can thus only speculate what the reason may be. Since, on the one hand, relatively large amounts of DXR are still excreted in the urine up to 48 h after i.v. application, and on the other hand, in the initial hours severe, and later on, moderate oliguria occurs, urine with a high drug concentration acts on the mucous membrane of the bladder for an extended period of time. A serious local toxicity (chemocystitis) may develop under these conditions. Damage to resorption-inhibiting structures (intact epithelial layer and intact basal membrane) may lead to increased diffusion of substance into the bladder wall. This theory is supported by the synchronous decrease of excretion in urine and concentration in the bladder wall. Because of the extremely high concentration in the bladder wall, assumption of a substance of organ-specific affinity does not appear to be a sufficient explanation.

Methotrexate. Therapy with a high dose of MTX followed by citrovorum factor rescue has been used against a number of neoplastic diseases since 1966 [9, 12, 15]. The indications for use and effectiveness of this therapy are limited. Only a few reports exist on the use of MTX for the treatment of kidney and bladder carcinomas [8, 18, 24].

The maximum serum concentration of MTX we found in dogs following infusion of 100 mg/kg body wt. was 324 mcmol/l. This is within the range determined in humans (100–1,000 mcmol/l) having received the same or a similar dose with varying infusion duration [4, 25, 28]. Likewise, the calculated elimination half-life time in serum of 90 min is similar to that of 115–157 min for humans given in published reports [22, 25, 28, 30].

In contrast, data on cumulative urinary excretion vary greatly. We found 31% of the administered dose in urine at 8 h, and 35% at 24 h. Thereafter, there was no noteworthy further excretion. Pratt et al. [25] found 41% after 6 h and even 95% at 30 h; Stoller et al. [28] determined 50% of the administered dose in urine at 12 h. These differences may be due to the use of different analytic methods (RIA versus dihydrofolate reductase method). Using a radioimmunoassay, van den Berg et al. [29] found only 26% of the dose in urine within 48 h, and concluded that extrarenal excretion or metabolism had taken place. Using the same method, Lenzhofer et al. [22] found practically no further measurable MTX-concentration in serum after 24 h; most of the applied dose had been excreted in the urine by 12 h. This data coincides to a large extent with our experiments.

We were unable to find any published reports on MTX concentrations in organs or interstitial fluids following application of high doses.

Our results show that serum concentrations allow no prediction of drug concentrations in tissue. The degree of cytotoxicity can only be predicted when it is known how much of the cytostatic drug reaches the target organ. Knowledge of the distribution of the drug is also necessary to keep toxicity for the entire organism at a minimum. The diffusion chamber method appears to be suitable for the continuous measurement of concentration of cytostatics in tissue or interstitial fluid. The technique is relatively simple, economical, and gives reproducible results.

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