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Influence of the antibiotics piperacillin, doxycycline, and tobramycin on the pharmacokinetics of methotrexate in rabbits*

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Summary. In rabbits methotrexate (MTX) plasma concentrations showed a triexponential decline after short-term infusion (12 mg/kg in 10 min). Probenecid (50 mg/kg PO 1 h before MTX) and piperacillin (100 mg/kg SC 10 min before MTX plus 50 mg/kg SC 4 h later) increased the plasma concentrations of MTX and its metabolite 7-hydroxy-methotrexate (7-OH-MTX) 40 min to 6 h after the end of MTX infusion. The total body clearance of MTX was reduced, while the elimination half-life and the drug distribution to the peripheral body compartments were unchanged. Doxycycline (5 mg/kg PO 30 min before MTX) had no influence on the pharmacokinetics of MTX. Tobramycin was ineffective when given either only on the day of MTX infusion (2 mg/kg SC, 5 min before MTX and 2 mg/kg SC 4 h later) or as a 5-day pretreatment (2 × 2 mg/kg SC daily). The influence of probenecid and piperacillin can be explained by a reduction of the renal elimination of MTX via the tubular transport mechanism for organic acids. This interaction occurred with therapeutic drug concentrations and thus may be clinically relevant.

Introduction

After IV administration of the cytostatic drug methotrexate (MTX) 10%–30% of the dose is metabolized to 7-hydroxy-methotrexate (7-OH-MTX) in man [5, 28] and in rabbits [7, 14]. An additional small amount is stored in the form of MTX polyglutamates, and the rest is excreted in unchanged form, mainly by the kidney. The renal excretion of MTX is controlled by glomerular filtration and tubular secretion and, at least in some species, also by tubular reabsorption [4, 8, 10, 13, 16, 26]. MTX is secreted by a saturable tubular transport system that eliminates all kinds of organic anions. Probenecid and PAH are transported by the same carrier mechanism and reduce the renal elimination rate of MTX [2, 4, 10, 13, 16, 26]. In principle, any acidic drug may similarly inhibit the secretion of MTX. Some case reports have indeed suggested that coadministration of phenylbutazone [1] or penicillin [18] can increase the toxicity of MTX by delaying its elimination. Such a drug interaction may be clinically relevant particularly in the case of antibiotics, which must often be given during cytostatic therapy. Penicillin inhibits the accumulation of MTX in renal slices of rabbits [17, 21] and monkeys [9]. This probably indicates a competitive block of the common transport mechanism. In experiments with penicillin [27] a bolus injection followed by a continuous infusion delayed the elimination of MTX given as a bolus injection. Another antibiotic, gentamicin, which can produce renal tubular necrosis, has aggravated the toxic effects of MTX in rats [25].

In the studies cited the effective amounts of the antibiotics were larger than the usual therapeutic doses, which casts some doubt on the clinical relevance of the results. In the present paper we examine the influence of therapeutic concentrations of three antibiotics, piperacillin, tobramycin, and doxycycline, on the pharmacokinetics of a single short-term infusion (12 mg/kg) of MTX.

Material and methods

1. Experimental procedure. A group of 12 male rabbits each received three to four consecutive infusions of MTX at 4-week intervals. The animals were starved for 12 h before 12 mg/kg MTX dissolved in physiological saline solution (10 ml/kg) was infused during 10 min into an ear vein by a Braun Infusomat (Braun, Melsungen, FRG). Blood samples (2 ml) were drawn from the contralateral ear artery at the end of infusion and 10, 20, and 40 min and 1, 2, 3, 4, 6, 8, 12, and 24 h later. The blood samples were centrifuged immediately for 2 min in an Eppendorf 3200 centrifuge and plasma was stored at −20 °C until analysis. The first infusion in each animal served as individual control, while drug effects on MTX kinetics were tested with the second to fourth infusions.

In six animals weighing 3.1 ± 0.1 kg the influence of probenecid, piperacillin, and doxycycline was analysed. Probenecid was dissolved in water (10 mg/ml) and 50 mg/kg was given PO via a gastric tube 1 h before the second MTX infusion. Piperacillin (100 mg/kg) was injected SC 10 min before the third MTX infusion, and a second dose (50 mg/kg SC) was given 4 h later. Doxycycline (5 mg/kg) was administered PO to three animals 30 min before the fourth MTX infusion.

The effects of a single dose of tobramycin and of a continuous tobramycin treatment on MTX kinetics were tested in six other rabbits (body weight 4.1 ± 0.1 kg). These animals received an injection of 2 mg/kg tobramycin SC 5 min before the second MTX infusion, followed by an

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identical second dose 4 h later. At 5 days before the third MTX infusion a treatment with tobramycin 2 mg/kg SC twice daily was begun, which was continued until the end of the blood collection period after the third MTX infusion.

2. Assay procedures. MTX concentrations were measured with an enzymatic assay [23], while 7-OH-MTX concentrations were determined by an HPLC procedure [14]. Tobramycin concentrations were measured with a commercial immunofluorescence assay kit (Amerifluor™, American Diagnostics, Monrovia, USA).

Piperacillin and probenecid plasma concentrations were determined by HPLC using a 0.4 × 25 cm Shandon MOS-column which was perfused at a rate of 1.2 ml/min with a mixture of 0.01 *M* phosphate buffer pH 7-methanol (50/50; v/v).

For piperacillin, sample cleanup consisted in protein precipitation achieved by adding 50 µl methanol to 50 µl plasma. Then 10 µl of the clear supernatant was injected onto the column, and the absorbance was monitored at 220 nm. Probenecid was extracted from 10 µl acidified plasma into 1 ml chloroform-isopropanol (9/1; v/v). Metaiodobenzoic acid served as internal standard. Of the organic phase, 0.8 ml was transferred to a clean vial and, after addition of 10 µl of 0.1 *N* ethanolic NaOH, the organic layer was evaporated at 50 °C with a stream of clean air. The residue was taken up in 0.1 ml 0.01 *N* NaOH, and 10 µl was injected onto the column. The absorbance was monitored at 242 nm.

3. Drugs. The following drugs were used in the study (all suppliers are located in the FRG): Methotrexate (Methotrexate-Lederle: Cyanamid-Lederle, Wolfratshausen); probenecid (Benemid; MSD-Pharma, München); piperacillin (Pipril; Cyanamid-Lederle, Wolfratshausen); doxycycline (Vibravenös Steraject; Pfizer, Karlsruhe) and tobramycin (Gernebcin; Eli Lilly, Bad Homburg).

4. Statistics. Geometric means of the drug plasma concentrations were calculated for the presentations in Figs. 1 and 2. For the evaluation of MTX kinetics a triexponential curve was fitted to the MTX plasma concentrations of each individual experiment. The pharmacokinetic parameters were calculated from these functions with allowance for the 10-min infusion time [11]. For 7-OH-MTX the area under the concentration-time curve was calculated by the trapezoidal rule for the individual experiments. The half-lives for the monoexponential decline of the plasma concentration of probenecid, piperacillin, and tobramycin were calculated by linear regression analysis, using the data from all six experiments together. Drug effects on the kinetic parameters of MTX were assessed by Friedman's analysis of variance with ranks and the multiple comparisons of Wilcoxon and Wilcox [22]. A *P* value ≤ 0.05 for the two-tailed tests was fixed as the criterion for acceptance of statistical significance.

Results

Figure 1 shows the mean plasma concentrations of MTX after three consecutive infusions in the first group of six animals. In all cases the decline in the MTX concentration could best be described by a triexponential function. A three-compartment model with drug elimination from the central compartment was therefore chosen for calculation of the pharmacokinetic parameters. The half-lives of the α-, β- and γ-phases and the distribution volumes were similar in the two control groups (Table 1). For unknown reasons, however, the total body clearance (Q_{TOT}) was significantly lower in the second group of animals, and the area under the MTX concentration-time curve (AUC) was larger.

After pretreatment with probenecid the MTX plasma concentrations 40 min to 6 h after the end of infusion were

Table 1. Influence of various drug treatments on the pharmacokinetic parameters of MTX (12 mg/kg IV)

Pretreatment	$t_{0.5\alpha}$ (h)	$t_{0.5\beta}$ (h)	$t_{0.5\gamma}$ (h)	Vc (ml/kg)	Vss (ml/kg)	Q_{tot} (ml/kg × min)	AUC_{MTX} (µg × h/ml)	$AUC_{7\text{-OH-MTX}}$ (µg × h/ml)
Control	0.19 ± 0.02	0.54 ± 0.04	7.42 ± 1.04	188 ± 10.8	252 ± 10.1	8.63 ± 0.32	23.35 ± 0.93	8.97 ± 2.06
Probenecid 50 mg/kg PO	0.21 ± 0.02	0.60 ± 0.04	6.54 ± 1.56	181 ± 8.4	264 ± 17.3	6.42 ± 0.15*	31.24 ± 0.76*	21.93 ± 4.28*
Piperacillin 100 mg/kg SC + 50 mg/kg SC	0.28 ± 0.04	0.66 ± 0.05	6.16 ± 1.05	159 ± 26.4	199 ± 24.2	4.76 ± 0.37*	43.46 ± 3.94*	22.14 ± 2.07*
Doxycycline 5 mg/kg PO	0.17 ± 0.02	0.61 ± 0.07	7.97 ± 2.46	142 ± 3.7	224 ± 26.8	7.80 ± 0.64	26.02 ± 2.30	15.60 ± 1.17
Control	0.23 ± 0.02	0.63 ± 0.01	8.64 ± 0.75	159 ± 13.3	225 ± 21.2	6.72 ± 0.37	30.18 ± 1.61	7.53 ± 1.21
Tobramycin 2 × 2 mg/kg SC on 1 day	0.19 ± 0.01	0.62 ± 0.01	10.74 ± 2.63	135 ± 13.4	203 ± 18.0	6.25 ± 0.19	32.13 ± 0.96	8.19 ± 2.00
Tobramycin 2 × 2 mg/kg SC on each of 6 days	0.18 ± 0.01	0.65 ± 0.03	7.12 ± 2.73	142 ± 11.1	220 ± 18.0	6.70 ± 0.49	30.71 ± 2.45	7.03 ± 0.45

Arithmetic means and their standard errors are shown. Six experiments were performed in each treatment group, except for doxycycline ($n=3$). Values that differ significantly from the corresponding controls ($P \leq 0.05$) are indicated by an *asterisk*

$t_{0.5\alpha}$, $t_{0.5\beta}$, $t_{0.5\gamma}$, half-lives of the α, β, and γ phases; v_c, volume of the central compartment; V_{ss}, distribution volume during steady state; Q_{tot}, total body clearance; AUC, area under the concentration-time curve

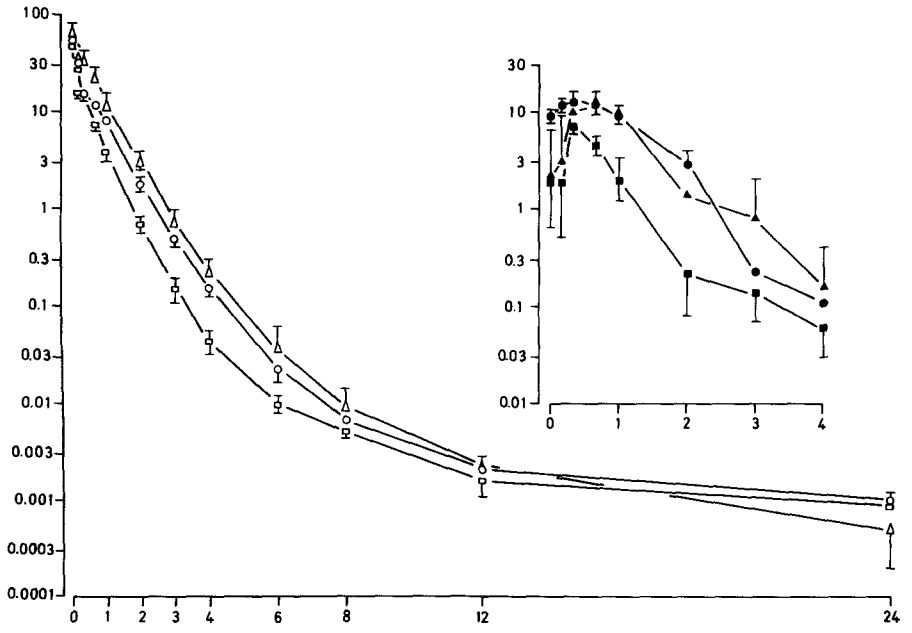

Fig. 1. Plasma levels of MTX *(open symbols)* and 7-OH-MTX *(insert; filled symbols)* after infusion of 12 mg/kg MTX in 10 min. Symbols indicate geometric means and their standard errors ($n=6$). Data are from three consecutive infusions in the same animals. ○, ●, first infusion, MTX alone; □, ■, second infusion, probenecid 50 mg/kg PO 1 h before MTX infusion; △, ▲, third infusion, piperacillin 100 mg/kg SC 10 min before MTX infusion and additional 50 mg/kg SC 4 h later. *Abscissae*, time after the end of MTX infusion (h); *ordinates*, plasma concentration of MTX or 7-OH-MTX *(insert)* (μg/ml)

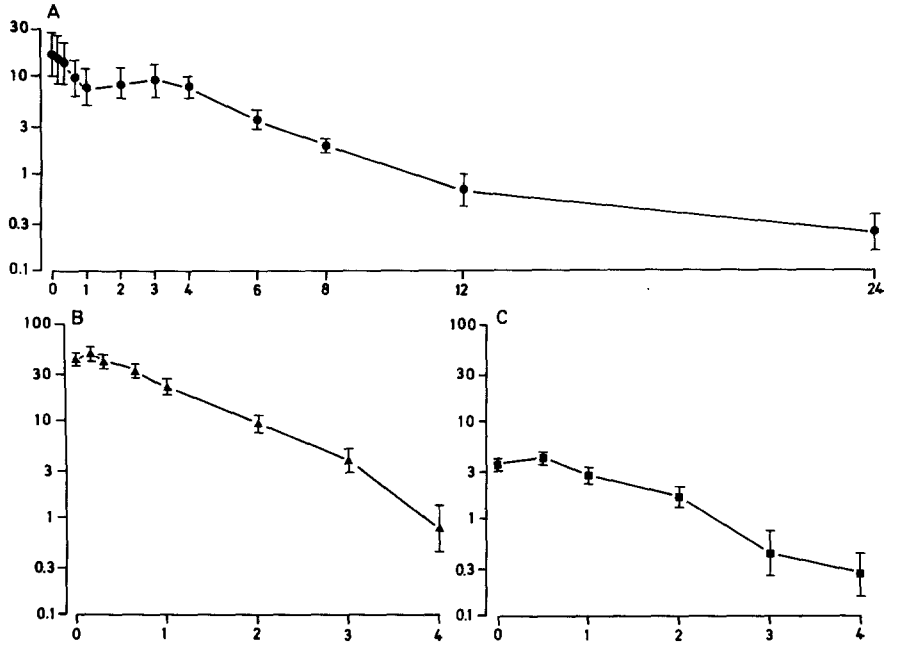

Fig. 2A–C. Plasma levels of probenecid **(A)**, piperacillin **(B)**, and tobramycin **(C)** *Symbols* indicate geometric means and their standard errors ($n=6$). Drug administration: probenecid 50 mg/kg PO 1 h before MTX infusion; piperacillin 100 mg/kg SC 10 min before MTX infusion; tobramycin 2 mg/kg SC 5 min before MTX infusion; MTX 12 mg/kg IV in 10 min. *Abscissae*, time after the end of MTX infusion (h); *ordinates*, drug plasma concentrations (μg/ml)

higher than in the control experiments (Fig. 1). Probenecid reduced the clearance of MTX and increased the AUC, but did not significantly change the half-lives of MTX kinetics (Table 1). The plasma concentrations of the main metabolite of MTX, 7-OH-MTX, and the area under its concentration-time curve were also increased by probenecid pretreatment.

As shown in Fig. 2A, the maximum plasma concentration of probenecid (16.5 μg/ml mean) was found in the first blood sample collected, i.e., immediately after the end of the MTX infusion, and the subsequent slow decline could be described by a monoexponential function with a half-life of 2.97 h. The irregularities during the first 3 h of observation were caused by large interindividual differences in the absorption of the drug from the gastrointestinal tract.

The influence of piperacillin on the kinetics of MTX resembled the effect of probenecid (Fig. 1, Table 1). The clearance of MTX was nearly halved; plasma concentrations of MTX and 7-OH-MTX and the corresponding AUCs were increased while the half-lives of the α-, β-, and γ-phases remained unaffected. Peak plasma concentrations (47.9 μg/ml mean) for piperacillin were registered 30 min after SC drug injection, i.e. 10 min after the end of MTX infusion (Fig. 2B). A half-life of 0.67 h was calculated for the subsequent decline.

No significant influence of doxycycline on MTX kinetics was found in three rabbits (Table 1). Plasma concentrations of doxycycline were not measured.

Single injections of tobramycin (2 mg/kg SC before and 4 h after MTX infusion) or a 5-day pretreatment (2 mg/kg twice daily) with the drug did not modify the

kinetics of MTX (Table 1). Plasma concentrations of tobramycin reached a maximum (4.2 μg/ml mean) 45 min after drug injection (i.e., 30 min after the end of MTX infusion; Fig. 2C) and declined with a half-life of 0.84 h.

Discussion

We observed a triexponential decline in MTX plasma concentrations after IV infusion. This is the usual finding in animal studies [3, 7, 24]. In a previous paper [14] we have shown that MTX pharmacokinetics in rabbits can be described by an open three-compartment mamillary model and appear to be linear over a wide dose range. The present results of the control experiments compare well with these earlier findings. However, the difference of the clearance value between the two series of control experiments is noteworthy (Table 1). We cannot offer a straightforward explanation. The result is not caused by an increase in the elimination half-life $t_{0.5\gamma}$; the γ-elimination phase contributes little to the total area under the curve (AUC) and thus hardly influences the clearance calculation. An obvious difference between the two groups of animals was the mean body weight, which was 3.1 kg in the probenecid-treated rabbits and 4.1 kg in the tobramycin-treated group. Probably the latter animals were somewhat older. Thus, an age-dependent decrease in the renal clearance may have contributed to the difference. Nevertheless, as we have also demonstrated [14] that repeated infusions of MTX at 4-week intervals do not modify MTX kinetics, we can safely conclude that all intraindividual changes of the pharmacokinetic parameters that were evaluated in the present paper were indeed caused by drug interactions.

Both probenecid and piperacillin decreased the total body clearance of MTX. The most likely explanation is a reduction of the renal excretion of MTX. Under control conditions renal excretion accounts for more than 50% of the total drug elimination [14]. A decrease in the renal MTX clearance with probenecid has already been demonstrated in rabbit [8], monkey [4], and man [2]. Being weak organic acids, probenecid and the penicillins are excreted by the common acid secretory mechanism in the kidney and competitively inhibit the transport of MTX in kidney slices [17, 21]. Thus, the reduction in MTX clearance is probably caused by an inhibition of the tubular secretion of MTX. This may lead the MTX clearance to fall even lower than the inulin clearance, because the tubular reabsorption of the drug is not impaired by probenecid [8, 13].

Paxton [20] has argued that probenecid, and possibly also other acidic drugs, can displace MTX from its plasma protein binding. Though only 46% of the total plasma MTX is bound to proteins [19], a decrease in this fraction could result in increased distribution of MTX to the peripheral compartments and prolongation of the elimination half-life. We could not detect an increase of the distribution volume or the terminal elimination half-life $t_{0.5\gamma}$ in the experiments with probenecid or piperacillin, and we do not believe that a reduced plasma protein binding is relevant to the decrease of the MTX clearance. It has been shown [15] that probenecid also inhibits the biliary excretion of MTX. In principle, piperacillin could have a similar effect. But in the rabbit only a very small amount of MTX is secreted into the bile [6; preliminary personal results]. It seems unlikely, therefore, that even total inhibition of this elimination pathway would result in a significant reduction of the total body clearance of MTX.

At least two factors may contribute to the increase of 7-OH-MTX plasma concentrations that was observed during probenecid and piperacillin administration. First, the reduced renal elimination of MTX could lead to a compensatory increase of the 7-OH-MTX production. In addition, 7-OH-MTX itself is a weak acid that is actively secreted by the kidney. This tubular secretion of 7-OH-MTX is also inhibited by probenecid [10].

Doxycycline is excreted mostly into bile and feces. Renal elimination accounts for only 10% of the total clearance. Like all other tetracyclines, the drug is filtered in the glomerulum but not actively secreted in the proximal tubulus [12]. The fact that doxycycline did not interfere with the elimination of MTX strengthens our assumption that the relevant influence of probenecid and piperacillin is an inhibition of the tubular secretion of MTX. The experiments with tobramycin were done because the aminoglycosides are known to be nephrotoxic [12]. Spector et al. [22] have shown that a single dose of gentamicin that is large enough to produce tubular necrosis can aggravate the toxic symptoms of an MTX injection in rats. In our study, however, even a 5-day pretreatment with therapeutic doses of tobramycin had no appreciable influence on the kinetics of MTX or on the renal drug excretion. We conclude, therefore, that the aminoglycosides do not interfere with MTX kinetics as long as the tubulotoxic side effects of these antibiotics can be avoided.

In summary, we have demonstrated that probenecid and piperacillin decrease the elimination of a single large dose of MTX, most probably by blocking the tubular secretion of the cytostatic. With piperacillin, this drug interaction occurred at therapeutically relevant drug concentrations. Our results confirm the hypothesis of Nierenberg and Mamelok [18], who concluded from a clinical case observation that the simultaneous administration of penicillin and MTX could increase MTX toxicity. Therefore, MTX plasma concentrations should be carefully monitored during a combined therapy with antibiotics, and the patients should be examined for signs of drug toxicity.

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