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The effects of antibiotics and uricosuric drugs on the renal elimination of methotrexate and 7-hydroxymethotrexate in rabbits

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Summary. In anesthetized rabbits, continuous infusion of methotrexate (MTX; 30 μg kg^{-1} min^{-1}) established steady-state plasma concentrations for MTX and the metabolite 7-hydroxymethotrexate (7-OH-MTX) within 40 min. Fifty percent of the infused dose was eliminated unchanged by the kidneys and the renal MTX clearance was slightly higher than the inulin clearance. Another 15%–30% was metabolized and excreted as 7-OH-MTX in the urine. Infusions of 7-OH-MTX, furosemide or benzarone had no influence on the clearance of MTX or 7-OH-MTX. Infusions of probenecid or piperacillin decreased the renal clearance of MTX and 7-OH-MTX mainly by reducing the tubular secretion of both compounds. In contrast, infusions of the antibiotics ceftriaxone and sulfamethoxazole increased the renal elimination of MTX and 7-OH-MTX. An increase was also observed during the infusion of the uricosuric drugs sulfinpyrazone and benzbromarone. These results are consistent with competition for tubular reabsorption between MTX, ceftriaxone and sulfamethoxazole. The pharmacokinetic drug interactions observed occurred with therapeutic drug concentrations and thus may be clinically relevant.

Introduction

Methotrexate (MTX) is eliminated mainly by the kidneys. In man, 50%–80% of a single i.v. dose is excreted unchanged with the urine [3, 16, 28]. Another 10%–30% is metabolized to 7-hydroxy-methotrexate (7-OH-MTX), which is also excreted in the urine [4, 6, 28]. Similar renal excretion data for MTX and 7-OH-MTX have been reported in monkeys [19] and rabbits [8, 18, 30]. The renal elimination of MTX and its metabolite is controlled by glomerular filtration, tubular secretion and tubular reabsorption. Tubular secretion is a saturable process [24] and can be reduced by probenecid, paraaminohippuric acid or penicillin [2, 15, 24, 31]. Thus, MTX and 7-OH-MTX seem to be excreted by the common transport mechanism for organic acids. In principle, any acidic drug might competitively block their elimination. Some case reports have indeed suggested that phenylbutazone [1] or penicillin and furosemide [26] can increase MTX toxicity in tumor patients by delaying its elimination. In a previous paper [17] we have shown that probenecid and piperacillin, in therapeutically relevant doses, reduce the total body clearance of MTX and 7-OH-MTX after a single i.v. injection. This influence was explained by an inhibition of the renal tubular secretion of MTX and its metabolite. These observations have been confirmed and extended in the present experiments by direct measurements of the renal drug clearance in anesthetized rabbits receiving a continuous MTX infusion. Furthermore, we present evidence that other uricosuric drugs and some antibiotics can also increase the renal elimination of MTX and 7-OH-MTX in this experimental model.

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Materials and methods

1. Experimental procedure. Twenty-six male rabbits were used in the study. Their body weight ranged from 3 to 5.5 kg on the day of the experiments. The animals had free access to tapwater and were fed Altromin standard diet. The latter was withdrawn 16 h before the beginning of an experiment. Up to three experiments with different drugs were performed with each animal at 4-week intervals.

The rabbits were anesthetized with an initial dose of 30 mg kg^{-1} pentobarbital i.v. and placed on an operation table. The rectal temperature was monitored by means of a digital thermometer (Bosch, Stuttgart, FRG) and was kept constant with the help of electrical heating. Anesthesia was maintained by injections of 3–6 mg kg^{-1} pentobarbital every 20–40 min. A loading dose of MTX (0.7 mg kg^{-1}) and inulin (15 mg kg^{-1}) was injected into an ear vein, and an infusion of MTX (30 μg kg^{-1} min^{-1}) and inulin (0.5 mg kg^{-1} min^{-1}) was started, which continued throughout the experiment. A second infusion, consisting of 0.9% NaCl solution, was given during the first 80 min. After that time a bolus injection of one of the test drugs was given and the saline infusion was replaced by an infusion of the test drug. The experiment was continued for another 80 or 120 min. The fluid volume of all bolus injections was 0.5 ml kg^{-1}. The solution containing MTX and inulin was infused at a rate of 0.2 ml min^{-1}. All other drugs were infused at a rate of 0.24 ml min^{-1}. All infusions were administered by means of Braun infusion pumps (Braun, Melsungen, FRG). The amounts of the test drugs given by bolus injection or continuous infusion are shown in Table 1, together with the steady-state plasma concentrations achieved.

About 2 ml blood was taken from an ear artery every 10 min. An 8-F Foley balloon catheter was placed in the

Table 1. Infusion rates, steady-state plasma concentrations and clearance data of the nine test drugs

Drug	*N*	ID	IR	SS	Q_{TOT}	Q_{REN}
7-OH-MTX	6	1.8	60	12.3 ± 1.29	–	5.28 ± 0.80
Furosemide	5	0.2	18	2.1 ± 0.29	9.66 ± 1.67	1.52 ± 0.43
Piperacillin	7	12	720	16.3 ± 3.70	45.25 ± 6.49	n.m.
Probenecid	7	12	300	124.6 ± 10.32	3.03 ± 0.55	0.11 ± 0.02
Ceftriaxone	6	7	53	91.4 ± 9.76	0.90 ± 0.19	0.74 ± 0.19
Sulfamethoxazole	6	20	300	113.9 ± 10.45	2.79 ± 0.34	0.08 ± 0.02
Sulfinpyrazone	7	1.5	15	6.0 ± 0.85	2.21 ± 0.28	2.36 ± 0.27
Benzbromarone	7	1	20	8.23 ± 0.70	2.53 ± 0.21	n.m.
Benzarone	6	1	20	n.m.	n.m.	n.m.

N, Number of experiments; ID, initial loading dose (mg kg^{-1}); IR, infusion rate (μg kg^{-1} min^{-1}); SS, steady-state plasma concentration (μg ml^{-1}); Q_{TOT}, total body clearance (ml kg^{-1} min^{-1}); Q_{REN}, renal drug clearance (ml kg^{-1} min^{-1}); n.m., not measured. Values given for drug concentration and clearance are arithmetic means and standard errors ($\bar{x} \pm$ SEM) of measurements recorded during the last 20 min of drug infusion

bladder and the urine was collected at 20-min intervals. At the end of each collection period, the bladder was carefully rinsed with 20 ml saline solution. Blood samples were centrifuged immediately, and plasma and urine were stored at −20° C until analysis.

2. Analytical procedures. Inulin concentrations in plasma and urine were determined with the anthrone reagent [14]. All other drugs were measured by HPLC procedures. The assay methods for MTX, the metabolite 7-OH-MTX, piperacillin and probenecid have been described in detail in two previous papers [17, 18]. Furosemide and sulfinpyrazone were determined by HPLC procedures previously published elsewhere [22, 25].

Ceftriaxone and sulfamethoxazole were quantitated by HPLC using the same procedure as for MTX and 7-OH-MTX. Though neither compound was detected at its UV optimum, the high concentrations in plasma made quantitation at 312 nm possible. Ceftriaxone eluted before MTX and was well separated, while sulfamethoxazole eluted from the column after 7-OH-MTX. Benzbromarone was extracted from 1 ml plasma with 5 ml chloroform-isopropylalcohol (9:1 by vol.). After centrifugation, 4.5 ml of the organic layer was evaporated to dryness and the residue taken up in 0.1 ml of the mobile phase, which was a mixture of methanole, acetonitrile, acetic acid-ethylester, acetic acid and water (72:3:1:1:23 by vol.). A Spherisorb ODS II 5-μm column was used, and the eluate was monitored at 240 nm.

3. Drugs and solutions. The following drugs were used: pentobarbital (Nembutal; Ceva, Bad Segeberg, FRG), methotrexate (Methotrexat-Lederle; Cyanamid-Lederle, Wolfratshausen, FRG), furosemide (Lasix; Hoechst, Frankfurt, FRG), piperacillin (Pipril; Cyanamid-Lederle, Wolfratshausen, FRG), ceftriaxone (Rocephin; Hoffmann-La Roche, Grenzach-Wyhlen, FRG), sulfinpyrazone (Ciba-Geigy, Basel, Switzerland), benzbromarone (Labaz, München, FRG), benzarone (Sanol Schwarz, Monheim, FRG), sulfamethoxazole (Serva, Heidelberg, FRG), probenecid (Serva, Heidelberg, FRG), inulin (Merck, Darmstadt, FRG), and polyethyleneglycol 400 (EGA-Chemie, Weinheim, FRG). 7-OH-MTX was prepared from MTX with enzyme from rabbit liver as described by Johns and Loo [20].

Benzarone and benzbromarone were dissolved in a mixture of 50% polyethyleneglycol 400 and 50% physiological saline solution. All other drugs were dissolved in 0.9% NaCl.

4. Calculations and statistics. For each animal, the mean drug plasma concentration for each 20-min infusion period was calculated from three consecutive measurements. Total drug clearance (Q_{TOT}) and renal drug clearance (Q_{REN}) were then calculated from the infusion rates, the plasma concentrations and the amounts excreted in the urine. To assess drug effects on the different parameters, the values obtained during the last 20 min before drug infusion were compared with the values measured during the last 20 min of test drug infusion. Student's *t*-test for paired data was used, and a P value ≤ 0.05 for the two-tailed test was fixed as the criterion for acceptance of statistical significance.

Results

1. Control values

Steady-state plasma concentrations of MTX, 7-OH-MTX and inulin were established during the first 40 min of MTX infusion, and little further change occurred during the rest of the 80-min control infusion period (Fig. 1). The clearance values that were obtained for the last 20 min before test drug infusion are listed in Table 2 (predrug values). At that time, the total body clearance of MTX varied between 5.64 and 8.92 ml kg^{-1} min^{-1} in the different series of experiments. About 50% of the infused MTX was excreted by the kidneys in unchanged form, and the renal clearance of MTX was usually somewhat greater than the inulin clearance. The latter varied between 2.76 and 4.51 ml kg^{-1} min^{-1} under control conditions and was not significantly influenced by any of the nine test drugs. Between 15% and 30% of the infused MTX was metabolized to 7-OH-MTX, which was excreted in the urine.

In six control experiments, the infusion of NaCl was not replaced by a test drug. No significant changes in the plasma concentrations or clearance values of MTX, 7-OH-MTX or inulin were observed during the second 80-min infusion period.

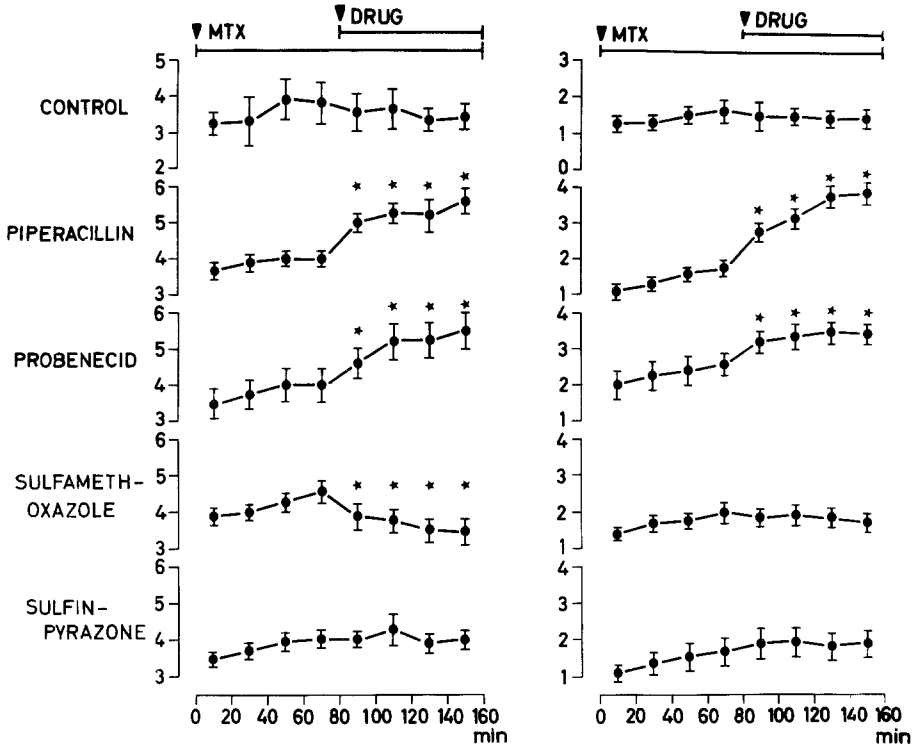

Fig. 1. Plasma concentrations of MTX (left) and 7-OH-MTX (right) during infusion of MTX as influenced by some of the test drugs. Infusion periods for MTX and the test drugs are indicated by the horizontal bars. The *points* indicate means and the *bars*, standard errors (n = 6–7). Drug-induced changes that are statistically significant are marked by *asterisks* ($P \leq 0.05$; t-test for paired data). *Abscissa* time after beginning of MTX infusion (min); *ordinates*, drug concentration (μg ml^{-1})

Table 2. Clearance values and renal excretion data for MTX, 7-OH-MTX and inulin as influenced by the different test drugs

		MTX			7-OH-MTX		Inulin
		Q_{TOT}	Q_{REN}	E_{REN}	Q_{REN}	E_{REN}	Q_{TOT}
Control	PRE	8.89±1.36	5.62±1.25	16.63±2.31	7.23±1.72	8.31±1.35	3.81±0.54
	Δ	+0.30±0.89	−0.43±0.30	−0.33±0.60	−0.70±0.75	−0.95±0.88	−0.21±0.25
7-OH-MTX	PRE	7.07±0.42	4.06±0.39	17.10±0.85	5.85±0.62	10.21±1.28	3.56±0.19
	Δ	−0.25±0.32	−0.23±0.19	−0.50±0.53	−0.56±0.31	+44.97±5.62*	−0.25±0.14
Furosemide	PRE	6.71±0.73	2.94±0.28	13.50±1.45	3.61±0.24	7.34±0.68	2.86±0.16
	Δ	−0.47±0.28	+0.18±0.50	+2.01±1.86	+0.59±0.62	+4.26±1.68	−0.26±0.14
Piperacillin	PRE	7.56±0.37	3.58±0.19	14.29±0.67	5.16±2.28	8.86±0.66	2.99±0.09
	Δ	−2.11±0.44*	−1.20±0.26*	+0.28±0.93	−2.88±0.45*	−0.16±0.95	−0.02±0.08
Probenecid	PRE	7.89±0.87	4.16±0.56	15.67±1.12	2.14±0.36	4.62±0.64	4.51±0.40
	Δ	−2.17±0.70*	−1.10±0.37*	−0.01±1.74	−0.33±0.39	+1.24±1.06	−0.50±0.23
Ceftriaxone	PRE	8.92±0.36	4.53±0.47	15.21±1.44	4.52±0.48	6.32±0.37	3.21±0.17
	Δ	+1.19±0.71	+1.84±0.33*	+3.71±1.36*	+4.84±1.19*	+3.93±1.52*	+0.02±0.08
Sulfamethoxazole	PRE	6.74±0.39	3.20±0.44	14.06±1.50	4.44±0.89	8.12±1.12	2.93±0.13
	Δ	+2.35±0.36*	+1.58±0.25*	+1.57±1.01	+2.99±0.81*	+3.11±0.78*	+0.18±0.19
Sulfinpyrazone	PRE	7.52±0.41	4.28±0.44	17.47±2.14	4.25±0.74	5.97±0.63	3.37±0.21
	Δ	+0.20±0.42	+1.00±0.31*	+3.39±1.35*	+1.32±0.55*	+3.38±1.10*	−0.05±0.07
Benzbromarone	PRE	7.09±0.66	4.19±0.78	17.38±2.10	4.25±0.54	7.16±0.38	3.20±0.19
	Δ	−0.21±0.58	+0.74±0.22*	+2.73±1.08*	+1.38±0.48*	+2.87±1.12*	−0.20±0.14
Benzarone	PRE	5.64±0.40	2.91±0.49	14.96±1.50	2.26±0.37	6.42±0.58	2.76±0.16
	Δ	+1.03±0.51	+0.46±0.28	+0.40±1.86	+0.74±0.35	+0.70±1.04	+0.17±0.08

Q_{TOT}, total body clearance (ml kg^{-1} min^{-1}); Q_{REN}, renal drug clearance (ml kg^{-1} min^{-1}); E_{REN}, renal drug elimination (μg kg^{-1} min^{-1}). Means ± SEM (n = 5–7) of values recorded during the last 20 min of the predrug infusion period (PRE) are given for all parameters, together with the mean changes ($\Delta\bar{x} \pm$ SEM) over the last 20 min of test drug infusion. Asterisks indicate changes that are statistically significant ($P \leq 0.05$; t-test for paired data)

2. Drugs without influence on MTX clearance

The infusion of 7-OH-MTX (60 μg kg^{-1} min^{-1}; Table 1) increased the steady-state plasma concentration of the metabolite from 1.76 ± 0.13 to 12.3 ± 0.15 μg ml^{-1}. The amount of 7-OH-MTX excreted by the kidneys rose proportionally, and thus there was no significant change in the renal 7-OH-MTX clearance. Similarly, the steady-state concentration and clearance values of MTX were not affected (Table 2).

Infusion of furosemide had a strong diuretic effect. The mean urine volume rose from 4.5 ml to 20 ml for a 20-min collection period. However, the amount of MTX or 7-OH-MTX excreted was not significantly increased, and the plasma concentrations and clearance values of both substances remained unchanged (Table 2).

The third drug without influence on MTX clearance was benzarone, which is believed to be an active metabolite of the uricosuric drug benzbromarone (Table 2).

3. Drugs decreasing MTX clearance

Immediately after the start of a piperacillin infusion (720 μg kg^{-1} min^{-1}; Table 1), the plasma concentrations of MTX and 7-OH-MTX began to rise. The steady-state concentrations increased from 4.03 ± 0.20 to 5.64 ± 0.34 μg ml^{-1} in the case of MTX and from 1.77 ± 0.20 to 3.84 ± 0.29 μg ml^{-1} for 7-OH-MTX (Fig. 1). The total clearance of MTX was decreased, and the renal clearance of MTX and 7-OH-MTX was depressed below the inulin clearance (Table 2), while the total amount of MTX and 7-OH-MTX excreted in the urine per minute remained unchanged.

A similar effect was obtained with probenecid. During probenecid infusion the plasma concentration of MTX increased from 4.05 ± 0.46 to 5.52 ± 0.52 μg ml^{-1} (Fig. 1) and there was a simultaneous decrease in the total and renal MTX clearance (Table 2).

4. Drugs increasing MTX clearance

An infusion of sulfamethoxazole decreased the MTX plasma concentration from 4.54 ± 0.31 to 3.45 ± 0.38 μg ml^{-1}, but did not change the 7-OH-MTX plasma concentration (Fig. 1). The total and renal clearances of MTX were increased, as were the renal clearance of 7-OH-MTX and the amount of 7-OH-MTX excreted per minute (Table 2). The drug effect developed quickly after the beginning of the infusion.

The remaining three drugs, the antibiotic ceftriaxone and the two uricosuric compounds sulfinpyrazone (Fig. 1) and benzbromarone, had no influence on the plasma concentrations of either MTX or 7-OH-MTX. However, all three drugs increased the renal clearance of MTX and 7-OH-MTX, and also the amount of MTX and 7-OH-MTX excreted in the urine (Table 2). With ceftriaxone and sulfinpyrazone, this effect became apparent soon after the onset of infusion. With brenzbromarone, on the other hand, it was 60–80 min before an increase of the renal MTX or 7-OH-MTX elimination was seen. Infusions of benzbromarone were therefore continued for 120 min, instead of 80 min, to establish a steady-state drug effect.

Discussion

In the present experiments the total MTX clearance ranged from 5.64 to 8.89 ml kg^{-1} min^{-1}. Fifty percent of the infused dose was excreted renally as MTX and an additional 15%–30%, as 7-OH-MTX. Similar excretion data have been reported in two earlier studies [17, 18] in awake and anesthetized rabbits. Steady-state clearance values for MTX, 7-OH-MTX and inulin changed little during the time-course of a given control experiment. All predrug clearance values, however, decreased by 20%–40% during the four consecutive experiments performed on any individual animal. This decrease explains, for the greater part, the considerable variation of the predrug clearance values in the different groups of experiments shown in Table 2. As the animals gained up to 60% body weight in the 4–6 months between the first and the last experiment, the product of body weight (kg) and clearance (ml kg^{-1} min^{-1}) remained nearly constant. We conclude, therefore, that repeated infusions of MTX at appropriate time intervals have no significant influence on the glomerular filtration rate or the total or renal clearance of MTX and 7-OH-MTX.

Before testdrug infusion, the renal clearance of MTX and 7-OH-MTX was usually greater than the inulin clearance, indicating that the two compounds were actively secreted into the tubular lumen. Probenecid and piperacillin decreased the renal clearance of MTX and its metabolite to values below the inulin clearance. A similar effect of probenecid on renal MTX elimination has frequently been described before [2, 8, 9, 15, 24]. Probenecid and piperacillin are both weak organic acids that are themselves secreted into the proximal tubulus. It seems plausible, therefore, to suggest that the two compounds compete for blocking the secretion of MTX and 7-OH-MTX by the same saturable transport mechanism. Such a block could, at least partly, explain the delayed elimination of MTX and its metabolite during simultaneous administration of probenecid or piperacillin in awake rabbits [17]. However, in the present experiments the reduction in total MTX clearance induced by probenecid or piperacillin was greater than the reduction in renal clearance. Thus, additional drug effects cannot be excluded. Paxton [29] has suggested that acidic drugs decrease the plasma protein-binding of MTX, thereby increasing its volume of distribution, an effect that could lead to delayed elimination. We do not think that this is a likely explanation in our case, because a decreased plasma-protein binding would not lead to an increase in the steady-state concentration of MTX. Inhibition of the biliary secretion of MTX and 7-OH-MTX [21] may be an additional effect of probenecid and piperacillin, though the importance of this elimination pathway for MTX in the rabbit is a matter of debate [7].

In tumor patients receiving high-dose MTX therapy, 7-OH-MTX plasma concentrations can exceed MTX plasma concentrations by a factor of 10–40 during the terminal elimination phase [5, 12, 23]. As 7-OH-MTX is secreted by the same transport system as the parent compound, some authors have suggested that the metabolite might increase the toxicity of MTX by delaying its renal elimination. Our results show that a 7-fold increase in the 7-OH-MTX plasma concentration does not influence MTX clearance. This confirms earlier observations [18] that the renal clearance of MTX and 7-OH-MTX remains constant up to plasma concentrations of 40 μg ml^{-1} for MTX and 13 μg ml^{-1} for 7-OH-MTX. Obviously, the transport capacity of the acid excretion system is quite large, and thus a competitive antagonism between MTX and its metabo-

lite at this transport site may not be therapeutically relevant. Furosemide, another acidic drug that is actively secreted in the kidney, did not reduce the elimination of MTX either. This coincides with observations in patients [27] and it is important to note it, because the diuretic is routinely used to increase the urine volume during high-dose MTX therapy. It seems likely that the low plasma concentrations of furosemide that are diuretically effective are not sufficient to block the transport capacity of the excretion mechanism. While furosemide does not accelerate MTX elimination, its diluting effect could prevent the precipitation of crystals of the poorly water-soluble 7-OH-MTX in the kidney and thus help to avoid nephrotoxicity in high-dose MTX therapy.

Four of the drugs, namely ceftriaxone, sulfamethoxazole, sulfinpyrazone and benzbromarone, increased the renal excretion of MTX and 7-OH-MTX. To the best of our knowledge, an increase of the MTX clearance by other drugs has not previously been reported. Sulfinpyrazone and benzbromarone are uricosuric drugs that block the tubular reabsorption of uric acid. For MTX, tubular reabsorption has been demonstrated in dogs [15], rabbits [8, 9] and man [24]. In our experiments, probenecid and piperacillin depressed the renal clearance of MTX and 7-OH-MTX below the inulin clearance (see above). This indicates that tubular reabsorption of both compounds occurs under our experimental conditions. We suggest, therefore, that the uricosuric drugs and also ceftriaxone and sulfamethoxazole block the reuptake of MTX and its metabolite from the renal tubulus. The increased renal elimination did not always lead to an increase in total MTX clearance. Apparently, the effect can partly be compensated by a decrease of the extrarenal loss of MTX.

With benzbromarone, the increase in renal MTX clearance took about 1 h to develop. The uricosuric effect of benzbromarone in patients appears with a comparable latency [32] and seems to be caused, at least in part, by an active metabolite. This metabolite is usually thought to be benzarone [10, 13]. In our experiments, however, benzarone was ineffective. Thus, since De Vries et al. [11] were unable to detect benzarone in human plasma during benzbromarone therapy, our results cause us to question whether the identification of benzarone as the major pharmacologically effective metabolite of benzbromarone is correct.

In summary, our results have confirmed earlier observations that probenecid and piperacillin decrease the renal elimination of MTX and 7-OH-MTX by blocking the tubular secretion of the latter compounds. Uricosuric drugs and the antibiotics cetriaxone and sulfamethoxazole increase the renal clearance of MTX and 7-OH-MTX, probably by preventing their reabsorption from the tubulus lumen. These pharmacokinetic drug interactions occur at therapeutically relevant plasma concentrations (Table 1) and thus may become clinically important during high-dose MTX tumor therapy.

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