Cancer Chemother Pharmacol (1990) 26: 139–143

Cancer Chemotherapy and Pharmacology

Cephalosporins increase the renal clearance of methotrexate and 7-hydroxymethotrexate in rabbits

Heiko Iven and Helmut Brasch

Department of Pharmacology, Medical University of Lübeck, Ratzeburger Allee 160, D-2400 Lübeck, Federal Republic of Germany

Received 4 April 1989/Accepted 30 October 1989

Summary. Anaesthetized rabbits were infused with methotrexate (MTX; 30 μg × kg^{-1} × min^{-1}) for 4 h. Constant plasma concentrations of MTX and its main metabolite 7-hydroxymethotrexate (7-OH-MTX) were achieved 40–60 min after the start of the infusion. In all, 50% of the infused MTX was eliminated by the kidney; another 15%–30% was hydroxylated and excreted as 7-OH-MTX in the urine. A concomitant infusion of penicillin G (3.96 mg × kg^{-1} × min^{-1}) decreased the renal clearance of MTX and 7-OH-MTX, probably by competitive antagonism at the common tubular secretion site. In contrast, the four cephalosporins ceftriaxone, ceftazidime, ceftizoxime and cefoperazone all increased the renal clearance of MTX and 7-OH-MTX. At similar plasma concentrations, ceftriaxone and ceftazidime were almost equipotent, ceftizoxime was less effective and cefoperazone seemed to have a biphasic effect, depressing the clearance of MTX and 7-OH-MTX at higher drug concentrations. The effects are best explained by an inhibition of the tubular reabsorption of the cytostatic and its metabolite. The results suggest that cephalosporins are a better choice than penicillin for antibiotic treatment during MTX therapy.

Introduction

Renal elimination accounts for about 50% of the total body clearance of methotrexate (MTX) in rabbits and man [3, 4, 12]. Another 10%–30% of the infused MTX dose is hydroxylated to 7-hydroxymethotrexate (7-OH-MTX), which is also eliminated by the kidney [12, 20, 23]. The renal excretion of both compounds is controlled by glomerular filtration, tubular secretion and tubular reabsorption [5, 14, 25]. Acidic drugs such as probenecid [2, 12] or penicillin [26] or nonsteroidal antiinflammatory drugs [18], which are actively secreted themselves, are known to delay the elimination of MTX. Several case reports [1, 6, 15, 19] suggest that these drug interactions may cause toxic side effects in patients on MTX therapy.

In a recent paper [11], we have shown that the uricosuric drugs sulfinpyrazone and benzbromarone increase the renal clearance of MTX and 7-OH-MTX; this was explained by a reduction in tubular reabsorption. A similar effect was observed with the cephalosporin antibiotic ceftriaxone. This might be a clinically relevant interaction, because cytostatic therapy often requires the additional prescription of antibiotics. Therefore, we investigated whether other cephalosporins that differ from ceftriaxone in half-life, plasma protein binding and main pathway of elimination would also increase the renal clearance of MTX and its metabolite.

Materials and methods

Experimental procedure. A total of 28 male rabbits weighing 3.6–6.3 kg at the start of the experiments were used in the study. They had free access to tap water and were fed Altromin standard diet, which was withdrawn 16 h before the beginning of an experiment. Up to three experiments per animal were carried out with different drugs at 4-week intervals.

The rabbits were anaesthetized with an initial dose of 30 mg × kg^{-1} i.v. pentobarbital and placed on a heated operation table. The rectal temperature was monitored with a digital thermometer (Bosch, Stuttgart, FRG) and was kept nearly constant throughout the experiment. Anaesthesia 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, followed by an infusion of MTX (30 μg × kg^{-1} × min^{-1}) and inulin (0.5 mg × kg^{-1}) lasting 240 min. Thus, the total dose given during an experiment was 7.9 mg × kg^{-1} for MTX and 135 mg × kg^{-1} for inulin. A second infusion, consisting of a 0.9% NaCl solution, was given during the first 80 min, after which a bolus injection of one of the test drugs was given and the saline infusion was replaced by an infusion (I) of the test drug. After a further 80 min, a second bolus injection of the same test drug was given and the amount infused per minute was increased 3-fold (II). The experiment was continued for another 80 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 carried out using Braun infusion pumps

Offprint requests to: H. Iven

Table 1. Loading doses, infusion rates, steady-state plasma concentrations and clearance values of the five antibiotics used. In all experiments MTX was infused at a rate of 30 μg × kg^{-1} × min^{-1} together with the respective antibiotic

Drug		ID	IR	SS	Q_{tot}	Q_{ren}
Penicillin G:	I	6.8	1,320	51.56 ± 3.64	26.38 ± 1.91	21.57 ± 0.77
	II	13.6	3,960	325.90 ± 52.38	13.69 ± 1.47*	8.45 ± 1.02*
Ceftriaxone:	I	7	53	83.08 ± 12.92	0.72 ± 0.09	0.40 ± 0.10
	II	14	159	209.76 ± 29.76	0.85 ± 0.12	0.75 ± 0.14*
Ceftazidime:	I	12.8	420	96.54 ± 8.44	4.79 ± 0.36	4.25 ± 0.26
	II	25.6	1,260	279.70 ± 21.61	4.88 ± 0.39	4.90 ± 0.41
Ceftizoxime:	I	11.8	460	71.18 ± 8.43	6.87 ± 0.73	5.44 ± 0.90
	II	23.6	1,380	210.57 ± 30.79	7.34 ± 1.14	5.55 ± 0.63
Cefoperazone	I	9.5	380	85.51 ± 3.20	4.48 ± 0.17	1.67 ± 0.21
	II	19	1,140	249.62 ± 10.48	4.62 ± 0.21	1.77 ± 0.29

Values shown for drug concentration and clearance represent the means ± SEM ($n = 7$) recorded during the last 20 min of each drug infusion period. 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})

* Clearance values for the second infusion period, which differed significantly from the corresponding values for the first period ($P \leq 0.05$; t-test for paired data)

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

Drug		MTX:			7-OH-MTX:		Inulin
		Q_{tot}	Q_{ren}	E_{ren}	Q_{ren}	E_{ren}	Q_{tot}
Control:	PRE	7.40 ± 0.42	3.67 ± 0.46	14.96 ± 0.70	4.36 ± 0.74	5.40 ± 0.37	4.07 ± 0.29
	Δ I	0.38 ± 0.80	1.04 ± 0.75	2.74 ± 1.38	0.53 ± 0.72	1.54 ± 0.78	−0.39 ± 0.18
	Δ II	1.28 ± 1.13	1.15 ± 0.67	0.24 ± 1.12	0.94 ± 0.62	1.14 ± 0.77	−0.52 ± 0.26
Penicillin G:	PRE	7.75 ± 0.56	4.10 ± 0.44	15.07 ± 0.55	5.08 ± 0.68	6.66 ± 0.28	4.77 ± 0.14
	Δ I	0.24 ± 0.62	0.15 ± 0.37	0.49 ± 0.51	−1.24 ± 0.66	1.69 ± 0.17*	−0.19 ± 0.18
	Δ II	−2.21 ± 0.45*	−1.49 ± 0.33*	−1.23 ± 0.89	−1.30 ± 0.64	0.71 ± 0.79	−0.43 ± 0.22
Ceftriaxone:	PRE	8.34 ± 0.58	4.54 ± 0.39	16.38 ± 0.97	3.59 ± 0.44	5.85 ± 0.57	4.89 ± 0.08
	Δ I	2.05 ± 0.42*	1.14 ± 0.27*	1.09 ± 0.46	1.72 ± 0.26*	1.85 ± 0.39*	0.06 ± 0.23
	Δ II	2.96 ± 0.98*	3.30 ± 0.86*	3.23 ± 1.35	3.36 ± 0.99*	2.56 ± 0.82*	0.02 ± 0.26
Ceftazidime:	PRE	8.40 ± 0.47	3.11 ± 0.21	14.25 ± 0.61	4.04 ± 0.82	4.93 ± 0.51	4.44 ± 0.38
	Δ I	0.88 ± 0.24*	0.84 ± 0.19*	1.57 ± 0.65	3.12 ± 1.24*	2.06 ± 0.84	−0.07 ± 0.15
	Δ II	1.17 ± 0.42*	1.61 ± 0.39*	3.80 ± 0.60*	5.13 ± 1.17*	2.52 ± 0.55*	0.07 ± 0.27
Ceftizoxime:	PRE	6.15 ± 0.48	2.68 ± 0.34	12.53 ± 1.13	3.78 ± 0.31	4.67 ± 0.51	4.28 ± 0.21
	Δ I	1.11 ± 0.55	0.66 ± 0.38	0.69 ± 0.86	1.45 ± 0.92	2.59 ± 0.43*	−0.07 ± 0.36
	Δ II	1.49 ± 0.58*	0.82 ± 0.24*	1.24 ± 1.13	2.59 ± 0.77*	3.42 ± 0.72*	0.01 ± 0.34
Cefoperazone:	PRE	8.07 ± 0.53	4.35 ± 0.46	16.09 ± 0.88	5.58 ± 0.50	9.40 ± 0.43	4.45 ± 0.25
	Δ I	1.71 ± 0.58*	0.87 ± 0.34*	−0.46 ± 0.67	3.21 ± 1.28*	1.72 ± 0.32*	−0.41 ± 0.23
	Δ II	0.15 ± 0.63	−0.02 ± 0.37	−0.76 ± 0.29*	0.84 ± 0.70	0.58 ± 0.40	−0.49 ± 0.20

Means ± SEM of values recorded during the last 20 min of the predrug infusion period (PRE) are given for all parameters, together with the means ± SEM of changes obtained during the last 20 min of the first (Δ I) and second (Δ II) drug infusion period. E_{ren}, renal drug elimination (μg × kg^{-1} × min^{-1}); all other parameters, as shown in Table 1

* Statistically significant changes ($P \leq 0.05$; t-test for paired data)

(Braun, Melsungen, FRG). The amounts of 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 bladder and 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.

Analytical procedures. Inulin concentrations in plasma and urine were determined with the anthrone reagent [8]. All other drugs were measured by HPLC. In most cases, 100 μl 0.08 *N* acetic acid was added to 200 μl plasma and the mixture was heated to 95° C for 10 min and subsequently centrifuged. A 50-μl aliquot of the supernatant was injected onto the column. For the analysis of cefoperazone only, 100 μl plasma was mixed with 200 μl methanol and centrifuged, and 100 μl supernatant was mixed with 150 μl 0.02 mol/l KH_2PO_4 buffer (pH 4.4) before injection. Urine samples were diluted 1/10 with double-distilled water before injection.

A Lichrospher 100 RP-18 column was used for the analysis of all drugs. In most cases, 0.01 mol/l KH_2PO_4 buffer (pH 6.8) containing 10%–20% methanol was used as the mobile phase (flow rate, 1 ml × min^{-1}), but for the analysis of cefoperazone, 0.02 mol/l KH_2PO_4 buffer (pH 4.4) with 30% methanol was found to be appropriate. The UV

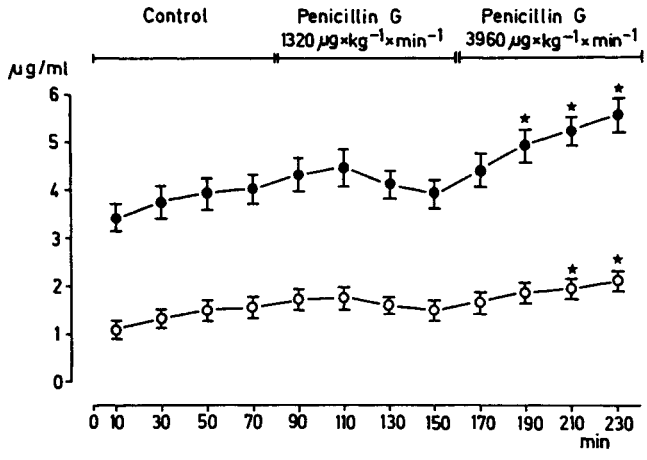

Fig. 1. Plasma concentration of MTX (●) and 7-OH-MTX (○) during infusion of MTX, as influenced by penicillin G. The durations of the control period and the two infusion periods with penicillin G are marked by the *horizontal bars on top*. The symbols indicate the means ± SEM (n =7). Drug-induced changes that are statistically significant are marked by asterisks ($P \leq 0.05$; t-test for paired data). *Abscissa*, time after beginning of MTX infusion; *ordinate*, drug concentration

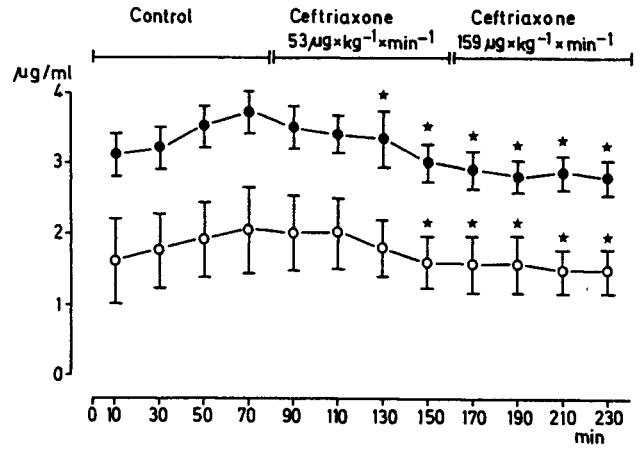

Fig. 2. Plasma concentration of MTX (●) and 7-OH-MTX (○) during infusion of MTX, as influenced by ceftriaxone. The durations of the control period and the two infusion periods with ceftriaxone are marked by the *horizontal bars on top*. The symbols indicate the means ± SEM (n = 7). Drug-induced changes that are statistically significant are marked by asterisks ($P \leq 0.05$; t-test for paired data). *Abscissa*, time after beginning of MTX infusion; *ordinate*, drug concentration

absorbance was monitored at 220 nm for penicillin G, 254 nm for cefoperazone, 308 nm for ceftizoxime and 312 nm for all other substances.

Drugs and solutions. The following drugs were used: pentobarbital (Nembutal; Ceva, Bad Segeberg, FRG), methotrexate (Methotrexat-Lederle; Cyanamid-Lederle, Wolfratshausen, FRG); inulin (Merck, Darmstadt, FRG), penicillin G (Grünenthal, Stolberg, FRG), ceftriaxone (Rocephin; Hoffmann-La Roche, Grenzach-Wyhlen, FRG), cefoperazone (Cefobis; Pfizer, Karlsruhe, FRG), ceftazidime (Fortum; Cascan, Wiesbaden, FRG) and ceftizoxime (Ceftix; Boehringer Mannheim, Mannheim, FRG). All drugs were dissolved in 0.9% NaCl.

Calculations and statistics. For each animal, the mean drug plasma concentration for a 20-min infusion period was calculated from three consecutive measurements. Total (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 test-drug infusion were compared with values measured during the last 20 min of each test-drug infusion period. Student's t-test for paired data was used, and a P value of ≤ 0.05 for the two-tailed test was fixed as the criterion for acceptance of statistical significance.

Results

During the 80-min control period, MTX and 7-OH-MTX plasma concentrations became constant. The clearance values and renal elimination data for the last 20 min before the beginning of the test-drug infusions are listed in Table 2. The total MTX clearance ranged between 6.15 and 8.4 ml × kg^{-1} × min^{-1} in the six groups of animals. About 50% of the infused dose was excreted unchanged by the kidneys; a further 15%–30% was hydroxylated to 7-OH-MTX and appeared in the urine. In most cases, renal 7-OH-MTX clearance was somewhat greater (3.59–5.58 ml × kg^{-1} × min^{-1}) than renal MTX clearance (2.58–4.54 ml × kg^{-1} × min^{-1}). In the control experiments, with 0.9% NaCl infused throughout, there was little change in these values over the rest of the experiment (Table 2). The inulin clearance, which ranged between 4.07 and 4.89 ml × kg^{-1} × min^{-1} at the end of the control period, showed little variation thereafter and was not significantly modified by any of the five different antibiotics.

Penicillin G was excreted mainly by the kidneys. At the lower dose, 81.7% of this antibiotic appeared in the urine, and the renal clearance of 21.57 ml × kg^{-1} × min^{-1} indicated tubular secretion (Table 1). Only 61.7% of the dose was excreted in the urine during infusion of the larger dose. The renal drug clearance was drastically reduced, which caused a similar decrease in the total drug clearance. During the second period of the penicillin infusion, the plasma concentrations of MTX and 7-OH-MTX increased significantly (Fig. 1) and the total and renal MTX clearance were reduced (Table 2).

The four cephalosporins increased the elimination of MTX and its metabolite. The influence of ceftriaxzone on the plasma concentrations of both compounds is shown in Fig. 2. A dose-dependent increase in both total and renal clearance is apparent from Table 2. Ceftriaxone itself had a very low total body clearance (Table 1); about 55% of the dose appeared in the urine during infusion of the lower dose. The renal clearance increased significantly when the infusion rate was tripled, and the renal excretion rose to nearly 88% of the infused dose (Table 1).

Ceftazidime was eliminated almost exclusively by the kidneys (Table 1), and its influence on the clearance of MTX and 7-OH-MTX was comparable with the effect of ceftriaxone (Table 2). Ceftizoxime also increased the total and renal clearance of the cytostatic and its metabolite, but its effect seemed to be somewhat smaller than that obtained with the two aforementioned cephalosporins and became statistically significant only at the higher infusion rate (Table 2). Ceftizoxime itself was eliminated by the kidneys at 76%–79% of the dose, and its renal clearance was greater than the inulin clearance (Table 1). Only 37%–38%

of the infused dose of cefoperazone appeared in the urine. This drug seems to have a biphasic effect on MTX and 7-OH-MTX elimination. A significant increase in all clearance values was seen at the lower infusion rate (Table 2), but the effect was not augmented by the larger drug dose; instead, a decrease back to the predrug values was observed.

Discussion

The clearance data for MTX, 7-OH-MTX and inulin that were obtained during the control period in the present experiments are in line with the results of previous investigations [11, 12]. Due to simultaneous tubular secretion and reabsorption, renal MTX clearance was not greater than inulin clearance. The renal clearance of 7-OH-MTX was greater than renal MTX clearance and, in most cases, also exceeded the inulin clearance. We suggest that this indicates that the metabolite has a lower affinity for the reabsorption mechanism than does the parent drug.

The depression of the renal clearance of MTX and its metabolite by penicillin G was most likely caused by an inhibition of tubular secretion, which has previously been observed in monkeys [26]. Penicillin G itself is actively secreted, as can be seen from its high renal clearance. A reduction in MTX clearance was seen at penicillin G plasma concentrations that saturate the carrier mechanism. This can be concluded from the drop in renal penicillin G clearance after the increase in the penicillin G infusion rate. Piperacillin causes a similar decrease in renal MTX and 7-OH-MTX secretion, but its effective concentrations are lower than those for penicillin G [10, 11].

Although cephalosporins, like the penicillins, are β-lactam antibiotics, tubular secretion is not believed to play an important role in their renal elimination [17]. Therefore, it is understandable that these drugs do not decrease the renal elimination of MTX or 7-OH-MTX; instead, they seem to inhibit the tubular reabsorption of both compounds, an effect comparable with that of some uricosuric drugs [11]. In our opinion, this is the best possible explanation for the observed increase in renal MTX and 7-OH-MTX clearance. The latter was increased to a greater extent (Table 2); this suggests that due to a lower affinity of 7-OH-MTX for the carrier mechanism (see above), the tubular reabsorption of the metabolite can be more easily inhibited than that of the parent compound.

Ceftriaxone is extensively bound to plasma proteins; this binding is saturable, and the free fraction increases with increasing drug plasma concentration [24]. As only the free fraction can be filtered in the kidney, the reduced protein binding could explain the increase in renal ceftriaxone clearance with increasing infusion rate. Alternatively, the observed increase might be caused by a saturation of the tubular reabsorption of ceftriaxone. MTX is also bound to plasma proteins, albeit to a lesser extent [21], and some preliminary results (our unpublished observations) suggest that ceftriaxone can competitively reduce MTX protein binding.

We do not believe, however, that an increase in the free fraction of MTX contributes much to the increased renal elimination of this drug. First, Paxton [22] has argued that a reduction in MTX protein binding would decrease rather than increase the renal clearance, due to an increased distribution of the drug to peripheral tissues. Second, the protein binding of ceftazidime is negligible [9], but the drug was equipotent to ceftriaxone in its influence on MTX clearance. Ceftizoxime is also not bound to plasma proteins [7], but its renal elimination can be delayed by probenecid [17]. This indicates tubular secretion, and, indeed, in our experiments renal ceftizoxime clearance was slightly higher than the inulin clearance. Compared with that of penicillin G, however, the difference was very small. A certain degree of inhibition of tubular secretion that partly compensates the inhibition of reabsorption could therefore be responsible for the slightly smaller overall effect of ceftizoxime vs the two other cephalosporins on renal MTX clearance.

In contrast to the drugs discussed thus far, cefoperazone is mainly secreted into the bile [16]; renal elimination accounts for only about 40% of its total clearance. It is therefore understandable that its influence on renal MTX and 7-OH-MTX clearance is only slight. However, no ready explanation can be offered for the biphasic nature of its effect. As cefoperazone is not actively secreted [16], it is unlikely to inhibit tubular MTX excretion.

With most drugs, the shift in total MTX clearance was greater than that in renal MTX clearance. As probenecid decreases the biliary secretion of MTX in rats [13], a similar mechanism could contribute to the effect of penicillin G. For the cephalosporins, no influence on extrarenal MTX transport mechanisms has thus far been described; however, based on our data, we cannot rule out such unknown drug effects.

In conclusion, the present experiments show that penicillin G decreases renal MTX and 7-OH-MTX clearance, whereas cephalosporins have the opposite effect. Therefore, cephalosporins may be the better choice for antibiotic therapy in combination with MTX administration.

Acknowledgements. The authors wish to thank Miss V. Naß for her skillful technical assistance, Mrs. M. L. Stolte for the preparation of the figures and Mrs. G. Mundhenke for typing the manuscript.

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