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SHORT COMMUNICATION

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Influence of piperacillin on the pharmacokinetics of methotrexate and 7-hydroxymethotrexate

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Abstract The influence of concomitant administration of piperacillin (PIP) on the pharmacokinetic parameters of methotrexate (MTX) and 7-hydroxymethotrexate (7-OH-MTX) was studied in rabbits. Six rabbits received an initial i.v. bolus (0.21 mg kg^{-1}) followed by a constant-rate i.v. infusion of the drug (5 μg min^{-1} kg^{-1}) for 240 min. The PIP dose (30 mg kg^{-1}) was repeated every 30 min until the end of the infusion period. The control group consisted of four rabbits treated the same way except for the addition of PIP. There were significant increases in the mean residence times found for MTX (MRT_{inf}) and 7-OH-MTX ($MRT_{m,inf}$) following PIP administration. Concomitant administration of PIP with MTX also produced significant 1.5- and 2.8-fold increases in the area under the curve of MTX and 7-OH-MTX, respectively. The total body clearance of MTX and the operative total body clearance of 7-OH-MTX significantly decreased, but in a less than proportional manner. The study demonstrates that the interaction between MTX and PIP is mainly due to the reduced clearance of both MTX and 7-OH-MTX combined with a slight increase in the formation clearance of the metabolite.

Key words Methotrexate · 7-Hydroxymethotrexate · Piperacillin · Pharmacokinetic parameters · Interaction

Introduction

Previous reports have suggested that piperacillin (PIP) may interact with methotrexate (MTX) and its major metabolite 7-hydroxymethotrexate (7-OH-MTX) [1–3]. Infusions of PIP in the anesthetized rabbit decreased the renal clearance of MTX and 7-OH-MTX, mainly by reducing the tubular secretion of both compounds [1]. This pharmacokinetic drug interaction was observed with therapeutic concentrations of MTX and may thus be considered of possible clinical significance. It has also been found that the administration of PIP at 10 min before and 4 h after MTX infusion increases the plasma concentrations of MTX and 7-OH-MTX at 40 min to 6 h after termination of the MTX infusion [2]. The investigators explained the interaction as the result of a reduction in MTX total body clearance in association with no change in the elimination half-life or the distribution to the peripheral body compartments. The renal elimination of MTX via the tubular transport mechanism for organic acids was also reduced. Despite the lack of consistent pharmacokinetic data, it is evident that serious complications can occur as a result of the use of either low-dose MTX or the antibiotic PIP [4–6].

This study was conducted to determine the effect of the coadministration of PIP on the pharmacokinetic parameters of MTX and its major metabolite 7-OH-MTX using statistical moment analysis in the effort to clarify the potential interaction between the two drugs and the mechanism involved.

Materials and methods

Animals and materials

Male New Zealand rabbits weighing from 3.30 to 5.23 kg were used. The marginal ear veins on both sides were cannulated with an i.v. catheter (Terumo, 24 × 3/4-inch, inside diameter 0.47 × 19 mm) for i.v. infusion of MTX in one ear and i.v. administration of PIP in the other one. For blood sampling, a third i.v. catheter was inserted in the central ear artery opposite the one used for MTX. MTX (1.2 mg ml^{-1}) was prepared in 5% dextrose solution containing 0.2% sodium bicarbonate and was infused in rabbits at a rate of 5 μg $kg^{-1}min^{-1}$. The volume of MTX solution infused was 36 ml. An Oximetrix Infusion pump Model 3 (Oximetrix, Inc., Mountain View, Calif. USA) was used for the MTX infusion study. The duration of infusion was 240 min. A calculated bolus dose of 0.21 mg kg^{-1} was injected before the start of the infusion. This dose

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represents the required MTX concentration multiplied by the volume of distribution at steady state. The PIP (Pipril, 2 g; Lederle Pharmaceuticals, Division of cyanamide GmbM, Germany) was reconstituted by the addition of 20 ml of water for injection (provided in the package) and was given i.v. using a second i.v. catheter after the first 60 min of MTX infusion. The PIP dose (30 mg kg^{-1}) was repeated every 30 min until the end of the experimental period.

Blood samples (2 ml) were collected every 10 min before and every 30 min after the PIP administration during the infusion. Additional postinfusion samples were collected at different intervals (245, 250, 260, 270, 300, and 360 min) up to the end of the study.

Assay of MTX and 7-OH-MTX

Concentrations of MTX and 7-OH-MTX in plasma were analyzed by a high-performance liquid chromatography (HPLC) method developed in our laboratory [7]. The metabolite 7-OH-MTX was isolated from the urine of rabbits according to the method of Redetzki et al. [8]. Male New Zealand white rabbits were placed in different metabolic cages. A MTX dose of 200 mg kg^{-1} was injected i.m.. Urine was collected for up to 26 h.

Pharmacokinetic analysis

The area under the curve from time zero to time t (AUC_{0-t}) was determined by the linear trapezoidal method with extrapolation to infinity by division of the last measurable plasma concentration by the absolute value of the terminal slope to produce the $AUC_{0-\infty}$. The areas under the curve of the first moment of the MTX plasma concentration-time curve from zero to the last measurable plasma concentration ($AUMC_{o-t}$) and from zero to infinity ($AUMC_{0-\infty}$) were calculated by the area under the curve of a plot of the product of concentration and time versus time. Similar techniques were applied to determine the $AUC_{m(o-t)}$ and $AUC_{(0-\infty)}$ from the 7-OH-MTX plasma concentration-time curve and the $AUMC_{m(0-t)}$ and $AUMC_{m(0-\infty)}$ from a plot of the product of 7-OH-MTX plasma concentration and time versus time. The i.v. mean residence time ($MRT_{i.v.}$) was calculated from the reciprocal of the absolute value of the terminal slope. The mean residence time of the infusion (MRT_{inf}) was calculated as:

$$MRT_{inf} = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}. \quad (1)$$

The mean residence time of 7-OH-MTX in the body was calculated using the following relationship [9–11]:

$$MRT_{m,inf} = \frac{AUMC_{m(0-\infty)}}{AUC_{m(0-\infty)}} - \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}. \quad (2)$$

The apparent volume of distribution at steady state $V_{d_{ss}}$ was determined according to Perrier and Mayersohn [12]. The total body clearance of the drug was determined from the quotient of the total dose (bolus + infusion) and $AUC_{0-\infty}$.

As the drug and metabolite levels reached steady state, the rate of the metabolite formation is equal to its rate of elimination:

$$CL_f.C_{ss} = CL_m.C_{ss,m}, \quad (3)$$

Where CL_f is the formation clearance of the metabolite, CL_m is the total clearance of the metabolite, and C_{ss} and $C_{ss,m}$ are the drug and metabolite plasma concentrations, respectively. Consequently, the ratio of the metabolite concentration to the drug concentration represents the ratio of the formation clearance to the total clearance of the metabolite. This ratio can also be expressed in terms of the area under the curve as follows:

$$\frac{CL_f}{CL_m} = \frac{AUC_{m,(0-\infty)}}{AUC_{0-\infty}}. \quad (4)$$

The total operative clearance of the metabolite (CL_m/f_m) was calculated from the quotient of the total dose and $AUC_{m(0-\infty)}$, where f_m is the fraction of the parent drug metabolized.

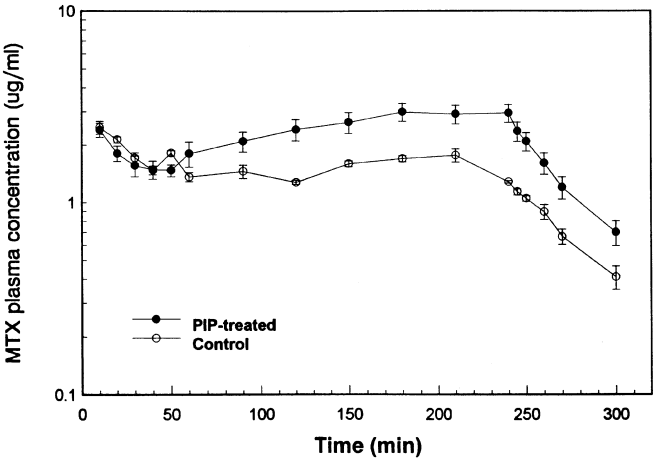

Fig. 1 Plasma concentration of MTX (mean ± SEM) measured after the administration of an i.v. bolus dose of 0.21 mg kg^{-1} followed by a constant-rate i.v. infusion of 5 μg kg^{-1} min^{-1} for 240 min with (*black circles*) or without (*white circles*) the addition of PIP (30 mg kg^{-1}) every 30 min starting at 60 min

Statistical analyses

Comparisons of pharmacokinetic parameters between PIP-treated and control groups were carried out using student's *t*-test for independent samples, assuming a homoscedastic or heteroscedastic model. The Wilcoxon rank-sum test/Mann-Whitney *U*-test were also used if the distribution of any pharmacokinetic parameter did not follow Gaussian distribution. The homogeneity of variance of the two groups was checked by Bartlett's test. The statistical level of significance was taken 0.05, and results were expressed as mean values ± SEM together with the 95% confidence interval and the actual *P* value.

Results

Figure 1 depicts the time course of plasma MTX levels in the presence absence of PIP. Although the decline in MTX levels after the end of the infusion was adequately described by a biexponential function, noncompartmental analysis was instituted. Higher plasma concentrations of MTX were detected in the PIP-treated group at each time point after 60 min (Fig.1). The average MTX steady-state plasma concentration found in the PIP-treated rabbits (2.66 ± 0.80 μg/ml) was significantly higher ($P < 0.05$) than that detected in the control group (1.76 ± 0.43 μg/ml). At the first 60 min of the experiment, no statistically significant difference in MTX plasma levels was observed between PIP-treated animals and control. Immediately after the commencement of PIP administration the levels of MTX rose dramatically, leading to significantly higher AUCs. This increase in steady-state plasma concentrations averaged 82.02 ± 7.05%.

The pharmacokinetic parameters calculated for MTX in the presence and the absence of PIP are shown in Table 1. The elimination rate constant (K_{el}) and the terminal half-life ($t1/2$) of MTX were almost identical in both groups. The MRT_{inf} follwing the infusion was

Table 1 Comparison of pharmacokinetic parameters of MTX given alone and in combination with PIP (*NS* Not significant, *S* significant)

Parameter	MTX + PIP group	MTX group	*P* value[a] Significance
K_{el} (min^{-1})	0.0187 ± 0.001 (0.017–0.021)[b]	0.0186 ± 0.002 (0.015–0.022)	0.9261 NS
$t_{½}$(min)	38.03 ± 2.49 (33.14–42.91)	38.30 ± 3.35 (31.74–44.86)	0.9497 NS
$AUC_{(0-t)}$(mg min l^{-1})	643.45 ± 62.01 (521.92–764.98)	421.45 ± 9.14 (403.53–439.37)	0.0334 S
$AUC_{(0-\infty)}$(mg min l^{-1})	682.85 ± 67.94 (549.69–816.01)	444.18 ± 11.38 (421.87–466.48)	0.0362 S
$AUMC_{(0-t)}$(mg min^2 l^{-1})	98017.6 ± 10047.0 (78325.9–117709.3)	56774.45 ± 1902.16 (53046.28–60502.62	0.0181 S
$AUMC_{(0-\infty)}$(mg min^2 l^{-1})	112136.9 ± 12230.3 (88166.0–136107.7)	64894.05 ± 2793.50 (59418.9–70369.2)	0.0243 S
$MRT_{i.v.}$(min)	54.84 ± 3.59 (47.80–61.87)	55.20 ± 4.83 (45.74–64.66)	0.9540[c] NS
MRT_{inf}(min)	162.88 ± 3.72 (155.58–170.17)	145.91 ± 2.83 (140.35–151.47)	0.0142[c] S
$V_{d_{ss}}$ (l)	0.568 ± 0.073 (0.425–0.710)	0.583 ± 0.040 (0.505–0.661)	0.8906 NS
$V_{zd_{ss}}$(ml kg^{-1})	133.05 ± 13.22 (107.14–158.96)	138.60 ± 6.14 (126.57–150.63)	0.7828 NS
CL(l h^{-1})	0.574 ± 0.079 (0.419–0.728)	0.799 ± 0.026 (0.748–0.850)	0.0255 S
CL (ml kg^{-1} min^{-1})	2.27 ± 0.31 (1.67–2.87)	3.18 ± 0.09 (3.01–3.35)	0.0208 S

[a] Student's *t*-test, for independent samples
[b] Numbers in parentheses represent 95% confidence intervals
[c] Mann-Whitney *U*-test/Wilcoxon rank-sum *W*-test

Table 2 Mean pharmacokinetic parameters calculated for 7-OH-MTX in MTX-treated versus MTX- and PIP-treated rabbits (*NS* Not significant, *S* significant)

Parameter	MTX + PIP group	MTX group	*P* value[a] Significance
K_{el} (min^{-1})	0.0108 ± 0.001 (0.008–0.014)[b]	0.012 ± 0.002 (0.007–0.016)	0.65914 NS
$t_{½}$(min)	73.30 ± 11.03 (51.69–94.91)	63.65 ± 9.42 (45.18–82.12)	0.58679 NS
$AUC_{m(0-t)}$(mg min l^{-1})	341.4 ± 27.29 (287.91–394.89)	140.33 ± 9.95 (120.82–159.83)	0.00053 S
$AUC_{m(0-\infty)}$(mg min l^{-1})	438.5 ± 37.3 (365.34–511.73)	159.1 ± 9.7 (140.1–178.0)	0.00045 S
$AUMC_{m(0-t)}$(mg min^2 l^{-1})	59029.2 ± 4920.1 49386.0–68672.5)	18832.28 ± 1436.05 (16017.7–21646.9)	0.00023 S
$AUMC_{m(0-\infty)}$(mg min^2 l^{-1})	100965.3 ± 13693.5 (74126.6–1127804.1	26285.95 ± 1457.23 (23429.8–219142.1)	0.00373 S
$MRT_{m,inf}$ (min)	61.8 ± 19.8 (42.4–81.3)	19.8 ± 6.1 (7.8–31.8)	0.0485[c] S
CL_m/f_m(l h^{-1})	0.85 ± 0.06 (0.72–0.97)	2.18 ± 0.14 (1.90–2.46)	< 0.00001 S
CL_m/f_m(ml kg^{-1} min^{-1})	3.42 ± 0.35 (2.73–4.10)	8.98 ± 0.61 (7.77–10.18)	< 0.00001 S

[a] Student's *t*-test for independent samples
[b] Numbers in parentheses represent 95% confidence intervals
[c] Mann-Whitney *U*-test/Wilcoxon rank-sum *W*-test

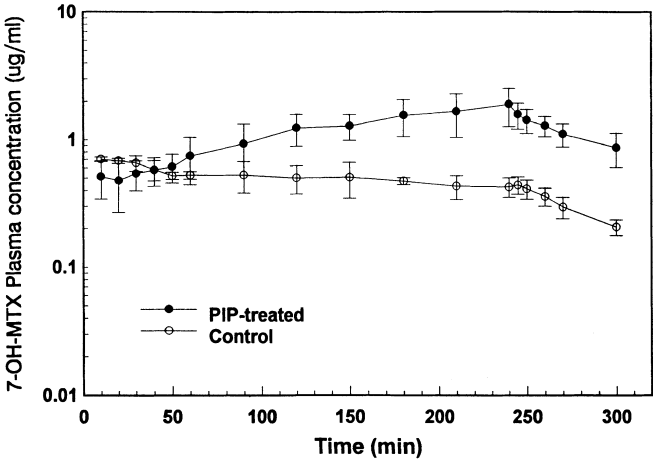

Fig. 2 Plasma concentration of 7-OH-MTX (mean ± SEM) measured after the administration of an i.v. bolus dose of 0.21 mg kg^{-1} MTX followed by a constant rate i.v. infusion of 5 μg kg^{-1} min^{-1} for 240 min with (*black circles*) or without (*white circles*) the addition of PIP (30 mg kg^{-1}) every 30 min starting at 60 min

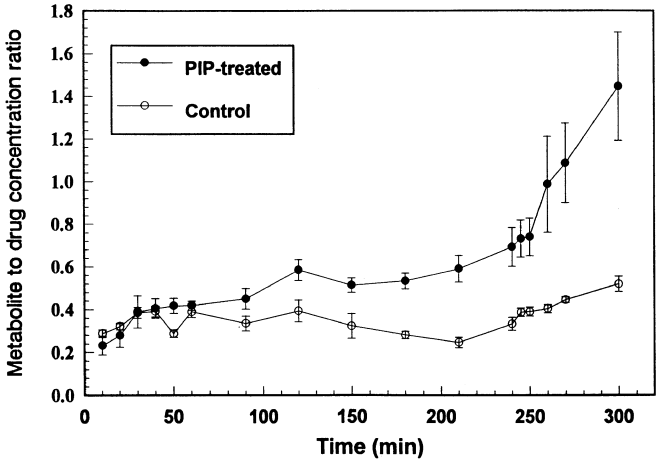

Fig. 3 Ratio of the plasma concentration of 7-OH-MTX to the plasma concentration of the parent drug MTX slotted as a function of time for the PIP-treated group (*black circles*) and the control group (*white circles*)

significantly increased ($P < 0.05$) in PIP-treated rabbits as compared with the control group. The AUC up to the last measured concentration (AUC_{0-t}) averaged 94.5 ± 0.66% and 94.9 ± 0.84% of the AUC from zero to infinity ($AUC_{0-\infty}$) calculated for the PIP-treated group and the control group, respectively, indicating adequate sampling time. The $AUC_{0-\infty}$ determined for PIP-treated rabbits was about 54% higher than that recorded for the control group, but clearance was 40% lower ($P < 0.05$). The clearance values were within the ranges reported by other investigators [13–15]. No statistically significant difference ($P < 0.05$) was observed in the $V_{d_{ss}}$ calculated for MTX between the PIP-treated group and the control group.

On the other hand, higher plasma concentrations of 7-OH-MTX were detected in the PIP-treated group at each time point after 60 min (Table 2, Fig. 2). The differences were not statistically significant before the addition of PIP. The steady-state plasma concentration of 7-OH-MTX detected in the PIP-treated rabbits averaged 1.43 ± 0.13 μg/ml as compared with the control value (0.58 ± 0.07 μg/ml). This significantly higher ($P < 0.01$) concentration of 7-OH-MTX resulted in an approximately 2.8-fold greater AUC and in a 2.6-fold decrease in the operative total body clearance. There was no statistically significant ($P < 0.05$) difference in the $t1/2$ and K_{el} values calculated for 7-OH-MTX between the two groups, whereas the $MRT_{m,inf}$ value was significantly higher ($P < 0.05$) in the PIP-treated group.

Discussion

Our results indicate that the total body clearance of MTX significantly decreased in the PIP-treated group as compared with the control group. These results are in agreement with previous findings. It is well known that only a small percentage of MTX undergoes hepatic metabolism to 7-OH-MTX, with the extraction ratio being smaller than 0.2. This means that MTX is eliminated mainly via renal excretion. The reason why the clearance of MTX did not change proportionally to the AUC may involve a compensatory mechanism, that is, as the renal clearance of MTX decreased, the metabolic clearance increased, resulting in a higher value for total body clearance than would have been expected from the AUC. These findings are supported by the observation of an approximately 3-fold increase in the plasma concentration of the metabolite. The elevation in metabolite plasma concentration can be attributed not only to the increase in metabolic clearance of MTX but also to the decrease in the total body clearance of the metabolite. The total body clearance of the major metabolite 7-OH-MTX in the PIP-treated rabbits significantly decreased to 65% of its value as compared with the control group.

Figure 3 depicts the ratio of the plasma concentration of 7-OH-MTX (C_m) to the plasma concentration of MTX (C) recorded for the PIP-treated group and the control group. No statistically significant difference in the ratio was observed between the two groups for up to 60 min before the addition of PIP. After this time the ratio C_m/C continued to be almost constant as a function of time, with insignificant change, if any, being seen in the control group. The slight rise observed in this ratio after 240 min was due to the stoppage of the MTX infusion and, hence, to the subsequent conversion of the drug to the metabolite. In contrast, the concentration ratio of the metabolite to the parent drug (C_m/C) noted in the PIP-treated group consistently increased as with time, with a sharp rise being seen after 240 min. The differences in this ratio observed between the two groups at between 90 and 300 min were highly significant ($P < 0.01$), indicating the accumulation of the metabolite 7-OH-MTX in the body due to the sharp decrease in its total body clearance.

The ratio $AUC_{m(0-\infty)}/AUC_{0-\infty}$ is equal to the ratio of metabolite formation clearance to the metabolite clearance from the body [16]. The area ratio of metabolite to drug significantly increased in the presence of PIP; thus, the exposure of the body to 7-OH-MTX increased relative to its exposure to MTX since the AUC is thought of as a measure of the body's exposure to a drug or substance. The area ratio can be converted to a percentage of increase in the body's exposure to 7-OH-MTX [16,17]. The body exposure almost doubled ($P < 0.01$) after the addition of PIP. The area ratio averaged 0.676 (± 0.077) for the PIP-treated group as compared with 0.357 (± 0.009) for the control group, which means that the exposure to 7-OH-MTX increased by 89.4% due to PIP.

Dealing with these results from another perspective, we can speculate that the possible mechanism of the increase in body exposure to 7-OH-MTX might have been due to the decrease in the total body clearance of the metabolite (CL_m) and/or to the increase in its formation clearance (CL_f) . Since there was a 2.8-fold increase in AUC_m and only a 1.5-fold increase in AUC_d in the PIP-treated group over the control group, one can say that both mechanisms might possibly be implicated. The increase in Cl_f may have been due to the increase in the rate of presentation of MTX to its metabolic sites. This is a direct consequence of the increase in plasma drug levels due to the decrease in its renal clearance. The toxic effects of this interaction could be augmented by a concurrent and more than proportional decrease in CL_m, which was manifested in the dramatic increase in AUC_m.

Iven and Brasch [1, 2] have pointed out that a decrease in the renal clearance of MTX and 7-OH-MTX is the major factor responsible for the toxicity observed on the concomitant administration of MTX and PIP since the influence of PIP on the pharmacokinetics of MTX resemble the effect of probenecid. Although we reached the same conclusion, our results emphasize that the accumulation of the metabolite in the body is another major factor in this interaction since 7-OH-MTX has cytotoxic activity in vitro and has caused renal toxicity in both humans and animals [18]. It has also been reported that 7-OH-MTX is considered to be potentially nephrotoxic because of its low water solubility [19].

There is consensus [18, 19] that it is unlikely that the observed MTX toxicity was due to the displacement of MTX from its plasma protein-binding sites since the volume of distribution of MTX does not exceed that of extracellular fluid [20]. It is well known that extracellular 7-OH-MTX is more highly protein-bound ($> 90\%$) than the parent drug (46%) [19, 20] and can be significantly displaced by other drugs, thereby increasing the concentration of free MTX in plasma. The toxic effect of MTX given concomitantly with other drugs cannot be explained solely by protein-binding displacement, and the possible mechanism involved in the case of PIP may be accumulation of 7-OH-MTX in the body.

Our results suggest that a decrease in the renal tubular secretion of MTX may not be the only explanation for MTX toxicity when the drug is given with other interactive drugs as shown in previous studies [1, 2]. It is more likely that the dramatic accumulation of the metabolite 7-OH-MTX is a major determinant of this interaction, even though 7-OH-MTX is 1 or 2 orders of magnitude less cytotoxic than MTX in vitro [21]. It is noteworthy that this interaction may not occur in every patient taking this combination. PIP has been used successfully in the treatment of patients who have received an accidental overdose of MTX [22]. The major clinical manifestations of MTX toxicity resulting from drug interaction are pancytopenia and renal failure. Therefore, the renal function of patients receiving this combination should be monitored with adequate fluid intake, especially in elderly patients because dehydration might accelerate the occurrence of toxicity.

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