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Effect of trimethoprim, paracetamol and cimetidine on trimetrexate metabolism by rat perfused isolated livers

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Trimetrexate (TMTX), a non-classical antifolate, is currently in clinical trial as an antineoplastic drug. In the rat perfused isolated liver, it undergoes extensive metabolism to two metabolites, M_1 and M_2, which are excreted primarily in the bile. The metabolites result from demethylation, and M_1 is also glucuronidated. We examined the effects of three commonly used drugs on the elimination of 1 mg of TMTX by the rat perfused isolated liver (perfusate volume was 100 mL). Co-administration of either 1 or 5 mg cimetidine, a well-known inhibitor of microsomal oxidation, caused an increase in TMTX terminal elimination half-life (69 and 100% at 1 and 5 mg, respectively) and a decrease in clearance (40 and 46% at 1 and 5 mg, respectively). Paracetamol (acetaminophen) was chosen for a possible interaction with TMTX because its major metabolic pathway is glucuronidation. Five mg paracetamol resulted in no change in TMTX pharmacokinetics, but M_1 concentrations were increased by 72% in bile, and M_2 was not present in perfusate. The third drug tested was trimethoprim, which has some structural similarities to TMTX; however, no effects were noted on the levels of TMTX, M_1 or M_2 after 1 mg trimethoprim. These results indicate that TMTX elimination can be inhibited by cimetidine, probably due to competition for microsomal enzymes, and that paracetamol may alter the metabolite profiles; trimethoprim had no effect on TMTX disposition under the conditions employed.

Trimetrexate (TMTX), a lipophilic inhibitor of dihydrofolate reductase (Jackson et al 1984), is currently used in clinical trials as an anticancer agent. Previous work has shown that it undergoes rapid biphasic elimination by the rat perfused isolated liver (Webster et al 1985a). It is excreted primarily in the bile as two metabolites (M_1 and M_2), both of which also inhibit dihydrofolate reductase and are present in man (Tong et al 1985). Both M_1 and M_2 are demethylated products and M_1 is also glucuronidated (McCormack et al 1986). Three commonly used drugs were selected, each with some potential for an interaction with TMTX metabolism: cimetidine, a well-known inhibitor of microsomal oxidation, paracetamol (acetaminophen), which has glucuronidation as a major metabolic pathway, and trimethoprim, which has some structural similarities to TMTX. These drugs were co-administered with TMTX in the rat perfused isolated liver, and the resulting TMTX pharmacokinetic parameters and metabolite concentrations were examined.

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Methods

Non-fasting, male Sprague-Dawley rats (190–260 g) were anaesthetized with sodium pentobarbitone (65 mg kg^{-1}). The livers were surgically isolated and perfused via the portal vein in a constant flow (15 mL min^{-1}) recirculating system at 37 °C (Webster et al 1985b). The bile duct was cannulated and bile was collected in preweighed vials. All drugs were added to the reservoir to simulate systemic administration, and samples for drug assay were removed from the reservoir. Perfusate lost through sampling was replaced by an equal volume of fresh perfusate to maintain a total circuit volume of 100 mL. Less than 4% of the dose was lost in samples.

In 13 control livers, 1 mg (2 μmol) TMTX was added to the perfusate reservoir and perfusate was sampled at 0 (pre-dose), 2, 4, 6, 10, 15, 20, 30, 45, 60, 90 and 120 min. In random order, and interspersed among the controls, the following drugs were administered 2 min before TMTX (n = 4 for each): cimetidine (1 mg or 5 mg), paracetamol (5 mg), or trimethoprim (1 mg). The same sample schedule was followed.

Trimetrexate, M_1 and M_2 were measured in perfusate or bile by a specific and sensitive high performance liquid chromatography (HPLC) assay (Webster et al 1985a; Ackerly et al 1985). The limit of detection for each compound was 50 ng mL^{-1}. Because cimetidine co-eluted with the metabolites, the assay was modified slightly by changing the solvent gradient from 10–40% to 6–40% acetonitrile. M_1, M_2 and cimetidine then had retention times of 7, 7·5 and 8 min. Metabolite results were incomplete for the high-dose cimetidine experiments and are not included.

Pharmacokinetic parameters for TMTX disposition were calculated using standard model-independent pharmacokinetic formulae (Gibaldi & Perrier 1982) with the aid of the computer program AUTOMOD (Gomeni & Gomeni 1979). The data were compared using Scheffe's method for multiple comparisons of independent groups of unequal size (Miller 1966) accepting $P < 0.05$ as statistically significant. All data are presented as mean ± s.e.m.

Results and discussion

The mean perfusate concentration-time profiles of TMTX for all experiments illustrate that TMTX elimination from the rat perfused isolated liver is biexponential (Fig. 1). The pharmacokinetic parameters (Table 1)

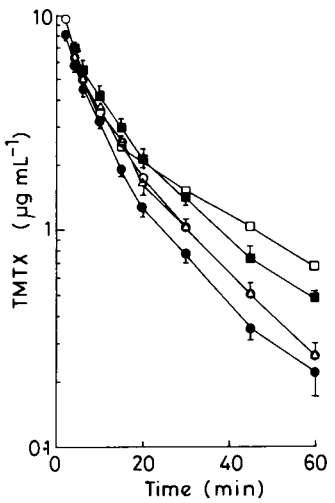

FIG. 1. Semilogarithmic plot of the elimination of 1 mg of TMTX from rat perfused isolated livers under control (●) conditions (n = 13), or after co-administration of (n = 4 each): 5 mg cimetidine (□), 1 mg cimetidine (■), 5 mg paracetamol (△) or 1 mg trimethoprim (○). Each point represents mean ± s.e.m.

indicate that none of the drugs influenced the initial elimination phase ($t_{\frac{1}{2}}\alpha$). This phase probably represents uptake and distribution, which are passive processes for most drugs (Thurman & Kauffman 1980) and would thus not be expected to be altered by competition from other drugs. The high $t_{\frac{1}{2}}\alpha$ after the low-dose cimetidine was not statistically significant and probably reflects an insufficient number of data points for accurate estimation of this rapid elimination phase; individual values for $t_{\frac{1}{2}}\alpha$ ranged from 1·3 to 5·9 min among all the experiments.

However, cimetidine at both 1 and 5 mg caused statistically significant increases in $t_{\frac{1}{2}}\beta$ of 69 and 100%, respectively, and a 40 and 46% decrease in clearance of TMTX. Cimetidine is a well-established inhibitor of microsomal mixed function oxidases (Reilly et al 1983), and in similar experiments it inhibited antipyrine clearance by 65 and 81% at 1 and 5 mg, respectively (Mihaly et al 1982). Heusner & Franklin (1985) used formaldehyde production as a measure of the demethylation of TMTX in a microsomal system and demonstrated competitive inhibition by cimetidine, thus providing additional evidence that microsomal enzymes contribute to TMTX metabolism. Trimethoprim and paracetamol appeared also to decrease TMTX clearance, but this effect did not reach statistical significance. Trimethoprim is reported to inhibit tolbutamide oxidation (Wing & Miners 1985), and may play a role in the increased concentrations of phenytoin and warfarin after trimethoprim-sulphamethoxazole co-administration (Wilcox 1981; Kaufman & Fauver 1980). The lack of effect of trimethoprim on TMTX metabolism may reflect differences in the affinity of these two compounds for cytochrome P450; it may also indicate metabolism by separate isozymes of this enzyme. Interactions of paracetamol with other drugs primarily involve an inhibition of its glucuronidation or sulphation, or an induction of its own oxidative metabolism. However, paracetamol itself can increase the elimination half-life of chloramphenicol (Buchanan & Moodley 1979), probably by competition for glucuronidation. Thus, it was plausible that paracetamol could inhibit TMTX metabolism to M_1, which is glucuronidated, and thereby decrease TMTX clearance.

The mean perfusate concentration time profiles for M_1 and M_2 are shown in Fig. 2. The most notable observation is the complete absence of M_2 after paracetamol co-administration. There was a concomitant rise in M_1 concentrations after the drug, but this did not reach statistical significance. This suggests a compensatory change in route of metabolism of TMTX in response to the presence of paracetamol, for which glucuronidation is the major route of metabolism,

Table 1. Pharmacokinetic parameters for the disposition of 1 mg TMTX in rat perfused isolated livers. Values are mean ± s.e.m.

	$t_{\frac{1}{2}}\alpha$ (min)	$t_{\frac{1}{2}}\beta$ (min)	Clearance (mL min^{-1})	Area under the curve (μg mL^{-1} min^{-1})	Volume of distribution (mL)
Control (n = 13)	2·4 (±0·4)	13 (±1)	11·4 (±0·9)	93 (±6)	215 (±30)
Cimetidine (5 mg, n = 4)	2·6 (±0·2)	26 (±2)*	6·2 (±0·2)*	162 (±5)*	235 (±20)
Cimetidine (1 mg, n = 4)	4·6 (±0·5)	22 (±2)*	6·8 (±0·4)*	149 (±9)*	220 (±30)
Trimethoprim (1 mg, n = 4)	2·1 (±0·3)	15 (±2)	8·5 (±0·7)	119 (±11)	180 (±30)
Paracetamol (5 mg, n = 4)	3·6 (±0·6)	15 (±1)	8·6 (±0·5)	117 (±6)	190 (±15)

* $P < 0·05$ compared with control.

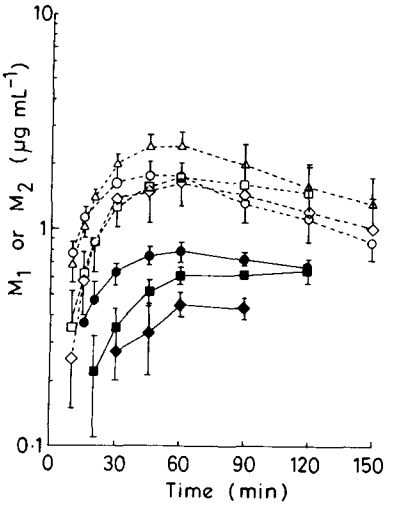

FIG. 2. Semilogarithmic plot of M_1 (open symbols) and M_2 (closed symbols) concentrations after administration of 1 mg TMTX to rat perfused isolated livers under control (○, ●) conditions, or after co-administration of 1 mg cimetidine (□, ■), 5 mg paracetamol (△), or 1 mg trimethoprim (◇, ◆). The vertical bars represent the standard error of the mean of 4 separate livers. M_2 was not present in the perfusate after paracetamol dosage.

although sulphation is the first to be saturated. The complete structure of M_2 has not yet been elucidated, but it appears to be neither glucuronidated nor sulphated (unpublished observations). The mechanism for the effect of paracetamol on M_2 is therefore not clear. No other changes in metabolite perfusate concentrations were statistically significant. However, for the first 60 min, cimetidine caused a decrease in M_1 and M_2 levels; this further supports the mechanism of inhibition by cimetidine of TMTX demethylation.

The total biliary excretion of trimetrexate during 2 h in the control studies was less than 2% of the dose. However, over half of the dose was present in bile as M_1 and 10% as M_2. The most important change occurred in the paracetamol group where biliary excretion of M_1 increased by 72% ($P < 0{\cdot}05$) while that of M_2 decreased by 39% ($P < 0{\cdot}05$). The presence of M_2 indicates that it was still being produced, and that its absence from the perfusate might be explained by the rate constant of excretion being greater than that for production. There were no changes in bile flow resulting from co-administration of any of the test drugs.

Initial perfusate concentrations of the test drugs (in μg mL^{-1}: 10 and 50 for cimetidine, 10 for trimethoprim and 50 for paracetamol) were selected on the basis of relevance to clinically effective concentrations, which are approximately 1, 10 and 20 μg mL^{-1} for cimetidine, trimethoprim and paracetamol, respectively. The drugs were all available to interact with TMTX for the duration of the experiments as evidenced by their co-elution on the TMTX chromatographs. Nonetheless, only cimetidine caused a statistically significant inhibition of TMTX elimination, and paracetamol had an apparent effect only on TMTX metabolites. The increase in AUC and $t_{\frac{1}{2}\beta}$ of TMTX indicates that this anticancer agent may interact with cimetidine.

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