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EFFECT OF INDOMETHACIN ON THE PHARMACOKINETICS OF METHOTREXATE IN RABBITS

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ABSTRACT

The interaction between indomethacin (IND) and methotrexate (MTX) was investigated in rabbits. The study was designed so as to evaluate the effect of IND (1 mg h^{-1}) during a continuous MTX infusion (1·2 mg kg^{-1}) over 240 min. IND was injected i.v. at hourly intervals after a steady state MTX concentration had been established. Plasma MTX concentration before and after IND did not differ significantly ($p > 0{\cdot}05$). The elimination half-life ($t_{½\beta}$) calculated during the washout interval (mean ± SD) was 47·4 ± 21·5 min which is close to that calculated in a reference group of rabbits. This excludes the possibility of delayed elimination as responsible for this toxicity. The toxicity of this combination was confirmed despite the absence of significant pharmacokinetic changes. It is possible that the toxic interaction was caused by enhanced cytotoxic effect of MTX.

KEY WORDS MTX IND NSAID Interaction Clearance

INTRODUCTION

MTX is the most widely used antimetabolite in the treatment of various malignant and nonmalignant diseases. The dose range is very broad; it ranges between 15 and 12 000 mg m^{-2} depending on the type of disease.[1,2] As a cytotoxic drug its use is usually associated with reversible side-effects particularly after high doses.[1,2] Interactions of MTX have been reported with a number of drugs.[3–10] However the most prominent interaction was observed in patients receiving one of the nonsteroidal anti-inflammatory drugs (NSAID) during the course of MTX therapy.[5–10] The proposed mechanism for this life-threatening interaction was delay in MTX clearance caused by competition at the elimination site of MTX.[5–7] The involvement of other mechanisms however could not be excluded since cellular toxicity of MTX (*in vitro*) was enhanced in the presence of IND.[11] Because the mechanism of this interaction has not been precisely

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determined the present study was performed to estimate the effect of IND on the clearance of MTX.

MATERIALS AND METHODS

Pharmacokinetic study

Seven male New Zealand white rabbits weighing between 3·5 and 4·5 kg were used. Two cannulas (Terumo, 22 Gx1, i.d. 0·60 × 25 mm) were inserted in the marginal ear veins, one in each ear, for MTX and IND administration. A third cannula was placed in the central ear artery, opposite to the one used for MTX administration and used for blood collection. MTX (Lederle, Cyanamid of Great Britain Ltd, UK) was infused in one ear and the samples collected from the second ear. The dose of 1·2 mg kg^{-1} MTX was prepared in 5 per cent dextrose solution containing 0·2 per cent sodium bicarbonate and infused at a rate of 5 μg kg^{-1} min^{-1}. In two of the rabbits the dose was 2·4 mg kg^{-1} infused at a rate of 10 μg kg^{-1} min^{-1}. The volume of fluids infused with each dose ranged between 36 and 84 ml. The infusion pump used was the 'Terfusion' pump model STC-502, and the duration of infusion was 240 min. Immediately before the beginning of each infusion a bolus dose was injected. The bolus dose was estimated from the required MTX concentration multiplied by the volume of distribution at steady state (0·2 l kg^{-1}). The interacting drug, IND (Confortid 50 mg, Dumex Ltd, Copenhagen, Denmark) was prepared in normal saline and injected hourly using a separate i.v. line. The initial dose (1 mg kg^{-1}) was injected immediately following the sample collected at 100 min post-infusion. This dose was repeated every hour until the end of the experimental period.

Blood samples (2 ml) were collected before and every 20 min after IND for 300 min post-infusion. Additional samples were taken every 30 min up to 390 min which is the end of interaction study.

Analytical procedure

A high performance liquid chromatograph (HPLC) was used to measure MTX concentration in plasma samples. The system consists of 720 system controller, 730 data module, 710B automatic injector (Wisp), and 481 UV detector (all from Waters associates). The assay, which was developed in our laboratory[12] combined the following steps. Initially plasma samples were deproteinized with trichloroacetic acid. The supernatant (100 μl) was then injected on to a C18 Novapak column. The separation was completed with a mixture of phosphate buffer, methanol, and acetonitrile (84:11:5), which was pumped throughout the column at a flow rate of 2·3 ml min^{-1}. The effluent was monitored at 313 nm where MTX and the internal standard (4-amino-acetophenone) appears at 5·3 and 8·8 min, respectively.

Data analysis

The infusion rates 5 and 10 μg kg^{-1} min^{-1} produced proportional steady state concentrations. It is known that the kinetics of MTX are linear over the low dosing range.[13,14] Because of this, the data obtained after 10 μg kg^{-1} min^{-1} (two rabbits) were normalized to the infusion rate of 5 μg kg^{-1} min^{-1}. The phamacokinetic parameters reported were the mean ± SD estimated in seven rabbits. The concentrations mean ± SD before and after IND were calculated using the data points of 60 to 100 and 120 to 240 min, respectively. The total body clearance (TBC) was calculated by dividing the infusion rate over the mean concentration at steady state. The decline in plasma concentration was fitted to a two-compartment model using a semi-log graph paper. The elimination rate constant was then determined from the slope of the terminal linear segment using least square linear regression analysis. The level of significance was determined using the Student's *t*-test.

RESULTS

The plasma concentration of MTX (mean ± SD) versus time is shown in Figure 1. Pharmacokinetic parameters and statistics are reported in Table 1. The steady state plasma concentration of MTX was obtained within 20 to 40 min of infusion. The mean ± SD steady state concentration was 1·1 ± 0·356 μg ml^{-1}. Despite the addition of IND, the concentration remained stable until the end of infusion (Figure 1). The individual means at 120 to 240 min were compared with the mean before the start of IND ($n = 7$). The difference was not significant ($p > 0·05$). The concentration (mean ± SD) during this interval was 0·96 ± 0·396 μg ml^{-1}. The corresponding total body clearances calculated before and after IND were 18·84 ± 4·57 and 21·62 ± 6·18 ml min^{-1}, respectively (Table 1). At the end of infusion, the MTX level declined biexponentially over the period until the drug was completely washed out (Figure 1). The distribution phase was brief. However, the elimination half-life for the terminal phase ($t_{½\beta}$) was 47·4 ± 21·5 min. The elimination half-life in the rabbits infused with MTX alone was 43·3 ± 7·6 min.

Table 1. Pharmacokinetic parameters obtained in seven rabbits injected with IND (1 mg h^{-1}) during the continuous infusion of MTX (5 μg kg^{-1} min^{-1})

Pharmacokinetic parameters	Experimental animals before IND	Experimental animals after IND	Controls	*p* value
Steady state (μg ml^{-1})	1·1 ± 0·36	0·96 ± 0·39	–	>0·05
Total body clearance (ml min^{-1})	18·8 ± 4·57	21·6 ± 6·18	–	>0·05
Elimination half-life ($t_{½\beta}$) (min)	–	47·4 ± 21·5	43·3 ± 7·6	>0·5

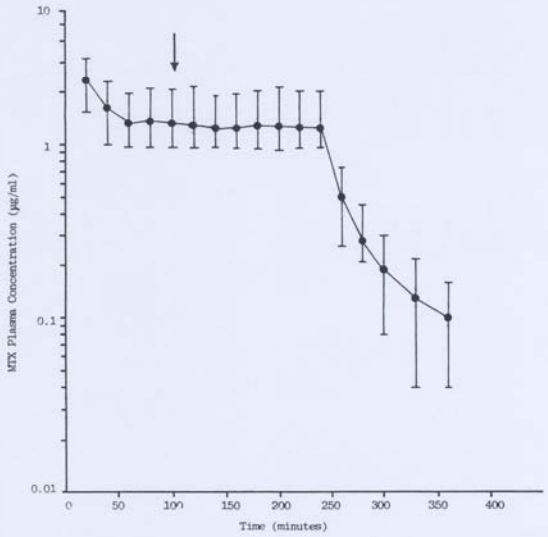

Figure 1. Plasma concentration of MTX after i.v. bolus of 0·24 mg kg^{-1} followed with i.v. infusion of 1·2 mg kg^{-1} over 240 min. At the arrow (IND) was injected i.v. and the injection repeated hourly until the end of experiment. The points represent the mean ± SD obtained in seven rabbits

DISCUSSION

The doses selected for MTX and IND were sufficient to cause toxicity in the experimental animals. Vomiting and weakness were observed and some of the animals died the following day. MTX was infused at a rate of 5–10 μg kg^{-1} min^{-1}. Higher doses were associated with more toxicity following the addition of IND.

The main finding of the present study is the absence of any significant change in MTX concentration after the addition of IND despite the toxic effect of the combination. The steady state achieved during infusion remained stable until the end of the infusion period. The difference in clearance before and after IND was not significant ($p > 0·05$) which rules out the possibility of competition at the elimination sites of MTX. The clearance data obtained in this study was within the range reported by other investigators in rabbits.[13,14] It is known that the plasma level of MTX is sensitive to the presence of drugs that interfere with

its renal elimination. A two-fold increase in MTX concentration was reported in rabbits in the presence of probenecid.[13]

Based on a literature survey this was probably the first study organized in animals to estimate the effect of IND on the elimination of MTX. The interaction between MTX and members of the NSAID group of drugs is well documented in clinical practice.[5–10] It is proposed that the NSAID compete with MTX on the elimination sites and/or induce protein displacement effect.[15] However, the involvement of other mechanisms cannot be excluded. For example, Gassen *et al.* (1985) had reported that IND enhanced the cytotoxicity of MTX (*in vitro*).[11] The interaction in humans is associated with an acute renal failure.[5–7] In one patient, the clearance of MTX was measured before and in the presence of IND and dicloxacillin.[16] The clearance was reduced during the combination period. The investigators denied the presence of any change in serum creatinine that may account for the decreased clearance. Very recently, Furst *et al.* determined the clearance of MTX after an i.v. bolus of 10 mg m^{-2} in a group of rheumatoid arthritis patients in the presence and absence of salicylate and sulindac (NSAID).[17] The difference in clearance was not significant which confirmed the finding obtained in this study. An important consideration in the cytotoxicity of MTX is the concentration and duration of exposure.[18] In this study the concentration of MTX and the duration of combination was probably below that required to observe renal toxicity that will change the serum level.

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