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Theophylline inhibits the elimination of flumazenil in rabbits

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Summary

The effect of theophylline on the elimination of flumazenil during constant infusion of 1 mg/kg per h over 240 min was investigated in 10 rabbits. Theophylline was introduced as a single intravenous (i.v.) dose of 5 mg/kg (5 rabbits) and 15 mg/kg (5 rabbits), 100 min postinfusion. The addition of theophylline was associated with a new steady state which is approx. 32 and 46% higher for the 5 and 15 mg/kg doses, respectively. The elimination half-life of the drug was also increased from 18 and 12 min to about 28 and 42 min, respectively, for the two doses. Despite the relatively wide margin of safety for flumazenil the changes observed in plasma concentration during concomitant administration of theophylline may be clinically important. The pharmacokinetic parameters obtained prior to theophylline injection in the 10 rabbits were 406 ng/ml and 15 min for the steady state and elimination half-life, respectively. The variations between subjects in these parameters were as much as 2-fold. A simple and sensitive HPLC procedure to measure flumazenil in plasma is also described.

Introduction

Flumazenil is a recently introduced specific benzodiazepine (BDZ) antagonist (Brodgen and Goa, 1988). It is mainly used for the reversal of BDZ-induced sedation during surgery or toxicity from overdose (Bodenham et al., 1988; Brodgen and Goa, 1988). Additional use as in epilepsy is still investigational (Hart et al., 1991). Little is known about its interactions with other drugs. However, the drug is extensively metabolised by the oxidative process of the liver, leading to a short half-life of less than 1 h (Roncari et al., 1986). Theophylline, on the other hand, as a non-specific central nervous system stimulant, is commonly used during BDZ toxicity and post-surgery (Kumar et al., 1987; Sibai et al., 1991). Because the two share the same metabolic pathway, changes in the elimination process of each is expcted during concomitant administration. This study was aimed at estimating the effect of theophylline on the elimination of flumazenil during continuous infusion. Since these interactions are usually dose-dependent, theophylline was tested at two different doses.

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Materials and Methods

Animal preparation

A total of 10 white New Zeland male rabbits, weighing between 3.5 and 5 kg, were included in

this study. Each was prepared on the day of experiment by inserting three cannulas (22 G × 1 inch, Terumo®) as follows: one in the central ear artery which was used for blood collection; the remaining two were placed in the marginal ear veins, one in each ear and used as a route for drug administration. The rabbits were infused with flumazenil via the cannula of the ear opposite to the one used for blood collection.

Pharmacokinetic study

Flumazenil was dissolved in 5 ml propylene glycol and diluted with normal saline to a final concentration of 1 mg/kg per 12 ml. The rabbits were divided into two groups (A and B) and each received a constant infusion of flumazenil (1 mg/kg per h) over a period of 240 min. The total volume infused during this interval was 48 ml. Theophylline was injected shortly after the sample collected 100 min postinfusion. The dose injected was 5 mg/kg (group A) or 15 mg/kg (group B) as a single dose, slowly over 2–3 min. Blood samples were collected at 0, 10, 20, 30, 40, 60 80, 100, 120, 140, 160, 180, 200, 220, 240, 250, 260, 280, 300, 310 and 320 min post-infusion. Plasma was immediately separated and kept at −30°C until the day of analysis.

Drug analysis

A simple and sensitive high-performence liquid chromatography (HPLC) method was developed to measure the levels of flumazenil in plasma. A mixture of 100 μl plasma and 10 μl carbamazepine (4 μg/ml) as internal standard was extracted twice each with 1 ml ether. The residue after evaporation was reconstituted with 100 μl of the mobile phase. The latter consisted of sodium dihydrogen phosphate (20 mM) and acetonitrile (70:30), the pH being adjusted to 3.6 with phosphoric acid. A 25 μl volume of this solution was injected into the HPLC system. The HPLC system consisted of a M45 solvent pump, a WISP 712B automatic injector, Lambda Max 484 spectrophotometer and a Novapak-C18 cartridge (10 × 8 mm i.d, 4 μm) column. The flow rate of the mobile phase was 2 ml/min and the eluent

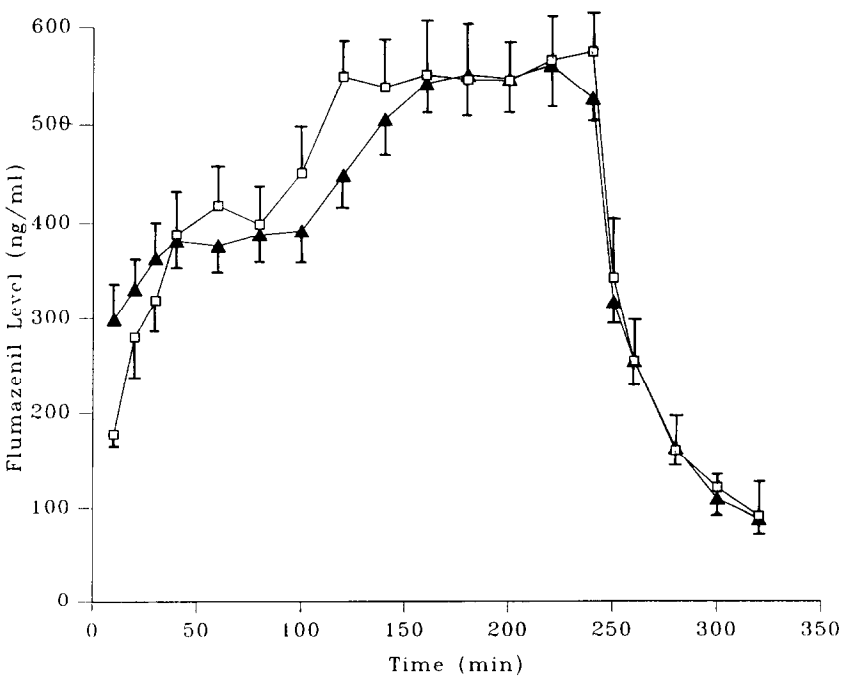

Fig. 1. The plasma concentration-time course of flumazenil in two groups of rabbits (A and B), infused with flumazenil at a rate of 1 mg/kg per h for 4 h. Each point represents the mean (±SD) of five rabbits. Theophylline was injected shortly after the sample taken 100 min post-infusion as a single dose of 5 and 15 mg/kg for the two groups, respectively.

was monitored at 245 nm. Retention times were 2.4 and 4 min for flumazenil and carbamazepine, respectively, and the detection limit was less than 10 ng/ml.

Pharmacokinetic analysis

The elimination of flumazenil was fitted to one compartment model. The rate constant was calculated from the linear segment of the log concentration-time data. The data collected during infusion to steady state C_{ss} were used in calculating the elimination rate constant by plotting $(C_{ss} - C)$ vs time (Gibaldi and Perrier, 1989). A minimum of three points were used in calculating the average steady state C_{ss}. The clearance was calculated by dividing the infusion rate by the average steady state. Data expressed as the mean ± SD were analysed using paired Student's *t*-test.

Results

Fig. 1 shows the plasma concentration-time curves for the two groups (A and B) of rabbits, during 240 min infusion and after the infusion had been stopped. Prior to the injection of theophylline, the steady states of flumazenil were 434 ± 88 and 377 ± 55 ng/ml and the elimination half-lives were 18 ± 3.4 and 12 ± 2.4 min, respectively, for group A and B. The administration of 5 and 15 mg/kg theophylline was associated with a new steady state which was about 32 and 46% higher for the two groups, respectively. The decline in plasma level following the end of infusion is biexponential. The terminal phase (β) is represented by the data points of 260 min post-infusion until the last sample. The line connecting the log concentration-time data is linear with elimination half-lives of 28 ± 5.1 and 42 ± 2.6 min, respectively, for group A and B. In both, the differences in steady state, elimination half-lives and clearance as a result of theophylline administration were statistically significant ($p < 0.005$). Table 1 shows the mean (±SD) for each group before and after theophylline. The pharmacokinetics of flumazenil in the 10 rabbits (group A and B) infused with 1 mg/kg per h were as follows. The steady state was in the range of 280–503 ng/ml and was reached within 30–60 min of infusion, the elimination half-life being in the range 9–22 min.

TABLE 1

The elimination half-life, steady state and the clearance of flumazenil before and after single i.v. injection of 5 mg/kg (group A) and 15 mg/kg (group B) theophylline

Parameter	Animal group	Before theophylline	After theophylline
Elimination	A	18 ± 3.4	28 ± 5.1
half-life (min)	B	12 ± 2.4	42 ± 2.6
Steady state	A	434 ± 88	572 ± 101
(C_{ss}) (ng/ml)	B	377 ± 55	552 ± 41
Clearance	A	158 ± 33	119 ± 21
(ml/min)	B	139 ± 33	93 ± 13

Data expressed as the mean (+SD) which was taken from five rabbits. The p value during all comparison is < 0.001.

Discussion

Conducting an interaction study during constant infusion as in this study eliminates the need for a control group. This type of studies is more suitable for drugs with a very short half-life, such as flumazenil, since the two phases of the steady state can be seen within a relatively short infusion period. In addition, flumazenil is frequently administered by i.v. infusion, so that the reversal of the BDZ effect can be controlled to the patient needs (Ahmad et al., 1991). As expected, the elimination of flumazenil was inhibited in the presence of theophylline. The steady state increased by approx. 32 and 48% when 5 and 15 mg/kg theophylline were injected, respectively. Because the two share the same metabolic pathway, competitive inhibition may represent the mechanism of this interaction. However, in a recent study in rats, it was shown that there also exists extrahepatic metabolism, mainly in red blood cells (RBC) (Mandema et al., 1991). The ratio of this metabolic pathway to the well-known hepatic elimination is undefined. Because theophylline diffuses poorly into RBC (Kern and Lipman, 1977), it is unlikely that the extrahepatic route of elimination is involved in this interac-

tion. The interactions which involve inhibition of the metabolic pathways are usually dose dependent. In this study the higher doses were associated with an increase in the steady state, which was insufficient, however, to be equivalent to the dose difference. Theophylline was injected as a single dose because of its relatively long (4 h) half-life in rabbits (El-Yazigi and Sawchuk, 1981). This means that sufficient concentrations of theophylline will be maintained for as long as the study period. Literature investigations on the effect of theophylline on the pharmacokinetics of other drugs are very limited (Upton et al., 1991). Most of the data available address the effects of other drugs on the pharmacokinetics of theophylline.

Because of the wide therapeutic margin for flumazenil (Broden and Goa., 1988) the effect of this interaction on the dosage regimen of flumazenil cannot be predicted from this study. However, because the two drugs act as antagonists to the effect of BDZ and their dosage requirements vary between patients, it is likely that this combination needs to be carefully administered to patients. The influence of interaction on the dosage regimen of orally administered flumazenil should be considered. This is because an improvement is expected in the bioavailability, which is usually very poor, being less than 16%, due to the extensive first pass effect (Roncari et al., 1986).

Interaction studies with flumazenil in the literature are very sparse. The effects of BDZ and ethanol on the elimination of flumazenil were studied and found to be insignificant (Hartmann et al., 1988; Breimer et al., 1991). In patients with liver cirrhosis, 50% reduction in the clearance of flumazenil was reported (Janssen et al., 1989), which closely approximates the effect of theophylline in this study. The pharmacokinetic parameters for flumazenil which were obtained from the 10 rabbits vary by as much as 2-fold. This is similar to the variations reported in humans (Brogden and Goa, 1988), which may not represent wide intersubject variations.

In conclusion, it appears that the concomitant administration of theophylline with flumazenil was associated with a significant increase in the plasma level of flumazenil. The influence of this interaction on the dosage regimen of flumazenil needs to be clinically investigated.

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