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COMMUNICATIONS

The effect of three H_2-receptor antagonists on the disposition of midazolam in the rat in-situ perfused liver model

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Abstract—The rat in-situ perfused liver model was used to investigate the effect of three H_2-receptor antagonists on the pharmacokinetic disposition of the short-acting benzodiazepine, midazolam. Perfusion experiments, using standard techniques, were carried out on four groups (one control and three H_2-receptor antagonist-treated groups) of male Sprague-Dawley rats (300–350 g). All animals received midazolam 1 mg; the three treated groups received cimetidine (8 mg), ranitidine (3 mg) or famotidine (0·4 mg). Perfusate and bile samples were collected and assayed for midazolam using gas chromatography. The perfusate data indicated that midazolam disposition was impaired at 10, 50 and 60 min of the experimental period following the addition of cimetidine, whereas ranitidine and famotidine produced an effect at 10 min only; midazolam levels in bile were not affected by the presence of an H_2-receptor antagonist. It was concluded that the limited inhibitory effect of cimetidine may be attributed to its lack of specificity for CYP3A, the isoenzyme responsible for the metabolism of midazolam.

Midazolam is a short-acting benzodiazepine, its effects being seen within 1–3 min following intravenous administration (Amrein & Hetzel 1990). The metabolism of midazolam results in the production of three metabolites; the main metabolite is 1-hydroxy midazolam, the two minor metabolites being 4-hydroxy midazolam and 1,4-dihydroxy midazolam (Heizmann et al 1983). This metabolic profile is observed in both rats and man (Allonen et al 1981).

The metabolic pathway of midazolam and other benzodiazepines may be affected by the presence of interacting drugs. One group which has been extensively studied in relation to the benzodiazepines is the H_2-receptor antagonists.

Klotz et al (1979) first reported that the elimination of diazepam was significantly reduced in the presence of cimetidine. Studies conducted by Desmond et al (1980) and Patwardhan et al (1980) demonstrated that cimetidine markedly impaired the clearance of chlordiazepoxide and the formation of its major metabolite.

Therefore, it would seem possible that cimetidine would interact with midazolam and most reports have suggested that this interaction does occur (Elliott et al 1984; Klotz et al 1985; Salonen et al 1986). However, the potential interaction between ranitidine and midazolam has proved to be less clear. A series of studies in volunteers suggested that the systemic bioavailability of midazolam was significantly increased after treatment with ranitidine (Elwood et al 1983; Elliott et al 1984; Fee et al 1987), whereas other work has suggested that ranitidine has no effect on midazolam disposition (Klotz et al 1985; Greenblatt et al 1986).

As the liver is the major organ responsible for metabolism of midazolam (Heizmann et al 1983), it was thought that the rat in-situ perfused liver preparation might prove useful for investigating drug interactions affecting the metabolism of this benzodiazepine. Therefore, this hepatic model was used to study the effect of cimetidine, ranitidine and famotidine on the pharmacokinetic disposition of midazolam.

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Materials and methods

The perfusion method used was based on a technique developed by Hems et al (1966) and adapted by Maguire et al (1984). Animals which had been starved overnight were anaesthetized using pentobarbitone (50 mg kg^{-1}, i.p.); the liver was exposed via a midline incision, and the vena cava, portal vein and bile duct cannulated. The preparation was placed in a perfusion cabinet maintained at 37°C and the cannulated blood vessels connected to the perfusion apparatus. Oxygenated medium (total volume 150 mL) consisting of Krebs–Henseleit buffer (pH 7·4), aged human red cells (2·5%) and bovine serum albumin (2·5 g%) was used to perfuse each liver at a flow rate of 20 mL min^{-1} using a recycling mode. Pre-weighed vessels were used to collect bile at 10-min intervals during an initial 30-min equilibration period. Throughout this time, liver viability was monitored in relation to amount and rate of bile flow, perfusion medium flow, pH of the medium, temperature at the surface of the liver and overall macroscopic appearance. These parameters did not differ between the four experimental groups.

In the control group (n = 5), midazolam 1 mg (2 mg mL^{-1}) was added to the perfusion medium at the end of the 30-min equilibration period; in each of the other groups (n = 5 in each case), the respective H_2-receptor antagonists, cimetidine 8 mg (100 mg mL^{-1}), ranitidine 3 mg (25 mg mL^{-1}) and famotidine 0·4 mg (famotidine injectable preparation 4 mg mL^{-1}) were added 5 min before midazolam. Dose levels for all drugs were calculated by extrapolating on the basis of body weight from the highest intravenous dose (mg kg^{-1}) used in a 70 kg man to an equivalent dose in a 300 g rat. In the case of midazolam, it was found necessary to select a higher dose of 1 mg; a dose less than this could not be detected after 15 min of perfusion. After the 30-min equilibration period, each experiment proceeded for 1 h; preliminary investigations had shown that midazolam could not be detected after this time in untreated preparations. Perfusate and bile sampling took place at 10, 20, 30, 40, 50 and 60 min; perfusate samples (1 mL) were taken from both sides of the liver close to the points of entry and exit (portal and venous). These samples were centrifuged and 0·5 mL samples of perfusate plasma and the bile samples (stored at −20°C for no longer than five days) were analysed for midazolam using a gas chromatography method (Heizmann & von Alten 1981). Concentrations of midazolam in the samples were calculated on the basis of calibration graphs constructed using the peak area ratios of standard samples analysed simultaneously with experimental samples. Assay variation was monitored during each analytical run using standards to calculate intra-day and inter-day variation.

Because samples were taken on either side of the liver, and the perfusion medium flow rate was measured at each sample time, it was possible to calculate a clearance value for midazolam at each time using the following equation:

$$\text{Clearance} = Q\frac{(C_a - C_v)}{C_a} \qquad (1)$$

where C_a = midazolam concentration on the portal side ($ng\,mL^{-1}$), C_v = midazolam concentration on the venous side ($ng\,mL^{-1}$) and Q = the flow rate ($mL\,min^{-1}$). The flow rate remained constant at $20\,mL\,min^{-1}$ throughout the experimental period.

Analysis of the data was by analysis of variance and Newman-Keuls multiple range test, unless otherwise stated.

Results and discussion

Midazolam portal perfusate data are shown in Fig. 1a. Statistical analysis showed there were no significant differences amongst the groups ($P > 0.05$). Fig. 1b illustrates midazolam venous perfusate results. Statistical analysis showed there was a significant difference between the groups ($P < 0.05$). A Newman-Keuls multiple range test indicated that this difference lay between the cimetidine-treated group and control group at 10, 50 and 60 min when the midazolam venous levels were significantly higher compared with controls ($P < 0.05$); at 20, 30 and 40 min, the difference between the two groups was not significant ($P > 0.05$). A statistically significant difference could only be found at 10 min in the ranitidine group and in the famotidine group when the midazolam venous concentrations were significantly higher compared with the control group ($P < 0.05$).

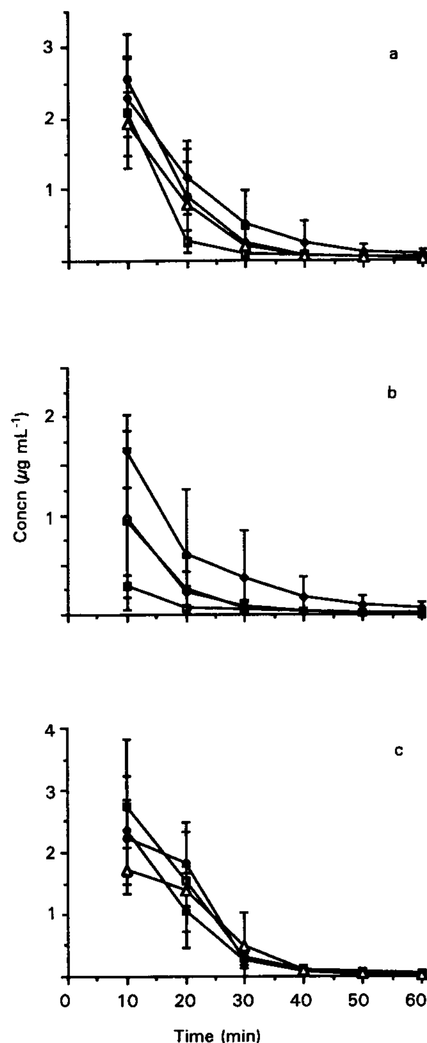

FIG. 1. Comparison of midazolam portal (a), venous (b) and bile (c) perfusate concentrations in the H_2-receptor antagonist-treated groups with those of the control group during a 1-h perfusion experiment. □ Control, ◆ cimetidine, ● ranitidine, △ famotidine.

Table 1. The effect of pre-treatment with H_2-receptor antagonists on mean midazolam clearance (± s.d., n = 5) measured at 10 min.

Group	Mean clearance ± s.d. ($mL\,min^{-1}$)
Control	17·22 ± 0·88
Cimetidine (8 mg)	5·32 ± 3·71
Ranitidine (3 mg)	13·24 ± 5·32
Famotidine (0·4 mg)	10·34 ± 6·29

The mean midazolam clearance values measured at 10 min are shown in Table 1.

At 10 min, the mean clearance value in the cimetidine group was significantly lower compared with the other three clearance values for the control, ranitidine and famotidine groups ($P < 0.05$). No other significant differences in clearance were found between the control and treatment groups at this or any subsequent times.

The mean concentrations of midazolam in bile for each group are presented in Fig. 1c. Statistical analysis indicated that there was no significant difference amongst the groups ($P > 0.05$).

Analysis of midazolam venous data showed there was a significant difference between the cimetidine-treated group and the control group at 10 min. The large inter-individual variability may be responsible for failure to observe significant differences in venous midazolam concentrations between 20 and 40 min. However, significant differences were observed at 50 min, when midazolam was detected in venous samples obtained from only two of the five control rats, and at 60 min when none was detected in the controls. In contrast to this, midazolam was detected in all cimetidine-treated rats at these times.

In the ranitidine- and famotidine-treated groups, the mean midazolam venous levels remained higher than the control levels for the first 20 min; however, this difference was significant only at 10 min. After 30 min, midazolam venous concentrations within these three groups were comparable. From 20 min onwards, the elimination half-life of midazolam, as determined from venous perfusate samples, was not significantly different amongst the groups; any effects that the H_2-receptor antagonists had on the perfused livers were of short duration. A possible explanation for the transient effect of ranitidine and famotidine seen on the venous concentrations of midazolam is that the initial high concentrations of H_2-receptor antagonists are sufficient to temporarily inhibit the metabolism of midazolam by the rat perfused liver.

The midazolam portal and venous data are in agreement with the results of Fee et al (1987), who concluded that following oral dosing, midazolam levels were highest with cimetidine pre-treatment and lowest after no pre-treatment; pre-treatment with ranitidine produced an intermediate effect. This is similar to the data presented here, with ranitidine and famotidine treatment having a less marked effect than cimetidine.

Comparison of the portal and venous data indicates that the inhibitory effect of cimetidine on midazolam metabolism is more clearly seen on the venous side of the liver. This may be explained by considering that a certain concentration of midazolam coming from the outflow side of the liver is returning to a relatively large volume of perfusion medium present within the reservoir and the resultant effect on the concentration of midazolam is minimal. Therefore, an effect on the level of midazolam entering the liver on the inflow (portal) side of the perfused organ from the reservoir will be less obvious. How-

ever, the reverse situation applies to the passage of midazolam through the liver; in this case, the drug is present within a relatively small volume in the perfused organ and a metabolic effect on midazolam will be more marked.

Midazolam clearance was significantly lower in the group treated with cimetidine when compared with the control group ($P < 0.05$). No other significant differences in clearance were found between the control group and other treated groups at this time or at 20 min. Comparison of areas under the curves of midazolam concentration in the venous perfusate vs time (0–60 min, measured by the trapezoidal rule) provided a further indication of an effect of cimetidine on the clearance of midazolam by the perfused liver. Significantly higher ($P < 0.05$, Kruskal-Wallis test) values were obtained in the cimetidine group (1040.7 ± 305.5 h ng mL^{-1}, mean $\pm$ s.d.) relative to the control group (630.5 ± 31.6 h ng mL^{-1}).

Analysis of the bile samples showed that initially, midazolam was being excreted by this route. High levels of the drug were detected over the first 20 min of each perfusion experiment. Thereafter, the concentration of midazolam decreased markedly. Statistical analysis of the data indicated there was no significant difference amongst the groups. Therefore, we conclude that the presence of an H_2-receptor antagonist had no effect on the biliary excretion of midazolam.

It is somewhat surprising that cimetidine did not have a more marked effect on the metabolism of midazolam, which was impaired only in the early stages of the experimental period, following the addition of cimetidine. Midazolam is predominantly metabolized by the hepatic mono-oxygenase system (Heizmann et al 1983; Clausen et al 1988) and cimetidine is a well-known inhibitor of this enzyme system (Speeg et al 1982). An explanation may lie in the multiplicity of the cytochrome P450 enzyme system and the specificity of cimetidine as a P450 enzyme inhibitor.

A number of studies have attempted to identify the specific cytochrome P450 isoenzyme responsible for the biotransformation of midazolam, and it has been designated as a member of the CYP3A sub-family (Fabre et al 1988a, b; Kronbach et al 1989; Gorski et al 1993). Similar work has identified the isoenzyme group which is selectively inhibited by cimetidine. Chang et al (1991, 1992a, b) concluded that cimetidine inhibited a member of the rat hepatic CYP2C sub-family, with little or no effect on CYP3A. In addition, other studies have shown that diazepam is primarily metabolized to its *N*-desmethyl metabolite by a CYP2C isoenzyme, identical to that which is inhibited by cimetidine (Neville et al 1993); this provides an explanation for the early studies which reported an interaction between diazepam and cimetidine. Thus, cimetidine appears to be a selective cytochrome P450 inhibitor, and its effect on the CYP3A sub-family is minimal. This selectivity of action may explain, in part, the limited effect on midazolam metabolism, as indicated by the data presented here.

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