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Screening for Cytochrome P450 3A in Man: Studies with Midazolam and Nifedipine

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Abstract

This report describes work directed towards the development of a screening technique for cytochrome P450 3A activity which should be valid for a variety of drugs metabolized by this enzyme.

A significant correlation ($P < 0.01$) was found between the ratio of the plasma concentration of nifedipine to that of its oxidized metabolite and the area under the time curve for the plasma concentration of midazolam.

It is suggested that the nifedipine : metabolite ratio might have general predictive value for the metabolism of orally administered cytochrome P450 3A substrates.

Cytochromes P450 of the CYP3A gene subfamily account for up to 25% of the total cytochrome P450 present in the liver in the adult man and most of the cytochrome P450 in the small bowel (Watkins 1994). Their particular interest for clinical pharmacologists and pharmacokineticists lies in their ability to oxidize a wide variety both of xenobiotics and natural substrates (Wrighton & Stevens 1992), in their up to 10-fold interindividual variation in activity (e.g. Schellens et al 1988) and in their importance in drug interactions (e.g. Varhe et al 1994).

For clinical studies with a panel of volunteers it would be of great interest to have a screening method for individual cytochrome P450 3A (CYP3A) activity which would give results valid for a variety of drugs. Such information could be used to exclude subjects who were in particular danger of developing side-effects; it could equally be applicable to the selection of metabolically homogenous subject groups or to the interpretation of study results. Unfortunately progress in this direction has been very limited.

No correlation could be found between the pharmacokinetic results from the pairs of CYP3A substrates cortisol and erythromycin (Hunt et al 1992; Watkins et al 1992; Kinirons et al 1993), dapsone and erythromycin (Kinirons et al 1993), dapsone and cortisol (Kinirons et al 1993), alfentanil and erythromycin (Krivoruk et al 1994), and midazolam and erythromycin (Lown et al 1994). One reason for these unsuccessful correlations could be the direct comparison between intravenously and orally administered drugs, which would then be distorted by the high levels of CYP3A in the small bowel (Kinirons et al 1993). A second reason might be the role of distinct members of the CYP3A family in the metabolism of different drugs. Although the most important forms are the almost indistinguishable CYP3A3 and CYP3A4, about 25 to 30% of adult liver specimens contain substantial levels of CYP3A5, which is known to have somewhat different metabolic specificity (Wrighton & Stevens 1992). Successful correlations have, in contrast, been established between the pairs of CYP3A substrates cyclosporin and erythromycin (Watkins et al 1990; Turgeon et al 1994a), cyclosporin and midazolam (Thummel et al 1994a), and OG37-325 and erythromycin (Turgeon et al 1994b). Unfortunately these studies all used patients, mostly critically ill, some of whom had been treated with potent enzyme inducers. The general validity of these results for healthy volunteers is therefore unclear (see Thummel et al 1994b).

We now report the first correlation between the pharmacokinetics of two CYP3A substrates in healthy subjects. The drugs used were two well-established substrates, nifedipine and midazolam (Wrighton & Stevens 1992). The pharmacokinetic parameters examined were the area under the plasma concentration against time curve (AUC) for midazolam and the ratio of the plasma concentrations of nifedipine to its oxidized metabolite (Renwick et al 1992). We deliberately restricted ourselves to orally administered drugs and to the measurement of plasma and not urine concentrations, to avoid distortions in the results arising from differential intestinal metabolism or kidney function.

Methods

Fifteen healthy adult subjects (7 male, 8 female) were included in the study, which was approved by a local ethics committee. The subjects were aged between 24 and 53 and weighed from 49 to 97 kg. After the subjects had given fully informed consent they were administered either a 15-mg capsule of midazolam (Dormicum, Roche) or a 10-mg capsule of nifedipine (Adalat, Bayer) on an empty stomach. Blood samples were taken immediately before drug administration and either at 8 h after (nifedipine) or at 0·5, 1, 2, 3, 4, 5, 6, 8, 12 and 24 h after (midazolam). Plasma samples were prepared and analysed for midazolam or for nifedipine and its pyridine metabolite.

Nifedipine and its primary pyridine metabolite were analysed simultaneously by gas chromatography (Schmid et al 1988). The ratio of the concentrations of nifedipine to metabolite in each sample was then calculated.

Midazolam was analysed by gas chromatography (Allonen et al 1981), with diazepam rather than Ro 21-2212 as internal

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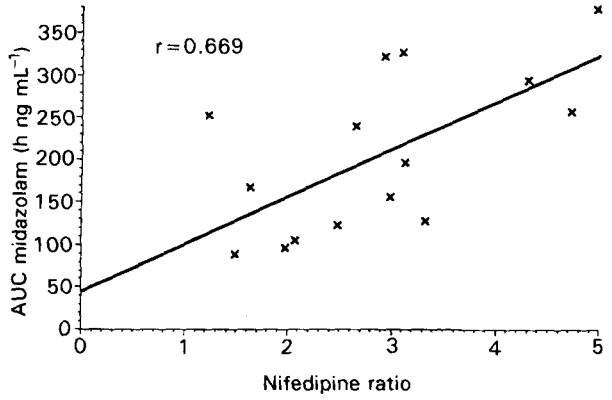

FIG. 1. Least squares linear regression of the individual values for AUC midazolam on the nifedipine metabolite ratio.

standard. The area under the midazolam concentration-time curve (AUC) was calculated by linear trapezoidal integration from zero time to the last time with a quantified concentration, adding the term C (last quantified)/k_{el} for the residual area. The best linear regression between the individual values of the AUC for midazolam and the metabolite ratio for nifedipine was fitted and the coefficient ratio of correlation calculated (SAS, Cary, NC, USA). The P value for the existence of a correlation was taken from Sachs (1992).

Results and Discussion

Fig. 1 shows the regression of the AUC for midazolam on the nifedipine metabolite ratio. The coefficient of correlation was calculated as $r = 0.669$, corresponding to $P < 0.01$ for the existence of a correlation. The calculation of the nifedipine metabolite ratio effectively normalizes for the differential absorption of the drug. Without this correction there is still a significant, but weaker correlation between nifedipine concentration and AUC midazolam, with $r = 0.455$ ($P < 0.05$). Normalization of the midazolam AUCs would also have been possible in principle, had the main metabolite, 1-hydroxymidazolam, been available to us. It is reasonable to expect that this would have resulted in a substantial improvement in the correlation found.

Much further work is necessary before it can be said that the measurement of the nifedipine metabolite ratio is of established value in clinical trials with CYP3A substrates. One important aspect is extension of the studies to other drugs, particularly those which are not substrates of CYP3A5 (as are nifedipine and midazolam). More attention should also be given to possible intraindividual variation in the nifedipine metabolite ratio. Because of the known role of CYP3A in steroid metabolism (e.g. Guengerich et al 1986) changes in activity during the menstrual cycle might be a real possibility.

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