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Comparative effects of antifungal agents on zidovudine glucuronidation by human liver microsomes

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Zidovudine (ZDV) is extensively metabolised by the liver to an inactive glucuronide (GZDV). Since ZDV is often administered with antimycotic drugs, we studied the effect of six systemic antifungal agents on the *in vitro* glucuronidation of ZDV by human liver microsomes. 5-fluorocytosine and itraconazole had no inhibitory effect whereas amphotericine B, ketoconazole, miconazole and fluconazole inhibited *in vitro* GZDV formation (K_i values were 0.13, 0.08, 0.18 and 1.4 mM respectively).

Keywords antifungal agents zidovudine liver microsomes glucuronidation

Introduction

Zidovudine (3'-azido-3'-deoxythymidine; ZDV) is widely used in human immunodeficiency virus infected patients and is often administered with other drugs including antimycotic agents.

ZDV is mostly metabolised to an inactive 5'-*O*-glucuronide; GZDV. Any inhibition of this detoxication pathway could result in enhanced toxicity or modified efficacy [1, 2]. In a previous study, we demonstrated that amphotericine B and miconazole are able to inhibit ZDV glucuronidation [3]. Sahai *et al.* [4] have reported that fluconazole increased ZDV serum concentrations and decreased its apparent oral clearance. In addition, imidazole agents are well-known potent inhibitors of cytochrome P-450 isozymes [5–8] whereas their effect on UGT (uridine diphosphoglucuronosyltransferase) is relatively unknown.

In this context, we studied the effect of six antifungal agents: 5-fluorocytosine, amphotericine B, ketoconazole, miconazole, fluconazole, and itraconazole on ZDV glucuronidation by human liver microsomes.

Methods

Chemicals and reagents

[^{3}H]-Zidovudine (3 Ci mmol^{-1}, radiochemical purity > 95%), UDPGA (uridine 5'-diphosphoglucuronic acid), ZDV, Tris HCl, Brij 58, miconazole, and amphotericine B, were obtained from Sigma (Saint Quentin Fallavier, France). Fluconazole, itraconazole, ketoconazole and 5-fluorocytosine were generous gifts from Pfizer (Orsay, France), Janssen (Val de Rueil, France) and Roche laboratories (Basel, Switzerland).

Human liver microsome preparation and ZDV-UGT activity determination

Microsomal fractions were obtained from one human liver by using a differential centrifugation technique as previously described [9, 10]. ZDV-UGT activity was determined as previously described [3]. All assay mixtures contained 0.2 M Tris/HCl buffer (pH 7.5), 10 mM $MgCl_2$, 3 mg ml^{-1} microsomal protein, 0.5 mg Brij 58/mg protein and 0.5 to 2 mM [^{3}H]-ZDV in a final reaction volume of 300 µl. Reactions were started by adding 4 mM UDPGA and stopped by 200 µl methyl alcohol. Aliquots of the supernatant were analysed by h.p.l.c. [11].

Inhibition studies

Prior to inhibition studies, the apparent K_m value for ZDV glucuronidation was determined in the microsomal sample used. Activity was measured for eight AZT concentrations in the 0.5–7.5 mM range. In preliminary experiments, ZDV concentration was 0.5 mM and antifungal concentrations were 0.1, 1 and 5 mM. Antifungal agents were dissolved in DMSO. It was necessary to slightly heat (40° C) the solution to obtain dissolution of ketoconazole. The percentage of DMSO never exceeded 1% (v/v) in the incubation medium. When total inhibition was observed for at least one concentration, we determined the apparent inhibition constant K_i by graphical analysis of Dixon

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plot. Each inhibitor concentration (0, 0.05, 0.1, 0.15, 0.25, 0.35, 0.5 mM) was tested with three ZDV concentrations (0.5, 1, 2 mM). All experiments were performed in triplicate.

H.p.l.c. techniques

ZDV and GZDV were separated by an h.p.l.c. method as previously described [11]. Reverse phase chromatography was carried out using a nuleosil C_{18} column (5 μm, 250 mm × 4.6 mm, Interchrom, Montluçon, France). The mobile phase was a 15:85 mixture of acetonitrile-ammonium phosphate buffer. Retention times for ZDV and GZDV were 10 and 6 min respectively. Detection of tritiated compounds was performed using a radio h.p.l.c. detector (Flow One A250 Packard instruments, La Queue les Yvelines, France).

Results

The K_m value for ZDV glucuronidation was 3.7 mM. The inhibitory effect of antifungals on ZDV glucuronidation by human liver microsomes is shown in Table 1. The results show that 5-fluorocytosine and itraconazole have no effect even at the highest concentration (5 mM). Fluconazole totally inhibited ZDV glucuronidation at the highest concentration (5 mM). Fluconazole totally inhibited ZDV glucuronidation at the highest concentration (5 mM); at 1 mM, a slight inhibition was observed and at 0.1 mM there was no inhibition. Ketoconazole, miconazole and amphotericine B produced marked inhibition at 1 mM. For these three drugs, we observed a more pronounced inhibitory effect than fluconazole at 0.1 mM.

Dixon plots for inhibition of ZDV glucuronidation by amphotericine B, ketoconazole, miconazole, and fluconazole, are shown in Figure 1. These plots were consistent with competitive inhibition. K_i values were 0.13, 0.08, 0.18 and 1.4 mM respectively. These results confirmed that ketoconazole was the strongest inhibitor of the six studied compounds.

The results indicate that the rank order of the drugs with respect to their *in vitro* inhibitory effect on ZDV glucuronidation was ketoconazole > amphotericine B > miconazole > fluconazole >> itraconazole and 5-fluorocytosine.

Table 1 Screening of different drugs as inhibitors of ZDV glucuronidation in human liver microsomes

	% of control enzyme activity at drug concentration		
Antifungal agents	*0.1 mM*	*1 mM*	*5 mM*
5-fluorocytosine	97.6 ± 3.1	99.7 ± 7.2	108 ± 5.6
Fluconazole	99.2 ± 13.5	86.5 ± 6.2	0
Ketoconazole	77.4 ± 11.7	0	0
Miconazole	73.6 ± 8.7	0	0
Amphotericin B	81.4 ± 4.1	0	0
Itraconazole	105 ± 6.2	106 ± 1.9	98.7 ± 10.8

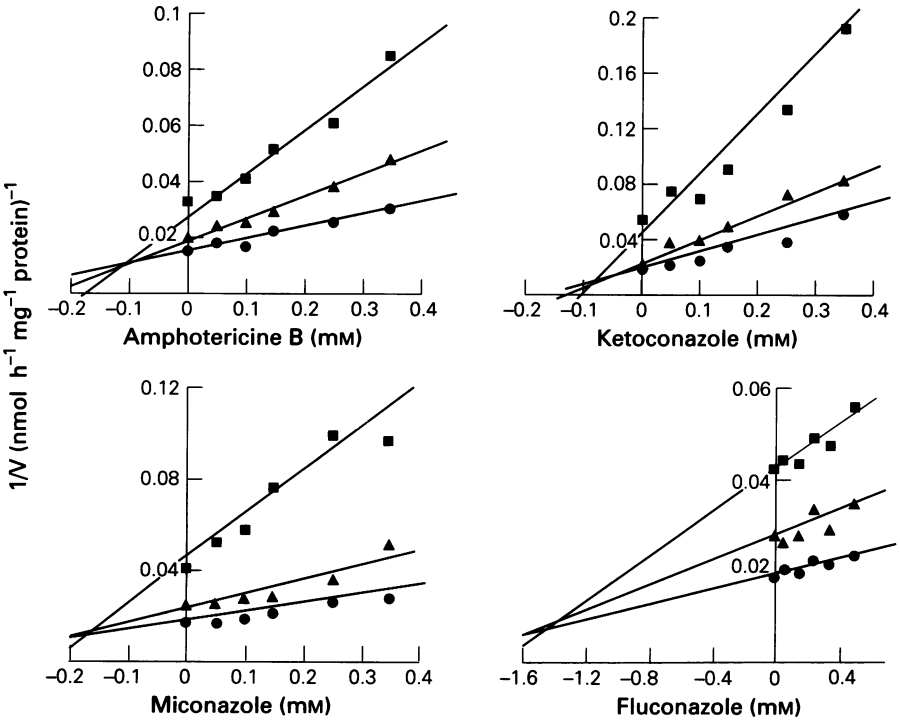

Figure 1 Dixon plots for inhibition of ZDV glucuronidation in human liver microsomes. Inhibitors were amphotericine B, ketoconazole, miconazole and fluconazole. ZDV concentrations were 0.5 (■), 1 (▲) and 2 (●) mM. Each point represents the mean value of three independent determinations.

Discussion

There is currently little information on the pharmacokinetics of ZDV when administered with antifungal agents. Sahai *et al.* [4] have recently shown that fluconazole, modified ZDV pharmacokinetics (increased C_{max}, $t_{1/2}$, AUC). The exact mechanism of the inhibition of ZDV glucuronidation is unknown. Animal data indicate that at a dose of 160 mg kg^{-1}, fluconazole slightly decreases UGT activity [12]. However, this is a much higher dose than that used in clinical practice. On the other hand, like ZDV, fluconazole forms an ether glucuronide although plasma concentrations are negligible [13].

Since fluconazole is only a weak *in vitro* inhibitor of ZDV glucuronidation and yet modified ZDV pharmacokinetics *in vivo*, we could hypothesize that miconazole and ketoconazole, which are more potent *in vitro* inhibitors, could also modify ZDV pharmacokinetics *in vivo*.

The present study confirms the lack of metabolic interaction between itraconazole and ZDV, as demonstrated *in vivo* albeit with limited data by Henriveau *et al.* [14]. It should be noted that in this latter study, a large intrasubject variability was observed and the pharmacokinetic profile was established using only four blood samples per subject. More than 30 metabolites have been described for itraconazole [15]. Glucuronidation has not been reported for this drug and this could explain the lack of interaction. However, we also demonstrated that miconazole is a strong inhibitor of ZDV glucuronidation and glucuronides have not been described for this drug [16]. Miconazole could therefore interact with UGT by an unknown mechanism (formation of inactive complex, steric hindrance, physical or chemical perturbation of the microsomal membrane, etc).

We recognize that the concentrations of antifungal drugs used were high. However, the K_m for ZDV glucuronidation is high (3.7 mM) and many studies on drug-drug interactions with ZDV [17,18] have used very high concentrations (1 to 10 mM). Since such concentrations are well in excess of plasma concentrations it may be considered that comparison between *in vitro* and *in vivo* data are highly speculative. However, interactions occur at the hepatic enzyme level where *in vivo* drug concentrations are unknown. In the particular case of azole compounds, tissue concentrations are sometimes higher than plasma concentrations [15,19]. Nevertheless, microsomal fractions are only an *in vitro* model and *in vitro* results must be extrapolated with caution with regard to the effect of drugs on ZDV glucuronidation *in vivo*.

From a structural point of view, it is interesting to note that the more recent azoles, itraconazole and fluconazole, which are triazole antifungals were less potent inhibitors than the diazole compounds miconazole and ketoconazole. It will be of interest to evaluate their effects on the metabolism of other glucuronidated drugs.

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