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# INFLUENCE OF PROBENECID AND PARACETAMOL (ACETAMINOPHEN) ON ZIDOVUDINE GLUCURONIDATION IN HUMAN LIVER *IN VITRO*

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## ABSTRACT

The effects of probenecid and paracetamol on zidovudine glucuronidation were investigated, *in vitro*, using human liver microsomal preparations. The presence of probenecid in the incubation medium significantly reduced the maximum reaction velocity for zidovudine glucuronide formation by more than 60 per cent, and the $K_m$ was reduced by 47 per cent, suggesting an uncompetitive inhibition of zidovudine glucuronidation. In contrast, paracetamol had no significant effect on zidovudine glucuronidation. The maximum reaction velocity for zidovudine glucuronide formation and the $K_m$ were unchanged when paracetamol (5 mM) was present in the incubation medium. The effects of probenecid and paracetamol on zidovudine metabolism *in vitro* correlates closely with those observed *in vivo*. The *in vitro* system of human liver microsomes may have a useful role in predicting the possible interaction of other drugs with zidovudine metabolism.

KEY WORDS Zidovudine Probenecid Paracetamol (acetaminophen) Human liver microsomes

## INTRODUCTION

Zidovudine (3′-azido-3′-deoxythymidine, Retrovir) has been shown to have beneficial effects in certain patients with acquired immunodeficiency syndrome (AIDS)[1,2] and it reduces the frequency of opportunistic infections.[3] In man zidovudine undergoes first-pass metabolism (30–40 per cent) and is extensively metabolized (60 per cent) to an ether glucuronide (3′-azido-3′-deoxy-5′-β-D-glucopyranurosylthymidine). Because of this rapid metabolism by glucuronidation zidovudine has a high clearance (1500 ml $min^{-1}$) and a short half-life of about 1 h.[4–6]

Probenecid has recently been reported to reduce the clearance of zidovudine by two-fold, when given concurrently to patients with AIDS. The urinary

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excretion of zidovudine glucuronide was also reduced in these patients.[7] Probenecid is an organic acidic compound, which has been shown to reduce the renal elimination of many acidic drugs.[8-10] Probenecid is also eliminated, in part, by glucuronidation[11] and has been demonstrated to reduce the formation of glucuronides of many drugs by inhibition of glucuronidation.[12,13] Therefore, apart from its effects on the renal system, probenecid may decrease elimination of zidovudine at least partly by inhibition of zidovudine glucuronidation.

Many AIDS patients may require paracetamol (acetaminophen) for relief of pain or fever. Paracetamol also undergoes conjugation with glucuronic acid and about 60 per cent of a therapeutic dose of the drug is converted to its glucuronide metabolite.[14] Paracetamol may therefore alter the pharmacokinetics of zidovudine by competing for conjugation with glucuronic acid.

In the present study, the mechanism of potential inhibitory effects of probenecid and paracetamol on zidovudine glucuronidation was investigated *in vitro*, using preparations of human liver microsomes.

## MATERIALS AND METHODS

### *Tissue preparation and incubation*

Human livers were obtained 2–4 h after death due to cerebral haemorrhage, from six Caucasian donors (five males, age range 32–69 years) who had, during life, normal conventional tests (bilirubin, aspartate transaminase, alkaline phosphatase) of liver function. Liver samples were immediately frozen at $-80°$. Liver homogenates were prepared at 4° with 4 volumes of 0·25 M sucrose using a glass-Teflon homogenizer. The microsomal fraction was separated by differential centrifugation.[15] The microsomal pellet was washed three times and resuspended with 0·1 M phosphate buffer, pH 7·4. The protein content of the microsomal fractions was determined according to the method of Lowry *et al.*[16] Incubations were performed in duplicate at 37°C in air for 30 min in a total volume of 250 µl, which contained 200 mM phosphate buffer pH 7·4, microsomes (2 mg protein $ml^{-1}$), $MgCl_2$ (10 mM), uridine diphosphoglucuronic acid (UDPGA, 3 mM), Triton X-100 (0·0025 w/v), probenecid or paracetamol (5 mM) and five different concentrations of zidovudine in the 0·25 mM–5·0 mM range. The reaction was initiated by the addition of UDPGA and was terminated by adding 30 per cent perchloric acid (50 µl). Following centrifugation (2500 × g, 5 min), the supernatant was stored at $-20°$C prior to analysis for zidovudine glucuronide by high performance liquid chromatography (HPLC). Zidovudine glucuronide formation was found to be linear up to 60 min for the above experimental conditions at all five concentrations of zidovudine used (data not shown).

### *Analytical procedures*

Measurement of zidovudine-glucuronide in the samples incubated with probenecid/paracetamol and zidovudine was carried out using a modified

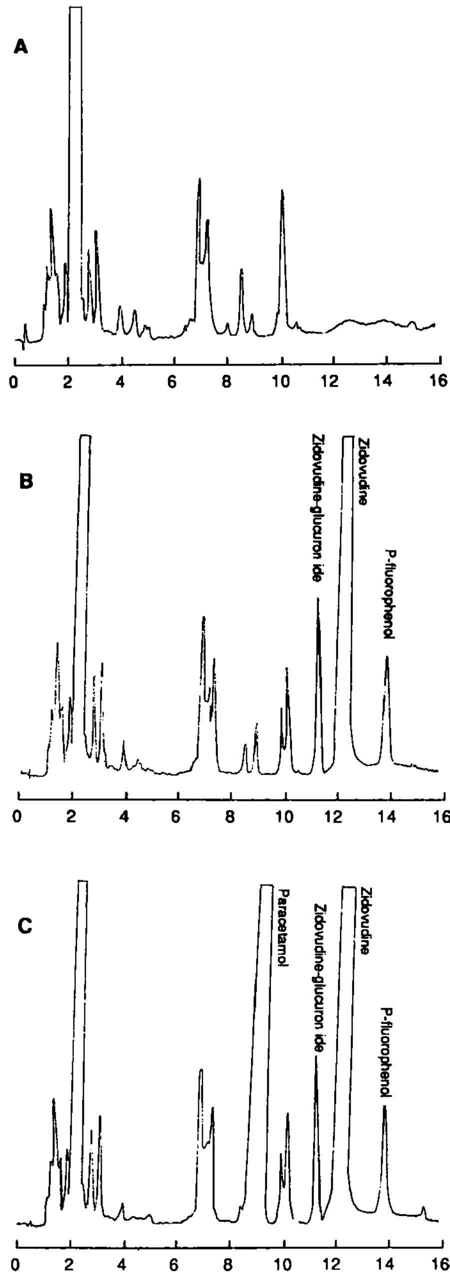


Figure 1. Elution patterns for (A) a blank microsomal sample following a 30 min incubation, (B) a microsomal sample following a 30 min incubation with zidovudine, and (C) a microsomal sample following a 30 min incubation with zidovudine and paracetamol

reverse-phase HPLC method[17] with *p*-fluorophenol as the internal standard. The modification involved the use of a solvent gradient, instead of an isocratic elution, in which the concentration of acetonitrile in the elution was varied from 5·25 to 21 per cent. This resolved the zidovudine glucuronide peak from other endogenous interfering peaks. The elution was started with 15 per cent solvent B (phosphate buffer, 20 mM, pH 2·7:acetonitrile [65:35]) and 85 per cent solvent A (phosphate buffer, 20 mM, pH 2·7). The concentration of solvent B was varied by a four-step gradient programme over a 15 min period as follows; 15–40 per cent in 7 min, 40–60 per cent in 3 min, at 60 per cent for 5 min, and 60–15 per cent in 5 min. The retention times for zidovudine glucuronide, zidovudine, and *p*-fluorophenol were 11·1, 12·5, and 14·1 min, respectively (Figure 1). The intra-assay coefficient of variation for all the measurements was less than 5 per cent. Paracetamol (Rt = 8·9 min) was also detected by the assay, but it did not interfere with the peaks of interest (Figure 1). Probenecid did not show an absorbance at the 267 UV wavelength.

### *Analyses of results*

The data were analysed by Direct Linear Plot[18] of the reaction velocity (pmole zidovudine-glucuronide formed $mg^{-1}$ protein $min^{-1}$) versus zidovudine concentration (mM). Statistical significance was tested using a paired Student's *t*-test. The value of $p<0{\cdot}05$ was taken to be statistically significant. Uncompetitive inhibition reduces both $V_{max}$ and $K_m$. The inhibitor–enzyme dissociation constant ($K_i$) for an uncompetitive inhibitor was calculated thus:

$$V^{I}_{max} = V_{max}/(1 + [I]/K_i).$$

Where $V^{I}_{max}$ is the maximum reaction velocity in the presence of the inhibitor and $[I]$ is the inhibitor concentration. The values are given as the mean ± SD.

## RESULTS AND DISCUSSION

A total of six different livers were used for each of the studies. The presence of probenecid in the incubation medium significantly decreased both the maximum reaction velocity (by 60 per cent) and the $K_m$ (by 47 per cent) for zidovudine glucuronidation (Table 1). The inhibitor-enzyme (probenecid-UDPG-transferase) dissociation constant $K_i$ was $2{\cdot}16 \pm 2{\cdot}24$ mM. Previous reports have indicated that probenecid reduces the rate of formation of glucuronide conjugates of many drugs as a direct result of inhibition of the glucuronidation pathway.[12,13] It has also been demonstrated that probenecid causes a considerable inhibition *in vitro* of UDPG-transferase activity in microsomal preparations.[19] The effect of probenecid on the maximum reaction velocity and the $K_m$ *in vitro* suggests that probenecid produces a strong uncompetitive

Table 1. The mean ($\pm$SD) maximum reaction velocity and the apparent $K_m$ for zidovudine glucuronidation with and without the presence of probenecid and paracetamol in the incubation medium ($n=6$)

| | $V_{max}$ (pmoles mg protein$^{-1}$ min$^{-1}$) | $K_m$ (mM) |
|---|---|---|
| Without probenecid | 372·7 ± 144·8 | 0·67 ± 0·15 |
| With probenecid | 136·3 ± 125·4* | 0·33 ± 0·12† |
| Without paracetamol | 363·0 ± 159·4 | 0·45 ± 0·15 |
| With paracetamol | 358·5 ± 177·5 | 0·56 ± 0·22 |

*$p<0.001$; †$p<0.05$.

inhibition of zidovudine glucuronidation. This may be due to probenecid combining with the glucuronyl transferase-zidovudine complex to produce an inactive enzyme-substrate inhibitor complex, which in turn cannot undergo further reaction to yield zidovudine glucuronide, or an interaction with an allosteric site on the glucuronyl transferase. An earlier study has demonstrated that probenecid is also an uncompetitive inhibitor of paracetamol glucuronidation.[20] The results of the *in vitro* study indicate that probenecid may also interfere with the elimination of zidovudine *in vivo*, by inhibiting zidovudine glucuronidation. This is supported by the finding of a reduction in urinary recovery of zidovudine glucuronide in AIDS patients who were administered zidovudine and probenecid concurrently.[7]

Like zidovudine, paracetamol is also extensively metabolized by conjugation with glucuronic acid. Paracetamol may therefore alter the pharmacokinetics of zidovudine by competing with zidovudine glucuronidation. The presence of paracetamol in the incubation medium had virtually no effect on either the maximum reaction velocity or the $K_m$ for zidovudine glucuronide formation (Table 1). This indicates that unlike probenecid, paracetamol has no significant inhibitory effect on zidovudine glucuronidation.

The effect of paracetamol on zidovudine disposition in man *in vivo* is unclear. Although an earlier study suggested a potentially adverse zidovudine-paracetamol interaction,[21] two recent investigations have found that paracetamol does not alter zidovudine pharmacokinetics.[22,23] The results of our study show that paracetamol has no effect on zidovudine metabolism in microsomes prepared from livers of donors with normal liver function, in concentrations similar to those observed in plasma during therapeutic dosing. Furthermore, the high $K_m$ of 13·4 mM for paracetamol glucuronidation in man[24] suggests that paracetamol is a poor substrate for glucuronyl transferase *in vitro*. However, many patients with AIDS have abnormalities of liver function secondary to previous infections such as hepatitis viruses. Furthermore, zidovudine has a narrow therapeutic index. Therefore, it remains possible for paracetamol to have a clinically significant effect on zidovudine disposition in AIDS patients with severely compromised hepatic drug metabolizing ability.

The kinetic data for zidovudine glucuronidation obtained in this study ($V_{max} = 327 \cdot 2$ pmoles min$^{-1}$ mg$^{-1}$, $K_m = 0 \cdot 67$ mM) are quite different to those reported in previous studies, using human liver microsomes: $V_{max} =$ 966 pmoles min$^{-1}$ mg$^{-1}$, Km = 2·7 mM,[25] $V_{max} = 2700$ pmoles min$^{-1}$ mg$^{-1}$, $K_m = 2 \cdot 6$ mM,[26] $V_{max} = 980$ pmoles min$^{-1}$ mg$^{-1}$, $K_m = 13$ mM,[27] and $V_{max} =$ 1133 pmoles min$^{-1}$ mg$^{-1}$, $K_m = 2 \cdot 6$ mM.[28] Variance in experimental techniques could explain these anomalies to some extent, but more appropriately could be due to differences in the nature and the quality of liver specimens used in the *in vitro* experiments. Factors such as age, sex, race, as well as previous treatment with drugs and the social background of the donors could play an important role in the drug metabolizing ability of the microsomal preparations obtained from liver biopsies. The method of collection and storage of liver specimens is also important in preserving the drug metabolizing enzymes. However, apart from one,[28] none of the other reports provide such details.

In conclusion, probenecid, but not paracetamol, inhibits zidovudine metabolism, *in vitro*, in human liver microsomes. The results of the present study indicate that the route of metabolism of zidovudine *in vitro* by human liver microsomes is a good predictor of the metabolism of zidovudine *in vivo*. It is anticipated that the *in vitro* system may provide a useful tool for elucidating the possible interactions of other drugs with zidovudine metabolism *in vivo*.

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