

Differential inhibition of cytochrome P450 isoforms by the protease inhibitors, ritonavir, saquinavir and indinavir

V. A. Eagling, D. J. Back & M. G. Barry

Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, UK

Aims To compare the inhibitory potential of the HIV protease inhibitors saquinavir, ritonavir and indinavir against CYP1A2, CYP2C9, CYP2E1 and CYP3A4 catalysed metabolic reactions in human liver microsomes *in vitro*.

Methods Microsomes from six human livers were utilized in this study. The probe substrates were phenacetin (CYP1A2), tolbutamide (CYP2C9), chlorzoxazone (CYP2E1) and testosterone (CYP3A4). Metabolites were analysed by high performance liquid chromatography. IC_{50} (concentration of inhibitor giving 50% decrease in enzyme activity) and, where appropriate, K_i values were calculated.

Results Ritonavir was a very potent inhibitor of CYP3A4 mediated testosterone 6 β -hydroxylation (mean $K_i = 0.019 \pm 0.004 \mu\text{M}$, mean \pm s.d.; $n = 6$) and also inhibited tolbutamide hydroxylation ($IC_{50} = 4.2 \pm 1.3 \mu\text{M}$, mean \pm s.d.; $n = 6$). Inhibition of phenacetin *O*-deethylation and chlorzoxazone 6-hydroxylation was negligible. Indinavir was an order-of-magnitude less potent in inhibiting CYP3A4 ($K_i = 0.17 \pm 0.01 \mu\text{M}$) and did not produce appreciable inhibition of the CYP1A2, CYP2C9 or CYP2E1 catalysed reactions. Saquinavir was the least potent CYP3A4 inhibitor ($K_i = 2.99 \pm 0.87 \mu\text{M}$) and produced some inhibition of CYP2C9 (approximately 50% at 50 μM).

Conclusions The HIV protease inhibitors have differential effects on CYP isozymes. There is obvious potential for clinically significant drug interactions particularly with ritonavir. Pharmacokinetic drug interaction studies are crucial to gain an overall understanding of the beneficial and potentially harmful effects of this important group of drugs.

Keywords: protease inhibitors, CYP450 isozymes, inhibition.

Introduction

The three major genes (*env*, *gag* and *pol*) of human immunodeficiency virus type 1 (HIV-1) encode polyproteins (gp160, gag and gag-pol) which undergo post-translational proteolytic processing by either cellular [1] or virally encoded proteinase enzymes [2]. Considerable effort has been expended in recent years in the development of inhibitors of the virally-encoded protease of HIV. If this enzyme is non-functional, immature non-infectious virions are produced. Initial clinical studies suggest that the protease inhibitors are very potent anti-HIV drugs with the three compounds licensed for use ritonavir, saquinavir and indinavir all reported to produce marked reductions in plasma viral load, particularly when used in combination with nucleoside analogue reverse transcriptase inhibitors [3–8]. However, there are important pharmacokinetic issues relating to the use of protease inhibitors. For example, bioavailability appears to be limited with several compounds, most notably saquinavir, due to substantial first-pass metabolism by CYP3A4 [9]. Both ritonavir and indinavir are also metabolised by CYP3A4 [10, 11] and in addition, ritonavir is a potent inhibitor of CYP3A-mediated reactions [10]

showing clinically significant interactions with other co-administered drugs [9, 12–14]. Ritonavir has recently been reported to increase the area under the plasma concentration-time curve of saquinavir by more than 20-fold [14] in a group of HIV + patients.

The aim of the present work was to compare the effect of ritonavir, saquinavir and indinavir on different CYP isoforms in human liver microsomes *in vitro*. The following probe substrates were used: phenacetin (CYP1A2 [15]), tolbutamide (CYP2C9[16]), chlorzoxazone (CYP2E1[17]) and testosterone (CYP3A4[18]).

Methods

Chemicals

Phenacetin, paracetamol, metacetamol, tolbutamide, chlorzoxazone, zoxazolamine, testosterone, 6 β -OH testosterone, 11 β -OH testosterone and β -NADPH (reduced form) were purchased from the Sigma Chemical Company (Poole, Dorset, U.K.). 6-Hydroxychlorzoxazone was purchased from Ultrafine Chemicals (Salford, UK). Chlorpropamide and 4-OH tolbutamide were gifts from Hoechst AG (Frankfurt, Germany); ritonavir was a gift from Abbott Laboratories (Illinois, USA); saquinavir was a gift from

Correspondence Professor D. J. Back, Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, L69 3GE, UK.

Roche Products Ltd. (Welwyn Garden City, UK) and indinavir was a gift from Merck (West Point, USA). H.p.l.c. grade acetonitrile (AcN), dichloromethane (DCM), methanol and ethyl acetate were purchased from Fisons plc (Loughborough, UK). All other reagents were of the highest grade possible.

Human liver samples

Histologically normal human livers were obtained from kidney transplant donors. Consent for their removal was obtained from the donor's relatives and Ethics Committee approval was granted for their use in this study. Liver tissue (10–20g portions) was frozen in liquid nitrogen and stored at -80°C until required. Washed microsomes (105,000g pellets) were prepared from human liver samples by the classical differential centrifugation technique and microsomal protein yield was determined by the method of Lowry *et al.* [19] using bovine serum albumin as standard.

Enzyme assays for CYP probes

Phenacetin O-deethylation A 500 μl reaction mixture typically contained 0.5 mg microsomal protein, phenacetin (20 μM), MgCl_2 (10 mM) and NADPH (2.5 mM) in 0.067 M phosphate buffer (pH 7.4). Metacetamol was added as internal standard and samples extracted with DCM (10 ml; 20 min) to remove unmetabolized phenacetin followed by ethyl acetate (10 ml; 20 min). Samples were evaporated to dryness and reconstituted in mobile phase (200 μl) prior to h.p.l.c. analysis. Paracetamol and metacetamol were separated using a mobile phase (flow rate 1 ml min^{-1}) consisting of 10% AcN: 90% sodium phosphate buffer (0.1M; pH 4.3; v:v) and a Spherex 5 μC_{18} column (25 cm \times 4.6 mm; Phenomenex, Macclesfield, UK) with u.v. detection at 254 nm. Paracetamol formation was quantified by interpolating peak height ratios of paracetamol and metacetamol from a standard curve of known paracetamol concentrations. Inter- and intra-assay coefficients of variation were 8.7% and 6.5% respectively determined at 100 pmol paracetamol. The lower limit of determination was 25 pmol.

Tolbutamide 4-hydroxylation A 500 μl reaction mixture contained 0.5 mg microsomal protein, tolbutamide (100 μM), MgCl_2 (10 mM) and NADPH (1 mM) in 0.067 M phosphate buffer (pH 7.4). Termination, extraction of samples and h.p.l.c. conditions were as previously described [20]. The inter- and intra-assay coefficients of variation were 3% and 1.3% respectively determined at 0.6 nmol hydroxytolbutamide. The lower limit of determination was 20 pmol.

Chlorzoxazone 6-hydroxylation A 500 μl incubation volume contained 0.2 mg microsomal protein, chlorzoxazone (100 μM), MgCl_2 (10 mM) and NADPH (1 mM) in 0.067 M phosphate buffer (pH 7.4). The reaction was terminated by the addition of zoxazolamine as internal standard and extracted with DCM (5 ml; 10 min). The organic phase was evaporated to dryness and reconstituted into mobile phase (200 μl) prior to h.p.l.c. analysis. Metabolite formation was quantified by interpolating peak height ratios of

6-hydroxychlorzoxazone and zoxazolamine from a standard curve of known 6-hydroxychlorzoxazone concentrations with u.v. detection at 295 nm. A 5 μC_{18} Spherex column (25 cm \times 4.6 mm; Phenomenex, Macclesfield, UK) was employed to separate chlorzoxazone, 6-hydroxychlorzoxazone and internal standard using a gradient mobile phase system. Initial chromatographic conditions were 28% AcN:72% ammonium acetate buffer (0.05 M; pH 3.3; v:v), followed by a linear increase of AcN to 33% between 10 and 15 min remaining so until 17 min then returning to the original run conditions at 20 min. This was followed by a 5 min re-equilibration period. The inter- and intra-assay coefficients of variation were 5.3 and 8.5% respectively determined at 3 nmol hydroxychlorzoxazone. The lower limit of determination was 100 pmol.

Testosterone 6 β -hydroxylation A 500 μl incubation contained 0.05 mg microsomal protein, testosterone, MgCl_2 (10 mM) and NADPH (2.5 mM) in 0.067 M phosphate buffer. The reaction was terminated by the addition of 11 β -hydroxytestosterone as internal standard and immediate extraction with DCM (10 ml; 20 min). The DCM layer was then evaporated to dryness and reconstituted with mobile phase (200 μl) prior to h.p.l.c. analysis. Briefly, a 50 μl aliquot was injected onto a 5 μC_{18} Prodigy column (15 cm \times 4.6 mm; Phenomenex, Macclesfield, UK) and separated from 11 β -hydroxytestosterone, testosterone and other metabolites by a gradient mobile phase system comprising of solvent mixtures: mixture A 35:64:1 methanol:distilled water:AcN (v:v:v) and mixture B 80:18:2 methanol:distilled water:AcN (v:v:v). Initial run conditions were 75% A: 25% B. Between 9 and 28 min there was a linear increase of solvent B to 75% (25% A) remaining so until 30 min. Between 30 and 32 min there was a linear decrease back to the starting run conditions (25% A: 75% B) with a 3 min re-equilibration period. U.v. detection was at 254 nm. The inter- and intra-assay coefficients of variation were 5.6% and 4.3% respectively, determined at 500 pmol 6 β -hydroxytestosterone. The assay had a lower limit of determination of 50 pmol.

Inhibitor studies A range of protease inhibitor concentrations (up to 100 μM) were screened against a single concentration of each probe substrate. IC_{50} values (i.e. concentration producing 50% inhibition of control enzyme activity) were determined (where appropriate) for each compound using an iterative program GRAFIT 3.0 (Erithacus Software Ltd). In addition, the apparent K_i was determined for each of the protease inhibitors with testosterone as substrate (concentrations of testosterone 50–400 μM) by substituting K_m and V_{max} values in the appropriate equations. All kinetic studies were performed under linear conditions with respect to time and protein concentration.

Results

The six human livers used in this study were from patients (4M; 2F) between 10–41 years old. The protease inhibitors had differential effects on the CYP isoforms (Table 1, Figure 1). Ritonavir was an extremely potent inhibitor of CYP3A4 catalysed testosterone 6-hydroxylation

Table 1 Inhibition of CYP activities in human liver microsomes by protease inhibitors.

Protease inhibitor	Phenacetin <i>O</i> -deethylation ^a (CYP1A2)	Tolbutamide 4-hydroxylation ^b (CYP2C9)	Chlorzoxazone 6-hydroxylation ^c (CYP2E1)	Testosterone 6 β -hydroxylation ^d (CYP3A4)	
	IC ₅₀ * (μ M)	IC ₅₀ * (μ M)	IC ₅₀ * (μ M)	IC ₅₀ * (μ M)	K _i (μ M) +
Ritonavir	> 100	4.2 \pm 1.3	> 100	0.034 \pm 0.013	0.019 \pm 0.004
Saquinavir	> 100	53.9 \pm 9.9	> 100	2.14 \pm 0.48	2.99 \pm 0.87
Indinavir	> 100	> 100	> 100	0.43 \pm 0.14	0.17 \pm 0.01

* IC₅₀ values obtained from incubations with microsomes from six livers; where appropriate data are mean \pm s.d. + Values represent mean \pm s.d. of K_i determinations in microsomes from three human livers ^aControl enzyme activity = 0.276 \pm 0.110 nmol mg⁻¹ min⁻¹ ^bControl enzyme activity = 0.103 \pm 0.026 nmol mg⁻¹ min⁻¹ ^cControl enzyme activity = 1.110 \pm 0.043 nmol mg⁻¹ min⁻¹ ^dControl enzyme activity = 2.71 \pm 1.66 nmol mg⁻¹ min⁻¹.

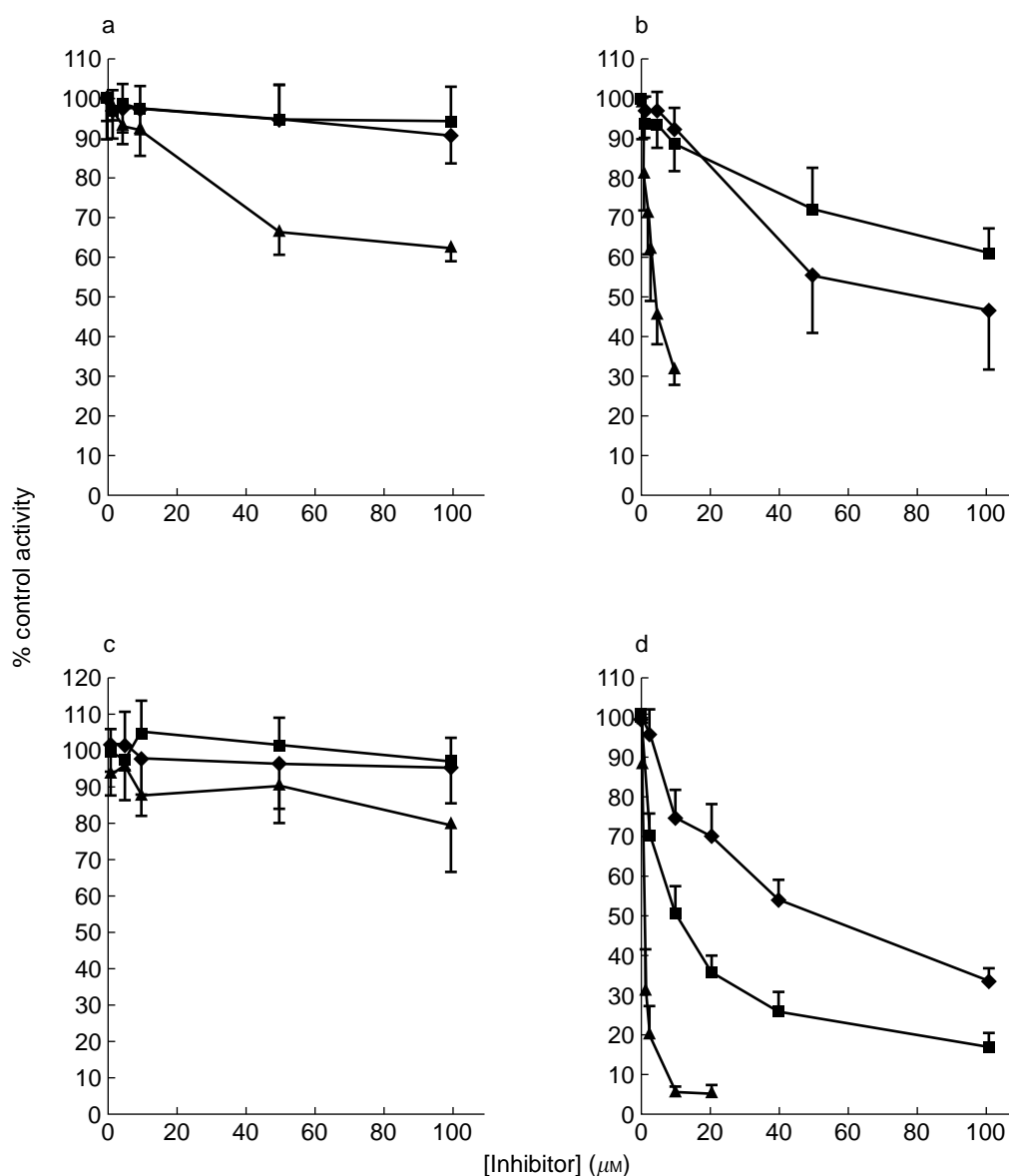


Figure 1 The inhibitory effect of saquinavir (◆), ritonavir (▲) and indinavir (■) on a) CYP1A2-mediated phenacetin *O*-deethylation, b) CYP2C9-mediated tolbutamide 4-hydroxylation, c) CYP2E1-mediated chlorzoxazone 6-hydroxylation, d) CYP3A4-mediated testosterone 6 β -hydroxylation by human liver microsomes. Each value represents the mean with s.d. from six livers.

(K_i = 0.019 \pm 0.004 μ M; mean \pm s.d.; *n* = 3, mixed inhibition) and also inhibited CYP2C9 (IC₅₀ for tolbutamide hydroxylation = 4.2 \pm 1.3 μ M; mean \pm s.d.; *n* = 6). Limited inhibition of phenacetin *O*-deethylation (CYP1A2) and chlorzoxazone 6-hydroxylation (CYP2E1) occurred at high concentrations (40% and 20% decrease in enzyme activity at 100 μ M respectively). Indinavir was an order of magnitude less

potent in inhibiting CYP3A4 (K_i = 0.17 \pm 0.01 μ M; mixed inhibition) and did not give appreciable inhibition of CYP1A2, CYP2C9 or CYP2E1 catalysed reactions. Saquinavir was the least potent CYP3A4 inhibitor (K_i = 2.99 \pm 0.87 μ M; mixed inhibition); it inhibited CYP2C9 with an IC₅₀ of 53.9 \pm 9.9 μ M but had negligible inhibitory effect on CYP1A2 or CYP2E1.

Discussion

The development of the protease inhibitors represents a significant advance in the treatment of HIV infection. Potent suppression of virus replication to levels below detection in plasma have been achieved [21, 22] and eradication of virus has now become an acceptable hypothesis to test [23]. However, there are important pharmacokinetic issues to be considered prior to the widespread use of these new anti-HIV drugs.

Ritonavir is one of the most potent inhibitors of CYP3A. Kumar *et al.* [10] reported IC_{50} values in human liver microsomes of 0.07, 0.14 and 2 μM for nifedipine oxidation, terfenadine hydroxylation and ethinyloestradiol 2-hydroxylation respectively. In the present study the K_i value for ritonavir against testosterone 6 β -hydroxylation was estimated to be 0.019 μM which is equal to or less than the reported K_i values for the well documented CYP3A4 inhibitor ketoconazole ie 0.015 μM for nifedipine oxidation [24], 0.73 μM for dexamethasone 6-hydroxylation [25], 0.3 μM for cyclosporin oxidation [26], 0.023 μM for triazolam 4-hydroxylation [27], 0.11 μM for amitriptyline *N*-demethylation [28]. Although an order of magnitude less potent an inhibitor of CYP3A4 than ritonavir, indinavir inhibited testosterone hydroxylation with a K_i of 0.17 μM . This is in line with the finding of Chiba *et al.* [11] who estimated the K_i of indinavir to be approximately 0.5 μM . Saquinavir was a further order of magnitude less potent ($K_i = 2.99 \mu\text{M}$) than indinavir against CYP3A4. A picture therefore emerges of a rank order of potency against CYP3A4 of ritonavir > indinavir > saquinavir.

In addition, and in agreement with Kumar *et al.* [10], ritonavir inhibited tolbutamide 4-hydroxylation (CYP2C9); saquinavir also inhibited this enzyme at high concentrations ($IC_{50} = 53.9 \mu\text{M}$) but indinavir was essentially non-inhibitory. Neither CYP1A2 or CYP2E1 mediated metabolism were inhibited to any marked degree by the protease inhibitors indicating the selectivity of P450 inhibition. However, previous work has shown ritonavir to be inhibitory against CYP2D6 ($K_i = 1.0 \mu\text{M}$; dextromethorphan as substrate [10]).

The potential for clinically relevant drug interactions in HIV+ patients is self-evident, particularly in patients receiving ritonavir and other drugs metabolized by CYP3A4 (and also to a lesser extent CYP2C9 and CYP2D6). To date, pharmacokinetic data have been reported on clarithromycin (77% increase in plasma concentrations), carbamazepine (>3-fold increase in AUC), rifabutin (>7-fold increase in AUC) and desipramine (2.5 fold increase in AUC) [9, 13, 29] but numerous drugs are stated to be contraindicated with ritonavir in the product information sheet. On the other hand it may be possible to gain therapeutic benefit from the metabolic inhibition produced by ritonavir. The greater than 20-fold increase in saquinavir AUC seen when ritonavir is co-administered to HIV positive patients [14, 22] means that the saquinavir dose can be significantly reduced and yet still maintain therapeutic blood concentrations of drug. Also, co-administration of ritonavir with dapsone and co-trimoxazole may reduce metabolite-mediated toxicity by reducing the formation of the toxic hydroxylamine [9]. On the other hand, since protease

inhibitors are substrates for CYP3A4 their metabolism may be induced by drugs such as rifampicin, rifabutin and carbamazepine [30].

There is an additional aspect to the drug interaction portfolio of ritonavir. Recent data suggest that in addition to being a potent inhibitor of CYP isozymes it also may induce CYP1A2 (as shown by a 43% decrease in steady state theophylline concentrations [31]) and glucuronyl transferase (as shown by a 40% decrease in ethinyloestradiol AUC [32]). It seems certain that with new protease inhibitors in various stages of clinical development (e.g. nelfinavir, 141W94), drug interaction studies will be crucial to gain an overall understanding of the beneficial and potentially harmful effects of this important group of drugs.

We are grateful to the MRC and Roche Products Ltd. for financial support.

References

- McCune JM, Rabin LB, Feinberg, MB, *et al.* Endoproteolytic cleavage of gp160 is required for the activation of human immunodeficiency virus. *Cell* 1988; **53**: 55–67.
- Debouck C, Metcalf BW. Human immunodeficiency virus protease: a target for AIDS therapy. *Drug Dev Res* 1990; **21**: 1–17.
- Moyle G, Gazzard B. Current knowledge and future prospects for the use of HIV protease inhibitors. *Drugs* 1996; **51**: 701–712.
- Collier AC, Coombs RW, Schoenfeld DA, *et al.* for the AIDS Clinical Trial Group. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine and zalcitabine. *N Engl J Med* 1996; **334**: 1011–1017.
- Danner SA, Carr A, Leonard JM, *et al.* for the European-Australian Collaborative Ritonavir Study Group. A short term study of the safety, pharmacokinetics and efficacy of ritonavir, an inhibitor of HIV-1 protease. *N Engl J Med* 1995; **333**: 1528–1533.
- Schapiro JM, Winters MA, Stewart F, *et al.* The effect of high dose saquinavir on viral load and CD4+ T cell counts in HIV-infected patients. *Ann Intern Med* 1996; **124**: 1039–1050.
- Notermans DW, de Wolf F, Foudraire NA, *et al.* The effects of an antiretroviral triple combination with ritonavir, AZT and 3TC. *AIDS* 1996; **10** (Suppl 2): S17.
- Gulick R, Mellors J, Havlir D, *et al.* Potent and sustained antiretroviral activity of indinavir (IDV), zidovudine (ZDV) and lamivudine (3TC). XI International Congress on AIDS. Vancouver, July 1996 (abstract No. Th.B 931).
- Barry M, Gibbons S, Mulcahy F, Back D. Protease inhibitors in patients with HIV disease: clinically important pharmacokinetic considerations. *Clin Pharmacokin* 1997; **32**: 194–209.
- Kumar GN, Rodrigues D, Buko AM, Denisson JF. Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. *J Pharmacol Exp Ther* 1996; **277**: 423–431.
- Chiba M, Hensleigh M, Nishime JA, Balani SK, Lin JH. Role of cytochrome P450 3A4 in human metabolism of MK-639, a potent human immunodeficiency virus protease inhibitor. *Drug Metab Dispos* 1996; **24**: 307–314.
- Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. *JAMA* 1997; **277**: 145–153.

- 13 Sahai J. Risks and synergies from drug interactions. *AIDS* 1996; **10** (Suppl 1): S21–S25.
- 14 Merry C, Barry MG, Mulcahy F, *et al.* Saquinavir pharmacokinetics alone and in combination with zidovudine in HIV infected patients. *AIDS* 1997; (in press).
- 15 Tassaneeyakul W, Birkett DJ, Veronese ME, *et al.* Specificity of substrate and inhibitor probes for human cytochromes P4501A1 and 1A2. *J Pharmacol Exp Ther* 1993; **265**: 401–407.
- 16 Relling MV, Aoyama T, Gonzalez FJ, Meyer UA. Tolbutamide and mephenytoin hydroxylation by human cytochrome P450s in the CYP2C subfamily. *J Pharmacol Exp Ther* 1990; **252**: 442–447.
- 17 Berthun F, Goasduff T, Lucas D, Dreano Y, Lebot MH, Menez JF. Interaction between 2 probes used for phenotyping cytochromes P4501A2 (caffeine) and P4502E1 (chlorzoxazone) in humans. *Pharmacogenetics* 1995; **5**: 72–79.
- 18 Waxman DJ, Lapenson DP, Aoyama T, Gelboin HV, Gonzalez FJ, Kozelkwa K. Steroid hormone hydroxylase specificities of eleven cDNA-expressed human cytochrome P450s. *Arch Biochem Biophys* 1991; **290**: 160–166.
- 19 Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein determination with the folin phenol reagent. *J Biol Chem* 1951; **193**: 265–275.
- 20 Back DJ, Tjia JF, Karbwang J, Colbert J. *In vitro* inhibition studies of tolbutamide hydroxylase activity of human liver microsomes by azoles, sulphonamides and quinolines. *Br J Clin Pharmacol* 1988; **26**: 23–29.
- 21 Perelson AG, Neumann AU, Markowitz M, Leonard JM, Ho, DD. HIV-1 dynamics *in vivo*: virion clearance rate, infected cell life-span, and viral generation time. *Science* 1996; **271**: 1582–1586.
- 22 Cameron DW, Hsu A, Granneman GR, *et al.* Pharmacokinetics of zidovudine-saquinavir combination therapy. *AIDS* 1996; **10** (Suppl 2): S16.
- 23 Hammer SM. Advances in antiretroviral therapy and viral load monitoring. *AIDS* 1996; **10** (Suppl 3): S1–S11.
- 24 Bourrie M, Meunier V, Berger Y, Fabre G. Cytochrome P450 isoform inhibitors as a tool for the investigation of metabolic reactions catalyzed by human liver microsomes. *J Pharmacol Exp Ther* 1996; **277**: 321–332.
- 26 Gentile DM, Tomlinson ES, Maggs JL, Park BK, Back DJ. Dexamethasone metabolism by human liver *in vitro*. Metabolite identification and inhibition of 6-hydroxylation. *J Pharmacol Exp Ther* 1966; **277**: 105–112.
- 27 Maurice M, Pichard L, Daujat M, *et al.* Effects of imidazole derivatives on cytochromes P450 from human hepatocytes in primary culture. *EASEB J* 1992; **6**: 752–758.
- 27 von Moltke LL, Greenblatt DJ, Harmatz JS, *et al.* Triazolam biotransformation by human liver microsomes *in vitro*: Effects of metabolic inhibitors and clinical confirmation of a predicted interaction with ketoconazole. *J Pharmacol Exp Ther* 1996; **276**: 370–379.
- 28 Schmider J, Greenblatt DJ, von Moltke LL, Harmatz JS, Shader RI. N-demethylation of amitriptyline *in vitro*: Role of cytochrome P4503A (CYP3A) isoforms and effect of metabolic inhibitors. *J Pharmacol Exp Ther* 1995; **275**: 592–597.
- 29 Bertz R, Cao G, Cavanaugh JH, Hsu A, Granneman GR, Leonard JM. Effect of zidovudine on the pharmacokinetics of desipramine. *XI International Congress on AIDS*, Vancouver, July 1996 (abstract No. Mo.B.1201).
- 30 Heylen R, Miller R. Adverse effects and drug interactions of medications commonly used in the treatment of adult HIV positive patients: Part 2. *Genitourin Med* 1997; **73**: 5–11.
- 31 Hsu A, Granneman GR, Witt G, Cavanaugh JH, Leonard J. Assessment of multiple doses of zidovudine on the pharmacokinetics of theophylline. *XI International Congress on AIDS*. Vancouver, July 1996 (abstract No. Mo.B.1200).
- 32 Ouellet D, Hsu A, Qian J, Cavanaugh J, Leonard J, Granneman GR. Effect of zidovudine on the pharmacokinetics of ethinyl estradiol in healthy volunteers. *XI International Congress on AIDS* Vancouver, July 1996 (abstract No. Mo.B.1198).

(Received 21 February 1997,
accepted 8 April 1997)