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Biotransformation and mass balance of tipranavir, a nonpeptidic protease inhibitor, when co-administered with ritonavir in Sprague–Dawley rats

Sreeraj Macha, Linzhi Chen, Stephen H. Norris, Elsy Philip, Yanping Mao, Helga Silverstein, Craig Struble, Wendy Beers

Abstract

In this study, tipranavir (TPV) biotransformation and disposition when co-administered with ritonavir (RTV) were characterized in Sprague–Dawley rats. Rats were administered a single intravenous (5 mg kg^{-1}) or oral (10 mg kg^{-1}) dose of [^{14}C]TPV with co-administration of RTV (10 mg kg^{-1}). Blood, urine, faeces and bile samples were collected at specified time-points over a period of 168 h. Absorption of TPV-related radioactivity ranged from 53.2–59.6%. Faecal excretion was on average 86.7% and 82.4% (intravenous) and 75.0% and 82.0% (oral) of dosed radioactivity in males and females, respectively. Urinary excretion was on average 4.06% and 6.73% (intravenous) and 9.71% and 8.28% (oral) of dosed radioactivity in males and females, respectively. In bile-duct-cannulated rats, 39.8% of the dose was recovered in bile. After oral administration, unchanged TPV accounted for the majority of the radioactivity in plasma (85.7–96.3%), faeces (71.8–80.1%) and urine (33.3–62.3%). The most abundant metabolite in faeces was an oxidation metabolite R-2 (5.9–7.4% of faecal radioactivity, 4.4–6.1% of dose). In urine, no single metabolite was found to be significant, and comprised <1% of dose. TPV when co-administered with RTV to rats was mainly excreted in feces via bile and the parent compound was the major component in plasma and faeces.

Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT, USA

Sreeraj Macha, Linzhi Chen, Stephen H. Norris, Elsy Philip, Yanping Mao, Helga Silverstein

Covance Laboratories Inc., Madison, WI, USA

Craig Struble, Wendy Beers

Correspondence: S. Macha, Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road/P.O. Box 368, Ridgefield, CT 06877, USA. E-mail: smacha@rdg.boehringer-ingelheim.com

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Introduction

Highly active antiretroviral therapy (HAART) has become the standard for treating HIV infection (Yeni et al 2004). Over the past 10 years, protease inhibitors (PI) have been used as a major component of the HAART regimens along with nucleoside and non-nucleoside reverse transcriptase inhibitors. The introduction of these regimens resulted in a dramatic decline in morbidity and mortality due to HIV infection (Palella et al 1998; Patick & Potts 1998). PIs bind to the active site of HIV protease and prevent cleavage of polyproteins necessary for maturation of infectious virions. Although highly potent, many PIs at standard dosing may be limited by high pill burdens, unfavourable pharmacokinetic profiles with relatively short elimination half-lives, and variable drug exposure (Moyle 2001). However, significant progress has been made in improving PI potency, tolerability and toxicity over the past several years. One approach is the use of boosted PI regimens, consisting primarily of low-dose ritonavir (RTV) in combination with another PI. The advantages of this approach include raising trough drug concentrations, minimizing inter-patient variability, prolonging drug elimination half-life and reducing pill burden (Moyle 2001; Cooper et al 2003).

Tipranavir (TPV), a nonpeptidic protease inhibitor, has been recently approved by both the FDA and EMEA for the treatment of HIV infection. The structure of TPV distinguishes it from all currently available peptidomimetic PIs (Turner et al 1998; Thiasrivongs & Strohbach 1999), resulting in a distinct resistance profile and activity against HIV isolates resistant to peptidomimetic PIs (Back et al 2000; Larder et al 2000). TPV is a sulfonamide-substituted dihydropyrone. Similar to other PIs, TPV binds directly to HIV aspartyl protease, disrupting the catalytic site of the enzyme and preventing protease-dependent cleavage of HIV gag and gag-pol polyproteins into smaller functional proteins (Turner et al 1998; Thiasrivongs & Strohbach 1999; Monini et al 2003). Clinical studies have demonstrated the significant

antiviral activity of TPV against HIV-1 in treatment-naïve and treatment-experienced patients at doses ranging from 300 to 1200 mg twice daily (Cahn et al 2004; Hicks 2004; McCallister et al 2004; Walmsley et al 2004). When combined with other antiretroviral agents, TPV proved to be more effective in suppressing HIV compared with another PI regimen in two pivotal studies, RESIST-1 and RESIST-2, involving patients selected based on the genotypic sensitivity of HIV.

TPV, similar to other PIs, is a substrate of CYP3A4 and has low systemic bioavailability. Clinical studies demonstrated that the bioavailability of TPV could be significantly enhanced by low-dose RTV boosting. RTV is a potent inhibitor of CYP3A4 (Kumar et al 1999; Ernest et al 2005) and co-administration of RTV with other PIs resulted in increased plasma concentrations with improved antiretroviral activity. Co-administration of TPV/RTV 500/200 mg twice daily in healthy subjects resulted in an 11-fold increase in AUC, 4-fold increase in C_{max}, and 45-fold increase in C_{min} (MacGregor et al 2004). TPV is currently co-administered with low-dose RTV to ensure adequate plasma concentrations are achieved in HIV-infected patients.

In this report, we present the mass balance and metabolite profile carried out in rats before conducting radiolabelled TPV studies in healthy subjects. In retrospect, we also evaluated whether rats, as one of the toxicology species, were exposed to the same metabolites as observed in man. The purpose of this study was to characterize the excretion and metabolite profile of TPV in rats when co-administered with RTV.

Methods

Materials

TPV, [R-(R*,R*)]-*N*-[3-[1-[5,6-dihydro-4-hydroxy-2-oxo-6-(2-phenylethyl)-6-propyl-2*H*-pyran-3-yl]propyl]phenyl]-5-(trifluoromethyl)-2-pyridinesulfonamide, [^{14}C]TPV and TPV glucuronide were synthesized at Boehringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, CT). Chemical identities of these compounds were established by HPLC, mass spectrometry and NMR. [^{14}C]TPV radiochemical purity was determined to be >97% by LC/radiochromatography. Aquasol-2 and Ultima FLO-M scintillation fluids were purchased from Packard Instrument Co., (Meriden, CT). Trifluoroacetic acid (TFA; 100%) was purchased from J. T. Baker (Phillipsburg, NJ). PEG 400 and propylene glycol were obtained from Sigma-Aldrich, Inc. (St Louis, MO).

In-vivo studies

All experimental procedures were approved by the local Institutional Animal Care and Use Committee. A total of 44 Sprague–Dawley rats (Crl:CD(SD)IGS BR) and 4 bile-duct-cannulated Sprague–Dawley rats from Charles River Laboratory (Portage, MI) were used for this study. The rats were assigned to different groups as follows: 16 rats were used for determining pharmacokinetics after intravenous (group 1) and oral (group 2) administration (4 rats/sex/group); 16 rats were used for the excretion mass balance study after intravenous (group 3) and oral (group 4) dosing (4 rats/sex/group); 4 bile-duct-cannulated males (group 5) were used for the biliary excretion study; and 12 rats (group 6) were used for the plasma metabolite ID/profiling study. During the test period, rats were housed as appropriate for sample collection: rats designated for collection of blood samples were housed in individual cages; rats designated for collection of excreta and expired air were housed in glass metabolism cages designed for the separation and collection of urine and faeces and collection of expired air; rats designated for collection of bile and excreta were housed in individual Nalgene cages designed for the separation and collection of urine and faeces and collection of bile. Certified Rodent Diet #8728CM (Harlan Teklad, Inc., Madison, WI) and water were freely available, unless otherwise specified. In bile-duct-cannulated rats, a solution of taurocholic acid (1.53 g L^{-1} in 0.9% saline) and 5% dextrose (for hydration of rats) was infused via the distal (duodenal) cannula at a rate of 1.35 mL h^{-1} starting the day before dose administration until the time of sacrifice.

For the intravenous and oral [^{14}C]TPV doses, appropriate quantities of radiolabelled and non-radiolabelled TPV were combined and formulated as a solution in PEG 400–water (80:20, v:v) to achieve concentrations of 5 mg mL^{-1} (100 μCi mL^{-1}) and 1 mg mL^{-1} (10 μCi mL^{-1}) TPV, respectively. For metabolite ID/profiling, the oral dose solution was prepared at a concentration of 1 mg mL^{-1} (40 μCi mL^{-1}) TPV. RTV was formulated as a solution in propylene glycol at a concentration of 5 mg mL^{-1}.

RTV was administered orally to all rats once daily for 5 days. The rats were fasted overnight before the fifth dose of RTV. Immediately following the fifth dose of RTV, each rat received an intravenous or oral dose of [^{14}C]TPV. The volume of radiolabelled dose formulation to be administered to each rat was calculated based on the body weight taken on the fifth day. The actual amount administered was determined by weighing the dose syringe before and after dose administration. The oral dose was administered via a ball-tipped gavage needle and the intravenous dose was administered via a tail vein. The overall mean doses administered to rats were 5.19 mg kg^{-1} (96.9 μCi kg^{-1}) and 5.30 mg kg^{-1} (98.9 μCi kg^{-1}) in the intravenous dose groups (groups 1 and 3, respectively), and 9.94 mg kg^{-1} (92.2 μCi kg^{-1}), 10.1 mg kg^{-1} (93.3 μCi kg^{-1}), 10.0 mg kg^{-1} (92.9 μCi kg^{-1}), and 9.94 mg kg^{-1} (435 μCi kg^{-1}) in the oral dose groups (groups 2, 4, 5 and 6, respectively).

For pharmacokinetics, blood samples (approximately 0.4 mL) were collected from a jugular vein and immediately transferred into tubes containing sodium heparin anticoagulant at 0.033, 0.25, 1, 2, 4, 8, 24 and 48 h post-dose from group 1 and at 0.5, 1, 2, 4, 8, 12, 24, 48, 72 and 96 h post-dose from group 2. Blood was placed on wet ice until centrifugation to obtain plasma. For mass balance, urine and faeces were collected at 24 h intervals up to 168 h from groups 3 and 4. After the last excreta collections, cages were wiped and washed first with 1% trisodium phosphate, and then 95% ethanol and gauze pads. The cage wash samples and gauze were collected into separate plastic containers and the weight of each cage wipe and cage wash sample was recorded. Expired air was passed through an activated charcoal trap to recover volatile organic compounds, then through a carbon dioxide trapping solution (2-ethoxyethanol–ethanolamine, 1:1) to collect

radiolabelled carbon dioxide. Expired air was collected at 0–4, 4–8 and 8–24 h post-dose. The weight of the CO_2 trapping solution sample was recorded. For biliary excretion, bile was collected at 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–24, 24–32 and 32–48 h post-dose. Urine and faeces were also collected at 0–24 and 24–48 h post-dose from group 5. For plasma metabolite ID/profiling, blood samples were collected at 4, 8 and 24 h post-dose.

Rats were euthanized with an overdose of halothane anaesthesia at the end of the study. The residual carcasses from groups 3 and 4 were retained for radioanalysis.

All samples, except blood, were stored at approximately −20°C before and after analysis. Blood was stored at approximately 5°C until sampled and centrifuged. Plasma was harvested and stored at approximately −20°C.

Sample preparation and analysis

Pharmacokinetics and mass balance

All sample combustions (blood and faecal homogenates) were done in a sample oxidizer (Model 307; Packard Instrument Company, Boston, MA) and the resulting $^{14}CO_2$ was trapped in a mixture of PermaFluor and Carbo-Sorb (Perkin Elmer, Boston, MA). Ultima Gold XR scintillation fluid was used for samples analysed directly (plasma, urine, bile, cage wash and cage wipe). Faecal samples were homogenized in water (at approximately 1:1, w:w). All samples were analysed for radioactivity using liquid scintillation counters (Model 1900TR and 2300TR; Packard Instrument Company, Boston, MA) for at least 10 min or 40 000 counts. All samples were analysed in duplicate if sample size allowed. If results from sample duplicates (calculated as ^{14}C d min^{-1} g^{-1} per sample) differed by more than 10% from the mean value, the sample was re-homogenized and re-analysed (if the sample size permitted). This specification was met for all sample aliquots that had radioactivity greater than 100 d min^{-1}. Scintillation counting data (counts min^{-1}) were automatically corrected for counting efficiency using the external standardization technique and an instrument-stored quench curve generated from a series of sealed quenched standards.

Metabolite profiling and identification

Rat plasma samples collected at 8, 12 and 24 h post-dose were pooled according to gender and time point. Six plasma pools were prepared, 8, 12 and 24 h pools each for males and females. Acetonitrile (MeCN) was added to the plasma and the mixture was sonicated for 5 min using a Branson 1200 Ultrasonic Cleaner (Branson Cleaning Equipment, Danbury, CT). The samples were centrifuged at 3750 rev min^{-1}, 4°C, for 10 min using a Beckman GS-6R centrifuge (Beckman, Fullerton, CA). After the supernatant was separated, the sediment was further extracted with MeCN and methanol. The supernatants were combined and dried at room temperature under a nitrogen stream in a Zymark Turbo Vap LV Evaporator (Zymark, Hopkinton, MA). After the sample was completely dried, the residue was reconstituted in a mix of 0.1% acetic acid–MeCN–MeOH, and then transferred to an autosampler vial for metabolite profiling and identification. The overall recovery of radioactivity from plasma was > 85%.

The urine samples were pooled according to gender. Only 0–24 and 24–48 h urine samples were used to make the pools, because the majority of radioactivity (≥80%) excreted in urine was found in the 0–48 h sample period, and the remaining radioactivity was spread in large volumes of urine across 120 h. Equal percentages (by volume) of the 0–24 and 24–48 h urine samples from each rat were combined. To 20 mL of the pooled urine, 1 mL of glacial acetic acid and 20 mL MeCN were added. The mixture was briefly vortexed with a Minivortexer (VWR Scientific, West Chester, PA) and centrifuged at 3750 rev min^{-1} for 20 min. Supernatant was separated and dried down. After the samples were dried down to about 2 mL, 6 mL of MeCN was added and the mixture was centrifuged at 3750 rev min^{-1} for 20 min. The supernatant was dried down to about 2 mL and then 1 mL of methanol was added. The mixture was vortexed briefly and centrifuged at 3750 rev min^{-1} for 5 min. The supernatant was transferred to autosampler vials for profiling. The overall recovery of radioactivity from urine was 94.0% for males and 90.0% for females.

The faeces samples were pooled according to gender. For males, samples collected up to 72 h were pooled, and this represented 97.3% of the total radioactivity ultimately recovered in faeces of males. For females, samples collected up to 120 h were pooled, and this accounted for 98.5% of the total radioactivity ultimately recovered in faeces of females. Equal percentages (by volume) of the faecal homogenates from each rat were combined across those time points. A 10-mL sample of pooled faecal homogenate was spiked with 10 μL TFA, vortexed and centrifuged at 3750 rev min^{-1} for 10 min. After removal of the supernatant, the pellet was extracted twice with 10 mL of MeCN and once with a mixture of 5 mL methanol, 5 mL water and 1 mL acetic acid. The extracts were combined with the supernatant and dried down at room temperature under a nitrogen stream. A fraction of the combined supernatant and extract was counted for radioactivity. The extraction recovery was 82.8% for the male pool and 88.5% for the female pool. After drying, the sample was reconstituted in 300 μL of 0.1% acetic acid and transferred into an autosampler vial for profiling. The recovery for the dry-reconstitution procedure was 89.0% and 92.6% for the male and female pools, respectively. The overall extraction recovery of radioactivity from faeces was 73.7% for the male pool and 82.0% for the female pool.

A rat bile sample pool was prepared by pooling samples across the entire 0–48 h sample collection period from all four male rats. The bile samples were pooled proportionately to the bile volume excreted at each collection interval. To 2.25 mL of the bile pool, 0.25 mL of acetic acid and 6.75 mL MeCN were added, and the mixture was vortexed briefly. The sample was centrifuged at 3750 rev min^{-1} for 10 min, and the supernatant was removed and dried down at room temperature under a nitrogen stream. After the sample was completely dried, the residue was reconstituted in 0.25 mL of 0.1% acetic acid–MeCN (95:5, v:v) and transferred to an autosampler vial for metabolite profiling and identification. The overall extraction recovery of radioactivity from bile was 93.7%. Metabolite profiling and identification was carried out with an LC/radiochromatography/MS/MS system. The system consisted of an Agilent 1100 series G1312A LC and a

G1367A autosampler (Agilent, Palo Alto, CA), with a Packard 525 Radiomatic flow scintillation analyser (Packard Instrument Co., Meriden, CT) and a Finnigan LCQ Deca XP Plus ion trap (Finnigan, San Jose, CA) serving as detectors. The chromatographic separation was achieved using a Waters xTerra MS C_{18} column (250×4.6 mm, 5 μm) (Waters, Millford, MA). The mobile phase A comprised of 0.1% acetic acid containing 5% MeCN (v:v), and the mobile phase B was MeCN containing 0.1% acetic acid (v:v). The gradient conditions were 18% to 50% of mobile B over 50 min and then increased to 100% over 20 min at 1 mL min^{-1}. The sample injection volumes were 10–70 μL.

The post column HPLC flow was split in a 1:20 ratio via an Acurate flow splitter (LC Packings, San Francisco, CA), with 20 parts to the flow scintillation analyser and one part going to the LCQ. The Radiomatic flow scintillation analyser was equipped with a 250 μL flow cell and the detection window was 4–100 keV. Packard Ultima FLO-M was used as the scintillation fluid at a flow rate of 2.5 mL min^{-1}. Radiochromatograms generated from the flow scintillation analyser were integrated and processed using Packard's FLO-ONE for Windows software.

MS and MS/MS were performed on the LCQ for metabolite structure elucidation. The LCQ mass spectrometer was equipped with an electrospray ion source and operated in positive mode. The key operating parameters included: spray voltage, 5 kV; sheath gas flow, 35 units; auxiliary gas flow, 0 units; capillary temperature, 300°C; and MS/MS collision energy, 40% with MS/MS wide excitation on.

Data analysis

Data tables were generated by Debra, Version 5.2a (LabLogic Systems Ltd., Sheffield, UK). Debra is an automated and validated data capture and management system for data collection in absorption, distribution and excretion studies using radiolabelled test articles. Debra captures data from balances and liquid scintillation counters. Non-compartmental analysis of plasma concentration–time data was carried out by using WinNonlin Professional Edition (Pharsight Corporation, v3.3). Pharmacokinetic parameters calculated included, but were not limited to, maximum concentration (C_{max}) in plasma, time to reach maximum concentration (t_{max}), half-life (t), area under the concentration–time curve from time 0 to the last quantifiable time point (AUC_{0-t}), and area under the concentration–time curve from 0 to infinity ($AUC_{0-\infty}$). The statistical significance between parameters was determined using analysis of variance (SAS JMP v6.0.2; SAS Institute Inc., NC). $P<0.05$ denoted significance in all cases unless otherwise stated.

Results

Plasma and blood pharmacokinetics

The blood and plasma concentrations versus time profiles of radioactivity following intravenous and oral administration of [^{14}C]TPV are presented in Figures 1 and 2, respectively. Concentrations of radioactivity in the blood and plasma tended to be higher in females than in males following both intravenous and oral dosing. There did not appear to be any significant association of radioactivity with red blood cells. The mean initial maximum concentrations of radioactivity in blood at the first sampling time at 2 min following an intravenous dose of [^{14}C]TPV (5 mg kg^{-1}) for male and female Sprague–Dawley rats (group 1) were 34 800 and 40 900 ng Eq mL^{-1}, respectively. Corresponding plasma concentrations following an intravenous dose were 65 500 and 71 200 ng Eq mL^{-1}, respectively. At 48 h after the intravenous dose, concentrations in blood and plasma declined to 112 and 140 ng Eq mL^{-1} in males and 413 and 555 ng Eq mL^{-1} in females, respectively. After oral administration of [^{14}C]TPV, the mean C_{max} of radioactivity in blood was 5925 and 8023 ng Eq mL^{-1}, at 6.5 and 10 h (mean t_{max}) for males and females, respectively. Corresponding plasma concentrations following the oral doses were 10 898 and 14 003 ng Eq mL^{-1}, at 6.5 and 8.5 h

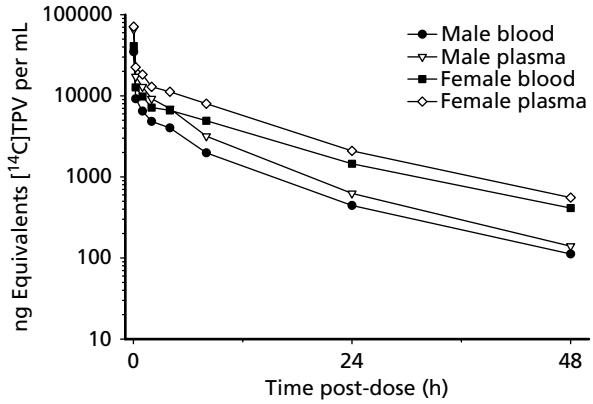

Figure 1 Mean concentration of radioactivity in blood and plasma following single intravenous doses of [^{14}C]TPV (5 mg kg^{-1}) after 5 consecutive daily oral doses of RTV (10 mg kg^{-1}) to male and female Sprague–Dawley rats.

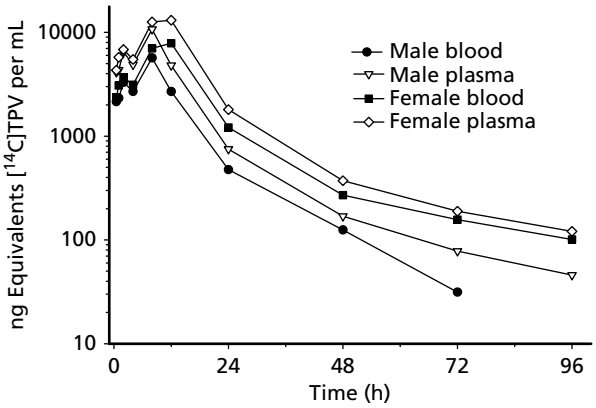

Figure 2 Mean concentration of radioactivity in blood and plasma following single oral doses of [^{14}C]TPV (10 mg kg^{-1}) after 5 consecutive daily oral doses of RTV (10 mg kg^{-1}) to male and female Sprague–Dawley rats.

(mean t_{max}), respectively. At 96 h after the oral dose, concentrations in blood and plasma declined to below the limit of quantitation (BLQ) and 45.8 ng Eq mL^{-1} in males and 101 and 121 ng Eq mL^{-1} in females, respectively.

The pharmacokinetic parameters for radioactivity in blood and plasma after intravenous (group 1) and oral (group 2) administration of [^{14}C]TPV are presented in Table 1. Because there was no significant association with red blood cells, only pharmacokinetic parameters for plasma are discussed below.

Following intravenous dosing, the terminal elimination half-life (t½) values of radioactivity in plasma were 9.2 and 10.4 h for males and females, respectively. The corresponding $AUC_{0-\infty}$, CL, and V_{ss} values were 111 443 and 226 788 ng Eq h mL^{-1}, 49.2 and 22.2 mL h^{-1} kg^{-1}, and 385 and 267 mL kg^{-1} for males and females, respectively. The oral absorption of [^{14}C]TPV was similar in males (59.6 ± 20.5%) and females (53.2 ± 18.9%). After oral dosing, the t½ and $AUC_{0-\infty}$ for radioactivity in plasma were 25.7 and 29.5 h and 132 766 and 241 133 ng Eq h mL^{-1} for males and females, respectively. The higher concentrations of radioactivity and AUC values found in females could be attributed primarily to the lower clearance of radioactivity in females compared with males.

Mass balance and excretion

The percentages of radioactive dose recovered in urine and faeces following intravenous and oral administration of [^{14}C]TPV are presented in Table 2. The main route of excretion after intravenous administration of [^{14}C]TPV was via faeces. Over the 168 h study a mean total value of 86.7% was excreted via faeces in males and 82.4% in females. In males, most radioactivity was excreted during the 0–24 h (49.3%) and 24–48 h (33.1%) collection intervals. For females, 24.1 and 34.8% were excreted during the 0–24 h and 24–48 h collection intervals, respectively, and an additional 14.3% was excreted over the 48–72 h interval. Excretion of radioactivity in urine was a minor route of elimination after intravenous dosing. A total of 4.06% and 6.73% was excreted by this route in males and females, respectively. Collection of expired air indicated that only a small fraction of radioactivity from [^{14}C]TPV was metabolized to $^{14}CO_2$. The total amount of radioactivity recovered in CO_2 traps during the first 24 h after dosing was 0.78% in males and 0.51% in females. No radioactivity was found in the organic volatile traps. The total mass balance after intravenous administration of [^{14}C]TPV was 92.3 ± 0.9% in males and 91.0 ± 0.6% in females. Only a small amount of radioactivity remained in rat carcasses at the completion of the 168 h study — 0.54% for males and 1.22% for females.

The results for excretion of radioactivity after oral administration of [^{14}C]TPV were similar to the intravenous results. The main route of excretion after oral administration was also via faeces. Over the 168 h study, a mean total of 75.0% was excreted via faeces in males and 82.0% in females. The majority of radioactivity was excreted within 48 h in males and it extended to 72 h in females. In males, 40.7% and 29.0% of radioactivity was excreted during the 0–24 h and 24–48 h collection intervals, respectively. For females, 15.5 and 47.2% were excreted during the 0–24 h and 24–48 h collection intervals, respectively, and an additional 13.1% was excreted over the 48–72 h interval. Excretion of radioactivity in urine was a minor route of elimination after oral dosing. A total of 9.71% and 8.28% of the radioactive dose was excreted by this route in males and females, respectively. Most of the radioactivity in urine (about 80%) was eliminated during the 0–24 h and 24–48 h collection intervals for both males and females. Collection of expired air after the oral dose indicated that only a minor fraction of radioactivity from [^{14}C]TPV was metabolized to $^{14}CO_2$. The total amounts of radioactivity recovered in CO_2 traps during the first 24 h after dosing were 0.69% in males and 0.49% in females. No radioactivity was found in organic volatile traps. The total mass balance after oral administration of [^{14}C]TPV was 91.6 ± 1.5% in males and 93.5 ± 0.9% in females. Only a small amount of radioactivity remained in rat carcasses at the completion of the 168 h study — 0.48% for males and 0.81% for females.

After oral administration of [^{14}C]TPV to male bile-duct-cannulated rats, a mean value of 30.9% of the radioactive dose was excreted in bile in the first 24 h after dosing. Total excretion in bile was 39.8% (± 11.5%) over a period of 48 h. Total excretion of radioactivity in faeces and urine of bile-duct-cannulated rats accounted for mean values of 26.7% (± 26.7%) and 14.1% (± 8.5%) of the dose, respectively. Total recovery of radioactivity from the bile-duct-cannulated rats was 86.1 ± 8.1% over a period of 48 h.

Metabolite profiling

For metabolite profiling and identification, the plasma samples from group 6, urine and faeces from group 4 and bile from group 5 were used. The contributions of unchanged TPV and its metabolites to matrix radioactivity and percent of dose are listed in Table 3. Representative radiochromatographic profiles from the plasma, faeces and bile are shown in Figure 3. The nomenclature for metabolites uses letter R (to designate a rat metabolite) and numbers according to their HPLC elution order.

In plasma at the 8, 12 and 24 h time points, TPV contributed 85.7–91.6% (males) or 87.2–96.3% (females) of the plasma radioactivity. Only a few minor metabolites were found. Among those minor metabolites, R-3 (a hydroxyl metabolite) was the most abundant metabolite for both males and females. R-3 represented 0.5–8.5% of plasma radioactivity. Another minor metabolite was tentatively identified as a dehydrogenation metabolite (R-4), and it represented ≤ 2.8% of plasma radioactivity. The remaining minor metabolites were less than 3.9% each and were not further identified.

For urine, the most abundant peak in the urine radiochromatograms corresponded to TPV, representing 62.3% and 33.3% of urine radioactivity (or 6.0% and 2.8% of dose) for males and females, respectively. Many minor metabolites contributed to the remaining radioactivity. Among these minor metabolites, R-3 was found to be present in the male urine pool at 2.3% of urine radioactivity (0.2% of dose). All the other metabolites were each less than 1% of dose and were not further identified.

For both male and female rats, the majority of faecal radioactivity was assigned to unchanged TPV, which accounted for 71.8% and 80.1% of faecal radioactivity (or 53.9% and 65.7% of dose), respectively. Many minor metabolites contributed to the remaining radioactivity. The most abundant

Table 1 Pharmacokinetic parameters for radioactivity in blood and plasma following intravenous (5 mg kg^{-1}) and oral (10 mg kg^{-1}) doses of [^{14}C]tipranavir after five consecutive daily oral doses of ritonavir (10 mg kg^{-1}) to Sprague–Dawley rats

Route	Matrix	Gender	C_{max}[a] (ng Eq mL^{-1})	t_{max} (h)	$AUC_{0\text{-}t}$ (ng Eq h g^{-1})	$AUC_{0\text{-}\infty}$ (ng Eq h g^{-1})	k_e (L h^{-1})	t ½ (h)	CL[b] (mL h^{-1} kg^{-1})	V_{ss}[c] (mL kg^{-1})	F (%)
Intravenous	Blood	M	42 930 ± 7408$^{\#}$	NA	64 500 ± 22 156*	66 101 ± 22 486*	0.0697 ± 0.00570	10.0 ± 0.9	82.6 ± 27.6*	763 ± 313	NA
		F	48 870 ± 2980‡	NA	134 301 ± 11 992*‡	141 140 ± 11 918*‡	0.0622 ± 0.00820	11.3 ± 1.5	35.6 ± 3.00*‡	479 ± 105‡	NA
	Plasma	M	80 866 ± 14 666$^{\#}$	NA	109 597 ± 37 837†	111 443 ± 38 247†	0.0757 ± 0.00520	9.2 ± 0.7	49.2 ± 17.0†	385 ± 149	NA
		F	84 835 ± 5865‡	NA	218 236 ± 21 003†‡	226 788 ± 21 238†‡	0.0674 ± 0.00930	10.4 ± 1.4	22.2 ± 2.10†‡	267 ± 58.0‡	NA
Oral	Blood	M	5925 ± 2348	6.50 ± 3.00	71 290 ± 20 773$^{@}$	73 091 ± 20 748$^{@}$	0.0495 ± 0.0108$^{@\%}$	14.5 ± 2.9$^{@\%}$	146 ± 45.0$^{@}$	3077 ± 1086	55.3 ± 15.7
		F	8023 ± 2475	10.0 ± 2.3	142 438 ± 39 824$^{@}$	147 757 ± 40 121$^{@}$	0.0198 ± 0.00470$^{@}$	36.9 ± 10.9$^{@}$	71.6 ± 19.4$^{@}$	3734 ± 1024$^{\&}$	52.3 ± 14.2
	Plasma	M	10 898 ± 4307	6.50 ± 3.00	131 068 ± 45 445	132 766 ± 45 726	0.0271 ± 0.00200$^{\$\%}$	25.7 ± 1.9$^{\$\%}$	82.5 ± 28.3	3060 ± 1074	59.6 ± 20.5
		F	14 003 ± 5841	8.50 ± 4.70	235 977 ± 85 628	241 133 ± 85 534	0.0235 ± 0.00160$^{\$}$	29.5 ± 2.0$^{\$}$	45.7 ± 15.9	1932 ± 634$^{\&}$	53.2 ± 18.9

Data are presented as mean ± s.d., n = 4. [a]For intravenous dose group the value represents C_0. [b]For oral dose group the value represents Clearance/F. [c]For oral dose group the value represents V_z/F. $*^{\dagger@\$}P < 0.05$ represent comparison of respective pharmacokinetic parameters between male and female rats. $^{\#\ddagger\%\&}P < 0.05$ represent comparison of respective pharmacokinetic parameters between blood and plasma.

Table 2 Mass balance and excretion of [^{14}C]tipranavir derived radioactivity following intravenous (5 mg kg^{-1}) and oral (10 mg kg^{-1}) administration after five consecutive daily oral doses of ritonavir (10 mg kg^{-1}) to Sprague–Dawley rats

Route	Gender	Urine (%)	Faeces (%)	Bile (%)	Total (%)[a]
Intravenous	Male	4.06 ± 0.43*†	86.7 ± 1.5*†	—	92.3 ± 0.9
	Female	6.73 ± 0.61*‡	82.4 ± 1.1*	—	91.0 ± 0.6
Oral	Male	9.71 ± 1.97†	75.0 ± 6.6†	—	91.6 ± 1.5
	Female	8.28 ± 0.80‡	82.0 ± 2.7	—	93.5 ± 0.9
Oral (bile)	Male	14.1 ± 8.5	26.7 ± 8.7	39.8 ± 11.5	86.1 ± 8.1

Data are presented as mean ± s.d., n = 4. [a]Total percent of radioactivity recovered includes cage wash, cage wipe, expired air, and carcass. *†‡$P < 0.05$.

Table 3 Radioactivity contribution of TPV and major metabolites in male and female rats after oral administration of TPV/RTV

Compound	Bile (group 5)		Plasma (group 6)			Faeces and urine (group 4)				
	%[^{14}C]	% Dose	8 h	12 h	24 h	Faeces		Urine		Faeces + urine
			%[^{14}C]	%[^{14}C]	%[^{14}C]	%[^{14}C]	% Dose	%[^{14}C]	% Dose	% Dose
Radioactivity contribution of TPV and major metabolites in male rats[a]										
R-1	40.6	16.2	—	—	—	0.3	0.2	—	—	0.2
R-2	2.7	1.1	—	—	—	5.9	4.4	—	—	4.4
R-3	—	—	3.1	3.4	1.3	—	—	2.3	0.2	0.2
R-4	—	—	1.0	2.8	1.5	—	—	—	—	—
TPV	3.8	1.5	91.6	85.7	89.4	71.8	53.9	62.3	6.0	59.9
Radioactivity contribution of TPV and major metabolites in female rats[a]										
R-1			—	—	—	—	—	—	—	—
R-2			—	—	—	7.4	6.1	—	—	6.1
R-3			3.2	8.5	0.5	1.6	1.3	—	—	1.3
R-4			—	1.5	0.8	—	—	—	—	—
TPV			96.3	87.2	95.2	80.1	65.7	33.3	2.8	68.5

[a]The remaining radioactivity in each matrix was contributed by a number of minor metabolites, each <3.9% of sample radioactivity in plasma, <1.4% of dose in bile, <2.5% of dose in faeces and <1% of dose in urine.

metabolite was R-2, a hydroxyl metabolite, which contributed 5.9% and 7.4% of faecal radioactivity (or 4.4% and 6.1% of dose) for males and females, respectively. In addition, a TPV glucuronide conjugate (R-1) was found in males and metabolite R-3 was found in females, at 0.3% and 1.6% of faecal radioactivity, respectively. The remaining metabolites were each less than 2.5% of dose and were not further identified.

Unchanged TPV was found to be a minor component in bile, only accounting for 3.8% of bile radioactivity. The major metabolite in bile was the TPV glucuronide conjugate (R-1), which represented 40.6% of bile radioactivity or 16.2% of dose. Metabolite R-2 was also present but contributed only 2.7% of bile radioactivity (or 1.1% of dose). The remaining metabolites were each less than 3.5% of the bile radioactivity (or < 1.4% of dose) and were not further identified.

Metabolite identification

The radiochromatographic peaks in the rat samples corresponding to TPV and the *O*-glucuronide conjugate (R-1) were confirmed by comparing their retention times and MS/MS patterns to authentic standards. Tentative identification of TPV metabolites R-2, R-3 and R-4 was based on HPLC retention time, radiochromatography and mass spectral analysis. MS/MS was performed for structure elucidation for representative samples.

R-2 The radioactive peak eluting at approximately 55.7 min was observed in faeces and bile. LC/MS analysis produced a protonated molecular ion at m/z 619, 16 mass units higher than that of TPV. This implied that R-2 is likely a single oxidation metabolite. As shown in Figure 4, MS/MS of R-2 showed daughter ions at m/z 575, 411, 385 and 343, similar to TPV. In addition, new fragments were observed at m/z 557 (-CO_2-H_2O), 513 (-C_6H_5CHO) and 495 (-C_6H_5CHO-H_2O). The formation of the daughter ions at m/z 513 and 495 suggested that oxidation took place on the benzyl moiety.

R-3 The radioactive peak eluting at approximately 63.0 min corresponded to a protonated molecular ion at m/z 619, 16 mass units higher than that of TPV, indicating another

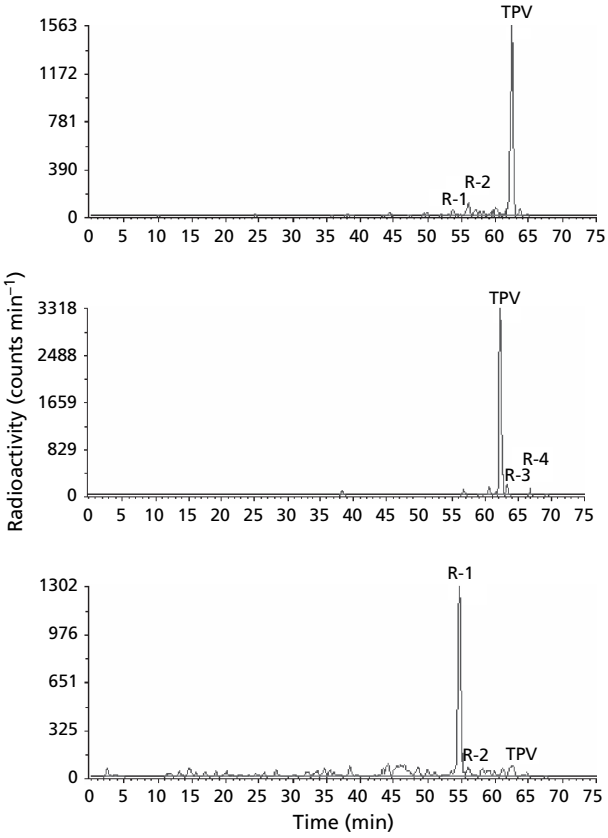

Figure 3 Representative radiochromatograms of male rat faeces (top), 12 h plasma (middle) and 24 h post-dose (bottom panel).

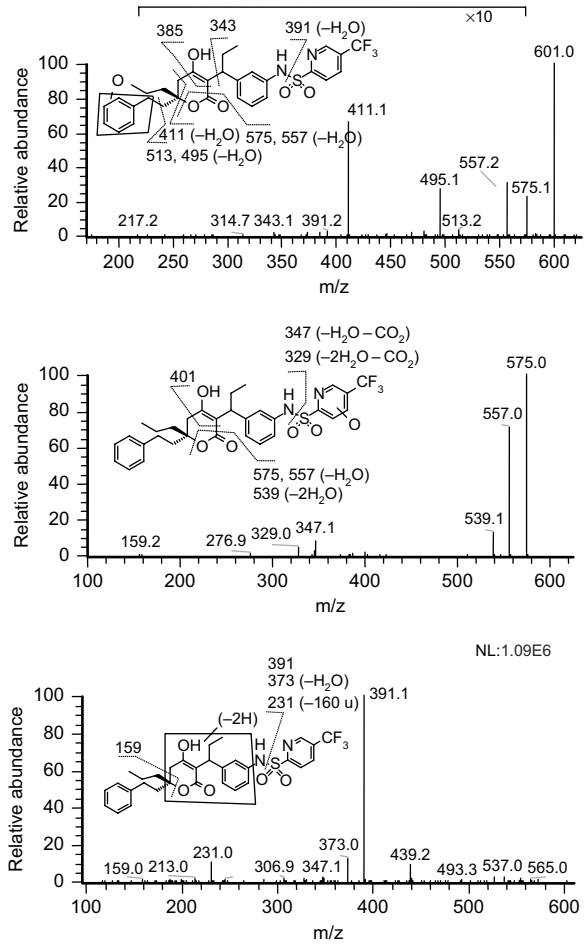

Figure 4 MS/MS of metabolites R-2, R-3 and R-4.

oxidation metabolite. As shown in Figure 4, MS/MS of R-3 produced daughter ions at m/z 575 (-CO_2), 557 (-CO_2-H_2O), 539 (-CO_2-2H_2O), 347 and 329. The formation of daughter ions at m/z 347 and 329 suggested that oxidation occurred on the pyridinyl ring.

R-4 The radioactive peak eluting at approximately 66.7 min was observed in plasma. LC/MS analysis showed that R-4 corresponded to a protonated molecular ion at m/z 601, 2 mass units less than that of TPV. Therefore, R-4 appeared to be a dehydrogenation metabolite. As shown in Figure 4, MS/MS of R-4 produced daughter ions at m/z 391, 373, 231 and 159. The MS/MS data suggested that the dehydrogenation took place in the middle moiety of the molecule, although the exact structure was not identified.

Discussion

HAART chemotherapeutic regimens containing a combination of reverse transcriptase inhibitors and PIs are currently the preferred therapeutic approach for treating HIV infection (Hoetelmans et al 1998; van Praag et al 2002; Yeni et al 2004). However, despite its ability to suppress viral load, HAART does not completely eradicate the virus. As a result, HIV-infected patients are faced with lifelong treatment with antiretroviral agents. Although there are a large and growing number of antiretroviral agents available, virologic failure and drug resistance continue to be serious problems for HIV-positive patients. Another approach for enhancing the potency of antiretroviral drugs is the use of dual PI regimens, including agents with divergent resistance profiles, which have been very effectively used in AIDS patients (Barry et al 1997). PIs are primarily metabolized by CYP3A4 in the liver and intestine and also act as competitive inhibitors of this isoenzyme. Combination PI regimens, usually including low-dose RTV, are currently being used that take advantage of favourable drug interactions (Moyle 2001). RTV is a potent inhibitor of intestinal and liver CYP3A4 and has been shown to boost systemic exposure of other co-administered PIs (Kumar et al 1999; Ernest et al 2005).

Previous studies suggest that metabolism of TPV occurs via the CYP3A pathway (MacGregor et al 2004; McCallister et al 2004). In the above study, to determine the net effect of TPV and RTV on CYP3A4 activity, the erythromycin breath test was used as a surrogate of CYP3A4 activity. Initially during the first 11 days of dosing with TPV alone, CYP3A4 activity increased, consistent with mild CYP3A4 induction by TPV. Upon RTV therapy commencement at day 11 and

co-administration for 7 days, the percentage of erythromycin metabolized by CYP3A4 per hour decreased to negligible levels, indicating substantial CYP3A4 inhibition by RTV. As a result, co-administration of TPV with RTV resulted in 20-fold or greater increases in TPV $C_{ss,min}$ and 4- to 13-fold increases in TPV AUC_{0-12}, but only a 3- to 4-fold rise in TPV $C_{ss,max}$ compared with TPV treatment alone (MacGregor et al 2004). In our study, with co-administration of RTV, TPV was the predominant drug-related species found in plasma (≥85.7% of plasma radioactivity) following oral administration in rats. Unchanged TPV was also the most abundant drug-related component excreted in faeces and urine, accounting for 59.8% and 4.4% of dose (male and female combined), respectively, after oral dosing. This study demonstrates that the majority of the administered dose of TPV remained unchanged in plasma. The lack of TPV metabolism, except for glucuronidation, following administration is attributed to the profound inhibition of CYP3A by RTV (Koudriakova et al 1998).

In-vitro inhibition of the metabolism of PIs by RTV was shown to be similar in rat liver microsomes and human microsomes (Shibata et al 2002). In-vitro metabolism of lopinavir was inhibited by RTV similarly in both rat and human liver microsomes (Kumar et al 2004). Co-administration of RTV with saquinavir, indinavir and nelfinavir resulted in a prolonged and substantial increase in plasma levels of these PIs in rats (Kempf et al 1997; Shibata et al 2002; Kumar et al 2004). Similar findings were observed when lopinavir was co-administered with RTV in man (Kumar et al 2004). These studies suggest that pharmacokinetic studies in rats might be useful in predicting CYP3A related drug interactions in man.

The proposed biotransformation pathway for TPV with co-administration of RTV in rats is shown in Figure 5. TPV underwent three metabolic pathways: direct glucuronidation to yield R-1, oxidation to form R-2 and R-3, and dehydrogenation to produce R-4. R-1 was the major metabolite in bile and present as a very small percentage of radioactivity in male faeces, but absent in female faeces. This suggests that β-glucuronidase hydrolysis of the biliary glucuronide conjugates occurs in the gut. No significant gender differences were observed in terms of excretion and metabolite profiles.

Absorption of TPV-related radioactivity was similar in males and females, with mean values ranging from 53.2% to 59.6%. The main route of excretion after intravenous and oral administration of [^{14}C]TPV was via faeces. There was substantial biliary excretion (39.8%) in bile-duct-cannulated rats dosed orally with [^{14}C]TPV. Excretion of radioactivity in urine was a minor route of elimination after intravenous dosing. Although urinary excretion was low, substantial biliary excretion indicated at least moderate absorption. Results for total elimination following both intravenous and oral administration were similar for males and females. Based on unpublished data, it was also shown that TPV excretion and metabolite profiles in rats were similar to those in man after

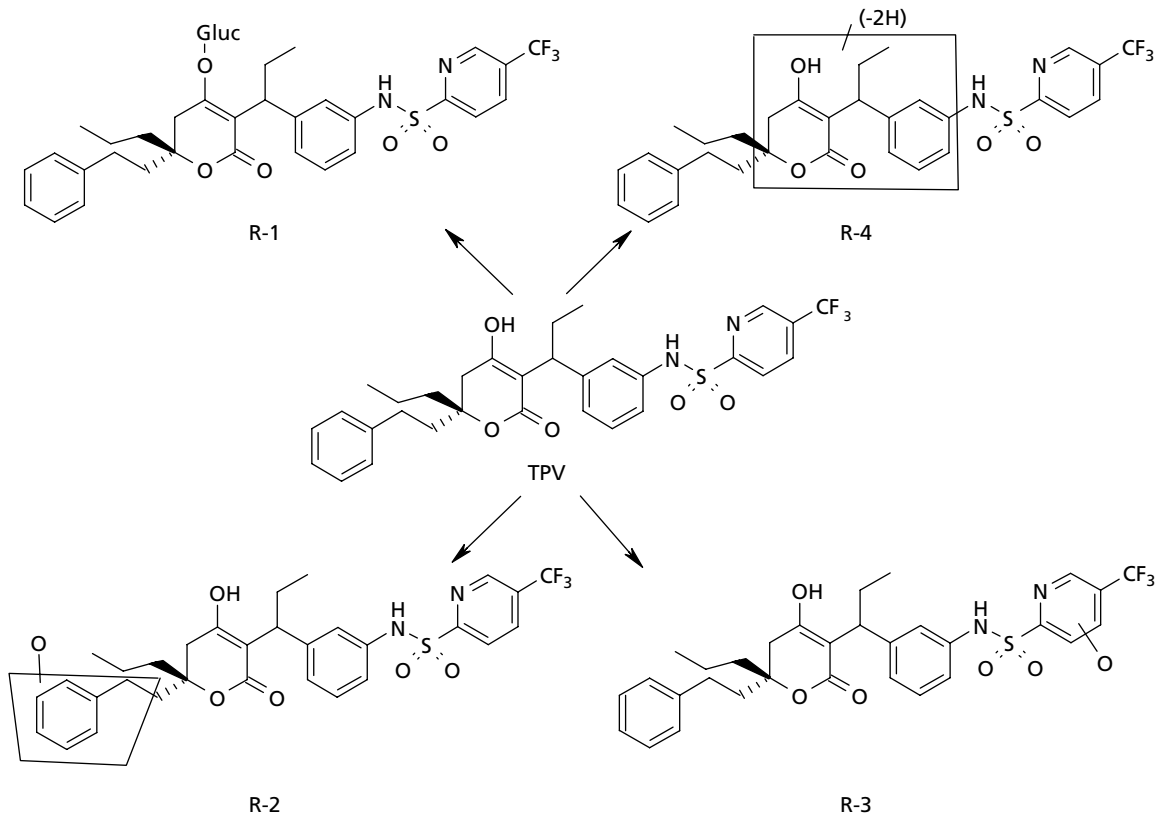

Figure 5 TPV metabolic pathways in rat with co-administration of RTV.

oral co-administration of RTV (Boehringer Ingelheim internal report; manuscript in preparation).

Conclusions

This study demonstrates that TPV is well absorbed following oral administration and is excreted mainly in faeces via bile. Overall elimination of total radioactivity following intravenous and oral administration was similar in male and female rats. TPV phase-I metabolism was minimal when co-administered with RTV in rats. There were no major gender-related differences in metabolite profiles of TPV.

References

Back, N. K. T., van Wijk, A., Remmerswaal, D., van Monfort, M., Nijhuis, M., Schuurman, R., Boucher, C. A. B. (2000) In-vitro tipranavir susceptibility of HIV-1 isolates with reduced susceptibility to other protease inhibitors. *AIDS* **14**: 101–102

Barry, M., Gibbons, S., Back, D., Mulcahy, F. (1997) Protease inhibitors in patients with HIV disease. Clinically important pharmacokinetic considerations. *Clin. Pharmacokinet*. **32**: 194–209

Cahn, P., RESIST-2 Study Team (2004) 24-week data from RESIST-2: Phase 3 study of the efficacy and safety of either tipranavir/ritonavir (TPV/r) or an optimized ritonavir (RTV)-boosted standard-of-care (SOC) comparator PI (CPI) in a large randomized multicentre trial in treatment-experienced HIV+ patients. 7th International Congress on Drug Therapy in HIV Infection, November 14–18, Glasgow, UK. Abstract PL 14.3

Cooper, C. L., van Heeswijk, R. P., Gallicano, K., Cameron, D. W. (2003) A review of low-dose ritonavir in protease inhibitor combination therapy. *Clin. Infect. Dis*. **36**: 1585–1592

Ernest, C. S., Hall, S. D., Jones, D. R. (2005) Mechanism-based inactivation of CYP3A by HIV protease inhibitors. *J. Pharmacol. Exp. Ther*. **312**: 583–591

Hicks, C. (2004) RESIST-1: a phase 3 randomized, controlled, open-label multicenter trial comparing tipranavir/ritonavir (TPV/r) to an optimized comparator protease inhibitor/r (CPI/r) regimen in antiretroviral (ARV) experienced patients: 24-week data. 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, October 31–November 2, 2004, Washington, DC. Presentation H-1137a

Hoetelmans, R. M., Reijers, M. H., Weverling, G. J., ten Kate, R. W., Wit, F. W., Mulder, J. W., Weigel, H. M., Frissen, P. H., Roos, M., Jurriaans, S., Schuitemaker, H., de Wolf, F., Beijnen, J. H., Lange, J. M. (1998) The effect of plasma drug concentrations on HIV-1 clearance rate during quadruple drug therapy. *AIDS* **12**: F111–F115

Kempf, D. J., Marsh, K. C., Kumar, G., Rodrigues, A. D., Denissen, J. F., McDonald, E., Kukulka, M. J., Hsu, A., Granneman, G. R., Baroldi, P. A., Sun, E., Pizzuti, D., Plattner, J. J., Norbeck, D. W., Leonard, J. M. (1997) Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir. *Antimicrob. Agents Chemother*. **41**: 654–660

Koudriakova, T., Iatsimirskaia, E., Utkin, I., Gangl, E., Vouros, P., Storozhuk, E., Orza, D., Marinina, J., Gerber, N. (1998) Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: mechanism-based inactivation of cytochrome P4503A by ritonavir. *Drug. Metab. Dispos*. **26**: 552–561

Kumar, G. N., Dykstra, J., Roberts, E. M., Jayanti, E. M., Hickman, D., Uchic, J., Yao, Y., Surber, B., Thomas, S., Granneman, G. R. (1999) Potent inhibition of the cytochrome P-450 3A-mediated human liver microsomal metabolism of a novel HIV protease inhibitor by ritonavir: a positive drug-drug interaction. *Drug. Metab. Dispos*. **27**: 902–908

Kumar, G. N., Jayanti, V. K., Johnson, M. K., Uchic, J., Thomas, S., Lee, R. D., Grabowski, B. A., Sham, H. L., Kempf, D. J., Denissen, J. F., Marsh, K. C., Sun, E., Roberts, S. A. (2004) Metabolism and disposition of the HIV-1 protease inhibitor lopinavir (ABT-378) given in combination with ritonavir in rats, dogs, and humans. *Pharm. Res*. **21**: 1622–1630

Larder, B. A., Hertogs, K., Bloor, S., van den Eynde, C., DeCian, W., Wang, Y., Freimuth, W. W., Tarpley, G. (2000) Tipranavir inhibits broadly protease inhibitor-resistant HIV-1 clinical samples. *AIDS* **14**: 1943–1948

MacGregor, T., Sabo, J. P., Norris, S. H., Johnson, P., Galitz, L., McCallister, S. (2004) Pharmacokinetic characterization of different dose combinations of co-administered tipranavir and ritonavir in healthy volunteers. *HIV Clin. Trials* **5**: 371–382

McCallister, S., Valdez, H., Curry, K., MacGregor, T., Borin, M., Freimuth, W., Wang, Y., Mayers, D. L. (2004) A 14-day dose-response study of the efficacy, safety, and pharmacokinetics of the nonpeptidic protease inhibitor tipranavir in treatment-naive HIV-1-infected patients. *J. Acquir. Immune. Defic. Syndr*. **35**: 376–382

Monini, P., Sgadari, C., Barillari, G., Ensoli, B. (2003) HIV protease inhibitors: antiretroviral agents with anti-inflammatory, anti-angiogenic and anti-tumor activity. *J. Antimicrob. Agents. Chemother*. **51**: 207–211

Moyle, G. (2001) Use of HIV protease inhibitors as pharmacoenhancers. *AIDS Read* **11**: 87–98

Palella, F. J., Delaney, K. M., Moorman, A. C., Loveless, M. O., Fuhrer, J., Satten, G. A., Aschman, D. J., Holmberg, S. D. (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N. Engl. J. Med*. **338**: 853–860

Patick, A. K., Potts, K. E. (1998) Protease inhibitors as antiviral agents. *Clin. Microbiol. Rev*. **11**: 614–627

Shibata, N., Gao, W., Okamoto, H., Kishida, T., Yoshikawa, Y., Takada, K. (2002) In-vitro and in-vivo pharmacokinetic interactions of amprenavir, an HIV protease inhibitor, with other current HIV protease inhibitors in rats. *J. Pharm. Pharmacol*. **54**: 221–229

Thiasrivongs, S., Strohbach, J. W. (1999) Structure-based discovery of tipranavir disodium (PNU-140690E): a potent, orally bioavailable, nonpeptidic HIV protease inhibitor. *Biopolymers* **51**: 51–58

Turner, S. R., Strohbach, J. W., Tommasi, R. A., Aristoff, P. A., Johnson, P. D., Skulnick, H. I., Dolak, L. A., Seost, E. P., Tomich, P. K., Bohanon, M. J., Horng, M. M., Lynn, J. C., Chong, K. T., Hinshaw, R. R., Watenpaugh, K. D., Janakiraman, M. N., Thaisrivongs, S. (1998) Tipranavir (PNU-140690): a potent, orally bioavailable nonpeptidic HIV protease inhibitor of the 5,6-dihydro-4-hydroxy-2-pyrone sulfonamide class. *J. Med. Chem*. **41**: 3467–3476

van Praag, R. M., Wit, F. W., Jurriaans, S., de Wolf, F., Prins, J. M., Lange, J. M. (2002) Improved long-term suppression of HIV-1 replication with a triple-class multidrug regimen compared with standard of care antiretroviral therapy. *AIDS* **16**: 719–725

Walmsley, S., Leith, J., Katlama, C., Arasteh, K., Pierone, G., Blick, G., Lazzarin, A., Johnson, M., Samuels, C., Jones, P., Chaves, R., Quinson, A., Kohlbrenner, V., McCallister, S., Mayers, D. L., Curry, K. (2004) Pharmacokinetics and safety of tipranavir/ritonavir (TPV/r) alone or in combination with saquinavir (SQV),

amprenavir (APV), or lopinavir (LPV): interim analysis of BI1182.51. The XV International AIDS Conference, July 11–16; Bangkok, Thailand. Abstract WeOrB1236

Yeni, P. G., Hammer, S. M., Hirsch, M. S., Saag, M. S., Schechter, M., Carpenter, C. C., Fischl, M. A., Gatell, J. M., Gazzard, B. G., Jacobsen, D. M., Katzenstein, D. A., Montaner, J. S., Richman, D. D., Schooley, R. T., Thompson, M. A., Vella, S., Volberding, P. A. (2004) Treatment for adult HIV infection: 2004 recommendations of the International AIDS Society-USA Panel. *JAMA* **292**: 251–265