Metabolism and Disposition of the HIV-1 Protease Inhibitor Lopinavir (ABT-378) Given in Combination with Ritonavir in Rats, Dogs, and Humans

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Received April 21, 2004; accepted May 9, 2004

Purpose. The objective of this study was to examine the metabolism and disposition of the HIV protease inhibitor lopinavir in humans and animal models.

Methods. The plasma protein binding of [^{14}C]lopinavir was examined *in vitro* via equilibrium dialysis technique. The tissue distribution of radioactivity was examined in rats dosed with [^{14}C]lopinavir in combination with ritonavir. The metabolism and disposition of [^{14}C]lopinavir was examined in rats, dogs, and humans given alone (in rats only) or in combination with ritonavir.

Results. The plasma protein binding of lopinavir was high in all species (97.4–99.7% in human plasma), with a concentration-dependent decrease in binding. Radioactivity was extensively distributed into tissues, except brain, in rats. On oral dosing to rats, ritonavir was found to increase the exposure of lopinavir-derived radioactivity 13-fold. Radioactivity was primarily cleared via the hepato-biliary route in all species (>82% of radioactive dose excreted via fecal route), with urinary route of elimination being significant only in humans (10.4% of radioactive dose). Oxidative metabolites were the predominant components of excreted radioactivity. The predominant site of metabolism was found to be the carbon-4 of the cyclic urea moiety, with subsequent secondary metabolism occurring on the diphenyl core moiety. In all the three species examined, the primary component of plasma radioactivity was unchanged lopinavir (>88%) with small amounts of oxidative metabolites.

Conclusions. Lopinavir was subject to extensive metabolism *in vivo*. Co-administered ritonavir markedly enhanced the pharmacokinetics of lopinavir-derived radioactivity in rats, probably due to inhibition of presystemic and systemic metabolism, leading to an increased exposure to this potent HIV protease inhibitor.

KEY WORDS: ABT-378; drug-drug interactions; HIV protease inhibitors; lopinavir; ritonavir.

INTRODUCTION

HIV protease inhibitors have revolutionized the treatment of HIV infection and acquired immune deficiency syndrome (AIDS) (1–6). Due to the limited oral bioavailability and poor pharmacokinetic characteristics of many of the currently used protease inhibitors, additional efforts have been made to design more potent protease inhibitors with improved pharmacokinetic properties. Lopinavir (LVR), an analog of HIV protease inhibitor ritonavir (RVR), was designed to minimize interaction of the inhibitor with valine-82 of the HIV protease, the predominant site of mutation selected during RVR therapy (7,8). LVR is a potent inhibitor of wild type and mutant HIV protease (K_i = 1.3-28 pM) and is also active against mutant HIV selected by RVR *in vivo* (EC_{50} = 0.06 μM). The oral bioavailability of LVR in animal models was low, probably due to high first-pass metabolism (9). Co-administration of RVR, a potent inhibitor of cytochrome P450 3A isoform, was found to result in significant increases in AUC and C_{max} of LVR in rats, dogs and monkeys (9,10).

In vitro investigations with human liver microsomes have shown that cytochrome P450 3A plays a predominant role in the metabolism of LVR, and RVR inhibited the metabolism of LVR potently, with a K_i of 13 nM (11). RVR is also a potent inhibitor of LVR metabolism in rat liver microsomes, with an IC_{50} of 36 nM (9). Based on *in vitro* studies, the pharmacokinetic enhancement is presumed to be due to inhibitory effect of RVR on the pre-systemic and possibly systemic metabolism of LVR. This formed the rationale for the therapy of HIV infection with LVR and small amounts of RVR, leading to increased and sustained plasma levels of this potent HIV protease inhibitor. In human clinical trials, increased exposure to LVR was observed on coadministration with RVR (12). More recently, the LVR:RVR combination (ABT-378/r, Kaletra) has been shown to be effective in the treatment of HIV infection and is approved for clinical use (13,14).

The data presented in this article examined the metabolism and disposition of [^{14}C]LVR given alone (in rats only) and in combination with RVR in Sprague-Dawley rats, beagle dogs and HIV-negative healthy male human volunteers. The animal experiments were conducted in accordance with the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985). For the human disposition study, written informed consent from the volunteers and approval by the Covance Clinical Research Unit Institutional Review Board were obtained.

MATERIALS AND METHODS

Drug

LVR (88.2 μCi/mg, >97% radiochemically pure) was labeled with carbon-14 in the carbonyl carbon beta to the 2,6-dimethylphenoxy group of the molecule. Human serum albumin and α_1-acid glycoprotein were obtained from Sigma Chemical Company (St. Louis, MO). All other reagents were of highest commercial grade available.

Plasma Protein Binding

Freshly obtained blood from male and female CD-1 mice, Sprague-Dawley rats, beagle dogs, Cynomolgus monkeys and healthy adult human volunteers (at least two of each sex) was used. The pooled heparinized blood samples were

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ABBREVIATIONS: HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; AUC, area under the plasma concentration-time curve; HSA, human serum albumin; AAG, α1-acid glycoprotein; LVR, lopinavir; RVR, ritonavir; IV, intravenous; ID, intraduodenal; PO, oral; CYP3A, cytochrome P450 3A.

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centrifuged to separate the plasma from the cells. Appropriate aliquots of ethanolic stock solutions were added to the plasma aliquots to give initial [^{14}C]LVR concentrations of approximately 0.1, 1.0, 10, 30 and 100 μg/ml. Binding to human serum albumin (HSA, 40 mg/ml) and α_1-acid glycoprotein (AAG, 0.8 mg/ml) was examined with 0.1–30 μg/ml of [^{14}C]LVR. The protein binding was determined in a Spectrum Equilibrium Dialysis System (Spectrum Medical Industries, Los Angeles, CA) using 1-ml cells and a Spectra/Por 2 dialysis membrane with a molecular weight cut-off of 12,000–14,000 Da. The cell carrier was rotated at about 20 rpm for 3 h in a water bath maintained at approximately 37°C. Preliminary experiments had established that the equilibrium was attained by 3 h. At the end of the designated time interval, samples were removed from each side of the dialysis cells and radioactivity was determined by liquid scintillation counting.

Dose Formulations

For intravenous administration to animals, [^{14}C]LVR was combined with unlabeled LVR and two molar equivalents of *p*-toluenesulfonic acid in 3:3:4 ethanol:propylene glycol:5% dextrose in water. For oral and intraduodenal administration to animals, [^{14}C]LVR was combined with unlabeled LVR and two molar equivalents of *p*-toluenesulfonic acid in 5:95 ethanol:propylene glycol. RVR dose solutions were also prepared as described above. For administration of the combination, dose solutions of [^{14}C]LVR and RVR were combined in a ratio of 2:1 just before dosing. The target doses are summarized in Table I. For human study, a liquid formulation containing 160 mg (40 μCi) of LVR and 40 mg of RVR/g in a proprietary formulation base was prepared. Just before dosing, this liquid was filled into hard gelatin capsules. Each human subject was administered multiple capsules and received a target dose of 400 mg of LVR (approximately 100 μCi) and 100 mg of RVR.

Rat Studies

The study design is summarized in Table I. To examine the tissue distribution of radioactivity, the combination dose solution was administered by gavage to cohorts of three male rats (Group 1). At several time points extending up to 72 h post-dose, blood and several tissues were harvested and the total radioactivity in each tissue was quantitated by combustion and liquid scintillation analysis. To examine the recovery of dose radioactivity, [^{14}C]LVR alone or in combination with RVR (Groups 2 and 3) were administered intravenously into the femoral vein or orally by gavage to male and female rats, and excreta were collected for three days. To examine the extent of biliary excretion and profile of biliary radioactivity, [^{14}C]LVR alone or in combination with RVR were administered intravenously (Group 4) or intraduodenally (Group 5) via the distal bile duct cannula to anesthetized bile-duct cannulated male rats. To examine the plasma radioactivity levels and composition, [^{14}C]LVR alone or in combination with RVR (Groups 6 and 7) were administered intravenously into the femoral vein or orally by gavage to male or female rats, and blood samples were withdrawn from the jugular vein at various time points up to 24 h.

Dog Studies

Since dosing LVR alone produced no detectable plasma drug levels in dogs (9), only combination dose solution was administered to dogs. For studying the biliary elimination, the combination dose solution was administered intravenously as a slow bolus over ~60 s (Group 8) or intraduodenally (Group 9) to anesthetized dogs and bile was collected for 6 h. Administration was done via intraduodenal route in lieu of oral route since dogs were anesthetized. Bile was collected for only 6 h, since it was not possible to maintain dogs beyond 6 h under anesthesia. After euthanasia, urine was aspirated from the bladder. To study the mass balance and metabolism, the combination dose solution was administered orally by gavage (Group 10). After a 2-week washout, a freshly prepared combination dose solution was administered intravenously to the same dogs as a slow bolus over 1–2 min (Group 11). Blood samples were withdrawn from the jugular vein. Urine and feces were collected daily for five days.

Table I. *In Vivo* Study Design

Study	Group	Route	Sex	n	LVR/RVR (mg/kg)	Duration (hours)	Samples collected
Rat*							
Tissue distribution	1	PO	M	3/time	10-5	72	Bl, T
Excretion	2	IV	M/F	2/2	10/0 & 10/5	72	U, F
Excretion	3	PO	M/F	2/2	10/0 & 10/5	72	U, F
Biliary excretion	4	IV	M/F	2/2	10/0 & 10/5	24	Bi
Biliary excretion	5	ID	M/F	2/2	10/0 & 10/5	24	Bi
Plasma radioactivity	6	IV	M/F	2/2	10/0 & 10/5	24	Bl
Plasma radioactivity	7	PO	M/F	2/2	10/0 & 10/5	24	Bl
Dog†							
Biliary excretion	8	IV	M	2	5/2.5	6	Bi, U
Biliary excretion	9	ID	M	2	5/2.5	6	Bi, U
Excretion and plasma radioactivity	10	IV	M/F	3/3	5/2.5	120	U, F, Bl
Excretion and plasma radioactivity	11	PO	M/F	3/3	5/2.5	120	U, F, Bl
Human‡							
Excretion and plasma radioactivity	—	PO	M	5	400/100‡	192	U, F, Bl

Bl, blood; T, tissues; U, urine; F, feces; Bi, bile; IV, intravenous; ID, intraduodenal; PO, oral.

* Target radioactive dose was 60 μCi/kg of ^{14}C-LVR.

† Target radioactive dose was 3 μCi/kg of ^{14}C-LVR.

‡ Total target dose with 100 μCi of ^{14}C-LVR for each human subject.

Human Disposition Study

Human disposition study in healthy male volunteers (n = 5, age: 20–43 years, 64–70 kg) was conducted at Covance Clinical Research Unit (Madison, WI).

Quantitation of Radioactivity

Radioactivity levels in bile, plasma, urine and cagewash samples were determined by liquid scintillation analysis. Feces were homogenized in 70% ethanol in water to prepare approximately 10% w/v fecal homogenates. Radioactivity in blood and fecal homogenates was determined by combustion and liquid scintillation counting.

HPLC Analysis

Plasma aliquots were combined with three volumes of acetonitrile, vortexed and centrifuged. The supernatant was concentrated to dryness under nitrogen, and the residue was reconstituted in HPLC mobile phase. Bile and urine aliquots were combined with an equal volume of acetonitrile. Aliquots of fecal homogenates were combined with an equal volume of acetonitrile, vortexed and centrifuged. The fecal pellet was resuspended in 1 ml acetonitrile, vortexed, centrifuged and supernatant was separated. The procedure was repeated until >80% of recovery of radioactivity was obtained. The pooled supernatants were filtered using syringe filters (Nalgene 25 mm PTFE syringe filters, 0.45 μm) and evaporated *in vacuo* at ambient temperature. The residue was reconstituted in HPLC mobile phase. Separations were achieved at ambient temperature with a Beckman Ultrasphere 5 μm 4.6 × 250 mm C_{18} column connected to an Alltech Ultrasphere 5 μm C_{18} cartridge guard column. A linear gradient of 25–55% acetonitrile in buffer (25 mM ammonium acetate, pH adjusted to 4.8) over 57 min was used as column eluent at a flow rate of 1 ml/minute. Radioactivity in the column effluent was monitored with a Flo-One/Beta Model A-500 radioactivity flow detector (Packard Instruments, Meriden, CO). Pharmacokinetic analysis of data was performed by non-compartmental analysis using WinNonlin (PharSight Corporation, Mountain View, CA). AUC values were calculated by linear trapezoidal method.

Mass Spectral Analysis

The chromatographic peaks corresponding to major metabolites in bile, urine and feces were isolated by fraction collection. Mass spectrometric analysis was performed as previously described, and the metabolites were identified by comparison of the fragmentation patterns to previously identified *in vitro* metabolites of LVR (15).

RESULTS

Plasma Protein Binding

Binding of LVR was high in the plasma of all five species and ranged from 97.6% to 98.9% in mouse, 95.9% to 99.8% in rat, 96.3% to 99.5% in dog, 95.4% to 98.3% in monkey, and 97.4% to 99.7% in human plasma (Table II). At all the five concentrations examined, monkey plasma had the highest unbound fraction. The extent of protein binding was found to decrease with increasing drug concentration in all five species.

Table II. Plasma Protein Binding of Lopinavir

LVR (μg/ml)	Mean percent bound (n = 2)				
	Mouse	Rat	Dog	Monkey	Human
0.1	98.9	99.8	99.5	98.3	99.7
1.0	98.6	99.8	99.5	98.0	99.7
10.0	98.2	98.5	98.8	97.4	99.5
30.0	98.2	97.6	97.9	96.9	98.7
100.0	97.6	95.9	96.3	95.4	97.4

The increase in unbound fraction over the 1000-fold concentration range was highest for rat (ca.16-fold) and lowest for mouse (ca. 2-fold). In human plasma, binding did not change appreciably between 0.1 and 10 μg/ml of LVR, averaging 99.6%, but was lower at 30 and 100 μg/ml. Between 10 and 100 μg/ml, the unbound fraction of LVR increased approximately 5-fold in human plasma. Since the peak plasma concentrations of LVR in humans after therapeutic doses are <15 μg/ml (16), a higher unbound fraction *in vivo* in humans is probably not likely. LVR binding to HSA ranged from 93.6% to 96.3%, and was found to decrease in a concentration-dependent manner in the 30 to 0.1 μg/ml concentration range. LVR was extensively bound to AAG at lower concentrations of the drug and the binding ranged from 72.5% to 99.6% in the 300-fold concentration range (30 to 0.1 μg/ml) examined. These results indicate that both HSA and AAG contribute to the binding of LVR and the concentration-dependent decrease of protein binding in human plasma could be due to saturation of binding sites of these two proteins.

Tissue Distribution of Radioactivity in Rats

Table III summarizes the peak radioactivity levels (C_{max}) and the area under the total plasma radioactivity versus time curve ($AUC_{72\ h}$) in plasma and tissue to plasma ratios (T/P) of these two parameters. The peak radioactivity levels in plasma were achieved at 4 h post-dose. At 72 h after dosing, only 0.13% of the dose radioactivity was recovered from tissues (including carcass) indicating a complete excretion of [^{14}C]LVR-associated radioactivity. As expected after oral dosing, a majority of the administered radioactivity was recovered from gastrointestinal tract. At 4 h after dosing, liver (tissue to plasma ratio, T/p = 22.52), adrenals (T/p = 2.07), and thyroid (T/p = 1.90) exhibited greater radioactivity levels than plasma. The radioactivity levels in the remaining tissues were lower than those in plasma, with the T/P ratios ranging from 0.02 to 0.94. The T/P ratio in brain was 0.02, indicating that [^{14}C]LVR-associated radioactivity poorly penetrated the blood-brain barrier. The levels of radioactivity in lymph nodes were moderate, with lumbar and submaxillary lymph nodes having T/P ratios of 0.19 and 0.55, respectively. The distribution of [^{14}C]LVR into lymph nodes is of clinical relevance because it is the most important site where anti-HIV action is needed (17).

Excretion of Radioactivity

Following intravenous or oral dosing to surgically unmodified male and female rats, with [^{14}C]LVR given alone or in combination with RVR, fecal route of elimination was the predominant excretory pathway with <2% excreted in urine.

Table III. Tissue Distribution of Radioactivity in Male Rats After an Oral Dose of [^{14}C]LVR and RVR

Tissue	C_{max}*	$AUC_{72\ h}$
	Total plasma radioactivity	
	2.32 μg eq/ml	14.5 μg eq · h/ml
	Tissue to plasma ratio†	
Adrenals	2.07	1.62
Blood	0.50	0.50
Bone marrow (femur)	0.13	0.30
Brain	0.02	0.02
Eyes	0.16	0.19
Fat (perirenal)	0.78	0.73
Heart	0.52	0.52
Kidneys	0.92	0.92
Large intestine‡	18.87	82.97
Liver	22.52	22.19
Lungs	0.51	0.54
Lymph nodes§	0.15	0.19
Lymph nodes¶	0.61	0.55
Pancreas	0.94	0.66
Plasma	1.00	1.00
Prostate	0.45	0.36
Skeletal muscle	0.25	0.20
Skin	0.29	0.36
Small intestine‡	41.97	40.75
Spleen	0.41	0.36
Stomach‡	81.54	84.83
Testes	0.17	0.16
Thymus	0.42	0.33
Thyroid	1.90	1.17
Urinary bladder	0.55	0.47

Mean of n = 3.
* C_{max} of plasma radioactivity was achieved at 4 h post-dose.
† Tissue to plasma ratio at plasma T_{max} (4 h post-dose).
‡ With contents.
§ lumbar.
¶ submaxillary.

A major portion of this radioactivity was excreted within 24 h after dosing. After intravenous dosing of [^{14}C]LVR in combination with RVR to dogs, the mean total recovery of radioactivity in male and female dogs by five days after dosing was 100.6 and 101.7%, respectively. The recoveries after oral dosing were 100.8 and 90.8% for male and female dogs, respectively. A major portion of this radioactivity was excreted in feces within first two days after dosing. In all cases, the excretion of radioactivity in urine accounted for <1% of the administered dose in dogs. In humans, 10.4 ± 2.3% of the dose radioactivity was excreted in urine within three days of dosing, with no detectable radioactivity present in urine thereafter (Fig. 1). The mean cumulative recovery of radioactivity in feces was 82.6 ± 2.5% of the dose radioactivity. Due to constipation, the recovery of radioactivity in feces was slow and erratic. The mean overall recovery in humans was 93.0 ± 0.9% of the dose radioactivity.

Biliary Excretion in Rats

After IV dosing, 69.5% and 66.8% of the dose radioactivity was excreted in the bile after dosing [^{14}C]LVR alone or with RVR, respectively, over a 24-h period (Fig. 2; Table IV). A majority of the biliary radioactivity was excreted during the first two hours after dosing of [^{14}C]LVR alone. However, only a minor fraction of the biliary radioactivity was excreted

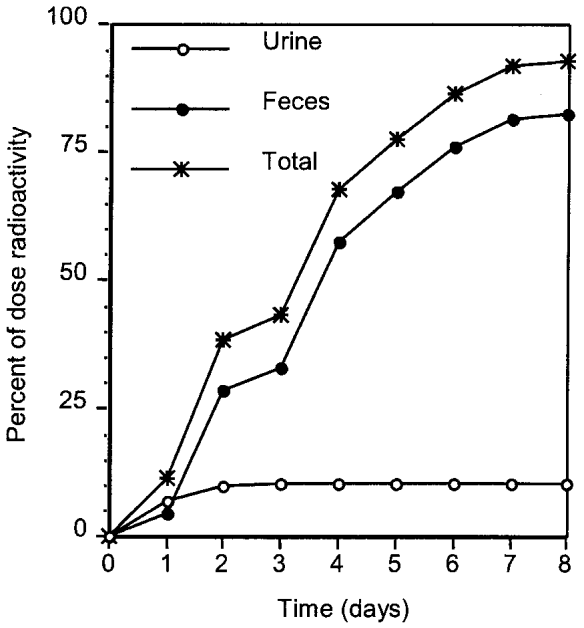


Fig. 1. Cumulative excretion of radioactivity after a single oral dose of 400 mg of [^{14}C]LVR with 100 mg of RVR to humans.

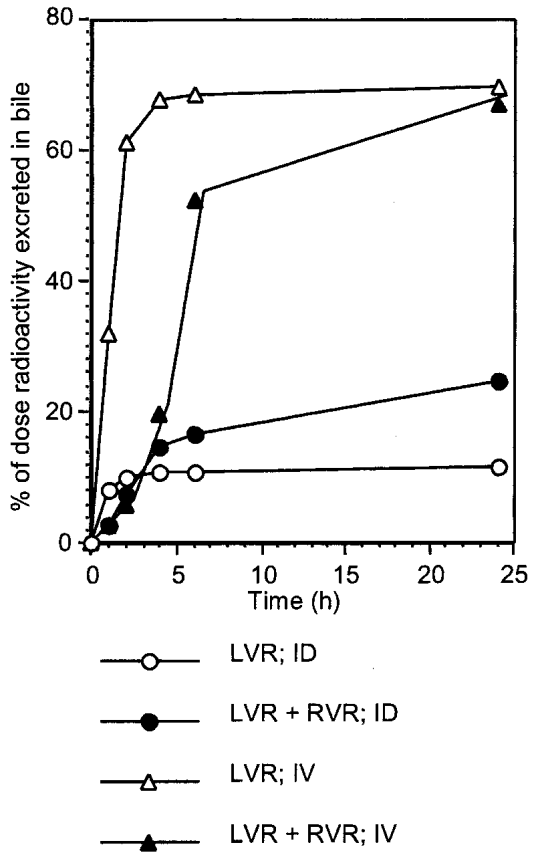


Fig. 2. Effect of RVR on biliary excretion of radioactivity after intravenous or intraduodenal administration of [^{14}C]LVR to bile duct-cannulated male rats.

Table IV. Distribution of LVR and its Metabolites in Rat, Dog, and Human Excreta

Species (route) (group)*	Dose LVR/RVR (mg/kg)	Sample (time)	% of dose excr.	Mean percent of radioactive dose							
				LVR	M-1	M-3/M-4	M-5	M-6/M-8	M-9/M-10	M-11–M-15†	Other
Rat (IV) (2)	10/0	Feces (0–3 days)	90.0	0.4	3.5	7.7	1.4	ND	6.4	5.3	65.4
Rat (IV) (2)	10/5	Feces (0–3 days)	99.5	28.0	5.3	21.6	1.3	ND	8.3	8.9	26.3
Rat (PO) (3)	10/0	Feces (0–3 days)	96.6	14.9	4.1	8.4	2.2	ND	4.6	5.9	56.5
Rat (PO) (3)	10/5	Feces (0–3 days)	107.1	56.7	4.5	19.8	0.8	ND	8.4	8.0	9.0
Rat (IV) (4)	10/0	Bile (0–24 h)	69.5	0.4	1.0	10.2	ND	ND	4.9	7.8	45.4
Rat (IV) (4)	10/5	Bile (0–24 h)	66.8	1.4	0.7	20.6	1.7	ND	3.1	5.8	33.5
Rat (ID) (5)	10/0	Bile (0–24 h)	11.7	<0.1	0.2	2.3	ND	ND	0.6	1.2	7.5
Rat (ID) (5)	10/5	Bile (0–24 h)	24.7	0.2	0.7	9.8	0.7	ND	1.1	2.8	9.4
Dog (IV) (8)	5/2.5	Bile (0–6 h)	19.6	1.4	0.5	7.5	0.2	0.5	1.0	3.6	5.0
Dog (ID) (9)	5/2.5	Bile (0–6 h)	8.1	0.1	0.1	1.2	0.1	0.2	0.9	1.7	3.9
Dog (IV) (10)	5/2.5	Feces (0–5 days)	98.4	4.2	3.6	12.7	3.4	8.4	17.8	23.6	24.6
Dog (PO) (11)	5/2.5	Feces (0–5 days)	94.3	1.4	2.0	6.4	1.6	3.9	8.6	12.5	58.0
Human (PO)	400/100‡	Feces (0–8 days)	82.6	19.8	3.2	10.1	ND	3.9	7.4	8.9	29.3
		Urine (0–8 days)	10.4	2.2	0.2	0.2	ND	0.1	0.1	0.1	7.5
		Total (0–8 days)	93.0	22.0	3.4	10.3	ND	4.0	7.5	9.0	36.8

ND, not detected.
* Data presented for male rats and dogs only, since no marked gender-related differences were observed.
† Sum of metabolites M-11, M-12, M-13, M-14, and M-15.
‡ Dose for humans was the total amount (mg) administered.

during the first two hours after combination dosing, with continuous excretion of radioactivity over the 24-h period. After ID dosing, 11.7% and 24.7% of the dose radioactivity was excreted in bile after dosing [^{14}C]LVR given alone or with RVR, respectively, over the 24-h period (Fig. 2; Table IV). A majority of the biliary radioactivity was excreted during the first two hours after dosing of [^{14}C]LVR alone. However, only a minor fraction of the biliary radioactivity was excreted during the first two hours after combination dosing, with a major portion of excretion occurring thereafter.

Plasma Radioactivity

The total plasma radioactivity levels in rats after oral dosing of [^{14}C]LVR with or without RVR are shown in Table V. Co-administration of RVR produced substantial increases in the C_{max} and AUC of plasma radioactivity in rats. After IV administration, RVR increased AUC values of total plasma radioactivity by greater 4-fold. After oral dosing, RVR increased the C_{max} and AUC values of total plasma radioactivity by greater than 7-fold in both male and female rats. In addition, C_{max} were attained at later time points (T_{max}) when

Table V. Mean Pharmacokinetic Parameters of LVR-Associated Radioactivity

Group	Route	Sex	LVR/RVR (mg/kg)	Mean pharmacokinetic parameters of total radioactivity					
				C_{max} (μg Eq/ml)	T_{max} (h)	AUC*,† (μg Eq·h/ml)	Half-life (h)	Fold-increase	
								C_{max}	AUC
Rats									
6	IV	M	10/0	NA	NA	4.50	2.07	NA	NA
6	IV	M	10/5	NA	NA	26.71	1.51	NA	5.9
6	IV	F	10/0	NA	NA	8.34	2.23	NA	NA
6	IV	F	10/5	NA	NA	35.09	4.50	NA	4.2
7	PO	M	10/0	0.172	0.25	1.12	1.50	NA	NA
7	PO	M	10/5	1.735	6.0	14.58	2.24	10.1	13.0
7	PO	F	10/0	0.161	0.5	1.25	1.53	NA	NA
7	PO	F	10/5	1.219	8.0	16.97	3.33	7.6	13.6
Dogs									
10	IV	M	5/2.5	NA	NA	48.40	2.24	NA	NA
10	IV	F	5/2.5	NA	NA	32.10	1.88	NA	NA
11	PO	M	5/2.5	3.50	2.0	22.20	2.07	NA	NA
11	PO	F	5/2.5	3.20	2.0	16.30	1.43	NA	NA
Humans‡	PO	M	400/100‡	9.0	6–12	136.4	NC	NA	NA

NA, not applicable; NC, not calculated; ND, not determined.
* AUC presented are for 24 h for rats, 72 h for dogs, and 48 h for humans.
† LVR accounted for ≥88% of the AUC of plasma radioactivity in all species.
‡ Total target dose.

RVR was coadministered with LVR in rats. Female rats exhibited higher exposure and slightly longer terminal half-life compared to male rats following oral dosing of the combination. In dogs, studies were conducted with the combination of [^{14}C]LVR and RVR only, since there were no detectable plasma levels following dosing LVR alone in dogs (9). Following IV dosing, the mean AUC observed was 48.4 and 32.1 μg eq · h/ml in male and female dogs, respectively. After oral doses of the drug combination, the mean C_{max} and AUC of plasma radioactivity were 3.5 and 3.2 μg eq/ml, and 22.2 and 16.3 μg eq · h/ml in male and female dogs, respectively. After oral dosing of [^{14}C]LVR in combination with RVR, the mean peak plasma radioactivity level attained in humans was 9.0 ± 1.6 μg eq/ml (range 8.6–10.6 μg eq/mL) and was achieved between 6 and 12 h after dosing (Fig. 3). The plasma radioactivity profile was also characterized by an initial absorption phase with peak levels reaching around 6 h after dosing, a stable peak phase lasting up to 12 h, after which the radioactivity declined rapidly, with negligible to no radioactivity levels present at 72 h after dosing. At 6 h after dosing, the mean blood to plasma ratio of total radioactivity was 0.44 ± 0.03, indicating that there is minimal distribution of LVR into the cellular elements of the blood.

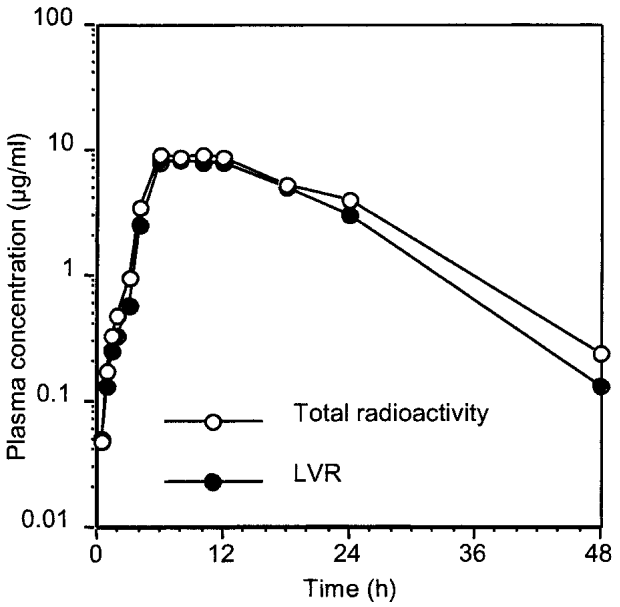
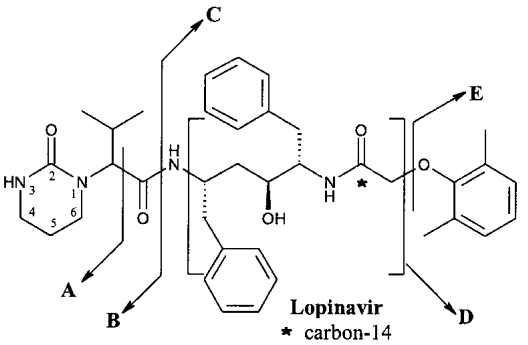


Fig. 3. Total plasma radioactivity and LVR levels in male human plasma after a single oral dose of 400 mg of [^{14}C]LVR with 100 mg of RVR.



	MH$^+$	Fragment ions A	B	C	D	E
LVR	629	155	183	447	310	120
M-1	643 (+14)	169	197	447	310	120
M-3, M-4	645 (+16)	171	199	447	310	120
M-5	645 (+16)	155	183	463	310	136
M-6,7,8	645 (+16)	155	183	463	326	120
M-9, 10	659 (+30)	169	197	463	326	120
M-11-15	661 (+32)	171	199	463	326	120

Fig. 4. Mass spectral fragmentation pattern of LVR and its metabolites (number in parenthesis indicates the change in the protonated molecular ion compared to LVR).

Metabolite Identification

The metabolites of [^{14}C]LVR in rat, dog and human matrices were identified by comparison of their HPLC retention times and mass spectral fragmentation patterns to those of previously identified liver microsomal metabolites (Figs. 4 and 5) (15). The mass spectral fragmentation patterns indicated transformations on cyclic urea (fragment A), central di-phenyl core moiety (fragment D) and 2,6-di-methyl-phenoxy moiety (fragment E) (Fig. 4). Six primary metabolites (M-3, M-4, M-5, M-6, M-7 and M-8), six secondary (M-1, M-11 to M-15) and two tertiary (M-9 and M-10) were identified. M-1 was identified as a 4-oxo derivative of LVR. Metabolites M-3 and M-4 were identified as an epimeric pair of 4-hydroxylated derivatives of LVR. The identities of the major metabolites M1, M3 and M4 were also confirmed by nuclear magnetic resonance spectroscopy previously (15) and by co-chromatography with authentic reference standards in the present study. Metabolite M-5 was found to be a hydroxylated derivative of LVR, with the hydroxyl group located on the 2,6-dimethyl-phenoxy moiety. Metabolites M-6, M-7 and M-8 were identified as mono-hydroxylated derivatives of LVR, with the hydroxyl group located on the central diphenyl core moiety. Metabolites M-9 and M-10 were identified as 4-oxo-hydroxy derivatives of LVR, with hydroxy group located on the diphenyl core moiety. Metabolites M-11, M-12, M-13, M-14 and M-15, were identified as hydroxylated derivatives of either M-3 or M-4, with the second hydroxy group located on the diphenyl core moiety. The quantitative data for groups of metabolites with similar structural features, just differing in the sites of hydroxylation on the di-phenyl core moiety, is presented together in Table IV.

Composition of Plasma Radioactivity

In all three species examined, plasma radioactivity was predominantly composed on unchanged LVR (≥88% of AUC). After both IV and oral dosing of [^{14}C]LVR, a majority of the rat plasma radioactivity was present as the parent compound, with only small amounts of M-1 and M-4 present. LVR accounted for 88 and 95% of the AUC of the total plasma radioactivity in male and female rat plasma, respectively. After IV dosing to male dogs, a majority of the plasma radioactivity was present as the parent compound (96% of AUC of the total plasma radioactivity), with only small amounts of metabolites M-3/M-4 present. In female dogs after IV administration and both male and female dogs after oral administration, the plasma radioactivity was present exclusively as LVR. In humans, the mean AUC were 121.2 ± 15.3 and 136.4 ± 17.1 μg · h/ml for LVR (determined by HPLC

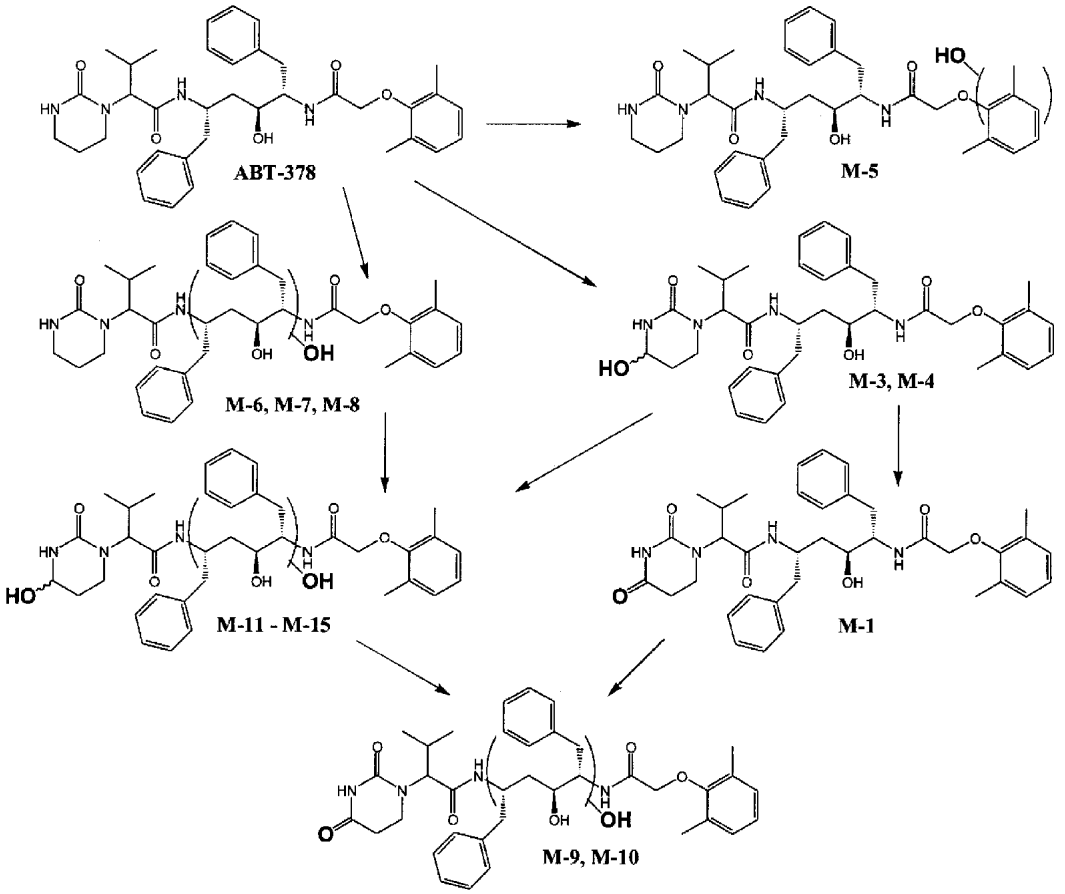


Fig. 5. Proposed metabolic pathways for LVR in rats, dogs, and humans.

with UV detection, Fig. 3) and the total plasma radioactivity, respectively, indicating that approximately 89% of plasma radioactivity was present as the unchanged parent drug. The remaining radioactivity was present as unknown polar metabolites, which eluted in the void volume.

Composition of Biliary Radioactivity in Rats and Dogs

In rat bile, after both IV and ID dosing of [^{14}C]LVR with or without RVR, <2.1% of the biliary radioactivity (<1.5% of radioactive dose) was present as unchanged parent drug (Table IV). The major components of biliary radioactivity were oxidative metabolites M-3/4 and M-9 through M-15. A substantial portion of the biliary radioactivity was present as numerous polar unknown metabolites, presumably tertiary and quaternary metabolites of LVR. The major difference in the biliary metabolite profile after dosing [^{14}C]LVR alone or in combination with RVR is the 2-fold higher quantity of the primary metabolites M-3/4 after combination dosing compared to dosing LVR alone (20.6% vs. 10.2% after ID dosing, Table IV). In dogs, LVR accounted for only 7.3% (1.4% of radioactive dose) and 1.3% (0.1% of radioactive dose) of biliary radioactivity after IV and ID dosing, respectively (Table IV). The major metabolites observed in dog bile were 4-hydroxy-LVR (M-3/M-4) and dihydroxy-LVR (M-11 to M-15).

Composition of Fecal Radioactivity

There were no significant gender-related differences in the distribution of metabolites in the feces of male and female rats or dogs (female data not shown in Table IV). Major metabolites present in all cases were M-1, M-3/4, M-9/M-10 and M-11 through M-15, with a substantial amount of fecal radioactivity present as numerous unknown metabolites, presumably tertiary and quaternary metabolites of LVR (Table IV). After IV dosing of [^{14}C]LVR to male rats, only traces of radioactivity were excreted as parent drug in feces, compared to 28% present as unchanged drug after the dosing of the combination. After oral dosing of [^{14}C]LVR to male rats, 14.9% of radioactivity was present as unchanged drug, compared to 56.7% present as unchanged drug after dosing of the combination. In dogs dosed with the drug combination, unchanged drug constituted less than 5% of fecal radioactivity, with 4-hydroxy-LVR (M-3/M-4), 4-oxo-hydroxy-LVR (M-9 and M-10) and dihydroxy-LVR (M-11 to M-15) as the major metabolites. In human subjects, LVR accounted for 24% of the fecal radioactivity (19.8% of radioactive dose), with M-1 (3.2%), M-3/4 (10.1%), M-6/7/8 (3.9%), M-9/10 (7.4%) and M-11-15 (8.9%) as major components. The remaining fecal radioactivity was composed of numerous polar metabolites. Unlike in animal models, urinary excretion of radioactivity was significant in humans (10.4% of radioactive dose) with LVR accounting for about 21% of the urinary radioactivity (2.2% of the radioactive dose) (Table III). Minor amounts of M-1, M-3/4, M-6/7/8 and some secondary metabolites were also observed in urine. Previous *in vitro* studies had indicated that there were no major inter-species differences in the metabolism of LVR (15). The results of these *in vivo* studies confirm that finding.

DISCUSSION

The results of these studies indicate that [^{14}C]LVR-derived radioactivity was predominantly cleared via the hepato-biliary route in humans as well as animal models. LVR was extensively metabolized before elimination, with carbon-4 of the cyclic urea moiety as the predominant site of biotransformation (Fig. 5; Table III).

The extent of protein binding has been shown to be critical to anti-HIV potency of protease inhibitors (18,19). The *in vitro* antiviral activity of weakly protein-bound indinavir (ca. 50% bound) (2) was attenuated only 2-fold in the presence of serum, whereas the attenuation observed with other highly protein-bound protease inhibitors such as LVR (5- to 10-fold), RVR (>20-fold), saquinavir and amprenavir (11- to 17-fold) was higher (19,20).

RVR, an inhibitor of CYP3A, inhibited *in vitro* metabolism of HIV protease inhibitors saquinavir and indinavir potently (20–22). When RVR was coadministered with these protease inhibitors, a dramatic increase in their exposures was observed (23–25). Due to the high metabolic rate of LVR, the oral bioavailability was low in animal models (9). *In vitro* metabolism of LVR is catalyzed by CYP3A and is potently inhibited by RVR (K_i = 13 nM) (11). As in human liver microsomes, RVR is also a potent inhibitor of LVR metabolism in rat liver microsomes, with an IC_{50} of 36 nM (9). Consistent with the *in vitro* findings, RVR increased both C_{max} and AUC of LVR in rats (twelve-fold increase in exposure) and dogs (from undetectable levels to good bioavailability) (9). In this study, co-administered RVR markedly increased the C_{max} and AUC of the total plasma radioactivity in rats, which is primarily composed of LVR. Similar pharmacokinetic enhancement has also been observed in humans (12,16).

In rats, 0.4% and 28% of the fecal radioactivity was present as LVR after IV dosing of LVR alone or in combination with RVR, respectively. This is interesting in light of negligible levels of LVR in bile and lack of any glucuronide conjugates of the parent drug after combination dosing. This data would suggest that there is a trans-intestinal route of elimination for LVR. However, the plasma levels of LVR are very low after dosing it alone, so the trans-intestinal elimination route might not be significant. However, in the presence of RVR, high plasma levels of LVR are sustained for up to 12 h after dosing, therefore trans-intestinal elimination apparently becomes an important route for the elimination of the unchanged LVR. It is not certain if this route of LVR elimination is attributable to the involvement of transporters (e.g., P-glycoprotein) and their modulation by RVR.

A time lag in excretion of radioactivity in bile was observed in rats after combination dosing. Further, biliary excretion was approximately 2-fold higher after ID dosing of the combination compared to dosing LVR alone. When biliary and fecal radioactivity profiles after mono and combination therapy are compared, the major difference is the higher content of the primary metabolites M-3/M-4. *In vitro* studies have shown that primary as well as secondary metabolism (i.e., the metabolism of M-3/M-4) is catalyzed by the CYP3A subfamily (data not shown). Thus further metabolism of the primary metabolites is also subject to modulation by RVR. Based on the biliary excretion data, it appears that intact LVR is not a good substrate for biliary elimination compared to its polar metabolites. Thus, as long as the CYP-dependent metabolism of LVR is minimal, which is dependent on RVR plasma concentrations, the biliary elimination is minimal. Once the RVR levels fall below a certain inhibitory level, LVR undergoes facile hepatic metabolism and subsequent biliary elimination. In humans, LVR levels were found to parallel RVR levels, indicating that the clearance of LVR was controlled by RVR plasma levels (12,16).

In contrast to rats and dogs (<2% of dose radioactivity excreted in urine), a higher amount of the radioactive dose (ca. 10%) was excreted in human urine. A similar observation was made with RVR (26). As in rats, metabolites M-3/M-4 and dihydroxy metabolites were also major components of fecal radioactivity in both dogs and humans. The metabolic profile of LVR in rats, dogs and humans is similar. In all the three species examined, the plasma radioactivity was present predominantly as unchanged LVR with small amounts of metabolites. The metabolism of LVR is essentially a deactivation reaction, because its major metabolites are less potent inhibitors of HIV protease than LVR (27).

In summary, LVR was extensively metabolized before elimination. The predominant site of metabolism was found to be the carbon-4 of the cyclic urea moiety, with subsequent secondary metabolism occurring on the diphenyl core moiety. RVR was found to increase the plasma levels of LVR-derived radioactivity in rats, probably due to inhibition of its presystemic and systemic metabolism. A similar effect of RVR on the first-pass metabolism of LVR in humans is the most likely cause for the pharmacokinetic enhancement observed, leading to an increased exposure to this potent HIV protease inhibitor.

ACKNOWLEDGMENTS

We thank Dr. A. Zutshi and Ms. N. Trigg of Battelle Memorial Institute for the rat tissue distribution study. We thank Ms. J. Hunter of Covance Laboratories, and Ms. M. Knipper, Dr. S. Ghosh and Ms. P. Ludwig of Abbott Laboratories for their help during the human disposition study. We also thank Drs. R. Bertz, A. Hsu and G.R. Granneman of Abbott Laboratories for helpful discussions.

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