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PHARMACOKINETICS AND DRUG DISPOSITION

Ritonavir increases loperamide plasma concentrations without evidence for P-glycoprotein involvement

Background: The antidiarrheal drug loperamide is frequently used to treat ritonavir-associated diarrhea in patients with human immunodeficiency virus. The absence of marked central opioid effects has been attributed to its low bioavailability and its poor penetration of the blood-brain barrier, both of which might be altered by ritonavir, a potent P-glycoprotein and cytochrome P4503A inhibitor.

Methods: A 16-mg dose of loperamide was administered to 12 healthy male and female volunteers together with either 600 mg of ritonavir or placebo. Detailed pharmacokinetics of loperamide and its metabolites were determined over 72 hours. Central opioid effects were measured by evaluation of pupil diameter, cold pressor test, and transcutaneous P_{CO_2} and P_{O_2}.

Results: Ritonavir caused a major pharmacokinetic interaction, increasing the area under the concentration-time curve of loperamide from 104 ± 60 h · pmol/ml after placebo to 276 ± 68 h · pmol/ml and delayed formation of the major metabolite desmethylloperamide (time to reach maximum concentration after drug administration [t_{max}], 7.1 ± 2.6 hours versus 19.6 ± 9.1 hours). The urinary metabolic ratio of loperamide increased 3 times whereas the total molar amount of loperamide and metabolites excreted

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in urine remained unchanged. No central pharmacodynamic effects were observed after coadministration of loperamide with either ritonavir or placebo.

Conclusion: **This study demonstrates a major metabolic interaction probably by cytochrome P4503A4 with no evidence of P-glycoprotein involvement. This might explain the lack of central effects after ritonavir. (Clin Pharmacol Ther 2001;70:405-14.)**

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Loperamide, a peripherally acting opioid receptor agonist with a methadone-like structure and antidiarrheal action, possesses no typical systemic opioid effects such as respiratory depression, analgesia, or changes in alertness. Studies in rats and mice suggested that loperamide causes central nervous system (CNS) effects only in doses many times exceeding those required for antiperistaltic actions and that, whereas it binds to opioid receptors in the gut and brain, it penetrates the CNS poorly.[1] This low penetration of the blood-brain barrier is regarded as a major reason for the lack of central opioid effects of loperamide.[2] The multidrug resistance transporter P-glycoprotein in the blood-brain barrier blocks the access to target tissues by extruding substrates such as loperamide from the brain endothelial cells.[2] Nevertheless, it was shown in mice that loperamide may act in an opioid-like manner when it reaches the brain.[2,3]

P-glycoprotein is situated in many excretory and absorptive tissues, such as, for example, the intestine, the renal tubule, and the blood-brain barrier, and in malignant cells, resulting in multidrug resistance. The encoding genes are thus labeled multidrug resistance genes, *MDR1* in humans and *mdr1a* and *mdr1b* in mice.[4] The absence of P-glycoprotein in *mdr1a*(–/–) mice resulted in a higher increase in plasma and brain loperamide concentration, about 2 and 13.5 times in comparison with the wild-type mice *mdr1a*(+/+), respectively.[2] Moreover, it was recently shown in humans that loperamide caused respiratory depression as a result of P-glycoprotein inhibition by quinidine, suggesting that brain penetration of loperamide was increased.[5]

Diarrhea is a very common side effect of all human immunodeficiency virus (HIV) protease inhibitors.[6] It can usually be controlled with antidiarrheal drugs such as loperamide or fiber supplements.[7] The HIV protease inhibitor ritonavir is also known to be a potent inhibitor of P-glycoprotein.[8-10] Among available protease inhibitors, ritonavir carries the highest risk of causing drug interactions as a result of inhibition of several cytochrome P450 (CYP) isozymes, which also catalyze its metabolism.[6,11,12] This could give rise to a major interaction between ritonavir and loperamide by inhibiting both P-glycoprotein and CYPs. The wide use of the over-the-counter drug loperamide requires systematic investigation of our hypothesis. We therefore investigated the detailed pharmacokinetics of loperamide and its metabolites, as well as the systemic opioid effects determined by measurement of changes in pupil diameter,[13] transcutaneous P_{CO_2} as a surrogate of respiratory depression,[14] and the cold pressor test responses for pain assessment,[15] assuming that an increase of brain loperamide concentration through P-glycoprotein inhibition by ritonavir would modulate these end points of opioid action.

METHODS

The study protocol was approved by the Ethics Committee of the University Hospital, Heidelberg, and was conducted at the Department of Internal Medicine VI, Clinical Pharmacology and Pharmacoepidemiology, in accordance with the Declaration of Helsinki and subsequent amendments. Written informed consent was obtained from each participant.

Study population. Twelve healthy nonsmoking male (n = 6) and female (n = 6) subjects (age, 21-45 years; body mass index, 19.9–27.5 kg/m^2) were enrolled in the trial. All volunteers were drug-free for at least 6 weeks before entry into the study. Subjects were ascertained to be healthy by clinical examination, electrocardiography, and routine laboratory tests. Women were required to undergo a pregnancy test and were enrolled if the result was negative and they were using a barrier contraceptive.

Study design. A randomized, double-blind, 2-way crossover study was carried out with single-dose administration of the study drugs. After an overnight fast, the subjects received a standard breakfast, consist-

ing of white bread, cheese, butter, and caffeine-free tea 30 minutes before oral administration of the study drugs. Alcoholic and caffeinated beverages were not allowed from 12 hours before drug administration until the last blood sample on the kinetic study day. Subjects stayed at the Clinical Research Unit of the department for 12 hours after drug administration; thereafter they returned at the required blood sampling times. Lunch and supper consisted of normal hospital meals. Subjects maintained their usual daily activities throughout the trial except on the days of the kinetic study.

After the cannulation of a forearm vein, all 12 volunteers received 600 mg ritonavir (6 capsules of Norvir 100 mg; Abbott Laboratories, Abbott Park, Ill) or placebo (6 capsules). Immediately thereafter, they took 16 mg loperamide (8 capsules of Imodium 2 mg; Janssen-Cilag, Neuss, Germany) orally together with 200 ml of mineral water. Placebo capsules were prepared by and ritonavir was obtained through the hospital pharmacy. After a washout period of 11 days after the last blood sample, the identical procedure was repeated with ritonavir and placebo interchanged.

Blood sampling and urine collection. Venous blood samples (7.5 ml) for determination of the concentrations of loperamide, its metabolites desmethylloperamide and didesmethylloperamide (Fig 1),[16] and ritonavir were drawn before loperamide administration and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 24, 30, 48, 54, and 72 hours after dosing. Blood samples were immediately centrifuged at 4°C, and separated plasma was stored at –20°C until analysis. Urine was collected at 4 intervals as follows: 0 to 8, 8 to 24, 24 to 48, and 48 to 72 hours after loperamide administration. An aliquot of 10 ml was kept frozen at –20°C until analysis.

Quantification of loperamide, ritonavir, and loperamide metabolites. Plasma concentrations of loperamide, desmethylloperamide, and didesmethylloperamide were assessed by liquid chromatography–tandem mass spectrometry.[17] In brief, the 3 analytes were extracted from alkalinized plasma with acetic acid ethyl ester and separated on a Zorbax Eclipse XDB-C8 narrow-bore column (Hewlett-Packard, Waldbronn, Germany) with a mixture of acetonitrile, methanol, and 4-mmol/L ammonium acetate (pH 4.6) as the mobile phase. A Turbo Ionspray was used for ionization. Molecular ions and several fragments characterized all compounds. Multiple reaction monitoring (MRM) was chosen for identification and quantification. Racemic methadone was used as internal standard. Calibration curves for the 3 compounds showed excellent linearity with correlation coefficients of $r = 0.999$ for loperamide and $r = 0.998$ for

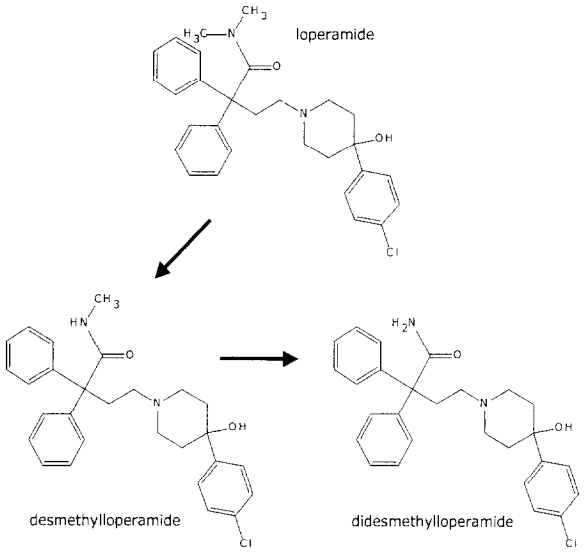

Fig 1. Chemical structure of loperamide and its metabolites.[16]

desmethylloperamide and didesmethylloperamide. Intra-assay variabilities obtained by analyzing replicate samples (n = 5) at two concentrations (419 and 4193 fmol/ml) were 4.2% and 2.1% for loperamide, 2.6% and 3.4% for desmethylloperamide, and 4.5% and 5.0% for didesmethylloperamide. Interassay variabilities obtained were 7.2% and 4.4% for loperamide, 5.3% and 6.3% for desmethylloperamide, and 5.7% and 6.0% for didesmethylloperamide.

Urine concentrations of loperamide and its metabolites were determined by liquid chromatography–tandem mass spectrometry with and without hydrolysis. Urine (300 µl) from pooled samples (0-72 hours) was spiked with internal standard solution (10 µl $^2H_5$-saquinavir, 17.4 pmol/L) alkalinized with 700 µl buffer, pH 10 (Merck, Darmstadt, Germany). Subsequently, *tert*-butylmethylether (4 ml) was added, shaken automatically (15 minutes), and centrifuged (10 minutes) at 2000*g*. The clear supernatant (organic layer) was evaporated to dryness (40°C) in a stream of nitrogen. Reconstitution was achieved with the mobile phase (400 µl). Conjugates of loperamide and its metabolites in urine were determined indirectly via alkaline hydrolysis. Therefore urine (100 µl) was alkalinized with 1-mol/L sodium hydroxide solution (200 µl) and incubated for 15 minutes at 50°C. Subsequently, the same extraction procedure was applied. Calibration curves for the 3 compounds showed linearity with correlation coefficients of $r = 0.999$. Intra-assay variability came to –9% to +1% for loperamide, –14% to +2%

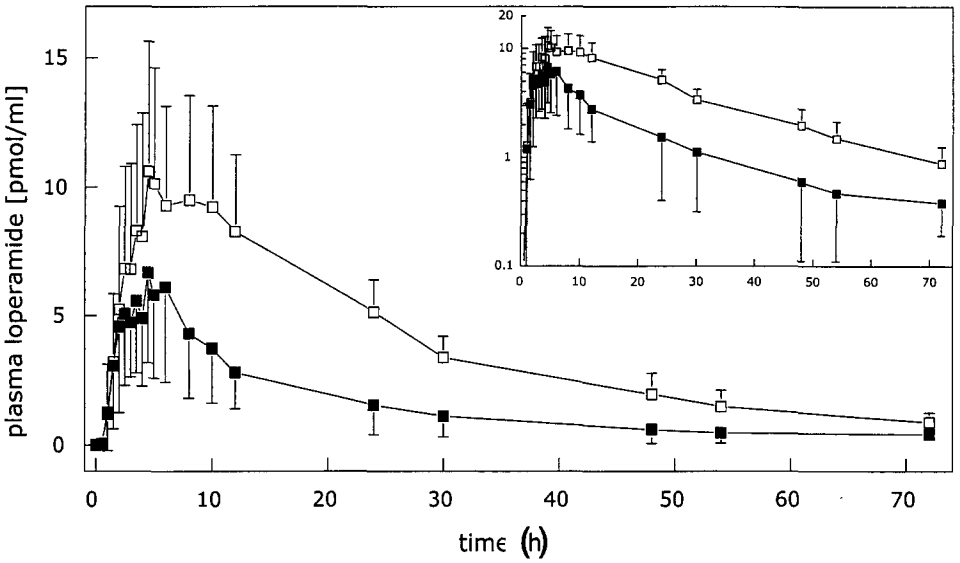

Fig 2. Mean ± SD plasma concentration time profiles of loperamide (16 mg) with placebo *(solid boxes)* or ritonavir *(open boxes)* (n = 12).

Table I. Pharmacokinetic data, median (range), of loperamide after single oral administration of 16 mg loperamide in combination with either placebo or 600 mg ritonavir in 12 healthy volunteers

Parameter	*Placebo*	*Ritonavir*	*Wilcoxon test*
C_{max} (pmol/ml)	8.6 (2.4-13.4)	10.1 (7.1-21.8)	$P < .01$
t_{max} (h)	4.5 (2.0-6.0)	7.0 (4.5-12.0)	$P < .01$
$t_{½}$ (h)	16.9 (7.9-21.0)	17.5 (15.4-27.8)	NS
AUC(0-∞) (h · pmol/ml)	85.3 (37.4-250.8)	275.5 (168.0-404.9)	$P < .01$
CL_{oral} (ml/min)	5720 (1921-11,975)	1741 (1193-2880)	$P < .01$
CL_{renal} (ml/min)	30.2 (14.1-59.6)	33.4 (12.2-129.1)	NS
Ae (nmol)	199 (46-347)	559 (213-1301)	$P < .01$

C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; AUC(0-∞), area under the plasma concentration-time curve; $t_{½}$, terminal elimination half-life; CL_{oral}, apparent oral clearance; Ae, amount of loperamide excreted in urine from 0 to 72 hours; CL_{renal}, renal clearance of loperamide; NS, not significant.

for desmethylloperamide, and −13% to +2% for didesmethylloperamide, respectively. The limit of quantification for loperamide and its metabolites in urine was 2.1 pmol/ml.

Ritonavir plasma concentrations were determined by liquid chromatography–tandem mass spectrometry after liquid/liquid extraction according to Burhenne et al.[18] The calibration curve for ritonavir showed linearity with a correlation coefficient of at least $r = 0.997$. Intra-assay and interassay variabilities were in the range of −10% to +12%, and the limit of quantification was 2.5 pmol/ml.

Clinical and pharmacodynamic assessments. Subjects were asked during the study period for any symptoms or effects they may have noticed. On study days the pharmacodynamic assessment was performed immediately after each blood sampling until 6 hours after dosing. Pupillometry, a transcutaneous P_{CO_2} and P_{O_2} analysis, and a cold pressor test for the assessment of pain sensitivity were performed. Before the study the subjects had to be familiarized with the study methods to avoid habituation effects. This was done by a training session on the day of the clinical examination.

Static infrared pupillometry with a compact Integrated Pupillograph (CIP; Amtech, Weinheim, Germany) was applied for assessment of static changes in pupil diameter. This noninvasive procedure enables the assessment of pupil diameter by an infrared camera

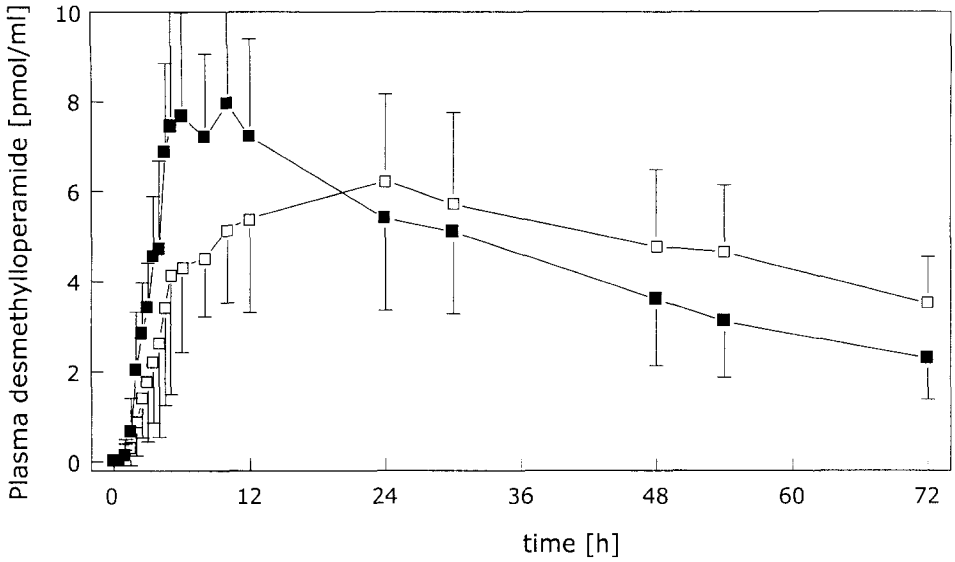

Fig 3. Mean ± SD plasma concentration time profiles of desmethylloperamide after administration of 16 mg loperamide with placebo *(solid boxes)* or ritonavir *(open boxes)* (n = 12).

Table II. Pharmacokinetic data, median (range), of desmethylloperamide after single oral administration of 16 mg loperamide in combination with either placebo or 600 mg ritonavir in 12 healthy volunteers

Parameter	*Placebo*	*Ritonavir*	*Wilcoxon test*
C_{max} (pmol/ml)	8.8 (5.8-13.9)	6.0 (4.2-10.7)	$P < .01$
t_{max} (h)	6.0 (4.5-12.0)	24.0 (5.0-30.0)	$P < .01$
$t_{½}$ (h)	35.7 (17.6-62.6)	48.9 (31.8-56.4)	$P < .01$
AUC (h · pmol/ml)	334.4 (183.6-534.4)	310.9 (234.0-598.6)	NS
CL_{renal} (ml/min)	68.6 (35.3-108.3)	79.4 (29.1-201.4)	NS
Ae (nmol)	1324 (458-2199)	1703 (478-3026)	NS

C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; AUC, area under the plasma concentration-time curve fron 0 to 72 hours; $t_{½}$, terminal elimination half-life; CL_{renal}, renal clearance of desmethylloperamide; Ae, amount of desmethylloperamide excreted in urine from 0 to 72 hours; NS, not significant.

under defined light conditions. A mean of 3 measurements with a minimum interval of 30 seconds was obtained. Immediately after this test, a transcutaneous P_{CO_2} and P_{O_2} analysis was performed with a Radiometer A/S device (TC100; Radiometer, Copenhagen, Denmark). The electrode was placed on the internal surface of the dominant forearm. Pain sensitivity and pain tolerance were assessed with the cold pressor test with two temperature-controlled water baths of 35°C ± 0.5°C and 1°C ± 0.5°C, respectively. The nondominant arm of the subjects was placed in the water bath of 35°C for 2 minutes. Fifteen seconds before the forearm was transferred into the cold water bath, a blood pressure cuff was inflated to a pressure of 20 mm Hg below the diastolic blood pressure to increase the stability of the cold pressor test as a result of better local hemodynamic control.[19] At this time the subject's eyes were covered with eye patches. The subjects then placed the forearm into the cold water bath in a constant position with the fingers wide apart for a maximum of 2 minutes. They were instructed to indicate the time point of the first pain sensation (pain threshold) and the time point of intolerable pain (pain tolerance), at which time the forearm was immediately removed from the water bath.

Calculations and statistics. Data are presented as median values and range. Noncompartmental analysis with WinNonlin 3.1 (Pharsight Corporation, Mountain View, Calif) was performed to determine

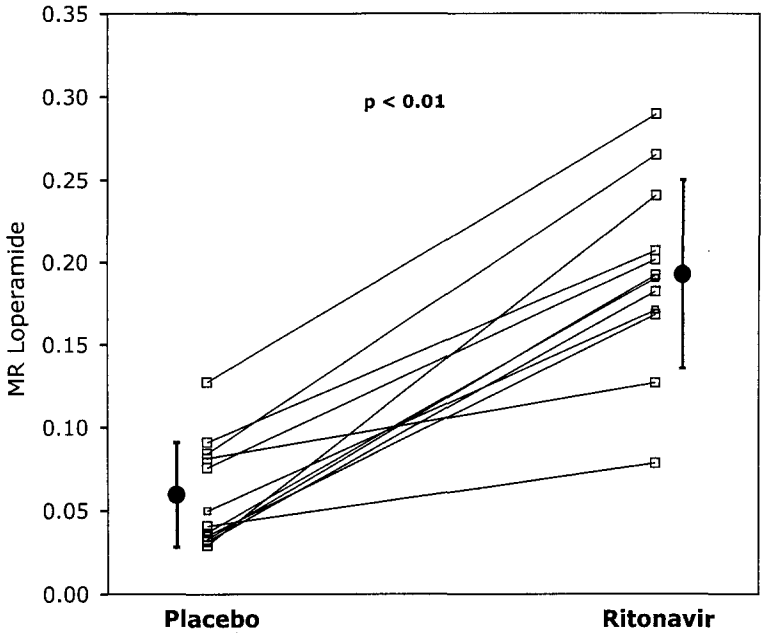

Fig 4. Urinary metabolic ratio of loperamide (16 mg) with placebo or ritonavir (n = 12).

pharmacokinetic parameters of loperamide and ritonavir. For loperamide and its metabolites the following pharmacokinetic parameters were calculated: maximum observed plasma concentration (C_{max}), time to reach C_{max} (t_{max}), area under the plasma concentration-time curve from time zero to the last measurable concentration (AUC), and area under the plasma concentration-time curve extrapolated to infinity [AUC(0-∞)] calculated with the linear trapezoidal rule and the terminal elimination half-life ($t_{½}$). The apparent oral clearance (CL_{oral}) was calculated as the dose of loperamide divided by the AUC(0-∞). Renal clearances of loperamide and its metabolites were determined as the amount excreted in urine divided by the respective AUC(0-∞) values. Partial metabolic clearances to loperamide metabolites were calculated as the amount of metabolite (corrected for molecular weight differences) excreted divided by the AUC(0-∞) of the parent drug. A urinary metabolic ratio for the primary metabolic step to desmethylloperamide was determined as the molar amount of loperamide excreted divided by the sum of desmethylloperamide, didesmethylloperamide, and their conjugates. Differences in these pharmacokinetic parameters between placebo and ritonavir treatment were assessed with the nonparametric Wilcoxon matched pairs signed-rank test.

To assess the pharmacodynamics of loperamide analyzed by pupillometry, transcutaneous P_{CO_2} measurement, and cold pressor test, the changes in the data relative to baseline values obtained before loperamide administration were calculated. A 2-way repeated-measures ANOVA was applied to test for significant changes over time and changes between placebo and ritonavir treatment. A P value < .05 was considered significant.

RESULTS

Pharmacokinetics. In the absence of ritonavir, only low loperamide plasma concentrations were observed in all subjects despite the high dose of 16 mg loperamide. Fig 2 depicts the pharmacokinetic profiles of loperamide with and without ritonavir treatment. Table I shows the respective resultant pharmacokinetic parameters. The median maximum loperamide plasma concentration was 8.6 pmol/ml. After concomitant ritonavir administration, the plasma loperamide concentration increased significantly (C_{max}, 10.1 pmol/ml; P < .01). The AUC of loperamide increased approximately 3 times during ritonavir treatment, and therefore the apparent oral clearance decreased correspondingly. However, the renal clearance of loperamide was low and unchanged during ritonavir (Table I). Plasma concentrations of the primary metabolite desmethylloperamide (see Fig 3) were in a range simi-

Table III. Partial metabolic clearances, milliliters per minute, median (range), of loperamide and its metabolites after single oral administration of 16 mg loperamide in combination with either placebo or 600 mg ritonavir in 12 healthy volunteers

Parameter	*Placebo*	*Ritonavir*	*Wilcoxon test*
$CL_{lop \rightarrow g}$	2 (0-37)	3 (0-136)	NS
$CL_{lop \rightarrow dmlop}$	608 (152-1191)	174 (46-678)	$P < .01$
$CL_{dmlop \rightarrow g}$	15 (4-96)	9 (1-175)	NS
$CL_{dmlop \rightarrow ddmlop}$	82 (31-150)	55 (13-120)	$P < .01$
$CL_{ddmlop \rightarrow g}$	1154 (473-2721)	780 (179-2347)	NS

$CL_{lop \rightarrow g}$, Partial metabolic clearance of loperamide to its conjugates (glucuronide); $CL_{lop \rightarrow dmlop}$, partial metabolic clearance of loperamide to desmethylloperamide; $CL_{dmlop \rightarrow g}$, partial metabolic clearance of desmethylloperamide to its conjugates (glucuronide); $CL_{dmlop \rightarrow ddmlop}$, partial metabolic clearance of desmethylloperamide to didesmethylloperamide; $CL_{ddmlop \rightarrow g}$, partial metabolic clearance of didesmethylloperamide to its conjugates (glucuronide); NS, not significant.

lar to that of loperamide (C_{max}, 8.8 pmol/ml) and were significantly reduced during ritonavir treatment (C_{max}, 6.0 pmol/ml; $P < .01$). A 4-fold prolongation of t_{max} was observed during ritonavir (Table II). Between placebo and ritonavir treatment no differences in the area under the plasma concentration-time curve from time zero to 72 hours [AUC(0-72)] for desmethylloperamide, renal clearance, and amount excreted in urine were observed. The secondary metabolite didesmethylloperamide was not detectable in the majority of plasma samples; therefore no pharmacokinetic parameters could be calculated. In the urine of all volunteers didesmethylloperamide was detected in small amounts (placebo, 52.0 nmol; range, 16.6-101.0 nmol; ritonavir, 46.2 nmol; range, 6.2-85.3 nmol; difference not statistically significant). The median urinary metabolic ratio of loperamide during placebo was 0.046 (range, 0.029-0.127), indicating substantial metabolism, and significantly increased during ritonavir treatment by a factor of 4 (Fig 4). Determination of the partial metabolic clearances revealed significant decreases of both *N*-demethylation pathways (desmethylloperamide and didesmethylloperamide) and no change of the conjugation pathways (Table III). The total amount of loperamide and its metabolites excreted in urine over a 72-hour period was 10.2% (range, 4.4%-23.4%) of the loperamide dose and was not changed by ritonavir administration (11.6%; range, 3.6%-31.2%).

Compared with loperamide, ritonavir had plasma concentrations that were about 400-fold higher observed after administration of 600 mg (C_{max}, 3.5 nmol/ml; range, 0.2-10.3 nmol/ml), resulting in an AUC of 32.4 h · nmol/ml (range, 3.8-79.6 h · nmol/ml). The terminal elimination half-life was 9.0 hours (range, 5.5-15.4 hours). There was no difference ($P > .1$) between the study periods for C_{max} (3.3 versus 4.1 nmol/ml), AUC (32.4 versus 34.6 h · nmol/ml), and $t_{1/2}$ (9.0 versus 8.8 hours).

Pharmacodynamics. During the 6-hour observation period after administration of loperamide with placebo, no changes in pupil diameter, pain threshold, pain tolerance, and transcutaneous P_{O_2} and P_{CO_2} were observed. Coadministration of ritonavir had no significant effect on any of these pharmacodynamic parameters.

Clinical end points. No serious adverse events occurred during the study. A total of 7 adverse effects were observed in the 12 subjects after administration of loperamide and placebo (2 reports of flatulence, 2 of mild abdominal pain, 1 of mild headache, 1 of nausea, and 1 subject had mild constipation). Eleven mild adverse effects were reported after loperamide and ritonavir (3 nausea, 2 dizziness, 1 headache, 1 vomiting, 1 drowsiness, 1 flatulence, 1 tiredness, and 1 diarrhea). There was no significant difference between the two groups (paired *t* test, $P = .394$). All of these adverse effects were defined as mild, because they were transient and easily tolerated by the subjects.

DISCUSSION

Loperamide is a full agonist at the opioid μ-receptor with the potential to induce CNS opioid effects if it reaches the brain.[2,5] However, for the following two reasons, loperamide reaches the CNS only under exceptional conditions: (1) Oral bioavailability is very poor; (2) penetration of the blood-brain barrier is low, because loperamide is a substrate of P-glycoprotein–mediated transport out of the brain. Both pathways are believed to be blocked by ritonavir,[5,20,21] a compound that frequently causes diarrhea and thus is likely to be coadministered with the over-the-counter drug loperamide. The aim of this study was therefore to evaluate both pharmacokinetics and pharmacodynamics of loperamide in the presence and absence of the HIV protease inhibitor.

Ritonavir produced a major pharmacokinetic drug-drug interaction that did not result in opioid CNS

effects. Loperamide bioavailability substantially increased and the major metabolic pathway decreased whereas renal clearance remained unchanged. Several factors have been discussed that may affect the extent of availability of a drug. The drug disposition as determined by the interplay between drug-transporting and drug-metabolizing systems is now well established.[22] In vitro, ritonavir is a potent inhibitor of CYP3A4,[11] CYP2D6,[23] and CYP2C9,[24] and in vivo, it significantly increases the AUC of drugs primarily eliminated by CYP3A4-mediated metabolism such as rifabutin, clarithromycin, and ketoconazole[25] or by CYP2D6 metabolism.[23] Without providing data, Sadeque et al[5] claimed that loperamide is being metabolized to desmethylloperamide by CYP3A. It is therefore not known whether CYP isozymes mediate loperamide *N*-demethylation to desmethylloperamide and further to didesmethylloperamide and, if so, which ones do so, although there is a likelihood of CYP3A contribution. The results of our study indicate a metabolic drug interaction between ritonavir and loperamide. The loperamide metabolic ratio as an index of the primary metabolic step was increased 4 times after ritonavir, both partial metabolic clearances involving *N*-demethylation were decreased, leading to delayed formation of desmethylloperamide as assessed by t_{max} prolongation, and its further metabolism was inhibited (see Fig 2). If CYP3A is indeed involved in the loperamide demethylation steps, this metabolic interaction is likely to take place in the intestine and the liver, because these two organs contain the highest amounts of CYPs, especially CYP3A.

In theory, there might also be a potential role for P-glycoprotein in limiting the intestinal absorption of loperamide. A major indicator for such an interaction is the total amount of urinary excretion of loperamide and its metabolites. If ritonavir inhibits intestinal P-glycoprotein[26] and loperamide is a substrate, one would expect an increase in loperamide absorption and therefore an increased amount of loperamide and its metabolites excreted in urine. In contrast to this expectation, the total amount excreted was unchanged. Loperamide was shown to be a substrate of P-glycoprotein, because after oral administration its plasma concentration was increased 2 times in knockout mice [*mdr1a*(–/–)], in comparison with the wild-type [*mdr1a*(+/+)].[2] It was also shown in cell experiments in vitro that loperamide is effectively transported by both *MDR1* and the *mdr1a* P-glycoprotein.[2] Ritonavir and other HIV protease inhibitors are also substrates for the *MDR1* multidrug transporter.[10] The absolute bioavailability of ritonavir has not been determined but has been claimed to be high[27] or at least 65%,[21] and conflicting data on the ability of P-glycoprotein inhibition exist.[28,29] With respect to the interaction with saquinavir there is increasing evidence that the increased bioavailability of saquinavir most likely results from reduced saquinavir metabolism and not from inhibition of intestinal P-glycoprotein.[30] Ritonavir might therefore only slightly, if at all, enhance bioavailability of other drugs for which P-glycoprotein is the limiting step of absorption.

Concomitant administration of loperamide and ritonavir in healthy volunteers was associated neither with detectable central pharmacodynamic effects nor with adverse events. This is in apparent contrast to the respiratory depression observed with quinidine after loperamide,[5] which was attributed to P-glycoprotein inhibition by quinidine, which increased loperamide penetration into the brain. This respiratory depression was independent of changes in plasma loperamide concentrations, which occurred only 2 hours after administration, whereas respiratory depression occurred as early as 30 minutes after dosing; these data suggest that loperamide penetration into the brain was increased (eg, by P-glycoprotein inhibition).[5] Hence this earlier study provides indirect evidence for changes in drug distribution that modulate the plasma concentration-effect relationship. The pharmacodynamic methods used in our study would have been sufficient to assess central opioid effects,[13,14,31] because they all have been applied to quantify the effects of codeine, morphine, and other opioids. In addition, it can be ruled out that plasma ritonavir concentrations insufficient for P-glycoprotein inhibition were achieved, because the pharmacokinetic parameters were similar to previously published data.[32] In *mdr1a*(+/+) mice with a loperamide dose of 1 mg/kg, plasma concentrations of 13.3 ± 3.4 ng/ml (±27.9 pmol/ml) were observed and increased 2 times in *mdr1a*(–/–) mice.[2] The maximum concentrations in our study were somewhat lower (8.6 pmol/ml) and increased during ritonavir to 10.1 ± 4.9 pmol/ml. However, brain concentrations of loperamide were concurrently 13.5 times higher in *mdr1a*(–/–) mice, which then showed opiate-like effects.[2] Therefore the brain concentrations of loperamide necessary to elicit opioid effects may not have been reached, even though a high single dose of 16 mg loperamide was used. Ritonavir did not increase the brain/plasma ratio of the HIV protease inhibitor nelfinavir in *mdr1a*(+/+), mice suggesting that P-glycoprotein is probably not involved in enhanced tissue bioavailability resulting from combined use of HIV protease inhibitors.[29] Very recently, it was shown that in knockout mice the brain entry of saquinavir is limited by P-glycoprotein activity and that access to the brain was still considerably restricted on coadministration of

ritonavir.[30] It appears likely, therefore, that ritonavir does not substantially inhibit P-glycoprotein at the blood-brain barrier and consequently does not facilitate brain entry of loperamide at the dose given in this study and generally used in patients with HIV infection.

It is interesting that both loperamide and desmethyl-loperamide plasma concentrations increased after quinidine administration, which was attributed to intestinal P-glycoprotein inhibition.[5] However, no detailed pharmacokinetic parameters of loperamide and metabolites have been obtained to further support this conclusion. Our data indicate that ritonavir, in contrast to quinidine, is not able to enhance bioavailability by inhibition of intestinal P-glycoprotein and predominantly exerts its effect on loperamide pharmacokinetics through inhibition of CYPs, possibly CYP3A.

Diarrhea associated with ritonavir has an incidence of 13% to 22%, which is in the range of 0%-50% for all protease inhibitors.[7] In view of the results of this study we believe it is safe to treat this very common side effect of ritonavir with loperamide, although we performed only a single-dose study and repetitive administration of ritonavir might alter the situation. It is not clear whether this holds true for the other protease inhibitors used in the treatment of HIV.

In conclusion, we observed a significant increase of loperamide bioavailability after oral administration of ritonavir, which is likely caused by substantial metabolic inhibition. The fact that no evidence was found for significant inhibition of drug transport systems or altered access of loperamide to the brain suggests that the combination of loperamide with ritonavir does not pose particular risks to the patient. Because the amount of active parent compound was substantially increased and because the site of antidiarrheal action is outside the CNS compartment, it appears likely that loperamide will be sufficiently effective even when reduced doses are administered. This, however, will have to be confirmed in studies in patients with diarrhea.

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