

Bioavailability of Generic Ritonavir and Lopinavir/Ritonavir Tablet Products in a Dog Model

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ABSTRACT: In this study, we explored the bioavailability in dogs and chemical potency of generic ritonavir and lopinavir/ritonavir tablet products manufactured by various pharmaceutical companies. Chemical potency of the products was examined by HPLC quantitation of ritonavir and lopinavir. Using a dog model, we determined point estimates for C_{\max} and AUC of ritonavir and lopinavir/ritonavir for eight generic products compared to Abbott's Norvir[®] capsule and Kaletra[®] tablet. Chemical potencies ranged from 79.0% to 104.6%. Point estimates for AUC in the generic tablet products ranged from 0.01 to 1.11, indicating that the relative bioavailability of these formulations was in the range of 1–111% compared to the branded products. This study showed significant variability in bioavailability in a dog model amongst generic tablet products containing the protease inhibitors ritonavir or lopinavir/ritonavir. The chemical potency of the generic products was not indicative of the plasma levels of ritonavir or lopinavir that were achieved. These results reinforce the need for human bioequivalence testing of generic products containing ritonavir or lopinavir/ritonavir to assure that efficacy in patients is not compromised prior to these products being made available to patients. Procurement policies of funding agencies should require such quality assurance processes. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: lopinavir/ritonavir; quality; antiretroviral; bioequivalence; bioavailability; HIV/AIDS; absorption; formulation; dissolution; solubility

INTRODUCTION

Human immunodeficiency virus (HIV) is the cause of a major worldwide epidemic that has resulted in the death of millions of people. In 2007, approximately 33.2 million people were living with HIV and an estimated 2.1 million died of AIDS and HIV-related illnesses.¹ Although the development of highly active antiretroviral therapy (HAART) has helped to decrease global HIV morbidity and mortality, the vast majority of people do not benefit from HAART due to limited

access and availability.^{2–5} While access to ART is increasing among low and low–middle income countries, such as those in sub-Saharan Africa, there remains an urgent need for treatment programs that provide affordable access to high-quality medicines.

Both branded and generic products have a role in the global fight against HIV. Some major pharmaceutical companies have made efforts to meet the needs of resource-limited countries through no-profit pricing, fixed-dose formulations, and the development of heat-stable drugs that eliminate the need for refrigeration. Generic drug companies have also played a significant role in the developing world by supplying cost-effective treatment regimens, particularly fixed-dose combinations used in first-line therapy.⁶ Stringent

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regulatory authorities such as the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the World Health Organization (WHO) prequalification program have implemented programs intended to unify the standards of acceptable quality, safety, and efficacy, including the requirement for products to demonstrate bioequivalence in humans.⁷⁻⁹ However, the requirements of national drug regulatory agencies vary, with some not requiring demonstration of bioequivalence in humans, allowing for potential variability in the quality of approved generics in local markets.

In this study, we explored the relative bioavailability of ritonavir and lopinavir/ritonavir tablet products manufactured by various generic pharmaceutical companies. In prior studies, it has been shown that maintaining adequate plasma levels of protease inhibitors is important to ensure treatment success.¹⁰⁻¹² Using an internally developed dog model, we compared generic ritonavir and lopinavir/ritonavir tablet products to Abbott's Norvir[®] capsule and Kaletra[®] tablet, respectively. The dog model is used frequently in formulation development and has shown utility for prediction of human bioavailability.¹³⁻¹⁴ The model used in these studies has been established and used extensively by Abbott to aid in the development of both Norvir[®] and Kaletra[®] formulations. In our model, the results for 12 of 16 experimental ritonavir formulations in dog mirrored those obtained in human (both bioequivalent and nonbioequivalent results) (Fig. 1). For the remaining four formulations, the good bioavailability observed in the dog was not achieved in humans. However, there were no instances in which a formulation that performed poorly in the

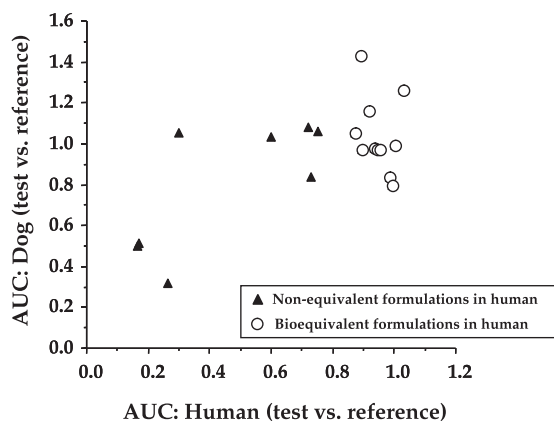


Figure 1. Ritonavir bioequivalence studies, dog versus human.

dog subsequently demonstrated bioequivalence in human. This model suggests that bioequivalence in dogs is necessary for bioequivalence in humans; however, bioequivalence in dogs does not guarantee bioequivalence in humans for these compounds (Fig. 1). Furthermore, the model suggests that formulations that perform poorly in the dog are unlikely to perform well in humans.

At the time of testing, Abbott's Norvir[®] capsule and Kaletra[®] tablet had FDA and EMA approval and WHO prequalification, whereas, none of the generic products tested had FDA tentative approval, EMA approval, or WHO prequalification.

METHODS

Products Tested

All of the generic products tested were manufactured in India. The tablet products (drug, manufacturer, expiration date) tested were: Empetus lot 01A06001 (ritonavir, Emcure, August 2008), Emletra lot 01A06001 (lopinavir/ritonavir, Emcure, August 2008), Ritomune lot K70556 (ritonavir, Cipla, March 2009), Lopimune lot K701172 (lopinavir/ritonavir, Cipla, December 2008), Ritocom lot RI70501 (lopinavir/ritonavir, Hetero, April 2009), Emletra lot EM80301 (lopinavir/ritonavir, Hetero, February 2010), Empetus lot EP80301 (ritonavir, Hetero, February 2010), and lopinavir/ritonavir lot 1004728 (lopinavir/ritonavir, Matrix, March 2010). All testing was conducted within the label-specified shelf life.

Bioequivalence Studies in Dog

All animal procedures were approved by the Institutional Animal Care and Use Committee at Abbott Laboratories. Bioequivalence studies were conducted using a randomized, two-period crossover study design in a group of 12 beagle dogs. A washout period of approximately 1 week separated each of the two dosing periods. In each of the two study periods, each dog received single oral doses of 100 mg of ritonavir or 200/50 mg of lopinavir/ritonavir (test [generic product] or reference [Abbott product]), for the ritonavir and lopinavir/ritonavir studies, respectively. All doses were administered under fasting conditions. Serial blood samples were obtained from each dog over a period of 24 h after dosing. Concentrations of lopinavir and ritonavir were determined

by high-performance liquid chromatography (HPLC)–MS/MS following liquid–liquid extraction of the plasma samples. Specifically, the components of interest (and an internal standard) were separated from plasma using liquid–liquid extraction with a mixture of ethyl acetate and hexane. The organic layer was evaporated to dryness at room temperature using a gentle stream of dry nitrogen. Samples were reconstituted with mobile phase. Spiked plasma standards were analyzed simultaneously with the samples. Lopinavir, ritonavir, and the internal standard were separated from coextracted contaminants on a 50 mm × 3 mm × 5 μm column with acetonitrile: 0.1% aqueous trifluoroacetic acid mobile phase (~56:44, by volume) at a flow rate of 0.4 mL/min. Analysis was performed on a Sciex API2000 Biomolecular Mass Analyzer with a turbo-ion spray interface. Analytes were ionized in the positive ion mode with a source temperature of approximately 450°C. Detection was in the multiple reaction monitoring (MRM) mode at m/z 629.4 → 155.1 for Lopinavir, m/z 721.4 → 286.1 for ritonavir, and m/z 747.3 → 140.0 for the internal standard (a closely related analog of ritonavir). Lopinavir, ritonavir, and internal standard peak areas were determined using Sciex TurboQuan software. For Kaletra formulations, the ritonavir standard curve ranged from 0.01 to 9.92 μg/mL, with mean percent accuracy for the analysis of replicate (minimally in triplicate) standards ranging from 90.2% to 105.5%; the lopinavir standard curve ranged from 0.017 to 33.96 μg/mL, with mean percent accuracy for the analysis of replicate standards ranging from 92.1% to 106.3% (representative example). For Norvir formulations, the ritonavir standard curve ranged from 0.03 to 59.5 μg/mL, with mean percent accuracy for the analysis of replicate standards ranging from 93.7% to 107.4%.

All samples for each dog were analyzed at the completion of both dosing periods. Peak plasma concentrations (C_{\max}) and the time to peak plasma concentration (T_{\max}) were read directly from the plasma concentration data for each dog. The area under the plasma concentration–time curve (AUC) from 0 to t hours (time of the last measurable plasma concentration) after dosing was calculated using the linear trapezoidal rule for the plasma concentration–time profiles. Point estimates for C_{\max} and AUC were calculated on a logarithmic scale for the comparison of the test versus the reference formulation.

Chemical Potency Assay

Tablets of each product were tested for chemical potency. The Active Pharmaceutical Ingredient (API) content was determined by HPLC with ultraviolet detection. Specifically, assay of lopinavir and ritonavir in lopinavir/ritonavir dosage forms was determined by HPLC. A reverse-phase C8 column with an eluent consisting of potassium phosphate monobasic solution, acetonitrile, methanol, and tetrahydrofuran (62.5:17.5:10:10) was used to isocratically elute lopinavir and ritonavir. The HPLC system used a flow rate of 1.5 mL/min and an ultraviolet detection wavelength of 215 nm. Quantitation was performed using the internal standard method with biphenyl as the internal standard. This method has been validated to accurately and precisely determine ritonavir and lopinavir content in Abbott drug products. Although this method is not specifically validated for the generic products tested in these studies, the assay procedure has been proven to be suitable for the analysis of drug substance and a wide variety of ritonavir and lopinavir/ritonavir formulation types and dosage forms, it was deemed to be acceptable for use to estimate the chemical potency of the generic products as part of this study.

RESULTS

Chemical potencies of ritonavir were 96.6%, 100.1%, and 101.2% for the ritonavir products, indicating that all labeled amount of ritonavir was contained in the test ritonavir tablets. For the lopinavir/ritonavir products, the ritonavir chemical potencies ranged from 89.0% to 102.0%, and the lopinavir chemical potencies ranged from 79.0% to 104.6%.

Table 1 summarizes the dog bioavailability data and chemical potencies for the generic products tested. Relative bioavailability is expressed as point estimates for ritonavir and lopinavir C_{\max} and AUC for test tablet products compared to the reference products. The point estimates for ritonavir AUC for generic tablet products compared to Norvir[®] soft gelatin capsule ranged from 0.01 to 0.87, indicating that the relative bioavailabilities of these formulations were in the range of 1–87% compared to the branded product. The point estimates for lopinavir AUC for generic lopinavir/ritonavir tablet products compared to Kaletra[®] tablet ranged from 0.04 to 1.11,

Table 1. Relative Bioavailability and Chemical Potency for Various Lopinavir/Ritonavir and Ritonavir Products

Product Name ^a	Manufacturer	RTV Point Estimates ^b		LPV Point Estimates ^b		Chemical Potency (Label Claim, %)	
		C_{\max} (90% CI)	AUC (90% CI)	C_{\max} (90% CI)	AUC (90% CI)	RTV	LPV
Emletra	Emcure	0.01 (0.004–0.051)	0.01 (0.003–0.030)	0.08 (0.04–0.16)	0.04 (0.02–0.06)	97.2	101.1
Lopimune	Cipla	0.75 (0.53–1.07)	0.92 (0.70–1.19)	1.00(0.88–1.15)	1.08 (0.90–1.31)	95.8	104.6
Ritocom	Hetero	0.70 (0.52–0.96)	0.71 (0.54–0.93)	0.78 (0.69–0.87)	0.84 (0.66–1.07)	89.0	79.0
LPV/r	Matrix	0.54 (0.33–0.74)	0.55 (0.38–0.72)	0.77 (0.71–0.82)	0.64 (0.53–0.75)	102.0	97.5
Emletra	Hetero	1.07 (0.93–1.22)	0.98 (0.85–1.12)	1.06 (1.00–1.12)	1.11 (1.03–1.20)	93.3	99.3
Empetus	Emcure	0.02 (0.008–0.03)	0.01 (0.003–0.02)	N/A ^c	N/A ^c	101.2	N/A ^c
Empetus	Hetero	0.86 (0.79–0.92)	0.87 (0.79–0.95)	N/A ^c	N/A ^c	100.1	N/A ^c
Ritomune	Cipla	0.50 (0.40–0.62)	0.36 (0.28–0.44)	N/A ^c	N/A ^c	96.6	N/A ^c

^aNorvir[®] and Kaletra[®] are trademarks of the Abbott Group of Companies. All other trademarks are property of their respective owners.

^bRelative to lopinavir and ritonavir from Kaletra[®] tablets (Abbott) for Emletra, Lopimune, LPV/r and Ritocom, and ritonavir from Norvir[®] capsules for Empetus and Ritomune.

^cThe ritonavir products were not tested for lopinavir point estimates and chemical potency.

indicating that the relative bioavailabilities of lopinavir from the generic products were in the range of 4–111% compared to the branded product. Point estimates for C_{\max} were similar to those for AUC.

The plasma concentration–time profiles for each of the products tested are shown in Figures 2 and 3, and demonstrate significant variability between products in plasma exposures of lopinavir and ritonavir.

DISCUSSION

The physical and chemical properties of lopinavir and ritonavir, specifically solubility and dissolution, make development of formulations providing acceptable bioavailability of these molecules particularly challenging. This study assessed the bioavailability of several lopinavir/ritonavir and ritonavir products in a dog model, and demonstrated broad variability in relative bioavailability of generic ritonavir and lopinavir/ritonavir tablet products compared to Norvir[®] capsule and Kaletra[®] tablet products, respectively.

Chemical potency of the generic products was not indicative of the plasma levels of ritonavir or lopinavir achieved after oral administration to dogs. For example, although the chemical potency testing for the Emletra (Emcure) product showed that 97.2% and 101.1% of the labeled amount of

ritonavir and lopinavir, respectively, were present in the test tablets, the relative bioavailabilities of ritonavir and lopinavir in dogs were 1% and 4%, respectively. These results demonstrate that *in vitro* testing methods, such as chemical potency, are not reliable predictors of bioavailability for ritonavir and lopinavir in the dog.

Over the course of the Kaletra[®] and Norvir[®] development programs, evaluation of the bioavailability of a variety of ritonavir and lopinavir/ritonavir test formulations were conducted in a dog model. The dog model has been used to evaluate and select formulations that have the highest probability of achieving bioequivalence in man. We have shown that formulations that are not bioequivalent in dog have not been bioequivalent in man. In addition, there are formulations that were bioequivalent in the dog, which failed bioequivalence testing in man. This model demonstrated that the dog may overpredict bioavailability in humans, and that formulations that were poor in the dog did not achieve bioequivalence in humans. Therefore, bioequivalence in the dog is necessary, but not sufficient, for bioequivalence in man (Fig. 1). The bioavailability of ritonavir and lopinavir in humans from the generic products tested in this work is not known. However, based on the previously described experience with this dog model, the results of these studies suggest that decreased bioavailability for some of these products may be expected.

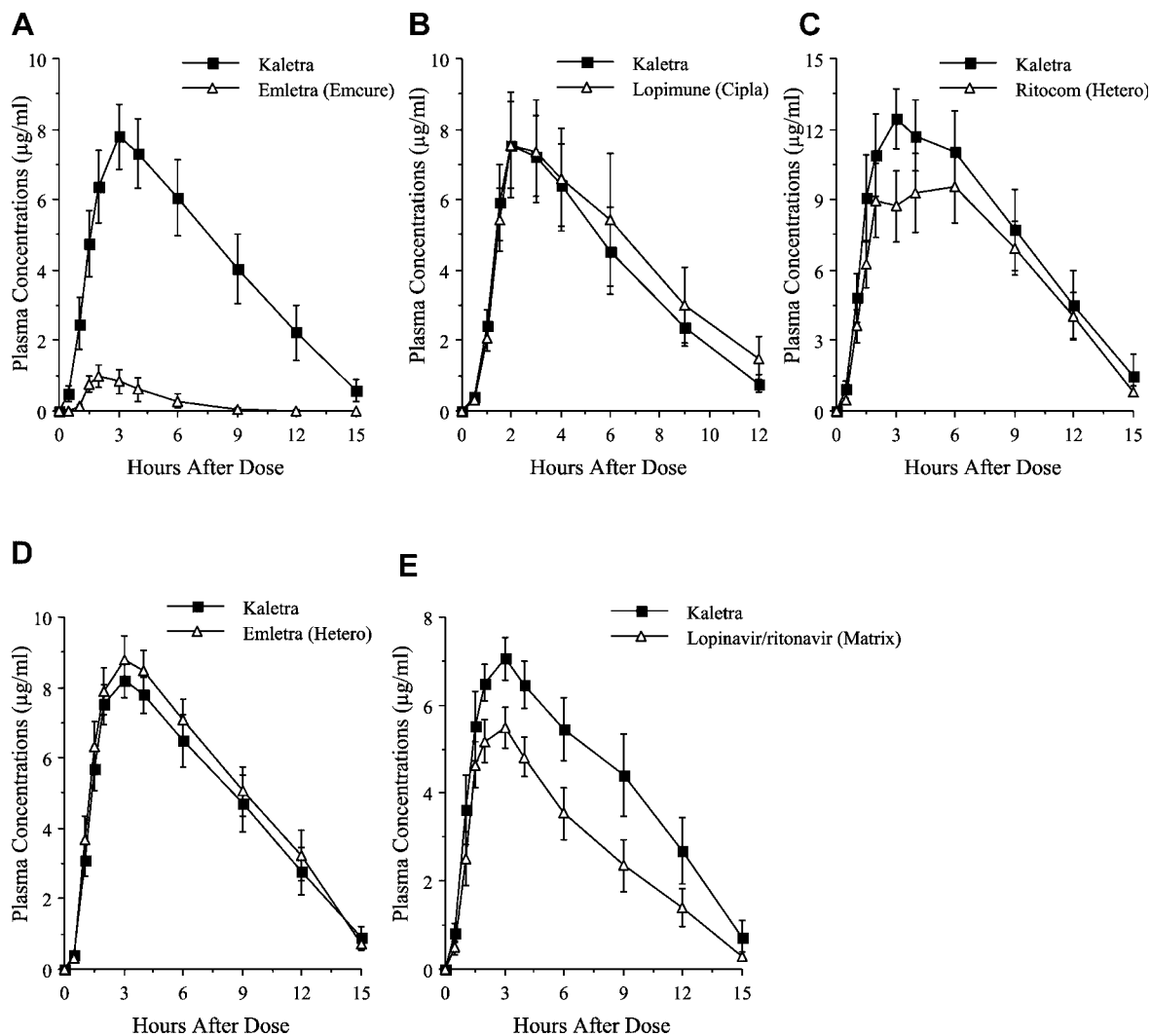


Figure 2. Lopinavir plasma concentrations following a 200/50 mg oral dose of lopinavir/ritonavir products in dog. A: Emletra (Emcure); B: Lopimune (Cipla); C: Ritocom (Hetero); D: Emletra (Hetero); E: Lopinavir/ritonavir (Matrix).

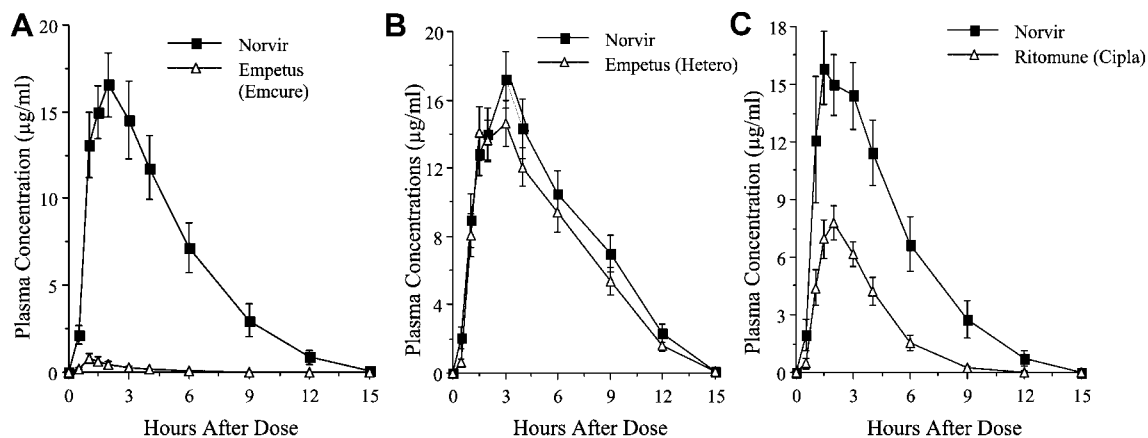


Figure 3. Ritonavir plasma concentrations following a 100 mg oral dose of ritonavir products in dog. A: Empetus (Emcure); B: Empetus (Hetero); C: Ritomune (Cipla).

The results obtained in this study suggest the need for human bioequivalence testing for generic tablet formulations of ritonavir and lopinavir/ritonavir to ensure that adequate plasma exposures of these agents are achieved. The long-term clinical and epidemiological implications that may result from the utilization of lopinavir/ritonavir or ritonavir products that have not met this requirement are currently unknown. However, reduced efficacy of the antiretroviral regimen and increased risk of protease resistance might reasonably be anticipated if bioavailability and subsequently drug concentrations are significantly reduced. Therefore, physicians prescribing such medicines should be cautious regarding a potentially adverse impact on efficacy, safety, and emergence of viral resistance. Finally, the procurement policies of funding organizations should consistently require that products be approved by stringent regulatory authorities, such as the FDA tentative approval process, or that products be evaluated through an internationally recognized quality process, such as WHO prequalification, which require evidence of bioequivalence in humans, prior to being made available to patients.

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