Poster PE9.7/4

Preclinical Evaluation of Gastrointestinal Tolerability of HIV Protease Inhibitors in a Ferret Model

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Abstract

Background: Despite the prevalence of adverse gastrointestinal (GI) events concomitant with the use of HIV protease inhibitors (PIs), there are few preclinical studies addressing this topic. In addition, the contribution of excipients such as oleic acid to GI intolerance has not been established. Ferrets (*Mustela putorius furo*) are small carnivores that have proven valuable for the prediction of clinical emetic and antiemetic activity of novel drugs.

Methods: Fasted male ferrets were administered lopinavir, nelfinavir, or atazanavir (each dissolved in propylene glycol/ethanol) by gavage at 10, 25, and 50 mg/kg; a 5 mg/kg boosting dose of ritonavir was co-administered with each PI. Additional experiments examined the tolerability of two formulations of lopinavir/ritonavir, an oleic acid-based solution and a tablet lacking oleic acid, administered at 10/2.5 or 25/6.25 mg/kg. After dosing, ferrets were observed for 4 hours for the occurrence of emesis and stereotypical behaviors believed to correlate with nausea. Defecatory events were also recorded and all loose feces were collected for fecal osmotic gap analysis. Plasma levels of each PI were determined from blood obtained 4 hours after dosing.

Results: The plasma levels for all PIs approximated respective clinical values. Lopinavir, nelfinavir, and atazanavir were essentially non-emetic over the tested dose range; however, each PI elicited a dose-dependent increase in the incidence of diarrhea. Osmotic gap analysis of fecal water was consistent with a drug-induced secretory, and not osmotic, diarrhea. The oleic acid solution formulation of lopinavir/ritonavir produced both emesis and diarrhea. Conversely, the tablet formulation of lopinavir/ritonavir evoked no emesis and essentially no diarrhea.

Conclusions: These results suggest that preclinical evaluation of potential GI adverse events is possible in ferrets, and that the tablet formulation of lopinavir/ritonavir recently approved by the FDA in the United States may have GI tolerability superior to the softgel capsule containing oleic acid.

Introduction

For some patients, adverse gastrointestinal (GI) events accompany the use of HIV protease inhibitors (PIs). GI disturbances are especially prevalent when starting PI therapy; this can impact patient adherence. A recent study on the reasons for discontinuation of nelfinavir- or indinavir-based HAART regimens by antiretroviral-naïve patients concluded that GI intolerance (nausea, vomiting, diarrhea) was the most frequent cause for therapy cessation (1).

Despite its widespread prevalence, the etiology of PI-associated GI disturbances remains ill-defined. Fat or other nutrient malabsorption may contribute to PI-associated diarrhea (2), while in vitro studies suggest that PIs can increase active ion secretion or impair epithelial barrier function, leading to either secretory or leak-flux diarrhea, respectively (3,4).

An *in vivo* animal model for assessing adverse GI events would be helpful for the discovery and development of novel PIs with a better GI tolerability profile. Ferrets (*Mustela putorius furo*) are small carnivores that are frequently used for assessing the emetic or antiemetic properties of novel therapeutics. We investigated the utility of this species for the identification of adverse GI events (emesis, diarrhea) of ritonavir (RTV), lopinavir (LPV), nelfinavir (NFV) and atazanavir (ATV). The GI tolerability of excipients used in some formulations of PIs, such as oleic acid used in the lopinavir/ritonavir soft gel capsule formulation, was also examined in this model.

Objectives

- Establish the predictability of the ferret model by comparing the GI tolerability of PIs in this assay with the known clinical GI effects of the PIs.
- Examine the contribution of the pharmaceutical excipient oleic acid to the adverse GI effects of lopinavir/ritonavir.
- Investigate possible mechanistic basis of PI-associated diarrhea.

Materials and Methods

Materials: Lopinavir and ritonavir were synthesized at Abbott. The marketed formulations of nelfinavir and atazanavir were purchased and extracted; purity of active PI was verified. Oleic acid was obtained from Cognis North America (Cincinnati OH), Cremophor EL from BASF. All other reagents were from Sigma-Aldrich Co. and were of analytical grade. Solution formulations were prepared in either propylene glycol/ethanol (95%/5%) or a lipid-based vehicle consisting of oleic acid/PEG400/Cremophor EL (81%/10%/9%).

Animals: Castrated male ferrets were obtained from Marshall BioResources (North Rose NY). Animals were housed in groups of 3 in a temperature (20°C) and humidity (45% RH) controlled environment. Ferrets were maintained on a 12-h light-dark cycle with lights on at 0600 hours. Food (Marshall Premium Ferret Diet; Marshall Pet Products, Wolcott NY) and water were available ad libitum up until 4–6 h prior to beginning a study, at which time food was withdrawn. All procedures involving animals were reviewed and approved by the Abbott Animal Care and Use Committee. United States Dept of Agriculture (USDA) regulations on the scientific use of laboratory animals were strictly observed.

GI tolerability assay: Emesis studies were carried out as previously described (5). Briefly, ferrets were placed in individual plastic cages with ventilated tops and allowed to acclimate to the testing environment for 30–60 min prior to dosing. For solution formulations, drugs were dissolved in vehicle and administered by gavage at 2 ml/kg body weight. For the tablet formulations, the appropriate amount of lopinavir/ritonavir solid tablet shavings (based on each ferret's body weight) were prepared in gelatin capsules (sizes #1–3 Coni-Snap, Capsugel, Greenwood SC) and administered with a pilling syringe to each ferret. Ferrets were observed for a 4 hour time period for the appearance of emesis, nausea behaviors, and diarrhea. Diarrheal output was collected for electrolyte analysis of fecal fluid. At the end of the study (4 h post dosing), a blood sample was obtained from each animal for determination of PI plasma concentrations.

Fecal osmotic gap analysis: Fecal water was prepared from the watery feces (diarrhea) with Centriprep YM-50 centrifugal filter devices (Millipore Corp., Bedford MA). Feces were loaded into the unit and centrifuged at 1,000*xg* for 30 min at 4°C. The concentrations of sodium, potassium, and chloride in the resulting fecal fluid was measured with an Abbott Aeroset clinical chemistry analyzer.

The fecal osmotic gap (FOG) was calculated using the equation:

$$FOG = 320\ mOsmol/kg - [2 \times (Na+ + K+)]$$

Data analysis: The incidence of emesis and diarrhea are expressed as a percentage of animals that had one or more emetic or diarrheal events. Fecal osmotic gap data are expressed as mean ± SEM. FOG data were analyzed by one-way ANOVA followed by Tukey's post-hoc test if a significant F value was obtained in the ANOVA.

Results

Model Validation I: Ritonavir

- Ritonavir produced a dose-dependent emetic response.
- The incidence of diarrhea also increased in a dose-dependent manner.
- The lower incidence of diarrhea at the highest dose may result from excessive emesis.
- Although mean plasma ritonavir concentrations were slightly higher in animals experiencing emesis, there was no strong correlation between plasma drug levels and emesis.

Figure 1. Model Validation with RTV

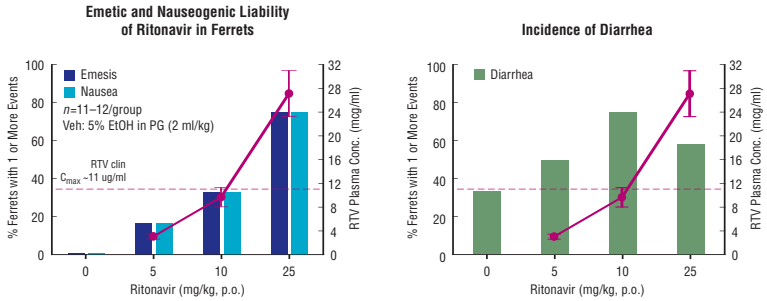
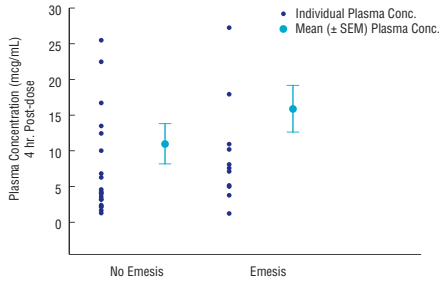


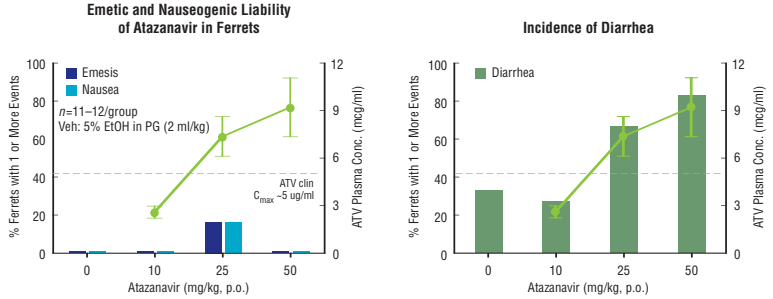
Figure 2. Incidence of Emesis as a Function of Ritonavir Plasma Concentration



Model Validation II: Atazanavir

- Atazanavir was better tolerated than full dose ritonavir with respect to emesis.
- Diarrhea was observed at higher doses at plasma concentrations above the average C_{max} in humans.
- Therefore, ferrets reasonably mimic the human GI response to HIV PIs.

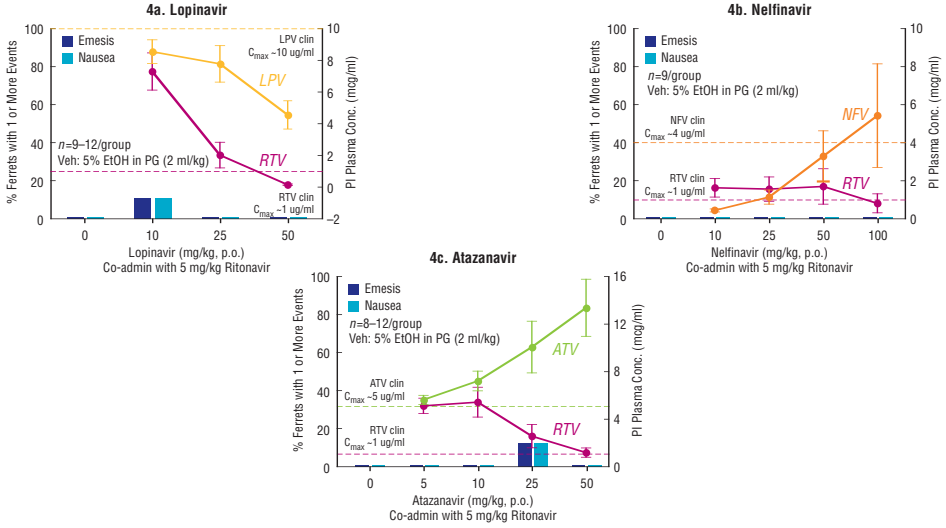
Figure 3. Model Validation with ATV



Comparison of Other HIV PIs – Emesis

- Lopinavir, nelfinavir and atazanavir were co-administered with a boosting dose (5 mg/kg) of ritonavir in order to maximize exposure.
- Plasma concentrations of the PIs approached or exceeded average C_{max} values in humans.
- The incidence of emesis and nauseogenic behavior was low with all three PIs.

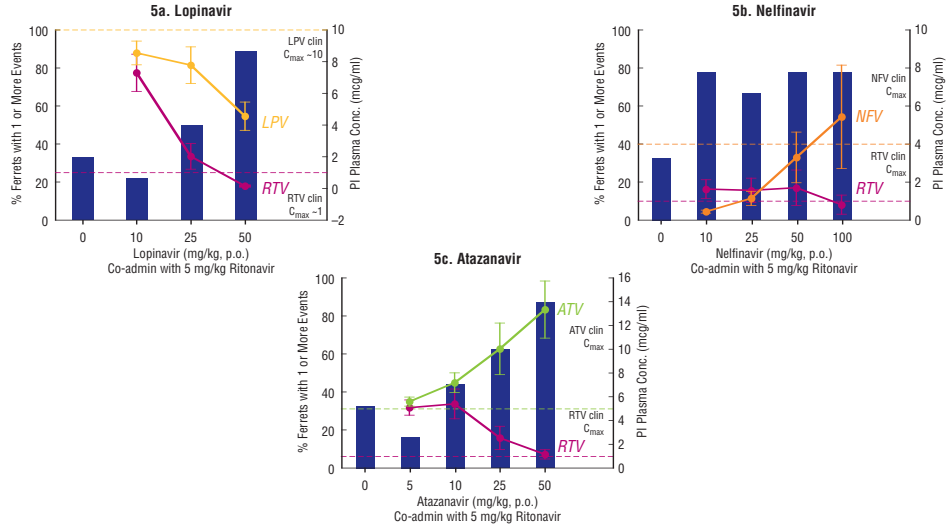
Figure 4. Emetic and Nauseogenic Liability of PIs in Ferrets



Comparison of Other HIV PIs – Diarrhea

- Ritonavir-boosted lopinavir and atazanavir produced a dose-dependent increase in diarrhea.
- Diarrhea was produced by all tested doses of ritonavir-boosted nelfinavir, even those resulting in sub-efficacious nelfinavir plasma concentrations.
- There was no clear relationship between PI plasma concentration and the incidence of diarrhea, suggesting that PI-elicited diarrhea may occur through a local effect within the gut.

Figure 5. Diarrhea Liability of PIs in Ferrets



Examination of the Effect of Lopinavir/ritonavir Formulation

- A new tablet formulation of lopinavir/ritonavir, based on melt extrusion technology, was compared to the oleic acid-based formulation of lopinavir/ritonavir capsules.
- Lopinavir/ritonavir was dosed at a constant 4:1 ratio rather than with a constant ritonavir boosting dose.
- A high incidence of both emesis and diarrhea was observed with the oleic acid-based (capsule) formulation, even in the absence of lopinavir/ritonavir.
- In contrast, very little emesis and only a modest incidence of diarrhea was observed with the tablet formulation.

Figure 6. Tolerability of Lopinavir/ritonavir Administered in a Lipid-based Formulation

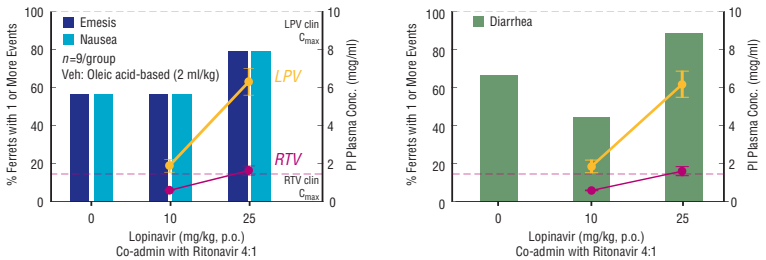


Figure 7. Tolerability of Lopinavir/ritonavir Administered in a Tablet Formulation

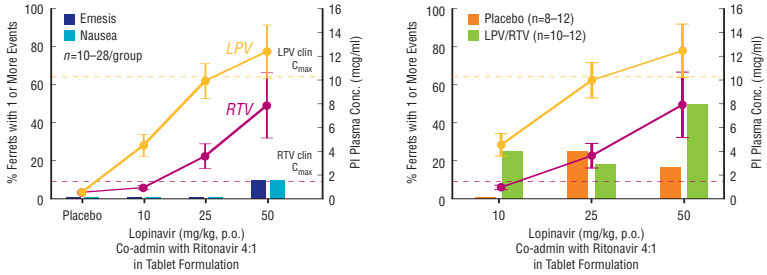
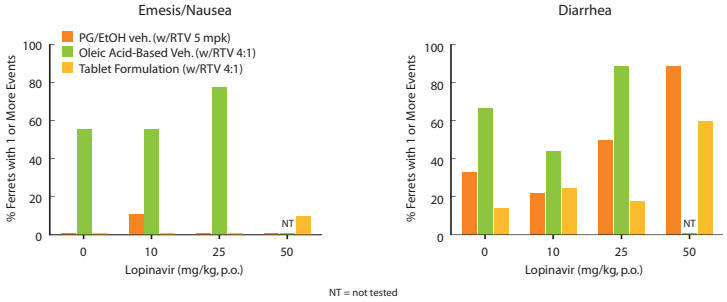


Figure 8. Summary Comparison of the GI Effects of Three Formulations of Lopinavir/ritonavir

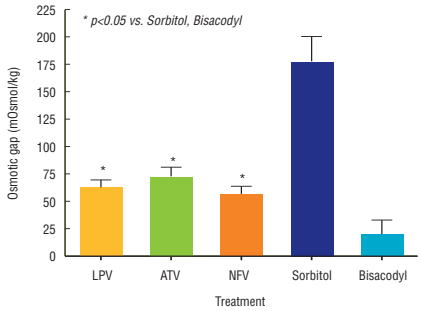


NT = not tested

Mechanism of Diarrhea with HIV PIs

- The mechanism of PI-induced diarrhea was probed using fecal osmotic gap analysis.
- Stool osmotic gap was consistent with production of secretory diarrhea rather than osmotic diarrhea.

Figure 9. Fecal Osmotic Gap Analysis of Fecal Fluid



Summary

- Ritonavir-boosted lopinavir, nelfinavir, and atazanavir did not produce a significant emetic response in ferrets. There were qualitative differences in the ferret diarrheal response to the PIs: lopinavir and atazanavir produced less diarrhea than nelfinavir. When expressed on a plasma-concentration basis, lopinavir was better tolerated with respect to diarrhea than was nelfinavir.
- Although ritonavir produced a dose-dependent increase in the incidence of emesis, its contribution to the production of diarrhea was likely minimal at the boosting dose employed in the coadministration studies.
- The solid tablet formulation of lopinavir/ritonavir was better tolerated than the lipid-formulation with respect to emesis and diarrhea.
- Mean osmotic gap values (range: 57–73 mOsmol/kg) obtained from PI-dosed ferrets are consistent with production of secretory diarrhea.
- Ferrets appear to be a useful model species for assessing the GI tolerability of PIs.

References

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