# Regiospecific Intestinal Absorption of the HIV Protease Inhibitor L-735,524 in Beagle Dogs

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***Purpose.*** To evaluate regional intestinal absorption and the feasibility of sustained release dosage form development for an HIV protease inhibitor, L-735,524. ***Methods.*** L-735,524 free base or sulfate salt was administered orally as suspension, solution or in solid dosage forms to fasted or fed Beagle dogs. Delayed-release dosage forms with "slow" or "fast" *in vitro* dissolution rates were evaluated *in vivo* to assess plasma concentration profiles. In addition, drug was administered directly into the jejunum or colon of animals, and drug concentrations determined in portal circulation to characterize absorption from these sites. ***Results.*** L-735,524 sulfate was well absorbed orally from a solution or capsule formulation if fasted animals' stomachs were preacidified with citric acid solution. A free base suspension, delivered in divided doses to fed animals, was also well absorbed. Prototype extended release dosage forms of L-735,524 produced a reduction in peak plasma levels but failed to prolong absorption and extend plasma concentrations compared to an immediate release capsule. Administration of L-735,524 sulfate solution (pH<3) as bolus solution or by infusion into the jejunum resulted in rapid but incomplete absorption compared to oral gavage. The free base suspension (pH 6.5) delivered into jejunal or colonic regions did not produce measurable systemic plasma concentrations. ***Conclusions.*** Extended release formulations did not prolong absorption of L-735,524 in dogs. Optimal L-735,524 absorption was dependent on solubility in an acidic environment in the duodenum.

**KEY WORDS:** HIV protease inhibitor; regiospecific absorption; canine absorption model.

## INTRODUCTION

Inhibition of the retrovirus HIV type 1 protease arrests virus replication and the spread of HIV infection in cell culture and offers promise for suppressing the pathogenicity of HIV in humans. A potent and orally bioavailable protease inhibitor, L-735,524 (Fig. 1), was developed for clinical studies. The compound exhibited adequate oral bioavailability in rats, dogs and monkeys when given as an acidic solution, with plasma concentrations reaching peak values between 30 to 60 min after dosing (1). Bioavailability in dogs was particularly promising (>70%); however, the plasma half-life was only 35 min. Since the 95% inhibitory concentration against acute infection of several HIV strains in cell culture was 100 nM (1), development of an extended release formulation that could sustain plasma levels above 100 nM compared to an immediate release formulation was desirable. This extended release dosage form would blunt peak to trough fluctuations between dosing intervals and also avoid potential adverse effect(s) that may be associated with peak plasma concentrations obtained after an immediate release capsule. Adequate drug absorption along the gastrointestinal (GI) tract must be demonstrated for any sustained release formulation effort to be successful. The availability of a chronic canine model permitted comparison of drug absorption from the jejunal and colonic regions and measurement of compound in the portal and systemic circulations *in vivo*. Data from these studies enabled evaluation of the *in vivo* performance of extended release dosage forms.

## MATERIALS AND METHODS

### Animals and Reagents

L-735,524 free base monohydrate and sulfate salt were synthesized in the Department of Medicinal Chemistry, Merck Research Laboratories.

Equilibrium solubility of L-735,524 free base monohydrate was determined in 0.1 M citrate buffers. Drug concentration in the buffer was determined by HPLC after filtration to remove undissolved drug. The final solution pH was recorded.

Experiments were conducted in a conscious Beagle dog model that allowed direct delivery of drug solutions into the upper jejunum or ascending colon via chronic cannulas implanted in these sites (2). Intestinal and portal vein cannulas were attached to vascular access ports (VAP, Access Technologies, Skokie, IL) which may be accessed from the backs of these dogs. Animals used were male, purebred Beagles (Marshall Farms, North Rose, NY) weighing between 9-11 kg and 8-10 months in age. Food was withheld at least 12 h prior to study and returned after completion of the study. Water was withheld until 4 hr after drug dosing. A total of nine animals were used over a period of 6 months. Experiments were conducted in a crossover fashion whenever possible and plasma concentrations from an oral reference dose (L-735,524 sulfate solution) were available for each animal.

### Dosing Regimens

#### *Immediate Release Formulations*

Relative bioavailabilities of L-735,524 from experimental dosing regimens were compared to a reference 200 mg (free base equivalent) L-735,524 sulfate solution (pH<3). A lactose-based gelatin capsule formulation of the sulfate salt served as the reference (immediate release) solid dosage form. The solution and all solid dosage forms were delivered to fasted dogs that had received 10 ml of 0.2 M citric acid immediately before drug dose.

A suspension of L-735,524 free base in 0.5% methylcellulose (pH 6.5) was also dosed orally in a repetitive manner,

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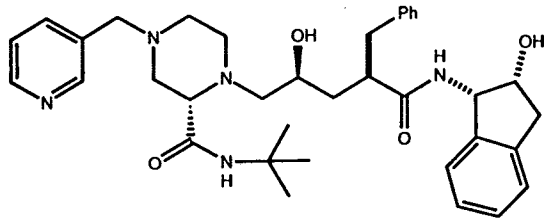


Fig. 1. Structure of L-735,524 (N-(2-(R)-hydroxyl-1-(S)-indanyl)-2(R)-phenyl-methyl-4(S)-hydroxyl-5-(1-(4-(3-pyridyl methyl)-2(S)-N'-(t-butyl carboxamido)-piperazinyl))-pentane amide).

20 mg every 15 min, to animals given Purina dog chow one hour before drug dose.

*Extended Release Formulations*

Four extended release dosage forms were manufactured, employing two different mechanisms for controlled drug delivery, each with a "fast" or "slow" *in vitro* dissolution profile. An uncoated, erosion-type tablet was based on a stearic acid matrix (SAS), and an osmotic tablet (Merck Osmotic Delivery System, MODS) was based on a rate controlling, semi-permeable membrane of cellulose acetate.

*In vitro* dissolution of dosage forms was performed using USP Apparatus 2 at 50 rpm with 900 ml of 0.1 N HCl as the medium at 37°C and measurement of absorbance at 260 nm.

*Direct Intestinal Delivery*

Drug absorption from the jejunum or colon was determined by direct injection of 10 ml of L-735,524 solutions (100 or 200 mg free base equivalent) into the respective sites *via* intestinal cannulas. The cannulas were flushed with 1 ml water.

"Sustained" drug release in the jejunum was simulated by constant rate infusion of L-735,524 sulfate in water or in 0.1 M citric acid using a CADD-LD mini syringe pump (Pharmacia Deltec, St. Paul, MN) at rates of 66 mg or 132 mg free base equivalent/hr. Total dose remained 200 mg in a volume of 6 ml.

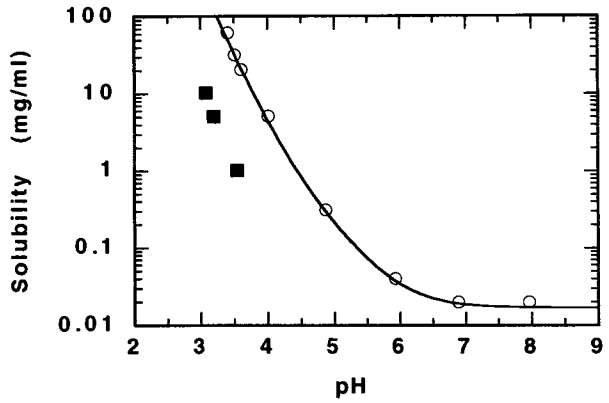


Fig. 2. pH-dependent solubility of the free base monohydrate (○) and sulfate salt (■) of L-735,524.

*Sample Preparation and Analysis*

Blood samples were collected from a peripheral vein and from the portal vein VAP at pre-dose and during study. Plasma was separated in Vacutainer tubes containing 100 U Na heparin as anticoagulant. Analysis of L-735,524 was performed by HPLC (3). The limit of detection was 5.2 ng (8.47 pmol) on column. Standard curves were linear from 50 nM to 15 μM (0.2 ml plasma) or 20 nM to 6 μM (0.5 ml plasma).

*Statistical Analysis*

Analysis of the influence of extended release formulations on peak plasma concentration (Cmax), time of Cmax (Tmax) and area under the plasma concentration-time curve (AUC), determined by linear trapezoidal rule, was performed using the General Linear Models procedure of SAS. Differences between means of these parameters was analyzed using Dunnett's T test with the immediate release capsule as reference.

## RESULTS AND DISCUSSION

### Oral Gavage

Crystalline L-735,524 free base monohydrate has a solubility in water of less than 0.02 mg/ml at neutral pH and increases three orders of magnitude below pH 3.5. At equivalent concentrations, the pH of a sulfate salt solution is almost one unit lower than a solution of the free base (Fig. 2). The aqueous solubility of the sulfate salt is greater than 500 mg/ml. Previous reports indicated poor and variable oral bioavailability of a free base aqueous suspension in 0.5% methylcellulose in dogs; however, absorption was vastly improved when the drug was dosed in solution with 50 mM citric acid (4).

Similar plasma concentration profiles were obtained after oral administration of a 200 mg dose of the sulfate solution or capsule formulation to fasted dogs predosed with citric acid to standardize stomach acidity (Fig. 3). Although

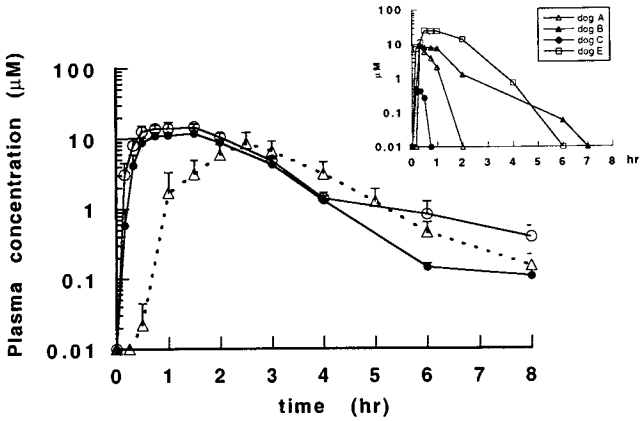


Fig. 3. Plasma concentrations of L-735,524 in Beagle dogs following 200 mg delivered as a sulfate solution (–○–), capsule formulation of the sulfate salt (–●–) or in repeated doses of 20 mg of the free base every 15 min over 2.5 hr (--△--). Shown are means ± sem (n = 3-6). Inset shows the variability in plasma profile from four dogs if animals received the capsule *without* citric acid pretreatment. Details of study in Methods.

mean fasting gastric pH in beagles is similar to humans, values are variable and can be as high as pH 6-7; moreover, basal gastric acid secretion in the dog is low (1% of maximal output *vs* 10% in man) (5,6). Administration of L-735,524 sulfate capsules to fasted dogs without citric acid pretreatment resulted in extremely variable absorption, with AUC values (0-8 hr) ranging from 0.2 to 52 μM•h (Fig. 3 inset). Meal stimulated acid secretion can decrease stomach pH by 4-5 units in dogs (6). Drug concentrations measured in plasma following a 200 mg free base suspension given in divided doses to fed animals are also shown in Figure 3. This regimen resulted in roughly a 40% and 50% reduction in mean Cmax and AUC values from the sulfate solution and capsule formulation, respectively (Table I); however, an extension of Tmax was attained due to drug administration in repeated doses and to slower gastric emptying in the fed state. The plasma profile obtained after the repeated dosing regimen, simulating controlled oral delivery, was desirable and further formulation efforts aimed to approximate this profile.

### Extended Release Devices

The performance of four sustained release dosage forms developed for human trials was initially evaluated in these animals. The "fast" and "slow" *in vitro* release profiles for these devices depended on the percentage of stearic acid in the SAS formulation or on the thickness of the cellulose acetate coating of the MODS device. The *in vitro* dissolution profile for the SAS and MODS formulations are shown in Figure 4, panels A and B, respectively, and compared to the complete dissolution of the immediate release capsule within 15 min. The fast and slow SAS formulations released 47% and 39%, respectively; and the fast and slow MODS devices released 57% and 33%, respectively, of drug dose in the first hour under dissolution conditions described in Methods. Mean Cmax, Tmax and AUC values obtained from animals given these formulations are presented in Table II. Peak plasma concentrations produced from the fast release devices were not significantly decreased, and plasma concentrations after 6 hr were not sufficiently greater than values obtained from the conventional capsule. The slow release SAS and MODS devices, with further reductions in the *in vitro* drug release rate, attenuated peak concentrations but produced significant and unacceptably diminished AUC values *in vivo*. None of the extended release devices produced

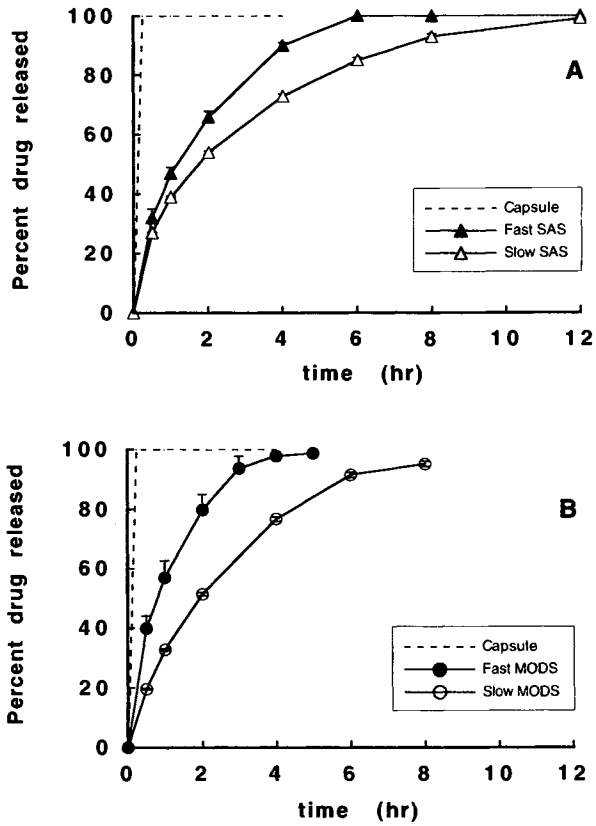


Fig. 4. *In vitro* dissolution of SAS (A) or MODS (B) type extended release dosage units for L-735,524, compared to the conventional capsule. Data are means ± RSD for six preparations.

a significant delay in Tmax, suggesting that drug released in the mid- and lower GI tract of dogs was not bioavailable due to regiospecific absorption of L-735,524 in the upper GI tract.

### Direct Intestinal Delivery

Administration of L-735,524 directly into the jejunum or colon allowed an unequivocal evaluation of the absorption and systemic availability from the mid- and lower sections of the GI tract.

Following bolus administration of the sulfate solution into the upper jejunum or ascending colon, drug absorption peaked immediately and declined rapidly, as shown by ap-

Table I. Absorption Kinetics of L-735,524 in Beagle Dogs[a]

| Formulation | Tmax (min) | Cmax (μM) | $AUC_{0-8}$ (μM · h) |
|---|---|---|---|
| Oral administration | | | |
| Sulfate solution, fasted dogs | 56 ± 12 | 18.1 ± 1.9 | 37.3 ± 3.45 |
| Conventional capsule, fasted dogs | 70 ± 10 | 13.7 ± 1.72 | 28.6 ± 3.07 |
| Repeated dosing, fed dogs | 150 | 8.45 ± 3.55 | 19.6 ± 8.85 |
| Intestinal delivery | | | |
| Jejunal bolus | 8.3 ± 1.7 | 6.2 ± 0.81 | 4.5 ± 0.86 |
| Colonic bolus | 96 ± 7.5 | 0.39 ± 0.08 | 1.79 ± 0.27 |

[a] Animals were given 200 mg free base equivalents by gavage or by direct intestinal delivery *via* vascular access ports. Fasted dogs received citric acid solution immediately before dose. Results are expressed as mean ± sem (n = 3-6).

Table II. Comparison of Extended Release Formulations of L-735,524 in Beagle Dogs[a]

| Formulation | Tmax (min) | Cmax (μM) | $AUC_{0-8}$ (μM · h) |
|---|---|---|---|
| Slow SAS | 65 ± 13 | 4.90 ± 0.27* | 8.80 ± 0.95* |
| Fast SAS | 110 ± 36 | 11.8 ± 1.75 | 26.2 ± 9.44 |
| Slow MODS | 75 ± 15 | 1.14 ± 0.15* | 2.94 ± 1.24* |
| Fast MODS | 65 ± 27 | 12.6 ± 2.91 | 21.7 ± 11.0 |

[a] Fasted dogs received citric acid solution immediately before drug dose. The sulfate salt (200 mg free base equivalent) was formulated in each dosage unit. Data are means ± sem (n = 3).

* Significantly different from corresponding Cmax and AUC values of conventional capsule (Table I) by Dunnett's T test, $p < 0.05$.

pearance of drug in the portal circulation as early as 2 min after dose (Fig. 5). Since drug solubility is strongly dependent on low pH, neutralization of the sulfate solution by lumenal fluids may promptly result in drug precipitation. Drug uptake from the jejunum was greater than from the colon, and was incomplete compared to oral gavage. Jejunal delivery of 100 mg or 200 mg L-735,524 sulfate produced peak systemic concentrations of 4.0 ± 2.05 μM and 6.2 ± 0.81 μM and AUCs of 2.06 μM•h (0-4 h) and 4.5 μM•h (0-8 h), respectively, which were significantly less than values from an oral dose (Table I). Systemic concentrations following 200 mg administered into the colon reached 0.39 ± 0.082 μM. Concentrations from the 100 mg dose were near the limit of quantitation at all time points.

Absorption from the ionized (sulfate) form of L-735,524 was better than from the insoluble free base suspension at all sites. One hundred milligram L-735,524 suspension delivered to the jejunum resulted in less than 0.3 μM concentrations in portal blood and systemic concentrations below the limit of quantitation. Colonic administration of L-735,524 free base suspension produced no measurable systemic plasma levels (data not shown).

Intestinal infusion studies were conducted to determine the maximum intestinal absorption and systemic availability of L-735,524 from a sustained release dosage form during its transit through the GI tract. A 20 mg/ml sulfate solution was infused at a constant rate into the proximal jejunum over 1.5 or 3 hr. Three hours was selected as a mean transit time through the small intestine and was also the period for release of over 80% of drug *in vitro* from the fast sustained release devices. These regimens produced pre- and post-hepatic plasma concentrations shown in Figure 6, panels A and B, respectively.

The following results were obtained from these infusion studies: 1) Three hr infusion (66 mg/h) of L-735,524 sulfate solution resulted in average portal concentrations of about 1 μM but did not produce systemic plasma concentrations greater than 0.1 μM. 2) Doubling the input rate (132 mg/h for 1.5 h) doubled pre- and post hepatic plasma concentrations. 3) Infusion of drug in 100 mM citric acid further increased circulating L-735,524 concentrations by more than two fold at both infusion rates. However, systemic concentrations greater than 1 μM were not achieved during infusion even with the coinfusion of citric acid to enhance absorption by increasing acidity of the lumenal environment.

While dogs are a popular model for dosage form evaluation, gastrointestinal pH is variable and higher in fasted dogs than in humans and mean intestinal transit time is slightly shorter (7). However, with preacidification the dog proved to be a predictive model for preclinical evaluation of sustained release dosage forms. Results from volunteers given SAS or MODS formulations of L-735,524 were in agreement with the animal data with respect to significant

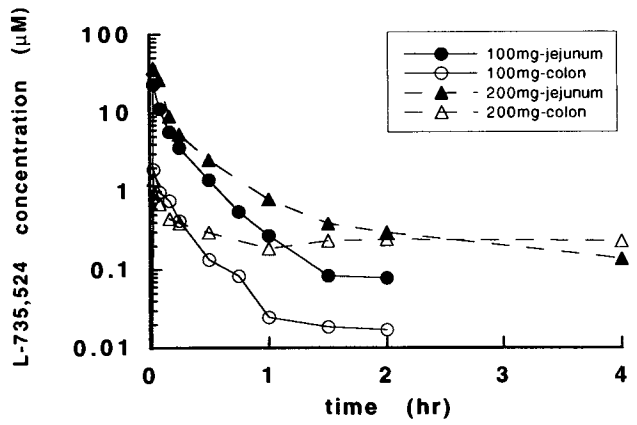


Fig. 5. Concentrations of L-735,524 in portal circulation following bolus doses of 524 sulfate solution delivered into the jejunum (solid symbols) or colon (open symbols) of dogs. Results shown are the means of four animals.

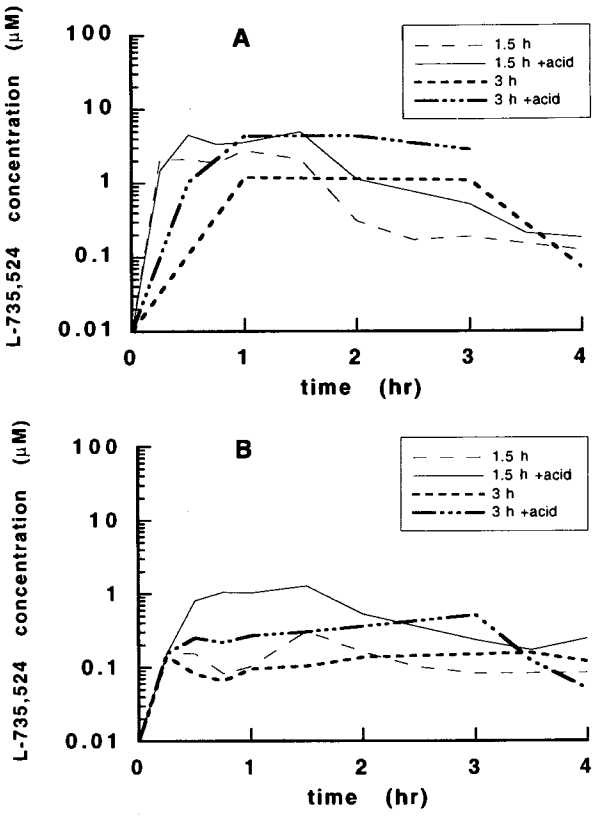


Fig. 6. Profiles of L-735,524 in portal (A) and systemic (B) circulation after infusion of 200 mg sulfate solution with or without 100 mM citric acid into the jejunum of conscious dogs. Rates of infusion were 66 or 132 mg/hr in a total volume of 6 ml. Results shown are the means of 3-4 animals.

reductions in Cmax and AUC values and failure to sustain plasma concentrations above 0.1 μM beyond 6 hr (data not shown).

Absorption of weakly basic pharmaceuticals with pH sensitive dissolution can be diminished in patients with various stages of hypochlorhydria, including the elderly and individuals taking medications that elevate gastric pH (8,9,10), and possibly in AIDS patients. We demonstrated the absorption of L-735,524, with pKa values of 5.9 and 3.7, is optimal in the relatively acidic environment of the duodenal segment favoring drug solubility. Drug released distal to the duodenum, even in acidic medium, was absorbed poorly, indicating regiospecific absorption. A reduction in peak plasma concentrations with extended release devices was achieved, but absorption could not be prolonged in the canine GI tract.

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