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Report

A Comparison of Oral and Rectal Absorption of L-Dopa Esters in Rats and Mice

Joseph A. Fix,[1,2] Jose Alexander,[1] Margot Cortese,[1] Karen Engle,[1] Paula Leppert,[1] and A. J. Repta[1]

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Short-chain alkyl esters of L-dopa were administered to rats and mice via oral and rectal routes. Plasma L-dopa esters and L-dopa were determined in the systemic and portal circulation by HPLC. A comparison of isopropyl, butyl, and 4-hydroxybutyl esters of L-dopa demonstrated significantly higher levels of the esters in both systemic and portal blood samples following rectal administration than following oral administration. In most cases, oral administration resulted in nondetectable (<0.01 μg/ml) levels of the esters in plasma. Correspondingly, the plasma levels of L-dopa itself were consistently higher following rectal administration. At very high oral doses (500 mg L-dopa equivalents/kg body weight), systemic plasma levels of the butyl ester could be detected (1.25 μg/ml at 10 min), which might indicate saturation of the esterase activity of the small intestine. These studies indicate that the systemic availability of L-dopa from short-chain alkyl esters of L-dopa may be best optimized by rectal administration, which avoids the relatively high esterase activity characteristic of the small intestine.

KEY WORDS: L-dopa esters; L-dopa; rectal; oral; bioavailability.

INTRODUCTION

L-Dopa, in combination with a peripheral decarboxylase inhibitor, has been the treatment of choice for Parkinson's disease for many years (1,2). Studies in animals have indicated that L-dopa is absorbed from the small intestine by a saturable amino acid transport system (3,4). Reproducibility of L-dopa absorption, therefore, is subject to nutrient amino acid competition and variations in intestinal transit. Recently, this laboratory reported studies describing the rectal absorption of short-chain alkyl esters of L-dopa which resulted in improved bioavailability of L-dopa in the systemic circulation when compared to L-dopa administration itself (5). Other workers have examined various esters of L-dopa, targeting primarily oral delivery (6–9). These oral studies have shown little, if any, improvement in L-dopa availability and efficacy as a result of oral L-dopa ester administration.

In this report, the rectal and oral absorptions of three esters of L-dopa were examined. Data have been obtained which suggest that L-dopa esters may be uniquely suited to rectal delivery systems as opposed to oral systems. Possible explanations for the differences observed in oral and rectal absorption are discussed.

MATERIALS AND METHODS

Chemicals. The butyl, 4-hydroxybutyl, and isopropyl esters of L-dopa were synthesized as hydrochloride salts and purified as previously described (5). All other chemicals were reagent grade.

Animals. Male Sprague–Dawley rats, 200–250 g, and male ICR mice, 20–30 g, were fasted 16–20 hr prior to use. For oral dosing, conscious animals received L-dopa esters in aqueous solution (0.9% NaCl with 0.1 mg/ml ascorbic acid, pH 5) by gavage (1.0 ml/kg body weight). For rectal dosing, animals were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories, 50 mg/kg i.p.) and received aqueous solutions (0.9% NaCl with 0.1 mg/ml ascorbic acid, pH 5) of the L-dopa esters administered at an intrarectal depth of 2.5 cm (rats) or 1.0 cm (mice). Volumes administered were 1.0 ml/kg body weight. The doses employed in the studies are given under Results and in the tables. At either 10 or 20 min, simultaneous systemic (cardiac) and portal blood samples were obtained from each animal (one to three animals per time point). For animals which were orally dosed, ether was used as an anesthetic prior to blood sampling. The blood samples were immediately added to centrifuge tubes containing solid NaF (3 mg/ml final concentration) to inhibit plasma esterase activity *ex vivo*. Plasma samples were isolated and immediately processed for high-pressure liquid chromatographic (HPLC) determination of the L-dopa esters or L-dopa. All plasma samples were assayed immediately after collection (within 15 min).

Analytical Procedure. L-dopa and L-dopa esters were analyzed by HPLC as previously described (5) using 3,4-dihydroxybenzylamine as an internal standard. Buffer or plasma samples were treated with H_3PO_4/acetonitrile, vortexed, and centrifuged. Compounds in the supernatant were

[1] INTERx Research Corporation, Subsidiary of Merck & Co., Inc., Lawrence, Kansas 66047.

[2] To whom correspondence should be addressed at INTERx Research Corporation, 2201 West 21st, Lawrence, Kansas 66047.

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separated on a Analytichem SCX column (4.6 × 100 mm) with a $NaClO_4$/EDTA/methanol mobile phase. Electrochemical detection (Environmental Science Associates 5100A detector) afforded a 0.01 μg/ml sensitivity limit for plasma L-dopa. Assay variability was less than 5%. Metabolites of L-dopa or the L-dopa esters were not observable under the assay conditions.

RESULTS

The systemic and portal plasma concentrations of L-dopa butyl ester (Table I) and L-dopa (Table II) are shown for oral and rectal administration of the ester to rats. Each animal received 147 mg L-dopa butyl ester/kg body weight (equivalent to 100 mg L-dopa/kg body weight). It is obvious from Table I that a trend toward higher plasma ester concentrations was observed after rectal dosing when compared to oral dosing. Differences in ester concentration were not observed when comparing systemic and portal samples for rectal administration. The plasma L-dopa data (Table II) also indicated greater absoprtion, and L-dopa availability, for rectal administration as compared to oral administration.

High-dose oral administration of the L-dopa butyl ester (500 mg equivalents L-dopa/kg body weight) did result in measurable plasma concentrations of the L-dopa ester. At 10 min postdosing, mean (n = 2) L-dopa butyl ester concentrations of 1.25 and 1.00 μg/ml were detected in the systemic and portal circulation, respectively. Plasma L-dopa was not evaluated in this study. These data compare to the reported L-dopa ester levels (Table I) of 0.37 and 0.39 μg/ml for systemic and portal plasma samples, respectively, following rectal administration of 100 mg equivalents L-dopa/kg body weight.

Very limited experiments were conducted with oral and rectal dosing of L-dopa 4-hydroxybutyl ester and L-dopa isopropyl ester to confirm the generality of the results seen with the butyl ester of L-dopa. Animals were dosed at 100 mg equivalents L-dopa/kg body weight, and systemic and portal blood samples collected at 10 and 20 min. Although only a single determination was made in these studies, it is clear from the data in Table III that both esters exhibit greater absorption following rectal administration than oral administration. The 4-hydroxybutyl ester was the only ester which was detected in significant amounts following oral administration.

In order to determine whether or not comparable ester absorption occurred in a second species, each of the esters was orally and rectally administered to mice at a dose of 100 mg equivalents L-dopa/kg body weight. The plasma L-dopa ester concentrations are shown in Table IV. Again, even though only single or duplicate experiments were performed, a clear trend was observed indicating greater intact ester absorption following rectal administration compared to oral administration.

Table I. Systemic and Portal Plasma Levels of L-Dopa Butyl Ester Following Rectal and Oral Administration to Rats[a]

Blood sampling site	Plasma L-dopa ester concentration (μg/ml) (mean ± SD; n = 3)			
	10-min sample		20-min sample	
	Rectal	Oral	Rectal	Oral
Systemic	0.37 ± 0.23	ND[b]	0.22 ± 0.18	ND
Portal	0.39 ± 0.18	ND	0.34 ± 0.22	ND

[a] Esters dosed at 100 mg equivalents L-dopa/kg body weight.
[b] Nondetectable (<0.01 μg/ml).

Table II. Systemic and Portal Plasma Concentrations of L-Dopa Following Rectal and Oral Administration of L-Dopa Butyl Ester in Rats[a]

Blood sampling site	Plasma L-dopa concentration (μg/ml) (range; n = 2)			
	10-min sample		20-min sample	
	Rectal	Oral	Rectal	Oral
Systemic	23.3–51.1	2.6–3.0	10.5–19.8	1.5–6.3
Portal	17.4–53.5	1.7–6.4	27.9–38.7	1.4–9.3

[a] Esters dosed at 100 mg equivalents L-dopa/kg body weight.

DISCUSSION

In a previous study (5), the systemic bioavailability of L-dopa was determined in rats and dogs following the rectal administration of a series of simple, short-chain alkyl esters of L-dopa (methyl, ethyl, isopropyl, hydroxypropyl, butyl, and 4-hydroxybutyl esters). In these studies, the L-dopa butyl ester afforded the highest L-dopa bioavailability in both the presence and the absence of a peripheral decarboxylase inhibitor. Based on these results, the L-dopa butyl ester was selected as the primary L-dopa prodrug for examining the comparative oral and rectal absorption of the ester itself.

Rectal administration of L-dopa butyl ester resulted in significant concentrations of the ester in both systemic and portal blood relative to concentrations following oral administration (Table I). In fact, the intact ester was not detected (<0.01 μg/ml) in the circulation following oral administration (Table I). Although the limited blood sampling (10 and 20 min) did not allow quantitation of the extent of ester absorption, the data clearly indicate distinct differences in intact ester absorption for oral and rectal administration. These results are consistent with data reported on intestinal esterase activity and L-dopa availability following oral administration of L-dopa esters. High levels of esterase activity have been reported for tissue of the small intestine (10). Extensive and rapid cleavage in the small intestine of the ester bond in the L-dopa butyl ester could account for the lack of the intact ester in portal and systemic blood following oral administration. Using a series of L-dopa esters and monitoring behavioral activity, Cooper *et al.* (6) have shown that oral administration affords activity not markedly different than L-dopa itself, indicating that the esters may be rapidly cleaved in the intestine to L-dopa, thereby eliciting behavioral responses similar to L-dopa. Administration of the L-dopa butyl ester by the rectal route would not expose the compound to the high esterase activity of the small intestine. This route, therefore, might allow significant absorption of the intact ester as seen in the present studies.

The data obtained for plasma L-dopa concentrations fol-

Table III. Systemic and Portal Concentrations of L-Dopa 4-Hydroxybutyl Ester and L-Dopa Isopropyl Ester Following Rectal and Oral Administration to Rats[a]

		Plasma L-dopa ester concentration[b] (μg/ml)			
		10-min sample		20-min sample	
L-Dopa ester	Route of administration	Systemic	Portal	Systemic	Portal
4-Hydroxybutyl	Oral	0.05	0.48	0.18	0.27
	Rectal	0.44	0.44	0.43	0.65
Isopropyl	Oral	ND[c]	0.01	ND	ND
	Rectal	0.10	0.02	0.49	0.86

[a] Esters dosed at 100 mg equivalents L-dopa/kg body weight.
[b] Single determination.
[c] Nondetectable (<0.01 μg/ml).

lowing oral and rectal L-dopa butyl ester administration confirm observations based on plasma L-dopa butyl ester analysis. Significantly higher plasma L-dopa concentrations were observed following rectal ester administration compared to oral administration. Since previous studies (11) have indicated that L-dopa itself is poorly absorbed from the rectum of rats, plasma L-dopa concentrations in these rectal studies likely represent postabsorption hydrolysis of the L-dopa butyl ester to L-dopa. The relatively low plasma levels of L-dopa observed after oral administration of the L-dopa butyl ester may reflect the limited availability of the ester for absorption following rapid intestinal conversion of the ester to L-dopa. Since the animals in this study were not pretreated with a peripheral decarboxylase inhibitor, intestinal aromatic amino acid decarboxylase activity (12,13) would also tend to decrease the amount of L-dopa itself which might be available for absoprtion from the small intestine. Decarboxylase activity is not a major factor in modifying L-dopa availability from the rectal compartment (11).

If the lack of intact L-dopa butyl ester in the circulation following oral administration was due to enzymatic cleavage of the ester in the small intestine, it should be possible to saturate this esterase activity by administration of a sufficiently high dose of the ester. In a very limited study, rats were orally administered 500 mg L-dopa equivalents/kg body weight. As reported, L-dopa butyl ester was detected in both the systemic and the portal circulation at a single sampling time. Without a complete plasma profile, relative estimates of bioavailability cannot be made. On a single sample point, however, a dose-adjusted comparison of rectal (100 mg equivalents/kg body weight) and oral (500 mg/kg body weight) ester absorption indicated 50–90% higher plasma ester levels after rectal administration than after oral dosing. The main conclusion from this experiment was that plasma L-dopa butyl ester can be detected after high-dose oral administration of the compound. The availability of L-dopa butyl ester for absoprtion in the high-dose study may be a result of saturation of intestinal esterase activity or bypassing of the enzymatic sites by excess compound presented to the intestinal lumen. In either case, it appears that the ester can be absorbed from the small intestine if present in sufficient quantities.

Although L-dopa butyl ester was employed as the primary model compound, limited studies performed with the 4-hydroxybutyl and isopropyl esters of L-dopa confirmed the results obtained with the butyl ester. Due to the confirmatory intent of these experiments and the technical difficulties of simultaneous systemic and portal blood sampling, only single determinations were made. However, the data clearly indicate that intact ester absorption is greater following rectal administration than oral administration. It was noted that the 4-hydroxybutyl ester was the only ester detected in sig-

Table IV. Systemic and Portal Concentrations of L-Dopa Esters Following Rectal and Oral Administration to Mice[a]

		Plasma L-dopa ester concentration (μg/ml)			
		10-min sample		20-min sample	
L-Dopa ester	Route of administration	Systemic	Portal	Systemic	Portal
Butyl[b]	Oral	0.01	0.01	0.01	ND[c]
	Rectal	0.25	0.28	0.24	0.20
4-Hydroxybutyl[d]	Oral	ND	0.14	0.01	0.01
	Rectal	0.06	0.07	0.06	0.07
Isopropyl[d]	Oral	ND	ND	ND	ND
	Rectal	ND	1.80	0.19	3.60

[a] Esters dosed at 100 mg equivalents L-dopa/kg body weight.
[b] Mean of two determinations.
[c] Nondetectable (<0.01 μg/ml).
[d] Single determination.

nificant quantities following oral administration. Previous studies in dogs had indicated that the 4-hydroxybutyl ester provided a more prolonged plasma profile of L-dopa following rectal administration (5). Although only indirect, these data may indicate that the 4-hydroxybutyl ester is somewhat more stable to enzymatic attack, thereby permitting greater absorption from the small intestine. Together with the more extensive L-dopa butyl ester experiments, the data from oral and rectal administration of the 4-hydroxybutyl and isopropyl esters of L-dopa provide evidence for preferential absorption of intact esters from the rectal compartment.

Significant species differences have been reported for esterase activity (10). Therefore, additional studies were conducted with mice to determine if ester absorption occurred which was comparable to that seen with rats. Oral and rectal administration of each of the three esters to mice afforded results in good general agreement with that observed for rats. The concentrations of L-dopa esters measured in systemic and portal plasma samples were consistently higher following rectal administration. Although general agreement was seen between the rat and the mouse data, there were some apparent isolated discrepancies. Of particular note were the results with the isopropyl ester of L-dopa. On an equivalent rectal dose per kilogram body weight basis, portal L-dopa isopropyl ester was much higher in the mouse than the rat (1.8 vs 0.02 and 3.6 vs 0.86 μg/ml for 10- and 20-min samples, respectively). Species differences in esterase substrate specificity may be involved, although a more exhaustive mouse versus rat pharmacokinetic study would be required to clarify these results. Regardless of this particular quantitative difference, the data from mice clearly corroborate the findings in the rat studies.

In nearly all cases, ester concentrations in the portal blood were comparable or slightly greater than those seen in the systemic circulation. This is consistent with the portal drug concentration being a function of absorption input rate and contributions from the general systemic circulation (following first-pass metabolism). The one notable exception was the isopropyl ester in mice, where following rectal administration, much greater levels were seen in the portal circulation. This may be the result of highly efficient hepatic extraction in mice of the isopropyl ester resulting in very low systemic levels. A definitive explanation is not possible with the present data, and other possible causes may be feasible.

In total, the data presented here clearly indicate that L-dopa esters may be uniquely suited to rectal delivery applications. With a superficial examination, one might expect L-dopa esters to be equally effective in the small intestine and rectal compartment. It appears, however, that the significant levels of esterase and decarboxylase activity present in the small intestine obviate any apparent advantages obtained by making the ester prodrugs. In the rectal cavity, where enzymatic activity is apparently less significant, the limiting factor is the permeability of the esters to the rectal mucosa. Once the esters reach the plasma compartment, *in vivo* conversion to the parent drug, L-dopa, can occur providing the active agent. The ultimate utility of rectal L-dopa esters in providing improved management of Parkinson's disease symptoms remains to be evaluated.

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