# A Feasibility Study of Differential Delivery of Levodopa Ester and Benserazide Using Site-Specific Intestinal Loops in Rats

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*Received 16 August 2008; accepted 18 April 2009*

*Published online 19 May 2009 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21822*

**ABSTRACT:** To study the effect of varied intestinal delivery of levodopa ester, levodopa and its butyl ester were administered as bolus or continuous infusions into site-specific, *in situ* ligated intestinal loops of rats. Benserazide, a carboxylase inhibitor, was not administered, coadministered with ester, or infused into the duodenal loop prior to ester dosing. While the proximal colon minimally absorbed levodopa itself, it substantially absorbed the ester. Coadministration of benserazide and ester at the colon did not increase absorption; however, prior infusion of benserazide into the duodenum enhanced the colonic absorption of ester. Compared to bolus infusion, continuous delivery of the ester resulted in a more sustained levodopa concentration in plasma, and less metabolism into dopamine. The results were repeated for methyl ester, and the relative differences between the results of methyl and butyl esters versus levodopa were similar. The overall results at the duodenum, jejunum, and ileum were also comparable, likewise were those for the proximal, middle, and distal colons. The results of the study are encouraging: a combination of the continuous delivery of levodopa ester with an immediate-release of benserazide optimizes levodopa bioavailability, potentially leading to a much more effective use of levodopa in the treatment of Parkinson's patients. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:227–233, 2010

**Keywords:** bioavailability; colonic drug delivery; gastrointestinal transit; intestinal absorption; prodrugs; site-specific absorption; metabolism; jejunum; absorption enhancer; preclinical pharmacokinetics

## INTRODUCTION

Levodopa (L-dopa) remains the most effective way to treat Parkinson's symptoms in patients. The problem is that levodopa-induced motor complications occur in many patients after 3–5 years of use.[1] However, tests have shown that a more consistent delivery of levodopa[2] has lessened motor complications. Numerous approaches have been attempted, including the use of ester prodrugs,[3–5] modified-release formulations,[6–10] or alternate routes of administration.[11–14] To date, these approaches have yet to demonstrate their effectiveness. Levodopa esters have been shown to be bioavailable after oral[4] and nasal[15] administrations. However, the use of an immediate-release ester levodopa may not be adequate, as demonstrated by a recent failure of a randomized controlled trial of an ethyl-ester levodopa formulation.[16]

In order to find a more effective means of delivering levodopa to patients so that they retain more consistent levodopa concentration in their

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Journal of Pharmaceutical Sciences, Vol. 99, 227–233 (2010)

systems, this study compared the levodopa availability of the butyl or methyl esters of levodopa, in the presence of a carboxylase inhibitor benserazide, using site-specific, *in situ* ligated intestinal loops of rats. To test the results, we measured plasma levodopa, its metabolite dopamine, and dopamine residues in the intestinal loops. Given that the absorption of benserazide (a polar compound) in the colon could be limited, benserazide was dosed at the duodenum prior to the ester dosing in the colon. In order to simulate a sustained delivery of levodopa, continuous infusions of levodopa were administered.

## MATERIALS AND METHODS

L-3,4-dihydroxyphenylalanine (levodopa, L-dopa), 3-hydroxytyramine hydrochloride (dopamine), L-3,4-dihydroxyphenylalanine methyl ester hydrochloride (levodopa methyl ester), benserazide hydrochloride, and 3,4-dihydroxybenzylamine hydrobromide were purchased from the Sigma–Aldrich (Tokyo, Japan). L-3,4-dihydroxyphenylalanine butyl ester hydrochloride (levodopa butyl ester) was synthesized as in the previous report.[15]

### *In Situ* Ligated Intestinal Loop and Assessments

Male Sprague–Dawley rats ($n = 3$–6 per group) weighing 230–280 g were used after overnight fasting. All experimental procedures in rats were approved by the Osaka University's Animal Experimentation Board. Abdominal incisions were made after the rats were anesthetized with intraperitoneal pentobarbital (40 mg/kg) and the intestines were exposed. A loop was prepared in each rat for the duodenum, jejunum, and ileum sections of the small intestine, and the proximal, middle, and distal colons as described below. The length of each loop was approximately 5 cm. For the duodenal loop, a ligature was placed at the pylorus portion after the stomach, while another ligature at 5 cm distally. The bile duct was tied off in order to prevent secretion. For the ileum loop, one ligature was placed at 12 cm from the pylorus and the other 5 cm distal, and prior to the cecum. The remaining jejunum and colonic loops were prepared from their respective intestinal sections. Only the results from the jejunum and proximal colonic loops for butyl ester were presented in this report, while those of methyl ester and the remaining results were summarized in the Discussion Section.

Levodopa (20 mg) was dissolved in 0.1 M hydrochloric acid (1 mL) and then neutralized with 0.1 M NaOH (approximately 1 mL) to pH 6.0. Total volume of the solution was adjusted to 2 mL with the addition of distilled water. Levodopa ester (20 mg molar equivalence to levodopa) was dissolved in 2 mL of distilled water. An aqueous solution of levodopa or ester (2 mL/kg) was administered through a polyethylene tubing cannula inserted at the proximal junction of each loop, and then the ligature was tightened to secure the cannula. The intestines were placed back into the abdominal cavity, and the incision was closed.

The benserazide dose used was 6 mg/kg for both bolus and continuous administration. For continuous administration into jejunum or colonic loop, the levodopa ester was dissolved in 480 μL of distilled water. The solution was infused into the loop at 8 μL/min for 1 h using a Jasco FAMICIC-100 HPLC pump (Tokyo, Japan). For concomitant administration with levodopa, a solution containing both benserazide and levodopa ester was administered into the intestinal loops. For pre-administration, benserazide dissolved in 1 mL of distilled water was administered into duodenum 30 min prior to the levodopa dosing at the colonic loop.

After the administration of levodopa or its ester, serial blood samples (120 μL) were collected from polyethylene tubing cannulated into femoral artery after the elapsed time for up to 3–4 h. After centrifugation (10,000 rpm for 3 min) the plasma was separated and stored in a freezer at −20°C prior to analysis.

To determine the residual amount of levodopa in the loop at the end of experiment, the loops were rapidly excised and washed with 0.1 M hydrochloric acid solution. Then, the tissue was minced and homogenized with aqueous 0.5 M perchloric acid solution (containing 1 mg of EDTA/mL). After centrifugation the supernatant was analyzed.

### Enzymatic Deconjugation and HPLC Analysis

Prior to analysis, conjugated dopamine in plasma was treated using a previously reported method,[17] where the plasma sample mixed with β-glucuronidase (25 U) and sulfatase (10 milliunits) was incubated for 1 h at 37°C. Purification of biological samples was carried out using an alumina