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# Dosage Form Design for Improvement of Bioavailability of Levodopa IV: Possible Causes of Low Bioavailability of Oral Levodopa in Dogs


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Received April 11, 1980, from the *Product Development Laboratories, Sankyo Company, Ltd., 1-2-58, Hiromachi, Shinagawa, Tokyo, Japan.* Accepted for publication September 30, 1980.



**Abstract** □ Several potential mechanisms for reduced levodopa bioavailability following oral administration to dogs and humans were investigated by studying the influence of the administration route on plasma levodopa levels after intravenous, hepatoportal, and duodenal administrations to dogs. The observed average areas under the plasma concentration–time curves (*AUC*) of levodopa following hepatoportal injection and intravenous injection were virtually identical; but following duodenal administration a decrease in the *AUC* of levodopa was observed with a concomitant increase in the *AUC* of total dopamine. The possible involvement of intestinal microorganisms in levodopa metabolism was explored in dogs that had been administered a combination of paromomycin and kanamycin to reduce intestinal microflora. Similar patterns of plasma level profiles and urinary excretion were observed between control and treated dogs. As measured by the release of [$^{14}$C]carbon dioxide from [$^{14}$C]levodopa, the distribution of levodopa decarboxylase enzyme activity in various parts of the intestine was studied in homogenates prepared from isolated intestinal segments of the duodenum and upper, middle, and lower parts of the jejunum and ileum. The jejunum showed the highest decarboxylase activity followed by the ileum and duodenum. These data indicate that the reduced bioavailability of orally administered levodopa occurs as a result of metabolism by levodopa decarboxylase enzyme in the gut wall.



**Keyphrases** □ Levodopa—bioavailability, effect of administration route, metabolism, intestinal microorganisms, levodopa decarboxylase □ Bioavailability—levodopa, effect of administration route □ Antiparkinsonian agents—levodopa, effect of administration route on bioavailability


Previous studies (1, 2) reported that, based on the measurement of levodopa and its metabolites recovered in the urine, the total amount absorbed, including levodopa metabolites, was 80–90% of the administered dose. However, the measurements of plasma levodopa concentrations after intravenous and oral administrations indicated that 20–40% of the administered dose reached the fluids of distribution intact.

The present study with levodopa was carried out in dogs to elucidate the mechanisms responsible for the low bioavailability of orally administered levodopa.

## EXPERIMENTAL

**Influence of Administration Route on Plasma Levodopa Levels and Its Metabolites in Dogs**—Nine healthy male beagle dogs, 10.5–13.2 kg, were fasted for ~16 hr and divided into three groups. They were anesthetized with 25 mg of pentobarbital sodium/kg iv. The first group was administered 20-mg doses of levodopa[1] into their brachial vein over 30 sec. After the dogs in the second group were fixed on their backs, a laparotomy was performed and levodopa solution[1] was administered directly into the hepatoportal veins over 30 sec. A laparotomy was performed in the third group, a 20-cm segment of the duodenum was ligated, and levodopa solution was administered into the ligated loop.

Blood samples were withdrawn from each animal with a heparinized syringe from the femoral or brachial vein at the time intervals indicated in Fig. 1. The blood specimens obtained were processed as described previously (1, 3). The third group was killed by exsanguination immediately after the last blood sample was collected, and the ligated duodenal loops were removed to determine residual levodopa and its metabolites in the duodenal loops where levodopa was administered. The contents of the duodenal loops were washed with saline and then three times with 0.04 *N* $HClO_4$ solution. The irrigating solutions were assayed for residual levodopa and its metabolites.

**Influence of GI Microorganisms on Oral Levodopa Absorption**—Two healthy male mongrel dogs, 6.0 and 11.8 kg, were orally administered a single capsule containing 110 mg of paromomycin and 100 mg of kanamycin. Two control dogs also were used. The dogs were anesthetized with intravenous injection of 25 mg of pentobarbital sodium/kg. After anesthetization, a laparotomy was performed and a series of intestinal loops were made by ligating segments of the stomach, duodenum, and jejunum. The contents of each segment were suspended in saline solution and diluted with 0.1% phosphate buffer (pH 7.2). The

[1] Dopaston Injection, Sankyo Co., Tokyo, Japan.




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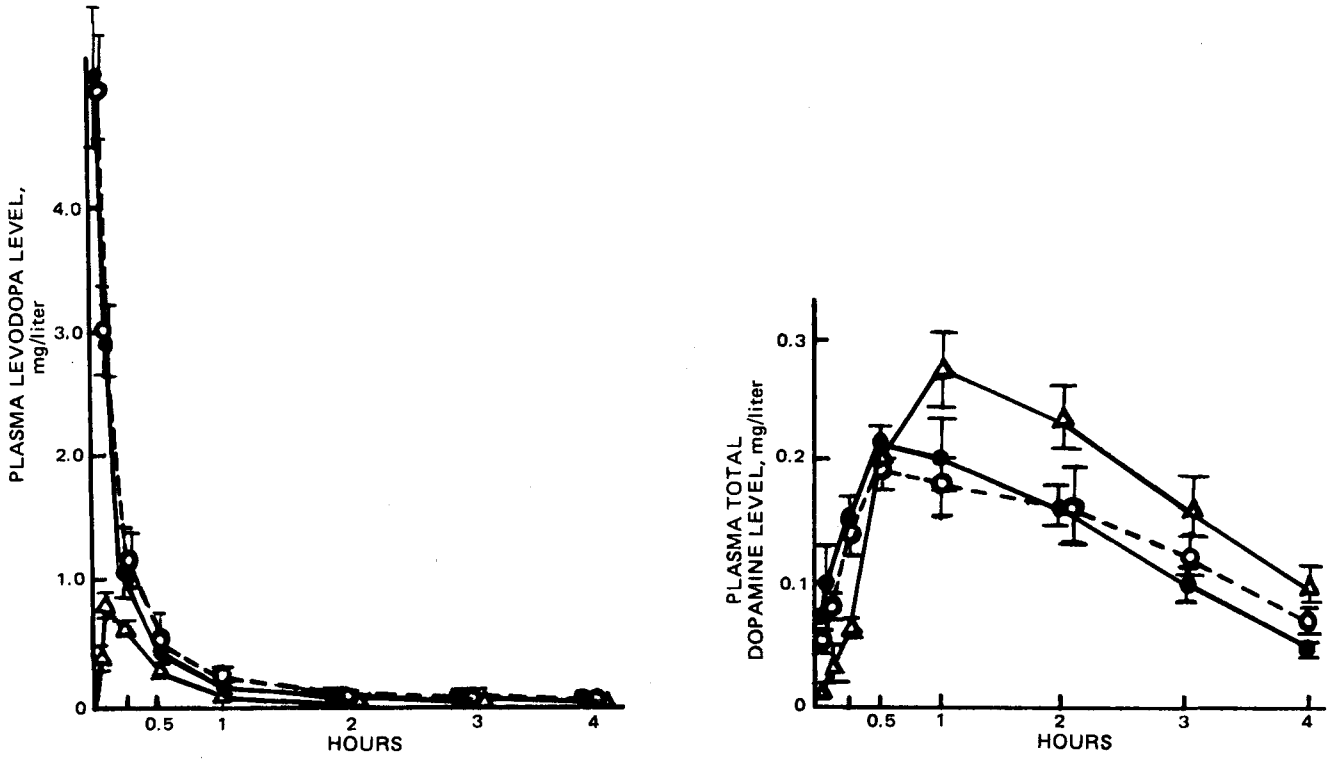


**Figure 1**—*Average plasma levels of levodopa (±SE) and total dopamine (±SE) following intravenous (●), hepatoportal (○), and duodenal (△) administrations of single 20-mg doses of levodopa to three dogs.*

diluted solution was smeared on the selective medium and cultured according to the conditions listed in Table I. After cultivation, the numbers of colonies were counted.

Following this initial study, six healthy male beagle dogs, 10.1–12.5 kg, were fasted for ~16 hr; levodopa in capsule form[2] was administered orally at a dose of 100 mg/dog with 10 ml of warm water. After 1 week, the same dogs were administered a single dose of the antibiotics as before, and levodopa in capsule form[2] was administered as previously described. Emesis was prevented by keeping the dogs' mouths closed by hand. Blood samples were obtained with a heparinized syringe at the intervals indicated in Fig. 2. The urine also was collected before and over 48 hr. The blood and urine samples were processed as previously described (1, 3).

**Distribution of Levodopa Decarboxylase Enzyme Activity in Intestinal Tract of Dogs**—Three healthy male mongrel dogs, 13.0–15.0 kg, were fasted for ~16 hr, anesthetized with 25 mg of pentobarbital sodium/kg iv, and then killed by exsanguination. Their abdomens were opened immediately, and sections of the duodenum and upper, middle, and lower parts of the jejunum and ileum were removed. The contents in each section were removed by washing three times with saline.

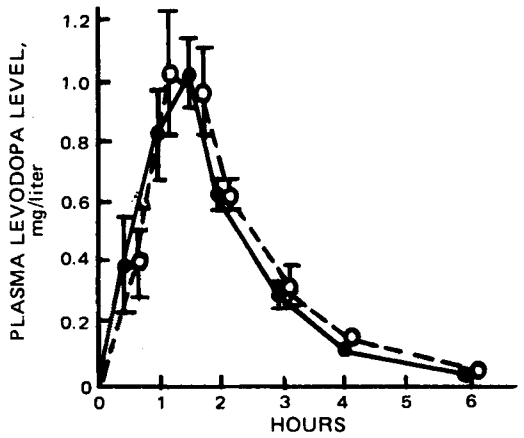


**Figure 2**—*Average plasma levodopa levels following oral administration of 100-mg doses of levodopa to six dogs treated with antibiotics (○) and to six control dogs without antibiotics (●) in a crossover fashion.*

An homogenate of each section of the intestinal tract was prepared in 0.05 *M* phosphate buffer (pH 6.8) cooled to 0°. The volume was adjusted to reflect the final wet tissue concentration of 100 mg/ml. Then 0.2–0.5 ml of each homogenate was added to the reaction mixture consisting of 0.2 ml of 0.2 *M* phosphate buffer (pH 6.8), 0.05 ml of 0.5% (w/v) pyridoxal phosphate solution, 0.1 ml of 8.1% levodopa solution [1 g of levodopa dissolved with 0.2% (w/v) sodium bicarbonate and 0.5% (w/v) polyvinyl acetate] and $^{14}$C-labeled DL-levodopa (0.05 $\mu$Ci/410 nmoles) and then incubated for 30 min at 37°. At 30 min after incubation, the reaction was stopped by adding 0.2 ml of 30% (w/v) $HClO_4$ solution into the reaction mixture.

The [$^{14}$C]carbon dioxide evolved from decarboxylation of [$^{14}$C]levodopa by levodopa decarboxylase in the homogenate was trapped completely in filter paper impregnated with benzethonium chloride solution[3]. The filter paper was immersed in a toluene scintillation mixture of 8 g of 2,5-diphenyloxazole and 0.2 g of 1,4-bis[2-(4-methyl-5-phenyloxazoly)]-benzene dissolved in 1000 ml of toluene. The trapped radioactivity was measured by the standard procedure.

**Assay of Levodopa and Its Metabolites in Plasma and Urine**—The assay of levodopa and its metabolites in plasma and urine was carried out as previously reported (3).

**Assay of Levodopa and Its Metabolites at Administration**—Residual levodopa and its metabolites were determined in the same manner as urine.

## RESULTS

**Influence of Administration Route on Plasma Levels of Levodopa and Its Metabolites in Dogs**—The average plasma levels of levodopa and total dopamine, one of the main metabolites of levodopa, are shown in Fig. 1 following intravenous, hepatoportal, and intraduodenal administrations to dogs. Visual inspection of average plasma levodopa level curves following intravenous and hepatoportal administrations indicates that their overall disposition profiles appear similar. However, the average plasma levodopa level curves following duodenal administration were lower than observed following intravenous or hepatoportal administra-

[2] Levodopa in capsule form was prepared in the same prescription as Dopaston capsules, Sankyo Co., Tokyo, Japan.

[3] This benzethonium chloride solution was prepared from a 10-fold dilution of Hyamine, Sankyo Co., Tokyo, Japan.

**Table I—Selective Medium and Cultivation Conditions**

| Bacteria | Medium | Cultivation Conditions |
|---|---|---|
| *Enterobacteriaceae* | MacConkey agar | 37°/24 hr |
| *Streptococcus* | Endo agar | 37°/48 hr |
| *Staphylococcus* | Mannitol salt agar | 37°/48 hr |
| *Lactobacillus* | LBS agar | 37°/72 hr |

tion. On the other hand, the average plasma total dopamine levels following duodenal administration were higher than following other routes.

The average *AUC* of levodopa and total dopamine and the average ratio of the *AUC* of total dopamine to the *AUC* of levodopa are summarized in Table II. The *AUC* plots of levodopa following intravenous and hepatoportal administrations were approximately identical, but the *AUC* of levodopa following duodenal administration was significantly different from that after the other forms of administration ($p < 0.05$, $t$-test); for the *AUC* of total dopamine, the situation was inverse. The higher ratio of the *AUC* of total dopamine to the *AUC* of levodopa following duodenal administration lends evidence for the postulation that this organ is involved in levodopa metabolism. In contrast, the hepatoportal ratio did not differ significantly from that observed after intravenous administration. In addition, the residual amounts of levodopa and the total of all its metabolites at 4 hr after administration in the duodenal site were <1%. Since only insignificant levels of levodopa and its metabolites were observed, levodopa was absorbed almost completely from the duodenum.

**Influence of GI Microorganisms on Oral Levodopa Absorption**—The aerobic bacterial counts in each part of the GI tract, including the stomach, duodenum, and jejunum, in dogs treated with a single dose of paromomycin and kanamycin in contrast to control dogs are compared in Table III. The treatment led to a decrease in the number of aerobic organisms in the gut tested.

The average plasma levodopa levels following oral administration to antibiotic-treated dogs and to control dogs in crossover fashion are shown in Fig. 2. Visual inspection of average plasma levodopa level curves following oral administration to those dogs indicates that their overall disposition profiles were essentially identical. Table IV summarizes the average urinary excretion of levodopa and its metabolites after oral administration of levodopa to those dogs.

These results show that there was no difference between the dogs with and without antibiotics. Unfortunately, the results are ambiguous since the observed reduction in intestinal bacterial flora may not have been sufficient to affect levodopa metabolism, and only the reduction in aerobic organisms in the stomach, duodenum, and jejunum was observed[4]. However, the flora in these areas are thought to be predominantly aerobic.

**Distribution of Levodopa Decarboxylase Enzyme Activity in Intestinal Tract of Dogs**—Figure 3 shows the distribution of levodopa decarboxylase activity responsible for levodopa metabolism in the different parts of the intestinal tract of dogs. The jejunum showed the highest decarboxylase activity, as measured by the release of [$^{14}$C]carbon dioxide from labeled levodopa, followed by the ileum and then the duodenum.

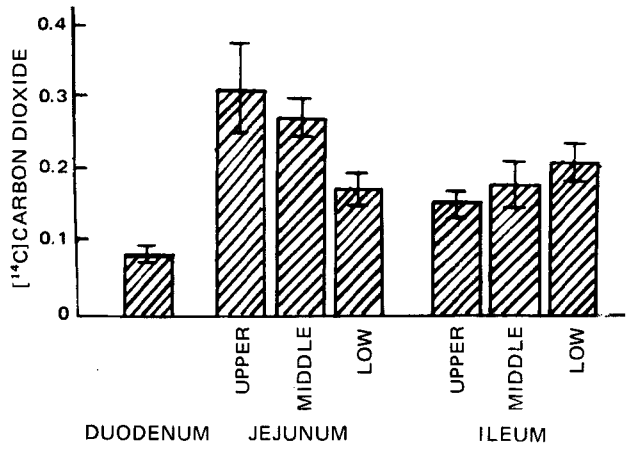


**Figure 3**—*Average distribution of levodopa decarboxylase activity in the different parts of the intestinal tract of dogs.*

[4] The duodenum will be shown to be the major absorption site in the next paper of this series.

**Table II—Average *AUC* of Levodopa and Total Dopamine and Ratio of *AUC* of Total Dopamine to Levodopa following Intravenous, Hepatoportal, and Duodenum Administrations to Dogs [a]**

| Route | *AUC* of Levodopa, (mg hr)/liter | *AUC* of Total Dopamine, (mg hr)/liter | Ratio of *AUC* of Total Dopamine to Levodopa |
|---|---|---|---|
| Intravenous | 1.70 ± 0.09[b] | 0.56 ± 0.14 | 0.34 ± 0.02 |
| Hepatoportal | 1.85 ± 0.10 | 0.57 ± 0.03 | 0.31 ± 0.01 |
| Duodenum | 0.65 ± 0.06 | 0.78 ± 0.07 | 1.20 ± 0.10 |

[a] The *AUC* from 0 to 4 hr was calculated using the trapezoidal rule. [b] Average ± *SE*.

## DISCUSSION

It was previously reported (1, 2) that the dose-dependent bioavailability of levodopa after oral administration to dogs and parkinsonian patients was probably due to metabolism during levodopa absorption. The plasma level profiles of levodopa and total dopamine were compared subsequent to intravenous, hepatoportal, and duodenal administrations to dogs. Analysis of the residual levels of levodopa and its metabolites in the duodenal loop indicated virtually complete levodopa absorption. However, plasma levodopa levels following duodenal administration were lower and plasma total dopamine levels were higher than those found after administration by other routes, leading to the highest ratio of the *AUC* of total dopamine to levodopa following duodenal administration. At the same time, the plasma level profiles of levodopa and total dopamine, the *AUC* of levodopa and total dopamine, and the ratio of the *AUC* of total dopamine to levodopa following the hepatoportal vein administration and intravenous administration were virtually identical.

These observations indicate that the first-pass metabolism of levodopa probably involves intestinal wall metabolism and, to a small extent, liver metabolism. This result is consistent with the conclusion reported previously (4, 5). Utilizing the isolated perfused rat liver technique, Mearrick *et al.* (4) reported that the major site of levodopa metabolism was the small intestine. Cotler *et al.* (5) administered [$^{14}$C]levodopa to dogs *via* hepatoportal, intravenous, and oral administrations on three separate occasions in crossover fashion. Comparisons of plasma levodopa level profiles *via* these administration routes led to the result that the physiologically impaired bioavailability of orally administered levodopa occurs virtually exclusively in the GI tract.

These results suggest the importance of levodopa metabolism in the intestine or the mucosal wall.

In addition, a similar degree of absorption and metabolism of levodopa was observed between control dogs and dogs whose numbers of intestinal bacteria decreased by treatment with antibiotics. Unfortunately, the results are not conclusive. Bakke (6) reported that levodopa was me-

**Table III—Comparison of Microflora between Dogs with and without Antibiotics**

| Group | Dog | Body Weight, kg | Segment of GI Tract | log (Number of Bacteria/Gram of Contents in GI Tract) |
|---|---|---|---|---|
| Control | 515 | 6.0 | Stomach | 7.0 |
| | | | Duodenum | 7.2 |
| | | | Jejunum | 6.8 |
| | 518 | 9.3 | Stomach | 6.6 |
| | | | Duodenum | 7.6 |
| | | | Jejunum | 7.3 |
| Treated | 114 | 8.6 | Stomach | 3.9 |
| | | | Duodenum | 3.2 |
| | | | Jejunum | 3.6 |
| | 51 | 11.8 | Stomach | 4.0 |
| | | | Duodenum | 3.7 |
| | | | Jejunum | 3.7 |

Table IV—Average Urinary Excretion of Levodopa and Its Metabolite after Oral Administration of Levodopa to Sterilized Dogs and Control Dogs

| Dogs | Total Levodopa[a] | Total Dopamine[b] | Total 3,4-Dihydroxyphenylacetic Acid[c] | Total Homovanillic Acid[d] | Total[e] |
|---|---|---|---|---|---|
| Control | 0.47 ± 0.07[f] | 8.9 ± 1.2 | 9.3 ± 1.4 | 30.4 ± 2.3 | 49.1 ± 2.4 |
| Sterilized | 0.47 ± 0.07 | 9.5 ± 1.6 | 10.1 ± 1.3 | 30.7 ± 2.5 | 50.7 ± 2.7 |

[a] Total levodopa = unconjugated levodopa + conjugated levodopa. [b] Total dopamine = unconjugated dopamine + conjugated dopamine. [c] Total 3,4-dihydroxyphenylacetic acid = unconjugated 3,4-dihydroxyphenylacetic acid + conjugated 3,4-dihydroxyphenylacetic acid. [d] Total homovanillic acid = unconjugated homovanillic acid + conjugated homovanillic acid. [e] Sum of total levodopa, total dopamine, total 3,4-dihydroxyphenylacetic acid, and total homovanillic acid. [f] Percent of dose excreted in 0–48-hr urine (average ± *SE*).

tabolized by intestinal microorganisms *in vitro* to *m*-hydroxyphenylacetic acid, 4-methylcatechol, and 4-methylguaiacol. Furthermore, levodopa was reported to be metabolized by intestinal microorganisms to *m*-hydroxyphenylacetic acid *in vivo* in conventional rats but not in germ-free rats (7–9). However, the small amount of metabolites formed by intestinal microorganisms reported by Bakke (6) and the fast absorption of levodopa from dogs observed in an *in situ* experiment[5] support the hypothesis that bacterial metabolism of levodopa may be insignificant.

The levodopa decarboxylase enzyme was widely distributed in the dog intestinal tract, with the greatest activity in the jejunum and the least activity in the duodenum. Taubin and Landsberg (10) suggested that catechol *O*-methyltransferase also played an important role in levodopa metabolism in the rat intestine. Administration of the levodopa inhibitor or benzerazide [*N*-*dl*-seryl-*N*-(1,2,3-trihydroxybenzyl)hydrazine], which cannot inhibit catechol *O*-methyltransferase, increased plasma levodopa levels (11–14). This finding implies that catechol *O*-methyltransferase plays a far less important role in intestinal metabolism of levodopa than levodopa decarboxylase.

The data presented here indicate that the reduced bioavailability of orally administered levodopa is due to metabolism of levodopa by levodopa decarboxylase in the intestine, with the greatest activity in the jejunum and the least activity in the duodenum.

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[5] Part V of this series, to be published.

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# Computational Problems of Compartment Models with Michaelis–Menten-Type Elimination


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Received April 8, 1980, from **PR&D Biostatistics, The Upjohn Company, Kalamazoo, MI 49001*, and ‡*Western Michigan University, Kalamazoo, MI 49008.* Accepted for publication December 2, 1980.



**Abstract** □ The Michaelis–Menten equation has been applied successfully in the study of enzyme kinetics. It usually is used to estimate $v_{max}$ and $k_m$ from observations of the initial rate of reaction, $v$, at various substrate concentrations, $C_s$. A variation of this expression recently was used in pharmacokinetics, where it was assumed that the elimination rate of drug from some compartment is $VC(t)/[K + C(t)]$, where $C(t)$ is the drug concentration. The meaning of $V$ and $K$ in this context is not clear. Attempts were made to estimate $V$, $K$, and other model parameters by fitting the model to observed drug concentrations at sampling times after dosing. This paper discusses the ill-conditioning of the estimation of parameters of a differential equation that includes the so-called Michaelis–Menten output. The solution of the equation is bound by the solutions to two first-order differential equations. Parameter values in an infinite region of the parameter space are shown to have solutions also lying within these two bounds. Simulations show that a minor change in the data (observations) or in the initial estimate of the parameters may cause a large change in the final estimates. In many cases, estimation and comparison of parameter values are meaningless.



**Keyphrases** □ Models, mathematical—compartment models with Michaelis–Menten-type elimination, computational problems, parameter estimation □ Michaelis–Menten equation—computational problems of compartment models, parameter estimation □ Compartment models—computational problems of models with Michaelis–Menten-type elimination, parameter estimation □ Pharmacokinetics—analysis, computational problems of compartment models with Michaelis–Menten-type elimination, parameter estimation


Linear compartment models have been used successfully in pharmacokinetics for the past 40 years. Like all mathematical models, they are an abstraction from the real biological system to a mathematical system and thus are