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ACKNOWLEDGMENTS

Supported in part by National Eye Institute Grant EY02328, by National Institute of General Medical Sciences Grant GM22357, and by Biomedical Research Support Grant SO7-RR-5606.

Dosage Form Design for Improvement of Bioavailability of Levodopa II: Bioavailability of Marketed Levodopa Preparations in Dogs and Parkinsonian Patients

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Received March 16, 1979, from the *Product Development Laboratories, Sankyo Company, Ltd., 1-2-58, Hiromachi, Shinagawa, Tokyo, Japan.* Accepted for publication September 14, 1979.

Abstract □ To estimate the absolute bioavailability of oral levodopa, plasma concentrations and urinary excretion of levodopa and its metabolites were determined in beagle dogs and in parkinsonian patients after intravenous and oral drug administration. The absolute bioavailability of orally administered levodopa was estimated to be about 35% in both dogs and patients; however, the total amount absorbed of intact drug and levodopa metabolites was estimated to be 80–90% of the administered dose. Due to the similarities of the pharmacokinetic characteristics of levodopa found in beagle dogs and in humans, beagle dogs can serve as a model to study bioavailability, absorption, and metabolic mechanisms.

Keyphrases □ Levodopa—bioavailability, dosage form design, dogs and humans □ Bioavailability—levodopa, dosage form design, dogs and humans □ Antiparkinsonian agents—levodopa, bioavailability, dosage form design, dogs and humans

Much higher oral doses than intravenous doses of levodopa are required to achieve a therapeutic effect in parkinsonian patients, and the resultant plasma levels after oral levodopa are markedly lower than those in patients receiving the drug intravenously.

Rivera-Calimlim *et al.* (1, 2) reported that when levodopa was administered orally to parkinsonian patients, a considerable portion apparently was metabolized *via* reactions in the stomach and intestines before it was absorbed. Other investigators (3–6) reported that 50–70% of the oral doses appeared to be metabolized in the intestinal mucosa of parkinsonian patients.

This paper reports part of a continuing study of levodopa to delineate mechanisms involved in the absorption profile in humans and to develop new dosage form designs to improve the bioavailability of levodopa. The absolute bioavailability of levodopa from marketed conventional preparations was determined by measuring the plasma levels and urinary excretion of levodopa and its metabolites after intravenous and oral administration of single doses to beagle dogs and to parkinsonian patients.

EXPERIMENTAL

Single Intravenous Doses in Dogs—After six healthy male beagle dogs, 10.3–12.5 kg, had been fasted for ~16 hr, levodopa[1] at a 50-mg dose was injected over 30 sec into the brachial vein. Blood samples were withdrawn with a heparinized syringe from the contralateral brachial vein. Samples were obtained 0, 2, 5, 15, and 45 min and 1, 1.5, 2, 3, 4, and 6 hr after dosing. Sodium metabisulfite solution (5% in a saline solution, 0.1 ml/5 ml of blood) and disodium ethylenediaminetetraacetate (2% in a saline solution, 0.1 ml/5 ml of blood) were added to the freshly drawn heparinized blood, and the plasma was separated immediately by centrifugation at 4°. All procedures were conducted in an ice bath.

Urine was collected before dosing and for 48 hr in bottles containing 5 ml of 6 *N* HCl and 3 ml of 0.2 *M* disodium ethylenediaminetetraacetate. The pH of the collected urine was adjusted to 2.0, and the urine was stored at −20° until assayed.

Single Oral Doses in Dogs—The same six beagle dogs used in the single intravenous dose studies were forcefully administered 250 mg of levodopa in capsule form[2] orally with 10 ml of warm water. The dogs' mouths were closed by hand to prevent emesis. Blood samples were withdrawn with a heparinized syringe 0, 0.5, 1, 2, 3, 4, and 6 hr after oral administration.

The blood samples were treated using the same procedures as in the intravenous study. The urine also was collected before dosing and for 48 hr after oral administration following the same procedure used for intravenous administration.

Single Intravenous Doses in Patients—Five parkinsonian patients (three men and two women) were studied. Their mean age was 63 years (range 51–71). Three days prior to the experiment, all levodopa preparations and other drugs that had been administered for the treatment of Parkinson's disease were withdrawn. Levodopa[1], 50 mg, diluted with 200 ml of physiological saline, was infused for 20 min at a constant rate into the brachial vein of these patients. Blood samples were withdrawn with a heparinized syringe at 0, 10, and 20 min during infusion and at 15 and 30 min and 1, 2, and 3 hr after infusion. The blood and urine samples were collected and processed in the same way as were those taken from the dogs.

Single Oral Doses in Patients—Six parkinsonian patients (three men and three women) were studied. Their mean age was 68 years (range 58–74). Three days before drug administration, all levodopa preparations and other drugs that had been administered for the treatment of Par-

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

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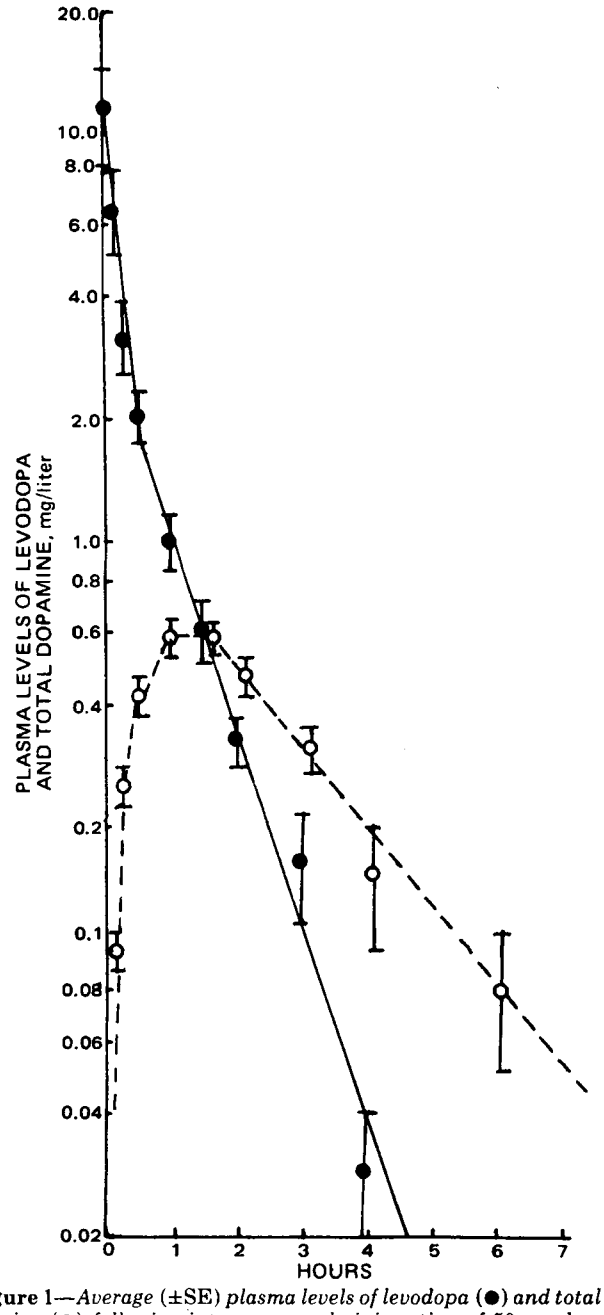

Figure 1—*Average* (±SE) *plasma levels of levodopa* (●) *and total dopamine* (○) *following intravenous administration of 50-mg doses of levodopa to six dogs.*

kinson's disease were withdrawn. A 1-g dose of levodopa in capsule form[2] was administered to the subjects with 1 cup of water at about 9:00 am.

On the day of the study, the subjects ate breakfast at about 7:00 am, lunch at about 12:00, and dinner at about 5:30 pm. Blood samples were withdrawn with a heparinized syringe before the drug was administered and at 0.5, 1, 2, 3, 4, and 6 hr after oral administration. The blood and urine samples were collected and processed using the same procedures as those used for the dogs.

Assay of Levodopa and Its Metabolites in Plasma and Urine—Levodopa and its metabolites in plasma and urine were assayed according to the methods reported previously (7).

Pharmacokinetics—The plasma levodopa concentration curves following intravenous administration to dogs were analyzed using the program of Hanano *et al.* (8). The plasma levodopa concentration curves following oral administration to dogs, infusion administration to patients, and oral administration to patients were analyzed using the BMDP-3R nonlinear regression program (9).

[2] Dopaston Capsules, Sankyo Co. Ltd., Tokyo, Japan.

Table I—Average Pharmacokinetic Parameters of Levodopa [a]

Parameter	Dogs[b], 50 mg iv	Patients[c], 50 mg iv
α, hr^{-1}	13.3 ± 3.4	11.7 ± 1.05
β, hr^{-1}	1.14 ± 0.11	1.12 ± 0.13
$0.693/\beta$, hr	0.58 ± 0.06	0.65 ± 0.069
k_{12}, hr^{-1}	6.5 ± 0.59	5.1 ± 0.52
k_{21}, hr^{-1}	3.3 ± 0.34	3.1 ± 0.29
k_{10}, hr^{-1}	4.5 ± 0.42	3.7 ± 0.35
V_1, liter/kg	0.29 ± 0.027	0.36 ± 0.037
AUC of levodopa, mg/kg/liter/hr	0.34 ± 0.025	0.0124 ± 0.0012
AUC of total dopamine, mg/kg/liter/hr	0.17 ± 0.014	0.0099 ± 0.00094
Clearance of levodopa, liters/kg/hr	1.21 ± 0.11	1.38 ± 0.19

[a] Average ± *SE*. [b] $n = 6$. [c] $n = 5$.

RESULTS

Absolute Bioavailability of Marketed Levodopa Preparations in Dogs—*Single Intravenous Doses*—The average plasma levels of levodopa and total dopamine[3] following intravenous administration of levodopa at a 50-mg dose to six dogs are presented in Fig. 1. Since the plasma levodopa concentration–time curve appeared to follow biexponential disposition characteristics, the data were fitted to the two-compartment open model using linear kinetics. Table I shows the results of the computer analysis. Visual inspection of the observed points around the fitted curve indicated satisfactory randomness of scatter. The average area under the plasma concentration–time curve (*AUC*) of levodopa corrected for body weight and the average plasma clearance of levodopa corrected for body weight also are shown in Table I to facilitate comparison between these parameters in dogs and in patients.

The average plasma levels of total dopamine formed by the metabolism of levodopa increased with time, reached a maximum value at ~1 hr after intravenous administration, and then decreased more slowly as compared to the elimination of levodopa in the plasma. The average *AUC* of total dopamine corrected for body weight is shown in Table I.

The intravenously administered levodopa was excreted in urine over 48 hr as total levodopa[4], total dopamine, total 3,4-dihydroxyphenylacetic acid[5], and total homovanillic acid[6], which represented 3.1 ± 0.43, 10.6 ± 0.92, 12.3 ± 1.24, and 37.3 ± 3.51% of the dose, respectively. Consequently, 63.3 ± 4.52% of the dose in terms of total urinary excretion[7] was eliminated within 48 hr after intravenous administration. The remainder of the drug probably was excreted in the feces or was undetermined metabolites.

Single Oral Doses—The average plasma levels of levodopa and total dopamine after oral administration of levodopa to six dogs are shown in Fig. 2. The maximum plasma level of 4.32 ± 0.42 mg/liter occurred 1 hr after oral administration. The levels then decreased rapidly to ~0.2 mg/liter 6 hr after oral administration, corresponding to 5% of the maximum plasma levels.

The average plasma levels of total dopamine changed more slowly than did those of levodopa. The elimination of total dopamine from the plasma also was slower compared to that of levodopa, and the plasma level of total dopamine 6 hr after oral administration was 0.42 ± 0.03 mg/liter, corresponding to about 25% of the maximum levels. The average *AUC* values of levodopa and total dopamine were 0.60 ± 0.053 and 0.56 ± 0.046 mg/liter/hr, respectively.

The orally administered levodopa was excreted in urine over 48 hr as total levodopa, total dopamine, total 3,4-dihydroxyphenylacetic acid, and total homovanillic acid, which represented 0.73 ± 0.93, 11.7 ± 1.21, 13.6 ± 0.91, and 25.6 ± 2.62% of the dose, respectively. Consequently, 51.6 ± 3.02% of the dose was excreted within 48 hr after oral administration.

Comparison of the absolute bioavailability of marketed levodopa capsules after oral administration with intravenous injection was accomplished using the plasma levodopa levels to estimate the intact amounts of levodopa absorbed into the circulation system, with the as-

[3] Total dopamine = conjugated dopamine + unconjugated dopamine.

[4] Total levodopa = unconjugated levodopa + conjugated levodopa.

[5] Total 3,4-dihydroxyphenylacetic acid = unconjugated 3,4-dihydroxyphenylacetic acid + conjugated 3,4-dihydroxyphenylacetic acid.

[6] Total homovanillic acid = unconjugated homovanillic acid + conjugated homovanillic acid.

[7] Total urinary excretion = total levodopa + total dopamine + total 3,4-dihydroxyphenylacetic acid + total homovanillic acid.

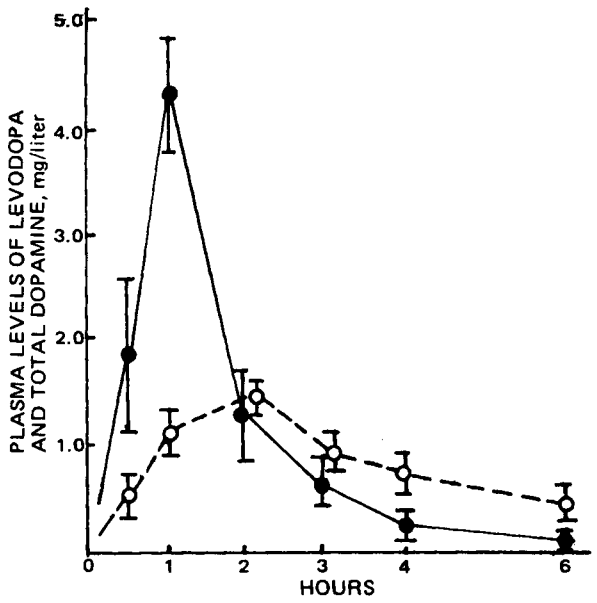

Figure 2—*Average* (±SE) *plasma levels of levodopa* (●) *and total dopamine* (○) *following oral administration of 250-mg doses of levodopa to six dogs.*

sumption that levodopa disposition in the body followed linear kinetics. On the other hand, the total amount absorbed from the GI tract, including levodopa and its metabolites, was calculated by the ratio of the total urinary excretion after oral administration to that after intravenous administration. The absolute bioavailability in the dogs and the total amount absorbed are shown in Table I. These results indicate that the difference between these values is due to presystemic metabolism and/or degradation of the drug.

Absolute Bioavailability of Marketed Levodopa Capsules in Parkinsonian Patients—*Single Intravenous Doses*—The average plasma levels of levodopa and total dopamine for 20 min following intravenous infusion of a 50-mg dose of levodopa to five parkinsonian patients are presented in Fig. 3. The plasma levodopa levels increased during the intravenous infusion and reached a maximum value of 0.89 ± 0.09 mg/liter at the conclusion of the infusion, *i.e.*, 20 min after the beginning of the intravenous infusion. The subsequent plasma levodopa levels decreased rapidly, and levodopa was almost undetectable in the plasma after 200 min. These plasma levodopa concentration–time curves were analyzed by appropriate procedures and linear kinetics presuming a two-compartment open model (Table I). The terminal half-life of levodopa in patients was 39 ± 4.1 min (mean ± *SE*). The *AUC* of levodopa corrected for body weight was 0.0124 ± 0.0012 mg/kg/liter/hr (Table II).

The plasma level of total dopamine increased with time and reached a maximum value of 0.18 ± 0.016 mg/liter 50 min after the intravenous infusion began. The levels then decreased more slowly compared to the elimination of levodopa in the plasma and were approximately 33% of the maximum plasma levels 200 min after the intravenous infusion began. Plasma levodopa and total dopamine were not detectable before dosing. The *AUC* of total dopamine was 0.0099 ± 0.00094 mg/kg/liter/hr.

The infused levodopa was excreted in urine over 8 hr as total levodopa, total dopamine, total 3,4-dihydroxyphenylacetic acid, and total homovanillic acid, which represented 2.5 ± 0.21, 11.2 ± 1.02, 11.8 ± 0.94, and 29.6 ± 2.43% of the dose, respectively. Consequently, 55.1 ± 4.24% of the dose was excreted within 48 hr after infusion.

Single Oral Administration—The average plasma levels of levodopa and total dopamine after oral administration of a 1-g dose of levodopa in capsule form to six patients are shown in Fig. 4. The maximum plasma levels occurred 1 hr after oral administration. The levels then decreased rapidly to <0.2 mg/liter at 6 hr after oral administration. The *AUC* of levodopa was 0.0826 ± 0.0105 mg/kg/liter/hr.

The average plasma levels of total dopamine reached a maximum after 2 hr and then decreased more slowly than did those of levodopa, with the concentration of 0.45 ± 0.05 mg/liter at 6 hr corresponding to about 33% of the maximum levels. The *AUC* of total dopamine was 0.104 ± 0.0102 mg/kg/liter/hr.

Orally administered levodopa was excreted in urine over 24 hr as total levodopa, total dopamine, total 3,4-dihydroxyphenylacetic acid, and total homovanillic acid, which represented 0.58 ± 0.065, 13.5 ± 1.25, 15.1 ± 1.62, and 18.2 ± 1.95% of the dose, respectively. Consequently, 48.4 ± 4.13% of the dose was excreted within 48 hr after oral administration.

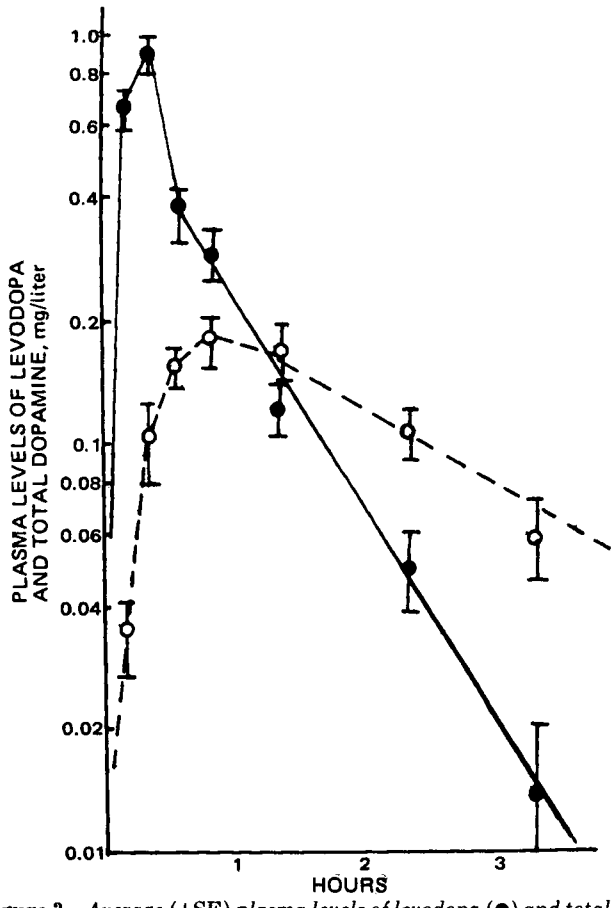

Figure 3—*Average* (±SE) *plasma levels of levodopa* (●) *and total dopamine* (○) *following infusion of 50-mg doses of levodopa to five parkinsonian patients.*

The absolute bioavailability and total percent of oral capsules that were absorbed by patients were calculated on the assumption that levodopa in the body obeys linear kinetics and that the two different population groups were comparable in this study. They are shown in Table II and were in good agreement with the values for the dogs.

DISCUSSION

The plasma concentration–time curves and the estimated pharmacokinetic parameters obtained in these studies compare well with the data obtained by Coutinho *et al.* (10) and Colter *et al.* (11) following intravenous administration of radiolabeled levodopa to dogs. However, Coutinho *et al.* (10) reported that ~75% of the intravenously administered dose, in terms of total radioactivity, was recovered in the urine within 72 hr. In contrast, ~65% of the intravenously administered dose, as the sum of total levodopa, total dopamine, total 3,4-dihydroxyphenylacetic acid, and total homovanillic acid, was excreted in the urine within 48 hr in the present studies. Both results seem to be consistent considering that the data of Coutinho *et al.* (10) involved total radioactivity measurements.

Table II—Absolute Bioavailability and Total Amount Absorbed, Including Metabolites, after Oral Administration of Levodopa to Dogs and Patients

Subject	Dose, mg	Absolute Bioavailability, %[a]	Total Amount Absorbed, %[a]
Dogs	250	36.3 ± 2.9[b]	81.0 ± 4.7
Patients	1000	33.3 ± 4.23	87.8 ± 7.4

[a] Based on the presumed linear kinetics using the dose-corrected *AUC* from the intravenous dose. [b] Average ± *SE*.

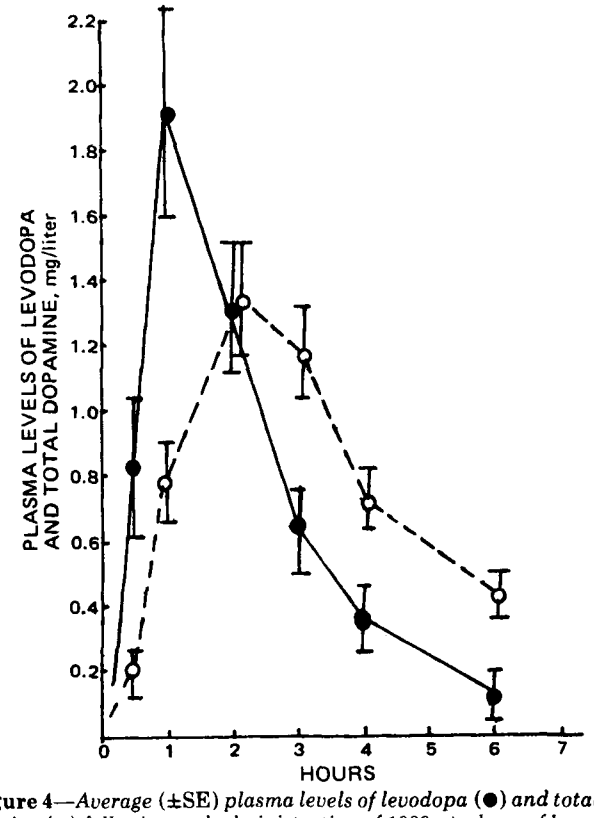

Figure 4—*Average* (±SE) *plasma levels of levodopa* (●) *and total dopamine* (○) *following oral administration of 1000-mg doses of levodopa to six parkinsonian patients.*

The profile of plasma levodopa levels and the pattern of urinary excretion obtained in this experiment following oral administration of levodopa, respectively, agree with the results and the observations reported previously (10–12). In the urine, 44–57% of the oral dose was excreted, including metabolites, but only small amounts of intact levodopa were excreted.

The absolute bioavailability and the extent of total oral absorption including levodopa and its metabolites following oral administration of levodopa to dogs were ~35 and 80%, respectively. These results showed that the bioavailability of levodopa was low but that the extent of total absorption of levodopa was good. It can be inferred that this difference is due to the degradation and/or metabolism of levodopa in the GI tract and liver during absorption. In fact, levodopa decarboxylase, believed to be responsible for metabolizing levodopa, is widely distributed in the liver (13–15), kidney (16), and GI tract (5, 6, 17). The activity of this enzyme is relatively high in the GI tract. Therefore, the proposal of gut and gut wall metabolism of levodopa by this enzyme probably is justified.

One way of evaluating the first-pass effect is to compare the ratio of the *AUC* of the metabolite, total dopamine, to that of the parent drug. These relationships may be represented as follows:

$$AUC_{\text{levodopa}} = FD/Cl_{\text{levodopa}} \qquad \text{(Eq. 1)}$$

$$AUC_{\text{total dopamine}} = fFD/Cl_{\text{total dopamine}} \qquad \text{(Eq. 2)}$$

where Cl_{levodopa} and $Cl_{\text{total dopamine}}$ represent the elimination clearance of the respective compounds, *FD* represents the levodopa dose absorbed, and *f* represents the fraction of the levodopa dose absorbed that is converted to total dopamine. The ratio of Eq. 2 to Eq. 1 gives:

$$\frac{AUC_{\text{total dopamine}}}{AUC_{\text{levodopa}}} = \frac{fCl_{\text{levodopa}}}{Cl_{\text{total dopamine}}} \qquad \text{(Eq. 3)}$$

Thus, the new ratio shown in Eq. 3 cancels out the *FD* term relating to the absorbed dose. The right side of Eq. 3 includes three parameters. The elimination clearance of dopamine, $Cl_{\text{total dopamine}}$, in the denominator should be unaffected by the administration route. Since Eq. 3 eliminates the effect of the absorbed dose, the observed elimination clearance for the parent drug, Cl_{levodopa}, should be unchanged as long as the system obeys linear kinetics.

Finally, Eq. 3 includes a term, *f*, in the numerator that represents the fraction of the parent drug converted to the metabolite, total dopamine. This term should increase after oral administration relative to an intravenous dose. Therefore, comparison of the *AUC* ratios obtained after oral and intravenous studies should reflect the increase in the metabolite formed *via* presystemic metabolism of the drug during oral absorption. The ratio in dogs was 0.56 ± 0.06 after intravenous injection *versus* 0.93 ± 0.10 after oral absorption. Therefore, the first-pass metabolism most likely results in more extensive metabolism of levodopa.

On the other hand, parkinsonian patients administered an intravenous dose of levodopa showed levodopa disposition kinetics similar to those observed in the dogs (Table I). As mentioned before, about 55% of the intravenous dose was recovered from the urine within 8 hr. Imai *et al.* (18) reported that following oral administration of levodopa to parkinsonian patients, >90% of the drug excreted within 24 hr was recovered from the urine within the first 8 hr. Therefore, even though these data are from urine collected within the first 8 hr after dose administration, it seems appropriate to assume that the results would be similar to the 24-hr data. In addition, Bronaugh *et al.* (19) determined the urinary excretion of total levodopa, total dopamine, total 3,4-dihydroxyphenylacetic acid, and total homovanillic acid separately after intravenous administration of radioactive levodopa to patients. They reported about 50% urinary excretion of these metabolites, which is in good agreement with our value. The plasma levodopa levels and the urinary excretion pattern following oral administration of levodopa in the parkinsonian patients in the present study were similar to previous (18, 20, 21) results.

The absolute bioavailability and the extent of total absorption calculated from results of intravenous infusion in parkinsonian patients were ~33 and 88%, respectively (Table II). These results show that bioavailability of levodopa was low in patients but that the extent of total absorption of levodopa was good, as in dogs. Also, the ratio of the *AUC* of total dopamine to the *AUC* of levodopa was 0.81 ± 0.06 after an intravenous dose in patients, while it was 1.26 ± 0.13 after oral administration. These values are consistent with the concept of first-pass metabolism, yielding a more extensive metabolism of levodopa during oral administration in patients as well as in dogs.

Furthermore, the results of these bioavailability studies using both dogs and patients indicate that beagle dogs and patients showed similar pharmacokinetic parameters following intravenous administration of levodopa (Table I) and similar bioavailability of oral levodopa (Table II). Therefore, this study supports the use of beagle dogs as model animals for pharmacokinetic and biopharmaceutical investigations to study the mechanism involved in the low bioavailability of marketed levodopa preparations. A subsequent paper of this series will discuss the possibility of nonlinear kinetics of levodopa in dogs and humans.

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ACKNOWLEDGMENTS

Presented in part at the Pharmaceutics Section, 97th Meeting of the Pharmaceutical Society of Japan, Tokyo, Japan, April 1977.

The authors are grateful to Dr. A. Noguchi for help in designing the protocol and supervising drug administration and blood and urine sampling in patients and to Mr. S. Yamane for technical assistance in the bioavailability studies in patients. The authors also thank Dr. T. Higuchi for valuable discussions; Dr. H. Negoro, Dr. S. Akagi, and Dr. T. Miki for the promotion of the research project; Dr. S. Riegelman for constructive comments on the manuscript; and Ms. Ellen Buoncristiani for typing the manuscript.

Polymers for Sustained Macromolecule Release: Procedures to Fabricate Reproducible Delivery Systems and Control Release Kinetics

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Received May 31, 1979, from the *Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139,* and the *Department of Surgery, Childrens' Hospital Medical Center, Boston, MA 02115.* Accepted for publication October 8, 1979.

Abstract □ Matrixes composed of ethylene–vinyl acetate copolymer are useful vehicles for the sustained release of macromolecules such as proteins. A new procedure for fabricating these polymeric delivery systems involved mixing the dry, powdered macromolecule with a polymer solution and casting the mixture at −80°. The resulting matrix was dried in two 48-hr stages, first at −20° and then at 20°. These polymer systems had uniform drug distribution, and their release kinetics were reproducible. Fabrication parameters such as drug particle size, drug loading, and matrix coating all significantly affected release kinetics.

Keyphrases □ Kinetics, drug release—effects of particle size, drug loading, and matrix coating on polymer–drug mixtures □ Polymers—use in sustained-release film formation, preparation methods of polymer–drug matrix, low temperature effects on polymeric matrix □ Controlled-delivery system—preparation methods, polymer–drug matrix, drug release kinetics

A previous study described the first biocompatible polymeric delivery systems capable of continuously delivering macromolecules (mol. wt. > 1000) such as proteins for prolonged periods (1). In that study, a polymer–macromolecule matrix was formed by mixing powdered macromolecules in a polymer solution. This mixture was poured into small, conical, glass molds, and the solvent was evaporated. The resulting pellets, when exposed to an aqueous medium, released macromolecules in biochemically active form for >100 days (2). Although sustained release was achieved, the reproducibility of the release kinetics was poor. Difficulties in obtaining reproducible release rates were compounded when larger volume polymeric delivery systems were fabricated.

Significant drug settling and redistribution occurred during casting and drying due to the insolubility of the incorporated macromolecules in the polymer solvent. At room temperature, the drug migrated vertically, and visible lateral motion was caused by currents (possibly thermal) in the mixture. The low temperature casting procedures described were developed to minimize this drug movement during matrix formation. The reproducibility of release kinetics of matrixes prepared using these low temperature methods was improved markedly.

The reproducible kinetics permitted the study of the effects of certain fabrication factors, including drug particle size, drug loading, and matrix coating, on macromolecule release. The significant effects of these factors on release kinetics suggest possible means of utilizing and modifying macromolecular release systems according to research or clinical needs.

EXPERIMENTAL

Matrix Preparation—Ethylene–vinyl acetate copolymer[1] (40% vinyl acetate by weight) was dissolved in methylene chloride[2] to give a 10% solution (w/v). Protein or another macromolecular powder was sieved[3] to give particles of <75, 75–250, or 250–425 μm. A weighed amount of powder from a single size range was added to 15 ml of the polymer solution in a glass vial[4], and the mixture was vortexed[5] for at least 10 sec to yield a uniform suspension. This mixture was poured quickly into the center of a leveled glass mold (7 × 7 × 0.5 cm), which had been cooled previously on dry ice for 5 min. During precooling, the mold was covered with a glass plate to prevent excess frost formation.

After the mixture was poured, the mold remained on the dry ice for 10 min, and the mixture froze. (The mold was covered again for the last 7 min of this stage.) The frozen slab was easily pried loose with a cold spatula, transferred onto a wire screen[6], and kept at −20° for 2 days. The slab then was dried for 2 more days at room temperature in a desiccator[7] under a mild, houseline vacuum (600 mtorr). Drying caused the slabs to shrink to ~5 × 5 cm. The central 3 × 3-cm square was excised with a scalpel[8] and a straight edge and divided further into nine 1 × 1-cm squares.

Kinetics—Each square was weighed, its thickness was measured with

[1] Elvax 40, DuPont Chemical Co., Wilmington, Del. This polymer also may be obtained from Dr. Langer at the Massachusetts Institute of Technology.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] American Standard sieves, Nos. 40, 60, and 200, Dual Manufacturing Co., Chicago, Ill.
[4] Wheaton Scientific Co., Millville, N.J.
[5] Scientific Industries, Bohemia, N.Y.; set at speed 10.
[6] Common steel mesh, 0.5-mm spacing.
[7] Bel Art, Pequannock, N.J.
[8] No. 10 blade, Bard-Parker, Rutherford, N.J.

0022-3549/80/0300-0265$01.00/0