Enhancement of the Systemic and CNS Specific Delivery of L-Dopa by the Nasal Administration of Its Water Soluble Prodrugs

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Purpose. To study the utility of the nasal route for the systemic delivery of L-dopa using water soluble prodrugs of L-dopa and to examine if this delivery method will result in preferential delivery to the CNS.

Methods. Several alkyl ester prodrugs of L-dopa were prepared and their physicochemical properties were determined. *In vitro* hydrolysis rate constants in buffer, rat plasma, rat brain homogenate, rat CSF, and rat nasal berfusate were determined by HPLC. *In vivo* nasal experiments were carried out in rats. Levels of L-dopa and dopamine in plasma, CSF, and olfactory bulb were determined using HPLC method with electrochemical detection.

Results. All the prodrugs showed improved solubility and lipophilicity with relatively fast *in vitro* conversion in rat plasma. Absorption was fast following nasal delivery of the prodrugs with bioavailability around 90%. Dopamine plasma levels did not change significantly following nasal administration of the butyl ester prodrug. Olfactory bulb and CSF L-dopa concentration were higher following nasal delivery of the butyl ester prodrug compared to an equivalent intravenous dose.

Conclusions. Utilization of water soluble prodrugs of L-dopa via the nasal route in the treatment of Parkinson's disease may have therapeutic advantages such as improved bioavailability, decreased side effects, and potentially enhanced CNS delivery.

KEY WORDS: nasal delivery; CNS delivery; L-dopa; Parkinson's disease; prodrugs.

INTRODUCTION

L-Dopa represents the most clinically useful drug in the treatment of Parkinson's disease. Unlike dopamine itself, L-dopa can be transported through the blood-brain barrier where it undergoes decarboxylation to dopamine within the brain and exerts its effect. The magnitude of improvement in Parkinsonism with L-dopa therapy cannot be surpassed by any other available anti-Parkinsonian agent (1).

Unfortunately, the clinical response to oral L-dopa is variable and unreliable because of its erratic oral absorption and first-pass metabolism. The oral bioavailability of L-dopa alone is estimated to be about 5 to 15% and less than 1% of the administered oral dose reaches the brain unchanged (1). The low bioavailabilty is attributed to site specific absorption in the duodenum (2), carrier-mediated transport absorption (3), and extensive metabolism in the GI tract (4). It is believed that the major peripheral side effects resulting from the oral administration of L-dopa are due to the formation of large amounts of dopamine during first-pass metabolism in the GI wall (3). These side effects include nausea, vomiting and cardiac irregularity. Inter and intra-individual variability in the degree of this first-pass effect is the main cause of the common difficulty of maintaining an effective therapeutic regimen with L-dopa. Decarboxylase inhibitors are co-administered with L-dopa to decrease its GI tract metabolism. The most notable effect of this is enhanced bioavailability (4) and a 75% reduction in total daily L-dopa dose required to produce clinical benefit (5,6). This will also decrease the peripheral side effects. However, the on-off fluctuation remains (7,8) because the oral absorption is still erratic and plasma concentrations still fluctuate. Intravenous infusion of L-dopa alone or in combination with a decarboxylase inhibitor was found to extend dramatically the duration of mobility and reduce the frequency of fluctuation and provide significant mobility improvement (8,9). The intravenous infusion, however, is impractical and inconvenient for routine clinical use.

Several approaches have been attempted to enhance the bioavailability and minimize the side effects of L-dopa such as super enteric coated tablets (10), controlled release formulation (11), and oral or rectal prodrug administration (12–14).

The nasal route is a very useful method of administration for many drugs that undergo extensive metabolism in the GI tract (15–17). In addition, several studies (18–21) have indicated that the nasal route might result in preferential absorption to the cerebrospinal fluid (CSF). These observations suggest that the nasal route could offer an attractive method for the administration of L-dopa. Unfortunately, L-dopa is not very water soluble. This makes the nasal administration of an effective dose (i.e., $>$ 10 mg in a volume of 0.1 ml) impractical. However the carboxylic group alkyl esters of L-dopa are very water soluble (12) due to change in the chemical structure from a zwitterion to an amine salt (Fig 1). These esters have been shown to exert a pharmacological response similar to that of L-dopa (13).

On the basis of the above considerations, several alkyl esters of L-dopa were synthesized in order to evaluate their potential as prodrug candidates for nasal delivery of L-dopa.

MATERIALS AND METHODS

Materials

L-3,4-Dihydroxyphenylalanine (L-dopa) and 1-heptanesulfonic acid sodium salt were purchased from the Sigma Chemical Company (St. Louis, MO). 3-Hydroxytyramine hydrochloride (dopamine) and ethylenediaminetetraacetic acid disodium salt dihydrate were purchased from the Aldrich Chemical Company (Milwaukee, WI). Alumina (Woelm neutral, activity grade I, 70–230 mesh) was activated by the method of A. H. Anton *et al.* (22). All other chemicals and solvents were of high purity and were used as received.

Synthesis of L-Dopa Esters

A modification of the procedure reported by Patel and Price (23) was used to prepare the prodrug esters of L-dopa.

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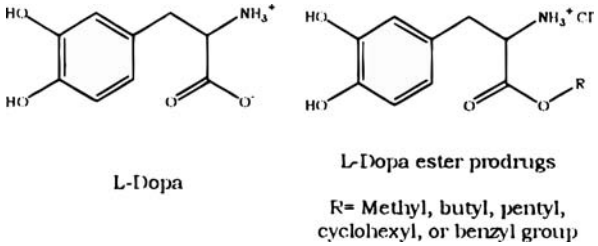


Fig. 1. Chemical structures of L-dopa and its ester prodrugs.

The method was modified by adding thionyl chloride to the appropriate alcohol before, rather than after, adding L-dopa. The structure and purity of each ester hydrochloride of L-dopa was confirmed by NMR spectra, HPLC, melting point, and elemental analysis.

HPLC Analysis

Chromatographic analyses for determining physicochemical properties (i.e., chemical stability, partition coefficient, etc.) were carried out on a system consisting of Applied Biosystems Solvent Delivery System 400, Spectroflow 757 Absorbance Detector, Spectra-Physics DataJet Integrator, Waters 712 WISP Autoinjector, Waters Nova-Pak C_8 column (3.9 mm × 150 mm). The mobile phase consisted of 0.05M phosphate buffer at pH 4.0 and acetonitrile. The acetonitrile portion was adjusted according to the ester. For L-dopa and its methyl ester, the portion of acetonitrile was 0. For other esters, the portion of acetonitrile was 25%. The flow rate was set at 1.0 ml/min. The UV wavelength was set at 280 nm. The limits of quantification was 10 μg/ml.

The HPLC system for the *in vitro* enzymatic studies also included: Applied Biosystems Fluorescence Detector 980, Whatman Partisil 5 SCX column (4.6 mm × 100 mm), Whatman CO:PEL ODS guard column (2 mm × 70 mm). The mobile phase consisted of 0.05M phosphate buffer at pH 2.6, and acetonitrile containing 20 mg/l ethylenediaminetetraacetic acid disodium salt dihydrate. The acetonitrile portion was adjusted as described above. The excitation wavelength was set at 282 nm and the emission wavelength was set at 310 nm. The limit of quantification was 0.05 μg/ml.

For the *in vivo* studies, L-dopa and dopamine were measured in plasma, brain, and CSF by a previously reported HPLC procedure using an electrochemical detector (24), with a slight modification. TOSOH TSK-GEL ODS-80Tm column (4.6 mm × 150 mm) and Whatman CO:PEL ODS guard column (2 mm × 70 mm) were used with BAS Amperometric Detector LC-4B (operated at +0.8 V vs. an Ag/AgCl reference electrode). The mobile phase consisted of 0.05M phosphate buffer at pH 2.9, heptane sulfonate sodium salt 500 mg/l, and ethylenediaminetetraacetic acid disodium salt dihydrate 15 mg/l. The flow rate was set at 1.5 ml/min. The limit of quantification was 0.01 μg/ml. The retention times were 13 minutes for L-dopa, 17 minutes for dihydroxyphenylanine (internal standard) and 29 minutes for dopamine.

Stability of the Ester Prodrugs in Aqueous Buffers

The reactions were initiated by preparing 0.2 mg/ml solutions of the appropriate ester prodrug in phosphate buffers at the desired pH and concentration. The solutions were kept in screw-capped culture tubes at 20°C or 37°C. At appropriate time intervals, samples were taken and kept on ice until analysis.

In Vitro Enzymatic Hydrolysis Studies

Rat brain homogenate was obtained by homogenizing one part of whole rat brain tissue with 5 parts of saline using a tissue grinder. Nasal perfusate was obtained from the rat nasal cavity by circulating 3 ml of saline into one nostril using polystaltic pump and collecting the solution from the other nostril (25). Circulating time was 3 minutes. The hydrolysis study was performed immediately following perfusion. The studies were conducted by adding five 200 μl aliquot parts of rat plasma, brain homogenate, or nasal perfusate to five 100 μl of 0.05M phosphate buffer solution pH 6.0 containing 1 mg/ml of the desired ester and the samples incubated at 37°C. The reactions were quenched at various times by adding 200 μl of acetonitrile. The samples were centrifuged for 2 minutes. The supernatant was filtered through a 0.45 μm filter and injected directly onto the HPLC.

For the rat CSF hydrolysis study five 50 μl aliquot parts of rat CSF were added to five 50 μl of a 0.05M phosphate buffer solution pH 6.0 containing 1 mg/ml of the butyl ester and the samples were incubated at 37°C. Samples were treated as described above.

In Vivo Studies

The nasal absorption studies of L-dopa, L-dopa prodrugs, and dopamine were conducted using an *in vivo* experimental technique described by Hussain *et al.* (15). All animal experiments adhered to the Principles of Laboratory Animal Care (NIH publication No. 85-23 revised 1985) and were approved by University of Kentucky Institutional Animal Care and Use Committee. Male Sprague-Dawley rats weighing 250–275 gm were used. All surgical procedures were performed under anesthesia (intraperitoneal injection of pentobarbital 40 mg/kg). The nasal cavity was isolated from the respiratory and gastrointestinal tracts. Blood samples were collected from a cannula inserted into the femoral artery. For intravenous administration, the jugular vein was cannulated for administering the dose. For nasal studies, the dose (100 μl) was administered into one nostril using a microsyringe. Since the two nostrils are connected, the solution enters both cavities. Although, the nasal cavityin humans is not isolated, human data correlates well with rat nasal data obtained using this procedure (25).

To determine the residual amount of dopamine in the nasal cavity at the end of experiment, the cavity was washed with 2 ml of 0.05M, pH 6.0 phosphate buffer at 120 minutes. Dopamine concentration was determined by HPLC.

CSF samples were obtained at the desired time by making an incision in the skin over the occipital bone and removing the first layer of the muscle. Samples (75 μl) were then obtained by a cisternal puncture with a sharp end needle. Sampling was stopped immediately upon observing any trace of blood and the last 25 μl of each sample were discarded to prevent blood contamination. The animal was then sacrificed and the brain was carefully removed. The olfactory bulb and striatum were carefully separated from the brain.

Table I. Physicochemical Properties of L-dopa, Dopamine, and L-dopa Prodrugs and Half-lives for the *In Vitro* Hydrolysis of L-dopa Ester Prodrugs in 0.05M Phosphate Buffer pH 7.4 (μ = 1.0 with NaCl), Rat Plasma, Rat Brain Homogenate, Cerebrospinal Fluid (CSF) and Rat Nasal Perfusate at 37°C

Compound	MW	MP(°C)	PC[a]	Solubility (mg/ml)[b]	Buffer $t_{1/2}$ (hr)	Plasma $t_{1/2}$ (min)	Brain $t_{1/2}$ (min)	CSF $t_{1/2}$ (min)	Nasal $t_{1/2}$ (min)
L-Dopa	197.19	276–278	0.01	1.65					
Dopamine	189.64	240–241	0.01	250					
Methyl ester	247.68	170–172	0.25	750	6.9	0.82	0.96	—	—
Butyl ester	289.76	134–137	7.17	660	29.4	0.63	0.76	33.0	144.0
Pentyl ester	303.85	143–146	31.56	31	23.9	1.25	1.56	—	—
Cyclohexyl ester	315.79	189–191	25.05	17	54.6	14.10	10.10	—	—
Benzyl ester	323.77	190–192	11.57	5	7.3	0.36	0.96	—	—

[a] Partition coefficient was measured at 20°C, octanol/pH 7.4, 0.05 M phosphate buffer.
[b] Solubility was measured in pH 7.4, 0.05 M phosphate buffer at 20°C.

Purification of biological samples for L-dopa and dopamine analysis was carried out using an alumina adsorption procedure (22) and the samples were then analyzed by HPLC.

RESULTS AND DISCUSSION

Physicochemical Properties

Several alkyl ester prodrugs of L-dopa were synthesized by a modification of the procedure reported by Patel and Price (23). Table I list their physicochemical properties (as hydrochloride salts) in comparison to L-dopa and dopamine. As evident from the data, the prodrugs are significantly more soluble and more lipophilic than L-dopa. This could be attributed to the change in the chemical structure from a zwitterion form to an amine salt. The methyl and the butyl esters are the most soluble but the butyl ester shows better lipophilicity.

Chemical Stability

The hydrolysis of all the esters in phosphate buffer at 37°C and pH 7.4 followed first-order kinetics for at least four half-lives. The half-lives for hydrolysis of all the esters under the same condition are listed in Table I. It is apparent that the butyl and cyclohexyl esters are the most stable prodrugs while the methyl and benzyl esters are the least stable.

Due to its favorable properties (solubility, lipophilicity, and solution stability), the butyl ester was chosen for further stability testing. The effect of buffer concentration and pH on the chemical stability of the butyl ester of L-dopa were investigated. A plot of the observed rate constants for the hydrolysis of the butyl ester of L-dopa against phosphate buffer concentrations (Fig. 2) at different pHs indicated that the hydrolysis is subject to specific as well as general acid- base catalysis. Specific acid- base catalysis is indicated by the different intercepts, while general acid- base catalysis is indicated by the different slopes.

The overall equation for the rate of decomposition of L-dopa esters in phosphate buffer can be written as follows:

$$\frac{-\,d[\text{Ester}]/dt}{[\text{Ester}]} = k_{obs} \qquad (1)$$

where:

$$k_{obs} = k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{H_2O} + k_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^{-2}}[HPO_4^{-2}] \qquad (2)$$

The k values represent the specific rate constants associated with the various catalytic species. Rearranging Eq. 2 and expressing the concentration of the buffer species in term of

Table II. Relationship of the Partition Coefficients of the Prodrug and L-dopa Levels in the Plasma, CSF, and Olfactory Bulb After 60 min of Nasal Administration

		L-Dopa concentration (μg/ml)		
Ester	P.C.[a]	Plasma	CSF	Olfactory bulb
Methyl	0.252	2.360 ± 0.200	3.250 ± 0.560	87.200 ± 20.570
Butyl	7.166	4.273 ± 2.310	13.920 ± 3.655	250.200 ± 182.100

[a] Partition coefficient was measured at 20°C, octanol/pH 7.4, 0.05 M phosphate buffer.

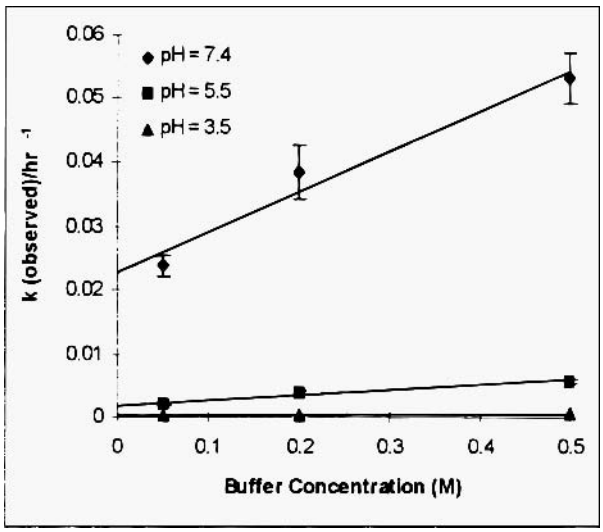


Fig. 2. Effect of buffer concentrations on the degradation rate constants of L-dopa butyl ester at 37°C at different pHs.

total buffer concentration and K_a the dissociation constant for $H_2PO_4^-$ Eq. 2 can be written as:

$$k_{obs} = k_0 + \left[k_{H_2PO_4^-} \frac{H^+}{H^+ + K_a} + k_{HPO_4^{-2}} \frac{K_a}{H^+ + K_a} \right] \times [\text{Buffer}]_T \quad (3)$$

where

$$k_0 = k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{H_2O} \quad (4)$$

k_0 represents the overall hydrolytic rate constant in the absence of buffer. According to Eq. 3, a plot of k_{obs} versus total buffer concentration $[\text{Buffer}]_T$ results in a straight line with:

$$\text{Slope} = k_{H_2PO_4^-} \frac{H^+}{H^+ + K_a} + k_{HPO_4^{-2}} \frac{K_a}{H^+ + K_a} \quad (5)$$

$$\text{Intercept} = k_0 = k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{H_2O} \quad (6)$$

From the linear regression equations of the three plots in Fig. 2 we can calculate the specific rate constants associated with each species. The values for the specific rate constants were:

$$k_{H^+} = 0.63 \text{ hr}^{-1} \text{ M}^{-1},$$

$$k_{OH^-} = 9.26 * 10^4 \text{ hr}^{-1\,\text{M}^{-1}}, \; k_{H_2O} \approx 0 \text{ hr}^{-1},$$

$$k_{H_2PO_4^-} = 7.50 * 10^{-4} \text{ hr}^{-1} \text{ M}^{-1},$$

$$k_{HPO_4^{-2}} = 0.10 \text{ hr}^{-1} \text{ M}^{-1}.$$

The data would suggest that the rate of degradation of the L-dopa butyl ester in the neutral pH range is determined by the concentration of the hydroxide ion and the buffer species. Based on the magnitude of the rate constants, it is evident that the stability of the butyl ester is influenced by the pH of the solution. At slightly acidic pHs (pH 3–5), the ester would have sufficient shelf-life stability to be formulated in a solution dosage form. The pH-rate profile calculated at zero buffer concentration using the specific second-order rate constants showed that the pH of maximum stability for the L-dopa butyl ester is 4.4. The stability data at 20°C and 37°C were used to calculate the apparent activation energy for the hydrolysis of the butyl ester. It was found to be 19.03 kcal/mole at pH 5.5 and 20.24 kcal/mole at pH 7.4. Using the above information, the estimated time for 10% decomposition (t_{90}) at pH 4.4, 0.05M phosphate buffer at 10°C was calculated to be 2.7 years. This is based on the assumption that the activation energy is independent of pH.

Enzymatic Stability

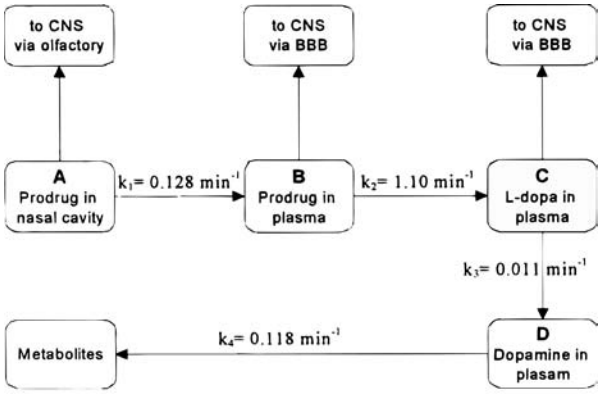
In order to verify the enzymatic conversion of these prodrugs to L-dopa in rat biological fluids, *in vitro* rates of hydrolysis were determined in plasma and brain homogenate. The rates of hydrolysis of the butyl ester in rat CSF and nasal perfusate were also determined. The hydrolysis of the prodrugs in rat biological fluids followed first-order kinetics. The rates of generation of L-dopa were very rapid in rat plasma and brain homogenate (Table I). However, the rate of generation of L-dopa from the butyl ester in rat CSF was much slower ($t_{1/2}$ = 34 min). The butyl ester was relatively stable in rat nasal perfusate with a half-life greater than 2 hours. Since nasal absorption is very rapid (18,19), a negligible amount of the prodrug is expected to hydrolyze in nasal cavity before it is absorbed into the systemic circulation.

In Vivo Studies with L-Dopa and Its Prodrugs

Since the L-dopa esters are converted to L-dopa very rapidly in rat plasma, analysis of L-dopa in the plasma following the nasal administration of the prodrugs should accurately reflect the absorption profiles of these esters. Figure 3A shows the plasma profiles following the nasal and intravenous administrations of the butyl ester at 20 mg/kg L-dopa equivalent dose. The bioavailability following nasal administration was obtained by comparing the areas under the curves after intravenous and nasal administrations and was found to be about 89.3%. Such complete and rapid absorption was also observed for L-dopa and its esters at 4 mg/kg L-dopa equivalent dose (Fig. 3B). The areas under the curve for 4 and 20 mg/kg L-dopa equivalent doses of the butyl ester (Fig. 3A) were calculated and were found to be 92.283, and 521.550 ($\mu g\ ml^{-1}\ min^{-1}$) respectively. The AUCs were proportional to the administered doses. The rate of absorption of the butyl ester from the nasal cavity and the rate of elimination of L-dopa from the plasma were calculated using the method of residuals and were found to be 0.128 min^{-1} and 0.011 min^{-1}, respectively

In Vivo Studies with Dopamine

In order to obtain a clear picture of the pharmacokinetic profile of the butyl ester following its nasal administration to rats, the absorption and elimination of dopamine in the same animal model were examined. Dopamine was found to be rapidly eliminated following intravenous administration as shown in Fig. 3C. The elimination rate constant was found to be 10 times faster than that of L-dopa and was estimated to be 0.118 min^{-1}. The nasal absorption of dopamine was found to be relatively slow and incomplete (Fig. 3C). At doses of 20 mg/kg, the absorption phase was long and at the end of the experiment, about 68% of the administered dose was recovered from the nasal cavity. It may be possible that dopamine retards its own absorption due to its vasoconstrictive effect. Previous studies (26) with phenylephrine (a vasoconstricting agent) showed that the compound inhibited the absorption of aspirin from the nasal cavity.



Scheme 1

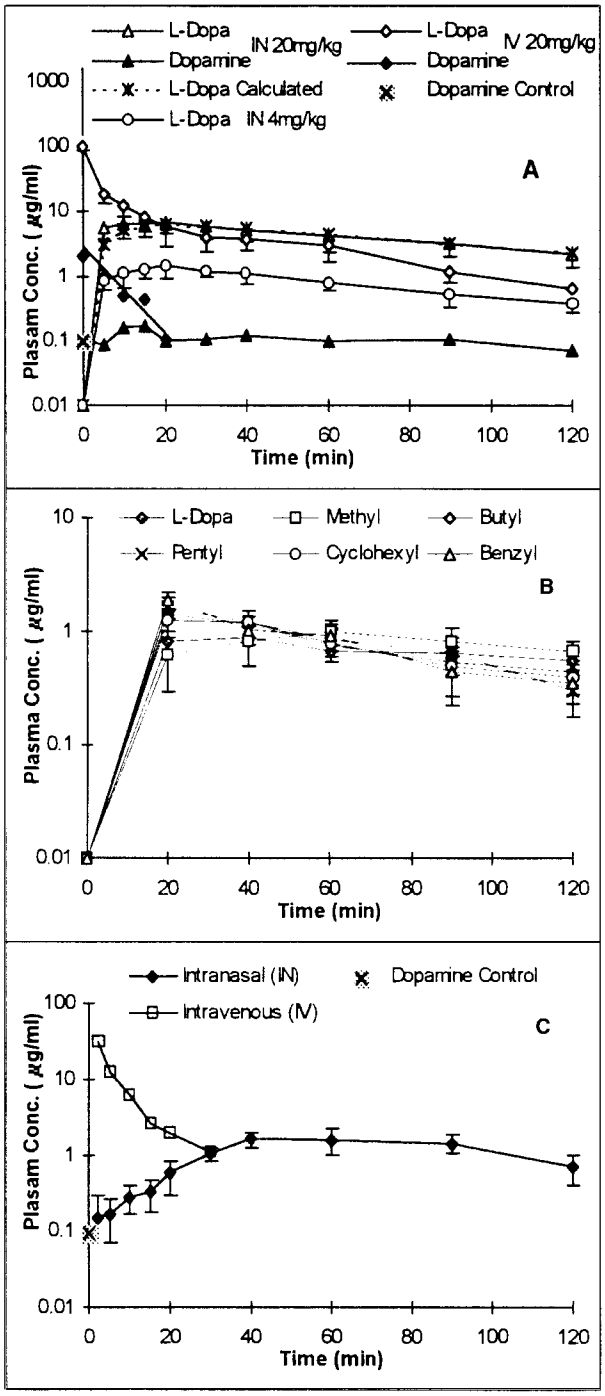


Fig. 3. A) Plasma dopamine and L-dopa levels following nasal and intravenous administrations of the L-dopa butyl ester at 20 mg/kg L-dopa equivalent dose and calculated plasma L-dopa levels for the nasal dose using Scheme I. The graph also shows plasma L-dopa levels following nasal administrations of the L-dopa butyl ester at 4 mg/kg L-dopa equivalent dose. B) plasma L-dopa levels following nasal administrations of L-dopa and L-dopa esters at 4 mg/kg L-dopa equivalent dose, C) plasma dopamine levels following nasal and intravenous administrations of dopamine at 20 mg/kg. Dopamine control represents the endogenous dopamine levels in normal rat blood.

Plasma Levels of Dopamine Following the Nasal Administration of L-Dopa Butyl Ester

Although L-dopa plasma levels were high following the nasal administration of the butyl ester, dopamine plasma levels were very low (Fig. 3A). This could be explained by the ten times faster rate of elimination of dopamine compared to L-dopa rate of metabolism. These rate constants are 0.011 min^{-1} and 0.118 min^{-1} for L-dopa and dopamine respectively. Using the kinetic model shown in Scheme I, ignoring absorption to the central nervous system, knowing the initial dose of the butyl ester, and assuming the same volume of distribution for B, C, and D, we could solve for the concentration of each component in the plasma according to the following equations:

$$\frac{dA}{dt} = -k_1A \tag{7}$$

$$\frac{dB}{dt} = k_1A - k_2 \tag{8}$$

$$\frac{dC}{dt} = k_2B - k_3 \tag{9}$$

$$\frac{dD}{dt} = k_3C - k_4 \tag{10}$$

solving for A, B, C, and D gives:

$$A = A_0e^{-k_1t} \tag{11}$$

$$B = \frac{-k_1A_0}{k_1 - k_2}\left[e^{(-k_1t)} - e^{(-k_2t)}\right] \tag{12}$$

$$C = \frac{k_1k_2A_0}{(k_1 - k_2)(k_1 - k_3)(k_2 - k_3)} \times \left[(k_2 - k_3)e^{(-k_1t)} - (k_1 - k_3)e^{(-k_2t)} + (k_1 - k_2)e^{(-k_3t)}\right] \tag{13}$$

$$D = \frac{k_1k_2k_3A_0}{(k_1 - k_2)(k_1 - k_3)(k_1 - k_4)(k_2 - k_3)(k_2 - k_4)(k_3 - k_4)} \times \left[-(k_2 - k_3)(k_2 - k_4)(k_3 - k_4)e^{(-k_1t)} + (k_1 - k_3)(k_1 - k_4)(k_3 - k_4)e^{(-k_2t)} - (k_1 - k_2)(k_1 - k_4)(k_2 - k_4)e^{(-k_3t)} + (k_1 - k_2)(k_2 - k_3)(k_1 - k_3)e^{(-k_4t)}\right] \tag{14}$$

A computer program in BASIC was written to solve for A, B, C, and D as a function of time using the initial dose of the butyl ester as A_0 and the values of the rate constant on Scheme I. The results are shown in Table III and Fig. 3a. It would appear from the data that the nasal administration of the butyl ester of L-dopa does not contribute significantly to dopamine plasma levels. This is due to the rapid rate of elimination of dopamine and relatively slow rate of metabolism of L-dopa in plasma. Thus by avoiding GI wall and liver metabolism we dramatically decreased the plasma concentration of dopamine. Since the peripheral side effects of oral L-dopa have been attributed to dopamine (3), administration of the L-dopa butyl ester prodrug nasally may minimize these side effects.

CSF and Olfactory Bulb L-Dopa Levels Following Nasal and Intravenous Administrations of the Prodrug

The cerebrospinal fluid and the olfactory bulb concentrations of L-dopa following the intravenous and nasal administration of the butyl ester at 20 mg/kg L-dopa equivalent dose are

Table III. Experimental and Calculated Plasma Levels (μg/ml) of L-dopa and Dopamine in the Rat Following the Administration of 20 mg/kg L-dopa Equivalent Dose of the Butyl Ester Prodrug

Time (min)	L-dopa (experiment)	L-dopa (calculated)	dopamine (experiment)	dopamine (calculated)
control	0.000	0.000	0.096	0.000
5	5.477	3.128	0.089	7.41×10^{-8}
10	6.314	5.135	0.155	2.39×10^{-7}
15	6.441	6.013	0.170	3.90×10^{-7}
20	6.763	6.301	0.097	4.98×10^{-7}
40	5.436	5.623	0.117	5.94×10^{-7}
60	4.273	4.564	0.100	5.08×10^{-7}
90	3.268	3.295	0.109	3.70×10^{-7}
120	2.234	2.376	0.070	2.69×10^{-7}

shown in Fig. 4. It is evident that the CSF and the olfactory bulb have higher concentrations of L-dopa following nasal administration than following intravenous administration. This suggests that the butyl ester can reach the CSF or olfactory bulb via a direct pathway. There are evidences in the literature that show that there is a communication among the nasal mucousa, the olfactory bulb, and the CSF. Johnson (27) showed that the spread of polio virus and the herpes simplex virus to the nervous system from the nasal cavity was via the olfactory pathway. Olfactory cilia are known to pick up substances from the surface by pinocytosis and transfer them into the brain. Czerniawska (28) showed that radioactive colloidal gold isotope ^{198}Au penetrates directly from the mucous membrane of the nasal olfactory region into the cerebrospinal fluid of the anterior cranial fossa. Preferential absorption of nasally delivered drugs into the CSF or into the olfactory bulb has been demonstrated for progesterone (20), antihistamines (21), cephalexin (18), sulfa drugs (19), and ^{125}I-labeled nerve growth factor (29). However, the olfactory region occupies a larger percentage of the rat nasal cavity compared to human. This significant anatomical difference between the rat and the human nasal cavity necessitate a cautious interpretation of this preferential delivery to the CSF.

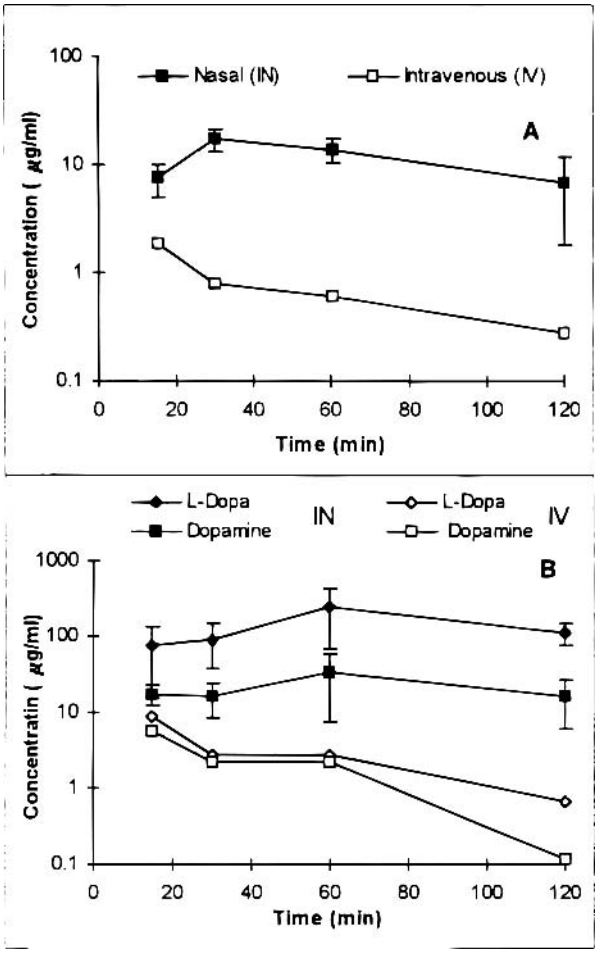


Fig. 4. Dopamine and/or L-dopa levels in: A) cerebrospinal fluid, B) olfactory bulb following nasal and intravenous administrations of L-dopa butyl ester at 20 mg/kg L-dopa equivalent dose.

It has been shown previously (19) that the concentration in the CSF of a series of sulfa drugs administered nasally is related to the lipophilicity of the compounds. To examine the above observation, two prodrugs of L-dopa (butyl and methyl esters) with different partition coefficients were given nasally at an equivalent dose. The levels of L-dopa in plasma, CSF, and the olfactory bulb after 60 minutes of administration were determined. The data in Table III shows that the more lipophilic drug (the butyl ester) resulted in higher L-dopa levels in both the CSF and the olfactory bulb than the methyl ester. It is noteworthy that for both esters, the olfactory bulb L-dopa levels are higher than the CSF L-dopa levels. This could support the idea that the pathway from the nasal cavity to the CSF is via the olfactory bulb.

CONCLUSIONS

The nasal administration of alkyl ester prodrugs of L-dopa resulted in rapid and complete absorption into the systemic circulation. Conversion of the prodrugs to L-dopa in the plasma was very fast as evident by *in vitro* and *in vivo* data. Furthermore, the nasal administration of the butyl ester prodrug did not result in significant formation of dopamine in the peripheral circulation. Since the peripheral side effects of oral L-dopa have been attributed to dopamine, administration of the L-dopa butyl ester prodrugs nasally may minimize these side effects. Finally, the nasal administration of the butyl ester prodrug of L-dopa resulted in an improved CNS bioavailability compared to that achieved from an equivalent intravenous dose. This is very important since the CNS is the intended site of action of L-dopa therapy. Considering all of the above, it would appear that the utilization of water soluble prodrugs of L-dopa via the nasal route may have therapeutic advantages in the treatment of Parkinson's disease.

REFERENCES

1. D. Standaert and A. Young. Treatment of Central Nervous System Degenerative Disorders. In A. G. Gilman, J. G. Hardman, L. E. Limbird, P. B. Molinoff, and R. W. Ruddon (eds.), *The Pharmacological Basis of Therapeutics*, McGraw Hill, New York, 1996 pp. 503–519.
2. K. Sasahara, T. Nitanai, T. Habara, T. Morioka, and E. Nakajima. Dosage form design for improvement of bioavailability of levodopa V: Absorption and metabolism of levodopa in intestinal segments of dogs. *J. Pharm. Sci.* **70**:1157–1160 (1981).

3. K. Sasahara, T. Nitanai, T. Habara, T. Morioka, and E. Nakajimai Dosage form design for improvement of bioavailability of levodopa III: Influence of dose on pharmacokinetic behavior of levodopa in dogs and parkinsonian patients. *J. Pharm. Sci.* **69**:1374–1378 (1980).
4. J. G. Nutt, W. R. Woodward, and J. L. Anderson. The Effect of carbidopa on the pharmacokinetics of intravenously administered levodopa: The mechanism of action in the treatment of parkinsonism. *Annal. Neurol.* **18**:537–543 (1986).
5. L. B. Wingard, T. M. Brody, J. Larner, and A. Schwartz. *Human Pharmacology*; Mosby Year Book, St Louis, 1991.
6. J. M. Cedarbaum. Anti-parkinsonian drugs. *Clin. Pharmacokin.* **13**:141–178 (1987).
7. J. G. Nutt and W. R. Woodward. Levodopa pharmacokinetics and pharmacodynamics in fluctuating parkinsonian patients. *Neurology* **36**:739–744 (1986).
8. R. Hardie, A. J. Lees, and G. M. Stern. On-off fluctuations in parkinson's disease. *Brain* **107**:487–506 (1984).
9. N. Quinn, J. D. Parkes, and C. D. Marsden. Control of on/off phenomenon by continuous intravenous infusion of levodopa. *Neurology* **34**:1131–1136 (1984).
10. K. Sasahara, T. Nitanai, T. Habara, T. Morioka, Y. Kawahara, and E. Nakajima. Dosage form design for improvement of bioavailability of levodopa IV: Possible causes of low bioavailability of oral levodopa in dogs. *J. Pharm. Sci.* **70**:730–733 (1981).
11. J. H. Hutton, J. L. Morris, and C. R. Gustavo. Treatment of chronic parkinson's disease with controlled-release carbidopa/levodopa. *Neurology* **45**:861–864 (1988).
12. N. Bordor, K. B. Sloan, T. Higuchi, and K. Sasahara. Improved delivery through biological membranes 4: Prodrugs of L-dopa. *J. Med. Chem.* **20**:1435–1445 (1977).
13. D. R. Cooper, C. Marrel, H. van de Waterbeemd, B. Testa, P. Jenner, and C. D. Marsden. L-Dopa esters as potential prodrugs: Behavioural activity in experimental models of parkinson's disease. *J. Pharm. Pharmacol.* **39**:627–635 (1987).
14. J. A. Fix, J. Alexander, M. Cortese, K. Engle, P. Leppert, and A. J. Repta. Short-chain alkyl esters of L-dopa as prodrugs for rectal absorption. *Pharm. Res.* **6**:501–505 (1989).
15. A. Hussain, S. Hirai, and R. Bawarshi. Nasal absorption of propranolol from different dosage forms by rats and dogs. *J. Pharm. Sci.* **69**:1411–1413 (1980).
16. C. H. Huang, R. Kimura, R. B. Nassar, and A. Hussain. Mechanism of nasal absorption of drugs II: Absorption of L-tyrosine and the effect of structural modification on its absorption. *J. Pharm. Sci.* **74**:1298–1301 (1985).
17. J. A. Faraj, A. A. Hussain, Y. Aramaki, K. Iseki, M. Kagoshima, and L. W. Dittert. Mechanism of nasal absorption of drugs. IV: Plasma levels of radioactivity following intranasal administration of ^{3}H-leucine enkephalin. *J. Pharm. Sci.* **79**:768–770 (1990).
18. T. Sakane, M. Akizuki, M. Yoshida, S. Yamashita, T. Nadai, M. Hashida, and H. Sezaki. Transport of cephalexin to the cerebrospinal fluid directly from the nasal cavity. *J. Pharm. Pharmacol.* **43**:449–451 (1991).
19. T. Sakane, M. Akizuki, S. Yamashita, T. Nadai, M. Hashida, and H. Sezaki. The transport of drugs to the cerebrospinal fluid directly from the nasal cavity: The relation to the lipophilicity of the drug. *Chem. Pharm. Bull.* **39**:2456–2458 (1991).
20. T. A. Kumar, G. F. X. David, A. Sankaranarayanan, V. Puri, and K. R. Sundram. Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion, or nasal spraying. *Proc. Natl. Acad. Sci.* **79**:4185–4189 (1982).
21. K. J. Chou and M. D. Donovan. Distribution of antihistamines into the CSF following intranasal delivery. *Biopharm. Drug Dispos.* **18**:335–346 (1997).
22. A. H. Anton and D. F. Sayre. A Study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the Analysis of catecholamines. *J. Pharmacol. Exp. Ther.* **138**:360–375 (1962).
23. R. P. Patel and S. Price. Synthesis of benzyl esters of α-amino acids. *J. Org. Chem.* **30**:3573–3577 (1965).
24. R. C. Causon, M. J. Brown, K. Leenders, and L. Wolfson. HPLC with amperiometric detection of plasma L-3,4-dihydroxyphenylalanine in parkinsonian patients. *J. Chromatography* **277**:115–123 (1983).
25. A. Hussain. Intranasal Drug Delivery. *Adv. Drug Deliv. Rev.* **29**:39–49 (1998).
26. C. H. Huang, R. Kimura, R. B. Nassar, and A. Hussain. Mechanism of nasal absorption of drugs. I: Physicochemical parameters influencing the rate of *in situ* nasal absorption of drugs in rats. *J. Pharm. Sci.* **74**:608–611 (1985).
27. R. T. Johnson and C. A. Mims. Pathogenesis of viral infectin of the nervous system. *New Eng. J. Med.* **278**:23–30 (1968).
28. A. Czerniawska. Experimental investigations on the penetration of ^{198}Au from nasal mucosa membrane into cerebrospinal fluid. *Acta Oto-Laryngol.* **70**:58–61 (1970).
29. J. Liu, W. H. Frey, and Y. E. Rahman. New approach to alzheimer therapy: Delivery of nerve growth factor (NGF) to the brain through the olfactory pathway and assessment of NGF integrity. *Pharm. Res.* **11**:S208 (1994).