Bioavailability and Pharmacokinetics of an Oral Dopamine Prodrug in Dogs

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Abstract □ The bioavailability and pharmacokinetics of an oral dopamine prodrug, *N*-(*N*-acetyl-L-methionyl)*O,O*-bis(ethoxycarbonyl)dopamine (**1**), were examined in dogs, and the mechanism of its absorption and bioactivation was discussed. Compound **1** showed a plasma dopamine concentration that was several times higher than that of dopamine (DA) following oral administration to dogs, while the plasma concentrations of dopamine-30-sulfate (DA-SO_4) and 3,4-dihydroxyphenylacetic acid (DOPAC) are lower in comparison with that of DA. The conversion of **1** to DA occurred in proportion to the dose administered. Compound **1** also showed a plasma DA concentration that was several times higher than that of other DA prodrugs reported hitherto. In dog plasma, in vitro, **1** was converted to its deethoxycarbonylated form, *N*-(*N*-acetyl-L-methionyl)dopamine (**2**), while other related compounds, *N*-(L-methionyl)dopamine (**3**), *N*-(L-methionyl)*O,O*-bis(ethoxycarbonyl)-dopamine (**4**), and *O,O*-bis(ethoxycarbonyl)dopamine (**5**), were rapidly converted to DA (however, **2** was stable in plasma). Bioavailability, based on the AUC of DA, **1**, **2**, and **5** following oral administration to dogs, increased in the following order: **1**, **2**, **5**, and DA. Thus, it was shown that the two protective groups introduced in **1** served to reduce the first-pass metabolism of the DA moiety in the absorption process. It was also confirmed that **1** is converted to **2** or DA in blood, liver, and intestine.

In a previous paper,[1] the bioavailability and pharmacokinetics of orally administered dopamine (DA) were studied, and remarkable first-pass metabolism in the intestine and liver were evidenced, with absolute bioavailability of ~3% in the dog. Several prodrugs of DA have appeared in the literature,[2,3] however, the improvement in their bioavailability was not sufficient for their potential use as oral substitutes to intravenous DA.

In an attempt to minimize the extensive first-pass metabolism of DA, a dopamine prodrug, *N*-(*N*-acetyl-L-methionyl)-*O,O*-bis(ethoxycarbonyl)dopamine (**1**), was synthesized in our laboratory;[4] the two main sites of metabolic inactivation in the DA molecule (amino group, catechol system) were both protected in **1**. The aim of the present study was to evaluate the bioavailability of the new compound and to describe the pharmacokinetics of its absorption and bioactivation in the dog.

Experimental Section

Materials—*Animals*—Beagle dogs were purchased from Yoshiki yakko Company and maintained on dog chow (Oriental Yeast). The dogs were fasted for 18 h prior to and 8 h after administration of drugs.

Chemicals—Dopamine hydrochloride (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and dopamine-30-sulfate (DA-SO_4) were used as described in the previous paper.[1] The dopamine prodrug *N*-(*N*-acetyl-L-methionyl)-*O,O*-bis(ethoxycarbonyl)dopamine (**1**) and its related compounds (**2**, **3**, **4**, **5**) were synthesized in our company (see Table I). L-DOPA was purchased from Nakarai Chemicals. Other chemicals were special grade reagents.

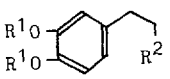
Animal Experiments—*Oral Administration*—One capsule containing 121 mg of DA or an equimolar amount of **1** was administered orally by compulsive swallow with 30 mL of water. In another experiment, a 10% lecithin solution of **1** and its related compounds (100 mg of DA equivalent) or a suspension of L-DOPA (100 mg of DA equiv) were administered, respectively. In all cases, blood samples were withdrawn at 0, 0.5, 1, 2, 3, 4, 5, and 7 h after oral administration.

Mesenteric Venous or Intraduodenal Administration—A 10% lecithin solution of **1** (10 or 100 mg of DA equiv) was administered into the mesenteric vein or the ligated upper intraduodenal loop (20 cm) of pentobarbital anesthetized dogs. Blood samples were withdrawn at 0, 2.5, 5, 10, 15, and 30 min for the former case and at 0, 10, 20, 30, 45, and 60 min for the latter case.

Determination of Plasma Concentrations—Plasma concentrations of DA, DA-SO_4 and DOPAC were determined by high-performance liquid chromatography (Shimadzu LC-3A), using an electrochemical detector (Yanaco Voltammetry Detector, VMD-501A), according to the previous paper.[1] The plasma concentration of **2** was determined according to the same assay method as for DOPAC. As described in a later section, **1** is converted to **2** in plasma by incubation for 30 min at 37 °C. Thus, the concentration of **1** in plasma was determined as the plasma concentration of **2** after the conversion of **1** to **2**. That is, the plasma concentration of **1** was determined by subtracting the plasma concentration of **2** from the total plasma concentration of **2** due to **1** and **2** in the plasma. Little **1** is detected in plasma even after intravenous administration of **1** (20 mg of DA equiv) to dogs.

Stability of 1 and Its Related Compounds in Dog Plasma or Blood In Vitro—Compound **1** or one of its related compounds was added to plasma or blood at the final concentration of 100 ng of DA equiv/mL, and the plasma or blood was incubated for 10–60 min at 37 °C. Then, the amount of DA or **2** in plasma was determined.

Table I—Dopamine Prodrug and Related Compounds



Compound	R^1	R^2
DA	H	NH_2
1[a]	C_2H_5OCO	$NHCOCHCH_2CH_2SCH_3$ $NHCOCH_3$
2[b]	H	"
3[c]	H	$NHCOCHCH_2CH_2SCH_3$ NH_2
4[d]	C_2H_5OCO	"
5[e]	C_2H_5OCO	NH_2

[a] *N*-(*N*-acetyl-L-methionyl)-*O,O*-bis(ethoxycarbonyl)dopamine. [b] *N*-(*N*-acetyl-L-methionyl)dopamine. [c] *N*-(L-methionyl)dopamine. [d] *N*-(L-methionyl)-*O,O*-bis(ethoxycarbonyl)dopamine. [e] *O,O*-bis(ethoxycarbonyl)dopamine.

Results and Discussion

Bioavailability of 1 in Dogs—Figure 1 shows the concentrations of DA and its metabolites in plasma after the oral administration of **1** and DA to dogs. Administration of **1**

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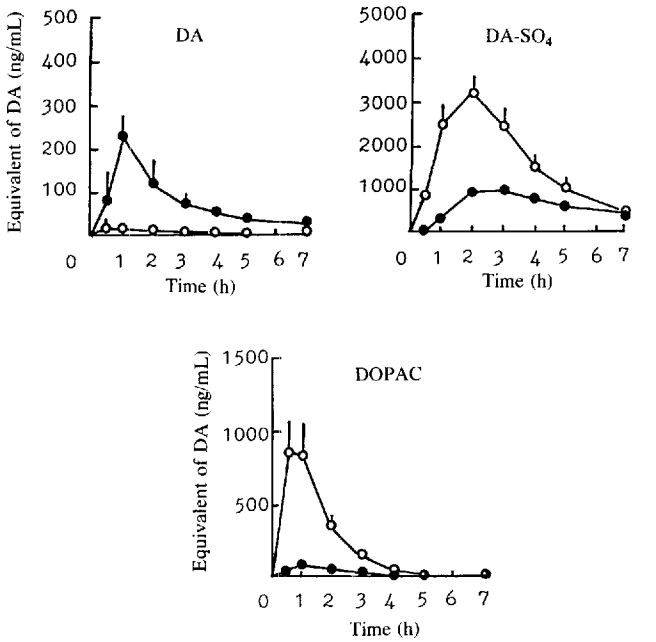

Figure 1—Plasma concentration of DA, DA-SO_4, and DOPAC in dogs after oral administration of DA or **1** at the dose of 121 mg of DA equiv. Key: (—○—) DA administration; (—●—) **1** administration. Each point represents the mean ± SE of five dogs. The points without vertical bars have smaller SE than the symbols.

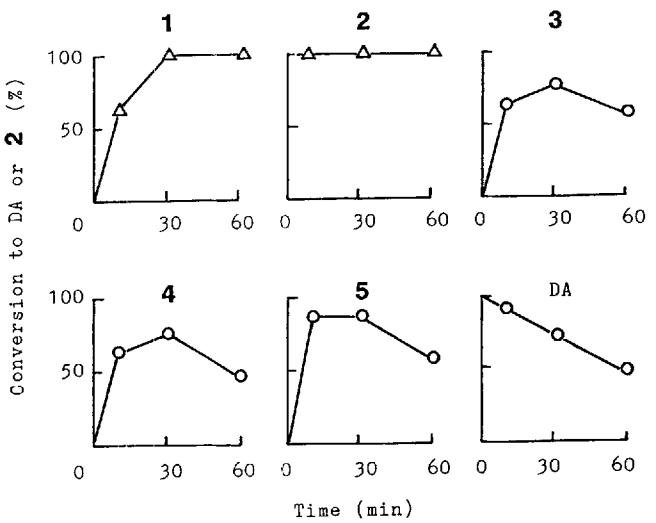

Figure 2—Stability of **1** and its related compounds (100 ng of DA equiv/mL, 37 °C) in dog plasma in vitro. Key: (—○—) DA; (—△—) **2**.

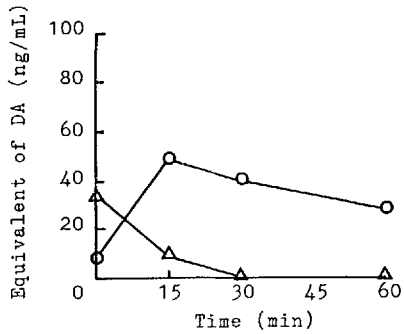

Figure 3—Conversion of **1** to **2** and DA in dog blood in vitro (100 ng of DA equiv/mL, 37 °C). Key: (—○—) DA; (—△—) **2**.

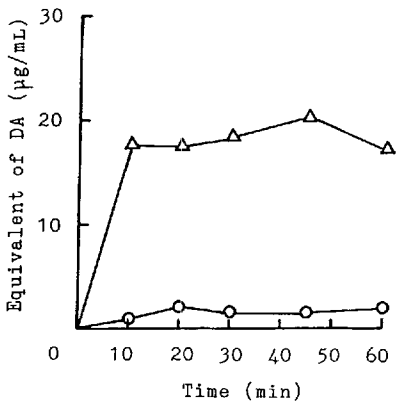

Figure 4—Conversion of intraduodenally administered **1** to **2** and DA in intestine in situ (dose = 100 mg of DA equiv). Key: (—○—) DA; (—△—) **2**. Each point represents the mean of two dogs.

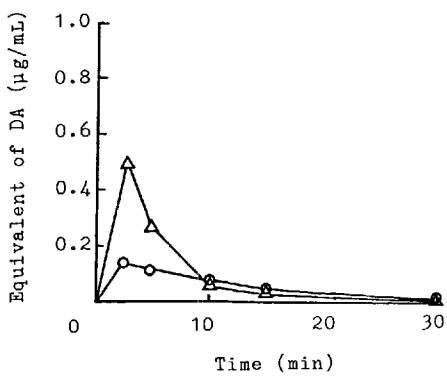

Figure 5—Conversion of mesenteric venously administered **1** to **2** and DA in liver in situ (dose 10 mg of DA equiv). Key: (—○—) DA; (—△—) **2**. Each point represents the mean of two dogs.

resulted in a several times higher plasma concentration of DA than that following DA administration, while plasma concentrations of DA-SO_4 and DOPAC were lower in comparison with that of DA. By comparing the area under the plasma DA concentration time curves for **1** with the area under the plasma concentration time curve for DA given intravenously,[1] the absolute bioavailability of **1** based on the AUC of DA was calculated to be ~30%, a value that is ~10 times the absolute availability (~3%)[1] of orally administered DA.

Compound **1** was converted to DA in proportion to the dose of administration within the dose range 150–600 mg of **1** (n = 3–5). From these results, it was deduced that two protective groups served to decrease the first-pass metabolism of DA and improve the bioavailability.

The bioavailability of **1** was also compared with both those of the DA prodrug, *O,O'*-bis-acetyl-*N*-(L-γ-glutamyl)dopamine (**6**) reported by Kyncl et al[3] or L-DOPA. The relative values of the AUC based on DA after oral administration of each compound compared with the AUC of DA after administration of DA were 467.8 ± 129.0, 97.6 ± 37.3, and 47.3 ± 28.5% (mean ± SE, n = 4) for **1**, **6**, and L-DOPA, respectively. Compound **1** gave a higher plasma DA concentration than these two compounds. It is considered that the DA prodrugs, having a free amino group, may be converted to DA in the absorption process too rapidly to escape first-pass metabolism.

Significance of the Two Protective Groups of 1—In order to make clear the significance of two protective groups of **1**, the bioavailabilities of **1** and its related compounds were compared. Before this experiment, the stability of these

compounds (**1**, **2**, **3**, **4**, **5**) in dog plasma was examined in vitro. As shown in Figure 2, **3**, **4**, and **5** were rapidly converted to DA, and **1** was converted to **2**. On the other hand, **2** was stable in the plasma and was not converted to DA under the conditions used in this study. Dopamine (DA) was slightly unstable in plasma at 37 °C.

On the basis of these observations, four compounds (**1**, **2**, **4**, and DA) which showed different behavior in dog plasma were selected and the bioavailability based on the AUC of DA of these compounds after oral administration to dogs was compared. The relative values of the AUC based on DA after oral administration of each compound to the AUC of DA after the administration of DA were 403.5 ± 58.7, 325.8 ± 67.8, and 152.5 ± 28.4% (mean ± SE, n = 4) for **1**, **2**, and **4**, respectively. The AUCs of DA increased in the order of **1**, **2**, **4**, and DA.

From these results, it was confirmed that the two protective groups of **1** served to increase the AUC of DA after oral administration of **1**, making it resistant to the first-pass metabolism in the absorption process.

Site of Bioactivation of 1 to Dopamine—In order to examine the bioactivation site of **1** to DA in vitro or in situ, conversion of **1** to **2** or DA was examined in blood, liver, and intestine. As shown in Figure 3, it was found that **1** is rapidly converted to **2** or DA in blood. Since **1** is not converted to DA in the plasma in vitro as described above, the conversion seemed to be caused by some enzymatic activity in the red blood cells.

As shown in Figure 4, **1** administration in the intraduodenal loop yielded **2** and DA in the mesenteric venous blood, showing the bioactivation of **1** to DA and **2** in the intestine. In the case of mesenteric venous administration of **1**, **2** and DA were determined in the systemic circulation (Figure 5). From these results, it was shown that **1** was converted to **2** or DA in blood, liver, and intestine.

In conclusion, it was found that the first-pass metabolism of DA was reduced by protecting both the catechol system and amino group of DA with an ethoxycarbonyl or *N*-acetyl-L-methionyl group, respectively. Therefore, the bioavailability of **1**, based on the AUC of DA, was improved. It is suggested that **1** may be considered as a potential useful prodrug of oral DA.

References and Notes

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2. Somani, P.; Hollinger, R.; Minard, F. N. *Am. Chem. Soc.* 165 Meeting, MEDI 11, 1973.
3. Kyncl, J.; Hollinger, R.; Ours, C. W.; Minard, F. N.; Jones, P. H.; Biel, J. H. *Am. Chem. Soc.* 172 Meeting, MEDI 19, 1976.
4. European Patent 7,441; assigned to Tanabe Seiyaku KK, Derwent 09514/C.

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