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ABSORPTION, METABOLISM AND DISTRIBUTION OF [^{14}C]-*O*-METHYLDOPA AND [^{14}C]-L-DOPA AFTER ORAL ADMINISTRATION TO RATS

LEONOR RIVERA-CALIMLIM

Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642

1 The absorption, tissue distribution, and metabolism of [^{14}C]-*O*-methyldopa were compared with those of [^{14}C]-L-DOPA after oral administration to rats.

2 Total radioactivity in the plasma and brain of rats treated with [^{14}C]-*O*-methyldopa was significantly higher (2 fold and 30-50 fold, respectively) than that of rats treated with [^{14}C]-L-DOPA.

3 Total radioactivity in the gut washings and intestinal tissue 2 h after oral administration was significantly higher in rats treated with [^{14}C]-L-DOPA than in rats treated with [^{14}C]-*O*-methyldopa. The reverse was observed in the stomach tissues.

4 Peripheral metabolism of [^{14}C]-*O*-methyldopa was much lower than that of [^{14}C]-L-DOPA; the major metabolite of [^{14}C]-*O*-methyldopa in the plasma is L-DOPA, whereas L-DOPA is mainly metabolized to phenylcarboxylic acids.

Introduction

One major problem in the clinical use of L-DOPA is the wide peripheral metabolism of L-3,4-dihydroxyphenylalanine (L-DOPA) by various tissues, including the gut (Rivera-Calimlim, Morgan, Dujovne, Bianchine & Lasagna, 1971), which drastically diminishes the available unchanged L-DOPA for brain penetration. 3-*O*-methyldopa has been observed to be a major metabolite of exogenous L-DOPA in the plasma and brain of both animals and man (Sharpless & McCann, 1971; Kuruma, Bartholini, Tissot & Pletscher, 1972). Studies show that parenterally administered 3-*O*-methyldopa accumulates in the brain, has a plasma half-life of 15 h, and undergoes demethylation in both rats and man (Bartholini, Kuruma & Pletscher, 1970; Chalmers, Draffan, Reid, Thorgeirsson & Davies, 1971; Kuruma *et al.*, 1972). The possibility that 3-*O*-methyldopa is a good and stable precursor of dopamine was tested in the treatment of parkinsonism. Clinical trials of 3-*O*-methyldopa in parkinsonian patients showed doubtful benefits (Gauthier, Ajuriaguerra, Geissbuhler, Simona, Constantinidis, Yanniotis, Krassoievitch, Eisenring & Tissot, 1971; Muenter, Dinapoli, Sharpless & Tyce, 1973) and, in some, neurological deterioration (Calne, Reid & Vakil, 1973). Chalmers *et al.* (1971) claimed that *O*-methyldopa would not have any advantage over L-DOPA in the treatment of parkinsonism because their studies suggested that demethylation of *O*-methyldopa occurs only in the gut and they were unable to show its *O*-demethylation by the liver and brain tissue, as had been reported by Bartholini *et al.* (1970).

Because of this conflicting evidence based on parenterally administered *O*-methyldopa, the absorption, distribution and metabolism of [^{14}C]-3-*O*-methyldopa and [^{14}C]-L-DOPA were studied and compared after oral administration to rats, since this is the route of administration in the clinical trials of 3-*O*-methyldopa.

Methods

Groups of four male Sprague-Dawley rats (200-250 g) were given the drugs orally after a 12 h fast. One group received [^{14}C]-L-DOPA, labelled at the β-carbon (Amersham-Searle Laboratories, Des Plaines, Ill.) in a dose of 50 mg/kg (2 μCi), and a matched group was given the same dose of [^{14}C]-3-*O*-methyldopa, with the same specific activity. Two hours later the rats were anaesthetized with ether, an abdominal incision was made, and blood was withdrawn from the inferior vena cava into a tube containing heparin. The blood was centrifuged and the plasma was separated. Ligatures were placed at the cardio-

oesophageal junction of the stomach, the pylorus, and the ileocaecal junction, and the intervening portions of gut were cut out, dissected free of mesenteric tissue and washed in 0.9% w/v NaCl solution. The stomach and small intestine were blotted dry, opened up by a longitudinal incision, washed with 0.01 N HCl three times, and the washings collected separately. The stomach, intestine, and brain were weighed and separately homogenized in 5 ml of 0.4 N perchloric acid; the homogenates were centrifuged, and the supernatants separated. Samples (0.2-0.5 ml) from the plasma, gastric and intestinal washings and extracts of stomach, intestines and brain were added to 10 ml Triton X-100-toluene scintillation liquid and assayed for total radioactivity in a Packard Tricarb liquid scintillation spectrometer. Counting efficiency for ^{14}C was 88-90%. All values for radioactivity were corrected for quenching and background.

Fractionation of the samples (plasma, gastric and intestinal washings, and stomach, intestine and brain homogenates) to separate 3-*O*-methyldopa and L-DOPA and their metabolites was by the ion exchange chromatographic technique described elsewhere (Rivera-Calimlim, Dujovne, Morgan, Bianchine & Lasagna, 1971). Portions of 1 ml from fractions consisting of (a) phenylcarboxylic acids (vanillylmandelic acid, homovanillic acid, 3,4-dihydroxyphenylacetic acid), (b) L-DOPA, (c) 3-*O*-methyldopa, (d) noradrenaline, adrenaline, normetanephrine and metanephrine, and (e) dopamine were assayed for radioactivity and expressed as a percentage of the total tissue radioactivity. Percentage recoveries of labelled standards from the columns ranged from 87 to 95%.

Results

Table 1 shows the values for [^{14}C]-L-DOPA and [^{14}C]-*O*-methyldopa in the plasma, brain, gut washings and the gut tissues of two groups of rats, 2 h after an oral dose of 5 mg (3 μCi) of either [^{14}C]-L-DOPA or [^{14}C]-*O*-methyldopa. The mean concentration of the radioactivity in the plasma of rats treated with [^{14}C]-*O*-methyldopa was over twice the mean plasma concentration of rats treated with [^{14}C]-L-DOPA. Total radioactivity in the brain (expressed as a percentage of dose) of rats that received [^{14}C]-*O*-methyldopa was 35-50 times as great as in rats treated with [^{14}C]-L-DOPA. Total radioactivity in the gut (stomach washings, intestinal washings and intestinal tissue) after [^{14}C]-L-DOPA, given orally, was two to three times that observed after [^{14}C]-*O*-methyldopa, except in stomach tissue, where the radioactivity of [^{14}C]-*O*-methyldopa treated rats was two to three times as high as that of the [^{14}C]-L-DOPA treated rats.

Tables 2 and 3 show the values for the parent compound and its metabolites in plasma, brain, and gut of rats treated with [^{14}C]-L-DOPA and of rats treated with [^{14}C]-*O*-methyldopa. After oral

Table 1 Absorption of [^{14}C]-L-DOPA and [^{14}C]-*O*-methyldopa after oral administration.

Sample		*[^{14}C]-L-DOPA*	*[^{14}C]-3-O-methyldopa*
		(mean d/min per ml ± s.e. mean)	
Plasma	(a)	4055 ± 160	9810 ± 98*
	(b)	2630 ± 68	7355 ± 92*
		(mean % of dose ± s.e. mean)	
Brain	(a)	0.004 ± 0.0001	0.22 ± 0.01*
	(b)	0.006 ± 0.0001	0.22 ± 0.02*
Stomach wash	(a)	0.50 ± 0.07	0.84 ± 0.50
	(b)	0.70 ± 0.4	0.49 ± 0.19
Stomach tissue	(a)	0.16 ± 0.02	0.44 ± 0.04*
	(b)	0.28 ± 0.15	0.51 ± 0.04
Intestine wash	(a)	9.50 ± 0.3	3.50 ± 0.15*
	(b)	8.20 ± 1.5	3.50 ± 0.22**
Intestinal tissue	(a)	4.80 ± 0.4	1.80 ± 0.14*
	(b)	3.60 ± 0.48	1.90 ± 0.03**

(a) and (b) denote separate runs of the experiment. $n = 4$.
Analysis of the difference between treatments was by Student's t-test: * $P < 0.01$; ** $0.01 < P < 0.05$. Other P values not significant. Dose of the labelled drugs was 50 mg/kg (2 μCi).

treatment with [^{14}C]-L-DOPA, only 3-9% of the plasma radioactivity was associated with unchanged L-DOPA, and 90-95% was in the form of metabolites. These data correlate well with plasma values obtained in the human studies (Bianchine, Rivera-Calimlim, Dujovne, Morgan & Lasagna, 1971). Brain homogenates from rats treated with [^{14}C]-L-DOPA were not fractionated because of the negligible amount of total radioactivity observed. Fractionation of the stomach (washings and tissue) radioactivity showed that only 30-65% was unchanged L-DOPA and the rest was in the form of metabolites. A much higher percentage of metabolites was observed when the intestinal washings and tissue were fractionated. Comparison of the values obtained after fractionation of

Table 2 Metabolism of [^{14}C]-L-DOPA, 2 h after oral administration in rats.

Sample		*L-DOPA*	*Metabolites**			
			PCA	*OMD*	*DA*	*A & NA*
Plasma	(a)	3.0 ± 0.8	62.0 ± 5.3	23.0 ± 1.4	7.0 ± 1.2	12.0 ± 2.3
	(b)	5.0 ± 2.3	37.0 ± 0.9	34.0 ± 4.0	5.2 ± 1.2	1.9 ± 0.5
Brain	(a)					
	(b)					
Stomach wash	(a)	47.0 ± 2.2	9.5 ± 1.8	14.4 ± 1.1	0.2 ± 0.2	0.6 ± 0.3
	(b)	66.6 ± 1.2	3.6 ± 1.5	19.0 ± 1.6	3.4 ± 1.2	11.1 ± 2.8
Stomach tissue	(a)	38.0 ± 1.0	12.3 ± 0.9	18.3 ± 1.6	15.0 ± 1.5	5.0 ± 1.2
	(b)	33.9 ± 1.7	34.9 ± 4.3	20.0 ± 2.4	11.2 ± 0.4	3.2 ± 1.4
Intestine wash	(a)	9.6 ± 0.6	21.6 ± 5.8	28.0 ± 1.1	2.3 ± 0.1	2.0 ± 0.5
	(b)	8.5 ± 0.6	44.6 ± 2.7	31.0 ± 2.2	1.2 ± 0.8	1.9 ± 0.1
Intestine tissue	(a)	10.0 ± 0.7	17.2 ± 1.9	50.0 ± 1.4	0.4 ± 0.1	0.7 ± 0.3
	(b)	11.2 ± 1.3	28.0 ± 6.4	38.0 ± 1.6	1.5 ± 0.2	0.9 ± 0.1

Values are expressed as mean % (± s.e. mean) tissue radioactivity. (a) and (b) denote two separate runs of the experiment. $n = 4$. * PCA = phenylcarboxylic acids: homovanillic acid, vanillylmandelic acid, dihydroxyphenylacetic acid and conceivably 5,6-dihydroxyindole and other melanin precursors; OMD = 3-O-methyldopa; DA = dopamine; A & NA = adrenaline, noradrenaline, metanephrine and normetanephrine.

Table 3 Metabolism of [^{14}C]-3-O-methyldopa 2 h after oral administration in rats.

Sample		*OMD*	*Metabolites**			
			L-DOPA	*PCA*	*DA*	*A & NA*
Plasma	(a)	73.0 ± 5.7	12.1 ± 0.1	8.7 ± 1.3	–	–
	(b)	76.0 ± 4.7	13.0 ± 0.8	10.8 ± 2.7	1.9 ± 1.0	1.5 ± 0.9
Brain	(a)	69.0 ± 2.3	0.6 ± 0.0	–	2.5 ± 0.5	2.2 ± 0.5
	(b)	68.2 ± 3.2	4.8 ± 1.0	5.1 ± 1.1	1.6 ± 0.3	4.4 ± 2.8
Stomach wash	(a)	53.0 ± 6.7	6.3 ± 5.7	4.0 ± 1.7	0.1 ± 0.1	0.6 ± 0.1
	(b)	72.0 ± 5.1	13.0 ± 4.5	5.9 ± 1.8	6.4 ± 2.6	3.4 ± 0.9
Stomach tissue	(a)	70.3 ± 1.1	7.5 ± 1.3	0.8 ± 0.4	0.7 ± 0.1	0.7 ± 0.3
	(b)	70.0 ± 1.6	19.0 ± 0.7	4.9 ± 1.8	0.9 ± 0.3	0.9 ± 0.3
Intestine wash	(a)	33.4 ± 1.2	8.6 ± 1.4	39.7 ± 6.6	0.9 ± 0.2	1.5 ± 0.4
	(b)	35.0 ± 5.1	6.8 ± 1.2	43.9 ± 6.9	0.9 ± 0.2	1.8 ± 0.2
Intestine tissue	(a)	63.0 ± 3.1	11.5 ± 1.6	11.3 ± 1.2	–	–
	(b)	51.4 ± 3.1	11.3 ± 2.4	22.0 ± 4.3	0.9 ± 0.1	0.4 ± 0.1

(a) and (b) denote two separate runs of the experiment. $n = 4$. Values are expressed as mean % (± s.e. mean) tissue radioactivity. OMD = 3-O-methyldopa; PCA = phenylcarboxylic acids (see note to Table 2); DA = dopamine; A & NA = adrenaline, noradrenaline, metanephrine and normetanephrine.

equivalent samples from rats treated orally with [^{14}C]-*O*-methyldopa (Table 3) suggests that 3-*O*-methyldopa is a poorer substrate for decarboxylase enzymes. Two hours after administration, about 75% of the radioactivity in the plasma was recovered in the fraction of 3-*O*-methyldopa. Because of the higher radioactivity recovered from brain homogenates of rats after oral treatment with [^{14}C]-*O*-methyldopa, we were able to fractionate the brain samples. The data show that 68-69% of the radioactivity was in the 3-*O*-methyldopa fraction. Similar metabolic profiles were observed for gut tissues. The presence of radioactivity in the L-DOPA fractions, phenylcarboxylic acids fractions, dopamine and other catecholamine fractions in all the tissues studied (Table 3) suggests that 3-*O*-methyldopa is demethylated into DOPA and further metabolized to its metabolites. One significant observation is the higher L-DOPA fraction in the plasma of rats treated with [^{14}C]-*O*-methyldopa as compared to rats treated with [^{14}C]-L-DOPA (see Tables 2 and 3).

Discussion

3-*O*-methyldopa was significantly better absorbed from the gut of rats than was L-DOPA, when measured 2 h after oral dosing. In tissues from 3-*O*-methyldopa-treated rats, radioactivity observed in the fractions of L-DOPA, dopamine, other catecholamines, and phenylcarboxylic acids, suggested that *O*-demethylation had occurred. Although our study did not localize the site of demethylation, whether liver, brain, or gut alone, a significant finding was the two- to three-fold increase in radioactivity in the DOPA fraction of plasma and brain of 3-*O*-methyldopa-treated rats over that of rats treated with an equivalent dose of L-DOPA. This observation could reflect the passage of L-DOPA from the demethylation of *O*-methyldopa in gut to the plasma, or from the plasma to the brain, or demethylation of *O*-methyldopa to DOPA by brain tissue. Whatever the source, the goal is achieved, i.e. to get L-DOPA into the brain.

The problem is that the optimum concentration of 3-*O*-methyldopa and L-DOPA in the brain required to obtain clinical benefits without adverse effects is unknown. Can high levels of both *O*-methyldopa and L-DOPA in the brain be deleterious and aggravate the symptoms of patients treated with high doses of 3-*O*-methyldopa? Claveria, Calne & Allen (1973) reported an association between episodes of neurological deterioration and exceptionally high plasma concentrations of L-DOPA; patients treated with L-DOPA combined with decarboxylase inhibitors showed an increase in 3-*O*-methyldopa in the plasma and an earlier and more frequent appearance of dyskinesias.

From the present study, it appears that if demethylation of 3-*O*-methyldopa in man is as efficient as in rats, 3-*O*-methyldopa given in a much lower dose than the usual dose of L-DOPA would attain adequate L-DOPA concentrations in plasma and brain and would also limit the concentrations of unchanged 3-*O*-methyldopa, which could be a competitive inhibitor of decarboxylation of L-DOPA to dopamine, the neurohumor thought to be missing in Parkinson's disease.

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