THE ABSORPTION AND CONJUGATION OF METHYLDOPA IN PATIENTS WITH COELIAC AND CROHN'S DISEASES DURING TREATMENT

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1 The absorption and elimination of a single 500 mg dose of methyldopa has been studied after oral administration to 10 normal volunteers, 10 patients with coeliac disease, who were on a gluten-free diet, and to five patients with Crohn's disease.

2 In patients with Crohn's disease the percentage of the dose recovered in urine (24 %) was about one-half of normal (50%). This decreased absorption was associated with reduced plasma concentrations of both free and conjugated methyldopa and a decreased pharmacological response.

3 In patients with coeliac disease the percentage of dose recovered in urine (43%) was similar to normals, indicating a normal absorption of the drug. However, the plasma concentrations of both free and conjugated methyldopa were significantly higher than normal, although there was no increase in drug-related effects.

4 These findings confirm the importance of using indices other than drug plasma concentrations in the assessment of bioavailability in disease states.

Keywords methyldopa coeliac disease Crohn's disease absorption conjugation

Introduction

Methyldopa [L-3-(3',4'-dihydroxyphenyl)-2-methylalanine] is prescribed widely for the control of hypertension and its fate in normal man has been well defined. It shows variable and incomplete absorption from the gut with 20-85% of an oral dose of [¹⁴C]-methyldopa recovered in the faeces (Buhs *et al.*, 1964; Au *et al.*, 1972). A further reduction in systemic bioavailability is brought about through its first-pass metabolism, by sulphate conjugation, which probably occurs within the intestinal wall (Saavedra *et al.*, 1975; Kwan *et al.*, 1976; Stenbaek *et al.*, 1977). [¹⁴C]-methyldopa also undergoes 3-*O*-methylation, decarboxylation to α -methyldopamine and 3-*O*-methyl- α -methyldopamine and oxidation to 3,4-hydroxyphenylacetone (Au *et al.*, 1972). The total amount of these metabolites excreted in the urine is approximately equal to the urinary elimination of free methyldopa (Stenbaek *et al.*, 1977), i.e. about 50% and 15% of the dose after intravenous and oral administration respectively.

Pharmacokinetic studies with a variety of drugs in patients with gastrointestinal disease (reviewed by Parsons, 1977) have revealed a variable effect on absorption, dependent on both the drug and the disease. Thus, in coeliac disease (gluten sensitive enteropathy) some drugs such as pivampicillin, amoxycillin (Parsons *et al.*, 1975) and practolol

(Parsons *et al.*, 1976) show evidence of decreased absorption, while others such as sulphamethoxazole, trimethoprim (Parsons, 1977) and propranolol (Parsons *et al.*, 1976) appear to show increased absorption. In Crohn's disease the appearance of the peak plasma levels of trimethoprim and sulphamethoxazole were delayed while the absorption of propranolol appeared to increase (Parsons, 1977). Since methyldopa is absorbed slowly across the intestinal wall (Renwick *et al.*, 1983) and probably undergoes sulphate conjugation in man at this site (Saavedra *et al.*, 1975), it represents a clinically important compound that might show considerable abnormalities in bioavailability in these disease states.

Methods

Ten normal subjects, 10 patients with coeliac disease and five patients with Crohn's disease were studied at various times after diagnosis and the initiation of treatments (see Table 1). The diagnosis of coeliac disease was based upon clinical evidence of malabsorption and upon the characteristic histological abnormalities of the jejunal mucosa with recovery to normal on withdrawal of dietary gluten. The patients had received gluten-free diet for various periods

Table 1 Subjects and their clinical status

<i>Normal</i>	<i>Age</i> (years)	<i>Sex</i> ^a	<i>Weight</i> (kg)	<i>Time since</i> ^b <i>diagnosis</i>	<i>Treatment</i> ^c ; <i>duration</i>
1	21	M	66		
2	22	M	67		
3	21	F	54		
4	34	M	76		
5	21	M	65		
6	23	M	89		
7	22	M	95		
8	35	M	73		
9	22	M	73		
10	21	F	56		
<i>Coeliac</i>					
11	64	F	41	2 y	GFD; 2 y
12 ^d	62	F	51	24 y (4 m)	GFD; 24 y
13	51	F	73	2 y	GFD; 2 y
14	38	F	70	2 y	GFD; 15 m
15 ^e	32	M	59	2 w	GFD; 1 w
16	58	F	57	4 m	GFD; 3 m
17	38	F	70	5 y	GFD; 5 y
18	48	F	48	1 y	GFD; 4 m
19	68	F	50	1 m	None
20	25	F	67	1 y	GFD; 2 m
<i>Crohn's</i>					
21 ^f	46	M	58	5 y	None
22	36	F	51	1 y	P; 1 y
23	33	F	59	15 y	P + S; 1 y(+)
24 ^g	32	M	64	10 y	P + S + Fe; 1 y(+)
25	62	M	47	30 y	S; 1 y(+)

^a M = male; F = female^b y = year; m = month; w = week^c GFD = gluten free diet; P = prednisone; S = sulphasalazine; Fe = ferrous sulphate^d Subject had received GFD for 24 y but diagnosis was confirmed by biopsy only 4 months before the study^e Subject also had ulcerative colitis for which he had received salazopyrine for 30 m. Subject also taking phenobarbitone and mestinon for 2–3 y^f Subject received prednisone until 3 m prior to the study^g Subject had undergone vagotomy and pyloroplasty 4 y prior to study

(Table 1) at the time of the study. Faecal fat was measured subsequently in five normal and six coeliac subjects using polyethylene glycol 4000 as an internal non-absorbable marker (Wilkinson, 1971) and a xylose tolerance test was performed (Rolles *et al.*, 1973). The diagnosis of Crohn's disease was made on the basis of clinical and radiological changes and characteristic histological abnormalities. Each subject gave informed consent to the study which had received the approval of the local ethical committee.

A single oral dose of methyldopa (500 mg) dissolved in water (50 ml) was given to each subject after an overnight fast. Blood samples (10 ml) were collected into heparinised tubes, prior to the dose, and subsequently at half-hourly intervals until 5 h, then hourly until 8 h after the dose. The plasma was separated and stored at -20°C prior to analysis.

Urine was collected for 48 h after the dose, and in some subjects (see Table 3) faeces were collected for 4 days after dosing. Prior to the dose and at hourly intervals throughout the study, the subjects were assessed for systolic and diastolic blood pressures, salivation (using weighed dental swabs) and sedation (using a 10 cm visual analogue scale from 'wide awake' to 'nearly asleep') (Dollery *et al.*, 1976). Methyldopa and its conjugates were measured by a method based on those of Saavedra *et al.* (1975) and Kwan *et al.* (1976) as described elsewhere (Renwick *et al.*, in preparation). The plasma pharmacokinetic data were analysed by least squares regression analysis and were described adequately by absorption and elimination from a single compartment. The binding of methyldopa to serum protein was assessed by equilibrium dialysis at 20°C for 18 h using an MSE Dianorm

for five normals, three coeliac patients and one patient with Crohn's disease. Each serum (0.5 ml) was equilibrated with [^{14}C]-methyldopa (supplied by Dr L.G. Dring) ($2.5\text{ }\mu\text{g}$; 3000 d/min in 0.1 M phosphate buffer (0.5 ml ; $\text{pH } 7.4$) containing ascorbic acid (1 mg)) and the total ^{14}C in serum and buffer determined.

Results

In normal subjects, methyldopa was absorbed slowly after a lag time of 0.9 h with an absorption half-time of 1.0 h . The concentration of free drug in the plasma reached a maximum of $3.1\text{ }\mu\text{g/ml}$ at 3.5 h after the dose (Figure 1). The terminal elimination half-life was 2.6 h and the area under the plasma concentration-time curve (AUC) was $13.4\text{ }\mu\text{g ml}^{-1}\text{h}$ (Table 2). In patients with coeliac disease the lag time (0.9 h), absorption half-time (1.0 h), time to peak (3.4 h) and elimination half-life (2.2 h) were very similar to normals, but the peak plasma concentration ($5.0\text{ }\mu\text{g/ml}$) and AUC ($20.5\text{ }\mu\text{g ml}^{-1}\text{h}$) were significantly increased ($P < 0.05$). Patients with Crohn's disease

had plasma levels lower than normals especially in the later part of the study; however, the overall AUC and elimination half-life were not statistically significantly lower.

The peak concentration of conjugated methyldopa in the plasma of normal subjects ($1.7\text{ }\mu\text{g/ml}$) (Figure 2) and the AUC ($7.9\text{ }\mu\text{g/ml h}$) (Table 2) were less than for the parent compound, while the time to peak (3.7 h) and elimination half-life (3.2 h) were similar. In coeliac disease the time to peak and elimination half-life were similar to normals but the peak plasma levels ($3.5\text{ }\mu\text{g/ml}$) and AUC ($15.7\text{ }\mu\text{g ml}^{-1}\text{h}$) were approximately double normal values ($P < 0.05$ and < 0.01). The data for patients with Crohn's disease were similar to normals.

The excretion of both free and conjugated methyldopa in the urine of normal subjects (Table 3) was almost complete within 24 h of dosing. A total of 49.7% of the dose was recovered in the urine, mostly (28% of the dose) as the parent drug. An additional 33.9% of the dose was detected in the faeces collected over 4 days by five subjects. In patients with coeliac disease the total recovery in urine (43.1% of the dose) and faeces (26.5% of the dose) was slightly lower than normals but the difference was not statistically significant. The extent of conjugation was almost identical to that seen in normals. However, in patients with Crohn's disease there was a statistically significant lower recovery of both free and conjugated methyldopa in the urine, especially during the first 8 h ($P < 0.01$). The ratio of conjugated to free was significantly higher than in normal subjects.

The 24 h faecal fat measurements in control subjects (1–5) were within the normal range, whilst of the coeliac patients who took part (11–14, 16, 17), only subject 12 showed abnormally high values, around the time when the pharmacokinetic study was performed. Of these same individuals (1–5 and 11–17) only subject 12 showed an abnormal xylose absorption test.

Measurements of the binding of [^{14}C]-methyldopa to plasma protein in five normal subjects, showed a wide range of values ($1\text{--}16\%$) with no indication of grossly altered protein binding in three patients with coeliac disease ($10\text{--}12\%$) or Crohn's disease (2%).

There was a decrease in salivation and an increase in sedation during the study but no significant change in diastolic blood pressure (Table 4). Whether these effects were drug related cannot be assessed due to the lack of a placebo. However, there were no major differences between the normals and coeliac patients, whilst subjects with Crohn's disease showed a reduced effect especially with respect to sedation.

Figure 1 Plasma concentrations of free methyldopa after a single oral dose in normal subjects (●) and patients with coeliac disease (○) and Crohn's disease (■). The values given are the mean with the bars denoting s.e. mean. * $P < 0.05$.

Discussion

The plasma concentration-time curves for free and conjugated methyldopa in normals were in good

Table 2 Plasma pharmacokinetic data for methyl dopa after a single oral dose in man

	Normal	Coeliac	Crohn's
<i>Free methyl dopa</i>			
Lag time (h) ^a	0.9 ± 0.4	0.9 ± 0.3	0.8 ± 0.1 ^d
Absorption half-time (h) ^a	1.0 ± 0.4	1.0 ± 0.4	0.7 ± 0.1 ^d
Time to peak (h) ^b	3.5 ± 0.6	3.4 ± 0.7	2.1 ± 0.7**
Peak concentration (µg/ml) ^b	3.1 ± 1.1	5.0 ± 2.2*	2.5 ± 0.8
Area under curve (µg ml ⁻¹ h) ^c	13.4 ± 4.9	20.5 ± 9.6*	8.7 ± 4.3
Elimination half-life (h) ^a	2.6 ± 1.0	2.2 ± 0.7	2.0 ± 0.6
<i>Conjugated methyl dopa</i>			
Time to peak (h) ^b	3.7 ± 1.1	3.6 ± 0.7	3.0 ± 0.6
Peak concentration (µg/ml) ^b	1.7 ± 0.4	3.5 ± 2.5*	1.8 ± 0.3
Area under curve (µg ml ⁻¹ h) ^c	7.9 ± 2.1	15.7 ± 7.5**	7.6 ± 1.8
Elimination half-life (h) ^d	3.2 ± 1.1	4.9 ± 2.9	2.4 ± 0.7

The results are the mean ± s.d. for 10 normals, 10 patients with coeliac disease and five patients with Crohn's disease (except where indicated).

^a values computed by least squares regression analysis

^b observed values

^c calculated by trapezoid rule for 0–8 h data

^d two subjects showed very rapid absorption but low blood levels so absorption could not be calculated accurately, and the results given are the mean of three subjects.

* < 0.05. ** *P* < 0.01 compared with normals by unpaired Student's *t*-test.

Figure 2 Plasma concentrations of conjugated methyl dopa after a single oral dose of methyl dopa in normal subjects (●) and patients with coeliac disease (○) and Crohn's disease (■). The values given are the mean with the bars denoting the standard errors. * *P* < 0.05, ** *P* < 0.01.

agreement with published data (Saavedra *et al.*, 1975; Kwan *et al.*, 1976). Similarly, the terminal half-life of the parent compound (2.6 h) agreed well with that published by Kwan *et al.* (1976) for 12 subjects (1.8 h) but was considerably less than that found by Stenbaek *et al.* (1977) who studied only three individuals (4.3–13.9 h). The average recovery of free and conjugated methyl dopa in the urine (28 and 22% of the dose) and free methyl dopa in the faeces (34% of the dose) also agreed well with published data (Buhs *et al.*, 1964; Au *et al.*, 1972; Kwan *et al.*, 1976; Stenbaek *et al.*, 1977). The range of total recoveries within 48 h (28–80% of dose) was similar to those found by other workers (Buhs *et al.*, 1964; Kwan *et al.*, 1976).

In patients with Crohn's disease, the plasma pharmacokinetics showed no indication of any major abnormality in the rate of absorption or elimination. However, the urine data indicate that only about one-half as much of the dose had been absorbed and excreted in the urine within 48 h. This was consistent with the reduced AUC for the parent drug in these patients. Patients with Crohn's disease showed a reduced pharmacological response at 7 h with respect to sedation and a smaller decrease in systolic blood pressure, which are in agreement with the lower blood levels of free drug in these patients. A greater proportion of the drug excreted in the urine of patients with Crohn's disease was present as conjugates. This result and the plasma concentration–time curve for the free drug are consistent with absorption being restricted mainly to the upper intestine, which has high levels of drug conjugating enzymes.

Table 3 The elimination of free and conjugated methyl dopa after an oral dose of methyl dopa (500 mg)

	% dose excreted		
	Normal	Coeliac	Crohn's
<i>Free methyl dopa</i>			
0-8 h urine	22.7 ± 7.0	21.3 ± 10.2	9.1 ± 6.7**
0-24 h urine	27.3 ± 8.8	24.4 ± 10.7	10.9 ± 8.4**
0-48 h urine	28.0 ± 9.2	25.1 ± 10.8	11.3 ± 8.4**
0-96 h faeces ^a	33.9 ± 11.7	26.5 ± 10.9	—
<i>Conjugated methyl dopa</i>			
0-8 h urine	15.6 ± 5.6	13.1 ± 6.8	9.3 ± 4.6*
0-24 h urine	21.0 ± 9.9	17.2 ± 6.9	12.2 ± 5.8*
0-48 h urine	21.7 ± 10.3	18.0 ± 6.7	12.4 ± 5.7
<i>Total recovered</i>			
0-48 h urine	49.5 ± 16.9	42.9 ± 16.9	23.6 ± 12.8*
% conjugation ^b	43.0 ± 7.6	42.8 ± 7.1	55.3 ± 11.8*

The results are the mean ± s.d. for 10 normal subjects, 10 patients with coeliac disease and five patients with Crohn's disease except:

^a where the results are for six normals and five coeliacs only

^b calculated as $\frac{\text{conjugates in 0-48 h urine}}{\text{total in 0-48 h urine}} \times 100$

* $P < 0.05$, ** $P < 0.01$ compared with normals by unpaired Student's *t*-test.

Table 4 Pharmacological responses to methyl dopa

Blood pressure (mm Hg)	Normal	Coeliac	Crohn's
<i>Systolic</i>			
a	119 ± 12	128 ± 15	118 ± 12
b	112 ± 15	114 ± 13**	118 ± 10
c	110 ± 4**	113 ± 14*	114 ± 5
<i>Diastolic</i>			
a	78 ± 6	81 ± 8	75 ± 4
b	76 ± 8	76 ± 9	78 ± 9
c	75 ± 10	76 ± 7*	74 ± 5
<i>Sedation</i> ⁺			
a	1.2 ± 1.5	1.2 ± 1.3	1.1 ± 1.1
b	1.4 ± 1.4	1.4 ± 1.1	2.3 ± 1.8
c	3.0 ± 3.3*	4.0 ± 3.4*	0.6 ± 0.5
<i>Salivation</i> [†]			
a	0.65 ± 0.17	0.73 ± 0.45	0.88 ± 0.73
b	0.61 ± 0.23	0.71 ± 0.45	0.73 ± 0.43
c	0.43 ± 0.13***	0.40 ± 0.27***	0.45 ± 0.19

The results are given as the mean ± s.d.

a—predose

b—at time of peak plasma concentration

c—at 7 h

+—sedation rating in cm on a 10 cm visual analogue scale.

0 = wide awake; 10 = nearly asleep

†—weight of saliva produced in g/min

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with predose values using Student's *t*-test for paired data.

Comparison of the plasma data for patients with coeliac disease and normal subjects showed that whilst the rate constants associated with absorption and elimination were almost identical, both the peak plasma level of free methyl dopa and the AUC were almost doubled. The peak plasma concentration and AUC of conjugated methyl dopa were also increased. Similar results would have been interpreted previously as evidence of an increase in the extent of drug absorption and conjugation in such patients (Parsons, 1977). However the excretion of methyl dopa in urine and faeces showed no evidence of increased absorption in patients with coeliac disease; indeed, the recovery in urine was, if anything, slightly reduced.

There is, therefore, a clear discrepancy between the plasma and urine data for the coeliac patients. Since these patients tended to be smaller than the normals the plasma concentrations and AUC values were recalculated adjusting for this variable. The significant differences reported above were still apparent, suggesting that body weight was not the explanation for the elevation in plasma concentrations. Similarly, the coeliac group was older than the normals but analysis of intra-group variations, and of all subjects published previously, has shown that age does not alter significantly the pharmacokinetics of methyl dopa.

The high plasma AUC:renal excretion ratio found in coeliac disease could be explained by either a relative decrease in renal excretion or an increase in the amount associated with the plasma. In normal subjects an intravenous dose of [^{14} C]-methyl dopa was recovered quantitatively in the urine (Stenbaek *et al.*, 1977), suggesting that non-renal elimination is restricted to metabolism for the parent compound and of minimal importance for the sulphate conjugate. In none of the patients was there clinical evidence of impaired renal function. Therefore, it seems likely that the increased AUC originates in a decrease in

distribution from plasma to tissues. This is consistent with the finding that the coeliac patients did not show an increased pharmacological response to the drug despite having elevated blood levels.

Abnormalities of drug distribution have been reported recently in patients with inflammatory conditions such as Crohn's disease and rheumatoid arthritis (Piafsky *et al.*, 1978; Waller *et al.*, 1982). These abnormalities were attributable largely to the elevated levels of α_1 -acid glycoprotein in these patients, and the increased protein binding in the patients' plasma samples. However, this would not explain the present results since analysis of the binding of methyl dopa to plasma protein showed that it underwent low, variable (1–16%) but similar binding in normals and patients with coeliac and Crohn's diseases. Indeed, the acidic sulphate conjugate of methyl dopa, which showed the more marked elevation in blood levels and AUC, would not be expected to bind to the acid glycoprotein. Therefore, it is likely that there are additional alterations to drug distribution in patients with coeliac disease.

In conclusion, it appears from the recovery of the drug in urine that methyl dopa is absorbed normally from the gut in patients with coeliac disease on treatment, but that absorption is reduced to about one-half in patients with Crohn's disease. In coeliac disease the plasma levels of free and conjugated methyl dopa were higher than expected based on the recovery in urine. This discrepancy between urine and plasma emphasises the potential pitfalls in assessing relative bioavailability and absorption solely on the basis of plasma data, without reference to other indices of absorption such as urine data or the use of an intravenous dose and measurement of pharmacological therapeutic effects.

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