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Variability of Urinary Dapsone/Creatinine Concentration Ratios in Leprosy Patients Fully Compliant with Dapsone Therapy

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A highly sensitive and reproducible assay procedure for the determination of dapsone (DDS) and hydrolizable metabolites in urine is described. DDS/creatinine (D/C) concentration ratios, which are used to monitor compliance with DDS therapy, have been determined on samples of all urine voided throughout a 24-h period by 7 leprosy in-patients fully compliant with their therapy. The D/C concentration ratios varied both within and between patients over the 24 h and the time-course of variation showed no closely predictable pattern. Urinary excretion of DDS over the 24 h was found to be 74.8%±5.7% (S.E.M.) uncorrected or 90.2%±6.8% corrected for recovery. Our results indicate an unreliability in the use of single urine samples to determine D/C ratios and hence compliance by individual patients with their DDS therapy.

Introduction

A quantitative method by which dapsone (DDS) self-administration in leprosy patients can be monitored has been evaluated (Ellard, Gammon, Helmy and Rees, 1974*a*). The ratios of DDS to creatinine (D/C) concentrations in urinary samples are determined and a comparison made between the D/C concentration ratios of patients taking DDS under strict supervision and those who administer their own medication. Ellard, Gammon and Harris (1974*b*), Low and Pearson (1974) and Huikeshoven *et al.* (1976) have applied this technique to determine the regularity of dapsone self-administration.

Prior to assessing in a large number of patients the regularity of DDS self-medication based on measurement of D/C concentration ratios, the reliability of the use of D/C concentration ratios obtained on single urine samples has been examined. This paper describes the results of determinations on samples of all urine passed in 24 h by 7 patients. All patients are resident in a leprosarium in England where their DDS administration is strictly supervised.

Methods

PATIENTS

Seven leprosy patients (6 male, 1 female), resident in a leprosarium, volunteered for this study. All had been on long-term DDS therapy and had

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received their current dosage regimen (25 mg or 50 mg once daily) for at least 6 weeks. Five of the patients (numbers 1, 2, 5, 6 and 7) were receiving other drugs listed in Table 1.

URINE SAMPLES

All urine was collected during one 24-h period. At each voiding the time was noted, a 25-ml sample separated and the remainder placed in a collection bottle. Creatinine and DDS were determined in each voiding and in the 24 h save.

CREATININE AND DDS DETERMINATIONS

Creatinine was determined by the alkaline picrate method (Jaffe, 1886), using an automated procedure (Technicon Method No. SE4–0011FH4, 1974). DDS, as total diazotizable compounds, was determined in duplicate by the following method which is similar to that recommended by Varley (1967) and is a modification of the Bratton and Marshall (1939) procedure. Two-ml portions of each sample were acidified by addition of 2 ml 2 N HCl and immersed in a boiling water bath for 15 min to ensure hydrolysis of dapsone metabolites (Ellard, 1966). After cooling, 0.4-ml volumes of 0.1% sodium nitrite, 0.5% ammonium sulphamate and 0.1% naphthylethylenediamine dihydrochloride (in 95% ethanol) were added successively at 5-min intervals. The optical density at 540 nm was measured 5 min after addition of the last reagent against a reagent blank using 5 μg/ml DDS standards. Urinary blank values were determined on each sample by substitution of water for sodium nitrite.

Recovery of DDS added to urine was 82.9% ± 2.8% (S.E.M.) (range 69–91%) and duplicate determinations showed satisfactory repeatability [Fisher's (1970) intrapair correlation coefficient $r > 0.98$]. Urine from 13 normal untreated subjects yielded apparent DDS/creatinine concentration ratios of 1.10 ± 0.20 (range 0.20–2.95) μg/mg.

All of the drugs listed in Table 1 and also the metabolites N-demethylclindamycin and desmethyldiazepam were tested for interference with the DDS assay procedure. None of the drugs, except desmethyldiazepam, was found to react.

Results

Total DDS and creatinine excretion in 24 h and D/C concentration ratios of these totals, together with the ranges found among all urine samples, are given in Table 1. Urinary DDS excretion over 24 h was found to be 74.8% ± 5.7% (S.E.M.) of the dose administered. After correction for an average recovery of 82.9% in the determination, this amounts to 90.2% ± 6.8% of the dose.

DDS/creatinine concentration ratios of different urine samples varied widely both between and within individual patients. The variability encountered is shown in Fig. 1; it ranged from 1.44-fold (patient no. 7) to 4.49-fold (patient

TABLE 1

Urinary excretion of DDS and creatinine and D/C concentration ratios in 7 patients: (a) 24 h excretion; (b) values for individual urine samples during 24 h

Patient	Sex	Age	DDS dosage (mg/day)	Other drugs	(a) 24 h excretion			(b) Range of individual values		
					DDS (mg)	Creatinine (g)	D/C ratio (μg/mg)	DDS (μg/ml)	Creatinine (mg/ml)	D/C ratio (μg/mg)
1	M	62	25	Diazepam	14.9	1.30	11.5	3.1–19.3	0.26–1.36	9.4–16.2
2	M	46	25	Erythromycin	21.5	1.39	15.5	6.4–28.7	0.40–2.10	7.1–21.0
3	M	82	25	nil	16.6	1.31	12.7	3.1–39.8	0.51–2.19	5.4–24.2
4	F	76	25	nil	23.0	0.74	31.1	8.2–61.0	0.28–2.27	19.5–33.3
5	M	50	50	Clindamycin Indomethacin	17.5	1.44	12.2	9.9–57.9	0.91–2.04	9.1–28.3
6	M	54	50	Diazepam	56.5	1.70	33.2	18.7–35.2	0.64–1.73	13.7–47.2
7	M	20	50	Clofazimine	36.8	1.21	30.4	23.0–58.4	0.86–1.52	26.6–38.3

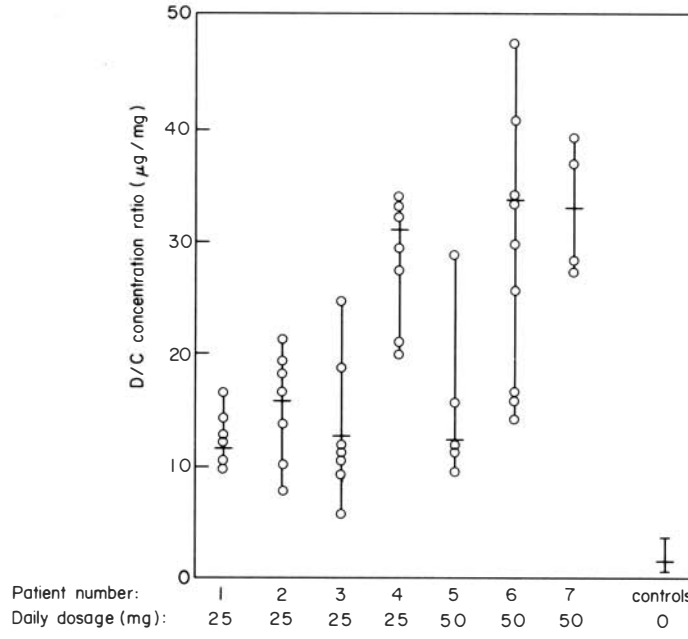

Fig. 1. Urinary DDS/creatinine concentration ratios (μg/mg) of all samples voided by 7 patients during 24 h. Horizontal lines indicate 24 h values.

no. 3). In one subject (patient no. 3) D/C concentration ratios were found to differ 3-fold in urine samples voided within one 2-h period. Analysis of variance, using a nested classification, revealed that the differences observed were highly significant: viz. between patients, $P < 0.01$; between times within patients, $P < 0.001$ (Table 2). Inspection of the time course of D/C ratios

TABLE 2
Analysis of variance of urinary DDS/creatinine concentration ratios. Values shown in Fig. 1

Source of variation	d.f.	Sum of squares	Mean square	*F*	*P*
Between doses	1	1871.92	1871.92	2.41	—
Between individuals within doses	5	3888.77	777.75	11.85	<0.01
Between 12-h periods within individuals	7	459.55	65.65	0.62	—
Between times within 12-h periods	36	3843.47	106.76	529.58	<0.001
Within times (error)	50	10.08	0.2016		
TOTAL	99	10073.79			

through the 24-h period of collection revealed no clear pattern of variation and there was no significant difference between ratios during the first and second 12-h periods following drug administration.

Discussion

A highly sensitive and reproducible assay procedure for the determination of DDS and hydrolizable metabolites in the urine has been described. Attempts were made in our laboratory to follow the adaptation of the Bratton and Marshall method described by Ellard *et al.* (1974*a*). The formation of a fine purple precipitate in standard solutions of 30 μg/ml and over led to interference with spectrophotometry, making the assay imprecise. If the precipitate is due to the insolubility of the azo dye so formed, then a certain amount of alcohol must be added with or just before the coupling component, as suggested by Bratton and Marshall (1939). Our modification of the assay procedure showed highly satisfactory reproducibility. Urine from untreated subjects yielded apparent D/C ratios of less than 3 μg/mg; some published figures using other modifications have yielded ratios up to 10 μg/mg. The present method therefore appears to be particularly applicable in studies of patients, such as ours, on low dosage regimens.

It was found that recovery of DDS added to urine samples of 10 normal subjects averaged 83%. The recovery rates varied between samples, however, to an extent which might be expected to influence DDS concentrations in clinical samples to a minor degree, calculated as less than 30%. No attempt has been made to measure recovery of the drug's acidic metabolites, which account for much of its total excretion (Ellard, 1966), so the clinical implication of this finding is at present incalculable.

DDS/creatinine concentration ratios of our patients were subject to substantial variability over the 24 h, the greatest difference between D/C concentration ratios within any one individual being $4\frac{1}{2}$-fold. This range is somewhat greater than that indicated by the repeat sample study performed by Low and Pearson (1974), and it is too great to be explained by differences in recovery in the method. No clear reason for such variation is apparent. In particular we found no consistent diurnal change such as would be expected from once daily drug administration.

Two of the patients (nos 1 and 6) were treated with diazepam, the first metabolite of which, desmethyldiazepam (Hillestad *et al.*, 1974) was found to yield colour with the assay procedure. Such interference is, however, unlikely to have contributed to the total variation in apparent DDS output because the metabolite has an elimination half-life of over 90 h and in such a case the urinary appearance of this compound and its further degradation products can change only minimally over the 24-h period.

Our results demonstrate that with determinations on single urine samples errors in classifying individual patients as taking their dapsone regularly or irregularly will arise in those who normally have quite variable D/C concentration ratios through the 24 h. The reliability of using one urine sample obtained

from the patient at a surprise visit to his home, as suggested by Ellard *et al.* (1974*b*) and carried out by Huikeshoven *et al.* (1976), is thus questioned. Our findings, however, do not invalidate the use of such samples in population studies from which general levels of patient compliance are deduced.

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