

Profile of urinary dapsone /creatinine ratios after oral dosage with dapsone

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Summary The ratios of the concentration of DDS plus its diazotisable metabolites to creatinine (D/C ratios) of successive urine samples collected after the ingestion of either single or consecutive daily doses of 100 mg DDS followed a pattern that was closely in accord with the hypothesis that once absorption is complete the rate of elimination of DDS and its diazotisable metabolites falls exponentially at a rate similar to the decline in plasma DDS concentrations. Diuresis influenced D/C ratios to only a minor extent. The results obtained indicated the validity determining dapsone compliance by determining D/C ratios of urine samples from either the individual patient or a group of patients self-administering their prescribed daily DDS medication, provided that the results obtained are compared with those achieved with fully supervised treatment.

Introduction

In 1974 my colleagues and I described a quantitative urine-test method for assessing the regularity with which leprosy patients self-administer their prescribed daily doses of dapsone (DDS).^{1,2} In this method the ratio of the concentration of DDS plus its diazotisable metabolites to creatinine in the urine is determined using simple colorimetric methods ('D/C ratio') and the D/C ratios of urine samples collected from out-patients self-administering their DDS treatment compared with those from in-patient controls given the same daily dose of DDS under strict supervision. We first used this method to investigate the regularity with which out-patients in Malawi ingested their prescribed treatment,² and it has subsequently been used by other investigators to monitor dapsone compliance among patients in Ethiopia,³ Tanzania,⁴ India,⁵⁻⁷ Kenya⁸ and Burma.⁹ Our method has however recently been criticized by Hagan and Smith¹⁰ who described an investigation that appeared

to indicate that the intra-individual variation in the D/C ratios of consecutive urine samples voided between repeated daily DDS doses may be so large and unpredictable as to make the determination of D/C ratios on single urine samples an unreliable measure of the compliance of individual patients. They also proposed an alternative modification of the Bratton Marshall procedure¹¹ for estimating DDS plus its metabolites that they claimed was more reproducible and likely to give lower "blank" values from urine samples from untreated controls than the modification¹ that we employed.

This report describes an investigation of the change with time in the magnitude of the D/C ratios of consecutive urine samples collected after the ingestion of single or repeated daily doses of DDS, and a comparison of the results obtained by the two modifications of the Bratton and Marshall procedure for determining DDS plus its metabolites.

Methods

Urine collections

The 10 healthy male adult volunteers who participated in these studies did so after giving informed consent. Subject A (the author) ingested consecutive daily doses of 50 and 25 mg DDS in Study I, single 100 mg doses of DDS in Studies II–IV, 6 consecutive daily doses of 100 mg DDS in Study V and 5 consecutive daily doses of 100 mg DDS in Study VI. The other 9 volunteers swallowed single 100 mg doses of DDS in Study IV. In order to ensure that traces of DDS or its metabolites were not carried over from one investigation to the next, each study was separated by an interval of at least a month. In Studies II–VI, the DDS doses were taken with a glass of water approximately 2 h after a light breakfast and 3 hr before lunch after first obtaining a pre-treatment urine collection.

In Study I complete urine collections were made from 0–2, 2–4, 4–6, 6–8, 8–10, 10–16 and 16–24 h on the day prior to the first DDS dose and after each of the two doses. In the second study urine collections were made at hourly intervals up to 4 h, at 2-hourly intervals from 4–12 h and at 12 hourly intervals from 12–96 h. In Study III urine collections were made at hourly intervals up to 4 h, at 2-hourly intervals from 4–16 h, from 16–23, 23–25 and 25–35 h, and thereafter at alternating 2- and 10-hourly intervals up to 169 h. In the fourth study complete urine collections were made from 0–2, 2–4, and 4–6 h, and hourly collections were then obtained at 1, 2, 5, 6, 7, 8, 9 and 14 days (i.e. from 23.5–24.5 h, 47.5–48.5 h, 119.5–120.5 h, etc.). In Study V hourly urine collections were made immediately before and after ingestion of the final DDS dose, and 2-hourly collections were then obtained from 1–11 h and at daily intervals up to 7 days (i.e. from 23–25 h, 47–49 h, etc.). In the

sixth study complete urine collections were made at 2-hourly intervals up to 8 h, from 8–20 h and then from 20–22 h and 22–24 h after the ingestion of each successive daily DDS dose. After measuring the volumes of the urine collections, samples were stored at -20°C prior to analysis.

Analytical methods

In Study I urinary concentrations of diazotisable compounds (as DDS equivalents) were determined using both Hagan and Smith's¹⁰ and our¹ modification of the Bratton and Marshall procedure. Thereafter only the latter modification was employed. Creatinine concentrations were estimated using an adaptation of the alkaline picrate method.¹

Results and Discussion

Comparison of Hagan and Smith's and our modification of the Bratton and Marshall procedure for the determination of DDS and its metabolites

Duplicate estimations were made using both modifications of the Bratton and Marshall procedure of the concentrations of diazotisable compounds in the 7 pre-treatment and 14 post-treatment urine samples collected in Study I. The concentrations of diazotisable compounds in the pre-treatment samples determined using Hagan and Smith's modification were consistently about 50% higher than those estimated by our procedure, mean pre-treatment D/C ratios averaging $4.4 \pm 0.7 \mu\text{g}/\text{mg}$ as compared with $2.9 \pm 0.3 \mu\text{g}/\text{mg}$ by our method. Despite their anticipation to the contrary, the increased 'blank' values obtained by Hagan and Smith procedure can readily be understood since in their method samples are acid-hydrolysed by boiling for 15 min prior to reaction, a step that could well result in the liberation of significant amounts of endogenous diazotisable amines.

There was however an extremely close correlation between the concentrations of DDS plus its metabolites determined among the post-treatment samples by the two modifications ($r = 0.96$), although Hagan and Smith's alternative gave slightly lower values (86%) than ours. The precision of both methods was excellent, duplicate errors averaging about $\pm 1.2\%$. Although in our modification of the Bratton and Marshall procedure¹ the azo dye formed from DDS settles out as an extremely fine precipitate after standing for 30–60 min, contrary to the experience of Hagan and Smith,¹⁰ we have never found that this led to inaccuracies in the measurement of optical densities 5 min after the addition of the N-naphthyl-ethylene-diamine reagent. It was therefore concluded that Hagan and Smith's modification of the Bratton and Marshall procedure for determining DDS and its metabolites is no more

reproducible, and probably less specific than the simpler procedure that we originally described.

Time profile of urinary D/C ratios after the ingestion of single doses of DDS

The results obtained after the ingestion of single doses of 50 mg and 100 mg DDS (Studies I–IV) are summarised in Table 1 and the data from the most extensive investigation (Study III) illustrated in Fig. 1. Urinary D/C ratios

Table 1. D/C ratios after the taking of single or multiple daily doses of DDS (Subject A)

Dose DDS (mg)	No. doses	Peak D/C ratio* ($\mu\text{g}/\text{mg}$)	Duration of > 90% Peak (h)	Pretreatment D/C ratio ($\mu\text{g}/\text{mg}$)	Asymptotic** D/C ratio ($\mu\text{g}/\text{mg}$)	Half-life** (h)
50	1	29	2–6	2.9	—	—
100	1	54	1–8	4.8	3.6	24
100	1	60	3–8	3.6	4.4	23
100	1	52	2–4	3.0	3.7	24
100	6	93	1–7	56 [†]	4.8 [‡]	29
100	5	97	0–2	60 [†]	—	—

* Ratio DDS equivalents/creatinine

** Estimated using an iterative procedure (see text).

[†] Prior to final DDS dose

[‡] After final DDS dose

reached near maximal values after 1–2 h and, after allowing for pretreatment blank values that averaged $3.7 \mu\text{g}/\text{mg}$, peak ratios were approximately proportional to dosage. From 8 h, when it was assumed that absorption of the drug must have been complete, D/C ratios of successive urine collections were closely similar (Fig. 1) to those calculated on the assumption that they could be represented by the sum of 2 components, one due to the elimination of DDS plus its diazotisable metabolites that fell exponentially with time, and the other representing natural diazotisable compounds whose magnitude did not change with time ('asymptotic' D/C ratio). An iterative computer program was used to calculate the best fitting curve to the observed data illustrated in Fig. 1. The decline in the rate of elimination of DDS plus its diazotisable metabolites was equivalent to a half-life of 24 h. Such a half-life was identical to that estimated for the urinary elimination of DDS plus its N-glucuronide after giving a further 100 mg dose of DDS (Study IV) and using a sensitive and specific high-pressure liquid chromatographic method to determine DDS plus acid-labile DDS.¹² Similar estimates of the half-life for the urinary elimination of DDS and its diazotisable metabolites and the asymptotic D/C ratio were obtained when the D/C ratios of consecutive urine samples collected after the ingestion of the other 2 single doses of 100 mg DDS (Studies II and IV) were analysed by the iterative computer program. (Table 1).

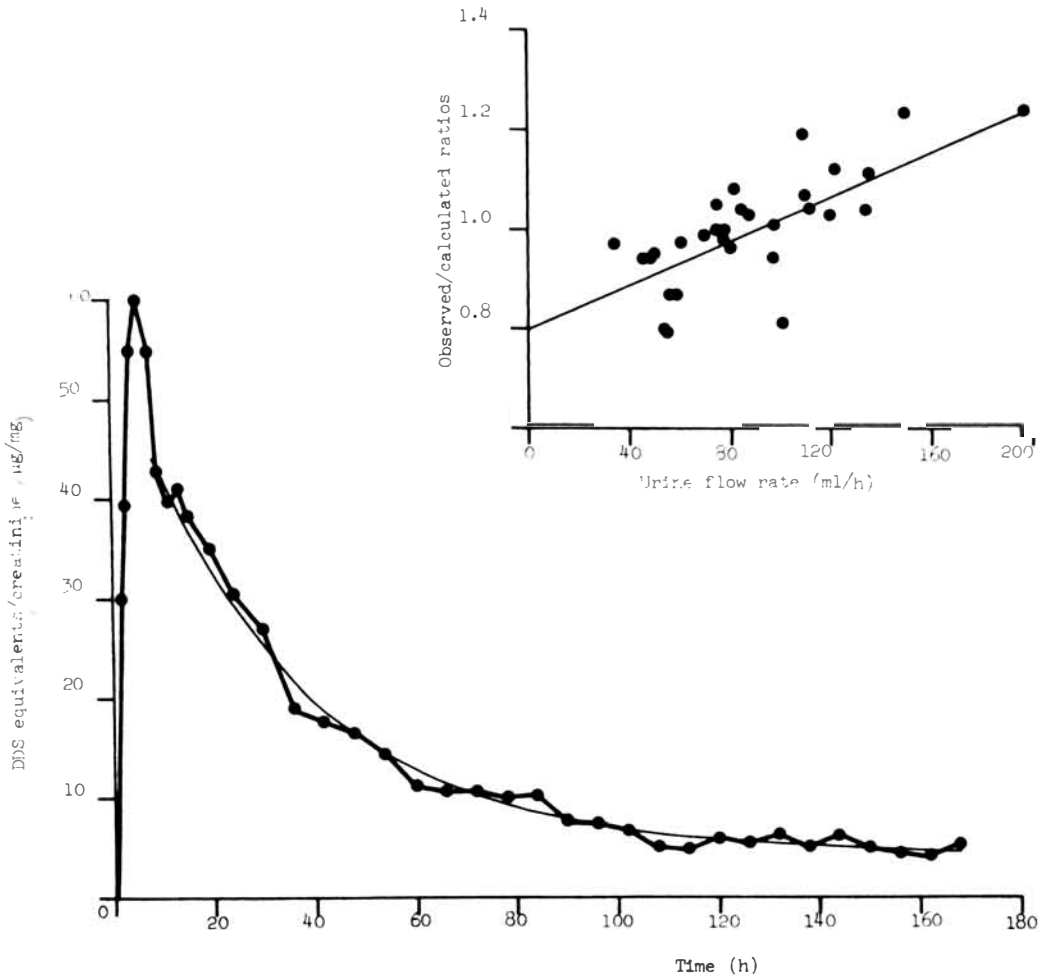


Figure 1. Time profile of urinary D/C ratios after the ingestion of a single dose of 100 mg DDS. Points represent the observed ratios, while the calculated ratios are shown as the smooth fine line. The insert shows the correlation between observed/calculated D/C ratios and urine flow rate.

Even though the average absolute difference between the individual observed and calculated D/C ratios after giving the second 100 mg DDS dose was only 8% (Fig. 1), there was a close correlation ($r = 0.73$, $p \ll 0.001$) between the ratios of observed to calculated values and urine flow rates (insert Fig. 1). This indicates that measuring D/C ratios does not compensate perfectly for the effect of diuresis on the excretion of DDS and its metabolites. Nevertheless the effect of urine flow on D/C ratios is small since it was estimated that an increase in urinary excretion from 30 to 120 ml/h would only result in D/C ratios increasing by about 25%.

The D/C ratios of the urine samples collected from the 10 volunteers pre-treatment and at varying times after the ingestion of single 100 mg doses of DDS (Study IV) are summarised in Table 2. In every case peak D/C ratios were

Table 2. D/C ratios after the taking of single doses of 100 mg DDS (10 subjects)

Time (h)	D/C ratios* ($\mu\text{g}/\text{mg}$)	Time (days)	D/C ratios ($\mu\text{g}/\text{mg}$)
0	3.0 (1.9–4.4)**	5	4.4 (3.1–6.7)
1	23.6 (9.1–41.8)	6	3.9 (2.6–5.1)
3	45.2 (28.2–70.7)	7	3.6 (2.2–6.3)
5	39.2 (25.0–54.8)	8	3.4 (2.0–4.9)
24	21.7 (15.1–26.9)	9	3.0 (1.4–4.1)
48	11.1 (6.6–15.8)	14	3.1 (1.7–4.0)

*Ratio DDS equivalents/creatinine

**Arithmetic mean and range

encountered at 3 h (2–4 h urine collections). Thereafter the D/C ratios steadily decreased until by 9 days they had reached pre-treatment values. From 24 to 48 h the component due to DDS and its metabolites declined on average at a rate equivalent to a half-life of 20 h, which is similar to a value of 23 h that may be calculated from the data set out in Table 2 of our original paper¹ for the D/C ratios of urine samples collected from Malaysian leprosy patients after the ingestion of a single dose of 200 mg DDS. An analysis of the data obtained from the 10 volunteers investigated in the Study IV indicated that the average absolute difference between the individual observed D/C ratios and the values predicted on the assumption that they were equal to the sum of an exponentially falling component plus a constant, probably lay between 11% and 20%. The similarity in these estimates of the average half-life for the urinary elimination of DDS plus its diazotisable metabolites and those published for the decline in plasma DDS concentrations^{2, 13–17} suggests that the rate of urinary excretion of DDS and its diazotisable metabolites is probably directly proportional to the concomitant plasma DDS concentrations.

Pattern of urinary D/C ratios after the ingestion of repeated daily doses of DDS

After giving 5 or 6 consecutive daily doses of 100 mg DDS (Studies V and VI) peak and trough D/C ratios gradually increased to about 95 and 58 $\mu\text{g}/\text{mg}$, respectively (Table 1). Such a pattern was to have been anticipated from the previously estimated half-life (about 24 h) for the elimination of DDS and its diazotisable metabolites. The results obtained in Study VI demonstrated that the D/C ratios of urine samples collected during any one 24-h period after giving successive daily doses of DDS varied over a very limited range (from

1.3- to 1.7-fold). This finding, coupled with the smooth and expected time-pattern of D/C ratios after giving a single 100 mg dose of the drug (Fig. 1), is in sharp contradiction to the results described by Hagan and Smith¹⁰ who reported D/C ratios ranging up to 4.5-fold over a period of 24-h among patients receiving supervised daily doses of DDS and concluded that the time course of their variation showed no closely predictable pattern. By contrast the findings of the present study support earlier evidence obtained by Low and Pearson⁴ for limited intra-individual variability in D/C ratios. Thus an analysis of the data illustrated in Fig. 3 of Low and Pearson's paper indicates that among the 12 Ethiopian patients they studied, the D/C ratios of urine samples collected immediately before the next daily supervised dose was swallowed, varied on average by only about $\pm 15\%$ from day to day. This evidence indicates that reliable information on individual patient compliance may be acquired by comparing the D/C ratios of single urine samples collected at either surprise home visits or out-patient clinic attendances with those of control samples obtained after a period of about 5 days supervised daily treatment. The chief limitation to monitoring individual compliance is therefore probably the impracticability of giving the supervised treatment in many situations rather than intra-individual variability in D/C ratios.

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