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Editorial

DRUG COMPLIANCE IN THE TREATMENT OF LEPROSY*

Recent publications in this journal concerning the regularity with which leprosy patients self-administer their dapsone treatment[1–3] and of improved techniques for monitoring dapsone compliance[4–7] make this an appropriate time to review the findings of previous investigations concerning the regularity of dapsone self-administration and to discuss their implications for the strategy of leprosy treatment.

Investigations concerning the regularity of self-administration in the treatment of other diseases indicates that poor compliance is an extremely widespread phenomenon.[8–13] Extensive studies of compliance of tuberculosis patients have been carried out in several Third World countries in situations where leprosy is also endemic using simple colorimetric urine-tests to monitor the ingestion of isoniazid.[14–19] In two of these studies[17, 18] significant correlations were demonstrated between irregularity in drug self-administration, as indicated by the proportion of urine samples giving negative tests, and inadequate therapeutic response. Investigations in India[20] and East Africa[21–23] have also demonstrated that regimens that are highly effective when used in controlled clinical trials are often much less effective in routine use, primarily because patients do not continue with their treatment for an adequate length of time. Such evidence concerning the prevalence and therapeutic importance of poor compliance provided the impetus for initiating controlled clinical trials to investigate the efficacy of supervised intermittent regimens and of short-course chemotherapy in the treatment of pulmonary tuberculosis.[24–6]

The dapsone/creatinine ratio method for monitoring the ingestion of dapsone

The most satisfactory method of monitoring patient compliance is by means of simple procedures to specifically detect the prescribed drugs or their metabolites

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in the urine. Since dapsone, and most other antileprosy drugs are prescribed for daily self-administration, a highly satisfactory urine-test procedure would be one that gave reliably positive results for up to 12–15 h after the ingestion of a standard daily dose of the drug and consistently negative results within 48 h. With such a test, a negative result would indicate unequivocally that a patient has omitted to ingest at least one of his or her prescribed doses.

Two factors frustrate efforts to devise such a simple test to monitor dapsone self-administration. Firstly, dapsone and its metabolites are eliminated very slowly in the urine at rates that parallel the decline in dapsone serum concentrations.[27] This decline is equivalent on average to a half-life of about 27 h.[28] Secondly, colorimetric procedures for detecting dapsone are unspecific. Thus the most satisfactory colorimetric method for estimating dapsone, in which its aromatic amino groups are diazotized and coupled with N-1-naphthyl-ethylenediamine, gives colours with a wide range of other compounds containing aromatic amino groups.

For these reasons qualitative urine-tests give positive results for several days after the ingestion of a single therapeutic dose of dapsone and attempts to read tests in such a way as to avoid classifying concentrated dapsone-free urine samples with substantial amounts of natural diazotisable compounds as positive are liable to result in categorizing dilute dapsone-containing samples as negative.[29] The effects of diuresis can, however, be largely overcome by quantitatively determining the ratio of the concentration of dapsone plus its diazotisable metabolites to creatinine in the urine using modifications of the Bratton and Marshall and alkaline picrate colorimetric methods, respectively.[27, 29, 30] Urine samples can be conveniently preserved by the addition of hydrochloric acid or thymol and do not need to be refrigerated.

Studies of dapsone compliance using this method have been reported from Malawi, Ethiopia, India, Burma, Tanzania and Kenya.[1–3, 30–8] Most outpatients are prescribed dapsone for daily self-administration. Estimates of their compliance were obtained by comparing the dapsone/creatinine ratios of urine samples collected during their visit to a rural, urban or hospital clinic with those of samples from supervised and non-treated controls, respectively. The samples from the supervised controls were generally collected from hospitalized patients 24 h after they had swallowed at least 4 consecutive supervised daily doses of dapsone of the same denomination as that given to the outpatients. The results of such investigations are summarized in Table 1 and indicate that irregular self-administration of dapsone is very widespread. In every study the dapsone/creatinine ratios of urine samples collected from the outpatients were significantly less than those from the supervised controls indicating that substantial proportions of the dapsone treatment prescribed for and collected by the outpatients were not being ingested. The overall level of self-medication as indicated by the mean dapsone/creatinine ratio of urine samples collected from outpatients could be interpreted in two ways. Firstly, if it is assumed that

the pattern of dapsone ingestion immediately prior to collection of the urine samples is typical of the patients' general compliance, the approximate percentage of prescribed dapsone doses being ingested can be calculated.[30] Such estimates calculated from the results obtained in investigations carried out in Malawi, Ethiopia, India and Burma are summarized in Table 1. Alternatively, the data can be analysed to provide an approximate estimate of the average length of time since patients had swallowed their last dose of dapsone.[1]

Table 1. Investigations of compliance using the dapsone/creatinine ratio method among outpatients prescribed dapsone for daily self-administration

Investigation	Clinic*	Number patients	Doses taken** (%)	Grossly irregular patients** (%)	Reference
Blantyre, Malawi (1974)	R	164	53	30	30
Addis Ababa, Ethiopia (1974)	U	89	42	11	31
Gudiyatham Taluk, India (1976)	R	100	87	11	32
Chinglepattu, India (1977)	R	90	59	–	33
Mandalay and Rangoon, Burma (1979)	H	170	74	24	34
	U and R	455	24	56	
Bombay, India (1979)	U	260	–	27	35
Gudiyatham Taluk, India (1981)	R	125	60	37	1
Mazuffarpur, India (1981)	R	44	–	25	2
Dichpalli, India (1981)	R	55	34	47	36
Addis Ababa, Ethiopia (1981)	U	368	78	11	3

* R = Rural, U = Urban, H = Hospital.

**For definitions see text.

The dapsone/creatinine ratios of many patients were clearly much less than those of the supervised controls and various definitions have been proposed for categorizing patients as being either 'irregular' or 'grossly irregular' in their dapsone self-administration. The proportions of patients classified as grossly irregular in Table 1 are those with apparent dapsone/creatinine ratios within the range found among samples from control subjects not receiving dapsone and varied from 11–56% of those studied. Such grossly irregular patients had probably not swallowed a tablet of dapsone for at least 4 days prior to the collection of the test urine sample. The question as to whether urine samples that have been collected from patients on the occasion of a visit to a clinic to replenish their stock of dapsone tablets are likely to be representative has been discussed previously.[30] Interestingly, in the one study where urine samples were collected by means of both surprise home visits and during routine clinic attendances, significant differences were not encountered between the results from the two sets of samples.[1]

Therapeutic implications of poor dapsone compliance

Substantial failure to self-administer prescribed dapsone-treatment results not only from patients failing to ingest the tablets that they have collected but also because a substantial proportion of patients default from treatment altogether.[39, 40] Thus a recent study from South India has shown that after their first year's treatment many lepromatous patients become increasingly irregular in collecting their drug supplies and in self-administering dapsone and that such poor compliance may account for the failure of many patients to achieve clinical and bacteriological quiescence despite prolonged periods of dapsone monotherapy.[36] However, because a single dose of 100 mg dapsone results in serum and tissue drug concentrations that are approximately 500-fold in excess of its minimal inhibitory concentration against *Mycobacterium leprae* and since the relatively long half-life of dapsone is such that inhibitory concentrations of the drug are probably maintained for about 10 days,[28] it is clear that the failure to self-administer dapsone must be extremely prolonged before it results in clinical and bacteriological relapse caused by the renewed multiplication of fully dapsone-sensitive *M. leprae*. Such a conclusion is strengthened by the observation that over the short term the response of a small group of patients treated with a dose of 1 mg dapsone/day was indistinguishable from that routinely observed with standard 50 or 100 mg daily doses.[41]

The most important therapeutic penalty of poor dapsone compliance is likely to be an enhanced risk that lepromatous patients will relapse after many years of treatment due to the emergence of dapsone-resistant strains of *M. leprae*. It is extremely difficult to prove an association between poor compliance and relapse with dapsone-resistant leprosy many years later because records of out-patient treatment attendance are rarely well documented over long periods and urine-testing to monitor dapsone ingestion has only been introduced relatively recently. Nevertheless, Shepard and his colleagues obtained strongly suggestive evidence indicating that irregular treatment with dapsone is likely to be a major factor encouraging the development of dapsone resistance,[42] while Jacobson reached a similar conclusion from his review of the case histories of over 75 patients with dapsone-resistant leprosy found over a 12-year period at the Carville United States Public Health Service Hospital.[43] Other evidence for the importance of maintaining high concentrations of dapsone to prevent the subsequent emergence of dapsone resistance comes from studies concerning the epidemiology of dapsone resistance in Malaysia and Ethiopia. In the Malaysian investigation it was found that lepromatous inpatients whose treatment was initiated with injections of solapsone (equivalent to approximately 10 mg dapsone/day) were three times more likely to relapse with dapsone-resistant leprosy than those whose treatment started with dapsone in full dosage.[44] In Ethiopia, where standard treatment during the period 1965–74 for outpatients consisted of weekly doses of

Table 2. Decline in the antileprosy activity of a 100 mg dapsone dose with time

Days	Dapsone serum concentration (% initial)	(μg/ml)	Inhibition *M. leprae* S*	low R**	medium R†
2	29	0.44	+	+	±
3	16	0.24	+	+	—
4	8.5	0.13	+	+	—
5	4.5	0.07	+	+	—
6	2.5	0.04	+	±	—
7	1.5	0.02	+	—	—
10	0.2	0.003	±	—	—

* Fully drug-sensitive.
**Resistant to 0.0001% dapsone in mouse diet.
† Resistant to 0.001% dapsone in mouse diet.

5 or 10 mg dapsone that were gradually increased to a maximum of 200–300 mg over 6–9 months,[45] the incidence of acquired dapsone resistance was alarmingly high (about 3% per annum) and resistance to dapsone developed more rapidly than in Malaysia.[46]

Standard doses of 50 or 100 mg dapsone taken regularly each day should not only completely prevent the growth of fully sensitive organisms but also that of mutants with low degrees of dapsone resistance,[47] while substantial interruption of treatment will permit such mutants to multiply. Repeated interruptions of treatment over many years can therefore be expected to result in the step-wise selection of mutants with increasing levels of dapsone resistance until organisms are completely insusceptible to inhibition by the highest tolerated dapsone doses. The bacteriological and pharmacological basis for such selection is set out in Table 2. In constructing this table it has been assumed that a single dose of 100 mg dapsone results in peak serum concentration of about 1.5 μg/ml, that serum and tissue dapsone concentrations then decline at a rate equivalent to a half-life of 27 h and that the minimal inhibitory concentration of dapsone against fully sensitive strains of *M. leprae* is about 0.003 μg/ml. The evidence for these assumptions and for the excellent tissue penetration of dapsone have been reviewed elsewhere.[28, 48] It will thus be apparent that if patients are prescribed 100 mg dapsone for daily self-administration, mutants with low degrees of resistance (minimal inhibitory concentration of about 0.03 μg/ml) might start to multiply after the omission of about 6 consecutive daily doses.

More sensitive methods for monitoring dapsone compliance

While the dapsone/creatinine ratio method has demonstrated the ubiquity of the failure of patients to take their prescribed dapsone treatment, the lack of

specificity of the Bratton and Marshall procedure for detecting dapsone and its metabolites, exemplified by the significant blank values of urine samples from untreated subjects, results in the method being insufficiently sensitive to identify omissions of more than about 4 days treatment. However, as indicated by the data set out in Table 2, it is probable that significant therapeutic penalties are likely to be incurred in the long run only when gaps in dapsone self-administration of 6 days or more occur. By this time dapsone serum and tissue concentrations and urinary excretion will have fallen to about 1–2% of that encountered if the prescribed treatment were being regularly ingested. There is therefore a need for more sensitive and specific assay methods to detect dapsone and its metabolites in the urine.

After a single dose of 100 mg dapsone, urinary concentrations of dapsone in excess of about 0.02 μg/ml are maintained for 7 days.[49] High pressure liquid chromatographic or thin layer chromatographic techniques of this order of sensitivity have been developed by several groups of workers,[50–3] but none of these methods is simple enough for ready application to comprehensive studies of dapsone compliance. It is for this reason that the pioneering investigations of Huikeshoven and his colleagues[4, 54–6] to develop simple enzyme-immunoassays of great sensitivity and specificity for detecting dapsone and its metabolites in body fluids are of such promise.

In the first of their two 'ELISA' (Enzyme-linked immunosorbent assay) methods,[4] aliquots of test urine samples and a solution containing a dapsone-specific immunoglobulin coupled to horseradish peroxidase are pipetted into the wells of a microtitre plate coated with a bovine serum albumin dapsone conjugate. After a washing step, the bound peroxidase is visualized using hydrogen peroxidase and 5-amino-salicylic acid, the amount of colour being inversely related to the concentration of dapsone and its metabolites in the test sample. When read by eye the method was capable of detecting concentrations of down to about 0.01 μg/ml dapsone in urine and gave reliably positive results when applied to urine samples collected from 9 of 10 volunteers for 8 days after the ingestion of single doses of 100 mg dapsone.

The second method described on page 125 of this issue, was approximately ten times more sensitive. In this method competition occurs between dapsone and a horseradish peroxidase dapsone conjugate for binding with antibody absorbed onto the microtitre well.[5] When this method was applied to the detection of dapsone in finger prick blood from two volunteers after the ingestion of single 100 mg dapsone doses, consistently positive results were obtained for 5–6 days.[6] Although such a finger prick blood method avoids the complication to the interpretation of urine assays caused by the effects of diuresis, it is likely to be considerably less sensitive since the concentrations of dapsone and its metabolites in the serum are much lower than those in the urine. Such tests are probably also less acceptable to patients. As pointed out by Rook in his letter on page 281, further increases in the sensitivity of these

enzyme-immunoassays are to be anticipated if the peroxidase substrate used in these investigations, 5-amino-salicylic acid, were replaced by the more recently discovered ABTS reagent (2,2′-azino-di-(3-ethyl benzthiazoline-6-sulphonic acid)).[57] The combination of 3-(dimethylamino) benzoic acid and 3-methyl-2-benzo-thiazolinone hydrazone hydrochloride monohydrate (MBTH) described by Ngo and Lenhoff[58] might also be investigated.

Improved enzyme immunoassay methods could be used to identify those outpatients whose dapsone compliance was so poor that dapsone urinary concentrations (or dapsone/creatinine ratios to allow for diuresis[49]) were less than 1–2% of those among samples collected from supervised controls receiving daily doses of 100 mg dapsone. Such exceedingly irregular patients, who, as will be seen from Table 2, would be most at risk of relapsing on account of the selection of dapsone-resistant strains of *M. leprae,* could then have their treatment specifically supplemented with acedapsone injections.

Monitoring the ingestion of other antileprosy drugs

The increasing prevalence of dapsone-resistant strains of *M. leprae* among lepromatous patients that has arisen as a result of past treatment with dapsone monotherapy has emphasized the importance of treating all new lepromatous patients with combinations of antileprosy drugs. The most promising drugs for use in combination with dapsone or for the treatment of patients with dapsone-resistant leprosy are rifampicin, clofazimine, ethionamide, prothionamide and thiacetazone.[28] The regularity with which such companion drugs are taken is likely to be a major factor in determining their potential value in preventing the emergence of dapsone resistance. An experimental investigation in the mouse foot-pad model has indicated that poor compliance would probably severely impair the efficacy of thiacetazone, since it is an inherently weak bacteriostatic drug,[59] and would compromise the bactericidal activities of ethionamide and prothionamide.[60]

The taking of a standard 600 mg dose of rifampicin can often be detected simply from the characteristic orange/brown colour that it imparts to urine samples collected within 6–8 h of ingestion. Rifampicin can, however, be reliably demonstrated in the urine for at least 12 h using a simple plate diffusion test with *Staphylococcus aureus,*[61] while an alternative microbiological procedure of far greater sensitivity has also been described.[62]

No method has as yet been described for monitoring the ingestion of clofazimine. Among light-skinned patients the degree of skin pigmentation may provide an indication of the extent to which the drug is being taken, but it is in just such patients that the clofazimine is likely to be least acceptable. A urine-test method for detecting the ingestion of ethionamide has been described by Eidus and Harnanansingh.[63] This method, which depends on the extraction and

concentration of its yellowish sulphoxide metabolite, is also applicable to detecting the ingestion of prothionamide and gives reliably positive results for at least 12 h after the ingestion of 250 mg doses of either drug.[63, 64]

No satisfactory colorimetric procedure exists for monitoring the ingestion of thiacetazone, but its excretion in the urine can be detected by extracting into chloroform/amyl alcohol and scanning the ultraviolet absorption spectrum of the extract to detect an absorption peak at about 333 mμ.[65] Reliably positive results may be anticipated for about 36–48 h after dosage with 150 mg of the drug.

Combined formulations of rifampicin plus isoniazid, ethionamide plus isoniazid, prothionamide plus isoniazid and thiacetazone plus isoniazid are commercially available for use in the treatment of tuberculosis while a combination of dapsone plus prothionamide plus isoniazid has been developed for use with rifampicin in the treatment of both leprosy and tuberculosis. The use of such isoniazid-containing combinations could considerably facilitate compliance studies concerning the ingestion of these drugs since their ingestion can be readily detected using colorimetric procedures to detect the presence of metabolites of isoniazid in the urine.

The Eidus and Hamilton procedure[66] for detecting acetylisoniazid is extremely simple. Twelve urine samples can be reacted at a time using a white procelain plate with hemispherical depressions with the dropwise addition of aqueous potassium cyanide and chloramine-T. A positive result is indicated by the appearance of a pink/red colour within 5 min. Urine samples collected after a 300 mg dose of isoniazid, the size of the isoniazid component of most of these combined formulations, would give reliably positive results for about 12 h and be uniformly negative after 48 h.[64, 67, 68]

The urine-test method for monitoring isoniazid ingestion based on detecting the metabolites isonicotinic acid and isonicotinylglycine[69] is slightly more elaborate involving the addition of pH 5 acetate buffer and the reagents potassium cyanide, chloramine-T and barbituric acid. A positive result is indicated by the formation of a blue/purple colour within 15–30 min. This method is much more sensitive than the acetylisoniazid procedure and gives reliably positive results for about 48 h after the ingestion of 300 mg isoniazid.[64] Such an approach was used to monitor the self-administration of thiacetazone in the Ethiopian compliance study reported on page 147.

Such standard therapeutic doses of isoniazid are very well tolerated so that the use of such combined formulations for relatively limited compliance studies poses no unacceptable hazard to the patients. The sensitivity of the isonicotinic acid method for detecting isoniazid ingestion is however so great that it is possible to use minute doses of isoniazid as an innocuous marker for monitoring the self-administration of medicaments for daily self-administration since over 99% of the urine samples collected within 18 h of ingesting 6 mg isoniazid gave positive results.[70] Such doses of isoniazid have been incorporated into capsules

or tablets containing dapsone, thiacetazone, ethionamide or prothionamide in preparation for future studies concerning the regularity of their self-administration by leprosy patients.

Supplementing daily self-administered dapsone with supervised intermittent treatment of other drugs

Just as the demonstration of irregular drug self-administration in the treatment of tuberculosis prompted the introduction of supervised chemotherapy, the evidence of poor dapsone compliance in leprosy illustrated by the investigations summarized in Table 1 has encouraged the advocacy of supplementing oral daily doses of dapsone with injections of 225 mg acedapsone given once every 3 months.[47, 71–5] Such injections, which release on average about 2 mg dapsone into the circulation each day and maintain dapsone serum concentrations in excess of about 0.03 μg/ml,[76–8] would prevent the multiplication of both fully sensitive *Mycobacterium leprae* and mutants with low degrees of dapsone resistance and would significantly augment the dapsone levels of patients whose compliance as defined above was exceedingly irregular.

In view of the extremely rapid and powerful bactericidal activity of rifampicin against *M. leprae*[79–81] and its high cost, the most advantageous way to supplement self-administered daily dapsone treatment would probably be to give single 600 mg doses of the drug once-monthly to outpatients on the occasion of a clinic visit to collect their stock of dapsone tablets.[75] An additional rifampicin dose could also be given for self-administration on the following day.[82] Supervised monthly doses of clofazimine might be given in a similar manner.[83] However experimental studies indicate that ethionamide, prothionamide and thiacetazone are probably unsuitable drugs for intermittent administration.[60]

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