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Spot Test for Detection of Dapsone in Urine: An Assessment of Its Validity and Interpretation in Monitoring Dapsone Self-administration[1]

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In 1965, de Castro, *et al.* ([2]) described a simple spot test for the detection of dapsone in urine, employing filter paper impregnated with a modified Ehrlich's reagent. Spots of urine on the impregnated paper show a yellow ring at the periphery caused by urea and an inner spot of orange when the drug is present. Noordeen and Balakrishnan ([6]) reported the test to be reliably positive in urine collected before 48 hours following the administration of 10 to 75 mg of dapsone to children. Ellard, *et al.* ([3]) could not affirm this high degree of sensitivity. In their hands, one day after giving single doses of 200 mg of dapsone to 34 patients only 23 (68%) urine samples were positive. Recently, however, Irudaya Raj, *et al.* ([5]) reported positive spot tests in the urine of 13 (59%) out of 22 patients six days after a last daily dose of 100 mg dapsone, while all urines were positive one day after the last dose.

The present study aims to clarify the sensitivity and, hence, the validity and interpretation of this simple test for monitoring dapsone self-administration.

MATERIALS AND METHODS

Urine samples. After giving their informed consent, 20 volunteers (15 men and 5 women) ingested four consecutive daily doses of 100 mg dapsone. Urine samples were collected at 9 a.m., immediately before the dapsone doses were taken, and at the same hour on each of ten days following the last dapsone intake; the urine used was never the first morning specimen. Dapsone was added to pairs of randomly selected, pretreatment urine samples of the volunteers to final concentrations of 1, 2, 3, 4, 5, 7.5, 10, 12.5, 15, and 20 μg/ml, respectively, to serve as internal standards. The specimens were preserved by thimerosal in a final concentration of 0.02%; they were coded and randomized prior to testing.

Spot tests. The method of de Castro, *et al.* ([2]) was employed with the following modifications. Chemicals for the impregnation of the paper were dissolved completely in 70% ethanol, thus giving brighter spots than before when very little nacconol was dissolved in absolute ethanol. The color intensities of 50 μl urine spots on the impregnated paper were compared with that of a standard spot using acidified urine containing 5 μg dapsone per ml (final HCl concentration = 0.1 N) both before and after the addition of 50 μl drops of 1 N HCl to the spots. Portions of the urine specimens were sent to Cebu City, Philippines, where a second observer (MGM) repeated the tests. Both observers repeated the tests once again on duplicate portions of the specimens.

D:C ratios and T½ values. The dapsone:creatinine (D:C) ratios in urine specimens were determined as described by Ellard, *et al.* ([3]). Individual values for the half-time (T½) of dapsone elimination were derived from the linear regression of the logarithm of the D:C ratios in the first six urines obtained after the last dapsone intake with deduction of the blank "D:C ratio" found in the pretreatment urine sample of each volunteer.

RESULTS

Results obtained with the 20 internal standards indicate that generally assessments were correct. However, in standards containing dapsone concentrations of (or near) the reference value of 5 μg/ml, the first observer was more inclined to positive assessments than the second observer. Table

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TABLE 1. *Variations between sets of spot test results in 296 urine specimens.*

Comparison	Variation	p[a]
Within 1st observer	1.4%	>0.6
Within 2nd observer	4.1%	>0.4
Between obs., 1st sets	4.7%	<0.001
Between obs., 2nd sets	6.1%	<0.001

[a] Indicates the significance of the difference between the two sets of results in the observed direction as assessed by McNemar's test for paired results.

1 shows low within observer variations which were not statistically significant, indicating that both observers were consistent in their assessments of urine specimens by the spot test. The between observer variations were low as well, but the first observer had significantly more positive results than the second observer in consistency with the findings in the internal standards.

In Table 2, the first observer's first set of results are summarized. On the first day after the fourth dapsone dose, all 20 urines were positive provided that acid was added to the spots. On the seventh day and thereafter, all urines were negative. In further results only the acidified spots will be presented.

Taking into consideration that sampling was done with a 24-hr interval and that, as a consequence, urines on the average may have been negative half a day earlier, the mean of the expected time of urine turning negative was 87 hr with a standard error (S.E.) of 8.6 hr, giving 95% confidence limits for the population mean of 69–105 hr (2.9–4.4 day). In terms of missed doses, this means that, on the average, a first negative spot test would be expected after 2–3 successive failures to take dapsone.

The T½-values for dapsone elimination ranged from 15.2 to 47.2 hr with a mean of 27.8 hr, in good correspondence with the literature ([1]). A positive but weak linear correlation was observed between the times for spot tests to turn negative and the individual T½-values ($r = 0.52$; $p < 0.02$). A slightly stronger positive linear correlation was found between the times for spot tests to turn negative and the average creatinine concentrations in each volunteer's urine specimens ($r = 0.65$; $p < 0.002$).

The average concentrations of dapsone plus its diazotizable metabolites in the volunteers' first negative urine specimens was 6.3 μg/ml, as estimated by corrected D:C ratios multiplied by creatinine concentrations.

TABLE 2. *Summary of first observer's first set of dapsone spot test results in urine of 20 volunteers compared with a standard containing 5 μg dapsone per ml of urine.*

Day after 4th dose	Urine only		Urine plus HCl	
	+	−	+	−
1	17	3	20	0
2	14	6	14	6
3[a]	12	7	13	6
4	5	15	7	13
5	1	19	5	15
6	1	19	1	19
7[a]	0	19	0	19
8	0	20	0	20
9[a]	0	19	0	19
10[a]	0	19	0	19

[a] No urine was received from one volunteer.

DISCUSSION

The addition of HCl to the spots appeared to improve the sensitivity of the test. Acidification of the urine spots was first practiced by Ellard, *et al.* ([3]) who employed 0.1 M citric acid for increasing the homogeneity and intensity of the central orange spot and decreasing the intensity of the yellow periphery. In a previously reported multi-center evaluation of the test, we showed that a 50 μl drop of 1 N HCl corrects both false-negatives due to high pH and false-positives due to crossreacting sulfonamides ([4]). In the same study, the advantage of dissolving nacconol completely in 50–70% ethanol was discussed. In the present study, using these modifications, urine specimens of all 20 volunteers taken 24 hours after their fourth dapsone dose were positive, if read against a standard of 5 μg/ml of dapsone in urine. Six days later all urine specimens were negative, and it was calculated that, on the average, urines may be expected to turn negative after 2–3 successive failures to take dapsone.

As could be expected, both the speed of dapsone elimination and the extent of diuresis affected the positivity of the spot tests, the second factor slightly more than the first. The influence of diuresis may at least partly

explain the less satisfactory results in the study of Ellard, *et al.* [3]. In their study, the creatinine concentrations in the urine samples ranged from 0.01 to 1.93 mg/ml and averaged 0.66 mg/ml; whereas in the present study, the range was 0.17–3.52 mg/ml with a mean of 1.46 mg/ml. On the other hand, in the study of Irudaya Raj, *et al.* [5] the spot test appeared to be considerably more sensitive than in our study; whereas the creatinine concentrations in their study averaged only about 0.85 mg/ml. We have no explanation for these contrasting results apart from the inherent subjectivity in the assessments by the naked eye. Note that we read the spots against a standard of dapsone in urine, whereas the authors referred to used a solution of dapsone in water. The latter gives a slightly different color which adds to the subjectivity of the reading.

In view of the large variations in diuresis in different populations, and because of the relatively small number of volunteers on which our results were based, no simple inferences can be made regarding the chances that a negative spot test might appear within 24 hours after the last daily 100 mg dapsone dose and that spots are still positive in urine collected six days later. However, for practical purposes in the field of leprosy control, any positive test could be interpreted as indicating that dapsone was very likely taken at least sometime during the last seven days and, on the average, not longer than four days previously, which would guarantee a blood level well above the minimal inhibitory concentration (MIC) against fully sensitive *Mycobacterium leprae* strains [1]. On the other hand, any negative test would indicate that dapsone was very likely not taken according to schedule, on the average not during the past four days, and possibly so long ago that the blood level has fallen below the MIC.

This broad interpretation may yet be of practical value in the management of leprosy patients in the field. A positive spot test may serve as an indicator of sufficient compliance in light of the treatment goal, whereas negative spots will create occasions for discussions of compliance with the patient(s). The test will thus be a tool both in the monitoring and in the management of satisfactory leprosy treatment.

SUMMARY

This study aimed to assess the validity and interpretation of the spot test for monitoring dapsone self-administration that employs filter paper impregnated with a modified Ehrlich's reagent. Urine specimens obtained from 20 volunteers, who took 100 mg dapsone for four days in succession, were investigated by this test. Findings indicate that spot tests will be negative after an average of three missed doses of dapsone, if compared with a standard of 5 μg dapsone per ml of urine. No negative spots are expected in fully compliant patients, and no positive spots are expected in patients who did not take dapsone for a week or longer. In the context of the treatment goal, it is argued that this degree of sensitivity makes the spot test a valid tool for the monitoring and management of patient compliance in leprosy control programs.

RESUMEN

Este estudio pretendió evaluar la validez y la interpretación de la prueba de la mancha (donde se usa papel filtro impregnado con un reactivo de Ehrlich modificado) que se utiliza para establecer la constancia en la auto-administración de dapsona. Se estudiaron muestras de orina de 20 voluntarios que habían tomado 100 mg de dapsona durante 4 días sucesivos. Los resultados (referidos a un estandar de 5 μg de dapsona por ml de orina) indicaron que la prueba de la mancha deberá ser negativa si se dejan de tomar, en promedio, 3 dosis de dapsona. No se esperan resultados negativos en pacientes que han sido constantes en su tratamiento y no se esperan resultados positivos en pacientes que han dejado de tomar la dapsona durante una semana o más. Se considera que el grado de sensibilidad de la prueba la hace una herramienta útil en el seguimiento y en el manejo de los pacientes en los programas de control contra la lepra.

RÉSUMÉ

Le but de cette étude est d'évaluer la validité et l'interprétation du "spot test" pour suivre l'auto-administration de dapsone. On a utilisé à cet effet du papier filtre imprégné avec un réactif d'Ehrlich modifié. On a étudié les résultats de cette épreuve, sur des échantillons d'urine recueillis chez 20 volontaires qui avaient absorbé 100 mg de dapsone pendant 4 jours successifs. Prenant un standard de 5 μg de dapsone par ml d'urine, les observations ont révélé que les épreuves deviennent négatives lorsqu'en moyenne trois doses de dapsone ont été manquées. On ne s'attend a aucune épreuve négative chez des malades qui suivent leur traitement

de façon adéquate. De même, on ne s'attend à ne trouver aucune épreuve positive chez des patients qui ne prennent pas de dapsone pendant une semaine ou plus. Dans la perspective des objectifs du traitement, on peut se demander si ce niveau de sensibilité fait de l'épreuve en question un instrument valable pour suivre et superviser l'assiduité du malade dans des programmes de lutte contre la lèpre.

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