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Dapsone Levels After Oral Therapy and Weekly Oily Injections in Ethiopian Leprosy Patients[1]

Ellen S. M. Modderman, Frans W. H. M. Merkus, Jan Zuidema,
Hans W. Hilbers, and Titia Warndorff[2]

In the 1950s oily injections of dapsone (DDS) were frequently used and many different formulations were tested by leprosy workers ([2, 5, 18]). Nowadays oral therapy has superseded the use of injections and the production of injections of dapsone in oily vehicles has been stopped. Nevertheless, stocks of these injections are still available in some places and are used for leprosy patients, e.g., in Ghana, Kenya, Pakistan, and Ethiopia. The type of injection used in these countries consists of a 25% suspension of DDS in ethylchaulmoograte, containing 4% guaiacol. These preparations were provided by UNICEF. The patient receives a weekly dose of 1.5 ml, which contains 375 mg DDS. Little is known about blood levels reached using this product in this dosage regimen. In the 1950s other doses and dosing intervals were used and only nonspecific colorimetric methods were available for analysis of blood levels.

Because this preparation is still used in leprosy therapy we studied 20 leprosy patients receiving weekly injections at ALERT (All-Africa Leprosy and Rehabilitation Training Centre) in Addis Ababa, Ethiopia. In this center weekly injections are given to out-patients when there are serious grounds for suspecting noncompliance with oral therapy. At the same time, we measured serum levels of DDS and monoacetyl DDS (MADDS) in a group of 16 patients receiving 100 mg dapsone daily in tablet form. We wanted to determine the steady state levels in Ethiopian leprosy patients receiving oral therapy to compare them with the levels obtained using the weekly injections.

The acetylation capacities of a group of 77 leprosy patients were calculated; the patients comprised the 36 patients in the above-mentioned trails plus 41 other leprosy patients on dapsone therapy. Methemoglobin levels were also measured, since methemoglobin formation is a side effect of dapsone and only a few reports on its formation are available from field studies.

METHODS

Patients on weekly injection therapy. The trail was performed in a group of 20 patients comprising 9 women and 11 men. The men's weight was 52.4 ± 4.1 kg (Mean ± S.D.) and the women's was 46.8 ± 7.7 kg. Their ages ranged from 20 years to 73 years. Blood samples (5 ml) and saliva samples (1–2 ml) were taken just before injection and 2, 4, and 7 days after. All patients in this trial had already been on weekly DDS injection therapy for at least six months.

Patients on oral therapy. This trial was performed in a group of 16 patients comprising 3 women and 13 men. The men's weight was 53.0 ± 6.2 kg and the women's was 43.7 ± 5.0 kg. Their ages ranged from 14 years to 49 years. Blood samples (5 ml) and saliva samples (1–2 ml) were taken just before intake of a 100 mg tablet and 3, 7, and 24 hr after. All patients had been taking dapsone tablets for several years before the start of this trial.

All volunteer patients in both trials had given their informed consent.

Analysis. Methemoglobin levels were determined in 0.5 ml EDTA blood within 1 hr of sampling by a slight modification of the method of Evelyn and Malloy ([4]). Methemoglobin levels were expressed as a percentage of total hemoglobin. Serum and sa-

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[2] E. S. M. Modderman, Pharm.D., Research Fellow; F. W. H. M. Merkus, Ph.D., Professor of Biopharmaceutics; J. Zuidema, Ph.D., Senior Scientist; H. W. Hilbers, Reserach Technician, Department of Biopharmaceutics, School of Pharmacy and School of Medicine, University of Amsterdam, The Netherlands. T. Warndorff, M.D., Staff Member, ALERT Hospital, Addis Ababa, Ethiopia.

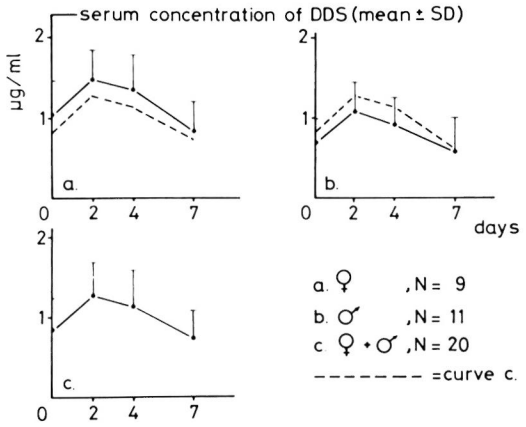

FIG. 1. Steady state serum level curves of DDS (Mean ± S.D.) after injection of 375 mg (=1½ ml) in an oily vehicle (UNICEF injection).

liva samples were frozen at −20°C, after centrifugation, and were transported to Amsterdam, The Netherlands by air. In Amsterdam, DDS and MADDS levels in serum and saliva were determined using the high-pressure liquid chromatographic (HPLC) method with fluorometric detection as published by Peters, *et al.* ([15]). Results were expressed as Mean ± S.D. Student's *t* test was used to evaluate the results. Linear regression analysis and determination of the 95% confidence region were used to demonstrate the relation between DDS levels and methemoglobin formation. Acetylation capacities were expressed as the ratio MADDS/DDS. Individuals with a ratio >0.35 were considered rapid acetylators; those with a ratio <0.35 were considered slow acetylators. The acetylation capacity was determined on the above-mentioned 36 patients and also on 41 other leprosy patients in a trial with long-acting DDS injections ([13]).

RESULTS

Weekly injections. We found a difference in serum levels between men ard women after UNICEF injections, women having slightly higher DDS levels (Fig. 1a and 1b). The mean serum levels of DDS of all 20 volunteers are shown in Figure 1c. The curves were very regular over a single week, without high peaks and with an average level of approximately 1 µg/ml. The differences between men and women were statistically significant ($p < 0.05$) at day 0 and day 2, but not at day 4 and day 7. The DDS levels in females were on the average 37% higher than in males.

TABLE 1. *Methemoglobin levels as percentage of total hemoglobin (Mean ± S.D.) after weekly UNICEF DDS injections of 375 mg.*

Group	Percentage methemoglobin	N[a]
Males	4.3 ± 1.0%	11
Females	5.3 ± 2.0%	9

[a] N = number of patients; four samples were obtained from each patient during a week.

The mean level in men over one week varied between 0.68 µg/ml and 1.07 µg/ml. In women the mean level varied between 0.81 µg/ml and 1.47 µg/ml. The highest DDS serum level measured in men was 1.75 µg/ml; in women it was 2.06 µg/ml. The lowest DDS serum level was 0.19 µg/ml in men and 0.21 µg/ml in women. In saliva samples 19.3 ± 6.1% of the DDS level in serum was found. Methemoglobin formation, shown in Table 1, was very slight and no levels of clinical importance were reached. Percentages in women were slightly higher, which is in agreement with their higher serum levels of DDS, but the differences were not statistically significant. The highest methemoglobin level measured was 12% in women and 7% in men.

Oral therapy. The mean serum level curves of DDS after oral therapy, in a dose of 100 mg, are shown in Figure 2. The levels are considerably higher than after the weekly, oily injections. Moreover, there appears to be a larger difference between the levels in men and in women. There were only three women in this group, but the difference was statistically significant at all sampling times ($t = 0$ hr, $p < 0.01$; $t = 3$ hr, $p < 0.001$; $t = 7$ hr, $p < 0.01$; $t = 24$ hr, $p < 0.001$). On the average the serum level of DDS in females was 61% higher than that in males. While the mean level in men varied during the day between 1.71 µg/ml and 2.88 µg/ml, the mean level in women varied from 2.61 µg/ml to 4.88 µg/ml. The lowest DDS level measured in men was 0.82 µg/ml and in women was 2.26 µg/ml. The highest DDS level in men was 3.90 µg/ml and in women

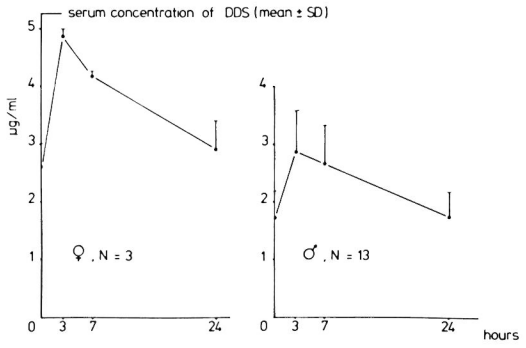

FIG. 2. Steady state serum level curves of DDS (Mean ± S.D.) after oral therapy with 100 mg DDS.

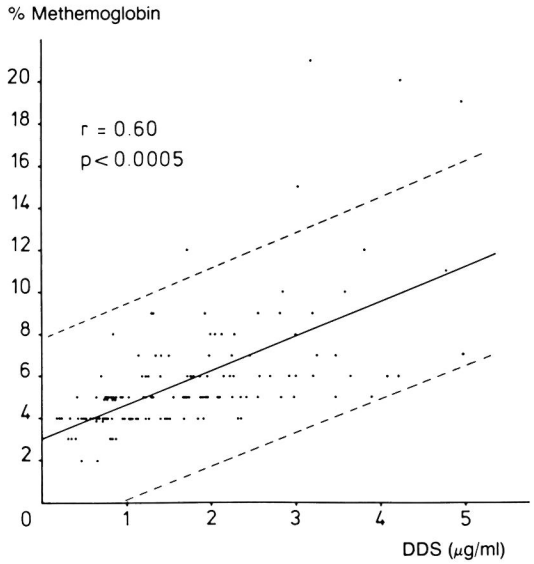

FIG. 3. Correlation between DDS serum level and methemoglobin percentage. The dotted lines indicate the 95% confidence region for this group of 36 patients (N = 135).

was 4.95 µg/ml. One woman with DDS levels during the day of 3.02 µg/ml to 4.95 µg/ml had a methemoglobinemia of 15% to 21%! On medical examination she proved to be cyanotic, and she reported having had a headache since starting dapsone therapy some years before. In this group of volunteers saliva DDS levels were 19.3% ± 7.6% of DDS serum levels.

An overview of methemoglobin formation is given in Table 2. In some of the patients, especially the female patients, the levels were clearly elevated. The highest level measured was 12% in men and 21% in women.

Methemoglobin formation. The correlation between DDS serum levels and methemoglobin formation in patients on oral therapy or weekly injections of DDS is shown in Figure 3. It is known that methemoglobin formation is caused primarily by N-OH-metabolites of DDS ([8, 11, 16]). These metabolites are not detectable in blood because of their rapid renal elimination ([17]). Because it is possible that there is a positive correlation between DDS serum level and the concentration of N-OH-metabolites ([3]) we used DDS concentrations for the abscissa. There appeared to be a positive correlation between DDS serum levels and methemoglobinemia ($r = 0.60$; $p < 0.0005$), but Figure 3 also shows a substantial inter-individual variation.

Acetylation. The acetylation capacities of 77 Ethiopian leprosy patients on dapsone therapy were calculated as ratios of MADDS/DDS and are shown in the histogram of Figure 4. This group of patients consisted of the 36 participants in the trials

TABLE 2. *Methemoglobin levels as percentage of total hemoglobin (Mean ± S.D.) after oral DDS therapy of 100 mg daily.*

Group	Percentage methemoglobin	N[a]
Males	6.2 ± 1.8%	13
Females	10.5 ± 6.5%	3

[a] N = number of patients; four samples were obtained from each patient during 24 hours.

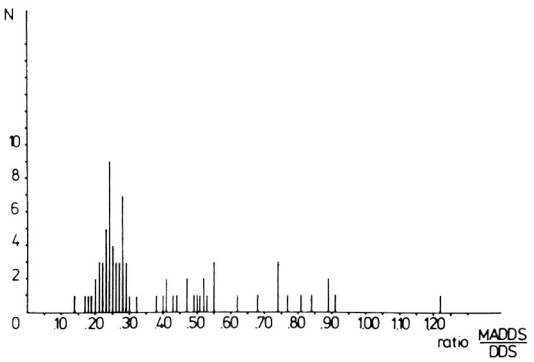

FIG. 4. Acetylation capacities in a group of 77 Ethiopian leprosy patients.

on oral therapy and weekly injections plus 41 patients receiving a recently developed, long-acting DDS injection. The ratio of MADDS/DDS is the mean of the values obtained from all serum samples from each patient. In this group, 62% of patients were slow acetylators (ratios of MADDS/DDS < 0.35).

DISCUSSION

The serum concentrations of DDS after the oily UNICEF injections in a dose of 375 mg each week are acceptable for both men and women. The difference of 37% in levels between men and women can be partly accounted for by a difference in body weight; the weight of the men was on the average 12% higher than that of the women. After correction for body weight the differences in serum levels are no longer statistically significant, but the levels of women always appear to be somewhat higher than those of men. Some other factors may also play a role here, for example, sex differences in absorption, distribution, or elimination. Peters, *et al.* ([14]) found no differences in the $t_{1/2}$ of DDS and MADDS between females and males. In a trial with monthly injections of dapsone, formulated as aqueous suspensions, female leprosy patients had lower levels in the first week but higher levels in the following weeks than did male patients ([13]). The lower levels in the first week are probably due to differences in absorption that may play a lesser role after oily injections. The higher levels, observed in the following three weeks, can be explained by the fact that in women a larger part of the dapsone depot is left, but other pharmacokinetic differences between men and women may have contributed. Sex differences in pharmacokinetics of drugs are not unusual ([6, 10]). However, to our knowledge, no research in this field has been done using dapsone.

Interviews with eight patients who used to receive weekly UNICEF injections revealed that the injections were considered painful by six of them. Two patients reported swelling at the injection site, and two other patients reported difficulty in walking or sleeping for some days after the injection.

After daily oral administration of 100 mg DDS, differences between men and women were more pronounced. The difference in serum level of 62% can also be partly accounted for by differences in body weight; the mean weight of the men being 21% higher than that of the women. After correction for body weight the statistical significance was less, but the levels in women were still always higher than in men. The differences were statistically significant at 3 hr ($p < 0.02$) and at 24 hr ($p < 0.05$), but not at 0 hr or 7 hr.

Other factors, mentioned above, may have attributed to the difference. The serum levels of DDS after oral therapy with 100 mg were rather high, but were consistent with pharmacokinetic calculations. After a single dose of 100 mg dapsone, a peak level of 1.5 μg/ml to 2 μg/ml is usually reached ([1]); however it can be calculated that with $t_{1/2}$ of 20 hr to 30 hr and a dosing interval of 24 hr, after repeated dosage, cumulation takes place to levels of 2.5 μg/ml to 4.5 μg/ml.

In some cases the levels were clearly too high, as illustrated by the female patient with a methemoglobinemia of around 20%. Her body weight was only 43 kg and 50 mg DDS per day would probably have been a better dosage regimen for her. In general, it is worthwhile to consider a lower dosage for persons with a low body weight. WHO takes into account the factor of body weight since it advises a dose of 1–2 mg/kg per day. Methemoglobin determination, which can be performed by a simple colorimetric method, could be a useful factor in deciding dosage levels for individual patients.

A positive correlation exists between DDS levels and methemoglobin formation but there is large inter-individual variation. The N-OH-metabolites, which are not detectable in serum, are the substances responsible for methemoglobin formation. There are reports that the amount of N-OH-metabolites, excreted in urine, varies from 10% to 50% of the dose ([7, 8, 17]). Different rates of formation of these metabolites and total or partial deficiencies of certain enzyme systems in individuals are possible explanations for the variations observed in Figure 3.

In a group of 77 Ethiopian patients 62% were slow acetylators. This figure is of the same magnitude as that determined in other countries of northeast Africa as reviewed by Karim, *et al.* ([9]), i.e., 65% in Libyans, 82% in Egyptians, 65% in (non-Arab) Sudanese,

56% in "East-Africans" (Kenyans, Ugandans, Tanzanians and Zambians).

Saliva from the 36 volunteers contained DDS concentrations which were 19.3 ± 7.0% of the serum levels. This is in agreement with a nonprotein-bound fraction of 17 ± 4% which we found in 35 other Ethiopian leprosy patients in ALERT in a trial of monthly injections. Our results are also of the same magnitude as that of Peters, *et al.* ([15]) who found 15%–20% of serum levels in saliva, and the results of Ahmad and Rogers ([1]) who found 27%, but are not consistent with the study of Lammintausta, *et al.* ([12]) who found a nonprotein-bound fraction of 50%, using an ultra centrifugation method.

The correlation coefficient for DDS levels in serum and saliva (r = 0.7542) was found to be much lower in our trials with Ethiopian leprosy patients than that found in laboratory trials using healthy volunteers ([1, 15]).

As was demonstrated by Peters, *et al.* ([15]) the determination of DDS in saliva is very easily achieved. There are, however, some drawbacks in field trials. Firstly, DDS concentrations in saliva are considerably lower than in serum, which means that the detection limit will be reached more quickly. Secondly, leprosy patients may easily mistake saliva for sputum, and the sampling has to be checked carefully to see that only saliva is given. This problem may have attributed to the standard deviation in our measurements. A third point is that saliva samples are prone to rapid deterioration and must be analyzed or frozen immediately. We agree with Peters and coworkers ([15]) that MADDS levels in saliva are too low to enable acetylator phenotyping using this fluid.

SUMMARY

In a trial with 20 leprosy patients in Addis Ababa, Ethiopia, serum levels of dapsone (DDS) and monoacetyl DDS (MADDS) were determined after weekly injections of 375 mg DDS in an oily vehicle (UNICEF injections). During the week DDS levels of approximately 1 μg/ml were maintained. Women had higher levels than men which could only partly be accounted for by differences in body weight. Methemoglobin levels were not significantly elevated.

In another trial with 16 leprosy patients receiving 100 mg DDS by mouth daily, serum levels of DDS and MADDS proved to be higher, varying between 0.82 μg/ml and 4.95 μg/ml. Women had significantly higher levels than men and the difference was still statistically significant after correction for body weight. Methemoglobin levels were elevated and not always without clinical symptoms. There proved to be a positive correlation between DDS serum levels and percentage methemoglobin ($r = 0.60$; $p < 0.0005$). In both trials saliva samples were analyzed and contained 19.3 ± 7.0% of the DDS level in serum.

RESUMEN

En un estudio con 20 pacientes con lepra realizado en Addis Ababa, Etiopía, se determinaron los niveles séricos de dapsona (DDS) y de monoacetil-DDS (MADDS) después de inyectar semanalmente 375 mg de DDS en un vehículo oleoso (Inyecciones UNICEF). Se encontró que durante la semana se mantuvieron niveles de DDS de aproximadamente 1 μg/ml. Las muneres tuvieron niveles más altos que los hombres pero ésto se explicó parcialmente en base a las diferencias en peso corporal. Los niveles de metahemoglobina no estuvieron significativamente elevados.

En otro ensayo con 16 pacientes con lepra que recibían 100 mg de DDS oral cada día, los niveles en suero de DDS y de MADDS resultaron ser mayores, variando entre 0.82 μg/ml y 4.95 μg/ml. Las mujeres tuvieron niveles mayores que los hombres y esta diferencia fue estadísticamente significante aún después de corregir por las diferencias en peso corporal. Los niveles de metahemoglobina estuvieron elevados y a veces asociados con síntomas clínicos. Se observó una correlación positiva entre los niveles séricos de DDS y el porcentaje de metahemoglobina ($r = 0.60$; $p < 0.0005$). En ambos estudios se analizaron muestras de saliva y se encontró que éstas contenían el 19.3% ± 7.0% del nivel de DDS encontrado en suero.

RÉSUMÉ

Lors d'un essai mené sur 20 malades atteints de lèpre à Addis Abeba, en Ethiopie, les taux sériques de dapsone (DDS) et de monoacetyl DDS (MADDS) ont été déterminés après des injections hebdomadaires de 375 mg de DDS dans un excipient huileux (UNICEF injections). Des taux de DDS d'approximativement 1 μg/ml ont été maintenus au cours de la semaine. Les femmes présentaient des taux plus élevés que les hommes, la différence du poids coporel ne pouvant expliquer cette différence qu'en partie. Les taux de methémoglobine ne présentaient pas d'augmentation significative.

Dans un autre essai portant sur 16 malades de la lèpre, qui avaient reçu 100 mg de DDS journellement

par voie orale, les taux sériques de DDS et de MADDS se sont révélés plus élevés, variant entre 0.82 μg/ml et 4.95 μg/ml. Les femmes présentaient des taux significativement plus élevés que les hommes, et la différence était encore statistiquement significative après correction pour le poids corporel. Lex taux de methémoglobine étaient élevés, et cette élévation n'allait pas toujours sans symptômes cliniques. On a mis en évidence une corrélation positive entre les taux sériques de DDS et le pourcentage de methémoglobine ($r = 0.60$; $p < 0.005$). Dans les deux essais, des échantillons de salive ont été analysés. Ils contenaient 19.3 ± 7.0% du taux de DDS du serum.

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