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Sustained Release Properties of an Intra-adiposely Administered Dapsone Depot Injection[1]

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For more than 30 years, dapsone has been the therapy of choice in the treatment of leprosy. The drug has proven to be effective and safe within the therapeutic concentration range and has the great advantage of being inexpensive. Because the elimination halflife of dapsone is about 24 hours ([4, 7, 15, 17]) a once-daily oral treatment, if executed properly, can be considered sufficient.

After the first proven case of dapsone resistance ([18]), many new reports appeared ([14, 21]). This resistance is probably caused by low-dosage dapsone therapy and noncompliance among leprosy patients ([1, 3, 5, 9, 14]). In most centers today, dapsone monotherapy is no longer considered adequate.

Patient noncompliance could be combated by the use of a dapsone depot injection. In its report in 1982, in which multiple drug therapy was recommended for the treatment of leprosy, the World Health Organization (WHO) stated that "a formulation of dapsone or a derivative of dapsone, that, on monthly administration, would provide bactericidal concentrations of dapsone in the tissues, without risk of toxicity, is desirable" ([21]). Dapsone injections have been studied in leprosy patients and healthy patients by Modderman, *et al.* ([10]). After intramuscular administration of this injection, differences in concentration/time profiles between men and women appeared to exist. In men, peak concentrations were higher (mean ± S.D. = 2.28 ± 1.06 mg/l) than in women (0.98 ± 0.42 mg/l). On the other hand, after 28 days dapsone concentrations on the average were considerably higher in women (0.42 ± 0.23 mg/l) than in men (0.11 ± 0.09 mg/l).

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The study by Cockshott, *et al.* ([2]) offered an explanation for this phenomenon. Skin-to-muscle distances at the gluteus maximus were measured using computerized tomography, and it was concluded that, especially in women, most of the injections intended to be intramuscularly administered are, in fact, delivered into the adipose layer overlying the gluteus maximus muscle because the length of the needle which is used is shorter than the thickness of that layer.

The previous study ([10]) with the dapsone depot injection yielded better depot properties in women than in men. The present study presents the sustained release results of the same injection after administration into the adipose layer overlying the gluteus maximus muscle in men and women. For this route of administration, the term "intra-adipose" is used after the suggestion by Morrison ([12]).

MATERIALS AND METHODS

The injection. A suspension of bipyramidally shaped crystals with a particle size of 38–63 μm in an aqueous vehicle, the injection was prepared according to the method described by Modderman, *et al.* ([10]), and contained 1000 mg dapsone per 4 ml suspension.

Subjects. The injection was administered to 15 male and 12 female healthy volunteers. Their ages ranged from 20 to 36 years (mean ± S.D. = 25.3 ± 5.0 years) and 19 to 28 years (mean ± S.D. = 23.6 ± 2.6 years), respectively. Body weights of the males were 62 to 85 kg (mean ± S.D. = 72.1 ± 6.1 kg); females weighed 53 to 67 kg (mean ± S.D. = 58.8 ± 4.9 kg). Skinfolds measured prior to the administration of the injection ranged from 9 to 45 mm (mean ± S.D. = 22.6 ± 9.9 mm) in men, and from 27 to 45 mm (mean ± S.D. = 37.0 ± 5.4 mm) in women.

Study design. After informed written consent and medical approval, the subjects received an oral dose of 100 mg dapsone to

avoid the possible occurrence of side effects after administration of the injection. Methemoglobin values were measured before and 24 hr after ingestion. After a two-week wash-out period, the injection was administered in the buttock. To establish the injection depth, the skinfold was measured at the injection site. A volume of 4.0 ml suspension, corresponding with 1000 mg dapsone, was injected at a depth equal to one third the thickness of the skinfold, being two thirds of the skin-to-muscle distance (Fig. 1). An 18G 1½-inch needle (Terumo Europe NV, 3030 Leuven, Belgium) was used to administer the injection. Prior to and 1, 3, 5, 7, 14, 21, 28, and 35 days after administration, 5 ml blood samples were taken to determine dapsone and monoacetyldapsone concentrations in the serum. To exclude the possibility of missing a peak concentration due to the sampling scheme used, the dapsone concentration course was more closely followed in two male volunteers during the first days after injection. At 1, 3, 6, and 48 hr after injection, blood samples were taken from these two subjects in addition to the normal scheme.

Determination methods. Serum samples were frozen at −20°C pending analysis. Dapsone and monoacetyldapsone concentrations were measured using the high-power liquid chromatography (HPLC) method with fluorometric detection according to Peters, *et al.* ([17]).

Pharmacokinetic and statistical analysis. Dapsone concentration/time curves in serum were constructed for each subject. Areas under the curve (AUC) from day 0 to day 28 were calculated using the trapezoidal rule. The dapsone concentration was corrected to a body weight of 70 kg ($conc_{70}$) using the equation ($conc_{70}$ = conc·BW 70), in which "BW" represents the subject's body weight and "conc" the measured dapsone concentration.

Rank sign tests according to Kendall were performed to investigate whether or not several parameters (body weight, acetylation capacity, skinfold) had an influence on the results. Wilcoxon rank sign tests were used to decide whether results in men and women differed from each other with statistical significance. Results were considered to be statistically significant if p values obtained were less than 0.05.

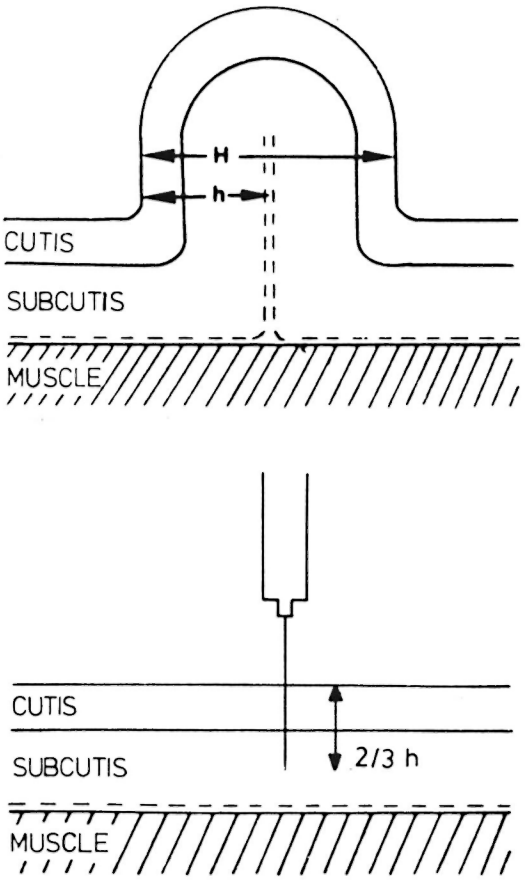

FIG. 1. Skinfold measurement and its relation to the injection depth. The injection is given at ⅓ H = ⅔ h.

RESULTS

In Figure 2, individual concentration/time curves of male and female volunteers are presented separately. The curves are all smoothly shaped. Peak concentrations in men ranged from 0.23 to 1.45 mg/l, with a mean value ± S.D. of 0.69 ± 0.40 mg/l; peak concentrations in women varied from 0.50 to 1.50 mg/l, averaging 0.84 ± 0.31 mg/l. After 28 days, between 0.02 and 0.36 mg/l dapsone (mean ± S.D. = 0.19 ± 0.09 mg/l) was detectable in men, and between 0.11 and 0.68 mg/l (mean ± S.D. = 0.27 ± 0.16 mg/l) was detectable in women. The Table presents the mean dapsone concentrations measured in men and women.

The mean AUC amounted to 10.5 ± 4.4 mg·day/l for men and 13.8 ± 5.2 mg·day/l for women.

Two volunteers were more closely fol-

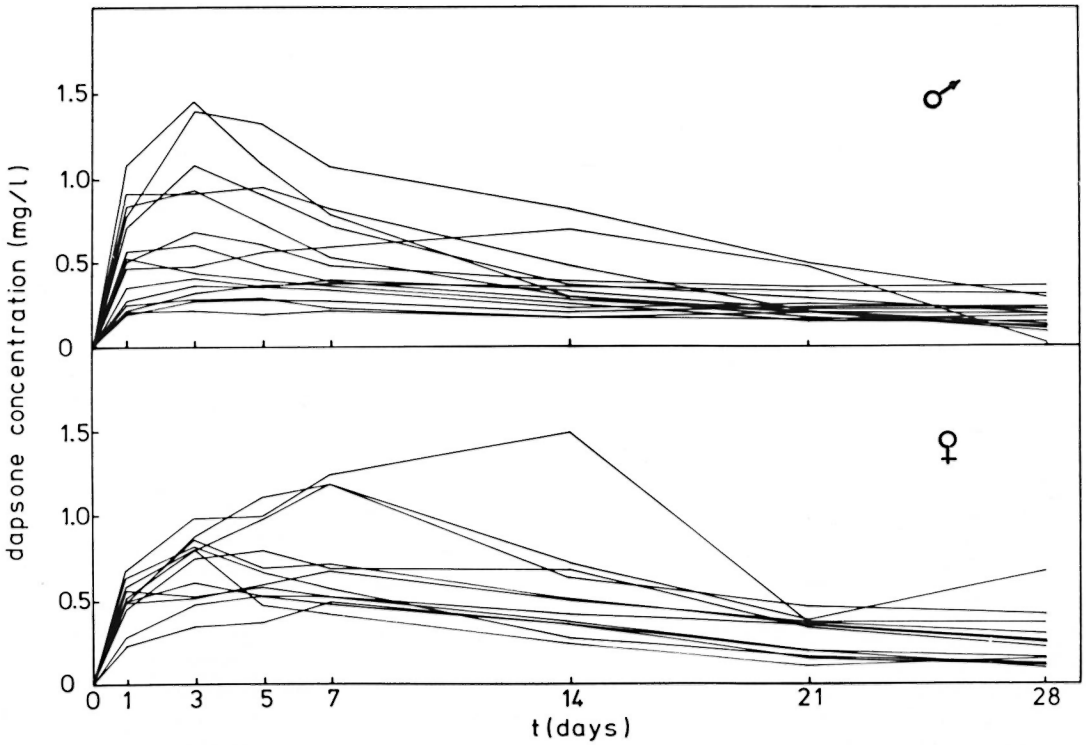

FIG. 2. Results of dapsone concentration course over 4 weeks after intra-adipose administration of 1000 mg dapsone in men (♂) and women (♀).

lowed in the first days after injection. A regular dapsone concentration/time profile was observed in both, with steadily increasing dapsone concentrations during this period. The occurrence of too high dapsone concentrations could be excluded.

The monoacetyldapsone:dapsone (M:D) concentration ratio was used to establish each subject's acetylation capacity. An obvious bimodal distribution pattern was found in the M:D ratio. According to the standards of Philip, *et al.* ([19]), 8 out of 15 male and 7 out of 12 female subjects were classified as rapid acetylators. Their M:D ratios ranged from 0.45 to 0.89, while in slow acetylators the M:D ratios varied from 0.16 to 0.23.

THE TABLE. *Dapsone concentrations*[a] *after intra-adipose administration of 1000 mg dapsone.*

Day	Males	Females
0	0	0
1	0.52 ± 0.29	0.50 ± 0.13
3	0.66 ± 0.41	0.70 ± 0.19
5	0.59 ± 0.34	0.70 ± 0.23
7	0.51 ± 0.25	0.73 ± 0.30
14	0.36 ± 0.19	0.55 ± 0.34
21	0.26 ± 0.11	0.29 ± 0.11
28	0.19 ± 0.09	0.27 ± 0.16
35	0.17 ± 0.11	0.22 ± 0.15

[a] mg/l; mean ± S.D.

None of the subjects experienced significant inconvenience from the injections, and the injection itself was not painful. In the period after injection, complaints were restricted to some tenderness at the injection site that never lasted more than 1 week. Two male volunteers participated in an earlier intramuscular trial ([11]). They both expressed their preference for the intra-adipose administration.

DISCUSSION

Differences between the sexes in absorption of drugs as found for dapsone were previously reported after intramuscular administration of penicillins ([6]) and cephradine ([20]). This could have been due to differences in skin-to-muscle distances between men and women, resulting in an

unintended intra-adipose injection in most of the female patients.

After deliberate intra-adipose administration of the dapsone depot injection, we found no differences between males and females (Fig. 2). The results for men and women in this trial are in agreement with the results of "intramuscular" administration of the injection in women ([10]). Statistically significant differences in dapsone concentrations between men and women could only be demonstrated at 7 and 14 days after injection. After correction for body weight, however, these differences disappeared. Differences in peak concentrations between men and women were not statistically significant. High peak concentrations, such as those occurring in men after intramuscular administration, did not occur. The day 28/peak concentration ratios, which can be considered as an indication of the sustained release properties of the injection, amounted to between 0.10 and 0.82 in all volunteers, with mean values for men of 0.38 ± 0.27 and for women of 0.32 ± 0.14. The difference is not statistically significant. As a comparison, after intramuscular injection, these ratios averaged 0.05 in men and 0.43 in women ([10]).

No statistically significant difference in mean AUC between men (10.5 ± 4.4 mg·day/l) and women (13.8 ± 5.2 mg·day/l) appeared to exist. In an earlier trial, the relation between AUC and the dose administered was established ([10]). In the present study, a statistically significant correlation between the dose (mg dapsone per kg body weight) and the AUC was found ($p < 0.01$).

No statistically significant correlations could be detected between the dapsone peak concentration, the day 28/peak concentration ratio, the AUC, or the concentration at different time points on the one hand and the thickness of the skinfold at the site of injection on the other. One would expect the skinfold thickness to influence these parameters if it significantly influenced the absorption rate of dapsone from the injection site.

The acetylation capacity did not influence results either. Earlier studies have already demonstrated the absence of an influence of the acetylation capacity on dapsone pharmacokinetics ([4, 7, 15]).

On close inspection, a tendency seems to exist toward a faster absorption of dapsone in men than in women. This is expressed by the somewhat higher concentration 1 day after administration (after body weight correction to 70 kg, 0.53 ± 0.28 mg/l in men and 0.42 ± 0.12 mg/l in women) and by the mean t_{max} values for men (5 ± 3 days) and women (6 ± 3 days). These differences are not statistically significant however.

The minimal inhibitory concentration of dapsone for *Mycobacterium leprae,* determined in mice and rats, is estimated to be around 3 μg/l ([8, 13, 16]). Our injection yielded much higher concentrations throughout the whole study period, which is important in preventing the development of dapsone resistance. Whether accumulation after chronic intra-adipose administration will lead to higher dapsone serum concentrations remains to be investigated.

The intra-adipose administration of the injection was well tolerated by the subjects in this study. This extra advantage should promote patient attendance at clinics. Cockshott, *et al.* ([2]) have already pointed out the fact that inadvertent intravenous and intra-arterial injection, muscle damage, tissue damage progressing to fibrosis, or nerve palsies are less likely to occur after intra-adipose than after intramuscular injection, and that deep implantation of bacterial infections are less damaging in fat than in muscular tissue.

The results of this study seem to confirm our assumption that the sex differences in the dapsone concentration/time profile found by Modderman, *et al.* ([10]) are caused by differences between men and women in the skin-to-muscle distance at the injection site.

The conclusion may be drawn that intra-adipose administration of the dapsone injection is preferable over intramuscular injection. A better depot effect is reached, reflected in lower peak concentrations and higher concentrations at day 28. Although it looks promising, further studies need to be performed to gain more experience with the long-term use of the injection before introduction into the WHO multiple drug therapeutic regimen can be considered.

SUMMARY

A dapsone depot injection, consisting of dapsone crystals of bipyramidal shape with

a particle size of 38 μm–63 μm suspended in an aqueous vehicle, appeared to result in different concentration/time profiles in men and women when delivered "intramuscularly." This phenomenon can be explained by the larger skin-to-muscle distance in women than in men. Injections intended to be delivered intramuscularly are, in fact, administered into subcutaneous fatty tissue in most of the women. Because sustained release properties were more satisfactory in women than in men, in this study the absorption of dapsone was investigated after administration of the same injection into gluteal fatty tissue. Via this route of administration, for which the term intra-adipose is used, 12 female and 15 male healthy volunteers received 1000 mg dapsone, after which blood samples were taken at regular intervals for 35 days to determine dapsone and monoacetyldapsone concentrations in serum using high-pressure liquid chromatography with fluorometric detection.

No important differences between men and women appeared to exist at any time point after injection. The peak concentrations were 0.69 ± 0.40 mg/l in men and 0.84 ± 0.31 mg/l in women. No important side effects were observed, either locally or systemically. Volunteers who previously received an intramuscular injection preferred the intra-adipose administration.

The good depot properties and better acceptance of intra-adipose dapsone administration are reasons to prefer this route of administration.

RESUMEN

La inyección de una suspensión de dapsona conteniendo cristales de forma bipiramidal, con un tomaño entre 38 y 63 μm, y un vehículo acuoso, pareció dar como resultado diferentes patrones de concentración y tiempo de liberación, dependiendo de si la inyección "intramuscular" se administró en hombres o en mujeres. Este fenómeno podría explicarse en base a la mayor distancia que hay entre la piel y el músculo de las mujeres. En realidad, las inyecciónes "intramusculares" en la mayoría de las mujeres son depositados en el tejido graso subcutáneo. Ya que la liberación sostenida de la droga fue más satisfactoria en las mujeres que en los hombres, en este estudio se investigó la absorción de la dapsona después de administrar la suspensión de la droga en el tejido graso de los glúteos. A través de esta ruta "intra-adiposa" de administración, 27 voluntarios (12 mujeres y 15 hombres) recibieron 1000 mg de dapsona para después donar muestras de sangre a intervalos regulares durante 35 días con el fin de determinar las concentraciones séricas de la droga y de su derivado monoacetil-dapsona por cromatografía de líquidos de alto presión y detección fluorométrica.

No parecieron existir diferencias importantes entre hombres y mujeres a ningún tiempo estudiado. Los picos de concentración fueron 0.69 ± 0.40 mg/l en los hombres y 0.84 ± 0.31 mg/l en las mujeres. No se observaron efectos colaterales importantes ni locales ni sistémicos. Los voluntarios que previamente habían recibido una inyección intramuscular prefirieron la inyección intra-adiposa.

RÉSUMÉ

L'injection intramusculaire d'un dépôt de dapsone, consistant en cristaux de dapsone de forme bi-pyramidale, avec une dimension des particules variant de 38 μm à 63 μm, en suspension en un milieu aqueux, entraînent des différences dans les concentrations au cours du temps entre les hommes et les femmes. Cette observation peut-être expliquée par le trajet plus long entre la peau et le muscle chez les femmes par rapport aux hommes. Chez la plupart des femmes, lorsqu'on veut procéder à une injection intramusculaire, c'est en fait dans le tissu adipeux sous-cutané que l'on délivre le produit. Puisque les profils caractérisant une libération prolongée étaient plus satisfaisantes chez les femmes que chez les hommes, on a étudié l'absorption de dapsone après administration d'une injection semblable dans le tissu adipeux fessier. Ce type d'injection a été qualifié du terme d'"intra-adipeux". Chez 12 femmes et chez 15 hommes adultes, tous volontaires et en bonne santé, on a administré par cette voie 1000 mg de dapsone. Des échantillons de sang ont été ensuite prélevés à intervalles réguliers pendant 35 jours, afin de déterminer les concentrations en dapsone et en monoacétyldapsone dans le sérum, par une technique de chromatographie liquide à haute pression avec détection fluorométrique.

Aucune différence notable liée au sexe n'a été observée, à aucun moment après l'injection. Les concentrations maximales se sont situées à 0,69 ± 0,40 mg/l, chez les hommes, et à 0,84 ± 0,31 mg/l chez les femmes. Aucun effet secondaire important, local ou systémique, n'a été observé. Les volontaires qui avaient reçu auparavant une injection intramusculaire, préféraient la voie intra-adipeuse.

La libération prolongée du produit, et le meilleur accueil fait par les sujets à l'administration intra-adipeuse de dapsone, militent en faveur de cette voie d'administration.

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