Possible Optimization of Sulphadimidine Dosage for Acetylator Phenotyping

Francis Hombhanje

Department of Basic Medical Sciences, Faculty of Medicine, University of Papua New Guinea, P.O. Box 5623, Boroko, Papua New Guinea

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ABSTRACT—The effect of different doses of sulphadimidine (250, 500, 750 and 1000 mg) on acetylation capacity in 10 normal individuals was investigated in a randomized cross-over study design. The subjects were initially phenotyped with an oral dose of 750 mg of sulphadimidine. Four weeks later, each subject was assigned four different doses of sulphadimidine. The acetylator phenotype was classified for each dose as rapid if the percentage of acetylated sulphadimidine in the post-dose 5–6 hour urine is more than 70% and as slow if less than 70%. The results indicate a clear separation into two phenotypes, even with the smallest dose of the drug. A slow acetylator in the 10 subjects showed a saturation of the acetylation capacity with increasing doses. This trend was not observed in the remaining subjects who were identified as rapid acetyulators regardless of the doses. An oral dose of 750 mg of sulphadimidine is suggested as a ‘standard’ or optimum dose for acetylator phenotype testing.

Several drugs are available for assessing the genetic acetylator polymorphism in man (1–4). Sulphadimidine (or sulphamethazine) is a widely used sulphonamide for acetylator phenotype determination. A conventional procedure for determining the acetylator phenotype with sulphadimidine for a population involves a standard dose of this test probe, usually based on body-weight, and subsequent assay of plasma or urine for the parent drug and its metabolite (5). This procedure is comparatively simple to perform, virtually free from side effects over the dosage range of interest. However, variation of this method has been used to determine an individual’s acetylator phenotype with different sulphadimidine dosage per body weight ranging from 5 mg/kg to 160 mg/kg in some studies (5, 6) and fixed single dose of 500, 750 or 1000 mg in others (7, 8).

Dosage schedules based on body-weight have the disadvantage of dose variation for a single population under study. Consequently, the acetylator phenotype status of individuals in a population is determined over a wide range of doses of the test drug. Dose-ranging studies using sulphadimidine, isoniazid and hydralazine have shown that acetylations of these drugs occur in a dose-dependent manner (9, 10). Furthermore, clear separation of acetylator phenotype becomes less bimodal (even mono-morphic) at the lower doses of hydralazine (10). However, there appears to be a lack of sufficient information on the use of an optimum oral dose of sulphadimidine, isoniazid, dapson or caffeine in the assessment of acetylator phenotype. The purpose of this study was (i): to assess acetylator phenotypes at the lowest possible dose of sulphadimidine and (ii): to determine an optimum dose-range of the test probe sulphadimidine for acetylator phenotyping.
For the chemical analysis, ammonium sulphamate, N-1-naphthyl-ethylene-diamine dihydrochloride and other chemicals including pure sulphadimidine powder were purchased from Sigma (St. Louis, MO, U.S.A.). All the chemicals were of analytical grade. Sulphadimidine powder was used to prepare standard solutions.

Sulphadimidine dosages as commercially available tablets (each tablet containing 500 mg of the drug) were orally administered for the phenotyping purpose.

Ten healthy volunteer subjects (staff and students), all Papua New Guineans by birth and lineage, from the Faculty of Medicine were recruited for the study. Their ages ranged from 20 to 35 years. A verbal consent was obtained, and none of them was on any medication, had any recent disease nor gave any history of sulphadimidine-induced adverse reactions.

All the subjects were initially phenotyped with an oral dose of 750 mg of sulphadimidine following an overnight fast. This dose was ingested in the morning, one and half hours before breakfast. The bladder was emptied completely at the 5th-hour post-dosing. Urine samples for analysis were collected on the 5th to 6th hour post-dosing. The samples were analyzed for total and free sulphadimidine using the standard colorimetric Bratton-Marshall procedure (5). Acetylated sulphadimidine was calculated as the difference between the total and free sulphadimidine and expressed as a percentage. The cut-off point or antimode for the phenotype classification was 70% of the sulphadimidine acetylated: slow acetylator (SA) when it is below 70% and rapid acetylator (RA) when it is above 70%.

After four weeks of the initial assessment of acetylator status, each subject was requested to take four different doses of sulphadimidine (250, 500, 750 and 1000 mg) at a 3- to 4-week interval in a randomized cross-over order. All urine samples for the 5th to 6th hour post-dose period were collected and treated for the assay in a similar manner as described above. Samples were stored at -20°C if not assayed immediately.

No alcohol or caffeinated beverages were allowed during the study period. The study was approved by the Research & Ethical Committee of the Faculty of Medicine, University of Papua New Guinea.

Individual data on the percentage of acetylated sulphadimidine in urine for the 4 different doses in this study are listed in Table 1. The 9 individuals were identified as RAs with a 70% or more of acetylated sulphadimidine regardless of the doses, while only one was a SA, in this small sample size. The ranging values for the RAs were from 76 to 95% and from 17 to 60% for SA. The extent of acetylation was markedly reduced with increasing

<table>
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<th>Dose</th>
<th>Baseline (750 mg)</th>
<th>250 mg</th>
<th>500 mg</th>
<th>750 mg</th>
<th>1000 mg</th>
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</thead>
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<tr>
<td>F.H.</td>
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<td>78.3</td>
<td>95.8</td>
<td>76.2</td>
<td>84.7</td>
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<tr>
<td>A.M.</td>
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<td>84.0</td>
<td>84.0</td>
<td>80.0</td>
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<tr>
<td>W.L.</td>
<td>90.0</td>
<td>86.2</td>
<td>84.5</td>
<td>90.1</td>
<td>80.1</td>
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<tr>
<td>J.M.</td>
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</table>

**Table 1.** Urinary excretion data on percentage of sulphadimidine acetylated in 10 normal individuals who were initially phenotyped with sulphadimidine in a 750-mg (baseline) dose and then with the 4 different doses on separate occasions.

RA = Rapid acetylator, SA = Slow acetylator.
dosages of sulphadimidine in the SA. As can be seen from Table 1, there are some intra-individual variations in the acetylation capacity for the RAs at the different doses of sulphadimidine. However, there were no effects on the phenotyping determination and no misclassification of the phenotypes (RA and SA) occurred in any of the individual.

From the data presented herein (Table 1), 90% of the subjects were RAs. This frequency is fairly consistent with the previous observations for the same population (11, 12).

These results indicate that the separation of acetylator phenotypes is not influenced by the different dose of sulphadimidine (Table 1). Thus, the distribution is clearly bimodal within the dose-range (250 to 1000 mg) studied. These findings further confirm the previous observation using 5 mg/kg of sulphadimidine (6). It was still possible to discriminate between the two phenotypes at the smallest possible dose of this test drug.

The acetylation pharmacokinetics of certain drugs such as sulphadimidine (6), isoniazid (9) and hydralazine (10) are known to occur in a dose-dependent manner in man. Saturation of the N-acetylation system with increasing the doses of these drugs have been suggested (6, 9, 10). However, in the present study, there were no clear-cut changes in the percentage of sulphadimidine acetylated over the doses studied for RAs despite a considerable variation in the absolute value for N-acetylated metabolite in the urine. In contrast, a SA showed a trend for the decreasing capacity to acetylate at the maximum dose of the test drug (Table 1). This possibly suggests a saturation of the N-acetylation enzyme, but such an observation is inconclusive because of the small sample size of the study subjects. As previously reported (11, 12), the frequency of SAs is extremely low in this population. Determination of whether saturation-type kinetics of sulphadimidine would occur only in the SAs with increasing doses is definitely required in further studies on Papua New Guineans as well as on other ethnic populations. For RAs, a greater dose range than tested in this study would also be needed to establish the threshold for a saturation of the N-acetyltransferase enzyme.

In conclusion, since there was no evidence for misclassifying the polymorphic expression of sulphadimidine over the dose-range studied, any dose, even as low as 250 mg, could be employed for the purpose of acetylation phenotype assessment. However, a single dose (say 750 mg of sulphadimidine) would be an ideal suggested dose, because this dose level has a discriminatory power of acetylator phenotypes. This contention may be supported by considering a possible enzyme saturation of N-acetyltransferase at the higher doses for SA phenotype and an erratic absorption with the smaller doses of the test drug for both phenotypes.

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REFERENCES

