ACETYLATOR PHENOTYPING OF SULFADIMIDINE IN A RANDOMLY MIXED POPULATION

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ABSTRACT

The world populations are classified into slow and fast acetylator phenotypes based on their genetic Lineage. Though some genetic intermixing exists in most populations, trend in genetic phenotypes in randomly mixed populations have not been reported. The population occupying the northwestern part of the Indian subcontinent (now Pakistan) is a unique example of prolonged and random mixing of divergent races. This study reports the genetic phenotyping in this randomly mixed populations and concludes that divergent phenotypes, randomly mixed, yield a perfect 50% distribution of slow and fast acetylators.

The metabolic acetylation status in the subject population was studied using Sulfadimidine as the marker at a dose of 3 mg/kg. The pharmacokinetics of Sulfadimidine and its acetylated metabolite were studied. The acetylation of Sulfadimidine was found to be bimodal with equal distribution between rapid and slow acetylators. The pharmacokinetics parameters of sulfadimidine and its acetylated metabolite, except renal clearance, differed significantly between the rapid and slow acetylators.

Keywords: Genetic phenotyping, pharmacokinetics of Sulfadimidine, acetylation of Sulfadimidine.

INTRODUCTION

The environmental conditions influence the genetic characteristics of the populations (Knudsen, et al., 1999; Marcus et al., 2000; Chen et al., 2001; Lilla et al., 2006; Sanderson et al., 2007; and Nothlings et al., 2009). These geonatical influences are characterized by physiological and biochemical manifestations which are peculiar to the population (Yu et al., 2000; Hein et al., 2000; Al-Yahyaee et al., 2007; Cottechio et al., 2008; Garcia-Martin, 2008; and Zupa et al., 2009). Geonal differences also influence the mechanism by which the drugs are biotransformed and removed from the body (Bell et al., 1993; Castaneda – Hernandez et al., 1995; Xie et al., 1997; Korrapati et al., 1997; and Cui xi et al., 2001). An important example of this phenomenon is drug acetylation polymorphism, where, on the basis of the extent of metabolism of the acetylatable drugs, the world populations are classified as rapid, intermediate or slow acetylators (Castaneda – Hernandez et al., 1995; and Zaid et al., 2007).

The highest percentage of fast acetylators is found among the populations of Eastern Asian origin and the lowest percentage is found in Egyptian and some western Europeans. (Castaneda – Hernandez et al., 1995; Zaid et al., 2004; Zaid et al., 2007; and Hall, 2009).

Several drugs with an aromatic amine functional group undergo the acetylation reaction. Examples include isoniazid, dapsone, hydralazine, phenelzine, procainamide, sulfonamides, nitrazepam, phenytoin, nitrates, allopurinol, acetophenetidin, phenylbutazone,

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antipyrine, phenacetidin, and coumarin and its related compounds (Lunde et al., 1985). To achieve the desired and therapeutically safe effects, the fate of these amine drugs in the body and the acetylation status of the population should be known. Polymorphism in drug acetylation is a prime consideration in the therapeutic management of patients using these drugs (Van der ven et al., 1994; Xie et al., 1997; Gross et al., 1999; Fatima et al., 2003; AL-Yahyee et al., 2007; Garcia – Martein, 2008; Matar et al., 2004). The pattern of polymorphic drug acetylation has not been reported in the South Asian Pakistani Population which represents a random mixture of several races.

South Asia, a native land of Dravidians, was later dominated by Aryans, covering its central and southeastern regions, where Symadic and Dravidian intermixing occurred. The Balochistan province of Pakistan was also under Aryan dominance with some Negroid populations, where racial intermixing took place mainly with Symadics and Turks. The northwestern region of the country is dominated by Symadics with Mongoloid, Aryan and Turks intermixing. The eastern and lower regions are dominated by the native Dravidians with Symadic, Aryan and Turk mixup. The people of Kashmir valley and the Kohistan areas are mainly Aryans and Symadic races, while Gilgit and Chital regions of Pakistan are influx of population took place from almost every part of the India, belonging basically to Dravidian, Mongoloid and Burmese racial mixtures with some Symadic and Turk influences, to the northwestern part of India which is now Pakistan, where now every ethnic group in the South Asian region is represented (Vree et al., 1980; Sarkar, 1981; Zaid et al., 2004; Zaid et al., 2007). Genetic mixing of divergent races in Pakistan represents an ideal population to study the effect of random racial mixing on the manifestation of phenotypes responsible for drug acetylation.

Sulfadimidine was chosen as a marker to study polymorphism in drug acetylation because of its safe therapeutic index, simple metabolism and ease of determination in the body fluids (Chapron, 1980; Xie et al., 1997; Zaid et al., 2004). Several studies have used sulfadimidine to type various world populations (Fristid et al., 1976; Talseth et al., 1977; Chapron, 1980; Jeyakumar & French, 1986; Ven der ven et al., 1994; Castaneda – Hernandez et al., 1995; Xie et al., 1997; Zaid et al., 2004; Hall, 2009).

MATERIALS AND METHODS

Human Studies

Ten healthy male volunteers were randomly selected. All subjects were in good health with normal hepatic and renal functions and normal blood counts. They were not taking any drug treatment for at least one month prior to the study. Their ages ranged between 23 to 28 years and weighed between 56 to 87.5 Kgs. Following overnight fasting, the volunteers were asked to empty their bladders and were given sulfadimidine 3 mg / kg body weight in gelatin capsules orally with 250 ml of water. Nothing was given by mouth to the volunteers for the next two hours followed by standard meals. Water was allowed ab-libitum.

Blood samples were collected just before the administration of the drug, after each hour for the next four hours and at 6th, 8th, and 12th hours in heparinized vacuum tubes. Urine voids were also collected just before the ingestion of the dose and at scheduled time intervals during 0.0-1.5, 1.5-2.5, 2.5-3.5, 3.5-4.5, 4.5-5, 5-7, 7-9, 9-11 and 11-12 hours.

Urinary pH and urine flow rate at different time intervals were also monitored.

Sulfamidine, sulfamethoxazole, N4-acetyl sulfadimidine and N4-acetyl sulfamethoxazole were all obtained from a commercial source (Sigma Chemicals, St. Louis, MO, U.S.A)

Assay Procedure

Determination of free drug and its acetylated metabolite in the body fluids was
Aslam et al. made by the methods described by Vree et al. (Frislid et al., 1976; Vree et al., 1979; Zar, 1984; Fatima et al., 2003; and Hall, 2009) using high performance liquid chromatograph (LC – 5A variable detector SPD – 2A, Shimadzu, Japan). Analytical column (C – 18 micropbondapk, waters Associates, USA) was used. The solvent system was a mixture of phosphate buffer (390 mL of 0.067 M potassium dihydrogen phosphate +10 mL of 0.067 M disodium hydrogen phosphate) and methanol (5:1) at pH 6.7. The solvent flow rate was 1.6 mL/min. The detection of drug and metabolite were performed at 254 nm. Sulfamethoxazole was used as an internal standard.

Plasma was separated by centrifugation at 4000 rpm for 5 minutes and kept at –4°C until assayed. Each sample was run in duplicate. To 0.2 mL of the separated plasma, 0.2 mL of a 10 µg/mL solution of sulfamethoxazole in 0.33 N perchloric acid were added, mixed, vortexed and centrifuged at 4000 rpm for 5 minutes. A 10 µL portion of the supernatant was injected onto the column. The volume and the pH of the urine voids were measured and a 15 mL portion was kept frozen until assayed. The specimens were diluted 10 – 100 times as required. To 0.5 ml of the diluted sample an equal volume of a 10 µg/mL solution of 0.33 N perchloric acid were added. The solution was then subjected to the same procedure as described for plasma.

Standard solutions for sulfadimidine and its metabolite were prepared in the pooled zero hour plasma and urine blanks and were treated in the same way as samples. Standard calibration curves were prepared and the precision and accuracy of the assay results were confirmed daily.

RESULTS AND DISCUSSION

The study subjects were classified as fast or slow acetylators based on their ability to metabolize sulfadimidine. The differentiation was made through profiles of the plasma concentration of sulfadimidine or acetylated sulfadimidine as function of time and excretion in the urine. The mean values of the pharmacokinetic parameters were compared statistically between the two groups using students t – test and ANOVA.

Figs. 1 and 2 show the plasma level profile of sulfadimidine and its acetylated metabolite as a function of time. The fast and slow populations could be easily differentiated and were distributed almost equally. The mean peak plasma concentration for sulfadimidine was substantially lower at 6.36 µg / ml in the fast and 9.60 µg/mL in the slow acetylators (P < 0.01) (Table, Fig. 1). The mean peak plasma concentration for acetylated sulfadimidine was substantially higher at 9.49 µg / mL in the fast and 3.85 µg/mL in the slow acetylators (P < 0.001) (Table, Fig. 2).

The plasma profiles of sulfadimidine and acetylated sulfadimidine were fitted to a non – compartmental model to calculate various derived parameters (Table). The elimination rate constant (K) calculated for sulfadimidine was 0.276 / hr (t½ : 2.56 hrs) in fast and 0.103 /hr (t ½ : 7.00 hrs) in slow acetylators showing a 2 to 3 – fold difference (p < 0.001) between the two groups. According to the published data (Frislid et al., 1976; Oudtshroom et al., 1972; Hekster & Vree, 1982; Van der ven et al., 1994; Alvaro et al., 2001; Cui xi et al., 2001; Garcia-Martin, 2008; Hall, 2009), the values for K in rapid acetylators ranged between 0.198 to 0.462 / hr (t½ : 1.5 to 3.5 hrs) while in slow acetylators K ranges between 0.099 to 0.315 / hr (t½ : 3 to 9 hrs), both values corroborating well with our findings.

The elimination rate constant (K) for acetylated sulfadimidine was 0.127 / hr (t½ : 5.8 hrs) in the fast and 0.047 / hr (t½ : 15.37 hrs) in the slow inactivators. Thus the demarkation between the two groups was quite clear for K. The published reports (Frislid et al., 1976; Oudtshroom et al., 1972; Hekster & Vree, 1982; Van der ven et al., 1994; Alvaro et al., 2001; Cui xi et al., 2001; Garcia-Martin, 2008; Hall, 2009), show the K value to range between 0.115 to 0.154 / hr (t ½: 4.5 to 6 hrs)
in fast and 0.057 to 0.115/hr. (t½: 6 to 12 hrs) in slow acetylators, also corroborating well with our data.

The area under plasma concentration: time curve from zero to 12 hours for sulfadimidine was 27.32 mg.hr/L in the fast and 71.25 mg.hr/L in the slow acetylators. For acetylated sulfadimidine it was 78.22 mg.hr/L in the fast and 39.18 mg.hr/L in the slow acetylators (p<0.001), with an inverse relationship for the free drug and its acetylated metabolite.

The difference between mean residence time (MRT) was also significantly different between the fast and the slow inactivator groups (4.74 hrs in the fast and 11.08 hrs in the slow acetylators). For acetylated sulfadimidine it was 10.33 hrs in the fast and 24.31 hrs in the slow metabolizers (p<0.001), showing a two-fold change, for both the parent drug and its metabolite between the two groups.

The mean area under moment curve (AUMC) evaluated for sulfadimidine was 110.00 mg.hr/L in fast and 386.75 mg.hr/L in slow metabolizers, the difference being highly significant (p<0.001). For acetylated sulfadimidine it was 46.58 mg.hr/L in the fast and

### Table

Biopharmaceutic and pharmacokinetic parameters of sulfadimidine and its metabolite in fast and slow acetylator population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fast Acetylators</th>
<th>Slow Acetylators</th>
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<tbody>
<tr>
<td>Peak Cone, µg/mL, sulfadimidine metabolite</td>
<td>6.36 ± 0.89</td>
<td>9.60 ± 0.29*</td>
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<tr>
<td></td>
<td>9.49 ± 0.18</td>
<td>3.85 ± 0.22**</td>
</tr>
<tr>
<td>Percent dose excretion, 12 hrs sulfadimidine metabolite</td>
<td>5.58 ± 0.43</td>
<td>9.84 ± 0.71*</td>
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<tr>
<td></td>
<td>57.04 ± 3.71</td>
<td>24.56 ± 2.49***</td>
</tr>
<tr>
<td>Half-life, hrs sulfadimidine metabolite</td>
<td>2.56 ± 0.19</td>
<td>7.00 ± 0.75**</td>
</tr>
<tr>
<td></td>
<td>5.80 ± 0.69</td>
<td>15.37 ± 1.66***</td>
</tr>
<tr>
<td>Mean Residence Time, hrs sulfadimidine metabolite</td>
<td>4.74 ± 0.23</td>
<td>11.08 ± 1.19***</td>
</tr>
<tr>
<td></td>
<td>10.33 ± 1.12</td>
<td>24.31 ± 2.97**</td>
</tr>
<tr>
<td>Renal Clearance, mL/min sulfadimidine metabolite</td>
<td>7.25 ± 0.61</td>
<td>5.14 ± 0.70 ns</td>
</tr>
<tr>
<td></td>
<td>25.27 ± 0.84</td>
<td>23.53 ± 2.54 ns</td>
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Key: * = P < 0.1; ** = P < 0.05; *** = P < 0.01

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![Fig. 1. Plasma level profile of sulfadimidine as a function of time.](image-url)
256.75 mg.hr/L in the slow acetylators, respectively.

The renal clearance of sulfadimidine and its metabolite did not differ between the slow and the fast acetylators (Table). Literature reports the renal clearance values from 2 to 14mL/min for sulfadimidine and 18 to 44 mL/min for acetylated sulfamidine in the two groups (Frislid et al., 1976; Hekster & Vree, 1982; Naizi, 1979). The present findings are therefore in good agreement with the published values.

The amount of acetylated sulfadimidine excreted in urine is an important criteria for phenotyping, especially in cases where only urinary data are available. Many researchers have reported that subjects excreting more than 70% of the total drug in urine as acetylated sulfadimidine are regarded as fast,
while subjects excreting less than 70% of the total drug as acetylated sulfadimidine form, are classified as slow acetylators (Oudshroom and Potgieter, 1972; Zar, 1984; Zaid et al., 2007). In this study, cumulative percent acetylated sulfadimidine excreted in urine was 91.07% in the fast and 71.47% in the slow acetylators (Fig. 3). These findings are in good agreement with the previously published data (Oudshroom and Potgieter, 1972; Vree et al., 1983; Zar, 1984; Xie et al., 1997, Zaid et al., 2004).

The amount of intact sulfadimidine excreted during the first 12 hours was not significantly different (5.58% in fast and 9.84% in slow acetylators). For acetylated sulfadimidine, it was 57.03% in fast and 24.56% in slow acetylators respectively (Table I). These values are comparable to reported values in other populations (Weber, 1984; Hekster and Vree, 1982; Vree et al., 1986; Pande et al., 2003). The excretion ratio of acetylated sulfadimidine to sulfadimidine show that the individuals with a value of 10 or higher could be classified as the fast and those with a 2-3 (or below 5) could be classified as slow acetylators.

CONCLUSION

The environmental and geonatical variations among various ethnic groups play an important role in drug metabolism and evaluation of optimum dosage regimen of drugs for achieving safe and effective blood levels. In a population with a known acetylation status one can predict the fate of a number of drugs, especially the aromatic amines, to achieve desire plasma levels.

This study on acetylator phenotyping, with sulfadimidine as a test substance, highlights biopharmaceutic and pharmacokinetic difference in the slow and the fast acetylators. The parameters based on metabolite were found to be more useful in characterizing the rapid and slow groups. The insignificant difference in the renal clearance between the two groups shows that in the two distinct phenotypes shows that the only significant differences lies in the extent of acetylated sulfadimidine formed.

A bimodal distribution ratio for rapid versus slow metabolizers was found as 50:50 in the randomly mixed population studied suggests that random racial mixing results in the distribution of the genetic phenotypes which is equally distributed for each type of gene.

REFERENCES


