Acetylation Pharmacogenetics and Renal Function in Diabetes Mellitus Patients

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Abstract Activities of human hepatic drug metabolizing enzymes *N*-acetyl transferase (NATS) had earlier been recognized as a cause of inter-individual variation in the metabolism of drugs. Therefore acetylation of many drugs in human exhibit genetic polymorphism. The aim of the study was to investigate if acetylator status predispose diabetic mellitus patients more to the complications of renal disease. One hundred and twenty (120) diabetics consisting of (50) Type 1 (T_1) and 70 Type 2 (T_2) diabetes mellitus patients and 100 healthy individuals as controls were classified as slow or rapid acetylator using sulphamethazine (SMZ) as an *in vivo* probe. The percentage acetylation, recovery of SMZ, creatinine clearance and presence of urinary albumin were determined. A significant difference ($P < 0.05$) was observed in the percentage of SMZ acetylated between slow and rapid acetylators in control, T_1 and T_2 subjects. The ratios of slow to rapid acetylators for T_1 , T_2 and control subjects were 1:4, 3:2 and 2:3 respectively. No significant differences were observed in the percentage of SMZ recovered in the urine of slow and rapid acetylators that are diabetics. The difference in creatinine clearance of slow and rapid acetylators in T_1 and T_2 were significant ($P < 0.05$). 29% out of 120 (24.2%) diabetics (T_1 and T_2) exhibited albuminuria out of which 25 (86.2%) had slow acetylator status. These findings suggest that slow acetylator status in diabetes mellitus could be a predisposing factor in the development of renal

complications. This underscores the need for a rapid pharmacogenetic testing and therapeutic drug monitoring in such patients. However this inference could be further validated with a larger sample size.

Keywords Diabetes mellitus · Pharmacogenetics · Toxicity · Renal function

Introduction

Variation in drug metabolism and drug response among individuals of the same body weight and on the same drug dose can be due to temporary causes such as transient enzyme inhibition, induction or permanent causes such as genetic mutation, gene deletion or amplification [1]. A genetic mutation frequency exceeding 1% of a population is considered as a genetic polymorphism [2]. Pharmacogenetic polymorphism can manifest at the pharmacokinetic and pharmacodynamic levels. The pharmacokinetic level deals with gene polymorphism that modify the concentrations of drugs and its metabolites at the site of their molecular action (Polymorphism of drug metabolizing enzymes, drug transporters) whereas the pharmacodynamics levels deals with polymorphisms of the genes associated drug effect and mechanism of action not related to its concentration (receptor's ion channels).

Pharmacogenetics explores the connection between genetic predisposition and drug efficacy. Thus allowing for the specific phenotypes to be identified.

According to drug metabolizing ability, genetic polymorphism is related to three phenotype classes; the extensive drug metabolizer phenotype (EM) is characteristic of the normal population; the poor drug metabolizer (PM) is a consequence of mutation and or deletion of both

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alleles and is associated with accumulation of specific drug substrates. The Ultra Rapid drug metabolizer (URM) phenotype is a consequence of gene amplification and results in enhanced drug metabolism. Genotype is the detailed gene structure of an individual whereas the more commonly measured phenotype is the outcome of metabolism of a drug in an individual [3]. Since genotype is the result of interactions between genetic make up and the environment, it is not always concordant with phenotype. Variable drug concentrations can produce a variety of clinical outcomes. Slow acetylators have more side effects with drugs like dapsone and procainamide and ultrafast acetylators have less reliable clinical responses with drugs like isoniazid [3].

Drug metabolism is conventionally described as consisting of phase I oxidation reactions primarily mediated by cytochrome P450 enzymes in the liver and phase 2 conjugations such as glucuronidation; sulphonation and acetylation. It is now known that *N*-acetyl transferase (NAT) is controlled by two genes (NAT 1) and (NAT 2) of which NAT 2 A and B are responsible for clinically significant metabolic polymorphism [4]. Many common drugs such as caffeine, isoniazid, nitrazepam and sulphonamides are acetylated. So also are aromatic and heterocyclic amine carcinogens which led to the concept of possible relationship between NAT genotypes and cancer susceptibility [4]. If Sulphamethazine, caffeine or another marker drug is given and plasma and urine drug concentrations are measured after a standard time interval, it is possible to separate individuals into two groups, fast acetylators who have only low concentrations of parent drug remaining in the blood and slow acetylators who have much higher concentrations [5]. Variations in acetylator status has been reported in different racial groups; Caucasians and Negro populations have approximately equal proportions of fast and slow acetylators whereas oriental groups have almost 90% fast acetylators [6]. Slow acetylator phenotype is preponderant among different Arab populations irrespective of geographical location of the country, whether it is in Asia (e.g. Saudi Arabia and United Arab Emirate) or North Africa (e.g. Egypt and Morocco) [7–9]. A third group whose status fall in between the rapid and slow metabolisers termed intermediate metabolisers had been observed in some workers using isoniazid as a marker [10]. In Nigeria, the relative incidence of the two acetylator phenotypes varies among different populations. For example, in Northern Nigeria, about 40% were found to be slow acetylators [11] while 41% were reported to be slow acetylators in both Eastern Nigeria [12] and South Western Nigeria [13]. Fast acetylators also have the potential for more side effects in situation where an active metabolite is responsible for toxicity. It is now possible to identify slow and fast acetylators by analyzing NAT 2 gene but this is

essentially a research procedure. However in routine laboratories, phenotype is determined by analyzing the drug metabolite ratio in urine after standard doses of drugs such as caffeine, isoniazid or sulphamethazine. This is commonly requested for in patients with tuberculosis.

Sulfonamide derivative oral hypoglycemics i.e. the sulfonyl ureas are among the most widely prescribed medications in the world for type 2 diabetics and examples include acetohexamide, chlorpropamide, tolazamide, glipizide, glyburide and glimerpirizide.

This study was therefore concerned with investigating if any relationship exists between acetylator status and increased risk of progression and development of renal complications in diabetes mellitus patients.

Materials and Methods

Patients

One hundred and twenty (120) patients consisting of 26 women and 94 men previously diagnosed with diabetes mellitus were enrolled for the study from the diabetic clinic of the University College Hospital, Nigeria after ethical committee approval and patient's consent.

The mean age and SD was 42.4 ± 13 years and the range was 18–65 years.

Fifty (50) were type I and seventy (70) were type II diabetic patients. One hundred (100) age and sex matched healthy volunteers were recruited from the student and staff community of the University College Hospital, Ibadan Nigeria as controls.

Metabolic Probe

Sulphamethazine tablets obtained from May and Baker were administered at a dose of 2,000 mg/subject for both diabetics and controls. Each tablet contains 500 mg of drug. Which means each subject consumed 4 tablets of the drug.

Sample Collection

Total body weight of all subjects were determined using Ohaus weighing balance.

Pre-drug loading urine samples were collected from both test and control subjects to serve as sample controls.

The control and Test subjects were free of drugs forty-eight (48) hours prior to the experiment. All subjects were well hydrated with an average of 1 l of clear drinking water and the urine voided over 6 h after dosing into a Winchester bottle.

Urinalysis

The volume of urine collected was determined using a litre measuring cylinder.

A dipstick urinalysis was performed on all samples using Combi 3 urinalysis strips.

Bratton and Marshall's Sulphamethazine Method

A Calibration Curve using 20 mg/ml of Standard Sulphamethazine was plotted using the following dilution 0, 4, 8, 12, 16, 20, 24 µg.

In the reaction, Sulphamethazine was diazotised with sodium nitrite in the medium, ammonium sulphamate was used to remove the excess sodium nitrite. The diazotized sulphamethazine was then coupled with *N*(1-naphthyl) ethylene diamine dihydrochloride (NED) and estimated Spectrophotometrically at 540 nm.

Each urine sample had 2 set of tubes labelled as F (free drug) T (Total drug).

A control sample tube of the urine was also set up for each experimental subject.

The absorbance was extrapolated on the standard curve and the concentrations of sulphamethazine determined by calculation (Fig. 1).

The percentage of sulphamethazine acetylated was given by the formula:

$$\frac{T - F}{T} \%$$

Percentage recovery of SMZ

$$= \frac{\text{vol of urine} \times \text{total SMZ in 6 h urine}}{2,000 \text{ mg}} \times \frac{100}{1} \%$$

Fig. 1 Standard curve relating absorbance to concentration of SMZ in urine

Fig. 2 Frequency distribution histogram of percentage of acetylated (SMZ) in urine of controls

Results were classified as slow acetylators if % of sulphamethazine recovered was between 0 and 49% and rapid acetylators if from 50% above (Fig. 2).

Creatinine Estimation in Plasma and Creatinine Clearance Test

Creatinine concentrations were determined in plasma and urine samples of tests and controls and creatinine clearance determined as a function of glomerular filtration rate (GFR) by a standard method [14].

Results

In this study, our *in vivo* probe revealed bimodal distribution: slow and rapid acetylation status of our study population. Among the controls, 40 representing 40% were slow acetylators while 60 representing 60% were rapid acetylators with a ratio 2:3. In type I diabetes patients, 10 (20%) were slow and 40 (80%) rapid acetylators with a ratio of 1:4 (Table 1) However, in type II diabetes patients, 42 (60%) were slow and 28 (40%) rapid acetylators with a ratio of 3:2 (Table 1). Significant differences ($P < 0.05$) were observed in the percentage of drug acetylated and the amount of drugs recovered in the urine between slow and rapid acetylators in the three groups studies. Renal function as assessed by creatinine clearance showed physiologically normal values in all the subjects studied but slow acetylators exhibited reduced function which was found to be significant in Type 1 diabetic patients (Table 2; Fig. 3). Furthermore, 8 out of 10 representing 72.7% of slow acetylators had albuminuria compared to 2 out of 40 (50%) of rapid acetylators in Type 1 diabetes (Table 2). However, 17 out of 42 representing 40.48% of slow acetylators had albuminuria compared to 2 out of 28 (7.14%) of rapid acetylators in Type II diabetes patients (Table 2; Fig. 4).

Table 1 Comparison of mean values and standard deviation of slow and rapid acetylators for sulphamethazine among diabetics and control subjects

Subjects	Acetylation (%)	Recovery in urine (%)	Ratio	<i>P</i> value
Control				
Slow (40)	30.69 ± 3.08**	16.31 ± 2.90	2:3	<0.05
Rapid (60)	69.50 ± 3.72	35.88 ± 5.00		
D.M. (TI)				
Slow (10)	22.60 ± 6.34**	13.90 ± 3.68	1:4	<0.05
Rapid (40)	64.80 ± 2.45	20.16 ± 3.14		
D.M. (TII)				
Slow (42)	25.50 ± 3.56**	14.47 ± 2.26	3:2	<0.05
Rapid (28)	65.14 ± 4.47	19.87 ± 6.07		

* Not significantly ($P > 0.05$) different from rapid acetylators** Significantly ($P < 0.05$) different from rapid acetylators**Table 2** Renal function tests of control and diabetic subjects in slow and rapid acetylators

Subjects	Creatinine clearance (ml/min/1.73 m ²)	Urinary albumin (no. of positive subjects)	<i>P</i> value
Control			
Slow (40)	127 ± 5.78*	Nil (0%)	>0.05
Rapid (60)	135 ± 4.02	Nil (0%)	
D.M. (TI)			
Slow (10)	114 ± 2.03**	5 (80%)	<0.05
Rapid (40)	132 ± 3.75	2 (5%)	
D.M. (TII)			
Slow (42)	130 ± 1.88*	17 (40.48%)	>0.05
Rapid (28)	139 ± 2.56	2 (7.14%)	

* Not significantly ($P > 0.05$) different from rapid acetylators** Significantly ($P < 0.05$) different from rapid acetylators **Fig. 3** Frequency distribution histogram of percentage of (SMZ) acetylated in urine of type I diabetes mellitus patients

Discussion and Conclusion

Sulphamethazine (SMZ) is mainly metabolized by polymorphic acetylation by the enzyme *N*-acetyl transferase

Fig. 4 Frequency distribution histogram of percentage of (SMZ) acetylated in urine of type II diabetes mellitus patients

and the stable soluble acetylated metabolite and the parent drug is excreted in urine. Evans and White [15] showed that SMZ acetylation exhibited bimodal distribution. Some other studies had described existence of intermediate metabolites [16]. The result of the present study is in consonance with previous studies which observed 40% of slow acetylators in the control group of subjects from south western Nigeria [13]. Our findings also showed that the distribution of rapid and slow acetylators among diabetics was comparable to the normal healthy subjects. However slow acetylators exhibited reduced renal function as evidenced by impaired creatinine clearance in type 1 but not in type 2 diabetics. This can be explained by the fact that patients with type 1 diabetes are at greater risk of developing microangiopathy of the kidneys and retina than the type 2 diabetics whose major complications are more apt to be related to disease of the medium sized and large arteries. Although the quality of control of diabetes most likely play an important role, complication cannot be attributed solely to poor control. The effect of diabetics control on the development of complications remains an unresolved controversy.

The observation that supposedly well controlled diabetics continue to have elevated levels of haemoglobin A₁C, a result of sustained hyperglycemia indicates the ineffectiveness of present conventional therapeutic methods of controlling hyperglycemia [17]. Although the haemoglobin A₁C estimation was not done in this study, its levels in relation to acetylator status could be a consideration for future studies.

The duration of diabetes in the type 1 patients studied was 10 years while that of type 2 diabetics was 15 years. The acetylator status could not be said to be influenced by the duration of the condition since it is a genetic phenomenon. It was also observed that significant differences occurred in the percentage of sulphamethazine acetylated in slow and rapid acetylators with T₁, T₂ diabetics and control rapid acetylators having higher values. Renal function especially glomerular filtration rate as assessed by the value of creatinine clearance test appeared to be within

physiological range in both diabetics and healthy subjects in this study. Although it is well documented that albuminuria is one of the complications of diabetes mellitus, it is not known whether the slow acetylator status could be a predisposing factor. In this study, 29 out of the 120 diabetic patients (Types I and 2) had albuminuria. 25 (86.2%) out of which were slow acetylator status. It therefore implies that a linear relationship might exist between slow acetylator status and development of albuminuria among the diabetes subjects studied. However, more expanded studies is required to ascertain whether slow acetylator status could be a contributing factor for the development of renal complications among diabetes mellitus patients. Furthermore, a relationship between acetylator phenotype and diabetic neuropathy had been observed by some other researchers [18, 19]. Polymorphic drug metabolism is no doubt an important area of therapeutics. However with the exception of in vivo procedures for thiopurine methyl transferase (TPMT), genotyping remains a research procedure [20]. Phenotyping should therefore be a routine procedure in large laboratories for acetylation and the best understood cytochrome P450 enzymes. *N*-acetyl transferase variants had also been known to have mutagenic potentials [21]. Clinical relevance of genetic polymorphism in cytochrome p450 system as contributing to adverse drug reactions should be recognized. The limitation of this study is the small sample size which may complicate the interpretation of genetics based studies.

The type 1 diabetes patients in this study were on insulin therapy, namely humulin R, humulin 1 and humulin 70/30 while their type 2 counterparts were being treated with sulfonylureas; namely glibenclamide and glicazide. Since acetylation is one of the pathways of sulfonylurea metabolism, it is logical to suggest that type 2 diabetics slow acetylators are more prone to acetylator status related adverse drug reaction than type 1 diabetes patients. However the latter cannot be totally excluded from such risks; as possibilities of their being treated with sulphonamides containing drugs and consumption of coffee still exist. It should be observed that adverse drug reaction which is likely acetylator status related has been reported with glipizide therapy [22]. It is also important to note that slow acetylator status has been associated with generically defective arylamine-N acetylation due to a parallel decrease in the quality of two structurally and functionally similar but kinetically distinct acetylating enzymes designated NAT-1 and NAT-2 [23, 24]. It is therefore not preposterous to suggest that slow acetylators diabetics are predisposed to adverse drug reactions in addition to known complications of the disease.

The results of this study which could be further validated with increased sample size underscores the need for

therapeutic drug monitoring in subjects phenotyped as slow acetylators. However, the cost implication viz a viz clinical benefits to the patients needs to be considered.

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