Assessment of cytochrome P450 2E1 activity in Hausa/Fulani of northwest Nigeria using chlorzoxazone as a probe determination of polymorphism

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Background and objective
High expression and activity of cytochrome P450 2E1 have been linked to non-alcoholic fatty liver disease; increased susceptibility to the gastric, nasopharyngeal, colorectal, urinary bladder and esophageal malignancies; and acetaminophen-induced hepatotoxicity. It plays key roles in activating procarcinogens to carcinogens, metabolism of xenobiotics, and hosts of endogenous compounds. This study aimed at determining the polymorphism of this highly polymorphic enzyme among Hausa/Fulani in northwest Nigeria.
Materials and methods
A total of 20 nonrelated Hausa/Fulani from Sokoto metropolis were selected by convenient sampling. A tablet of 250 mg chlorzoxazone was administered orally to them with 100 ml of distilled water after an overnight fast, and 3 h after dosing, urine was collected. HPLC equipped with a UV detector was performed for simultaneous estimation of chlorzoxazone and its metabolite 6-hydroxychlorzoxazone. Metabolic ratio index method was used for each participant The data generated were analyzed using Statistical Package for the Social Sciences version 20 (SPSS-20) by constructing frequency histogram and probit plots. A trend line was added to the probit plot, and polynomial equation obtained was resolved to get antimode. Participants with antimode greater than or equal to value of intercept on logMR were regarded as poor metabolizers, whereas those with less were extensive metabolizers.
Result and conclusion
Anti-mode was found to be –1.8, and only 7 of 20 participants were extensive metabolizers (35%, odds 0.54, 95% confidence interval: 0.22–1.3). Although convenience sampling was used, the findings are worrisome considering the highly polymorphic and the procarcinogenic nature of the enzyme.

Keywords:
chlorzoxazone, cytochrome 2E1, Hausa/Fulani, polymorphism

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Introduction

Cytochrome P450 2E1 is expressed mainly in the liver, nasal mucosa, kidney, lungs, and brain and serves as an important source of reactive oxygen species [1]. It is mapped to chromosome10q24.3-qter, spans 11 kb containing 9 exons, and encodes a membrane-bound protein of 493 amino acids [2,3].

High expression and activity of cytochrome P450 2E1 have been linked to non-alcoholic fatty liver disease and increased susceptibility to the gastric, nasopharyngeal, colorectal, urinary bladder, and esophageal malignancies among a host of others [4,5]. The activity of this enzyme undoubtedly also influences acetaminophen metabolism among different age groups. Ethnicity, apart from age, has been demonstrated to significantly determine cytochrome P450 2E1 gene expression. Higher level was found in Northern Europe-Americans and Hispanics than in African-Americans [6].

It plays key roles in activating procarcinogens to carcinogens, metabolism of xenobiotics, and hosts of endogenous compounds [7]. It metabolizes acetaminophen, isonicotinic acid hydrazide, chlorzoxazone, acetone, alcohol, aniline, vinylchloride, benzene, *N*-nitrosodimethylamines, and styrene. CYP 2E1 belongs to phase I DMEs, and though it metabolizes only ~3% of clinically used drugs, it is highly polymorphic, and this polymorphism corresponds to the replacement of cytosine and thymine at position –1019 [1–8].

Polymorphism of this enzyme and allelic distribution frequencies vary within and between populations. Increased susceptibility to breast cancer has been

reported to be linked to the polymorphism of this enzyme (*CYP 2E1*6*) as well as coronary artery lesions in patients with Kawasaki disease [9,10]. This study aimed at determining the polymorphism of this all-important drug-metabolizing enzyme among Hausa/Fulani in northwest Nigeria. To the best of our knowledge, this is the first attempt to assess cytochrome P450 2E1 activity among the Hausa/Fulani ethnic group, which forms the most populous ethnic group in Nigeria.

Materials and methods

Study population, exclusion criteria, and ethical consideration

Only participants who were declared of Hausa/Fulani descent and identified by one of the researchers (MTU) were involved in the study by convenient sampling from Sokoto metropolis in northwest Nigeria through their expressed consents. It was an exploratory study.

All volunteers who consumed alcohol, smoke tobacco, or with suspicion of hypersensitivity to chlorzoxazone (2E1 probe) were excluded. Other criteria were taking any prescription or herbal medicines within 2 weeks before the study or any over-the-counter medication within 1 week before the study. Similarly, all pregnant and lactating women, as well as children were excluded [11,12].

All participants read and signed a study-specific informed consent form before participating in the study procedures. The study was approved by the Ethics Committee of Sokoto State Ministry of Health.

Samples taking, preparation, and chromatography

Participants were asked to refrain from consuming caffeine 48 h before chlorzoxazone administration in addition to satisfying inclusion criteria. They fasted overnight for 11 h. At 8 a.m. on the day of the sample taking, the participants emptied their bladders, and a tablet of 250 mg chlorzoxazone was administered orally to them with 100 ml of distilled water. Three hours after dosing, urine was collected in a universal sample tube. The participants were observed for 8 h after the dose for any untoward effects and were discharged uneventfully.

Assay of chlorzoxazone and 6-hydroxychlorzoxazone in urine was carried out using Stiff *et al.* [13] method.

HPLC equipped with a UV detector was performed for the simultaneous estimation of chlorzoxazone and its metabolite 6-hydroxychlorzoxazone in urine using the method [13]. Urine samples (diluted 1 : 500) were treated with β-glucuronidase before analysis. The detection of components was on the wavelength of 270 nm.

Statistics

The data were analyzed by obtaining urine chlorzoxazone and 6-hydroxychlorzoxazone (metabolite) concentrations from the chromatograms generated by the HPLC. The metabolic ratio (MR) was calculated for each participant from these concentrations and logarithmic values determined. Statistical Package for the Social Sciences IBM* version 25, Armonk, NY, IBM Corp. USA, 2017 (SPSS-25) was used to construct frequency histogram using number of participants and logMR. Probit values (standard normal deviates) were obtained from *Z*-table and were plotted on *Y*-axis against logMR on *X*-axis (Scatter chart). A trend line was added to the probit plot and polynomial equation obtained. Anti-mode was determined as the intercept of *X*-axis, where *Y*=0 from logMR. Participants with antimode greater than or equal to value of intercept on logMR regarded as poor metabolizers, whereas those with less than values were considered extensive metabolizers. The outcome was reported as proportions with 95% confidence intervals. Polymorphism was determined graphically as the deviation of the probits values from the line of fit on the graph (Figs 1 and 2).

Results

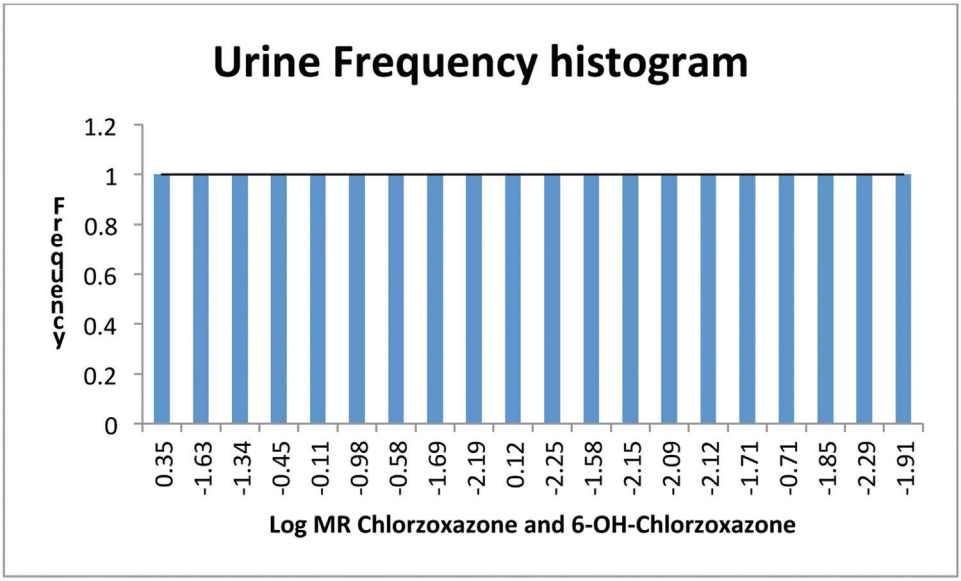
The age range and median are shown in Table 1. MRs for each participant and metabolic status are displayed in Table 2. Nonparametric statistics were used because the sampling was by convenience. Nonetheless, the means, standard deviations, and *P* values are also shown simply to identify trends, although they may be considered inappropriate for nonrandom data.

Discussion

Cytochrome P450 phenotype studies provide useful information on the instantaneous activity of drug-metabolizing enzymes by the use of specific probes of which chlorzoxazone is one [14]. Most drugs in clinical use are efficacious in only ~25–60% of patients mainly owing to polymorphism of cytochrome P450 enzymes, which may be up to 50-folds between individuals as previously cited [14].

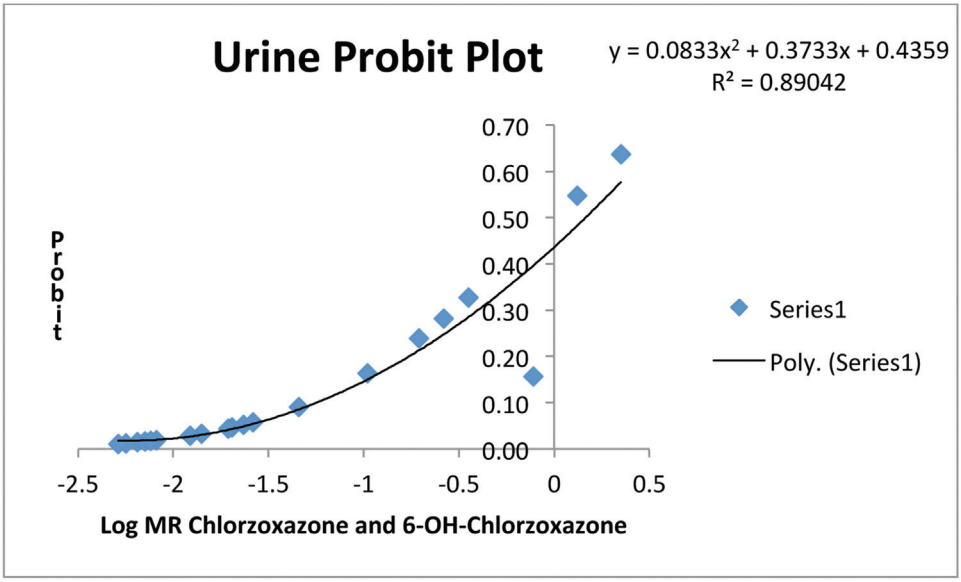
Probe drug's characteristics and MR measurements are critical in the evaluation of phenotype cocktails, which serve as a reservoir for future application in personalized therapy. Except for cumbersomeness and vulnerability to errors owing to multiple points of sample collection, the

Fig. 1



Urine frequency versus logMR histogram of participants.

Fig. 2



Urine probit vs LogMR plot of participants. Scatter (*XY*) chart showing the Trend line with the best linear fit to the data and polynomial equation. At *X*-intercept where *Y*=0, the equation becomes $0.083x^2+0.373x+0.435=0$. The values of *X* were −1.8 and −2.9 and therefore −1.8 was considered as the antimode. The probits that do not fit to the trend line are indicative of polymorphism.

formation clearance method appeared to be the most suitable approach for the measurement of drug-metabolizing enzyme activity *in vivo* [14]. However, metabolic indexes (MR) in which coefficients between probes and metabolites are calculated from probits and logarithms of MRs are generally used to circumvent drawbacks in the formation clearance method and achieve the desired outcome [15].

Chlorzoxazone hydroxylation to its metabolite, 6-hydroxychlorzoxazone, is a recognized measure of the in-vivo cytochrome P450 2E1 activity. Because the metabolism of this drug has fairly low interindividual variability at a dose of 250 mg, single sample taking is enough to assess cytochrome P450 2E1 activity *in vivo* [16,17]. Chlorzoxazone is a standard probe for cytochrome 2E1 that is used in establishing phenotypes of individual subjects [18]. Between-subjects variability in the enzyme's activities and consistent ethnic variations in the gene expression have been demonstrated previously [19,20]. The variability of cytochrome P450 2E1 expression

Table 1 Demographic characteristics of participants (*n*=20)

Variables	Range	Median	Mean	SD
Age (years)	19–46	23.0	26.5	8.4
Weight (kg) 35–131	59.5	66.3	23.3	
Height (m) 1.5–1.8	1.71	1.7	0.9	
BMI (kg/m^2)	13.7–37.2	20.9	22.4	6.3

Table 2 Hausa/Fulani urine phenotype parameters (*n*=20)

ID	Chlorzoxazone (mg/l)	6-OH-chlorzoxazone (mg/l)	LogMR	Probit	MS
1	11.67	5.213	0.35	0.64	PM
2	0.61	26.16	–1.63	0.05	PM
3	0.915	20.09	–1.34	0.09	PM
4	4.713	13.18	–0.45	0.33	PM
5	16.75	21.57	–0.11	0.16	PM
6	0.241	2.325	–0.98	0.16	PM
7	0.634	2.413	–0.58	0.28	PM
8	0.095	4.636	–1.69	0.05	PM
9	0.153	23.73	–2.19	0.01	EM
10	2.793	2.095	0.12	0.55	PM
11	0.128	23.02	–2.25	0.01	PM
12	0.064	2.452	–1.58	0.06	PM
13	0.159	22.33	–2.15	0.02	EM
14	0.207	25.75	–2.09	0.02	EM
15	0.081	10.68	–2.12	0.02	EM
16	0.407	21.02	–1.71	0.04	PM
17	0.328	1.665	–0.71	0.24	PM
18	0.304	21.65	–1.85	0.03	EM
19	0.135	26.51	–2.29	0.01	EM
20	0.177	14.42	–1.91	0.03	EM

Seven of 20 participants were extensive metabolizers (35%, odds 0.54, 95% CI: 0.22–1.3) anti-mode=–1.8; ID, participant identity LogMR ranges from –2.29 to 0.35. CI, confidence interval; EM, extensive metabolizers; MS, metabolic status; PM, poor metabolizer.

between persons is noteworthy and is interrelated with its enzymatic activity [21].

The occurrence of deviations from the line of fitness observed in the probit plots in this study was suggestive of polymorphisms among the participants [22]. This finding further support what was reported by Kim *et. al.* [23]. The correlations of determination of the plots revealed an outstanding relationship between the variables of probits and logMR as only 14% variations were unexplainable from polynomial expressions studied.

Cytochrome P450 2E1 enzyme activity among the participants was categorized phenotypically into poor and extensive metabolism. A good number of the participants were classified as poor metabolizers based on the anti-mode derived from urine probit versus logMR plots. This observation may be worrisome, as poor activity of the enzyme results ultimately to the toxicity of the agents being metabolized by the enzyme with wider pathological implications.On a long-term basis, this is manifested in the population as vulnerability to pathogenesis of nasopharyngeal, colorectal, stomach, esophageal, liver, lungs, and bladder malignancies [24,8–25]. While on a short-term range, fulminant hepatic failure from acetaminophen ingestion affecting millions of people globally remained in focus [26–28].

Conclusion

Although convenience sampling was used, the findings are worrisome considering the highly polymorphic and the procarcinogenic nature of the enzyme.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Zanger UMU, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Therapeut 2013; 138:103–141.

2 Lakkakula S, Maram R, Munirajan AK, Pathapati RM, Visweswara SB, Lakkakula BV. EPHX1 gene polymorphisms among south Indian populations. Mol Cell Toxicol 2013; 9:219–225.

3 Leung TM, Nieto N. CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease. J Hepatol 2013; 58:395–398.

4 Tang K, Li X, Xing Q, Li W, Feng G, He L, *et al.* Genetic polymorphism analysis of cytochrome P4502E1 (CYP2E1) in Chinese Han populations from four different geographic areas of Mainland China. Genomics 2010a; 95:224–229.

5 Yao K, Qin H, Gong L, Zhang R, Li L. CYP2E1 polymorphisms and nasopharyngeal carcinoma risk: a meta-analysis. Eur Arch Otorhinolaryngol 2017; 274:253–259.

6 Johnsrud EK, Koukouritaki SB, Divakaran K, Brunengraber LL, Hines RN, McCarver DG. Human hepatic CYP2E1 expression during development. J Pharmacol Exp Thera 2003; 307:402–407.

7 Rendic S, Guengerich FP. Contributions of human enzymes in carcinogen metabolism. Chem Res Toxicol 2012; 25:1316–1383.

8 Chaaben A, Ben Abaza H, Douik H, Chaouch L, Ayari F, Ouni N, *et al.* Polymorphisme génétique du cytochrome P450 2E1 et le risque du cancer du nasopharynx. B Cancer 2015; 102:967–972.

9 Chang LS, Hsu YW, Lu CC, Lo MH, Hsieh KS, Li SC, *et al.* CYP2E1 gene polymorphisms related to the formation of coronary artery lesions in Kawasaki disease. Pediatr Infect Dis J 2017; 36:1039–1043.

10 Lu Y, Zhu X, Zhang C, Jiang K, Huang C, Qin X. Role of CYP2E1 polymorphisms in breast cancer: a systematic review and meta-analysis. Cancer Cell Int 2017; 17:11.

11 Tortorici MA, Toh M, Rahavendran SV, Labadie RR, Alvey CW, Marbury T, *et al.* Influence of mild and moderate hepatic impairment on axitinib pharmacokinetics. Invest New Drug 2011; 29:1370–1380.

12 Devarakonda K, Morton T, Margulis R, Giuliani M, Barrett T. Pharmacokinetics and bioavailability of oxycodone and acetaminophen following single-dose administration of MNK-795, a dual-layer biphasic IR/ER combination formulation, under fed and fasted conditions. Drug Des Devel Ther 2014; 8:1125.

13 Stiff DD, Erye RE, Branch RA. Sensitive high-performance liquid chromatographic determination of chlorzoxazone and 6-hydroxychlorzoxazone in plasma. J Chromatogr 1993; 613:127–131.

14 Keller GA, Gago MLF, Diez RA, Girolamo GD. In vivo phenotyping methods: cytochrome P450 probes with emphasis on the cocktail approach. Curr Pharm 2017; 23:2035–2049.

15 Chao P, Uss AS, Cheng K. Use of intrinsic clearance for prediction of human hepatic clearance. Expert Opin Drug Met Toxicol 2010; 6:189–198.

16 Frye RF, Adedoyin A, Mauro K, Matzke GR, Branch RA. Use of chlorzoxazone as an in vivo probe of cytochrome P450 2E1: choice of dose and phenotypic trait measure. J Clin Pharmacol 1998; 38:82–89.

17 Ernstgård L, Warholm M, Johanson G. Robustness of chlorzoxazone as an in vivo measure of cytochrome P450 2E1 activity. Br J Clin Pharmacol 2004; 58:190–200.

18 Witt L, Suzuki Y, Hohmann N, Mikus G, Haefeli WE, Burhenne J. Ultrasensitive quantification of the CYP2E1 probe chlorzoxazone and its main metabolite 6-hydroxychlorzoxazone in human plasma using ultra-performance liquid chromatography coupled to tandem mass spectrometry after chlorzoxazone microdosing. J Chromatogr B Analyt Technol Biomed Life Sci 2016; 1027:207–213.

19 Bolt HM, Roos PH, Thier R. The cytochrome P-450 isoenzyme CYP2E1 in the biological processing of industrial chemicals: consequences for occupational and environmental medicine. Int Arch Occup Environ Health 2003; 76:174–185.

20 Marchand L, Le Wilkinson GR, Wilkens LR. Genetic and dietary predictors of CYP2E1 activity: a phenotyping study in Hawaii Japanese using chlorzoxazone. Cancer Epidemiol. Biomarkers Prev 1999; 8:495–500.

21 Ohtsuki S, Schaefer O, Kawakami H, Inoue T, Liehner S, Saito A, *et al.* Simultaneous absolute protein quantification of transporters, cytochromes P450, and UDP-glucuronosyltransferases as a novel approach for the characterization of the individual human liver: comparison with mRNA levels and activities. Drug Metab Dispos 2012; 40:83–92.

22 Varshney E, Saha N, Tandon M, Shrivastava V, Ali S. Prevalence of poor and rapid metabolizers of drugs metabolized by CYP2B6 in North Indian population residing in Indian national capital territory. SpringerPlus 2012; 1:34.

23 Kim RB, O'Shea D, Wilkinson GR. Interindividual variability of chlorzoxazone 6-hydroxylation in men and women and its relationship to CYP2E1 genetic polymorphisms. Clin Pharmacol Thera 1995b; 57:645–655.

24 Danko IM, Chaschin NA. Association of CYP2E1 gene polymorphism with a predisposition to cancer development. Exp Oncol 2005; 27:248–256.

25 Lin YC, Wu X, Zhou XQ, Ren R, Su ZX, Liu CX. Cytochrome P450 2E1 RsaI/PstI polymorphism is associated with urologic cancer risk: evidence from a meta-analysis. Int J Clin Exp Med 2015; 8:8927–8937.

26 Lee WM. Recent developments in acute liver failure. Best practice & research. J Clin Gastroenterol 2012; 26:3–16.

27 Jozwiak-Bebenista M, Nowak JZ. Paracetamol: mechanism of action, applications and safety concern. Acta Pol Pharm 2014; 71:11–23.

28 Brune K, Renner B, Tiegs G. Acetaminophen/paracetamol: a history of errors, failures and false decisions.Eur J Pain 2015; 19:953–965.