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OXIDATIVE HYDROXYLATION OF OMEPRAZOLE IN HEALTHY SUBJECTS OF NIGER DELTA REGION BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Background: Profiling individuals with a deficient capacity for oxidative drug metabolism is important in ensuring rational drug therapy. Cytochrome P450 2C9 isozyme (CYP2C19) accounts for the biotransformation of 10-15% of drugs in clinical use. Omeprazole (OME) is commonly employed as a probe drug to characterize individual expression of a specific CYP2C19 isozyme. The Thin Layer Chromatography technique described in the present study is an inexpensive and rapid alternative analytical method that can be applied in assessing metabolic profiles of omeprazole (OME) and its metabolites, 5-hydroxyomeprazole (5-HME) in different population. **Method:** Thirty healthy volunteers from the Niger Delta Region of Nigeria participated in the study by ingesting 40mg of OME and cumulative urine voided was collected for the ensuing 8 h. Urine samples were analysed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) in accordance with the method of Iuga *et al*. (2008). Phenotype assessment was based on the relative colour intensities of the unchanged drug and its metabolites in the urine and their reference standard on spotting, greater intensity of the unchanged drug (OME) in the urine relative to the metabolite (5-HME) indicates a poor metaboliser (PM) phenotype, an equal intensity of spot between OME and 5-HME indicates an Intermediate metaboliser (IM) phenotype whereas a far less intensity of OME relative to 5-HME and in situation where no OME spots were observed indicate an extensive metaboliser (EM) phenotype. **Results:** From the intensity of the spots on TLC plates phenotypes were assigned such that 13.3% (4) of the individuals were poor metabolisers,

36.7% (11) of the individuals were intermediate metabolisers, 50% (15) of the individuals were extensive metabolisers. The phenotype assignments made by TLC were verified by comparison with the quantitative HPLC method and complete agreement was found for both methods. **Conclusion: P**henotype characterization in oxidative metabolism for substrates of polymorphic enzyme such as CYP2C19 in clinical practice could be realized with this simple and cost-effective TLC technique.

KEYWORDS: CYP2C19 genetic Polymorphism, Omeprazole, Phenotyping, Thin Layer Chromatography.

INTRODUCTION

Pharmacogenetics is the branch of sciences that deals with the genetic basis for inter-individual or inter-ethnic differences in drug response. Generally, pharmacogenetics embodies pharmacogenomics, which utilizes techniques for surveying the entire genome to assess multigenic determinants of drug response. Prior to the advances in molecular biology of the last few decades, pharmacogenetics proceeded using a forward genetic, phenotype-to-genotype approach.[1,2] Variability in drug response between individuals is due to genetic and environmental effects on drug absorption, distribution, metabolism or excretion (pharmacokinetics) and on target protein (receptor) or downstream protein signalling (pharmacodynamics).[3] The study of variation in drug responses under hereditary control is also known as pharmacogenetics.

Mutation results in a change in the nucleotide sequence of DNA giving rise to Single nucleotide polymorphisms (SNPs), which are very common.[3] They may change the function or level of expression of the corresponding protein. (Not all single nucleotide variations change the coded protein because the genetic code is 'redundant' – i.e. more than one triplet of nucleotides codes for each amino acid – so a change in one nucleotide does not always change the amino acid coded by the triplet, leaving the structure of the coded protein unaltered).[4]

Cytochrome P450 2C19 (CYP2C19) is an enzyme which belongs to Cytochrome P450, family 2, subfamily C, polypeptide 19. CYP2C19 gene is located on long arm of chromosome 10 and twenty one mutant alleles of CYP2C19 have been reported.[5-8] The CYP2C19 enzyme is responsible for the metabolism of 10 – 15% of clinically available drugs.[9] Polymorphisms on the CYP2C19 gene have the potential to affect a drug adverse

effect, efficacy, and drug-drug interaction[10] Genetic polymorphism of CYP2C19 occurs with varying frequency among different ethnic groups namely, 12-22.5 % in Asians,[11-14] 2.8-5.2 % in Africans,[15,16] 0.95 to 7 % in Caucasian Europeans,[17,18] 2 % in African-Americans,[19] 2.4- 2.6%in Caucasian-Americans[20,21] and 70 % in Vanuatu islands of Melanesia.[22] On the basis of genetic polymorphism a population may be divided into two or more groups namely extensive metabolizers (EMs) demonstrating normal metabolism of the drugs, Intermediate metabolizers (IMs) demonstrating reduced capacity for drug metabolism, Ultra-rapid metabolizers (UMs) demonstrating an excessive capacity for drug metabolism and poor metabolizers (PMs) demonstrating impaired metabolism of drugs due to the deficiency of CYPs.[2] The first PM of mephenytoin, a substrate for CYP2C19, was reported by Kupfer *et al*. (1986)[23] giving rise to the characterization of the genetic basis of CYP2C19 PM trait.[24]

Omeprazole, a proton pump inhibitor (PPI) is metabolized primarily via the CYP2C19 and CYP3A4 isoenzymes; its activity is influenced both by exogenous and endogenous (pharmacogenetic) factors and this causes large individual pharmacokinetic variations. Differences in the CYP2C19-mediated metabolism can produce marked inter-patient variability in acid suppression, in drug-interaction potential and in clinical efficacy.[25] Examining this alteration may help clinicians optimize Omeprazole therapy and administer individual treatment, especially to non-responder patients with its indication or after failed eradication therapy.[25] The analysis of Omeprazole (OME) and its metabolites 5-hydroxylomeprazolesulfone (5-HME) and Omeprazole sulfone (OMES) have been encountered in both clinical and forensic settings. Omeprazole is metabolised in the liver and excreted in urine as glucuronide conjugates.[26]

Phenotyping of CYP2C19 activity using OME may be carried out in urine, plasma or saliva samples and the results from these biological samples have a good correlation with each other.[27] The Omeprazole/metabolites metabolic ratio can be measured in urine by HPLC, GC or HPTLC methods.[28] The use of urine samples as biomatrix allows for repeatability and testing of samples since it is usually voided in large volume.

Omeprazole is metabolised to the O – hydroxylated form and to its sulphone metabolite via CYP2C19 enzyme pathway (Fig 1). The intensity and metabolic ratio of OME and its metabolites demonstrates the CYP2C19 enzyme activity based on the method used to classify an individual as either a poor (PM), intermediate (IM), extensive (EM) or ultrarapid (UM) metabolisers. The metabolism of a number of drugs such as Clopidrogel, Proguanil,

Diazepam, Mephenytion, and Propranolol is known to be affected by this same genetic polymorphism.[29] The diminished biotransformation of these compounds in the PM phenotype can lead to excessive drug accumulation, increased peak values, or in some cases, decreased formation of active metabolites for prodrugs or untoward effects from recommended doses of some compounds that rely on the deficient isoenzyme for metabolism.[30,31] However, routine clinical screening of subjects to identify this metabolite deficiency is lacking. This may be partly due to the techniques presently available for this purpose. Moreover, the analytical methods currently used to categorise phenotypes using Omeprazole are too cumbersome and time consuming to be cost – effective measures in a clinical setting, it is a problem probing for genetic screening.[32]

The availability of a simple method for phenotype identification should encourage the incorporation of pharmacogenetics screening into drug development strategies and therapeutic drug monitoring. The TLC procedure in this study represents one of the few simplified methods to screen for the deficiency in oxidative metabolism. In addition to facilitating large scale screenings for pharmacogenetic studies of experimental interest, this technique should also be attractive to clinical studies.[33] TLC is very effective in that it can always come in handy when emergency results are needed for example in drug abuse, drug overdose, drug adverse events or forensic cases. However, the method was verified by comparison with the quantitative results obtained from HPLC analysis.

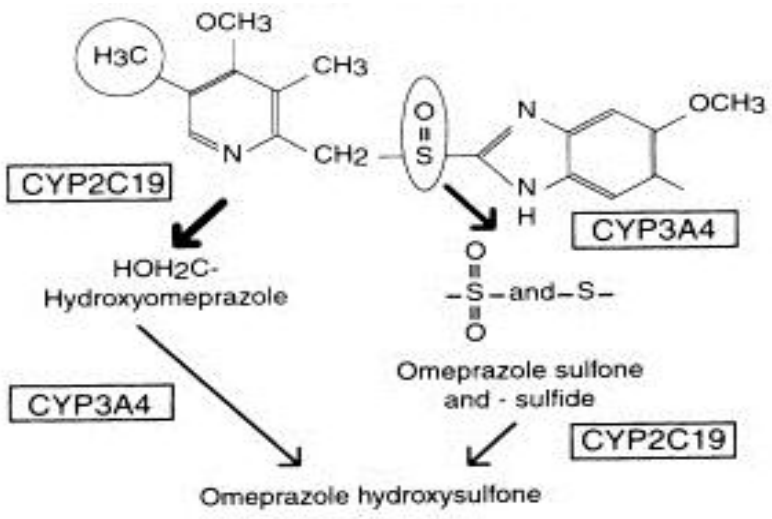


Fig 1: Metabolic pathway of Omeprazole

MATERIALS AND METHODS

Materials

Reagents

Omeprazole (OME), 5- hydroxyomeprazole (HME) and phenacetine (PHE) were purchased from Sigma (St. Louis, MO, USA). Omeprazole capsule 20mg (Ugazole ®) (Schwitz Biotech), Methanol (HPLC grade), triethylamine, orthophosphoric acid 85%, ethyl acetate, chloroform, dichlomethane, glacia acetic acid, diethyl ether, Iodine, hydrochloric acid. All solvents were from Sigma Aldrich India. Reagent-grade water obtained from a Millipore Milli-Q system was used throughout the experiments. All other reagents were of analytical grade.

Apparatus

Analytical weighing balance (Ohaus), TLC silica gel 60 F254 plates (Merck, Germany), TLC tank, non – EDTA tube bottles, HPLC (Agilent Technologies, USA), Sample calibrated containers, Height measuring apparatus, Human weighing scale, Droppers, Surgical gloves, Burettes, Fume cupboard, Micropipettes (eppendorf), Whirlmixer (Fischer Scientific), Test tube rack, Measuring cylinders, Conical flasks, Volumetric flasks, Beakers, Pasteur pipettes, Iodine tank, centrifuge tubes, Heparinised capillary tubes, 2L plastic containers, spatula, syringes, chest freezer, Autoclave, Oven and UV Light machine.

Chromatographic conditions

The liquid chromatographic system used was an Agilent 1100 series instrument, (Agilent Technologies, USA) made up of quaternary pumps, a gradient mixer (Agilent, Germany) with a system purge and a variable wavelength (200-800nm) UV-VIS detector model CE1100 (Agilent, Japan) with an 18µL flow cell and detection was done at the maximum wavelength of 302nm. Injection was by a Rheodyne model 7725 valve (Cotati, California, U.S.A.) fitted with a 20µL loop and an on-line vacuum degasser (Agilent, Japan). The HPLC was connected to a HP computer system and a printer**.** The column used was a reverse-phase (C-18) ultra sphere silica with 5µm particle size and 250 x 4.6 mm I.D (Beckman, USA). The mobile phase was CH_3OH:H_2O (55:45 v/v) containing triethylamine (TEA) 1% at pH=7 with H_3PO_4 85% was pumped through the column at a flow rate of 1ml/min in accordance with the method of Iuga *et al*. (2008)[34] and the analytical run was performed at ambient temperature.

Methods

Questionnaire development

A self-administered questionnaire was developed containing different items grouped into the following categories

Section A: Demographic data consisting of the age, sex, marital status, ethnicity, parents and grandparents ethnicity, weight and height.

Section B: consisting of the individuals social history including herbal medication, smoking, alcohol, or taking any oral contraceptive.

Section C: consisting of information on any concurrent illness and other drug use.

Study Population

Thirty healthy volunteers from the Niger Delta Region of Nigeria aged between 18 to 29 years comprising of 20 males and 10 females participated in the study after they all gave their informed consent. The pre-study screening was done by (interviewing both orally and filling a questionnaire providing vital information) and physical examination. A thorough screening process, which include physical examination and laboratory tests such as liver and kidney function tests were undertaken to ensure that subjects were healthy and to exclude presence of infections and use of the possible inhibitors of CYP2C19 and CYP3A4. Participants were appropriately monitored at least a month prior to the date of commencement of the study to ensure that they adhered to the conditions. None of the subjects had a history of alcohol addiction and drug abuse or dependence, and subsequently they did not have any medical condition that needed treatment. The volunteers were advised not to take any medication (natural herbal or over the counter drugs) two to three weeks before the study to avoid drug interaction. They were also asked to refrain from alcohol intake at least one to two weeks before the test and also refrain from ingesting products containing grapefruit throughout the study. All volunteers were asked to report any intake of any medication throughout the study. At the time of entry the study all volunteers that participated were assessed and were healthy. The study was approved by the Ethics and Research committee of the Niger Delta University.

Drug Administration and sample collection

An overnight fasting was ensured and carried out by all the volunteers prior to drug administration and food intake was not allowed for a period of 4 h after drug administration. The study was conducted using a parallel single dose design. The thirty (30) subjects were each administered with 40mg Omeprazole orally after which they were advised to take

enough quantity of water. Urine sample was collected pre–drug administration and the urine voided within 0 - 8 h was collected post-drug administration. The total volumes of the urine collected were immediately measured and recorded. A 5ml aliquot was then taken into fresh calibrated non – EDTA tube bottles and stored frozen at -20^{o}C before analysis.

TLC Analysis

Preparation of Standard Solution

Stock solution of standard of omeprazole and 5-hydroxyomeprazole (2mg/ml)

Reference standard of omeprazole and hydroxyomeprazole, 20mg each of powder was carefully weighed in a sample bottle on an analytical balance and dissolved in methanol in a 10ml volumetric flask. Thereafter the solution was made up to a final volume of 10ml with methanol.

Stock solution of omeprazole capsule (2mg/ml)

Omeprazole, 25mg of powder was weighed out and then transferred into a small beaker and triturated with methanol because it was in pellets form. After triturating it was filtered using a filter paper into a small 25ml volumetric flask and the final volume made up to the 12.5ml mark with methanol.

Determination of Unchanged Drug in the Urine

The urine stored in the 5ml non EDTA containers were brought out and arranged in order of labelling from 1-30. TLC plates where trimmed into different sizes for the analysis. TLC plates were activated by placing then in a hot air oven at 80^{o}C for about 15-30 min prior to analysis. Stock solutions of standard omeprazole and 5-hydroxylomeprazole and the stock solution of the omeprazole capsules were spotted on the TLC plate with the aid of capillary tubes. The plate was then taken into the already prepared saturated chromatographic tank and kept for some minutes to enable partitioning. Upon bringing out the plate was air dried and then viewed using the UV light at λmax of 254nm and further developed in an Iodine tank. The colour intensity of the spots and Rf of the omeprazole capsule was compared with that of the reference standard.

Determination of Omeprazole and 5-hydroxyomepazole In Urine Using Acid Hydrolysis

1ml of urine was each placed in an extraction tube and concentrated HCl (300µL) was then added to each tube. The tubes were capped and vortexed and then heated for 1.5h at 100ºC. At the end of the incubation, the pH of the samples was raised to 11 with concentrated

NH_4OH (1ml) after which dichloromethane (3ml) was added .The samples were then mixed properly and centrifuged .After centrifuging, the aqueous phase was aspirated off and the organic layer containing the drug was transferred to a fresh tube and evaporated to dryness. To the residue 2 drops of dicholoromethane (DCM) was added and the entire volume was spotted onto the origin of a thin layer plate. The plate was developed over a distance of 6.5cm in a compatible solvent system explained below. The TLC plates were then viewed under UV light (254nm) and by placing in an iodine tank. The spots intensities of the Drug and metabolites were noted as well as their R_f values. At all instances the same concentration, duration and intensity of spraying (with iodine in the iodine tank), type of silica used were all kept constant to minimize errors.

Development of TLC mobile phase

The plate was developed over a distance of 6.5cm to 8.1cm in a solvent system, which was found to be compatible with the work. Development of the mobile phase was done in the following ratio: Ethyl acetate: Methanol: Glacial acetic acid 55:40:5 vol/vol/vol/(ml) in a chromatographic tank. After the solvent had successfully reached the solvent front it was carefully brought out of the tank, allowed to air dry. Thereafter the plate was placed in an iodine tank for proper identification and viewing of intensity of spots. Drug and metabolite spots appeared brown, their respective intensities were noted and their R_f values properly calculated. Samples were spotted alongside the reference standard drugs for appropriate comparison and to lend credence to the results. Reference standards were also spotted separately on their own and the intensity and R_f values noted.

High Performance Liquid Chromatographic (HPLC) Analysis of Omeprazole

The total concentrations of Omeprazole (OME) and its major metabolite, 5-hydroxyomeprazole (5-HME) were analysed in urine by a reversed-phase HPLC with UV detection using the method of Iuga *et al*. (2008) [34] and subsequent hydrolysis of glucuronidated HME. Standard curves were analyzed in the concentration range 0.1 to 5.0µg/ml for OME and 0.1 to 5.0µg/ml for HME. The internal standard (Phenacetin, PHE) in a final concentration of 20µg/ml was added before incubation with β-glucuronidase (Sigma Aldrich, USA).

Calibration curve for omeprazole and 5-hydroxyomeprazole in urine

Blank urine (1ml) sample was each placed in six different extraction tubes and varying amounts of the stock solutions (100µg/ml) of OME and 5-HME were added to give

concentrations of 0.2µg/ml, 0.5µg/ml, 1.0µg/ml, 2.0µg/ml, 4.0µg/ml, and 5µg/ml for both OME and 5-HME. 20µL of the stock solution of the internal standard, phenacetin (1mg/ml), was added to each tube. The samples were rendered alkaline with 0.1M NaOH (0.5 ml) and whirlmixed for 1 min. 3ml of chloroform: isopropanol (6.5:3.5) mixture was added to each of the tubes and whirlmixed for 1min after which the tubes were centrifuged at 2500 rpm for 10 min. The organic layer was transferred into another extraction tube. The extraction process was repeated and the pooled extract was evaporated to dryness in a water bath at 40^{o}C. The residue was reconstituted in 100 µL of 0.1N HCl and whirlmixed before injecting 20 µL onto the HPLC. The peak area ratio was plotted against the concentration of each of the compounds injected. The regression analysis was carried out using Microsoft excel version 2007.

Determination of drug and metabolites in urine

To 1 ml of urine sample in a centrifuge tube, 20 µL of the internal standard was added and the mixture was incubated for 18 h with 0.5ml of 10, 000 IU β-glucuronidase (Roche Biochemicals). After the incubation, OME and its metabolite, 5-HME were extracted under alkaline conditions as previously described. The residue was reconstituted in 100 µL 0.1N HCl and whirlmixed before 20 µL was injected onto the HPLC.

The metabolic ratio (MR) was evaluated as the molar concentration ratio of Omeprazoze/5-hydroxyomeprazole in a 0-8 h urine collection and was used to estimate CYP2C19 activity.

Statistical analysis

The statistical analysis was performed using Stata intercooled version 9.0. The normality of metabolic ratio (MR) distribution was analyzed by the Shapiro-Wilk W-test with *p*-value <0.05 as the level of significance.

RESULTS

TLC Results

The intensity of the spots on TLC plate using UV Light at 254nm wavelength and placing in an iodine tank were observed and recorded. The criterion used for the phenotyping assignment using thin layer chromatography is the colour intensities of the unchanged drug omeprazole (OME) and its metabolites, hydroxyomeprazole (HME) in the urine as compared to the standards. This involves the virtual observation and determination of the intensities of the sample. If the intensity of the unchanged drug, OME in the urine spot is greater than the

spot for its metabolites, HME the subject was assigned a poor metabolisers (PM) phenotype. On the other hand, if the metabolite (HME) spot was of equal intensity to the parent drug (OME) spot, the subject was deemed to be an intermediate metaboliser (IM) and if the intensity of the unchanged drug (OME) spot was less intense than HME and in situation were no spot of OME was observed, the individual phenotype was seen to be an extensive metaboliser (EM). The Rf values of omeprazole ranged from 0.85 to 0.97.

Phenotype Assignment

Based on the intensity of the spot on the TLC, phenotypes were assigned such that 13.3% (4) of the individuals were poor metabolisers, 36.7% (11) of the individuals were intermediate metabolisers, 50% (15) of the individuals were extensive metabolisers, as shown in Table 1 and the frequency of each phenotype is shown in Fig 2.

Table 1: TLC Results and Phenotype Assignment.

Code	Intensity	Phenotype
ND1	V	PM
ND2	I	IM
ND3	L	EM
ND4	L	EM
ND5	L	EM
ND6	N.S	EM
ND7	I	IM
ND8	N.S	EM
ND9	N.S	EM
ND10	L	EM
ND11	I	IM
ND12	N.S	EM
ND13	I	IM
ND14	I	IM
ND15	V	PM
ND16	I	IM
ND17	V	PM
ND18	I	IM
ND19	I	IM
ND20	L	EM
ND21	N.S	EM
ND22	I	IM
ND23	L	EM
ND24	N.S	EM
ND25	N.S	EM
ND26	L	EM
ND27	V	PM
ND28	I	IM
ND29	I	IM
ND30	N.S	EM

Keys: V = Very intense I = Intense spots L= Less intense spots N.S= No spot observed EM= Extensive metaboliser PM= Poor metaboliser IM= Intermediate metaboliser

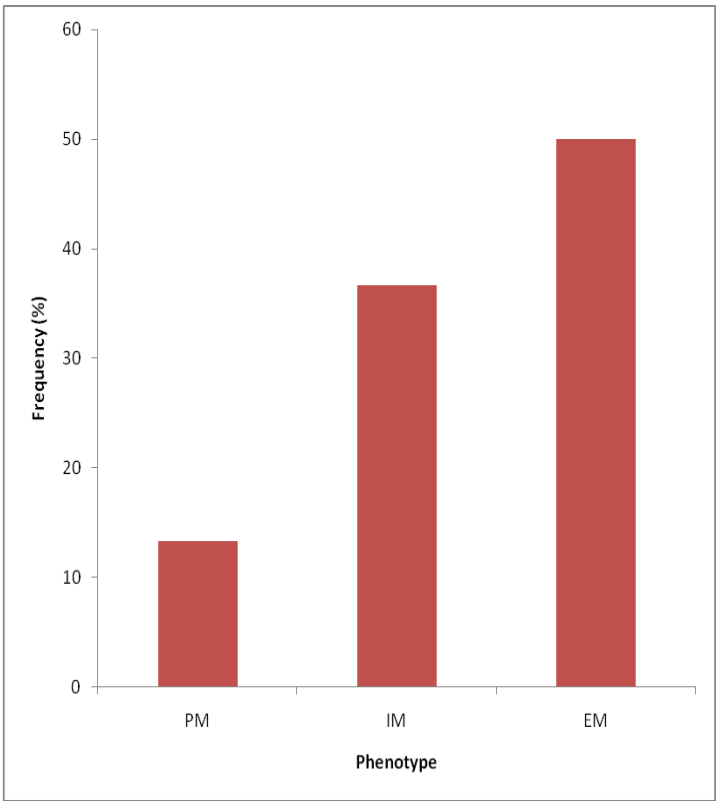


Fig. 2: Bar Chart showing the Frequency Phenotype in the studied population.

HPLC analysis of probe drug and metabolite

The retention time for OME and its metabolites 5-HME and the internal standard, Phenacetin (PHE) were found to be approximately 9, 5 and 4 mins, respectively, as shown in Fig 3A. A representative chromatogram of 8 h test urine sample after administration of 40mg of OME to subjects is shown in Fig 3A depicting an extensive metabolizer (EM) phenotype. The retention times of OME, HME and PHE in the test chromatograms corresponded to those obtained from the reference compounds by direct injection.

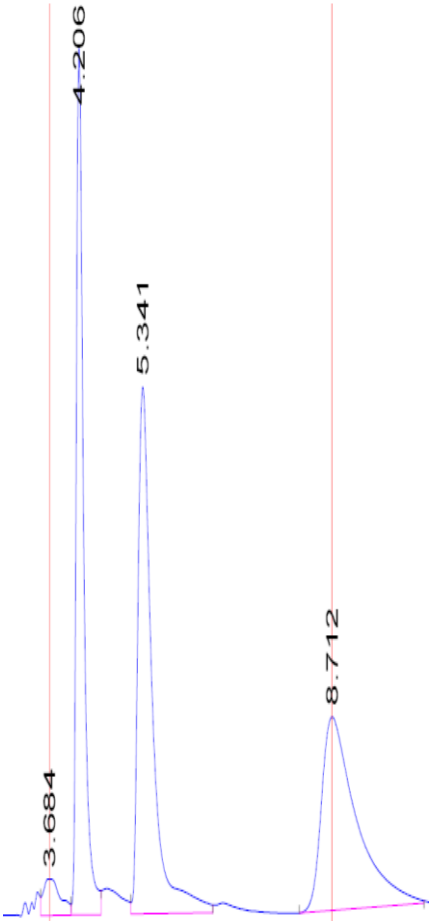
Quantitative analysis

Calibration curves for OME and HME in urine

The calibration plot was linear over the concentrations of 0.2, 0.5, 1.0, 2.0, 4.0 and 5.0 µg/ml for OME and 5-HME in urine. The regression coefficients (R^2) for the mean standard curve of six different lots of urine (set 1) were 0.992 and 0.995 for OME and HME, respectively. Typical equations for the calibration curves for OME and HME were $y = 1.54x - 0.12$ and $y = 0.72x - 0.25$, respectively.

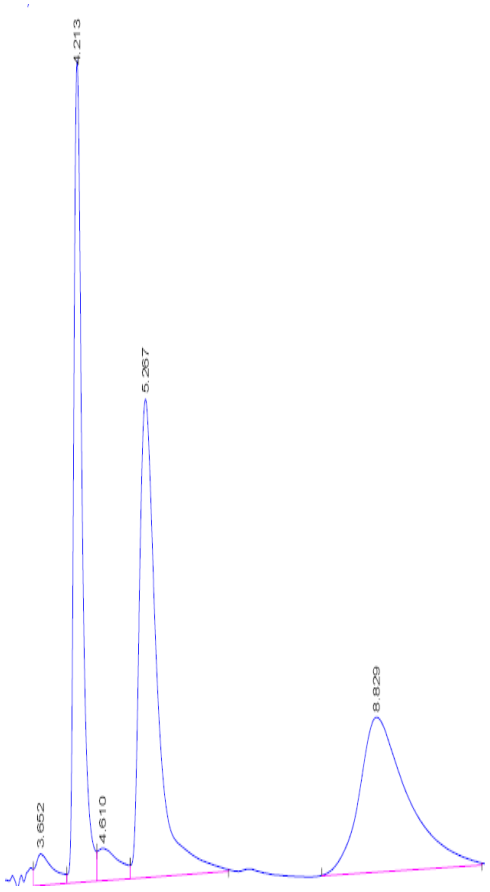
Frequency distribution of the metabolic ratios obtained from HPLC analysis

The OME/5-HME metabolic ratios (MRs) from HPLC analysis results of the subjects ranged from 0.79 to 2.65 with a median of 1.71. Visual inspection and the probit transformation of the MRs provided the predicted phenotype frequencies for OME hydroxylation by CYP2C19 are shown in Table 2.



A

Fig. 3A: HPLC chromatogram following direct injection of sample containing 2µg/ml of Omeprazole (OME; Ret time ~ 9min), 5-Hydroxyomeprazole (5-HME; Ret time ~5min) and Morphine (PHE; Ret time ~ 4 Min) as internal standard.



B

Fig 3B: HPLC chromatogram of an extract of test urine obtained 8 h after the administration of a 40mg dose of Omeprazole to a subject depicting an Intermediate metaboliser (EM) phenotype.

Table 2: Predicted phenotype frequency of Omeprazole hydroxylation by CYP2C19 using HPLC method;

Metabolic ratio	CYP2C19 Phenotype	CYP2C19 genotype	Participants (n)	Frequency (%)
2.13 - 2.65 (Median= 2.36)	PMs	*2/*2	5	16.7
1.32 – 1.96 (Median= 1.65)	IMs	*1/*2 *1/*3 *2/*17 *3/*17	10	33.3
0.79 - 1.25 (Median = 1.12)	EMs	*1/*1	15	50.0
	Total		30	100.0

Abbreviations: PM, Poor Metaboliser; IM, Intermediate Metaboliser; EM, Extensive Metaboliser

DISCUSSION

Many approaches of OME Pharmacogenetics have been explored since it was established that polymorphic hydroxylation may be responsible for marked inter-individual variability in Omeprazole 5-hydroxylation.[31,32] There are very few phenotyping methods in literature for Omeprazole hydroxylation in urine. Some studies include a 6–12 h period for blood and urine samples collection for pharmacokinetics and were relatively expensive in costs negating their application and incorporation to rountine clinical practice.[35] In some of the studies, omeprazole concentration in urine could not be measured because of unsuitable buffer that caused omeprazole degradation in urine samples.[36,37] However, some studies were able to evaluate omeprazole and 5-hydroxyomeprazole in urine by liquid chromatography tandem–mass spectrometry (LC-MS/MS) method. Omeprazole and 5-hydroxyomeprazole blood samples were used for CYP2C19 phenotyping, whereas urine samples with all time points were acquired from only two volunteers.[38] These methods are cumbersome and expensive for routine clinical operations and there are no reports of OME hydroxylation evaluation using any facile method such as the thin layer chromatography (TLC) employed in this study. Therefore, this study has demonstrated that a significant time and cost could be saved with the TLC technique. A cohort of fifty (50) participants can easily be screened by TLC within 12 h. In the same vein quantitating metabolic ratios using HPLC in this same number of subjects could take not less than 3 to 4 days to perform and may require the utilization of huge quantum of facilities and resources.

The cleavage of the 5-hydroxyomeprazole glucuronide conjugates was carried out using by acid hydrolysis,[39,40] However, the acid hydrolysis time was shortened by the use of elevated temperature rather than the 6 h required for complete conjugate cleavage, which remains a limiting factor to rapid phenotyping by TLC. Accurate phenotype characterization of most subjects using TLC and HPLC were made possible by the hydrolysis of the glucuronide conjugates either by acid hydrolysis or hydrolysis by the enzyme β-glucuronidase.[39,40] It was observed that complete hydrolysis of 5-hydroxyomeprazole glucuronide was achieved with conc. HCl at 100^{o}C in 1.5 h with no detectable degradation of OME and 5-HME based on TLC analysis. The phenotype assignments following TLC profiles after acid hydrolysis are in agreement with those obtained after treatment with β-glucuronidase for HPLC method with no significant difference ($p<0.05$). This invariably means that accurate qualitative phenotyping of omeprazole can be achieved more efficiently after the glucuronide conjugates are hydrolyzed with acid.

In this study, visual comparisons of the relative intensities of the spots on the TLC were employed as qualitative approach rather that their actual quantitation. Based on this approach, the apparent phenotypes of the subjects were characterized with little or no difficulty. The hydroxylation of Omeprazole in our study population occurs at a frequency comparable with data described in previous studies in African population.[41,42] The poor metabolizer phenotype of 13% in this study is relatively high when compared to previous studies in Nigerian population.[42] This may be due to the small number of participants in this study as well as the study design coupled with the heterogeneity of the population in the Niger Delta region of Nigeria. In the PMs It was noticed that the intensity of the OME spots were higher than the 5-HME spots. This implies that there was little or no CYP2C19 function. It could also be due to a co-morbid condition. Not anticipating that patients may be PMs is potentially dangerous. Any drug that is primarily metabolised by CYP2C19 and ingested by a PM will have a delayed metabolism. A drug may also be less effective for a PM at CYP2C19 if the drug is a prodrug and needs to be activated by CYP2C19. PMs would usually require a lower dose of the drug to achieve desired effects. The drug may be secondarily metabolised by another CYP450 isoenzyme that is higher in capacity but has lower affinity for the drug or substrate. Often alternative is CYP3A4 and this shifting to a less efficient enzyme leads PMs to have higher drug levels of the parent compound.[43] Participants in whom the relative intensity of OME spot and that of 5-HME are almost similar are termed intermediate metabolizers. Individuals who are IMs have decreased activity of CYP2C19*1/*2, CYP2C19*1/*3, CYP2C19*2/*17 and CYP2C19*3/*17, which is associated with Omeprazole metabolism.[44,45] Such individuals have their enzyme activity between poor and extensive metabolisers. Usually they need just a slight dose changes and are often even merged with extensive metabolisers as their response to drugs is not really very different. Half of the subjects that participated in the study were extensive metabolizers. Individuals who are EMs have normal activity of CYP2C19*1/*1 and hence normal CYP2C19 activity, which will ensure the metabolism of Omeprazole occurs at the normal rate.

Phenotyping methods based on single blood or urine intake are more practicable in clinical practice. Blood sampling for phenotyping study is invasive and needs equipment for centrifugation and the participants are often not very comfortable with the process. These limitations are avoided for urine samples as a bio-matrix for phenotyping, thus making the method simpler for rountine clinical practice. However, drug and metabolite concentrations in urine are less precise compared to those in plasma, mostly because of partial intestinal

excretion with no optimal time point found for urine sampling in literature for CYP2C19 phenotyping.[43] Therefore, the phenotyping method been presented is based on single-dose omeprazole intake with cumulative collection of urine sample voided. Furthermore, the ability to screen for this genetic polymorphism in drug oxidation can have profound positive impact on drug development stage and clinical practice. If polymorphic metabolism of a drug can be recognised during its design and development, dosing recommendations could be proposed, before its widespread use. Besides, if phenotypes are evaluated before the inception of therapy with drugs that are substrate of CYP2C19 isozyme, their clinical effectiveness might be enhanced and possible toxicity averted, in poor oxidative metabolisers.[46]

The main limitations of the study are a small sample size, heterogeneity of the study population, absence of plasma omeprazole and 5-hydroxyomeprazole concentrations and single CYP isoenzyme phenotyping. In addition, CYPs such as CYP3A4, CYP3A5 and CYP1A2 may also impact on the results of the study in conditions of reduced CYP2C19 activity, OME metabolism may go through other CYPs.[25] CYP1A2 induction in multiple omeprazole dosing was also implicated in previous studies.[43] However, the utilization of a simple and cost-effective method for phenotype identification should encourage the incorporation of pharmacogenetics screening into drug development strategies and therapeutic drug monitoring. The TLC procedures presented in this work represents the most simplified means available to screen for the deficiency in omeprazole metabolism. This technique can also be found applicable and attractive in clinical areas to ensure rational use of drugs and improve clinical outcomes of the drug whereas also minimising adverse reactions in patients.

CONCLUSION

From the findings, urine concentrations of omeprazole as a probe drug for CYP2C19 isoenzyme and its major metabolite, 5-hydroxyomeprazole are simplified techniques for evaluating the functional status of CYP2C19 enzyme. It can thus be concluded that phenotyping is a very useful technique for identifying genetic polymorphism in drug oxidation as well as in individual treatment with drugs that are substrate for CYP polymorphic enzymes. Besides, the use of TLC technique as phenotype identification tool could adequately determine inter-individual or inter-ethnic phenotype expressions with Omeprazole as the probe drug.

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