Evaluation of pharmacokinetic and bioequivalence of brands of sulphadoxine-pyrimethamine tablets used in intermittent preventive therapy for pregnant women in Nigeria

A Ogbonna, C Ogbonna, B Ogbonna, A Enyi, J Uneke

Abstract

Objectives: The aim of this study is to investigate the pharmacokinetic and relative bioavailability of three tablet formulation containing sulphadoxine pyrimethamine (SP) used for intermittent preventive therapy in pregnant women in Nigeria to see whether there is need for dose adjustment.

Methods: Twelve healthy volunteers(pregnant women at their fourth month of pregnancy) attending antenatal clinic (ANC)were randomised to receive a single oral dose of three SP tablets each containing 500mg sulphadoxine (XD)and 25mg pyrimethamine (PY) in form of A (innovator product) and B,C(locally manufactured SP tablet formulation),after an overnight fasting. Several blood samples (100µl) were collected from a finger prick in duplicates up to ten days and dried on a Whatman® filter paper. The samples were analysed for DX and PY using the High Performance Liquid Chromatography (HPLC) method. The pharmacokinetic parameters assessed were maximum plasma concentration (Cmax), area under curve (AUC), elimination half life (t1/2), time to attain maximum concentration (tmax) and relative bioavailability using the single compartment model.

Results: Sample formulation B was significantly lower than samples A and C ($p < 0.1$) in mean plasma concentration (Cmax), area under curve (AUC).

Conclusion: The difference shows in vivo inequivalence between the products, and calls for caution in using these products, however the pharmacokinetic results shows that there is no need for dose adjustment of SP in pregnancy since they attain therapeutic concentration in vivo, indicating that their kinetics is not altered in pregnancy.

INTRODUCTION

Malaria is a public health problem of global concern because of its high economic burden on the nation, high mortality in children, pregnant women and non immune individuals. It is a major cause of morbidity and mortality in Nigeria where it is holoendemic. Resistance of anti-malaria drug by plasmodium species has continued to create a burden in the management of malaria in endemic areas; the major causative factor in Sub-Saharan African is treatment with poor-quality drug preparation causing suboptimal dosing [1].

The World Health Organization (WHO) designated intermittent Preventive Treatment (IPT) as the preferred approach to reduce the number of malaria parasites in pregnant women during the critical period of greatest fetal gain[2]. IPT during pregnancy provides significant protection against low birth weight, maternal anemia, preterm delivery and maternal mortality [3, 4, 5, 6, 7, 8]. WHO has recommended the use of Sulphadoxine-Pyrimethamine (SP) for intermittent preventive Therapy in pregnant women living in endemic area (irrespective of their peripheral parasite status) (2)

Full treatment dose is given to all women at specified interval during the 2nd and 3rd trimester of their pregnancy. Poor in-vitro quality in over 50% of generic SP marketed in Nigeria have been reported [9].

Over 80% of approximately 10,000 prescription drugs

available in 1990 were obtained from more than one source, and variable clinical responses to these dosage forms supplied by two or more drug manufacturers is documented ([10]).

These variable responses may be due to formulation ingredients employed, method of binding, packaging and storage and even the rigors of in-process quality control, thus the need to determine their therapeutic equivalence in order to ensure interchangeability. SP pharmacokinetic has been investigated widely in non-pregnant population ([11], [12], [13], [14]).

There is no published data for SP pharmacokinetics in pregnant population in most part of Sub-Saharan Africa including Nigeria. There is evidence that the pharmacokinetics of several antimalaria drugs (chloroquine, mefloquine and artesunate) is altered in pregnancy and doses used in non pregnant population are not adequate in pregnancy ([15], [16],[17]). Pregnancy comes with many physiological changes which may have some effect on metabolism and pharmacokinetics of drugs ([19])

This study is therefore aimed at determining the relative bioavailability of locally manufactured SP generic in comparison with innovator SP tablet formulation and also their Pharmacokinetics in pregnant population in Nigeria, to determine whether adjustment of dose is necessary in this special population.

MATERIALS AND METHOD

SP maunfactured in Nigeria.

Three brands of sulphadoxine-pyrmethamine (A, B, C), (selected previously from eight different brands that underwent in vitro dissolution test) were obtained from different retail outlets in Abakaliki, South eastern Nigeria. Out of these three selected, A is the innovator product that passed the in vitro test, B is the local product that failed the in vitro test, while C, also a local product that passed the initial in vitro test. The drugs obtained had more than 1 year of shells life remaining and are all used in intermittent preventive therapy (IPT) in Nigeria.

SUBJECTS

Twelve apparently healthy pregnant women attending ANC (antenatal clinic) at the Federal Medical Centre, Abakaliki were recruited for this study after obtaining informed consent. All participants were in their fourth month of pregnancy (Quickening stage). The mean age, body weight and body mass index were determined and are reflected in table I

Figure 1

Table 1: Demographic characteristics of volunteers

Mean age(yrs)	Age range(yrs)	Mean body weight(Kg)	Body weight range(Kg)	Mean height (cm)	Height range (cm)	Mean body mass index	Range body mass index
38.6	26 - 46	70.4	55 - 86	164	150-174	19.8	17.4-22.8

CLINICAL PROCEDURE

A single blind randomized Latin square crossover method was employed. One month wash-out period separated the drug administrations. Subjects whose blood samples prior to drug administration contained active component as co-trimoxazole and SP as confirmed by Lignin color text and by HPLC method ([20], [21]) were excluded.

The participants were instructed to refrain from taking grape juice or alcohol, two days before and during the study ([22]), as well as not to take any medication 2 weeks before as well as during the study period. Those who got malaria during the study period were treated with Artesunate (AT) and not with SP or any other sulphonamide. Screening before the start of the study involved medical history, physical examination and laboratory test (blood chemistry, alanine, aminotransferase and aspartate aminotransferase and a complete hematological status). Blood pressure and pulse rate were determined before medication and 8hr after drug intake. Physical and laboratory test were repeated during the last week of wash out.

The participants were informed in advance about the possibility of dizziness, nausea, vomiting, headache, skin rash, general malaise and were advised to report to the project clinician in case of any serious effects.

DRUG ADMINISTRATION AND BLOOD SAMPLE COLLECTION

Using the HPLC method (20), levels of sulphadoxine and sulphamethoxazole in the blood samples were tested prior to drug administration. Following an overnight fast, 12 healthy volunteers were randomized to receive a single oral dose of three SP tablets,

(each containing 500mg and 25mg as DX and PY respectively) in form of either formulation A, B or C. The tablets were swallowed with a glass (200ml) of water under supervision in the morning. A low fat content breakfast and standardized lunch was served 3 and 5 hr after drug intake

respectively. The drug administration was repeated after a wash-out period one month. Blood samples were collected pre-dose and at 1-2, 2-5, 8, 10, 24, 46, 72, 96, 120, 168, and 240 hr after drug intake (14).

The blood samples (100ul) were collected from finger prick in duplicate into heparinized precision capillaries transferred onto whatman® filter paper and dried at room temperature. The samples were kept in polythene folders until assay.

ANALYTICAL METHODS

For assay of sulphadoxine (DX) blood samples, an HPLC method as described (20) was used. Pyrimethamine (PY) was quantified using another HPLC method developed by Minze et al (21).

The two methods enabled quantification of DX and PY from 100ul whole blood dried in spots on filter paper.

PHARMACOKINETIC CALCULATION

Pharmacokinetic data of DX and PY were determined using single compartment method ($_{21}$, $_{23}$). The maximum plasma concentration (C_{max}) and (t_{max}) time to attain C_{max} were estimated from observed plasma concentration versus time data. The elimination rate constant (Kel) were determined by least-squares regression analysis of the log drug concentration versus time curve. Half lives of DX and PY were calculated from the ratio of 0.693/Kel (14, 21).

The total area under plasma concentration versus time curve (AUC) of DX and PY was determined as AUC_{0-240} + $AUC_{240-\infty}$. AUC_{0-240} was estimated by the linear trapezoidal rule, and $AUC_{240-\infty}$ was estimated as $C/_{Kel}$ in which C is the last time concentration point and Kel is elimination rate constant estimated by linear regression of 3-5 last points of plasma concentration time curve. $AUC_{0-\infty}$ was obtained by the summation of these two values. The relative bioavailability test of formulation A with respect to DX and PY were calculated by dividing AUC_{0-240} to the corresponding AUC_{0-240} of formulation B. According to international Guideline, the two formulation are bioequivalent if the relative bioavailability of A at a 90% confidence interval lie within the range of 0.80 – 1.25 and if the ratio as C_{max} of A formulation to C_{max} of B formulation is between 70 – 143% (25)

STATISTICAL ANALYSIS

SPSS software (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Data from the SP formulations were analyzed for DX and PY based on Cmax, AUC_{0-240}, $AUC_{0-\infty}$, T_{max} and $t_{1/2}$. Assessment of difference between the tests A,B,C, and reference formulation for these pharmacokinetic parameters was performed using students t-test. Results are presented as means± standard deviation (SD).

RESULTS

The twelve subjects were in good health as assessed by clinical and laboratory screening. Also none of the subjects had taken sulphadoxine-pyrimethamine or any sulpha drug previously as indicated by the test prior to drug administration. Two subjects experienced headache 5 hr after intake of formulation A, B and C, they also experienced tachycardia second day after drug intake. No danger sign associated with drug intake was reported. One volunteer who got malaria during the study was treated with artesunate. There were no clinical significant alteration in blood pressure and respiratory rates.

PHARMACOKINETICS

The mean plasma concentration – time profiles of DX and PY showed that the concentration of the test formulation B were significantly lower than those of the formulation C and reference formulation A. Lower C_{max}, AUC and lower T_{max} were observed in formulation B for DX. The same applies for PRY except for lower $t_{1/2}$ for B. The relative bioavailability (F_{rel}) of DX and PY for formulate B/A and C/A using AUC up to the last Sampling time (240 hr) was 0.63, 1.02; 0.37, 1.02. (See tables 2 and 3)

Figure 2

Table 2: Pharmacokinetic profiles of sulphadoxine (DX) after a single oral intake of three tablets of SP by pregnant women, presented as mean ± coefficient of variation (CV), n = 12 in a crossover study.

	A	B	C
C_{max} μgmL^{-1}	118	74	119
t_{max} (h)	8	10	8
$T_{1/2}$ (h)	228.7	252.0	236.5
AUC_{0-240}	19530	12343	19986
$AUC_{0-\infty}$	39352.0	26524.8	41145.9

Significant difference(student's t- test p < 0.1)

Relative bioavailability (Frel) using $AUC_{0-240,}$ B/A = 0.632, C/A = 1.020

Figure 3

Table 3: Pharmacokinetic profiles of pyrimethamine (PY) after a single oral intake of three tablets of SP by pregnant women, presented as mean ± coefficient of variation (CV), n = 12 in a crossover study

	A	B	C
C_{max} µgmL^{-1}	470	175	482
t_{max} (h)	5	5	5
$T_{1/2}$ (h)	108.6	90.2	108.5
AUC_{0-240}	45408	16789	46347
$AUC_{0-\infty}$	60141.54	20044.20	61213.98

Relative bioavailability (Frel) using AUC_{0-240}, B/A = 0.369 , C/A = 1.020

DISCUSSION

As reported by Aubouy et al, for SP to exhibit treatment success, the concentration of DX and PY in the body, 72 hr after drug intake should be ≥100 µg/ml and ≥175 ng/ml, respectively ([23]).

From this study, formulation A and C exhibited adequate DX and PY concentration of 99 µg/ml and 242 ng/ml; and 100 µg/ml and 252 ng/ml respectively, but much lower levels of DX and PY is 60 µg/ml and 94 ng/ml of formulation B. This study goes to confirm low in-vivo performance of locally manufactured formulation B compared with formulations A and C. The observed difference in C_{max}, T_{max} and AUC of formulation B compared to that of A and C could not meet the criteria for bioequivalence. Plasmodium falciparum in sub-Saharan Africa is particularly life threatening especially in pregnant women and children under five and adequate concentration of an effective drug is essential. Reliable and adequate drug absorption after oral administration has to be ensured in order to achieve prompt and adequate systemic exposure to a drug. Both DX and PY are components of SP drug combination have antifolate activity and when combined in such a fixed drug combination, thier activity is enhanced by a synergistic mechanism of action. Therefore, their effectiveness depends on the bioavailability of both components after oral administration. The WHO recently compiled reports from selected African counties in which SP had high dissolution failure rates ranging between 75% and 100% especially with respect to PY ([24]), Predicting poor bioavailability of the drug. As locally manufactured medicines are cheaper and can be delivered to the target population than the imported product, it is very important to promote local pharmaceutical industries on good manufacturing process and internal quality control mechanism, so as to insure quality assurance.

In Nigeria, the regulatory body National Agency Food Drug Administration and Control (NAFDAC) had really done much to sanitize drug manufacture and distribution. From this study, it shows that more still need to be done in ensuring total quality of drugs. Continuous use of SP with poor bioavailability has lead to emergence of resistance Plasmodium falciparum strain against SP ([11], [12]).

The pharmacokinetics of medications can be modified during pregnancy; by physiological changes such increased total body water and levels of sex hormones ([26], [27]) and these in turn may affect the activity of the different cytochrome P450 enzymes ([28], [29]), which are involved in the metabolism of endogenous compounds and drugs. It is interesting to note that formulations that previously passed in vitro dissolution test, equally passed the in vivo test and that the therapeutic concentration was equally achieved with the same dosage in pregnant women. This goes to suggest that the dosage of SP for administration in pregnant women needs no adjustment.

The formulation B in 72 hr failed to measure up to the concentration ≥100 µg/ml for DX and ≥175 ng/ml for PY. Such formulations could thwart the effort of NAFDAC, Federal ministry of health and WHO in ensuring better outcome of pregnancy in Nigeria.

CORRESPONDENCE TO

A. Ogbonna Department of Pharmaceutical Services, Federal Medical Centre, Abakaliki, Nigeria P.O.Box 472, Abakaliki,Nigeria Tel: 234-08059506961; E-mail: annyogbonna@yahoo.com

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Author Information

A. Ogbonna, M. Pharm
Dept. of pharmaceutical services,, Federal Medical Centre

C.I.O. Ogbonna, B.Pharm
Dept. of pharmacology and toxicology, University Of Nigeria

B.O. Ogbonna, M.Pharm
Dept. of clinical pharmacy, College Of Medicine, University Of Lagos

A.A.U. Enyi, M.D.
Dept of obstetrics and gynaecology, Federal Medical Centre

J.C. Uneke, M.Sc.
Dept. of medical microbiology, Faculty Of Clinical Medicine, Ebonyi State university