Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

EFFECTS OF SICKLE CELL ANAEMIA ON THE PHARMACOKINETICS OF SULPHADOXINE AND PYRIMETHAMINE IN CHILDREN

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Received: 19 Jun 2012, Revised and Accepted: 25 Aug 2012

ABSTRACT

Introduction: The study investigated the disposition of sulphadoxine-pyrimethamine (SP) in sickle cell Anaemic (SCA) patients, with a view to establishing the desirability of the usage of SP for malaria chemoprophylaxis in SCA patients.

Method: Fifteen SCA children aged 12 ±1.3 y were recruited at the Sickle Cell Out-Patient Clinic of Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife. Subjects were administered two tablets of sulphadoxine-pyrimethamine, Fansidar© containing 1000mg sulphadoxine (SDX) and 50 mg pyrimethamine (PYR). Plasma drug concentrations were determined with a reversed phase high performance liquid chromatographic (RP-HPLC) method consisting of a C18 stationary phase and a mobile phase of acetonitrile/ 0.67M ammonium acetate (30:70). Sample detection was with a variable UV detector set at 220nm while proguanil was used as the internal standard.

Result: There were wide inter-individual variations in the pharmacokinetics of the drugs. The time to reach maximum plasma concentration (T_{max}) for SDX was 7±1 h and 6.8±1.327 h for PYR. Values for maximum plasma drug concentration C_{max} were 142. ± 18.76 µg/ml and 488.87±32.06 ng/ml for SDX and PYR respectively, while the corresponding area-under curve of the plasma concentrations-time curve $(AUC)_{0-\infty}$ values were 16057.38±27334.73 µg/ml.h and 26040.06±11019.92 ng/ml. h for SDX and PYR respectively. Elimination half-life ($T_{1/2}$) of SDX was 179.75±17.46 h while that for PYR was 97±22 h. The mean residence time (MRT) for SDX and PYR were 290.24±32 h and 123.71±36.58 h respectively. There was no statistically significant difference between the pharmacokinetic parameters C_{max}, T_{max}, $AUC_{0-\alpha}$ and $T_{1/2}$ of SDX and PYR obtained in the sickle cell children and those of other reported values for age-matched healthy subjects.

Conclusion: The result of this study suggests that sickle cell anaemia does not significantly alter the pharmacokinetics of SP in children. Thus adjustment of the drug does not appear to be necessary in this group of children.

Keyword: Sulphadoxine, Pyrimethamine, Sickle Cell anaemic patients, and chemoprophylaxis.

INTRODUCTION

Malaria and Sickle Cell Anaemia (SCA) are major health problems in the world amongst children especially below age five. Malaria is one of the most important public health problems in the world especially in the tropics and Africa in particular. It affects more than 240 million people worldwide with 120 million infections every year (1). In Nigeria, it accounts for over 25 % of under-5 year's mortality; while children aged greater than 5 years (about 24 million) have 2 to 4 attacks annually (2). On the other hand, sickle cell anaemia is an inherited blood disorder characterized by defective haemoglobin which affects their ability to carry oxygen. Sickle cell disease is known to affect those of Middle East (3), Africans and African descent. In many areas of sub-Saharan Africa, up to 2% of all children are born with the condition. In West African countries such as Ghana and Nigeria, the frequency of the trait is 15% to 30% (4) In Nigeria, the incidence of sickle cell trait and anaemia is 20% and 5% respectively (4).

SCA patients are known to be prone to several haemolytic crises and these conditions can result in stroke, organ failure like the kidneys and spleen (5). Although several conditions are known to trigger haemolytic crises the most common are infections and malaria. Falciparum malaria is believed to be a key factor at precipitating sickle cell crisis and increases the risk of death in children with sickle cell anaemia. Konotey-Ahulu (6) reported that malaria is the commonest precipitating cause of crises in sickle cell disease in countries where malaria is endemic. Malaria and other infectious diseases like pneumonia are factors which trigger off severe haemolytic crisis and anaemia but predominantly falciparum malaria seems to be major cause of mortality. Mortality and morbidity are also increased in people with both sickle cell disease and malaria (5,7,). Maharajan (8) reported that malaria parasites were the commonest infecting organism in people with homozygous sickle cell disease requiring hospitalizations in Nigeria. Anaemic crises in paediatric patients with sickle cell anaemia are major causes of morbidity and mortality, Juwah (7) and Nzeogwu (9) reported that some of these children die of severe anaemia before blood transfusion is administered at hospitals' emergency rooms or paediatric wards and malaria appeared to have played a role in precipitating some of the hyper-haemolytic episodes resulting in these deaths. In another study, malaria infection was reported as the precipitating cause in 133 of 848 consecutive admissions for crises in homozygous sickle cell disease in a hospital in Ghana (6). Fleming (10) observed that haemolytic and infective crises are the major cause of morbidity and mortality and malaria is the commonest cause of these crises. Glikman (11) also reported that patients who have sickle cell disease and are infected with malaria are prone to hyperhaemolytic crisis. These claims were reviewed by Oniyangi and Omari (12) and recommended that maintenance of health at sickle cell clinic must include anti malaria prophylaxis. It is beneficial to give a lifelong routine malaria chemoprophylaxis in sickle cell disease in areas where malaria is endemic.

Many drugs are used in malaria chemoprophylaxis in SCA, these include proguanil, chloroquine, mefloquine, pyrimethamine etc. However several, factors have to be considered when starting life-long malaria chemoprophylaxis. Poor adherence may occur as it is difficult to take drugs regularly. Adverse drug effects may develop, such as hair loss and mouth ulcers with proguanil, and neuropsychiatric reactions with mefloquine (13). The development of natural immunity to malaria (particularly in children) could be impaired by chemoprophylaxis with the potential risk of severe malaria on stopping the treatment (14,). Drug resistance may develop increase in the cost of treating patients, since newer antimalarials are more expensive (13). It is therefore important to assess the benefits and harms of this life-long intervention carefully.

In view of the above considerations, this study considered suitability of sulphadoxine-pyrimethamine among other drugs in malaria

chemoprophylaxis in sickle cell anaemia. This was based on the success of SP as intermittent preventive treatment in some vulnerable population like pregnant women and could also provide a substitute to proguanil presently in use for malaria prophylaxis amongst sickle cell patient (15). Pharmaco-economic advantage of SP over proguanil is also a factor as it makes for a cheaper monthly expense for patient. The study ascertained a safe dose bearing in mind the effects of SCA on organs responsible for drug disposition because there is a wide range of pathophyisological changes that are associated with sickle cell disease which can result in alteration of drug disposition hence the necessity of dosage adjustment for malaria chemoprophylaxis (16, 17).

MATERIALS AND METHOD

Chemicals

Pure powder of sulphadoxine and pyrimethamine were obtained from Swipha Nigeria Plc. Tetrabutyl ammonium sulphate and bromide were purchased from Sigma Adrich (UK), while Sulphadoxine and Pyritmethamine (Fansidar) tablets from Swipha Nigeria Plc. Were purchased from a retail pharmacy outlet. Other chemical reagents including methanol, diethylether, acetonitrile, triethylamine and Perchloric acid were obtained from Sigma Adrich (UK), while hydrochloric acid, sodium hydroxide and ammonium acetate crystals, were obtained from BDH (Poole, UK).

Hplc

The High Performance Liquid Chromatographic system consisted of Mersham Pharmacia Biotech IP-900 liquid chromatography (USA) (AKTA) fitted with a variable UV detector (Model P-900). The stationary phase was a reversed phase C18 column (Eclipse-XDB C-18 100 x 4.6 mm i.d). The injector system consisted of Rheodyne 7725 manual injector with a 100 µl sample loop mounted on a stand. Whirl mixers (Fission), precision pipettes (MLA), table centrifuge (Gallenkamp) and digital sonicator (Gallenkamp) were used for extraction procedure.

Ethical Approval

Ethical approval for the study was obtained from Ethical Committee of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Ile-Ife and the study was carried out with collaborative effort of Sickle Cell Clinic of Paediatrics Department of the Hospital.

Extraction Procedure

To 1 ml of plasma in a 15 ml extraction tube, 1 ml of acetonitrile and 20 µl of 1mg/ml of proguanil (internal standard) were added and centrifuged for 20 min at 3000 g to precipitate the plasma protein. The supernatant was separated into clean and dry extraction tubes. 1 ml of 0.2 M tetrabutyl ammonium hydrogen sulphate was added along with 2ml of 1M sodium hydroxide and 4 ml of diethyl ether and the content was vortexed for 2 min and later centrifuged for 20 min. The organic layer was separated into clean tapered-end tubes and 400 µl of 0.1M HCl was added. The content was vortexed for 1 min and centrifuged for 10 min at 3000 g. 100 µl of the aqueous phase was injected into the HPLC.

Hplc Analytic Procedure

The mobile phase was adapted from a validated method (18) and it consisted of 30% acetonitrile and 70% 0.67M ammonium acetate. To 1000 ml of the mobile phase, 6ml of Triethylamine was added with 2 ml perchloric acid to adjust the pH to 6.5. The mobile phase was pumped at ambient temperature at a flow rate of 1.5 ml/min. The separation was achieved using C18 column (Eclipse-XDB C-18 100 x 4.6 mm i.d) and UV detection wavelength set at 220 nm.

Drug Administration

Fifteen Sickle Cell (HBss) patients with mean age 12±1.3 y attending Children Outpatient Department of Obafemi Awolowo University Teaching Hospital, Ile-Ife Nigeria (OAUTHC) were recruited. Each subject was subjected to routine physical and laboratory investigations (haematological and biochemical) before drug administration and all were certified fit for the study. Informed consent was obtained from the parents of each of the subjects. Single dose of Fansidar© containing 1000 mg sulphadoxine and 50 mg pyrimethamine was administered to each of the subjects. They were admitted for observation for 24 hours and blood sampling was also carried out during the period at predetermined time points of 0,1,2,4,6,8,12,24 hrs, 72 hour, 120 hour, 168 hour, day 14, day 21 and day 28 following drug administration, and 3 ml of blood was withdrawn from the arm vein into heparinised tubes. Plasma samples were extracted and properly labelled were stored in the freezer at -20°C until analyzed. Four out of the subjects failed to report at predetermined time and one was dropped because of anaemia (PCV less than 20%)

RESULT

Ten sickle cell patients completed the study. Table I showed mean derived pharmacokinetic parameters for sulphadoxine and pyrimethamine. The pharmacokinetic parameters obtained were compared with some values obtained in literatures by authors who worked on SP pharmacokinetics in non sickle cell patients that are age matched with normal haemoglobin.

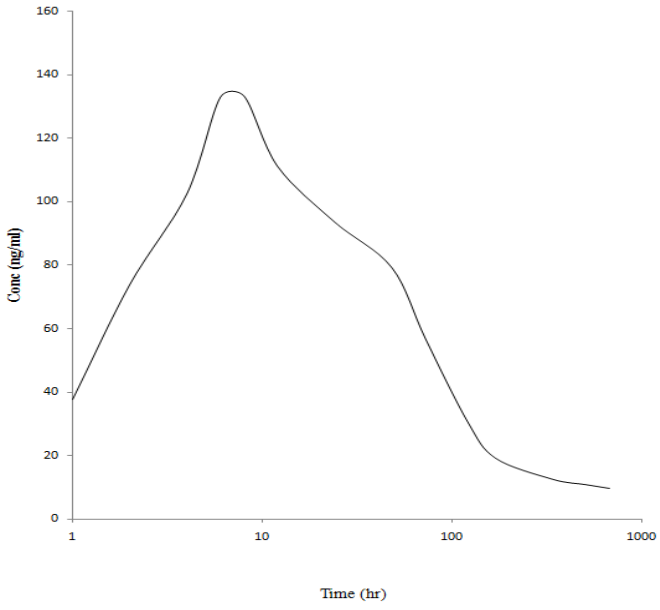

Fig. 1: Mean Pyrimethamine concentration versus time curve

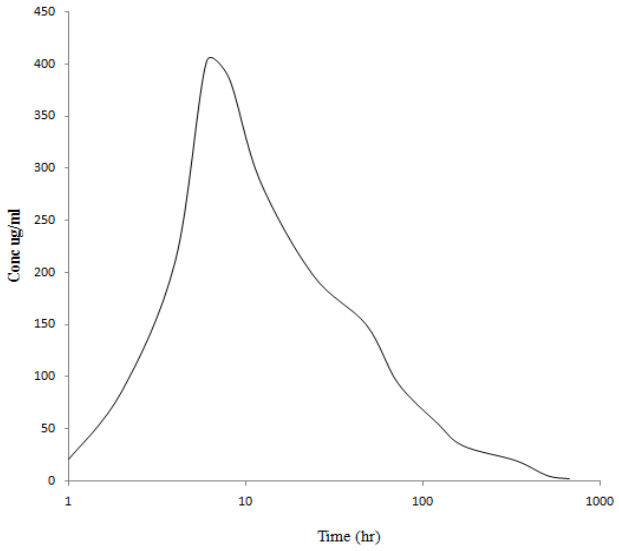

Fig. 2: Mean Sulphadoxine concentration versus time curve

Table 1: Mean of derived pharmacokinetic parameters for Sulphadoxine and Pyrimethamine

	Tmax (hr)	Cmax (µg/ml)	$T_{1/2}$ (hr)	$AUC_{0-\alpha}$ (µg,ng/ml.hr)	MRT (hr)	K_{el} (/hr)	Cl/F(L/hr)	Vd/F(L)
SDX mean	7	142.4	179.75	16,057.38	290.2	0.004	0.063	16.63
SD	1	18.76	17.46	2,734.73	32	0.0004	0.001	3.21
PYR	6.8	488.87	97.01	26,040.06	123.71	0.0075	2.34	314.93
Mean								
SD	1.33	32.06	22	11,019.92	36.58	0.0018	1.05	138.49

The mean Concentration versus time graph for both pyriemthamine and sulphadoxine is shown in Figure I and II for PYR and SDX are respectively.

DISCUSSION

The pharmacokinetic parameters T_{max}, elimination half-life $T_{1/2}$, C_{max} and AUC_T are in general agreement with findings of other workers. (Table II).

Table 2: Comparison between the study and the selected studies by other workers.

Drug	Cmax	Tmax	$AUC_{0-\infty}$	$T_{1/2}$	Author
SDX	79±22 ug/ml	13.5±15.5 hr	20013±12218 µg/mlhr	116±65 hr	Winstanley et al 1992.(19)
PYR	533±242 ng/ml	12±14 hr	62573±38867 ng/mlhr	81±32 hr	
PYR	488.87±32	6.8±1.33 hr	26040.057±11019 ng/ml.hr	97±22 hr	This study
SDX	142.40±18.76 µg/ml	7±1.0 hr	16057.38±2734.73 µg/ml.hr	179.75±17.46 hr	
SDX	p<0.05	p>0.05	p>0.05	P>0.05	
PYR	p>0.05	p>0.05	p>0.05	p>0.05	
SDX	118 ug/ml	8hr	39352 ug/mlhr	228 hr	Ogbonna et al 2008(15)
PYR	470 ng/ml	5 hr	60141ng/mlhr	104 hr	
SDX	p>0.05	P<0.05	P<0.05	P<0.05	
PYR	P>0.05	p>0.05	P<0.05	P<0.05	
SDX	63.2 µg/ml	3.7 ±1.7 hr	14168±4532 ug/ml hr	184.1±32.8 hr	Weidekamm et al 1982(20)
PYR	214 ± 0.065 µg/ml	4.9 ± 2.7 hr	191000 ng/ml hr	95.5±30.6 hr	
SDX	p<0.05	p<0.05	p>0.05	p>0.05	
PYR	p<0.05	p<0.05	p<0.05	p>0.05	

However, the study shows a long absorption period evident in T_{max} of PYR of 6.8 hr and SDX of 7 hr which is characteristic of these drugs as T_{max} reported in most studies range from 4 to 8 hours for SDX and 5 to 9 hours for PYR. This may be attributed to formulation factors and other physiological conditions. However, Winstanley (19) reported T_{max} of 13 hours for SDX and 12 hours for PYR which are significantly different from that of this study p<0.05 and other studies as well.

For SP to exhibit treatment success, the concentration of SDX and PYR in the body, 72 hr after drug intake should be ≥100 µg/ml and ≥175 ng/ml, respectively (21). In addition Dzinjalamala (22) also reported that for effective therapeutic efficacy, a mean value of 175 ng/ml for PYR and 69 µg/ml for SDX on day 3 of SP oral administration must also be attained. The day 3 concentrations in this study ranged between 48.1 µg/ml to 81 µg/ml for SDX and 40 ng/ml to 162 ng/ml for PYR. The study revealed wide inter-individual variations in the drug disposition. It is worthy to note that plasma PYR-SDX concentrations are unpredictable even when the dose is standardized for body weight (23). Obua (24) also revealed that body weight plays significant roles in plasma concentration profiles.

These values may be sufficient for expected therapeutic concentrations expected for this class of subjects. Degree of SCA in individuals may be responsible for observed difference and variations noticed in this study.

The C_{max} of 143±18.76 µg/ml for SDX obtained in this study significantly different ($p< 0.05$). from that obtained by Winstanley (19) but PYR with Cmax of 488.87±32 against 533±24 ng/ml did not show a significant difference from aged matched studies ($p>0.05$). Ogbonna (15) also reported C_{max} of 118 µg/ml and 470 ng/ml for SDX and PYR respectively. The values are not significantly different from corresponding values obtained for this study $p>0.05$. Generally, SDX and PYR plasma concentrations in this study were in the ranges required to maintain therapy and prophylaxis. This showed absence of influence of SCA features on some of the pharmacokinetic parameters of SP. The absence may be as a result of the age of the subjects. It is worthy to carry out further studies in adult population with SCA to ascertain the effect of SCA on drug disposition, because the organs responsible for disposition deteriorate with age.

The total AUC_T for SDX (16057±2734.73 ug/ml hr) is comparable with that obtained by Winstanley (24,) (20013±12218 µg/ml hr). Also AUC of PYR is not significantly different ($p>0.05$) (26040.057±11019 ng/ml hr against 62573±38867 ng./ml hr) ($p>0.05$). The values obtained in Ogbonna (15) studies were significantly different from this study because they reported 39352 µg/ml hr for SDX and 60141 ng/ml hr. The elimination half life of PYR (97±22 hours versus 179.75±17.46 showed no significant difference from compared study ($p>0.05$). Similarly SDX $T_{1/2}$ in both studies are comparable ($p>0.05$) The reported elimination half life of 95.5 ± 30.6 hr for PYR and 184.1 ± 32.8 for SDX these values are not significantly different from values obtained in this study.

CONCLUSION

In the course of the study, no adverse reactions were observed or reported in this small group of young children, indicating that the drug concentrations were within safe limits. The disposition of the drugs compared favourably with other healthy subjects therefore it can be safely administered in sickle cell anaemia. It has also been demonstrated that SP dosage of 25mg/kg body weight of sulphadoxine 1.25 mg/kg body weight of Pyrimethamine is adequate for chemoprophylaxis and sickle cell anaemia does not appear to have any significant effect on the disposition of sulphadoxine/pyrimethamine.

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