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Full Length Research Paper

The efficacy of urine data in comparative bioavailability of proguanil after oral and rectal administration in man

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The bioavailability of proguanil formulated as suppository, was compared to the tablet formulation in a bid to evaluate the utility of the suppository dosage form as means of administering proguanil in children and high-risk groups, such as sickle cell patients, who may not tolerate oral route of administration. The study was a completely randomized cross over involving administration of 200 mg of proguanil suppository and tablet to twelve healthy volunteers. Biological fluids such as urine and blood were collected before and at predetermined time intervals following administration of the drug. The biological samples were analyzed for the unchanged proguanil using an earlier reported method. The relative bioavailability of proguanil suppository as compared to the tablet dosage form was found to be about 61% from both urine and plasma data. The findings showed for the first time that proguanil suppository could be sufficiently bioavailable and may therefore be useful in chemoprophylaxis of malaria in sickle cell patients and children, particularly under such conditions that made oral route become impracticable.

Key words: Proguanil, tablets, suppositories, plasma and urine data, bioavailability.

INTRODUCTION

Proguanil (PG) (Figure 1) is a synthetic biguanide derivative of pyrimidine (1-(p- chlorophenyl)-5-isopropylbiguanide) with a marked effect on the primary tissue stages of *Plasmodium falciparum, P. vivax* and *P. ovale*. Its effect on the primary tissue stage of *P. malariae* is unknown. PG in combination with atovaquone has been developed for the treatment of *P. falciparum* infection. A series of controlled clinical trials conducted around the world, including areas of multidrug resistance, have shown that the combination of atovaquone plus proguanil hydrochloride is both highly efficacious and safe in the treatment of acute, uncomplicated falciparum malaria (Canfield et al., 1995; Radloff et al., 1996; de Alencar et al., 1997). Some studies have shown that it is efficacious for the treatment of *P. falciparum* malaria when given in combination with dapsone (Lapdap®) (Black et al., 1973).

PG is widely used in Nigeria as a prophylactic agent against malaria infection. It is administered chronically, on daily basis when daily prophylaxis is required in the suppression of malaria in sickle cell patients, particularly children (Onyeji et al., 1989). There are occasions when such patients including children may not be able to tolerate anything per oral. A non-invasive alternative route of administration may be useful in the circumstance. The rectal route may represent the practical alternative, since rectal administration is now well accepted for delivery a broad range of drugs (Hermann, 1995).

For a long period of time the rectal route was used only for the administration of local anesthetics, anti-haemorrhoidal, vermifugal and laxative agents. In recent times, some natural and synthetic drugs are also formulated in the form of suppositories to produce a systemic effect. The elimination of drugs subject to the

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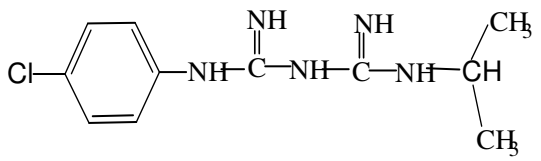

Figure 1. The Chemical Structure of Proguanil.

first-pass effect in the liver and/or in the gastrointestinal tract may be partially avoided by rectal administration (Kurosawa et al., 1985; Jonsson et al., 1988; Ogiso et al., 1991; Babul et al., 1992). Suppository may be useful as a sustained-release formulation for the long-term treatment of chronic diseases like essential hypertension, asthma, diabetes, HIV-AIDS and anemia (Kurosawa et al., 1993; Reynolds, 1993).

Previous work on the rectal absorption of some antimalarials suggested limited bioavailability. A study on the bioavailability of chloroquine following oral and rectal administration in man gave limited bioavailability (Onyeji et al., 1996) but on incorporation of absorption enhancers increased the *in vitro* release of the chloroquine suppository (Onyeji et al., 1999). It was reported that *in vitro* availability of amodiaquine from polyethylene glycol (PEG) suppository base was superior to that from tablet dosage form when compared under the same experimental conditions (Akala et al., 1991). The bioavailability of artemisinin suppository relative to the tablet formulations was evaluated (Koopmans et al., 1999). It was shown that therapeutic blood concentration of artemisinin could be reached after rectal dosage but the dose after rectal administration should probably be higher than oral administration; doubling or tripling the rectal dose may be necessary. Other studies on artemisinin include those carried out by (Arnold et al., 1990), in which they found out that artemisinin and its derivatives in a suppository form quickly reduced parasitaemia and lower recrudescence in hospital in-patients.

Generally, there are three broad methods of estimating bioavailability: pharmacokinetic, clinical and pharmacological methods. In this study we employ the use of pharmacokinetic method for the bioavailability studies. In the pharmacokinetic method, a completely randomized balanced crossover study in a group of subjects is usually employed although a parallel study is undertaken sometimes. In a crossover study, every subject in the group acts as its own control. After a period of time between treatments, the administration is reversed so that in the end every subject in the group would have received each of the test products. In a parallel study, the subjects are divided into groups equal to the number of test products in a parallel manner, each of the groups receiving only one of the test products.

The parameters that are used in describing the bioavailability of a drug from a drug product can be derived from the following:

Plasma data: When the blood concentration time curves are plotted, three parameters are used for evaluating bioavailability. These are peak concentration (Cmax), the time of peak plasma concentration (tmax) and the area under the plasma concentration-time curve (AUC).

Urine data: When urinary drug excretion data is to be used for the estimation of bioavailability, three parameters are important. These are; the cumulative amount of drug excreted in the urine (Du), the rate of drug excretion in the urine (dDu/dt) and time for maximum urinary excretion (tmax) (Leon and Andrew, 1996). In order to obtain valid estimates using urinary excretion data, the drug must be excreted in significant quantities in the urine and adequate sample of urine must be collected. Urinary excretion of PG is known to be almost 70% of the whole drug and therefore, it will be a good product for bioavailability studies using urine data. Also, urine sampling, a non-invasive method of sampling from volunteers, may be useful for ease of compliance in recruiting volunteers to participate in bioavailability studies.

The study therefore, set out to investigate the relative bioequivalence of PG between a suppository and tablet formulation using urinary excretion data in order to evaluate the utility of the suppository dosage form as means of administering PG in children and high-risk groups, such as sickle cell patients, who may not tolerate oral route of administration.

MATERIALS AND METHOD

Chemicals

Reagents used were proguanil hydrochloride powder (Sigma Aldrich, USA), proguanil hydrochloride tablets (Astra Zeneca Pharmaceutical Ltd, Sweden), cycloguanil powder (Sigma Aldrich, USA), 4-chlorophenylbiguanide powder (Sigma Aldrich, USA), pyrimethamine powder (Sigma Aldrich, USA), ammonium acetate (BDH, UK), perchloric acid 60% W/W (BDH, UK), diethyl ether (BDH, UK), HPLC grade acetonitrile (Sigma Aldrich, USA), HPLC grade methanol (Sigma Aldrich, USA) and acetone (BDH, UK).

Instruments/Apparatus

The Liquid chromatographic system used consisted of a Cecil 1100 series instrument (Cecil Instrument, Cambridge, England) made up of binary pumps fitted with a gradient mixer (Cecil instrument, Cambridge, England) with a system purge and a variable wavelength (200-800nm) ultraviolet-visible detector model CE1200

Table 1. Derived pharmacokinetic parameters of proguanil following oral and rectal administration of 200mg dose of proguanil tablet and suppository formulations to the subjects –urine data

Subjects	Body wt (Kg)	Ae (mg) Tab	Ae (mg) Sup	λ t ½ (hr) Tab	λ t ½ (hr) Sup	T max (hr) Tab	T max (hr) Sup	Cmax (mg/ml) Tab	Cmax (mg/ml) Sup	BA (%)
AAA	78	72.52	38.76	10.4	13.1	5	5	4.49	3.24	53.4
BUE	75	39.72	21.74	12.9	18.6	3	3	5.44	2.05	54.7
UAO	58	50.14	34.37	17.2	11.9	3	3	6.58	3.675	68.6
OOO	65	81.86	46.49	15.1	13.6	5	5	9.715	5.685	57.2
AEA	65	92.03	47.08	21.6	29.7	3	3	6.845	3.08	51.1
DOO	62	58.54	41.84	17.1	28.5	3	3	4.735	4.06	71.5
TEA	63	55.27	28.63	12.9	14.1	3	3	5.17	3.25	51.8
BDH	66	58.74	34.33	16.0	17.0	3	3	6.445	3.85	58.4
MAO	57	25.07	16.17	12.5	12.7	3	3	4.2	2.365	64.6
JMO	73	74.18	56.73	14.4	17.2	5	3	7.45	4.145	76.5
KDU	66	59.8	36.3	14.8	12.8	3	3	6.55	4.06	60.7
ASP	71	63.5	37.5	15.3	13.5	3	3	7.68	4.53	59.1

(Cecil instrument, Cambridge, England) with a 18 µL flow cell. Injection was by a Rheodyne model 7725 valve (Cotati, California, U.S.A.) fitted with a 20 µL loop. The detector output is linked to a Computer via a brain-box inter-phase (Cecil Instrument, Cambridge, England), which transforms signals from the detector to the computer that eventually records the chromatograms. The computer system is connected to an LX 300 printer (Epson). The column used was a Zorbax (SB-C18) 5 µm particle size and 250 x 4.6 mm I.D, reversed phase stainless steel (Agilent Technologies, USA). 220V Ultra sonicator (Branso), whirlmixer (Scientific Industries Inc., USA), table centrifuge (Gallenkamp), Eppendorf pipettes, Pasteur pipettes and extraction tubes. Glasswares were cleaned by soaking overnight in detergent solution, after which they were washed, rinsed with distilled water and 0.1 M HCl, methanol and then acetone before being oven-dried.

Subjects

Twelve healthy male volunteers between the ages of 20 and 33 years and weighing between 52 and 72 kg participated in the study. Informed consent was obtained from all the volunteers. None had history of liver disease or cardiac disease and all had normal physical examination, electrocardiogram and biochemical / haematological laboratory reports. The participants were instructed to abstain from other medications and alcohol one-week prior to and during the period of the study. All the subjects were non-smokers. The Ethical Committee of the Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria approved this study.

.**Preparation of the suppositories**

The suppositories were prepared by fusion method following earlier described methods (Akala et al., 1991; Onyeji et al., 1996) using witepsol as the suppository base. The suppository formulations were subjected to pharmaceutical quality assessment such as uniformity of appearance, weight and content, as well as the disintegration and dissolution tests were evaluated following the British Pharmacopoeia (1998) tests.

Drug administration and sample collection

Prior to drug administration all subjects fasted overnight and were not allowed food for a period of 4 h following drug administration. From then food intake was not regulated. The study was performed using a completely randomized crossover design, that is, the ten subjects were divided into two groups of five each. The first group received two tablets of 100 mg PG hydrochloride (Paludrine®) with 200 ml of water. The second group received one suppository of 200 mg PG by careful placement into the anus. After a washout period of two weeks, the administrations of the drugs were reversed. The first group received one suppository containing 200 mg PG by careful placement into the anus while the second group received two tablets of 100 mg PG orally accompanied by 200 ml of water.
Urine samples were collected from the volunteers just before the drug administration and thereafter all urine voided was collected at 2, 4, 6, 8, 12, 24, 48, and 72 h following drug administration. The total volume of urine was measured immediately after collection and the pH determined. Aliquots of urine samples were then placed in fresh tubes and stored frozen at –20ºC before analysis.

Table 2. Derived pharmacokinetic parameters of proguanil following oral and rectal administration of 200mg dose of proguanil tablets and the suppository to the subjects – plasma data.

Subjects	Body Weight (KG)	λt ½ {hr}		AUC_{0-t} (ng/ml. hr)		T max (hr		C. Max(ng/ml)		BA (%
		Tab	Sup	Tab	Sup	Tab	Sup	Tab	Sup	
AAA	78	9.7	12.7	1595.4	902.4	3	3	140.2	72.9	56.6
BUE	75	12.5	13.2	2264.8	1463.4	3	3	155.5	92.5	64.6
UAO	58	16.4	15.8	2447.4	1340.2	3	3	164.2	85.1	54.8
OOO	65	14.4	14.6	1406.3	922.6	3	3	139	80.6	65.6
AEA	65	15.3	14.9	2156.4	1334.5	5	5	146.5	89.5	61.9
DOO	62	15.3	17.9	2351.9	1537.2	5	5	140.8	88.4	65.4
TEA	63	14.9	14.7	2460.7	1587.7	3	3	174.3	111.5	64.5
BDH	66	13.9	14.0	1767.9	1085.7	3	3	134.9	80.9	61.4
MAO	57	15.4	14.2	2497.5	1456.4	3	3	163.7	106.4	58.3
JMO	73	16.6	15.3	2583.8	1584.6	5	5	156.1	96.9	61.3
KDU	66	15.6	15.2	2464.8	1429.6	3	3	146.9	85.2	58.0
ASP	71	14.8	15.1	2368.4	1444.7	3	3	138.5	84.5	61.0
MEAN±SD	**66.6 ± 6.5**	**14.6±1.9**	**14.8± 1.3**	**2197 ± 390.2**	**1341 ± 240.9**	**3.5±0.9**	**3.5±0.9**	**150.1±12.5**	**89.5±11.0**	**61.1 ± 3.6**

Blood samples (5 ml) were withdrawn with the aid of a catheter into lithium heparin tubes prior to drug administration and thereafter at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48 and72 h. Plasma was separated immediately by centrifuging at 3000 g for 10 min. The plasma were then placed in fresh tubes and stored frozen at –20°C before analysis.

Quantitative analysis

The urine and plasma samples were analysed in accordance with the method of Ebeshi et al. (2005).

Data Analysis

The individual amounts of PG excreted (Ae) and the areas under the plasma drug concentration time curves (AUC_{0-t}) following oral and rectal administration of the drug were determined using WinNonlin- pharmacokinetic programme, which uses non-compartmental methods for analyzing pharmacokinetic data. The time to reach peak urinary excretion rate (tmax) and the maximum urinary excretion rate (dDu/dt)max as well as the tmax and Cp max from urine and plasma data, respectively, were determined using the above mentioned programme. Also, the relative bioavailability for all the subjects was computed using the data obtained above.

Statistical analysis

Graphpad prism programme was used to determine the 90% Confidence Interval of the Test-Reference ratio of AUC_{0-t},, Ae and C_{max}. T-test was used to compare the various pharmacokinetic parameters derived in this study rejecting the null hypothesis if $p < 0.05$.

RESULTS AND DISCUSSION

The relative bioavailability of PG from the suppository (Tables 1 and 2) compared to tablet formulation as indicated by the Test-to-Reference ratio (T/R) ratio: $Ae_{(rectal)}/Ae_{(oral)}$ x 100% and $AUC_{(rectal)}/AUC_{(oral)}$ x 100% were 60.6 ± 8.1 and 61.1 ± 3.6% (Mean ± S.D) from urine and plasma data, respectively.

The 90% Confidence Interval of discrepancy of the Test-to-Reference ratio (T/R) of AUC_{0-t},, Ae and C_{max} for both urine and plasma data fall outside the stipulated range of 80 – 125%. For two drug products to be considered bioequivalent, it is required that the 90% confidence interval (CI) of the Test-to-Reference ratio (T/R) of AUC_{0-t},, Ae and C_{max} fall completely within the 80-125% boundary (FDA, 1992). The comparable bioavailability values obtained (i.e. about 61%) from urine and plasma data shows that urinary excretion data can be used in the assessment of bioavailability of the drug from the suppository. The use of such non-invasive method of estimating bioavailability will enable easy recruitment of volunteers for further studies.

The low bioavailability of 61% obtained in this study is sufficiently high for the use of the formulation in malaria chemoprophylaxis given the benefits earlier outlined but proportional increase in the dosage of the suppository may be necessary for the attainment of similar therapeutic level as the tablet formulation. In any case, a new antimalaria drug, artesunate, which has been formulated as suppository, has a bioavailability of 30% (Koopmans et al., 1999) and is being recommended for use in the treatment of malaria. Indeed, clinical trials are currently being conducted in some countries in Africa on this artesunate suppository. This implies that the PG suppository with a bioavailability greater than 60% can be found useful in the chemoprophylaxis of malaria in sickle cell patients and children.

The maximum urinary excretion rate, (dDu/dt) max, and the time of peak urinary excretion (tmax) (Figure 1) are important indicators of the rate of drug absorption and excretion. The tmax from urinary excretion rate is

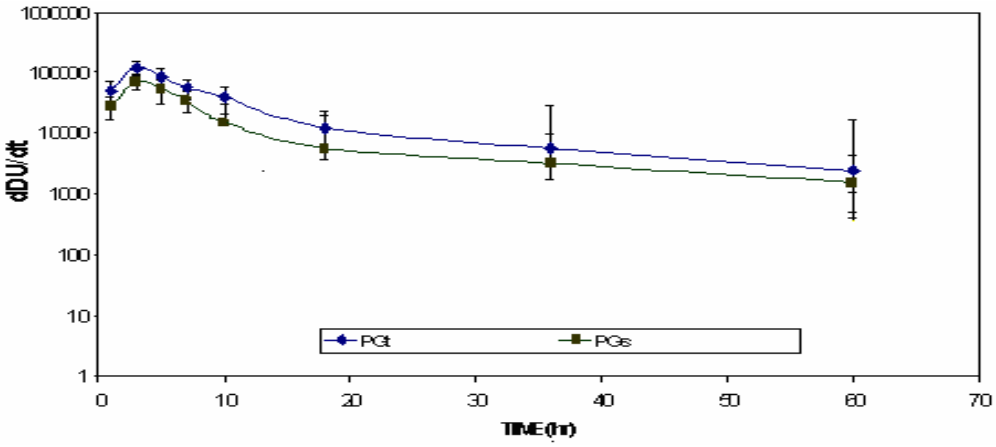

Figure. 2 Mean urinary excretion rate profile of PG after oral and rectal administration
PGt: Proguanil tablet

comparable with the tmax from plasma concentration-time profile (Figure 2). Since, tmax is a true measure of the rate of absorption, it could be said that the plasma data are in agreement with the urine data. Furthermore, analysis of the data in Tables 1 and 2 revealed no significant difference ($p< 0.05$) between the tmax of tablet and suppository formulations. Hence, it can be asserted that the rate of release of the drug from the suppository is similar to that from the tablet. The significant difference ($p> 0.05$) in the bioequivalence of the two dosage forms could have resulted from the extent of absorption (Cmax). The values of tmax obtained in this work are comparable to the tmax of about 4.0 h reported for PG by Ritschell et al. (1978) from plasma data and Onyeji et al. (1989) from saliva data.

The mean elimination half-life (λt ½) of PG was found to be 15.0 ± 3.0 and 16.9 ± 6.1 h for the tablet and suppository, respectively, whereas, the λt ½ for plasma data was 14.6 ± 1.9 and 14.8 ± 1.3 h for the tablet and suppository, respectively. There was no significant difference between the two formulations with respect to the elimination half-life of the drug ($p< 0.05$). However, there was considerable inter-subject variability, in agreement with previous report (Hussein et al., 1996). The values of elimination half-life obtained in this study for the tablet and suppository formulations were in agreement with the previously reported values of about 15 h by Ritschell et al. (1978) and White (1985) generated from plasma concentration-time profile (Table 3).

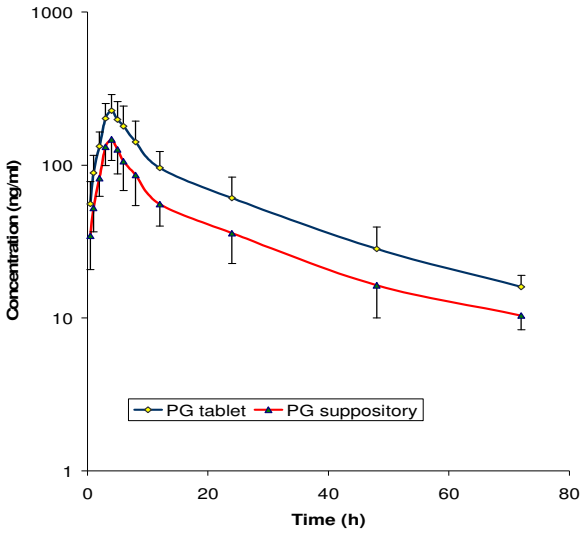

Figure 3. Mean plasma concentration time profile of PG after Oral and Rectal administration

This study demonstrates for the first time that PG is absorbed in man from the suppository formulation of the drug. The results of this study suggest that PG suppository can be found useful in chemoprophylaxis of malaria in sickle cell patients and children. This formulation can be a vital alternative to the tablet dosage form in these high-risk groups when oral route of administration becomes impracticable. Further studies on the PG suppository formulation may be necessary with the aim of optimizing the rectal absorption and consequently the bioavailability of the drug.

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