Comparative Studies of the Pharmacokinetic Parameters of Dihydroartemisinin with its Disulphide Derivative.

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ABSTRACT

Malaria parasite has developed resistance to readily available and affordable drugs. Artemisinin and its derivatives are challenged by short systemic half life. The disulphide derivative of dihydroartemisinin was synthesized and confirmed of having antiplasmodial efficacy *in vivo* and is effective against gram negative bacteria and some fungi species *in vitro*. This work was aimed at assessing the pharmacokinetic parameters of the disulphide derivative of dihydroartemisinin in comparison with pure dihydroartemisinin. Sixty six rats of both sexes weighing between 180g to 220g were divided into twelve groups of five each. Therapeutic doses of dihydroartemisinin and the disulphide derivative were administered orally to each group. At interval of 1.0, 2.0, 4.0, 8.0, 12 and 24 hours, a group were sacrificed, and blood collected by cardiac puncture. The plasma obtained from the blood was spiked with benzene diazonium chloride and analyzed using UV spectrophotometer. The results obtained gave the pharmacokinetic parameters for the pure and disulphide derivative respectively as follows: AUC 9 mg.h/ml and 28.50 mg.h/ml, clearance 2.5 x 10^{-4} L/h/kg and 1.08 x 10^{-4} L/h/kg, volume of distribution 1.1 x 10^{-3} L/kg and 1.55 x 10^{-3} L/kg, elimination rate constant 0.24 h^{-1} and 0.070 h^{-1} and halflife 2.88 h and 9.9 h. The disulphide derivative with a halflife of 9.9h.,if proven clinically useful as a therapeutic agent could be used as a sustained release and dosed once daily for treatment of susceptible bacteria, fungi and malaria.

Keywords: Dihydroartemisin, Disulphide dihydroartemisin, pharmacokinetic parameter.

INTRODUCTION

Artemisinin is the antimalaria principle isolated from *artermisia annua* L, it is a sesquiterpene lactone with an endoperoxide bond (Efferth 2006; WHO,2015). Artemisinin is poorly soluble in water, but its active metabolite and synthetic derivative dihydroatemisin (DHA) is soluble and is a more potent blood schizonticide than the parent compound (Coker *et al*.,2001). It is used in the treatment of uncomplicated and chloroquine resistant malaria (Cumming, 1997; Nosten and White, 2007; Baird, 2013;White, 2014). The clinical use of artemisinin and other derivatives as therapeutic agent is limited by several factors which include; short half-life, and high rate of recrudescence, partly due to the presence of the peroxide bond which is challenged by endogenous susperoxide dismutase (Wallem and Plowe, 2001; Bartoloni,2012).

The disulphide derivative of dihydroartemisin was synthesized by removal of the peroxide bond in dihydroatemisinin using hydrogen gas generated from Zn/HCl *in situ* and reoxidized using sulphide ion (S^{2-}) from hydrogen sulphide produced by the action of ferrous sulphide and HCl(aq) The synthesized disulphide derivative (SDHA) of DHA was confirmed to show antiplasmodial activity similar to that of DHA using rats (Etim, *et al*., 2017).

A drug must be present in appropriate concentrations at its sites of action to produce its desired effect. For orally administered drugs, it must be absorbed, from the gastrointestinal tract to an extent and at a rate that will ensure adequate blood levels to elicit pharmacological response of desired magnitude and duration (Rang, *et al*., 2007; Robert, 2012). Pharmacokinetics provides a mathematical basis to assess the time course and the effect of drugs in the body (Walker,2004; Eseyin *et al*., 2012). The present study is aimed at assessing the pharmacokinetic parameters of the synthesized disulphide derivative of dihydroartemisinin so as to know if proven clinically useful how best it can be formulated and the dosage regimen in comparison to the parent therapeutic DHA.

MATERIALS AND METHODS:

Ethical approval

Permission and approval for animal studies were obtained from the College of Health science, Animal Ethics Committee, University of Uyo. Chemicals: All Chemical used in the study were of analytical grade. Pure dihydro artermisinin was obtained from May and Baker Nigeria Plc. Lagos Nigeria. All the reagents were purchased from Sigma Aldrich Germany and BDH Chemicals – Poole England through their Nigeria representatives. A Cecil Spectrophotometer model number CE 7200 was used for the UV-analysis.

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Animals: A total of sixty six health albino rats of both sexes (wister strain) weighing between 180g – 220g were used in the study. They were maintained under standard environmental conditions and had free access to food and water at the animal house, Department of Pharmacology and Toxicology University of Uyo.

Preparation of standard calibration curve for dihydroartemisinin and sesquiterpene lactol endodisulphide

Dihydroartemisinin (pure grade, 10mg) and the synthesized disulphide derivative (10mg) were dissolved separately in 10ml of chloroform. Aliquots of 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml, 3.0ml 3.5ml, 4.0ml 4.5ml and 5.0ml of the stock solutions were measured into 10ml volumetric flash and appropriate volume of chloroform was added to make up 10.0ml. The absorbance of each of these mixtures was measured 320 for DHA and 360 for SDHA against chloroform as blank. A calibration curve was obtained.

Synthesis of sesquiterpene lectol endodisulphide: Dihydroartemisinin powder was dissolved in chloroform and the solution reduced with hydrogen gas generated *in situ* by the reaction of zinc dust and hydrochloric acid. The chloroform phase was separated, allowed to dry and dissolved in dimethylsulphoxide. Hydrogen sulphide gas was bubbled into the solution to form the disulphide solution which was freeze dried to obtain yellowish white powder the disulphide derivative (SDHA) (Etim, *et al*., 2017)

Determination of Median Lethal Dose (LD_{50}): The up and down method as described by (Rispin, *et al.*, 2002) was used.

Administration of test material: The rats were fasted overnight prior to the testing. The animals were divided into thirteen groups (A – M) with five rats per group. Group M (the thirteenth group) consisted of 6 rats and served as control. Groups A – F were administered an oral dose of 2.2 mg/kg of synthesized SDHA, while group G – L were given 3.1 mg/kg of the pure DHA orally, based on 30% of their respective $LD_{50.}$ Group M (control) were given distilled water.

Collection of Plasma Sample

At the stipulated time interval for each group (1hr, 2hr, 4hr, 8hr, 12hr and 24hr), the animals were anaesthetise using chloroform. They were sacrificed and the blood collected by cardiac puncture using 5ml syringes into already labeled EDTA sample tubes and swirled. The whole blood collected was allowed to stand for 5 minutes to cool and equilibrate with the anticoagulant. The blood was centrifuge for 20 minutes at 4000gpm. The plasma was aspirated into sterile samples tubes and labeled accordingly. The plasma samples were stored in a refrigerator at -10^{o}C for subsequent analysis.

Blank determination was done for the rats to which no drug was administered. The blood samples were collected and stored in the same manner.

Analysis of Samples

Two milliliter of the plasma collected was diluted with 1.0ml of distilled water and spiked with 0.01ml of benzene diazonium chlorine (Emmanuel, *et al.*,2016) and their absorbance measured at 320mm for DHA and 360mm for SDHA respectively, against blank dilute plasma. The concentration of the drugs in plasma was extrapolated from the various calibration curves earlier prepared

RESULTS AND DISCUSSION

The lethal median dose (LD_{50}) using up and down method for pure and synthesized DHA are 547.70 and 346.40 µg/kg, respectively. LD_{50} is a measure or an indicator of toxicity and it is inversely proportional to the toxicity of the drug (Rang, *et al.*, 2007). From the table, the LD_{50} for the pure drug was higher than that of the disulphide derivative which implies that the pure drug is less toxic than its synthesized disulphide derivative.

Table 1: Concentrations of Pure DHA and Synthesized SDHA (mg/ml) Extrapolated From Calibration Curve

TIME	ABSORBANCE	CONCENTRATION	
		DHA (mg/ml)	SDHA(mg/ml)
1 HOUR	0.053	0.58	2.91
2 HOURS	0.107	1.10	4.62
4 HOURS	0.164	1.80	2.40
8 HOURS	0.146	1.60	2.26
12 HOURS	0.052	0.57	1.12
24 HOURS	0.013	0.20	0.99

The maximum concentration ($C_{max.}$), and time to attain it ($T_{max.}$) was obtained from graph plotted with data from table 2, concentration against time. Specific pharmacokinetic metrics were calculated from the concentration-time graphs of both the pure and disulphide drugs (table 1)

Table 2:Pharmacokinetic Parameters of Pure Dihydroartemisinin and its Synthesized Disulphide Derivative.

PHARMACOKINETIC PARAMETER	PURE DIHYDROARTEMISININ	SULPHIDE DERIVATIVE
C_{max}	1.8 mg/ml	4.6 mg/ml
T_{max}	4 h	2 h
C_{min}	0.2 mg/ml	2.1 mg/ml
T_{min}	24 h	12 h
AUC (Trapezium method)	9 mg.h/ml	28.5 mg.h/ml
Clearance (CL)	2.5×10^{-4} L/h/kg	1.08×10^{-4} L/h/kg
Volume of distribution (V_d)	1.1×10^{-3} L/kg	1.55×10^{-3} L/kg
Elimination rate constant (K_e)	0.24 h^{-1}	0.07 h^{-1}
Half-life ($t_{1/2}$)	2.88 h	9.9 h

From the table, C_{max} was found to be 1.8 mg/ml and 4.6 mg/ml for the pure and disulphide drugs respectively with corresponding T_{max} at 4h and 2h. The C_{min} for both drugs was 0.2mg/ml and 2.1 mg/ml respectively with corresponding T_{min} as 24h and 12h.

The area under the curve (AUC) was found to be 9 mg.h/ml for the pure drug and 28.50 mg.h/ml for the disulphide derivative. The AUC is a measure of how long a drug stays in the systemic circulation, therefore from the results, the disulphide derivative will stay longer in the system compared to the pure drug (Shargel and Yu, 1998).

Clearance is a measure of the volume of the plasma that is cleared of a drug per unit time. The clearance was found to be 2.5×10^{-4} L/h/kg for the pure drug and 1.08×10^{-4} L/h/kg for the disulphide derivative. This indicates that the plasma is cleared of the pure drug faster than it is cleared of the disulphide derivative (Rang, *et al*., 2007). The volume of distribution for the pure and disulphide dihydroartemisinin was found to be 1.1×10^{-3} L/kg and 1.55×10^{-3} L/kg respectively, hence the disulphide has a higher volume in which it is distributed as compared to the pure drug (Walker, 2004).

The elimination rate constant (K_e) describes the rate at which a drug is removed from the body and is equivalent to the fraction of a substance (drug) eliminated per unit time. K_e for the pure drug was calculated to be 0.24 h^{-1} and 0.07 h^{-1} for its disulphide derivative. This implies that the pure drug is eliminated at a faster rate from the body when compared to its disulphide derivative.

The half-life of both drugs was calculated based on its inverse relationship with the elimination rate constant. $T_{1/2}$ for the pure and disulphide drug was calculated to be 2.88 h and 9.9 h respectively implying that the disulphide derivative has a much longer half-life than pure DHA which is an advantage as artemisinins are particularly noted for having a short systemic half-life. The half-life of 2.22 hours obtained from this research varied slightly from recent work which places the half-life of dihydroartemisinin at a range of 1- 2 hours (WHO, 2015).

CONCLUSION

Through physicochemical analysis, the physical and chemical properties of both DHA and SDHA were assessed and it was confirmed that the disulphide derivative was indeed different from the pure drug as both compounds were found to have distinct R_f values and melting points which are unique and characteristics of a particular chemical compound (Etim, *et al*., 2017). The different pharmacokinetic parameters evaluated here were also found to be different in both the pure dihydroartemisinin and the disulphide derivative. When compared to the pure drug, the disulphide had a lower clearance and elimination half-life and a longer half-life than the pure drug. It can be concluded that the disulphide derivative of dihydroartemisinin if proven clinically useful as a therapeutic agent could be used as a sustained release with a once daily dosage regimen for treatment of susceptible bacteria, fungi and malaria.

ACKNOWLEDGEMENT

The authors are greatful to Mr Nsikan Malachy, laboratory technician of the animal house of the department of Pharmacology and Toxicology,University of Uyo, and Mrs. Ekaete Umoh, Chief Technologist of the Department of Pharmaceutical and Medicinal Chemistry, University of Uyo for their technical assistance.

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