Journal of Applied Pharmaceutical Science Vol. 5 (08), pp. 090-094, August, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50814
ISSN 2231-3354

Pharmacokinetics of Chloroquine and Metronidazole in Rats

Kudirat Bola Mustapha[1*], Moji T Bakare-Odunola[3], Garba Magaji[3], Obiageri O. Obodozie-Ofoegbu[1], David D Akumka[2]

[1]Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development Idu, Abuja, Nigeria. [2] Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences. University of Ilorin, Ilorin. Nigeria. [3]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, A.B.U., Zaria, Nigeria.

ARTICLE INFO

Article history:
Received on: 12/05/2015
Revised on: 22/05/2015
Accepted on: 14/06/2015
Available online: 28/08/2015

Key words:
Pharmacokinetic, Chloroquine, Metronidazole, HPLC

ABSTRACT

The single oral dose pharmacokinetics of chloroquine (5mg/kg body weight) and metronidazole (7.5mg/kg body weight) were studied in rats' serum. Chloroquine and metronidazole concentrations were measured using high-performance liquid chromatography (HPLC) method developed earlier in our laboratory. The data were fitted into a WinNonlin standard non-compartmental programme. The Maximum serum concentration C_{max} (µg/ml) of chloroquine was 5.70 ± 1.41 while that of metronidazole was 3.13 ± 0.30, Time to peak concentration t_{max} was 1.00 ± 0.00 (h) and that of metronidazole was 0.83 ± 0.27, Volume of distribution V_d (L) 1.33 ± 0.26 for metronidazole 2.39 ± 0.28; Elimination half-life $t_{1/2\beta}$ (h) 10.05 ± 3.01 for metronidazole 4.05 ± 0.46. The values were comparable with the works of other authors. Compounds that show very high activity *in -vitro* may not have *in vivo* activity, or may be highly toxic using *in- vivo* models due to undesirable pharmacokinetic properties, and toxicity may result from formation of reactive metabolites. This study assures the quality of the brands of the drugs and encouraged the use of animal model in determining pharmacokinetic properties especially in drug design.

INTRODUCTION

Pharmacokinetics denotes the movement of drugs through the body over time, and addresses the absorption from the site of administration, distribution throughout the body, metabolism of the drug, and its elimination from the body. One of the main applications of pharmacokinetics in drug design industry is to use pharmacokinetics parameters to plan dosage regimens of drugs. A dosage regimen is the key to a drug producing the desired therapeutic effects. A regimen can be described in terms of the dose of the drug to be used, the frequency with which it should be established, the rate of administration and the formulation to be used. Malaria is an infectious disease that continues to be associated with considerable morbidity and mortality and significant social and economic impact on developing societies. According to the World Health Organization (WHO), malaria is endemic in 91 countries, predominantly in Africa, Asia, and Latin America, with about 40% of the world's population at risk (WHO, 1996).

Chloroquine (CQ) and other 4-aminoquinolines, like amodiaquine and its metabolite desethylamodiaquine, attack the malaria parasite in its intra erythrocytic stages when it digests hemoglobin in its lysosome, releasing haematin, potentially toxic to the parasite. Chloroquine is still in used for the treatment of malaria in an area where there is no chloroquine resistance.

About 400 million doses of chloroquine are taken annually (Nicholas, 1999). Metronidazole (MET) is a 5-nitronimidazole derivative with activity against anaerobic protozoa and anaerobic bacteria; it also has a radio sensitizing effects on hypoxic tumor cells. Its mechanism of action is thought to involve interference with DNA by a metabolite in which the nitro group of metronidazole has been reduced. Metronidazole is active against several protozoa including Balantidium coli, Blascyst hominid, Entamaeba histolytic, Giardia intestinals and Trichomonas vaginalis. Metronidazole administered orally will produce an effective concentration of the drug in the blood and urine. Pharmacokinetics of antimalarials and metronidazole has mostly been studied in humans (Gannon and Phillips, 1982; Lau *et al.*, 1992; White, 1992; Ducharme and Farinotti, 1996; White, 1997). This work reports the pharmacokinetic profiles of chloroquine, its metabolite and metronidazole in rat.

* *Corresponding Author*
Kudirat Bola Mustapha, Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development Idu, Abuja, Nigeria. Email: bolakud@yahoo.com

MATERIALS AND METHODS

Materials

Chloroquine phosphate reference standard, Lot 51H0255 (Sigma Chemical CO.), Metronidazole Lot 1 (USA, Rockville), Papaverine Lot 88F0589 (Sigma Chemical Co.), Desethylchloroquine (CQM)(Sigma Aldrich). All reagents were analytical grades and were obtained from British Drug House (BDH) Chemicals Limited, Poole, England. The liquid chromatographic system used to analyse chlororquine, desmethylchloroquine and metronidazole was Agilent 1100 series instrument (U.S.A.), consists of quarternary pumps (G131/A) - series with an online vacuum degasser (G1322A) series JP 03924111 and a variable wavelength (200-800nm) ultraviolet - visible detector model 1100 series (Agilent instrument), injector was fitted with a 20ul loop. The detector output was connected to a computer system and HP 1200 laser jet printer. The column used was an ODS dp 5µ, 2.0mm x 25cm Ultrasphere (Beckman, USA), reversed phase stainless steel.

Animals

Male adult Wister rats, with body weight range of 150-250g were used. The animals were housed in plastic cages 7 days to the experiment. They were maintained on standard laboratory diet (Ladokun Nigeria Limited, Ibadan). The animals were maintained at room temperature and water was allowed ad libitum.

METHODS

Study Protocol

Adult Wister rats were randomized into groups of twelve rats per sampling time of 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 for hour chloroquine while for metronidazole 0, 0.5, 1, 2, 3, 6 and 8h. Drugs were administered orally in solution to the rats using gastric cannula. The group A received aqueous solution of chloroquine (5mg/kg). Group B received aqueous solution of metronidazole (7.5mg/kg) alone, while test groups received the same dose of metronidazole. Blood sample (2ml) was collected from each rat at times 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours; through retro-orbital sinus puncture (Lhote etal., 1991) using 0.1 ml capillary tubes.

The blood samples were centrifuged at 2500 rpm for 10 minutes. The sera were separated and kept in the refrigerator at 20^0C until analyzed

Analysis

Chromatographic system

The Chloroquine and desethylchloroquine levels in the serum samples were determined by a modified reversed phase method of Ademowo *et al* (2000). The mobile phase was 0.02 mol/L Sodium dihydrogen phosphate, methanol, and acetonitrite in ratio of 65:30:5 respectively. 1ml/100ml of perchloric acid was added to provide counter ions. The pH of the solvent system was 2.8. The stationary phase was a silica–based column (5µm). Wavelength of detection was 254 nm and the mobile phase was pumped at flow rate of 0.8ml per min at ambient temperature. For metronidazole the mobile phase was 0.02 mol/L Sodium dihydrogen phosphate, methanol, and acetonitrite in ratio of 65:30:5 respectively. 1ml/100ml of perchloric acid was added onto the column to provide counter ions. The pH of the solvent system was 2.8.

The stationary phase was a silica–based column (5µm). Wavelength of detection was 254nm and the mobile phase was pumped at flow rate of 0.8ml per min at ambient temperature (Mustapha *et al.*,2006).

Analysis of serum samples

Chloroquine

To 0.5ml of serum sample was added 10µl of papaverine (10µg/ml). The mixture was rendered alkaline with 0.5ml of 2M NaOH and 2ml of diethyl ether was added. The resulting mixture was then vortex mixed for 1 min, then centrifuged at 2,500rpm for 10 min and the ethereal layer was transferred to fresh tubes to which 100µl of 0.1N hydrochloric acid was added. This was vortex mixed for 1min and the organic layer was clarified from the aqueous layer by centrifugation.

The organic layer was discarded. A 20µl aliquot of the aqueous layer was injected into the system. A calibration curve was constructed by spiking drug- free rat serum in duplicate with standard solution of chloroquine and desethylchloroquine to give 0-10 µg/ml.

Metronidazole

To 0.2ml of serum sample was added 10µl of sulphamethoxazole. The mixture was rendered alkaline with 0.5ml of 2M NaOH and 2ml of dichloromethane was added. The resulting mixture was then vortex mixed for 1 min, then centrifuged and the aqueous layer aspirated off. The organic layer was evaporated to dryness on a water bath at 40^0C. The dried residue was dissolved in 100 µl of 0.1N HCL, and 20 µl of the solution was injected onto the column.

A calibration curve was constructed by spiking drug- free rat serum with standard solution of metronidazole to obtain a concentration range of 0-2 µg/ml. The standard curves were constructed by plotting the peak area ratios of the drugs against the concentrations in the standard samples. Each sample was analysed in duplicate via HPLC and following the analytical procedure. The concentrations of the samples were then generated from the standard curve.

Pharmacokinetic Analysis

The pharmacokinetic parameters were obtained using WinNonlin® standard non-compartmental programme. A residual method was used to obtain lag-time using serum time curves as base data. The absorption rate constant (k_{ab}) and the elimination rate constant (k_{el}) were calculated from the absorption half-life ($t_{1/2ab}$) and elimination half-life ($t_{1/2el}$) respectively.

Data Analysis

The results were expressed in terms of mean ± S.E.M., and were analysed for statistical significance by Student's t-test for paired data, with P<0.05 being considered as significant in all cases.

Results And Discussions

Following oral administration of 5mg/kg chloroquine and 7.5mg/kg metronidazole the mean serum concentrations obtained in rats are shown in Table 1 and Table2. The peak serum concentration ranged from 5.61 to 0.47µg/ml in CQ and ranged from 3.01 to 0.77µg/ml in MET. The metabolite, CQM was detected after 0.5h and peak concentration was 0.96 µg\ml. The mean serum concentrations of chloroquine (CQ) (A) & desethylchloroqune (CQM) (AB) versus time plots following oral administration of 5mg/kg of chloroquine and mean serum concentration-time curve of metronidazole in rats following oral administration of 7.5mg/kg metronidazole are shown on Figures 1 and 2 respectively. Some derived pharmacokinetics parameters following the administration of CQ and MET are shown in table 3. The absorption half lives for CQ and MET were 4.05 ± 1.30 and 1.79 ± 0.08 h respectively. The elimination half lives were 10.05± 3.01, 13.44±3.07and 4.05 ± 0.46 h for CQ, CQM and MET respectively. The values were comparable with the works of other authors using humans. The data therefore justified the use of animal model in determining pharmacokinetic parameters of drugs.

This will be employed in drug design to minimize undesirable pharmacokinetics properties and toxicity which may result from formation of reactive metabolites. The serum concentrations of CQ estimated was 20% of the value determined in humans while the pharmacokinetic parameters were 25% of the human profiles determined by Dua *et al.*,(2002) and Frisk-Holmberg *et al.*,(1984).

The serum MET concentrations determined in the rats were 20% of the average levels estimated in humans following single oral dose. The pharmacokinetics parameters determined were also 20% of the profile in humans estimated by other researchers (Davies, 1967; Taylor *et al.*,1970; Welling and Munro, 1972; Ralph *et al.*,1974; Usman *et al.*,2007). The results therefore present a correlation of 1:5 for the values in rat and man serum concentrations and pharmacokinetics except CQ pharmacokinetics which is ratio1:4 respectively. Thus the values of pharmacokinetics from animal model can be extrapolated for the determination of pharmacokinetics in clinical trials. This study assures the quality of the brands of the drugs and encouraged the use of animal model in determining pharmacokinetic properties especially in drug design.

Table 1: Serum concentrations of chloroquine (CQ, A) and desethylchloroquine (CQM, AB) following oral administration of 5mg/kg of chloroquine

Time (h)	Serum Concentration of (µg/ml) ± SEM	
	A	AB
0	-	-
0.5	2.5001±0.598	-
1	5.6184±1.477	0.5111±0.123
2	2.3349±0.351	1.0086±0.502
4	1.7000±0.850	0.8659±0.268
8	1.5195±0.4199	0.6444±0.167
12	0.8991±0.581	0.2324±0.085
24	0.4712±0.302	0.3689±0.247

SEM ± Standard Error of Mean

Table 2: Serum concentrations of metronidazole following oral administration of 7.5mg/kg of metronidazole (MET, E) in rats

	Serum Concentration of (µg/ml) ± SEM
Time (h)	MET E
0	-
0.5	2.4459±0.536
1	3.0140±0.364
2	2.4251±0.469
3	1.9420±0.360
4	1.5671±0.326
6	1.0985±0.100
8	0.7696±0.365

SEM ± Standard Error of Mean

Table 3: Derived pharmacokinetics parameters of chloroquine (CQ) and desethylchloroquine (CQM) in rats following oral administration of 5mg/kg of chloroquine and of 7.5 mg\kg metronidazole (MET).

Mean parameters ± SEM	CQ	CQM	MET
Absorption half-life $t_{1/2a}$ (h)	4.0492±1.30	-	1.7572±0.20
Absorption rate constant Ka (h^{-1})	0.1971±0.04	-	0.3987±0.05
Maximum serum concentration Cmax (µg/ml)	5.6980±1.41	1.1272±0.38	3.1257±0.30
Time to peak concentration tmax (h)	1±0.0	4.8±3.03	0.8±0.27
Area under the curve.$AUC_{0\rightarrow 24}$ µg.h/ml)	30.6647±4.35	9.8147±1.49	13.0893±1.11
Volume of distribution Vd (L)	1.3311±0.26	6.7194±1.13	2.3895±0.28
Clearance Cl_T (L/h)	0.1641±0.01	0.3543±0.07	0.4272±0.05
Elimination half-life $t_{1/2\beta}$ (h)	10.0513±3.01	13.4433±3.07	4.0474±0.46
Elimination rate constant k_β (h^{-1})	0.0996±0.06	0.0533±0.01	0.1731±0.02

SEM = Standard Error of Mean

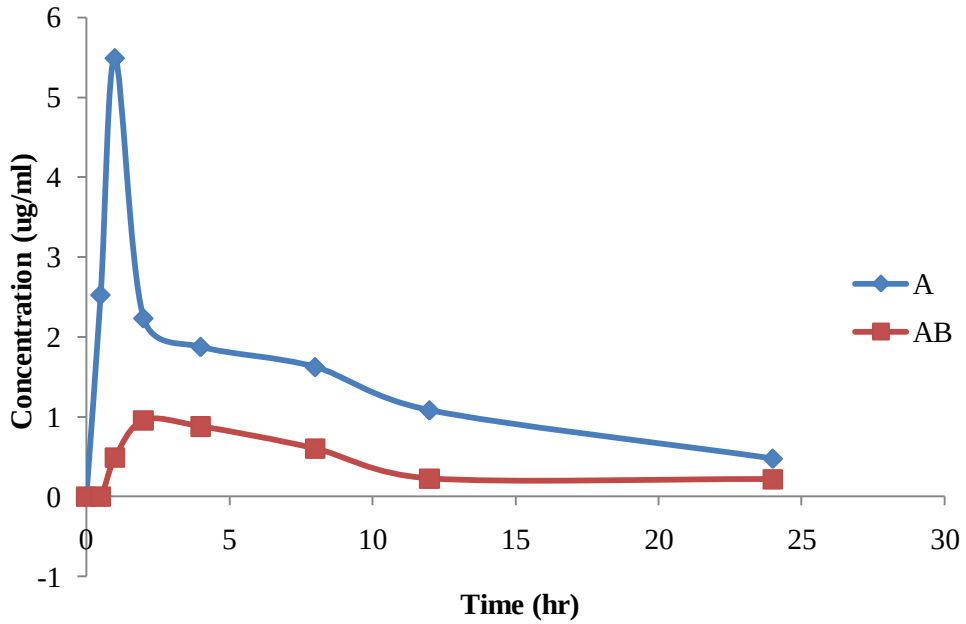

Fig. 1: Mean serum concentration – time curves of chloroquine (CQ) (A) and desethylchloroqune (CQM) (AB) in rats following oral administration of 5mg/kg of chloroquine.

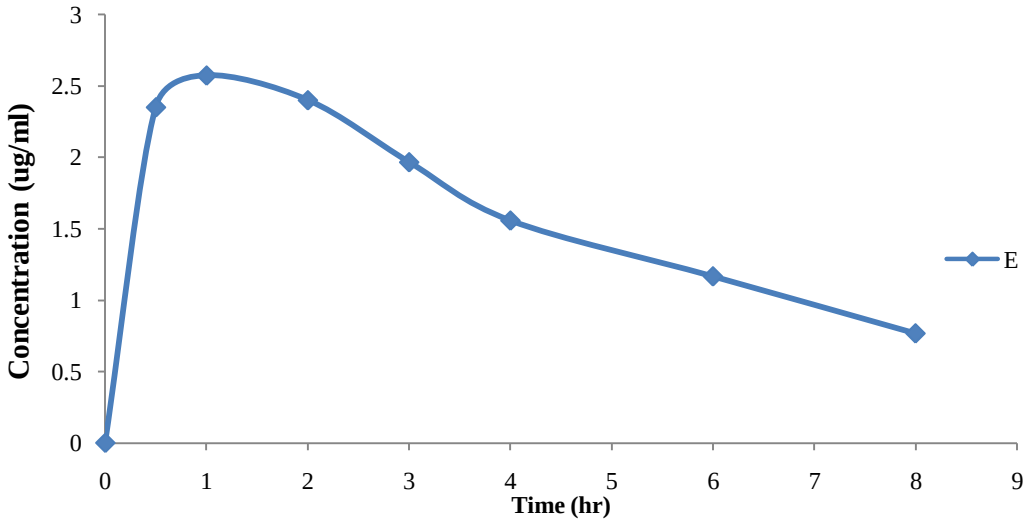

Fig. 2: Mean serum concentration-time curve of metronidazole in rats following oral administration of 7.5mg/kg metronidazole.

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How to cite this article:

Kudirat Bola Mustapha, Moji T Bakara-Odunola, Garba Magaji, Obiageri O. Obodozie-Ofoegbu, David D Akumka., Pharmacokinetics of Chloroquine and Metronidazole in Rats. J App Pharm Sci, 2015; 5 (08): 090-094.