

Full Length Research Paper

The Influence of Chloroquine on Chlorpropamide Pharmacokinetics in Healthy Volunteers

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The effect of chloroquine on the pharmacokinetics of chlorpropamide was investigated in human volunteers. The study was carried out by administering chlorpropamide alone on healthy volunteers, and then chlorpropamide was co-administered with chloroquine. High performance liquid chromatography (HPLC) was used for the analysis of the plasma levels of chlorpropamide. The solvent used was acetonitrile/water (6:4) at pH 6.7. Ultrasphere ODS column, 4.6 × 25 cm, USA was used. The result from the chlorpropamide plasma level analysis shows that the absorption half-life ($t_{1/2a}$) and the time to maximum peak concentration (t_{max}) increased by 10% ($P < 0.04$) and 20% ($P < 0.01$), respectively. The absorption rate constant (K_a) and the maximum concentration (C_{max}) decreased by 30 and 16% ($P < 0.02$), respectively. The area under the curve (AUC_{0-168}) decreased significantly by 50% ($P < 0.03$). The plasma clearance of chlorpropamide increased significantly by 86% ($P < 0.01$). The volume of distribution (V_d) and the elimination half life ($t_{1/2el}$) decreased by 10% ($p < 0.03$) and 47% ($P < 0.01$), respectively, while the elimination constant (k_{el}) increased by 100% ($P < 0.02$). Co-administration of chlorpropamide with chloroquine significantly ($P < 0.05$) impaired the absorption and elimination of chlorpropamide.

Key words: Pharmacokinetics, chlorpropamide, chloroquine, high performance liquid chromatography, half-life, absorption rate constant, elimination constant.

INTRODUCTION

Pharmacokinetics is the study of the time course of drug movement in the body during absorption, distribution, metabolism and elimination (excretion and bio-transformation) (Sadée and Beelen, 1990; Coker, 2001; Evans et al., 2000).

Chloroquine is a 4-aminoquinoline, which is used in the treatment of malaria. It has been used as the most common antimalarial drug in all developing countries for many years. Chloroquine is also used for the treatment of extra intestinal amoebiasis, juvenile rheumatoid arthritis, amoebic hepatitis and abscess, discoid and systemic lupus (Rollo, 1980; Neuvonen et al., 2004).

Chlorpropamide on the other hand, is a member of the

first-generation sulphonylureas, which is used in the treatment of Type 2 diabetes mellitus (Gillian, 2001; Ferner and Neil, 2000). The co-administration of chlorpropamide with some other drugs was shown to affect its pharmacokinetic parameters (Macris and Levy, 2005). Fabre et al. (2000) reported of the half-life of chlorpropamide being doubled when it was administered together with chloramphenicol. According to the study carried out by Pronskey (2007), when magnesium supplements were administered together with chlorpropamide, the absorption of chlorpropamide was increased. Ilomuanya et al. (2011) carried out an *in vitro* study where metronidazole and activated charcoal were used in the treatment of diarrhea caused by *Escherichia coli*. Samuel et al. (2009) also carried out a study on the pharmacodynamic effect of piperazine citrate on the blood pressure of anaesthetized cat. An assay of the pharmacokinetics of cyclooxygenase inhibitor etoricoxib

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in rats was carried out using a stability indicating high performance liquid chromatography (Mahasen et al., 2009).

Some drugs tend to produce excess of sugar in the blood (hyperglycemia), when such drugs are administered with chlorpropamide to a patient, the patient should be closely observed for loss of control. The patient should be observed closely for hypoglycemia when any of such drugs are withdrawn (RxList, 2006; Benet et al., 2003).

Chloroquine as an antimalarial drug may be co-administered together with chlorpropamide in diabetic patients. This study was designed to evaluate the effect of chloroquine on the pharmacokinetics of chlorpropamide when taken together orally in tablet form.

MATERIALS AND METHODS

All the chemicals and reagents used for this work were of analytical grade. Chloroquine tablets and reference standard powder were obtained from May and Baker, Nigeria, Ltd.

Chlorpropamide tablets and reference standard powder were obtained from Neimeth International Pharmaceuticals, Plc, Nigeria. Tolbutamide, which was used as an internal standard (IS), was supplied by Alpharma, Ltd, UK.

Subjects

Six male adults human subjects of a height range of 1.40 to 1.95 m (mean 1.72 ± 0.04 m), a weight range of 65 to 80 kg (mean 69 ± 0.83 kg) and age range of 24 to 38 years (mean 29 ± 0.93 years) were used for the study.

Study protocol

The study was approved by the Ethical Committee in Research. The protocol of the study was explained to the volunteers and they were made to sign a consent letter. No drugs, alcohol or kolanut was allowed two weeks before the study. Healthy volunteers were used for the study. No subject used any medication before and during the course of the study.

Each volunteer was administered with 250 mg chlorpropamide tablet with 200 cm³ of water in a fasting state during the first study. Blood samples (5 cm³) were collected at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 144 and 168 h, using an indwelling cannula for earlier samplings. The volunteers were allowed to eat after 3 h of the study.

Plasma was obtained by centrifuging blood samples at a speed of 2000 g for 10 min, after allowing them to stand for some time. The plasma was transferred into some screw capped sample bottles and stored in a deep freezer for analysis.

The volunteers were administered with 250 mg chlorpropamide tablet and 4 tablets of 250 mg chloroquine each during the second study. Blood samples were collected and treated as earlier stated.

Analyses

A high performance liquid chromatographic (HPLC) method (Uzama et al., 2008) was adopted for the determination of the chlorpropamide levels in the plasma samples. The HPLC equipment consisted of a CECIL model CE1200 with CE1100 liquid

chromatography pump CECIL 1100 series. CE1200 high performance variable wavelength detector, model CECIL 1100 series. Ultrasphere ODS column, 4.6 × 25cm, USA was used for the analysis. The solvent system consisted of acetonitrile/water (6:4) at pH 6.7 and was delivered at a flow rate of 1.0 cm³/min.

Plasma (1.0 cm³), tolbutamide (1.0 cm³) and 50 µl CuSO₄ (5%) were mixed together with acetonitrile (2.5 cm³) on an auto mixer for 30 s, centrifuged at 2000 g for 3 min. 25 µl of the clear supernatant upper layer was injected into the column.

Calibration curve and validation of the method

The calibration curve was prepared by spiking blank plasma with a standard solution of chlorpropamide (1 mg/cm³) to obtain a concentration range of 2 to 40 µg/cm³. Following the analytical procedure, the samples were analysed using HPLC. The standard curve was generated of peak height ratios against the concentration of chlorpropamide. The concentrations of the samples were generated from the calibration curve.

Pharmacokinetic analysis

The pharmacokinetic parameters were obtained by using Winnonlin standard non-compartmental programme (Winter, 2006). Area under curve (AUC) was calculated by trapezoidal rule. A residual method was used to obtain lag-time.

Data analysis

The results were expressed as mean ± standard error of the mean (SEM), and were analyzed for statistical significance by student's t-test for paired data. The differences among the data were compared by analysis of variance (ANOVA) and the significant points were analysed using the least square difference (LSD) where applicable.

RESULTS

The solvent system used for the analysis of chlorpropamide in plasma using HPLC was acetonitrile/water (60:40). The calibration curve (Figure 1) for chlorpropamide determination in plasma was linear with a correlation coefficient of 0.999.

The limit of detection was 1.45×10^{-4} µg/cm³. The coefficient of variation (CV) values that established the precision of the method of analysis determined for 6 samples at 5 concentration level for within-day and between-day assay were 1.66 and 2.24%, respectively. The results are shown in Table 1.

The chromatographic conditions for the analysis were flow rate of 1.0 cm³/min, pressure of 410 bars and pH = 6.7. The retention time of chlorpropamide was 2.7 min while that of tolbutamide was 4.2 min. The chromatograms of chlorpropamide and tolbutamide when extracted from a test sample obtained from human subjects following oral administration of chlorpropamide are as shown in Figure 2.

The mean pharmacokinetic parameters of chlorpropamide in the absence (A) and in the presence (B) of chloroquine are as shown in Table 2.

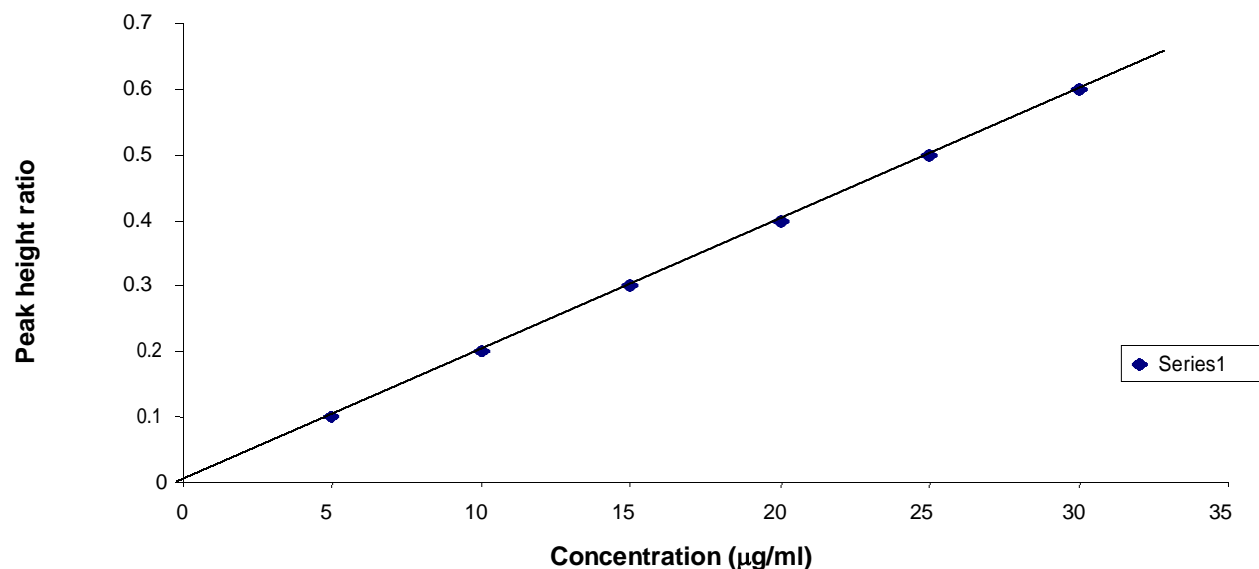


Figure 1. Calibration curve for chlorpropamide in plasma using HPLC.

Table 1. Precision of the method of analysis using HPLC method for within-day and day-to-day.

	Concentration (µg/ml)	C.V (%)	n
Within-day run	5	2.5	6
	10	2.0	6
	20	1.5	6
	30	1.2	6
	40	1.0	6
Day-to-day run	5	2.8	6
	10	2.5	6
	20	2.2	6
	30	1.9	6
	40	1.6	6

DISCUSSION

Pharmacokinetics of chlorpropamide when co-administered with chloroquine

When chlorpropamide was given alone (A), peak concentration (C_{max}), area under curve (AUC), the apparent volume of distribution (V_d) and the half-life of absorption ($t_{1/2a}$) and other pharmacokinetic parameters were in agreement with the reports of previous studies (Odunola et al., 1994). Following concurrent administration of chlorpropamide and chloroquine (B) in the crossover study (Table 2), the absorption rate constant (k_a) decreased significantly ($P < 0.02$) by 30%. The absorption half-life ($t_{1/2a}$) increased significantly ($P < 0.04$) by 10%. The maximum concentration (C_{max}) and the area under curve (AUC) of chlorpropamide were

significantly reduced ($P < 0.02$ and $P < 0.03$), respectively. 16% decrease occurred in the C_{max} while the AUC decreased by 50%. There was a significant increase ($P < 0.01$) in the time for maximum concentration (t_{max}) by 20%.

The significant reduction in the AUC could be due to the reduction in k_a , since there is a limit to which the k_a can be reduced without affecting the AUC. The delayed k_a could also have led to delayed t_{max} and C_{max} .

The significant increase in $t_{1/2a}$ and t_{max} indicated impaired absorption of chlorpropamide by chloroquine. The decrease in the C_{max} of chlorpropamide may be attributed to the interaction via liver cytochromes 3A4, 2C8, 2C9 or CYP450 isoenzymes. Chlorpropamide being a sulphonylurea is metabolized by CYP450 isoenzyme. Specific family and sub-family of the CYP450 isoenzyme of chlorpropamide has not been elucidated (Oette et al.,

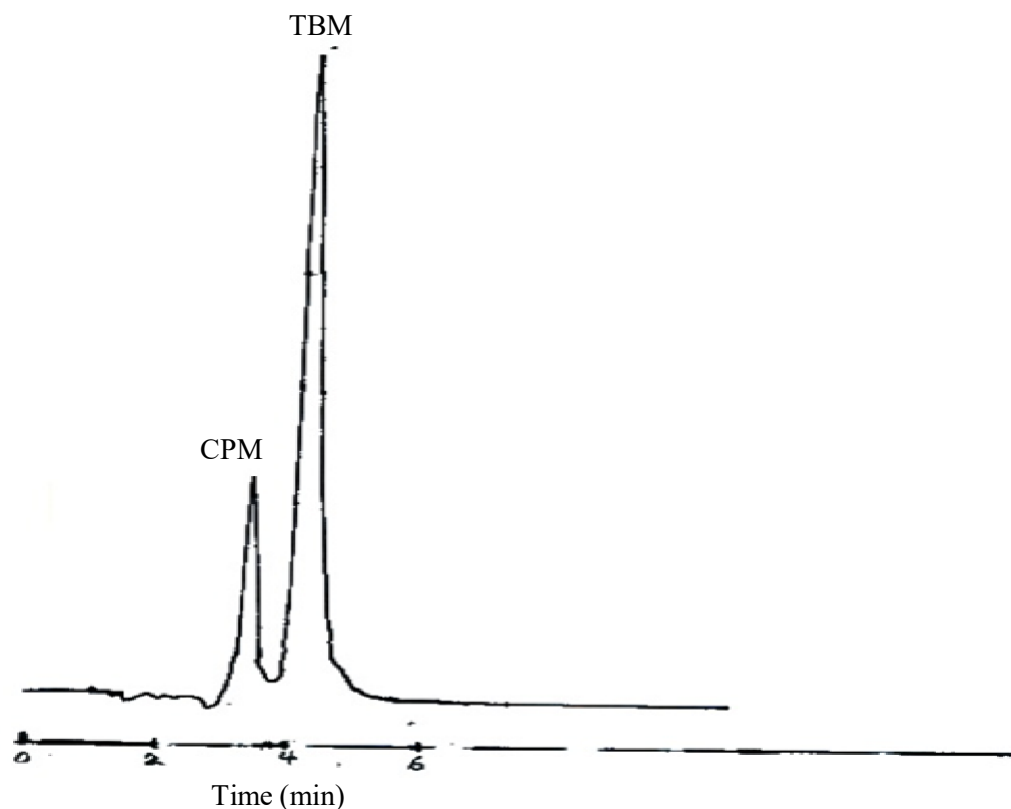


Figure 2. High performance liquid chromatograms of an extract of test sample obtained from human subjects 4 h following oral administration of chlorpropamide.

Table 2. Mean (\pm SEM) pharmacokinetic parameters of chlorpropamide following oral administration of chlorpropamide (A) with chloroquine (B) in six volunteers.

Mean Pharmacokinetic parameter (\pm SEM)	A	B	Student's t-test for paired data
Lag time (h)	0.41 \pm 0.22	0.53 \pm 0.13	P < 0.05
Absorption half-life [$t_{1/2a}$ (h)]	0.168 \pm 0.05	0.174 \pm 0.01	P < 0.04
Absorption rate constant [k_a (h^{-1})]	5.80 \pm 1.37	4.12 \pm 0.33	P < 0.02
Maximum concentration [C_{max} (μ g/ml)]	27.75 \pm 4.57	23.33 \pm 2.37	P < 0.02
Time to peak concentration [T_{max} (h)]	4.00 \pm 0.00	4.67 \pm 0.42	P < 0.01
AU C_{0-168} [μ g/ml.h]	1616.05 \pm 403.63	906.29 \pm 157.49	P < 0.03
AU C_{0-00} [μ g/ml.h]	1661.30 \pm 414.93	917.17 \pm 159.38	P < 0.03
Clearance [Cl (1/h)]	1.23 \pm 0.15	2.29 \pm 0.17	P < 0.01
Volume of distribution [$V_d(1)$]	10.44 \pm 2.59	9.45 \pm 0.93	P < 0.03
Elimination half-life [$t_{1/2el}$ (h)]	5.61 \pm 0.69	2.97 \pm 0.41	P < 0.01
Elimination rate constant K_{el} (h)	0.133 \pm 0.01	0.267 \pm 0.05	P < 0.02

2005; Wilkinson, 2001). The presence of chloroquine may induce the liver enzyme activity including CYP450 isoenzyme system. Increased CYP450 enzyme activity might have increased the meta-bolism which may lead to increased plasma clearance (Clr) (P < 0.01) by 86% and reduced the elimination half-life ($t_{1/2el}$) (P < 0.01) by 47% of chlorpropamide.

The resultant effect as observed was a significant (P < 0.02) increase in the elimination rate constant (k_{el}) by 100%. Reduction in C_{max} and AUC, according to Miners et al. (1984) chloroquine induces the tubular secretion of chlorpropamide.

One drug can increase the excretion rate of another drug thereby lowering its therapeutic effectiveness.

Conversely, decreasing the excretion rate of a drug can increase its toxicity (Graham, 2004).

Conclusion

The absorption parameters k_a , $t_{1/2a}$, C_{max} , t_{max} and AUC of chlorpropamide were significantly altered when it was co-administered with chloroquine.

The elimination phase was also impaired by co-administering chlorpropamide with chloroquine. Conclusively, the co-administration of chloroquine with chlorpropamide impaired the absorption and the elimination of chlorpropamide in healthy human subjects. Effect of such significant interaction on absorption phase may lower therapeutic effectiveness, and the decreasing rate of elimination can increase the toxicity of drugs like chlorpropamide having long half-life.

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