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Effect of paracetamol on the plasma protein binding of quinine sulphate

Sunday A. Adelusi, Festus E. Aitokhuehi and Henry A. Okeri*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City. Nigeria.

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Abstract

The co-administration of antimalarial and antipyretic drugs is a common practice in the treatment of malaria. Paracetamol, which is a majorly used antipyretic drug, has proved useful in the management of some common feverish effects such as pains and headache associated with most antimalarial drugs. This study was done to investigate if the co-administration of quinine and paracetamol could have an effect on the protein binding of quinine. Exactly 5ml of plasma contained in a dialysis sack was placed into 20ml of varying concentrations (2 - 10µg/ml) of test sample at 37°C. 2ml of the dialysate was basified with 20%NaOH after 8 hours above equilibrium time of 5 – 7 hours, and then extracted with diethyl ether and 0.1M HCl. The absorbance of the resultant solutions was taken at the wavelength of 350nm. The results showed that the albumin concentration in plasma was 4.04 ± 0.03% and the time at which equilibrium was attained was between 5 and 7 hours. The degrees of binding of quinine to plasma proteins for the concentration between 2 – 10 µg/ml in the absence and presence of paracetamol were respectively, 75.5 – 62.7% and 64.7 – 56.0%. A statistically significant decrease ($P < 0.05$) was observed. From this study, paracetamol has been shown to decrease the protein binding of quinine to both plasma proteins and 4.04% albumin.

Keywords: Co-administration; Equilibrium dialysis; Concentration; Quinine; Paracetamol

INTRODUCTION

In many parts of malaria endemic areas and particularly the African region, one of the effective methods of preventing mortality and reducing the morbidity caused by the disease is through the use of antimalarial drugs (Olaniyi, 2005). Quinine is one of the naturally occurring antimalarial drugs for the management and treatment of severe malaria (Yakoub *et al*., 1995), with endo-erythrocytic schizonticidal activity on the *Plasmodium* parasite.

Quinine belongs to the aryl amino alcohol group of drugs. It is an extremely basic compound and is, therefore, always presented as a salt (Macomber and Sprinz, 1967). Various preparations of quinine exist. These include the hydrochloride, dihydrochloride, sulphate, bisulphate, and gluconate salts. Dihydrochloride and sulphate preparations of quinine are the most widely used. Quinine reaches peak concentrations within 1–3 hours after oral and parenteral administration (Pagola *et al*., 2000) and it is rapidly absorbed and highly protein bound

* Corresponding author. *E-mail*: hokeri1@yahoo.com *Tel*: +234 (0) 8060444600
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(Mihaly, 1987). Several pharmacokinetic characteristics of quinine differ according to the age of the subject and are also affected by malaria. The volume of distribution is less in young children than in adults, and the rate of elimination is slower in the elderly than in young adults. In patients with acute malaria, the volume of distribution is reduced and systemic clearance is slower than in healthy subjects and these changes are proportional to the severity of the disease. As a result, plasma quinine levels are higher in patients with malaria. The binding of quinine to plasma protein in patients under malaria condition tends to increase due to the increase in the level of the acute alpha-1-acid glycoprotein in plasma (White, 1987).

Antipyretic drugs are usually co-administered with antimalarial drugs during malaria treatment to help manage some of the common feverish conditions such as pains and headache. Since the pharmacokinetics of a drug depends on its physicochemical properties, it is important to investigate, through *in vitro* studies, the effect of paracetamol on the plasma protein binding of quinine when they are co-administered.

EXPERIMENTAL

Albumin concentration in plasma was determined by bromocresol green method as described by Doumas *et al.* (1971). The time at which quinine attained equilibrium was determined using the method described by Adelusi and Ogonor (1987). A concentration of 6 µg/ml of quinine was used and thereafter confirmed by using 2 µg/ml and 10 µg/ml.

Plasma protein binding was determined by equilibrium dialysis method as described by Adelusi and Ogonor, (1987). 5ml of plasma contained in a dialysis sack was placed into 20ml of varying concentrations (2 - 10µg/ml) of test sample at 37^{o}C. 2ml of the dialysate was basified with 20% NaOH after 8 hours above equilibrium time of 5 – 7 hours, and then extracted with diethyl ether and 0.1M HCl. The absorbance of the resultant solutions was taken at the wavelength of 350nm. Blank determination was made. The solution of the buffer became turbid after about 8 hours during the dialysis experiment and was suspected to be due to protein leakage from the sack. Therefore, the buffer solution was tested for albumin using the BCG method. The blank and the sample gave the same reading, which showed that the turbidity was not due to protein leakage.

To study the effect of paracetamol on the degree of protein binding of quinine, paracetamol was added with quinine to the buffer solution at a ratio of 5:3 (which is equivalent to the preparation of 1000mg of paracetamol and 600mg of the quinine which is usual dosage regimen in which they are co-administered), but using the same concentration range of sample (2 – 10 µg/ml). The above procedure was then carried out to determine the percentage of quinine bound to albumin in the presence of paracetamol. The percentage of test samples bound to plasma was calculated from the following relationship:

$$\% \text{ drug bound} = \{[D_a] - [D_f] / [D_a]\} \times 100\% \quad \ldots \text{eqn. 1}$$

$$\% \text{ drug bound} = \{[D_b] / [D_a]\} \times 100\% \quad \ldots \text{eqn. 2}$$

where $[D_b] = [D_a] - [D_f]$ and $[D_b]$ is the amount of drug bound to protein which is obtained by subtracting D_f (the concentration of free drug) from D_a (concentration of drug available for binding).

A control experiment was conducted, with the dialysis bag containing the buffer solution instead of the plasma. This helped to correct for the adsorption onto the dialysis sack (Klotz *et al.*, 1946). The percentage recovery was about 90 % of quinine added to the buffer.

RESULTS

The value of albumin determined in the human plasma was 4.04 ± 0.03% (mean ± S.E.M.). This was interpolated from the calibration curve in Figure 1. The time at which quinine attained equilibrium under the condition of this study was between 5 and 7

hours. A graph of quinine concentrations (µg/ml) against time (hr) is shown in Figure 2. The results showed that the degree of binding of quinine to plasma proteins for concentrations between 2–10 µg/ml was found to be 75.5 – 62.7 % whereas, in the presence of paracetamol, quinine had percentage binding to plasma proteins of 64.7 – 56.0 % (Table 1). The binding to 4.04 % albumin for quinine in the absence of paracetamol was 58.5 – 48.5 % while in the presence of paracetamol, it was 43.7 – 36.6 % bound to 4.04 % albumin (Table 2). A statistically significant decrease ($P < 0.05$) was observed in the binding of quinine when combined with paracetamol. The number of binding sites per molecule of protein for quinine was found to be 3 and this was obtained by the use of a Scatchard plot as shown in Figure 3.

DISCUSSION

The effect of paracetamol on the protein binding of quinine was investigated. The ratio of 5:3 (paracetamol : quinine) used, was derived from the fact that two tablets of paracetamol (equivalent to 1000 mg of paracetamol) are usually administered with two tablets of quinine (equivalent to 600 mg of quinine).

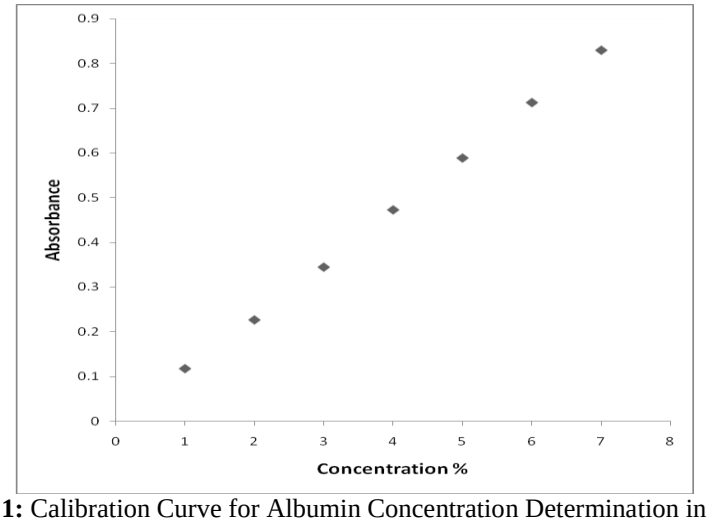

Figure 1: Calibration Curve for Albumin Concentration Determination in Plasma

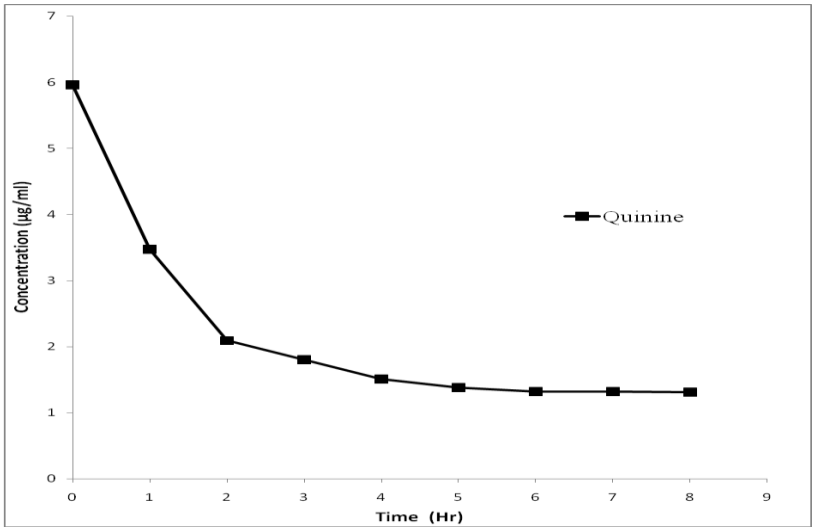

Figure 2: Determination of Equilibrium Time

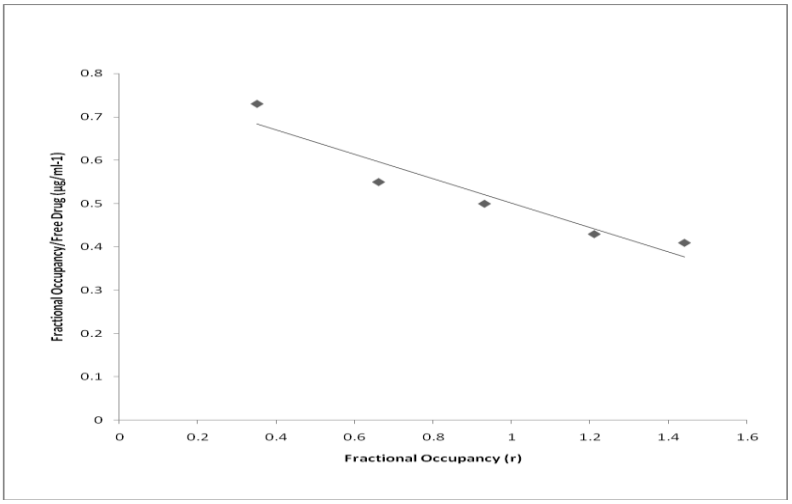

Figure 3: Number of Binding Site per Molecule of Protein for Quinine

Table 1: Degree of Binding of Quinine to plasma protein ± S.E.M

Concentration of Quinine (µg/ml)	Concentration of paracetamol (µg/ml)	Percentage Bound (%)
2	+	75.5 ± 0.71
2	-	64.7 ± 0.28
4	+	69.0 ± 0.43
4	-	61.9 ± 0.61
6	+	66.8 ± 0.09
6	-	58.2 ± 0.41
8	+	63.1 ± 0.15
8	-	56.9 ± 0.32
10	+	62.7 ± 0.25
10	-	55.7 ± 0.50

*Data indicate mean ± SEM *P < 0.05 (in comparison to the absence of paracetamol)

Table 2: Degree of Binding of Quinine to 4.04% Albumin ± S.E.M

Concentration of Quinine (µg/ml)	Concentration of paracetamol (µg/ml)	Percentage Bound (%)
2	+	52.9 ± 0.69
2	-	43.7 ± 0.29
4	+	48.6 ± 0.43
4	-	41.4 ± 0.38
6	+	45.8 ± 0.08
6	-	37.7 ± 0.14
8	+	43.3 ± 0.11
8	-	37.1 ± 0.27
10	+	42.5 ± 0.25
10	-	36.6 ± 0.33

*n = 4 *P < 0.05 (in comparison to the absence of paracetamol) + = presence of paracetamol
– = absence of paracetamol S.E.M = Standard Error of Mean

The result from this study of plasma protein binding was similar to that reported by Wanwimolruks and Denton, (1992) as 69 – 92.1 %, for quinine. The plasma protein binding of quinine in the presence of paracetamol was shown to have decreased significantly. This could possibly be explained by the presence of paracetamol,

which may competitively displace quinine from its original binding sites on plasma protein and may expose the antimalarial drug to faster metabolism. The decrease in the percentage binding of quinine to 4.04 % albumin when compared to that of plasma protein showed that albumin was not the only protein responsible for binding in plasma. Other proteins such as α_1-acid-glycoprotein and lipoproteins could also be involved in the binding of the drug. According to Gbotosho *et al.* (2008), the binding of drugs to plasma proteins, especially albumin and acute phase protein (α_1-acid-glycoprotein), is known to decrease antimalarial drug efficacy. From this study, it was observed that the co-administration of quinine with paracetamol probably increased the bioavailability of quinine. The clinical and therapeutic implication of this increase is that the antiparasitic effect of quinine may be increased, since it is well established by Jullien *et al*. (2010) that the effect of a drug is dependent on the unbound concentration of the drug at the site of action.

Conclusion. The results of this study shows that the protein binding of quinine to plasma proteins and 4.04% albumin decreased in the presence of paracetamol. The technique used in this study has proved advantageous in the area of availability, cost, usage, precision, and accuracy over other techniques that have been used in the determination of protein binding of the drug of study.

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