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CHARACTERIZATION OF CHLOROQUINE PLASMA PROTEIN BINDING IN MAN

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Chloroquine protein binding was determined by equilibrium dialysis of purified plasma proteins and plasma samples from 20 healthy subjects and 14 patients with rheumatoid arthritis. The mean binding was 61 ± 9% in plasma from healthy subjects (range 46–74%) and 64 ± 7% in plasma from rheumatoid arthritis patients (range 55–79%). Albumin and α_1-acid glycoprotein at physiological concentrations bound chloroquine to an approximately equal extent. Protein binding is unlikely to be an important determinant of chloroquine pharmacokinetics or response.

Introduction

We have investigated the binding of the basic drug chloroquine to α_1-acid glycoprotein, albumin and γ-globulin and have also studied its binding in plasma from healthy subjects and from patients with rheumatoid arthritis.

Methods

Plasma samples

Blood was obtained by venepuncture from 20 healthy adults aged 29–50 years (mean 33 years). The blood was collected into Venoject heparin tubes and the plasma was separated and stored at −20°C. A large volume of plasma was obtained from one volunteer and stored in aliquots to assess inter-assay variation and the effect of storage. Plasma was also obtained from 14 patients aged 14–74 years (mean 49 years) with classical or definite rheumatoid arthritis. Plasma albumin and α_1-acid glycoprotein concentrations were available for 12 of these patients. Most patients were taking several drugs, most commonly chloroquine, salicylates and allopurinol.

Materials

[^{14}C]-chloroquine with a specific activity of 30 mCi/mmol was obtained from New England Nuclear Corporation (Boston Mass.). It was purified by thin layer chromatography to a radiochemical purity of > 99%. Human serum albumin (HSA) and γ-globulin were obtained from Hoechst. The α_1-acid glycoprotein was obtained from the Sigma Chemical Company.

Equilibrium dialysis

Equilibrium dialysis was carried out by a modification of the method of Ehrnebo *et al.* (1971). The modifications included the use of Teflon dialysis chambers and standard Technicon (Type A) membranes. Chloroquine protein binding was determined by dialysing 0.5 ml of buffer containing [^{14}C]-chloroquine and unlabelled chloroquine against 0.5 ml of protein solution or plasma. The dialysis buffer consisted of: 4g $Na_2HPO_4 \cdot 2H_20$, 0.775g $NaH_2PO_4 \cdot 1H_20$ and 5.58g NaCl in 1 l distilled H_2O. The pH was 7.40 and ionic strength 0.168. The radioactivity in 0.1 ml aliquots of buffer and protein was measured by liquid scintillation counting. Initial experiments showed that equilibium was reached within 2 h and a dialysis time of 3 h was routinely used. Unless otherwise specified the initial chloroquine concentration in the buffer was 100 ng/ml.

The percentage of bound drug was determined from the relationship

$$\% \text{ bound} = \frac{C_t - C_f}{C_t} \times 100$$

where C_t is the chloroquine concentration in the plasma or protein solution after dialysis and C_f is the chloroquine concentration in the buffer after dialysis.

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All binding experiments were carried out in duplicate or occasionally in quadruplicate at 21°C or at 37°C as indicated. Statistical analyses were performed with Student's *t*-test of independent variables. Linear regression analysis was done if applicable.

Results

The range of binding for the reference plasma was 62–68% in 6 different experiments (C.V. 4.5%). There was no systematic change in percent bound with storage of plasma at −20°C for up to 6 weeks. There was a small decrease in chloroquine protein binding when the experiment was done at 37°C as compared to 21°C ($P < 0.001$, $n = 18$). As the decrease was small (mean 3%) all further experiments were carried out at 21°C (room temperature). There was no systematic difference between chloroquine protein binding in plasma (mean 61%) and serum (mean 57%) in samples obtained at the same time from three individuals. Chloroquine protein binding was measured over a range of 30 to 2000 ng/ml in the plasma of three individuals (Figure 1). No obvious concentration dependency was seen. Chloroquine binding in plasma ranged from 46% to 74% (mean 61%) in healthy subjects and from 55% to 79% (mean 64%) in rheumatoid patients ($P > 0.1$).

At a physiological concentration of purified HSA (40 g/l) chloroquine was 31% bound. Chloroquine was also weakly bound to α_1-acid glycoprotein, the binding being 18% at an α_1-acid glycoprotein concentration of 0.7 g/l. The binding in a mixture of physiological concentrations of HSA (40 g/l) and α_1-acid glycoprotein (0.7 g/l) was 40% (Table 1). At a physiological concentration of γ-globulin (20 g/l) chloroquine binding was 8%.

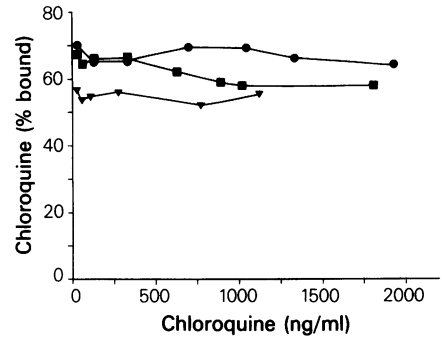

Figure 1 Plasma protein binding (%) of chloroquine (duplicate) in the range of 30 to 2000 ng/ml in samples from three healthy subjects (● ■ ▼).

Discussion

Many basic drugs show predominant binding to α_1-acid glycoprotein and their binding is affected by changes in the concentration of this acute phase reactant. This has been shown clearly for alprenolol and imipramine (Piafsky & Borga, 1977).

The high volume of distribution of chloroquine (Gustafsson *et al.*, 1983) indicates extensive tissue binding but it is weakly bound in plasma, 46–74% in healthy individuals. This is in agreement with a previous report by Buchanan & van der Walt (1972).

Our present results with purified protein fractions indicate weak binding to α_1-acid glycoprotein and to albumin. A mixture of these protein fractions at physiological concentrations did not give as high binding as plasma indicating some minor binding to other proteins, probably lipoproteins, as well. Due to solubility limits for chloroquine and weak binding, a formal analysis for albumin and α_1-acid glycoprotein was not possible.

Table 1 The binding of chloroquine to plasma from healthy volunteers ($n = 20$), from patients with rheumatoid arthritis ($n = 14$) and *in vitro* binding to purified protein fractions (duplicate or quadruplicate).

		Chloroquine (% bound)
Healthy subjects		61 ± 9 (range 46–74)
Rheumatoid arthritis subjects		64 ± 7 (range 55–79)
Human serum albumin (HSA)	(40 g/l)	31
α_1-acid glycoprotein (AAG)	0.3 g/l	3
	0.5 g/l	16
	0.7 g/l	18
	1.0 g/l	26
γ-globulin (20 g/l)		9
HSA (40 g/l + AAG (0.7 g/l)		40

There was a weak correlation between chloroquine binding ratio and concentration of α_1-acid glycoprotein ($r = 0.56$, $P < 0.05$) but not for albumin ($r = 0.43$, $P > 0.1$) in plasma from patients with rheumatoid arthritis. The moderate degree of binding and the high volume of distribution make differences in protein binding unimportant for the disposition of the drug and for the clinical evaluation of total plasma concentrations.

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