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PROTEIN BINDING OF CHLOROQUINE IN THE PRESENCE OF ASPIRIN

The pharmacokinetics of chloroquine are characterised by extensive tissue binding and very slow elimination from the body. In the case of the liver, spleen, kidney or lung, a tissue/plasma concentration ratio of over 500 can be obtained after a single dose of 300 mg chloroquine base in man. This fact, together with a plasma half-life of over 5 days in man (McChesney McAuliff, 1961; McChesney *et al.*, 1962; McChesney *et al.*, 1967) suggests that chloroquine might be extensively bound to some blood or tissue fraction. The few previous studies on the binding of chloroquine to plasma proteins have shown that it is, in fact, less than 50% bound (Parker & Irvin, 1952; Buchanan & Van der Walt, 1977). The studies of Buchanan & Van der Walt (1977) also showed that in kwashiorkor serum in which there is lower albumin than normal, more chloroquine was bound, but the binding occurred mainly at the gamma-globulin fraction. Aspirin is usually administered with chloroquine as an antipyretic and analgesic during treatment of malaria. Since aspirin is highly bound to plasma albumin (Aarons *et al.*, 1980), the resultant reduction in effective protein binding capacity might produce the same effect as that of the reduced plasma albumin of kwashiorkor which would have implications for efficacy and safety of dosage. It was to examine this hypothesis that we decided to determine the binding of chloroquine to normal plasma and to bovine serum albumin *in vitro*, in the presence and absence of aspirin.

Protein binding was determined by equilibrium dialysis using dialysis sacks (Sigma). Plasma was obtained from the hospital blood bank. It contained no detectable chloroquine and had an albumin content of 4.01 g/100 ml. Phosphate buffer pH. 7.4 (10 ml) containing 2, 4, 6 or 8 μg/ml chloroquine was put into 25 ml Quickfit tube. Plasma (5 ml) was put into the dialysis sack, tied at both ends and immersed in the buffer solution. The tubes were then stoppered and placed on an electrically driven roller. Dialysis was allowed to proceed at room temperature (25°C) for 16 h, preliminary tests having shown that equilibrium was reached in 12 h. The above procedure was repeated using 4% bovine serum albumin in the dialysis sack in place of plasma. We demonstrated, in preliminary experiments that the sacks did not leak protein under the experimental conditions. To determine the effect of aspirin on the degree of binding of chloroquine, acetylsalicylic acid and chloroquine were added to the buffer solution in the Quickfit tube in the ratio of 1:1 by weight. Chloroquine was estimated by a modification (Adelusi & Salako, 1980) of the fluorimetric method of Rubin *et al.* (1965) and correction was made for adsorption to the sack in calculating the percentage of protein-bound drug (Klotz *et al.*, 1946).

The results are summarised in Table 1. In both plasma and bovine serum albumin (BSA), the presence of aspirin did not significantly alter the percentage chloroquine binding. At all concentrations the percentage of bound chloroquine was significantly higher in plasma than in BSA. Possible dependence of binding in plasma or BSA on chloroquine concentration was tested for using one way analysis of variance. This showed that at the 0.05 level of significance the percentages of bound chloroquine in plasma or in BSA were not significantly different with the four chloroquine concentrations used in the study.

The results showed that aspirin did not affect the protein binding of chloroquine. The lower binding to BSA is in keeping with the earlier suggestion of Buchanan & Van Der Walt (1977) that chloroquine is bound not only to albumin but also to non-albumin

Table 1 Percentage binding of chloroquine to plasma protein and 4% bovine serum albumin (BSA) in the presence and absence of aspirin. Values are given as mean ± s.e. mean of 4 determinations.

Concentration of chloroquine (μg/ml)	*Concentration of aspirin (μg/ml)*	*Bound drug (%)* *Plasma*	*4% BSA*
2	–	46.8 ± 0.8	30.5 ± 0.7
2	2	46.2 ± 0.3	29.0 ± 0.5
4	–	44.5 ± 0.6	25.1 ± 0.4
4	4	42.6 ± 0.8	25.6 ± 0.7
6	–	43.1 ± 0.3	24.2 ± 0.4
6	6	42.3 ± 1.0	24.3 ± 0.3
8	–	43.1 ± 0.4	24.3 ± 0.4
8	8	42.7 ± 0.5	24.0 ± 0.5

fraction of plasma. Other observations (Adelusi & Salako, unpublished observations) have also shown that the aspirin did not affect the rate of absorption or the plasma level of chloroquine in children, and with the present result it is clear that adverse interaction between aspirin and chloroquine is unlikely to affect the total or free plasma concentration of chloroquine in man.

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FREE SODIUM VALPROATE MONITORING

Plasma anticonvulsant monitoring has contributed significantly to the management of epileptic patients. In most instances, only total drug concentration is measured, both for technical reasons and because it is assumed that the total value reflects the free (unbound—pharmacologically active) drug concentration. Considerable effort has been expended in attempting to define the therapeutic range of serum sodium valproate. Results to date have been disappointing, for although a range of 300–700 μmol/l has been proposed (Schobben *et al.*, 1975), there is little relationship between serum drug concentration and seizure control. Evidence exists that the free serum sodium valproate fraction shows some considerable variation during a single dosing interval at steady state (Bowdle *et al.*, 1980) and that there is marked intersubject variation in the free:total concentration ratio (Patel *et al.*, 1980). Due to the poor