PROTRIN BINDING OF DRUGS IN MALNUTRITION AND PARASITIC INFECTION

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SUMMARY

Plasma protein concentrations and protein binding of phenytoin and of propranolol were measured in Nigerian subjects with protein-energy malnutrition (PEM) and with 2 parasitic diseases, namely malaria and Guinea worm. Comparisons were made with Israeli blood bank plasmas. Although plasma albumin concentrations did not show large differences between the groups, plasma α₁ acid glycoprotein (AAG) levels were about 3-fold higher in all Nigerian groups compared with Israeli controls. Mean plasma prealbumin was lower in one of the Nigerian PEM groups and in malaria patients compared with Israeli controls, but significantly higher in Guinea worm-inflicted patients. The binding of propranolol was significantly higher in all groups of Nigerian patients, compared with Israeli plasmas. Surprisingly, phenytoin binding was also higher in Nigerian PEM and parasite-inflicted patients than in Israeli plasmas. It is concluded that PEM and parasitic infection exert substantial influences on plasma protein concentrations and result in significantly higher plasma protein binding of both propranolol and phenytoin. Such alterations in the distribution of drugs may influence other pharmacokinetic parameters and necessitate changes in dosing recommendations for large patient populations in the developing world.

KEY WORDS

protein-energy malnutrition; parasitic disease; drug protein binding; propranolol; phenytoin; α₁ acid glycoprotein.
It is estimated that some 100 million children, mainly in the developing world, suffer from significant malnutrition. Even larger populations, comprising a very significant fraction of the world population, are inflicted with severe parasitic diseases. Moreover, malnutrition and parasitosis often occur in association, because malnutrition predisposes to infection and, vice versa, severely ill patients are wasted (Isliker and Schurch 1981; Poskitt 1972).

The effects of many drugs, as well as their adverse reactions, depend to a large extent on the interaction of these drugs with the host. Among the variables affecting the distribution and fate of drugs, protein binding is a major contributor. Certain drugs are very avidly bound to plasma proteins, leaving only a small fraction as free, and therefore active, moiety. The extent of plasma protein binding exerts a substantial effect on the drug's volume of distribution, and may have variable influences on the clearance of drugs and their half lives. The major binding protein in plasma is albumin, which has a remarkable capacity for many drugs, mainly acidic in nature. In recent years more interest has been focused on α₁ acid glycoprotein (AAG) as a drug binding protein (Piafsky and Borga 1977; Paxton 1983). AAG has been shown to possess a high-affinity binding site for basic drugs (Muller and Stillbauer 1983), although some acidic drugs have also been observed to bind to this protein (Urlen et al 1982).

The concentrations of AAG and other acute phase proteins are
usually elevated in protein-energy malnutrition (PEM) (Schelp et al 1978; Patwardhan et al 1971). On the other hand, plasma albumin and to a larger extent prealbumin concentrations are characteristically low and correlate with the degree of malnutrition (Ingenbleek et al 1975; Ogunshina and Hussain 1980). Likewise, in patients inflicted with parasitic diseases the plasma protein pattern may undergo dramatic changes. Plasma albumin concentrations may be markedly reduced, while immune globulin concentrations are elevated (Sadun et al 1966). Also, acute phase proteins may display marked augmentation in patients during parasitic infection (Friedman 1983; Murphy et al 1972; Stadnyk and Gauldie, 1991). Such alterations in the concentrations of drug binding proteins are expected to affect actual protein binding parameters. Unfortunately, few studies have been reported on the protein binding of drugs in the undernourished or in patients with parasitic disease, which is the subject of the present investigation.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board of the Soroka Medical Center, in accordance with the Helsinki Declaration. Blood samples were obtained from a total of 129 Nigerian subjects. Ages ranged from 2 months to 65 years, with a median of 4 years; weights ranged from 3.5 to 100 kg, median 10.2 kg. Fifty eight subjects had malaria and 13 were inflicted with Guinea worm (Dracunculus medinensis). All others were assigned a percentile according to their age and weight (Heimendinger 1964a;
Although these tables may not apply to Nigerian standards, we used them as a relative scale. Forty seven subjects were in a weight percentile lower than 10% for their age and were arbitrarily designated as PEM I. Eleven who were in a percentile higher than 10% and were judged clinically well were arbitrarily designated PEM II. The relevant anthropometric data for each group are summarized in Table 1.

Blood was collected by venepuncture and transferred into EDTA-containing plastic test tubes. Plasmas were separated within several hours of collection by centrifugation at 2000 RPM for 10 min, transferred into glass test tubes, sealed and frozen at -20°C. Plasmas were then shipped with dry ice to the laboratory in Beer-Sheva. Eleven Israeli blood bank (BB) plasmas were used as reference throughout the study.

Albumin concentrations were analyzed spectrophotometrically with the bromcresol green reagent. AAG and prealbumin concentrations were measured by rocket immunoelectrophoresis according to Laurell (1977).

Plasma protein binding was assessed by equilibrium dialysis. Plasma (0.5 ml) was dialysed against 5.5 ml distilled water containing labeled phenytoin or propranolol at 40°C with continuous stirring. Equilibrium was achieved within 24 hours, at which time aliquots of the plasma and water were prepared for scintillation counting. The free fraction of the drug was calculated as the ratio of free drug concentration (in the water)
and total (in the plasma) multiplied x100.

Materials that were used: dialysis tubing (Thomas, Philadelphia, PA); diphenylhydantoin 5,5-[phenyl-4-\(^3\)H(N)], specific activity 47.2 Ci/mmol (New England Nuclear, Boston, MA); DL-[4-\(^3\)H] propranolol HCl, specific activity 20 Ci/mmol (Amersham, UK); anti human prealbumin and anti human AAG (Bio-Yeda, Rehovot, Israel). All reagents were of analytical grade.

As no assumptions could be made on the distribution of the populations in the different groups, non-parametric statistics were used. Comparisons between groups were made using a two-tailed Mann-Whitney U test. Single and multiple correlations were carried out by linear least squares regression. The significance of multiple correlations was examined by F-test, whereas in simple correlations the two-tailed Student's t-test was applied. A 5% level of significance was applied.

RESULTS

Although plasma albumin concentrations did not show important differences among the groups, the malaria group had somewhat higher levels than those observed in the BB samples (p<0.05, Figure 1). On the other hand, prealbumin (Figure 2) and AAG (Figure 3) levels showed marked differences as depicted in the respective figures. Prealbumin concentrations were low in the malaria and PEM II groups, compared with BB plasmas (p<0.01). On the other hand, prealbumin concentrations were elevated in the Guinea worm group compared with blood bank references (p<0.01). In
this group of patients prealbumin correlated positively with age 
(r = 0.98). The mean AAG concentration was some 3 fold higher in 
all Nigerian groups, compared with BB samples (p<0.01). 
Prealbumin levels correlated positively with albumin 
concentrations (r = 0.49, p < 0.05). On the other hand, AAG showed 
a negative correlation with albumin in adult subjects, but not in 
infants (< 2 years).

Drug protein binding also showed major differences between 
the populations. Phenytoin binding was significantly higher in 
the PEM and Guinea worm groups than in BB samples. The free 
fraction thus decreased by as much as 27% (Figure 4). Propranolol 
binding showed similar elevations in all Nigerian groups, the 
free fraction being only about half that of BB samples (p<0.05, 
Figure 5). There was a positive correlation between propranolol 
binding and AAG concentrations in infants < 2 years (r = 0.44), 
but not in other age groups. Phenytoin binding did not correlate 
with albumin concentrations, but showed variable degrees of 
correlation with AAG levels in the different groups.

DISCUSSION

The results show 3-fold increases above normal in AAG levels 
in all groups of Nigerian patients that were studied. This was 
associated with decreased prealbumin levels except in the Guinea 
worm group, and normal or slightly elevated albumin 
concentrations. The finding of increased AAG concentrations is not
unexpected in a group of patients who are malnourished or are parasitized and, in addition, may be in one stage or another of some other infectious disease. AAG levels are expected to rise in parasitized hosts as well as in PEM (Patwardhan et al. 1971; Schelp et al. 1978), possibly in relation to the increased susceptibility of malnourished individuals to infection (Isliker and Schurch 1981; Bondestan et al. 1988). AAG is actually thought to play a specific role in inhibiting the invasion by malaria parasites (Friedman 1983). Serum albumin concentrations decrease only in severe cases of PEM (Shetty et al. 1979), while prealbumin is considered a more sensitive index of PEM (Ingenbleek et al. 1975). Both are transport proteins and are negative acute phase proteins, i.e. are expected to decrease in inflammation and infection. Therefore, the observation that albumin concentrations in our Nigerian patients were not decreased is indeed surprising. However, the reduced concentrations of prealbumin in the malnourished and malaria patients is compatible with current knowledge. The increased prealbumin levels that were observed in Guinea worm patients are of interest, although the significance of this observation remains to be established. Other plasma protein concentrations, most notably immune globulins (Neumann et al. 1975), could be affected, but were not measured in the present study. Serum lipoproteins are elevated in experimental rodent malaria (Maurois et al. 1980) and may exhibit changes in parasitized humans and in PEM.

The increased binding of propranolol to the plasma proteins of malnourished and parasitized hosts is compatible with the high
levels of AAG that were recorded in both groups of patients. The magnitude of this phenomenon is certainly impressive. Drug binding to AAG exhibits considerable variation between, as well as within, patients (Piafsky 1980). Numerous reports also show a positive correlation between the extent of binding of basic drugs and increased AAG levels that are observed in a variety of disease states. Such data are available for cancer patients (Abramson et al 1982), acute illness (Paxton and Briant 1984), acute myocardial infarction (Sager et al 1981) etc. This subject was recently reviewed (Kremser et al 1988). The present data are, to the best of our knowledge, the first showing increased binding of drugs to the plasma proteins of patients with malaria and Guinea worm. For PEM, this investigation supports results by Jagadeesan and Krishnaswamy (1985) showing, in a group of malnourished subjects, increased plasma binding of propranolol that was associated with elevated AAG levels.

Previously, Krishnaswamy et al (1981) reported that the protein binding of phenylbutazone decreased in malnutrition. Data suggesting decreased protein binding of tetracyclin in malnourished adults was also presented (Raghuram and Krishnaswamy 1981). In a series of papers Buchanan and colleagues reported either reduced binding, no change or altered distribution among the binding proteins in the plasmas of individuals with PEM. Thus, salicylate (Eyberg et al 1974) and digoxin (Buchanan et al 1976) were bound to a lesser degree in kwashiorkor than in normal serum. Warfarin (Buchanan and van der Walt 1977a) and chloramphenicol (Buchanan and van der Walt 1977c) did not exhibit significant
binding differences. By contrast, chloroquine was bound more to kwashiorkor than to normal serum (Buchanan and van der Walt 1977b). In these studies pooled kwashiorkor sera were used, thus masking any variability within the population. Also, electrophoresis was performed under non-equilibrium conditions, thus favoring unbinding of the drug-protein complex.

The increased phenytoin binding that was presently observed, in the face of unaltered albumin concentrations, is intriguing. Moreover, phenytoin binding correlated with AAG concentrations, rather than with albumin. Phenytoin, an acidic drug, is conventionally considered to bind to plasma albumin. However, recent studies have shown significant binding of some acidic drugs to AAG (Schley and Mueller-Oerlinghausen 1983; Urien et al 1986; Urien et al 1982). Specifically for phenytoin, Israili and El-Attar (1983) showed that in vitro, at physiological protein concentrations, AAG bound as much as half the amount of drug as albumin. However, the clinical significance of these data remains to be explored. Our present data on patients with PEM and parasitic diseases, in whom increased plasma AAG was associated with significantly elevated binding of phenytoin could suggest that binding of this and possibly other acidic drugs to AAG assumes particular importance in clinical situations with increased concentrations of AAG.

Apart from the two major drug-binding proteins, other serum proteins have also been implicated in drug binding. Thus, highly lipid-soluble drugs bind to plasma lipoproteins (Chen and Danon
1979; Danon and Chen 1979). Other drugs may bind to plasma globulins (Aladjemoff et al 1958; Olsen 1973). Alterations in drug protein-binding that may occur in PEM or in parasitic infection could therefore be of a complex nature. While binding to one protein species could change in one direction, the association with another protein could increase, decrease or show no change. Thus, measurement of overall plasma binding may not reveal discrete changes among the various proteins and precludes precise assessment as to the nature of any change in binding that is observed. The coefficients of determination ($r^2$) that were obtained in the present investigation indicate that the regressions have accounted for only a fraction of the total variability in the observed binding data, even in those cases where the correlations were statistically significant.

Other factors may also contribute to the altered binding such as was documented in this study. Protein binding could be influenced by the presence of ligands in the plasma that may either compete with certain drugs for binding or produce some allosteric effect on protein binding. The concentrations of endogenous competing substances, such as free fatty acids, are expected to change in malnutrition, thus affecting the binding of drugs. In addition, one can envisage the possibility of specific parasitic metabolites present in the plasmas of patients infected with such organisms, which could act to displace drugs from binding proteins. Both helminthic and protozoan parasites are active metabolically (Howells et al 1972) and may alter the chemical composition of the plasma, not only by using up
nutrients, but also by the generation of parasitic waste products. The release of such materials from ruptured erythrocytes during the life cycle of the malaria parasite plays a dominant role in the clinical manifestations of the disease. Likewise, the clinical picture of other protozoan and helminthic disease depends not only on the presence of the parasites, but also on their secretions and excretions. Whether and to what extent such parasitic products can interfere with drug protein binding remains to be investigated.

For drugs that are highly protein bound, such as phenytoin and propranolol, even minor alterations in binding means significant changes in the free fraction. This, in turn, determines the pharmacological activity of the drug in question and also influences its volume of distribution and hepatic clearance (Wilkinson and Shand 1975). The half-life of such drugs could therefore change in either direction, depending on the magnitude of each of these effects (for review, see Koch-Weser and Sellers 1976). Thus one can predict that the prominently increased binding of both model drugs that is presently reported could affect the pharmacokinetic behavior of these drugs in patients with PEM, malaria or Guinea worm infection. Because available dosage recommendations stem from our knowledge of the pharmacokinetics of drugs in healthy or even ill patients, predominantly in the developed world, they may not apply to large populations in the developing world. Significant alterations in plasma protein binding in patients with PEM or parasitic disease and who receive recommended dosages, may expose them to undue toxicity, or on the contrary, to subtherapeutic drug levels. Thus,
the present data of altered protein binding in malnourished and parasitized patients, who comprise a large fraction of the world population, could warrant a search for new dosage recommendations for this population.

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FIGURE LEGENDS

Figure 1. Plasma albumin concentrations (mg/ml) in Blood Bank and in Nigerian patients (mean ± SEM).

Figure 2. Mean (± SEM) plasma prealbumin levels (mg/ml) in Blood Bank and in Nigerian Patients.

Figure 3. Mean (± SEM) plasma AAG levels (mg/ml) in Blood Bank and in Nigerian patients.

Figure 4. Phenytoin (DPH) binding to plasma in the different groups. Mean (± SEM) percent free fractions are shown.

Figure 5. Mean (± SEM) percent free propranolol in Nigerian patients compared with Blood Bank samples.
Table 1. Anthropometric data of Nigerian patients in each group

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<th>PEM II</th>
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<th>Guinea worm</th>
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</table>
**FIG 1.**

![Diagram showing plasma albumin mg/ml levels for Blood Bank, PEM I, Malaria, and Guinea worm samples.](image)

**FIG 2.**

![Diagram showing plasma prealbumin mg/ml levels for Blood Bank, PEM I, PEM II, Malaria, and Guinea worm samples.](image)

* p<0.01 vs. Blood Bank
**FIG 2**

![Graph 1](image1)

![Graph 2](image2)

*p<0.01 vs. Blood Bank*
*p<0.01 vs. Blood Bank