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Short Communication

Alterations of Phenytoin Protein Binding with in Vivo Haemodialysis in Dialysis Encephalopathy

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Summary. Protein binding of phenytoin was assessed in one patient with dialysis encephalopathy before and after haemodialysis. Phenytoin concentrations were measured by radioimmunoassay and continuous ultrafiltration was used to assess phenytoin binding. At a serum concentration of 60 μmol.l^{-1} the percentage of phenytoin bound to serum albumin was considerably lower in the patient serum (79.95% predialysis; 92.09% postdialysis) than that in three normal sera (97.90 ± 0.17%). Analysis of Scatchard plots indicated two classes of binding sites. In class I both the affinity and capacity for binding phenytoin were reduced in the pre and post-dialysis serum, whereas in class II the capacity of the uraemic serum was increased although the intrinsic association constant was greatly reduced. It was concluded that in vivo haemodialysis is associated with large fluctuations in the protein binding of phenytoin, in which the concentration of endogenous dialysible metabolites are strongly implicated.

Key words: phenytoin, dialysis encephalopathy; protein binding, continuous ultrafiltration.

The dialysis encephalopathy syndrome is, at present, one of the major causes of death in patients on regular haemodialysis therapy. Established encephalopathy is virtually irreversible but patients will often require anticonvulsant therapy to control the troublesome seizures which occur in this disorder. Phenytoin is a commonly used anticonvulsant for which there is good presumptive evidence of a relationship between elevated levels of unbound drug in serum and toxicity [1]. Uraemic serum has been previously shown to exhibit depressed phenytoin binding [2, 3] and in vitro dialysis restores phenytoin protein binding towards normal [4].

We have recently had occasion to study the protein binding of phenytoin in a patient with dialysis encephalopathy. We wish to report evidence that the improvement in the protein binding of phenytoin after haemodialysis is due to the reduction in dialysible metabolites, so that in vivo dialysis is accompanied by enhanced phenytoin protein binding such as occurs following dialysis in vitro.

Patient and Methods

Following a progressive glomerulonephritis a 30 year old male developed chronic renal failure and started haemodialysis therapy in 1972. In 1976 he began to show early features of dialysis encephalopathy with speech disturbance, myoclonus and generalised convulsions. Phenytoin therapy appeared to be associated with some diminution in the frequency of convulsions but the regulations of dosage proved difficult in this setting of progressive neurological deterioration where manifestations of phenytoin toxicity would not have been readily apparent.

The protein binding of phenytoin by this patient's serum was assessed by continuous ultrafiltration [5] at 37 °C and pH 7.4 using Amicon XM50 Diaflo ultrafiltration membranes, phenytoin concentrations being measured by radioimmunoassay [6].

Drug recoveries were measured and indicated that the binding of drug to these membranes was negligible (< 1.0%). Sulphosalicylic acid (1M) was used routinely to check for protein leakage through the membranes but it was never detected in the effluent.

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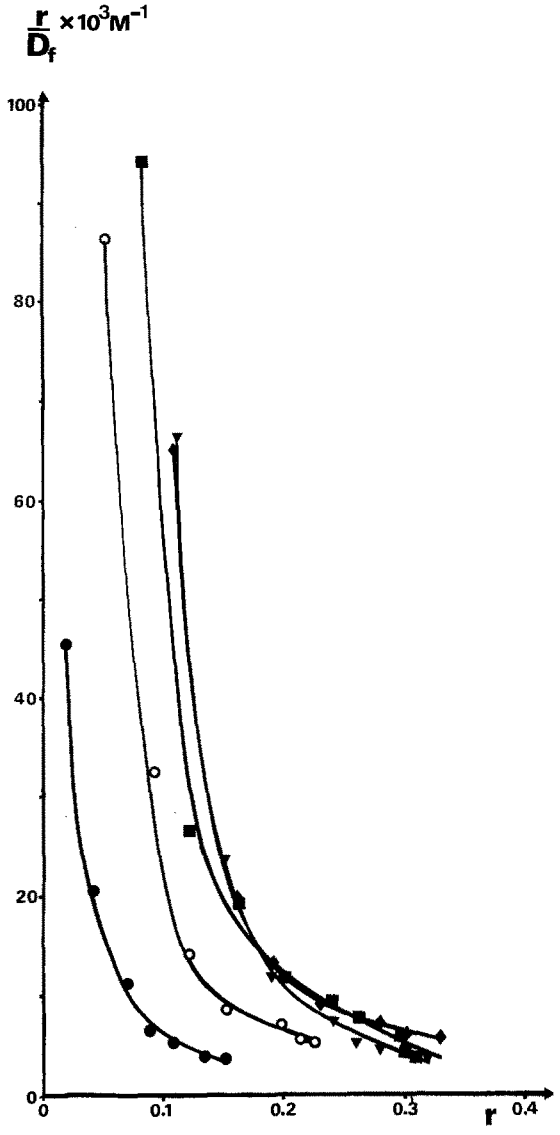

Fig. 1. Scatchard plots for three normal sera (■, ▼, ◆) and patient serum (predialysis ●; postdialysis ○). Df: concentration of free drug (mol. l^{-1}) r.: Moles of drug bound per Mole of albumin

Results and Discussion

At the mid-point of the therapeutic range (60 μmol l^{-1}) 79.95% of the drug was bound by the patient's serum. Following haemodialysis phenytoin protein binding was considerably increased, the drug being 92.09% bound, although even this value was lower than that determined in three normal subjects (97.90 ± 0.17%). Analysis of Scatchard [7] curves (Fig. 1) indicated two groups of binding sites in serum from both controls and patient (Table 1).

Thus, analysis of the Scatchard plots showed that abnormal drug protein interaction occurred in uraemia, being particularly marked pre-dialysis. A real reduction in the ability of serum albumin to bind phenytoin, independent of hypoproteinaemia was therefore indicated. Further reduction in methyl orange protein binding after in vivo haemodialysis has been reported which was attributed to 'non-dialysible' non-esterified fatty acids arising from the use of heparin [8]. Spin label studies have indicated that fatty acids can cause perturbations of albumin molecules [9] allosterically affecting protein binding properties. The protein binding of phenytoin has been reported to be sensitive to the presence of fatty acids which can cause its displacement from albumin molecules [10]. However, in the present study, the protein binding capacity was *enhanced* post-dialysis so that in vivo the effect of dialysis (commonly accompanied by increased free fatty acid levels) is probably attributable to reduction in metabolites which interfere with phenytoin binding in uraemia.

Albumin metabolism has also been shown to be altered in chronic renal failure [11] including changes in primary protein structure [12]. It is not readily conceivable that aberrations in the primary structure of the major part of the circulating albumin would have occurred during the time course of haemodialysis. The albumin concentration before and after haemodialysis remained constant.

Table 1. The number of binding sites (N_1 and N_2) and intrinsic association constants (K_1 and K_2) for controls and patient sera

	N_1	$K_1 \times 10^6\ M^{-1}$	N_2	$K_2 \times 10^3\ M^{-1}$
Controls				
K. McC	0.12	3.57	0.53	10.50
W. S.	0.08	9.47	0.29	43.20
P. M.	0.14	1.67	0.42	8.44
Mean ± SD	0.11±0.02	4.88±2.36	0.41±0.07	20.7±11.27
Patient				
Pre-dialysis	0.07	0.68	1.0	2.08
Post-dialysis	0.10	1.58	2.29	1.38

It could be postulated that phenytoin metabolites may be responsible for some displacement of the parent drug from serum albumin in vivo. To be an important factor in the change of protein binding, however, the clearance of the metabolites during haemodialysis would need to be grossly different from phenytoin, which seems unlikely. Aluminium toxicity has been implicated in the aetiology of dialysis encephalopathy and elevated serum aluminium concentrations are known to occur in the dialysis encephalopathy syndrome [13]. While aluminium is highly protein bound in serum [14] it seems unlikely that aluminium binding would have any bearing on the binding of phenytoin. Aluminium concentrations were in any case very similar before and after dialysis in our patient, being 300 $\mu g\ l^{-1}$ in the pre-dialysis serum and 267 $\mu g\ l^{-1}$ in the post-dialysis specimen.

In summary, in vivo haemodialysis is associated with large fluctuations in the protein binding of phenytoin, in which the concentration of endogenous dialysible metabolites are strongly implicated. While deterioration in the neurological condition, concomitant with dialysis, is part of the syndrome the observations recorded here suggest that phenytoin is not the ideal drug for seizure control in dialysis encephalopathy.

References

1. The Boston Collaborative Drug Surveillance Program: Diphenylhydantoin side-effects and serum albumin levels. Clin. Pharmacol. Ther. **14**, 529–532 (1973)
2. Blum, M. R., Riegelman, S., Becker, C. E.: Altered protein binding of diphenylhydantoin in uremic plasma. N. Engl. J. Med. **286**, 109 (1972)
3. Hooper, W. D., Bochner, F., Eadie, M. J., Tyrer, J. H.: Plasma protein binding of diphenylhydantoin. Effects of sex hormones, renal and hepatic disease. Clin. Pharmacol. Ther. **15**, 276–282 (1974)
4. Andreasen, F.: Protein binding of drugs in serum from patients with acute anuria. Acta Pharmacol. Toxicol. (Kbh.) **34**, 284 (1974)
5. Campion, D. S., Olsen, R.: Measurement of drug displacement by continuous ultrafiltration. J. Pharm. Sci. **63**, 249–252 (1974)
6. Paxton, J. W., Rowell, F. J., Ratcliffe, J. G.: Production and characterisation of antisera to diphenylhydantoin suitable for radioimmunoassay. J. Immunol. Methods **10**, 317–327 (1976)
7. Scatchard, G.: The attraction of proteins for small molecules and ions. Ann. N. Y. Acad. Sci. **51**, 660–672 (1949)
8. Dromgoole, S. H.: The effect of haemodialysis on the binding capacity of albumin. Clin. Chim. Acta **46**, 469–472 (1973)
9. Soltys, B. J., Hsia, J. C.: Fatty acid enhancement of human serum albumin binding properties. J. Biol. Chem. **252**, 4043–4048 (1977)
10. Geigler, R., Shoeman, D. W., Azarnoff, D. L.: Effect of in vivo elevation of free fatty acids on protein binding of drugs. Pharmacology **12**, 160–165 (1974)
11. Grossman, S. B., Yap, S. H., Shafritz, D. A.: Influence of chronic renal failure on protein synthesis and albumin metabolism in rat liver. J. Clin. Invest. **59**, 869–878 (1977)
12. Boobis, S. W.: The alteration of plasma albumin in relation to decreased drug binding in uremia. Clin. Pharmacol. Ther. **22**, 147–152 (1977)
13. Elliott, H. L., Dryburgh, F., Fell, G. S., Sabet, S., Macdougall, A. I.: Aluminium toxicity during regular haemodialysis. Br. Med. J. **1978/I**, 1101
14. Kaehny, W. D., Alfrey, A. C., Holman, R. E., Shorr, W. J.: Aluminium transfer during haemodialysis. Kidney Int. **12**, 361–365 (1977)

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