Effect of Phenytoin on Protein Binding of Valproic Acid

J. BRUNI, J.M. GALLO and B.J. WILDER

SUMMARY: *In vitro experiments using the equilibrium dialysis technique were performed to determine the binding of valproic acid to plasma components in the absence and presence of therapeutic concentrations of phenytoin. The free fraction of valproic acid was found to be dependent on the total valproic acid concentration. Phenytoin did not influence valproic acid protein binding.*

RÉSUMÉ: *Nous avons fait des expériences in vitro en employant la technique de dialyse équilibrée afin de déterminer la liaison de l'acide valproique aux composantes plasmatiques en présence ou absence de concentrations thérapeutiques de phenytoin. Il fut démontré que la fraction libre de l'acide valproique est dépendante de la concentration totale de l'acide valproique. Le phenytoin n'influence pas la liaison protéinique de l'acide valproique.*

From the Department of Neurology, College of Medicine and College of Pharmacy, University of Florida, Gainesville, Florida.

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Reprint requests to Dr. J. Bruni, Jones Building, Wellesley Hospital, 160 Wellesley St. East, Toronto, Ontario, M4Y 1J3, Canada.

Adequate in vivo and vitro experimental data are available to support the hypothesis that valproic acid (VPA) displaces phenytoin (DPH) from plasma protein binding sites (Patsalos and Lascelles, 1970; Jordan et al., 1976; Patsalos and Lascelles, 1977; Mattson et al., 1978; Lecchini et al., 1978; Wilder et al., 1978). This interaction results in a transient decrease in the total DPH plasma level with a corresponding increase in the free unbound portion of DPH. After a variable period, total DPH plasma levels return to pre-valproic acid plasma levels in the majority of patients (Bruni et al., 1979). Less data are available on displacement of valproic acid by phenytoin. Experimental data suggest that VPA is predominantly bound to albumin. Whether one or two classes of binding sites are involved is not clear. Gugler et al (1978) suggested that two classes of binding sites are involved. Patel and Levy (1979) concluded that the protein binding interaction involves only one class of binding sites.

In an earlier communication we reported that the protein binding of valproic acid in epileptic patients is not influenced by the presence of other anticonvulsants (Gallo et al., 1979). In vitro experiments using the equilibrium dialysis technique have shown that phenytoin, phenobarbital, and carbamazepine do not affect valproic acid binding to human serum albumin (Patel and Levy, 1979). These data are relevant when a patient is receiving valproic acid and one of these antiepileptic drugs is added to the treatment regimen. This study investigated the effect of phenytoin on the protein binding of VPA in human plasma.

MATERIALS AND METHODS

Protein binding of VPA was studied with an equilibrium dialysis system (Dianorm, Diachema Ag, Puschulkon, Switzerland). Two each of the following plasma samples of valproic acid at 40, 85, 95, and 160 ug/ml alone and with 10/ug/ml and 20 ug/ml of phenytoin were dialyzed against a modified physiological phosphate buffer. Two ml of the sample solutions were dialyzed against 2 ml of phosphate buffer for 6 hours at 37° C. It has been previously found that a dialysis time of six hours is sufficient and that no significant membrane adsorption occurs (Gallo et al., 1979). The buffer compartment was analyzed at the end of each dialysis run and assayed for VPA as previously described (Bruni et al., 1978). The donor plasma had a total protein concentration of 8.0 grams/100 ml and an albumin concentration of 5.1 gm/100 ml.

RESULTS

The binding of VPA to plasma proteins was studied over the concentration range 40 ug/ml - 160 ug/ml. Free fractions of VPA were determined in the absence and in the presence of phenytoin. The table demonstrates the free fraction of VPA under the various experimental conditions. The free fraction of valproic acid ranged from 0.042 at the lowest concentration (40 ug/ml) to 0.152 at the highest concentration (160 ug/ml). The difference in binding at the highest concentration was statistically significant ($P < 0.05$) (Duncan's Multiple Range Test) from the binding at the lower concentrations. The addition of phenytoin in concentrations at the extremes of the accepted therapeutic plasma range (10 ug/ml and 20 ug/ml)

TABLE I

The Effect of Phenytoin on Valproic Acid Binding

EXPERIMENT	TOTAL VPA	FREE FRACTION VPA			p
	ug/ml	VPA Alone	+DPH 10 ug/ml	+DPH 20 ug/ml	
1	40	0.042±0[1]	0.039±0.004	0.039±0.004	N.S.[2]
2	85	0.097±0.005	0.095±0	0.102±0.003	N.S.
3	95	0.075±0.02	0.058±0.004	0.070±0.003	N.S.
4	160	0.152±0.002	0.143±0	0.156±0.02	N.S.

1. Mean ± S.D.
2. Not significant at p= 0.05 (F test)
VPA — Valproic Acid
DPH — Phenytoin

did not significantly alter valproic acid binding to plasma components at any of the valproic acid plasma concentrations studied (F test).

DISCUSSION

In vitro results support the clinical observation that DPH does not significantly influence valproic acid binding to plasma components. This suggests that valproic acid has a higher affinity for binding sites and/or that there are more protein binding sites for valproic acid. When DPH is added to valproic acid therapy no dose adjustment of valproic acid is required as a result of this lack of interaction. Valproic acid binding to plasma components in the absence or presence of DPH is in the range 84.8 - 95.8 percent. This lack of effect of phenytoin on valproic acid binding is in agreement with observations using human serum albumin (Patel and Levy, 1979).

Since valproic acid has a small volume of distribution any protein displacement interaction could be significant, although it may be only transient. This is due to VPA undergoing restrictive hepatic elimination whereby increases in free drug concentration are equalized by an increase in hepatic clearance. Thus, other highly protein bound drugs such as salicylates, phenylbutazone, warfarin, and bishydroxycoumarin may affect VPA protein binding.

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