

LETTERS

Factors Affecting the Determination of Unbound Carbamazepine Concentrations in Plasma

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Free drug concentrations in plasma or serum may represent a better guide to optimize drug dosages than total concentrations when drugs are highly bound to plasma proteins (1, 2). The usefulness of three ultrafiltration systems suitable for routine drug monitoring was tested in determining free concentrations of carbamazepine in plasma in comparison with equilibrium dialysis: Centriflo CF25, Centriflo CF50A, and Ultrafree. The free fraction of carbamazepine in plasma was reported to be 0.10–0.35 (3, 4). The Centriflo cones (Amicon, Danvers, Massachusetts) are centrifuged at 1000 g in order to obtain the ultrafiltrate, while the Ultrafree filters (Millipore, Freeholt, New Jersey) are used in combination with a plunger-syringe based on vacuum suction.

Ultrafiltration was carried out using 2, 3, or 5 ml plasma to which carbamazepine had been added. Carbamazepine concentrations were measured in serial fractions of plasma ultrafiltrate. The concentrations measured in the first fractions were low, probably as a result of drug binding to the ultrafiltration system. The concentrations of carbamazepine gradually increased until reaching a constant value after approximately 1.8 ml, 1.2 ml, and 0.3 ml of ultrafiltrate was obtained with Centriflo CF25, CF50A, and Ultrafree, respectively. This plateau concentration is considered to correspond to the free drug concentration in plasma (5). Therefore, the first 1.8 ml and 1.2 ml of ultrafiltrate, for Centriflo CF25 and CF50A, respectively, must be discarded (centrifuging

time for both: 15 min), and the free concentration of carbamazepine can be determined in the ultrafiltrate obtained in the next 15 min of centrifugation.

With the Ultrafree filters, discarding the first fraction of ultrafiltrate was not necessary and not practical, because reconnecting a syringe often led to leakage and loss of vacuum. Approximately 0.5 to 0.6 ml Ultrafree filtrate must be collected for reproducible results (collection time: 2 h). When collecting 0.8 ml ultrafiltrate or more, plasma proteins began to pass through the filter membrane, resulting in overestimates of free carbamazepine concentrations. To obtain a reliable plateau concentration the minimal plasma sample volume was 5 ml, 3 ml, and 2 ml for Centriflo CF25, CF50A, and Ultrafree, respectively.

Using these ultrafiltration methods unbound carbamazepine concentrations were measured in plasma from four volunteers. To the plasma of each volunteer carbamazepine had been added in concentrations of 2.5 µg/ml, 5.0 µg/ml, 7.5 µg/ml, and 10.0 µg/ml. Each sample was measured five times. Results were compared with free concentrations determined with equilibrium dialysis of plasma against an isotonic phosphate buffer, pH 7.4 (Table I). A good correlation was found between the three ultrafiltration methods and equilibrium dialysis: $r = 0.96, 0.97$ and 0.98 ($p < 0.001$) for Centriflo CF25, CF50A, and Ultrafree, respectively. However, the Centriflo filters produced systematically and significantly lower free carbamazepine concentrations, although great care was taken in measuring only the drug's plateau level in each ultrafiltrate. These problems must be considered with the determination and interpretation of free drug levels in plasma.

Table I. Free Fraction of Carbamazepine at Different Plasma Concentrations, Determined with Equilibrium Dialysis and 3 Ultrafiltration Systems.

	Free Fraction of Carbamazepine ^a			
	2.5 µg/ml ^b	5.0 µg/ml ^b	7.5 µg/ml ^b	10.0 µg/ml ^b
Equilibrium dialysis	0.30 ± 9.1 %	0.30 ± 5.5 %	0.28 ± 6.5 %	0.30 ± 4.4 %
Centriflo CF25	0.18 ± 4.2 %**	0.18 ± 2.6 %**	0.17 ± 3.1 %*	0.18 ± 1.9 %***
Centriflo CF50A	0.22 ± 3.6 %**	0.24 ± 1.7 %**	0.23 ± 0.8 %	0.24 ± 1.4 %*
Ultrafree	0.30 ± 2.4 %	0.30 ± 1.9 %	0.29 ± 2.6 %	0.30 ± 2.6 %

^a Mean free fraction ± mean coefficient of variation of the method (four plasma samples, each determined five times).

^b Plasma containing the indicated concentrations of carbamazepine.

* Significant differences compared with equilibrium dialysis $0.01 < p < 0.05$.

** $0.001 < p < 0.01$.

*** $p < 0.001$.

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