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Evaluation of fluorescence polarization immunoassay as a method for the determination of carbamazepine in saliva*

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Introduction

Carbamazepine is particularly effective in the treatment of partial seizures. Clinical trials have been performed in order to establish the relationship between serum levels and the therapeutic effects of carbamazepine. However, such monitoring is usually based on total serum concentration, which includes both the bound and non-protein-bound fractions, thus interpatient variability in serum protein binding of drug may complicate the interpretation of the data.

Measurement of the concentration of drug in saliva has been shown to be an excellent indicator of the unbound fraction [1, 2] pharmacologically active form of drug. Consequently salivary sampling provides a better basis for anticipating the overall pharmacological effects of a drug. Furthermore this method is convenient when a large number of samples are required because no loss of blood or exposure of the subject to the distress of venipuncture is involved.

Fluorescence polarization immunoassay is used increasingly for drug analysis owing to its rapidity, specificity and precision. The aim of the present study was to evaluate the technique using a patient population taking carbamazepine alone and in association with other anticonvulsant co-medication, and to correlate the concentration of free carbamazepine in serum to that in saliva.

Also it was decided that the possible influence of the polypharmacy associated in the values obtained in the group undergoing multi-therapy should be investigated.

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Experimental

The study involved 33 out-patients subject to epilepsy. The subjects were children, ages 5–12 years, who were being treated with carbamazepine (Table 1). Seventeen patients received carbamazepine as the only drug of the antiepileptic therapy and 16 patients were in addition receiving phenobarbital, valproic acid or phenytoin (Table 1).

Saliva and blood samples were drawn just before the morning dose. Samples were separated immediately by centrifugation and stored at −20°C until required for analysis (always within 10 days of sampling). This handling procedure would not be expected to alter the binding of carbamazepine to serum proteins [3]. In order to separate the free carbamazepine from the total carbamazepine a "Free Level System", Syva Palo Alto, CA was used. Then the total and free carbamazepine in serum and carbamazepine in saliva were determined by FPIA (TDX System, Abbot Diagnostics, North Chicago, IC). The concentration of free fatty acid in serum was determined by spectrophotometrically as described by Duncombe [4] before ultrafiltration. Serum protein concentrations were determined by use of the biuret reagent. The pH of the samples was measured immediately after collection with a Beckman pH meter.

Results

The mean pH values observed for saliva were 7.18 (SD 0.26) versus 7.22 (SD 0.20) for the mono- and multi-therapy groups, respectively. In all subjects the total serum protein and free fatty acids were within the normal reference intervals: 73.8 (SD 3.9) versus 72.2 (SD 2.0) g l^{-1} and 330 (SD 180) versus 410 (SD 150) μmol l^{-1} for the mono- and multi-therapy groups, respectively.

Comparison of carbamazepine concentrations obtained by FPIA revealed a statistically significant difference ($P < 0.001$) between saliva and serum ultrafiltrate levels in the two groups studied. There was no statistically significant difference in either saliva or serum ultrafiltrate between the mono- and multi-therapy groups (Table 1). Also, a statistically significant difference ($P < 0.05$) was observed between the mono- and multi-therapy groups, for total serum carbamazepine (see Table 1), this is in agreement with the findings of other authors [5, 6]. Values for percentages of unbound fraction ratios of carbamazepine by the two groups are shown in Table 1.

Discussion

The results of the present study indicate that the values obtained by FPIA for carbamazepine in saliva, tended to be higher than for ultrafiltrate serum in the two groups studied.

These higher values for carbamazepine in saliva, were also observed by the EMIT (Syva) system [1, 6] and may be due to a cross reactivity of the principal metabolite, the 10,11 epoxide. This interference is less significant with serum ultrafiltrate measurements since the mean free carbamazepine fractions: 21.2 versus 24% for mono- and multi-therapy groups, respectively, were similar to that previously described by other authors [7, 8] (see Table 1).

The multi-pharmacy associated to carbamazepine appears to have not any influence on metabolism of carbamazepine since there is no significant difference between saliva and serum ultrafiltrate values from the two groups studied.

Table 1
Carbamazepine concentration measured, mg l^{-1}

Subject	Total dose (mg)	Co-medication dose (mg)	Saliva	Serum ultrafiltrates	Serum total	Unbound fraction (%)
1	800	—	3.6	2.6	11.7	22.2
2	600	—	2.1	1.5	6.4	23.4
3	800	—	2.4	1.7	7.1	23.9
4	600	—	1.9	1.3	6.3	20.6
5	400	—	2.1	1.3	6.8	19.1
6	600	—	3.0	2.0	9.7	20.6
7	600	—	2.6	1.6	7.5	21.3
8	500	—	2.9	1.7	9.3	18.2
9	450	—	2.8	1.7	8.7	19.5
10	300	—	1.6	1.3	5.4	24.1
11	500	—	2.1	1.4	5.9	23.7
13	800	—	2.0	1.3	5.8	22.4
13	600	—	1.7	1.3	6.1	21.3
14	600	—	2.3	1.6	7.6	21.0
15	600	—	1.8	1.5	8.7	17.2
16	400	—	1.2	1.4	7.8	17.9
17	600	—	1.7	1.2	5.1	23.5
Mean ± SD			2.22 ± 0.58*‡	1.55 ± 0.33‡	7.40 ± 1.70†	21.17 ± 2.14
1	600	VPA 600	3.4	2.2	8.6	25.5
2	300	PB 75	1.2	0.9	3.4	26.4
3	800	PB 150	2.1	1.4	7.0	20.0
4	400	VPA 600	3.6	1.5	6.8	22.0
5	900	PB 200	1.8	1.2	6.1	19.7
6	700	PB 100	3.8	1.6	6.4	25.0
7	300	VPA 400	1.9	1.5	5.4	27.7
8	900	DPH 300	2.2	1.5	5.3	28.3
9	400	PB 100	1.5	1.0	4.2	23.8
10	300	PB 75	1.2	1.1	4.0	27.5
11	1100	VPA 1500, DPH 275	2.1	1.5	5.4	27.7
12	1600	DPH 450, PD 750	1.7	1.1	5.9	28.6
13	600	DPH 250	1.6	1.1	5.4	20.4
14	800	VPA 1000	2.6	1.5	6.5	23.0
15	1000	VPA 1200	4.3	2.5	10.0	25.0
16	1000	PB 100	2.1	1.5	6.5	23.0
Mean ± SD			2.31 ± 092*	1.44 ± 0.40	6.05 ± 1.59	23.96 ± 3.10

VPA, valproic acid; PB, phenobarbital; DPH, phenytoin; PD, primidone.
*Significantly different in both groups from serum ultrafiltrates values ($P < 0.001$).
†Signficantly different from polytherapy group values ($P < 0.05$).
‡Not significantly different with respect to values for polytherapy groups. For statistical analysis (Student's *t*-test for paired data).

It may be concluded that FPIA is not a sufficiently specific method for the determination of carbamazepine in saliva to be recommended as a replacement for chromatographic methods.

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