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CSF CONCENTRATIONS AND SERUM PROTEIN BINDING OF CARBAMAZEPINE AND CARBAMAZEPINE-10, 11-EPOXIDE IN EPILEPTIC PATIENTS

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1 Serum and CSF samples of patients receiving chronic carbamazepine treatment were analysed.

2 Daily fluctuations in serum levels of carbamazepine and carbamazepine-10,11-epoxide did not appear to be related to the dosage schedule, but some patients tended to have lower fluctuations when the carbamazepine was given more frequently.

3 The epoxide/carbamazepine serum ratios varied greatly from patient to patient, and also fluctuated during the day for the same patient.

4 Carbamazepine and carbamazepine-10,11-epoxide were present in CSF in concentrations ranging from 19 to 34% and 26 to 71% of the serum concentrations, respectively.

5 There was a significant relationship between the free fraction of both drugs evaluated *in vivo* and the CSF/serum ratios.

6 The need for a careful evaluation of the possible clinical effect of the epoxide is stressed.

Introduction

Protein binding may for several reasons be an important determinant of drug therapeutic and toxic effects (Curry, 1970: Gillette, 1973; Dayton, Israili & Perel, 1973; Wardell, 1974). Modification of plasma protein binding due to diseases or displacing effects of other drugs has been shown to alter dramatically the toxic effects of several compounds (Reidenberg & Affrine, 1973; Anton, 1973). Recent data (Booker & Darcey, 1973; Glassman & Perel, 1974; Glassman, Hurwic, Kanzler, Shostak & Perel, 1974) tend to suggest that, at variance with previous results (Borgå, Azarnoff, Forshell & Sjøqvist, 1969), important inter-individual differences in plasma protein binding may be observed in patients undergoing chronic treatment, in the absence of specific phenomena such as displacement, or alteration in proteinemia. Such conditions may be of importance for the therapeutic outcome and give an explanation of the considerably large therapeutic range observed for several drugs. In the field of anticonvulsants, very little data on carbamazepine plasma protein binding *in vivo* in patients undergoing chronic treatment are available, although detailed studies in patients have been carried out with phenytoin.

The available data derived from observation on CSF/serum ratios (which is considered to be a reliable index of drug-serum protein binding) (Johannessen & Strandjord, 1973, 1975; Morselli, Gerna, De Maio, Zanda, Viani & Garattini, 1975) indicate that the free fraction of carbamazepine may range from 16-31% with a mean value of about 25-26%. However, Hooper, Dubetz, Bochner, Cotter, Smith, Eadie & Tyrer (1975) recently reported a wider range of variability (7.9-60%) in the free carbamazepine fraction in patients. Furthermore, no data are available on *in vivo* serum protein binding and the CSF/serum ratios of carbamazepine-10,11-epoxide, an active metabolite which has been shown to be constantly present during chronic treatment with carbamazepine (Christiansen & Dam, 1975; Morselli *et al.*, 1975) and of which, in volunteers, about 45-50% is bound to plasma proteins (Morselli, 1975; Morselli *et al.*, 1975).

In the present report we describe observations on the *in vivo* serum protein binding and on

CSF/serum ratios of both carbamazepine and its epoxide in epileptic patients undergoing chronic treatment with carbamazepine.

Methods

Fourteen male in-patients, aged 14-44 years, with epilepsy and without other relevant diseases were included in the study. Twelve patients received carbamazepine alone, one patient also acetazolamide and one patient also warfarin.

Carbamazepine was given twice to four times daily (Table 1). The dosages of carbamazepine and other drugs were kept constant for 4 weeks prior to the study to ensure steady state serum levels of carbamazepine. Routine clinical chemical analyses were within normal limits for all patients.

The daily fluctuation in levels and protein binding of serum carbamazepine and its main metabolite, the 10,11-epoxide, were studied in all patients. Blood samples for analyses were drawn before the morning dose was given, and subsequently seven times throughout the day and night; mainly at 0, 2, 4, 7, 9.5, 11.5, 13.5 and 24 h after the first dose.

The distribution of carbamazepine and the epoxide between serum and CSF was studied in ten patients. Lumbar puncture was performed before the morning dose of carbamazepine was given. CSF and serum specimens were kept frozen until analysis.

Serum and CSF concentrations of carbamazepine and carbamazepine-10,11-epoxide were determined by gas chromatography according to a procedure described previosly (Morselli, Biandrate, Frigerio, Gerna & Tognoni, 1973) with minor modifications. In contrast to the previous method, solvent extraction was carried out at pH 9, and the internal marker was 4-(3,4,5-trimethoxy-benzoyl) -tetrahydro-1,4-oxazine (ISF 2003). The minimal detectable amounts under our conditions, were 0.5 μg for carbamazepine and 0.25 μg for carbamazepine-10,11-epoxide. Volumes of 1-2 ml of serum and 2 ml of CSF were used for the drug quantitations. Serum protein binding was evaluated on patient serum samples by ultrafiltration at 22° C as described by Di Salle, Pacifici & Morselli (1974).

Results

Serum levels of carbamazepine-10,11-epoxide

Serum concentrations of carbamazepine and carbamazepine-10,11-epoxide observed in the fourteen patients undergoing chronic treatment are presented in Table 2. Serum carbamazepine levels ranged from 2.9-18.1 μg/ml and from 0.3-3.9 μg/ml for carbamazepine-10,11-epoxide. The doses varied from 9.1-36.0 mg/kg, but there did not seem to be any relationship between the daily carbamazepine treatment and the morning

Table 1 Vital data and carbamazepine dosage schedule of patients included in the study

Patient	*Age (years)*	*Body weight (kg)*	*Total dose (mg)*	*Dose (mg/kg)*	*Daily doses*	*Divided doses (mg)*	*Time interval (h)*
1§	19	63	600	9.5	2	300-300	11.5-12.5
2	20	72	700	9.7	2	350-350	11.5-12.5
3	25	70	1000	14.3	2	500-500	11.5-12.5
4	17	69	1100*	15.9	2	500-600	11.5-12.5
5§	29	82	1200	14.6	2	600-600	12-12
6	33	69	1400	20.3	2	700-700	11.5-12.5
7	33	70	1800	25.7	2	900-900	12-12
8	24	60	1800	30.0	2	900-900	11.5-12.5
9	22	66	600	9.1	3	200-200-200	3-5.5-14.5
10	22	66	1600	24.2	3	600-400-600	4.5-5-14.5
11	28	68	1800	26.5	3	600-600-600	7.5-6.5-10
12§	14	50	1800	36.0	3	600-600-600	7-6.5-10.5
13	44	75	1600**	21.3	4	400-400-400-400	4.5-5-4.5-10
14§	21	66	2000	30.3	4	500-500-500-500	4.5-5-4.5-10

§ no CSF sample.
* additional drug acetazolamide (750 mg) daily
** additional drug warfarin (13.5 mg) daily

Table 2 Serum levels (μg/ml) of carbamazepine and carbamazepine-10,11-epoxide in epileptic patients treated chronically with carbamazepine

		Sampling time (h)															
	Dose	*08.00*		*10.00*		*12.00*		*15.00*		*17.30*		*19.30*		*21.30*		*08.00*	
Patient	*(mg/kg)*	*CBZ*	*Epox*	*CBZ*	*Epox*	*CBZ*	*Epox*	*CBZ*	*Epox*	*CBZ*	*Epox*	*CBZ*	*Epox*	*CBZ*	*Epox*	*CBZ*	*Epox*
1	9.5	11.1	1.4	8.8	0.3	11.7	0.6	10.7	0.4	9.9	0.9	8.6	2.1	9.8	0.7	8.9	0.7
2	9.7	5.5	1.7	7.3	1.6	8.8	1.6	5.4	1.6	5.2	2.7	5.4	1.7	7.8	0.5	9.5	1.3
3	14.3	5.7	0.7	3.9	1.6	4.9	0.8	6.6	1.8	5.2	1.5	4.3	1.1	8.3	1.3	5.6	0.6
4	15.9	12.6	0.8	13.4	1.8	11.1	0.5	12.8	1.1	11.5	0.4	13.6	0.3	11.2	1.1	10.2	1.2
5	14.6	5.2	1.5	9.0	1.3	7.8	1.5	8.2	2.5	6.4	1.4	6.0	1.9	9.2	2.4	6.2	2.5
6	20.3	8.2	0.7	10.5	0.8	9.9	1.1	9.3	0.9	8.1	0.8	9.6	0.9	7.2	0.8	6.3	0.8
7	27.7	8.6	1.5	5.7	0.6	13.1	2.5	13.5	2.1	8.6	1.5	12.5	1.4	13.6	2.4	6.6	1.1
8	30.0	7.1	2.6	6.7	2.5	11.6	3.3	9.6	3.7	13.1	2.7	8.7	2.8	9.0	2.9	8.3	3.0
9	9.1	2.9	1.5	–		4.2	2.1	5.5	1.0	4.2	1.1	–		6.1	1.3	4.3	0.8
10	24.2	13.1	1.9	10.7	1.0	14.2	2.0	15.1	2.2	15.6	1.6	17.4	2.4	18.1	3.0	10.5	1.9
11	26.5	10.5	2.4	9.9	2.2	10.5	2.6	13.5	3.0	10.7	2.7	12.4	2.6	8.2	1.8	10.6	2.6
12	36.0	11.9	0.9	11.0	1.1	11.8	0.9	10.9	1.1	10.4	2.0	10.5	0.7	12.0	1.8	11.3	1.4
13	21.3	10.8	2.2	10.2	3.2	9.4	1.1	11.8	1.8	10.4	3.2	10.3	3.0	11.2	3.5	10.1	2.4
14	30.3	8.6	2.7	9.2	2.9	8.0	3.9	9.2	2.9	9.7	3.5	9.1	3.4	11.9	2.8	7.1	2.6

Patients nos. 1,2,3,4,5,6,7,8 received carbamazepine twice a day at 08.00 and 19.30, patients nos. 9 and 10 three times a day at 08.00, 12.00, and 17.30, patients nos. 11 and 12 three times a day at 08.00, 12.00, and 19.30, patients nos. 13 and 14 four times a day at 08.00, 12.00, 17.30, and 21.30.

Table 3 Serum carbamazepine and carbamazepine-10,11-epoxide fluctuations over a 24 h period

		Carbamazepine			*Carbamazepine-10,11-epoxide*		
Patient	*Daily doses*	*Difference between low and high levels (μg/ml)*	*Mean serum levels (μg/ml)*	*Fluctuation (%)*	*Difference between low and high levels (μg/ml)*	*Mean serum levels (μg/ml)*	*Fluctuation (%)*
1	2	3.1	10.1	31	1.8	0.9	200
2	2	4.3	6.8	63	2.2	1.6	137
3	2	4.4	5.5	80	1.2	1.9	109
4	2	3.4	12.0	28	1.5	0.9	166
5	2	4.0	7.2	56	1.2	1.9	63
6	2	4.2	8.6	49	0.4	0.8	50
7	2	7.9	10.2	77	1.9	1.6	119
8	2	6.4	9.2	69	1.2	2.9	41
9	3	3.2	4.5	71	0.7	1.1	64
10	3	7.6	14.3	53	2.0	2.0	160
11	3	5.4	10.7	50	1.2	2.5	48
12	3	1.6	11.2	14	1.3	1.2	108
13	4	2.4	10.5	23	2.4	2.5	96
14	4	4.8	9.1	53	1.3	3.1	42

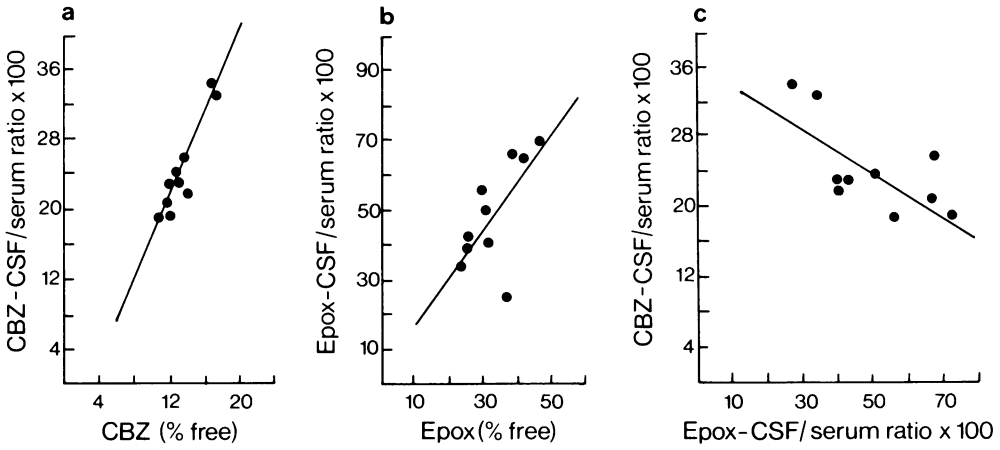

Figure 1 (a) Relationship between carbamazepine (CBZ)-CSF/serum ratio and the % free carbamazepine in serum measured by ultrafiltration ($r = 0.95$, $P < 0.01$, $n = 10$). (b) Relationship between epoxide-CSF/serum ratio and the % free epoxide in serum measured by ultrafiltration ($r = 0.64$, $P < 0.025$, $n = 10$). (c) Relationship between carbamazepine and epoxide-CSF/serum ratios ($r = -0.70$, $P < 0.025$, $n = 10$).

serum levels or daily mean levels for the two compounds.

Daily fluctuations of serum carbamazepine for individual patients varied from 14% (case No. 13) up to 80% (cases Nos. 3 and 7). For both carbamazepine and the epoxide the daily fluctuations in serum levels did not appear to be directly related to dosages and numbers of doses, although in two patients with three or four daily doses, the fluctuations tended to be less (Table 3). The ratio between carbamazepine-10,11-epoxide and carbamazepine varied considerably for individual patients, and also fluctuated to a great extent during the 24 hours of observations (Table 4).

CSF concentrations of carbamazepine and carbamazepine-10,11-epoxide and protein binding in vivo

Data related to CSF concentrations of carbamazepine and its metabolite are shown in Table 5 together with the data on serum protein binding.

Carbamazepine concentrations in the CSF ranged from 1.1-3.1 μg/ml, equivalent to 19-34% of the corresponding serum levels.

Carbamazepine-10,11-epoxide was constantly present in the CSF, and the ratio of CSF to serum concentration ranged from 34-71%. Values obtained with ultrafiltration at 22°C gave a free

Table 4 Daily fluctuations of carbamazepine-10, 11-epoxide/carbamazepine ratios in epileptic patients

	Sampling time (h)							
Patient	*08.00*	*10.00*	*12.00*	*15.00*	*15.30*	*19.30*	*21.30*	*08.00*
1	0.12	0.03	0.05	0.03	0.09	0.24	0.07	0.08
2	0.31	0.22	0.18	0.30	0.52	0.31	0.06	0.14
3	0.12	0.41	0.16	0.27	0.29	0.25	0.16	0.11
4	0.06	0.13	0.04	0.08	0.03	0.02	0.10	0.12
5	0.29	0.14	0.19	0.30	0.22	0.32	0.26	0.40
6	0.08	0.08	0.11	0.10	0.10	0.09	0.11	0.13
7	0.17	0.10	0.19	0.15	0.17	0.11	0.17	0.17
8	0.37	0.37	0.28	0.38	0.21	0.32	0.32	0.36
9	0.52	–	0.26	0.18	0.26	–	0.21	0.19
10	0.08	0.09	0.14	0.15	0.10	0.14	0.17	0.18
11	0.23	0.22	0.25	0.22	0.25	0.21	0.22	0.24
12	0.08	0.10	0.08	0.10	0.19	0.07	0.15	0.12
13	0.20	0.31	0.12	0.15	0.31	0.29	0.31	0.24
14	0.31	0.31	0.49	0.31	0.36	0.37	0.23	0.37

Table 5 Data on carbamazepine and carbamazepine-10,11-epoxide free fractions measured by ultrafiltration at 22°C and by the CSF/serum ratio

	Carbamazepine					*Carbamazepine-10,11-epoxide*				
Patient	*Pooled serum (μg/ml)*	*% free at 22° C*	*Morning serum (μg/ml)*	*CSF (μg/ml)*	*CSF/serum (×100)*	*Pooled serum (μg/ml)*	*% free at 22° C*	*Morning serum (μg/ml)*	*CSF (μg/ml)*	*CSF/serum (×100)*
1	8.7	14	11.1*	–	–	1.3	38	1.4*	–	–
2	6.8	14	6.7	1.5	22	1.6	25	1.0§	0.4	40
3	5.1	11	5.6	1.1	19	0.7	28	0.7§	0.4	57
4	11.5	12	14.1	3.1	21	1.0	40	1.2§	0.8	66
5	7.3	18	6.2*	–	–	1.8	33	1.5*	–	–
6	9.9	12	8.6	1.7	19	0.9	44	0.7	0.5	71
7	7.8	14	8.7	2.3	26	1.6	37	1.5	1.0	66
8	7.1	17	7.1	2.4	33	2.9	24	2.6	0.9	34
9	4.1	17	2.9	1.0	34	1.1	36	1.5	0.4	26
10	12.5	13	13.2	3.1	23	1.6	25	1.9	0.8	42
11	10.2	12	10.5	2.4	23	2.3	30	2.4	1.0	41
12	11.3	14	11.9*	–	–	1.8	44	0.9*	–	–
13	9.5	13	10.8	2.6	24	2.0	29	2.2	1.1	50
14	9.7	17	8.6*	–	–	3.5	25	2.7	–	–
Mean	8.7	14	9.0	2.1	24	1.7	33	1.6	0.7	49
± s.d.	2.4	2	3.1	0.8	5	0.8	7	0.7	0.3	15

* Not included in the mean.

§ Blood and CSF sampling performed on a different day from that reported in Table 1.

fraction ranging from 11-18% for carbamazepine, and a free fraction ranging from 24-44% for the epoxide. An evaluation of the possible relationships between the CSF/serum ratios and the % free drug measured by ultrafiltration at 22°C is presented in Figure 1a and 1b. The two parameters appear to be very significantly related by a direct linear relationship ($P < 0.01$ and $P < 0.025$) both for carbamazepine and the epoxide. It should also be emphasized that an inverse relationship between carbamazepine and epoxide CSF/serum ratios was observed (Figure 1c) ($P < 0.025$).

Discussion

According to the data reported here, fluctuations in carbamazepine and carbamazepine-10,11-epoxide concentrations appear to be independent of the number of daily doses administered. In the eight patients receiving carbamazepine (600-1800 mg/day) in two daily doses, the fluctuations in carbamazepine levels ranged from 28-80%, and in the group receiving the drug three times a day (600-1800 mg/day) the fluctuations were from 14-71%. Less variability was observed in the two patients receiving carbamazepine four times a day. The relatively lower fluctuations observed in the latter cases deserve further study and cannot be taken as significant on the basis of only two cases. Fluctuations in carbamazepine-10,11-epoxide levels were even greater (up to 200%), and such large fluctuations correspond well with the fact that the epoxide is metabolized faster than the parent compound (Morselli *et al.*, 1975). Another factor which has to be considered is that the ratio between the epoxide and its parent compound may vary considerably between individuals, indicating different metabolic rates.

Carbamazepine CSF concentrations were 24 ± 5 (s.d.)% of the serum levels, and were significantly related to the serum binding values. This finding confirms previous observations by Johannessen & Strandjord (1973) suggesting a linear relationship between antiepileptic drug CSF levels and serum protein binding.

The epoxide was constantly present not only in serum, but also in CSF at concentrations of 49 ± 15(s.d.)% of the serum levels, and this is in good agreement with *in vitro* binding data (Morselli, 1975). The data reported represent the first evidence that the epoxide may cross the blood-CSF barrier and are suggestive of a possibility of penetration into the brain. Preliminary data indicate that the epoxide may be present in human brain tissues at concentrations of the same order as those in serum (Gerna & Morselli, unpublished results). In our series of observations we did not register the wide inter-individual variability in carbamazepine serum protein binding described by Hooper *et al.* (1975). The reasons for the wide inter-individual variability for either anticonvulsants, or other drugs, may include factors such as the methodology used, the presence of other drugs or compounds capable of a displacing effect, and different groups of patients studied. Differences in free fatty acid levels and bilirubin, as well as alterations in albumin or other protein levels could also explain large variability in drug protein binding.

In the cases in this study, the clinical chemical analyses were, for all the patients, within normal limits, and with the exception of cases Nos. 4 and 13 no other drugs were administered. The fact that the free fraction observed by ultrafiltration at 22°C was lower than the CSF/serum ratio can be explained by the temperature effect on carbamazepine binding. The data are in good agreement with previous reports of Di Salle *et al.* (1974) and Hooper *et al.* (1975) on carbamazepine, and of Lunde *et al.* (1970) on phenytoin, showing that there may be a 50% increase in the free fraction on increasing the temperature from 22°C to 37°C.

Hooper *et al.* (1975) described a reduction in carbamazepine binding with increasing serum concentrations. In our experience this does not seem to be the case, at least for concentrations of carbamazepine up to 18 μg/ml and for carbamazepine-10,11-epoxide levels up to 4μg/ml.

We have no explanation for the inverse relationship observed between carbamazepine and epoxide CSF/serum ratios. A possible interaction of the two compounds at the level of serum protein binding could be excluded on the basis of *in vitro* data (Morselli, 1975). The situation could, however, be completely different *in vivo* and further data are needed to clarify this point.

The relatively low protein binding of the epoxide, together with its low lipid-water partition coefficient (Gagneux, 1976) agree well with the fact that its apparent plasma half-life is shorter than that of carbamazepine (Morselli, 1975). Furthermore these factors can explain why epoxide levels are constantly lower than those of the parent compound, and that less accumulation of the epoxide seems to occur in the course of chronic treatment.

The epoxide has been shown to possess remarkable anticonvulsant activity in the experimental animals (Frigerio & Morselli, 1975). The data reported here and the preliminary observation on its presence in human brain in concentrations similar to those in serum (Gerna & Morselli, unpublished results) suggest that the epoxide

could play a role in the anticonvulsant effect of carbamazepine in man.

In conclusion, the daily fluctuations in carbamazepine serum levels do not appear to be strictly related to the dosage schedule, and there is a very close relationship between CSF/serum ratios and serum protein binding, both for carbamazepine and carbamazepine-10,11-epoxide. The inter-individual variability in binding was limited and in agreement with our previous studies. Theoretically, the degree of serum protein binding of carbamazepine and its epoxide would not appear to be an important determinant for the therapeutic outcome. In fact, for a drug with an apparent V_d of about 2 litres/kg and a binding of 50% for the active metabolite, the impact of small variations in the free fractions should be a minor one.

Finally, the epoxide serum levels should always be taken into consideration when relating carbamazepine serum levels to the clinical efficacy in the course of long-term treatment.

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