SALIVARY CONCENTRATIONS AND PLASMA PROTEIN BINDING OF CARBAMAZEPINE AND CARBAMAZEPINE 10,11-EPOXIDE IN EPILEPTIC PATIENTS

J. J. MacKICHAN*, P.K. DUFFNER & M.E. COHEN

Division of Child Neurology, Children's Hospital of Buffalo and the Clinical Pharmacokinetics Laboratory, Millard Fillmore Hospital, Departments of Neurology and Pharmaceutics, Schools of Medicine and Pharmacy, State University of New York at Buffalo, New York, USA

- 1 The relationships between saliva, free and total plasma concentrations of carbamazepine (CBZ) and carbamazepine 10,11-epoxide (CBZ-EP) were studied in 24 chronically medicated epileptic patients. Four patients were taking CBZ alone, while 20 were taking one or more additional anticonvulsant drugs.
- 2 The free fraction of CBZ in plasma ranged from 0.19 to 0.33 (mean, 0.24) while the saliva:plasma (S:P) concentration ratios ranged from 0.20 to 0.35 (mean, 0.27). The free fraction of CBZ-EP in plasma ranged from 0.16 to 0.50 (mean, 0.32), while the S:P ratios ranged from 0.14 to 0.70 (mean, 0.43). The plasma protein binding and S:P ratios of these compounds appeared to be independent of age, sampling time and concurrently administered anticonvulsant drugs.
- 3 Significant linear relationships between saliva and total plasma concentrations and between saliva and free plasma concentrations were observed for both compounds ($P < 0.001$). However, salivary concentrations of CBZ and CBZ-EP were significantly more reliable as predictors of their respective free plasma concentrations than of their respective total plasma concentrations ($P < 0.01$).
- 4 It is concluded that measurement of CBZ and CBZ-EP in the saliva of chronically medicated epileptic patients provides a more reliable estimate of the pharmacodynamically active, free concentrations of these compounds in plasma.

Introduction

Carbamazepine (CBZ) is an anticonvulsant drug that has been shown to be as effective as phenytoin or phenobarbital in the treatment of generalized tonic-clonic and complex partial seizures (Cereghino *et al.*, 1974). Because there is a poor correlation between CBZ dosage and therapeutic efficacy (Morselli, 1975), adjustment of CBZ dosage in patients with convulsive disorders is often guided by measurements of plasma CBZ concentrations. Optimum seizure control is reported to occur when plasma CBZ concentrations are maintained in the range of 4 to 12 $\mu\text{g/ml}$ while concentrations greater than 12 $\mu\text{g/ml}$ have been associated with minor and major toxicities (Kutt, 1978).

The relationship between CBZ plasma concentra-

tions and therapeutic effect in humans may be complicated by the formation of a major metabolite, carbamazepine 10,11-epoxide (CBZ-EP). In rats, this metabolite has been shown to possess anticonvulsant properties comparable to those of the parent drug (Frigerio & Morselli, 1975). Although this activity has not yet been confirmed in man, it has been suggested that measurement of both CBZ and CBZ-EP in plasma would be more useful in the clinical management of epileptic patients than measurements of the parent drug alone (Eichelbaum *et al.*, 1975; Westenberget *et al.*, 1978).

Another factor which may complicate the interpretation of plasma CBZ concentrations is the variability in protein binding of CBZ and its metabolite. The degree to which a drug is bound to plasma proteins is a major determinant of its therapeutic and toxic effects. Since only the unbound fraction of a drug is pharmacologically active (Brodie,

* Present address: 217 Lloyd M. Parks Hall, 500 W. 12th Avenue, Ohio State University, Columbus, Ohio, USA 43210

1967), the use of total (bound and free) plasma drug concentrations as a guide to adjusting dosage in patients is confounded unless there is little inter-individual variability in the free fraction. The free fraction of CBZ in plasma of normal subjects and epileptic patients is reported to vary from 8 to 35% (Bertilsson, 1978) while the free fraction of the epoxide metabolite varies from 24 to 52% (Morselli *et al.*, 1975; Johannessen *et al.*, 1976). Because of the large interindividual variation in plasma protein binding of these compounds, adjustment of CBZ dosage may be better guided by monitoring free plasma CBZ and CBZ-EP concentrations.

Measurement of unbound plasma drug concentrations by methods such as ultrafiltration or equilibrium dialysis is time consuming, expensive, and therefore feasible only as a research procedure. An alternative approach to measuring free concentrations of drugs such as CBZ and CBZ-EP is to measure their respective drug concentrations in 'natural ultrafiltrates' of plasma, such as CSF or saliva. Several studies (Pynnönen, 1977; Rylance, Butcher & Moreland, 1977; Westenberg *et al.*, 1978) have demonstrated correlations between salivary and total plasma concentrations of CBZ. In this report, we have investigated the relationships between salivary, total and free plasma concentrations of both CBZ and its epoxide metabolite in epileptic patients and have assessed the reliability of salivary concentrations as an index of free and total plasma concentrations. The effects of anticonvulsant co-medication and total CBZ and CBZ-EP concentrations on the plasma protein binding of these compounds were also investigated. Concentrations of CBZ and CBZ-EP in saliva were found to provide a reliable estimate of the free, pharmacodynamically active fractions of these drugs in plasma, thus providing a convenient alternative to the measurement of these compounds in plasma.

Methods

Subjects

The 18 female and 6 male subjects of this study were routine patients of the Neurology Clinic of the Children's Hospital of Buffalo and ranged from 5 to 20 years of age. Four patients received CBZ alone, and 20 patients were taking one or more additional anticonvulsant drugs including sodium valproate, phenytoin, phenobarbitone, primidone, clonazepam and acetazolamide.

CBZ was given two to three times daily, at dosages ranging from 8 to 30 mg kg⁻¹ day⁻¹. The dosages of CBZ and other drugs were kept constant for at least 3 months prior to the study to ensure steady-state CBZ

concentrations. Compliance rate was assessed and confirmed by questioning the patients or the parents of the patients. The parents were informed of the purpose of this study before collection of samples and their consent was obtained.

One set of venous blood and saliva samples was obtained from each patient between 1 and 5 h following the administration of CBZ. Salivary flow was stimulated by chewing a short length of waxed film (Parafilm®) and saliva was collected by expectoration into a glass tube until at least 3 ml had been obtained. This procedure usually required less than 5 min. Whole blood (10 ml) was collected in heparinized tubes, centrifuged and the plasma separated. All samples were stored in glass tubes at -20°C until analyzed. Prior to analysis, saliva samples were centrifuged to separate mucous and cellular debris from supernatant saliva.

Saliva and total plasma concentrations of CBZ and CBZ-EP were analyzed in duplicate according to the liquid chromatographic method of MacKichan (1980). The ratio of salivary concentration to total plasma concentration (S:P) was calculated for each compound.

Protein binding

The binding of CBZ and CBZ-EP to plasma proteins was determined in duplicate by equilibrium dialysis. The system consisted of acrylic dialysis cells separated into two 1-ml chambers by a dialyzing membrane (Dialya-Por®), National Scientific, Cleveland, Ohio, U.S.A.). A volume of 0.8 ml of plasma was placed in one chamber and dialyzed against an equal volume of isotonic phosphate buffer (pH 7.4). The cells were gently shaken in a waterbath at 37°C for 24 h. Previous studies had indicated this to be of sufficient duration for attainment of equilibrium. At the end of each dialysis run, plasma and buffer were assayed for CBZ and CBZ-EP by liquid chromatography (MacKichan, 1980). The highest inter- and intra-day coefficients of variation obtained using this method were 7% for CBZ and 6.3% for CBZ-EP.

The fraction of CBZ or CBZ-EP unbound in each plasma sample was calculated as follows:

$$\text{Free fraction} = 1 - ((C_p - C_b) / C_p)$$

where C_p is the drug concentration on the plasma side and C_b is the concentration on the buffer side of the dialysis cell. Preliminary experiments using pooled human plasma had demonstrated constant protein binding of CBZ and CBZ-EP between 1 and 30 µg/ml. Free plasma concentrations were therefore calculated by multiplying the pre-dialysis total plasma concentration by the free fraction value determined in equilibrium dialysis experiments.

Table 1 CBZ and CBZ-EP concentrations (mean $\pm$ s.d.) in plasma and saliva of epileptic patients

Compound	Total plasma concentration ($\mu\text{g/ml}$)	Free plasma concentration ($\mu\text{g/ml}$)	Saliva concentration ($\mu\text{g/ml}$)	Free plasma fraction	S:P _a
CBZ (<i>n</i> = 24)	8.36 (2.55) (3.00–13.4) ^b	2.03 (0.687) (0.880–3.80)	2.31 (0.796) (0.640–4.10)	0.243 (0.036) (0.191–0.328)	0.270 (0.041) (0.207–0.349)
CBZ-EP (<i>n</i> = 23) ^c	3.38 (1.34) (0.56–5.72)	1.11 (0.550) (0.300–2.44)	1.49 (0.784) (0.300–2.98)	0.318 (0.073) (0.164–0.497)	0.427 (0.127) (0.135–0.714)

^a S:P = Saliva:Plasma concentration ratio^b Range^c CBZ-EP was not detected in the saliva or plasma dialysate of one subject.

Results

Table 1 summarizes the plasma protein binding and salivary concentration data of CBZ and CBZ-EP in 24 epileptic patients. Only 3 out of 24 patients had total plasma CBZ concentrations outside of the designated therapeutic range of 4 to 12 $\mu\text{g/ml}$. Concentrations of CBZ-EP were not detected in the saliva or plasma dialysate of one subject. Mean salivary concentrations of CBZ and CBZ-EP were 14% and 31% higher than their corresponding mean free plasma concentrations ($P < 0.001$). As a result, the calculated S:P ratios for CBZ (0.27) and CBZ-EP (0.43) were higher than their corresponding free plasma fractions, 0.24 and 0.32 respectively ($P < 0.001$).

Free plasma fractions and S:P ratios of CBZ and CBZ-EP were not related to sampling time, sex or age of the patients. The presence of concurrently administered anticonvulsants also did not appear to affect the protein binding of these compounds. The mean free plasma fractions and S:P ratios of CBZ and CBZ-EP were not significantly different between those patients taking CBZ alone ($n = 4$) and those taking CBZ with other anticonvulsants ($n = 20$). There was a slight trend indicating higher free fractions and S:P ratios of CBZ and CBZ-EP at higher total plasma concentrations of these compounds. The increases were not significant, however, over the plasma concentration ranges studied.

The relationships between saliva and total plasma concentrations of CBZ and CBZ-EP are shown in Figures 1a and 1b. Using the Student's *t*-test the intercepts of these regression lines were not found to be different from zero. The regression line slopes (0.28 for CBZ and 0.51 for CBZ-EP) agree with the mean S:P ratios of 0.27 and 0.43 for CBZ-EP reported in Table 1.

The relationships between salivary CBZ and CBZ-EP concentrations and their corresponding free plasma concentrations are shown in Figures 2a and

Figure 1 The relationships between saliva concentrations and total plasma concentrations of CBZ (a) and CBZ-EP (b) in epileptic patients. The dotted lines represent the least-squares regression lines of best fit. a) $y = 0.28x - 0.08$, $r = 0.91$, $P < 0.001$. b) $y = 0.51x - 0.26$, $r = 0.88$, $P < 0.001$.

Figure 2 The relationships between saliva concentrations of CBZ (a) and CBZ-EP (b) in epileptic patients. The dotted lines represent the least-squares regression lines of best fit. a) $y = 1.07x + 0.11$, $r = 0.94$, $P < 0.001$. b) $y = 1.29x + 0.06$, $r = 0.91$, $P < 0.001$.

2b. These variables were related for CBZ by a slope of 1.07 which was not significantly different from unity. Saliva and free plasma concentrations of the epoxide metabolite were related by a slope of 1.3, which is consistent with the finding that average salivary concentrations were 31% higher than free plasma concentrations (Table 1).

The reliability of using salivary drug concentrations to predict free and total plasma concentrations was assessed for both compounds. Using the appropriate regression equations (Figures 1 and 2), free and total CBZ and CBZ-EP plasma concentrations were calculated using their corresponding salivary concentra-

tions. These calculated values were then compared to the measured values and a percentage deviation obtained as follows:

$$\% \text{ Deviation} = \frac{((\text{Calculated} - \text{Measured}) / \text{Measured}) \times 100.}$$

Selecting 15% as the maximum acceptable deviation, the prediction of total plasma concentrations by salivary concentrations was poor. About one-third of the calculated total plasma concentrations for CBZ and CBZ-EP respectively, deviated more than 15% from measured values. In contrast, salivary concentrations were better predictors of free plasma concentrations for both compounds. In only 2 out of 24 (8%) patient samples did the calculated free plasma CBZ concentrations deviate more than 15% from measured values, thus demonstrating considerable accuracy. Prediction of free CBZ-EP plasma concentrations was somewhat less reliable as 5 out of 23 (22%) calculated values deviated more than 15% from measured values. These differences in predictability (percentage deviation values) were significant at $P < 0.01$ using the Wilcoxon Rank Sum test (Remington & Schork, 1970).

A direct relationship between the free plasma fractions of CBZ and its epoxide metabolite was observed in this study ($r = 0.50$, $0.50 < P < 0.025$). A similar relationship was also seen between the S:P ratios of these compounds ($r = 0.68$, $P < 0.001$). Although interindividual differences in total protein or albumin concentrations were sought as an explanation for these relationships, inadequate residual plasma sample volume prohibited protein concentration measurements in these samples.

A relationship between concurrently administered anticonvulsant drugs and the ratio of epoxide metabolite to parent drug in plasma was observed. The mean CBZ-EP:CBZ plasma concentration ratio was 0.24 ± 0.07 (mean $\pm$ s.d.) in 4 subjects taking CBZ alone and 0.45 ± 0.020 in 20 patients taking CBZ in combination with one or more additional drugs ($P < 0.001$). Ten of those 20 patients were taking CBZ with sodium valproate only. The mean plasma CBZ-EP:CBZ ratio in this subgroup was 0.41 ± 0.11 and also significantly higher than the mean ratios in those patients taking CBZ alone ($0.005 < P < 0.001$). Because of the limited patient population, other drugs or drug combinations could not be tested for their contribution to the observed phenomenon.

Discussion

The results of this study demonstrate strong relationships between salivary, free and total plasma concentrations of CBZ and its epoxide metabolite in epileptic patients. Similar relationships have been demonstrated by other investigators for CBZ

(Pynnönen, 1977; Rylance *et al.*, 1977; Westenberg *et al.*, 1978; McAuliffe *et al.*, 1977) and CBZ-EP (Christiansen & Dam, 1977). The agreement between our results and those of others supports the reliability of our methodology.

Salivary concentrations of CBZ and the epoxide metabolite were higher than their respective free plasma concentrations. Similar findings were reported by Troupin & Friel (1975) for CBZ and by Christiansen & Dam (1977) for CBZ and CBZ-EP. There are several possible explanations for this. Contamination of saliva by drug that has been retained in the mouth following oral intake has been reported for CBZ (Rylance *et al.*, 1977) as well as for other drugs. This situation usually yields much higher and more sporadic salivary concentrations than were observed in this study, and also does not explain why epoxide metabolite salivary concentrations were also high relative to free concentrations in plasma. It could also be postulated that salivary CBZ and CBZ-EP concentrations were elevated due to the presence of proteins in whole saliva that can bind these compounds. Measurement of CBZ and CBZ-EP in a specific salivary fraction, such as parotid fluid, may allow a more reliable estimate of free drug concentration. The simplest explanation for high salivary drug concentrations relative to free plasma concentrations is evaporation of saliva in the mouth during the collection procedure.

Salivary concentrations of CBZ and CBZ-EP were found to be poor predictors of their corresponding total plasma concentrations despite significant correlations between these variables. Quantitation of the deviation of predicted plasma concentrations from measured values provides a more rigorous means of assessing the clinical utility of saliva measurements as predictors of plasma concentrations. Observations that salivary concentrations are better predictors of free than of total plasma concentrations may be explained by the interindividual variation in plasma protein binding observed for CBZ (19 to 30%) and CBZ-EP (16 to 50%). According to theory, the salivary concentration of a drug like CBZ depends almost entirely upon its free fraction in plasma (Mucklow *et al.*, 1978). Measurements of CBZ and CBZ-EP in saliva consequently should provide a better estimate of free drug in plasma than of total drug, especially when large interindividual differences in protein binding exist.

The range of CBZ free fractions reported in this study of epileptic patients is similar to the ranges reported in studies of normal adults (Rawlins *et al.*, 1975; Hooper *et al.*, 1975). In contrast to our results, Hooper *et al.*, (1975) reported a much wider range of free CBZ percentages (8 to 60%) in the plasma of 54 epileptic patients. Because characteristics such as age, sex, concurrent diseases and concurrent drug therapy were not reported for these 54 patients

(Hooper *et al.*, 1975), their contribution to the observed interindividual variability cannot be evaluated or compared to the results of our study. There are few reports of CBZ-EP protein binding in epileptic patients. Morselli *et al.*, (1975) reported free CBZ-EP percentages of 47 to 53% in a group of normal subjects while Johannessen *et al.* (1976) reported a larger range (26 to 71 %) based on the determination of CSF:serum concentration ratios of the epoxide metabolite in 14 patients treated with CBZ alone. This is in good agreement with the range of saliva:plasma concentration ratios reported in this study (13.5 to 65.5%) as well as the range of free CBZ-EP percentages in plasma (16 to 50%).

The free fractions of CBZ and CBZ-EP in plasma were constant over the concentration range studied. This agrees with the results of *in vivo* (Johannessen *et al.*, 1976) and *in vitro* (Rawlins *et al.*, 1975) studies of these compounds over similar concentration ranges and indicates that saturation of protein binding is unlikely to occur within the therapeutic concentration range for this drug. Non-linear protein binding may occur, however, at supra-therapeutic concentrations of CBZ. In an *in vitro* study of CBZ concentrations ranging as high as 50 µg/ml, Hooper *et al.* (1975) reported that a reduction in percentage of CBZ bound occurred as total serum concentrations increase. This may account in part for the slight trend towards higher free drug fractions with higher plasma concentrations observed in this study. The protein binding of CBZ and CBZ-EP also did not appear to be affected by co-administration of other drugs. Because these compounds are not highly protein bound, it is unlikely that other drugs would compete with them for binding (Meyer & Guttman, 1968). This is supported by the results of *in vitro* studies with CBZ (Morselli *et al.*, 1975; Hooper *et al.*, 1975) which showed that the presence of other drugs, including phenytoin, phenobarbital, clonazepam and sodium valproate, did not appreciably alter its protein binding.

The plasma protein binding of CBZ paralleled that of its epoxide metabolite in this study, as shown by a direct relationship between the free fractions of these compounds in plasma. This indicates, for example, that when the free fraction of CBZ is high in a given individual, the free fraction of epoxide metabolite is also relatively high. This relationship might be explained on the basis of interindividual differences in total protein or albumin concentrations. According to theory (Jusko & Gretch, 1976), compounds having weak to intermediate association constants for albumin may show changes in binding which are somewhat proportional to plasma protein concentrations. Since the association constants of CBZ and CBZ-EP are similar and relatively weak (DiSalle, Pacifici & Morselli, 1974; Morselli, 1975), the protein binding of both compounds may be a function of

plasma protein concentrations, thus resulting in the observed parallel binding pattern. Hooper *et al.* (1975) observed no relationship between albumin concentrations and the *in vitro* protein binding of CBZ in the plasma of 25 patients with hepatic disease. This situation could, however, be completely different *in vivo* and further data are needed to clarify this point.

The epoxide to parent drug concentration ratios were larger and more variable in those patients taking CBZ with other anticonvulsant drugs as compared to those taking CBZ alone. Similar findings have been reported by other investigators (Rane, Höjer & Wilson, 1976; Westenberg *et al.*, 1978) and have been attributed to induction by other anticonvulsant drugs of enzymes responsible for the metabolism of CBZ (Rane *et al.*, 1976; Pymönen *et al.*, 1978). The induction of CBZ metabolism by drugs such as phenobarbitone and phenytoin has been demonstrated (Christiansen & Dam, 1975). In contrast, animal and human studies have demonstrated a lack of significant enzyme-inducing properties by sodium valproate (Jordan, Shillingford & Steed, 1976; Perucca *et al.*, 1979). Our finding that sodium valproate in combination with CBZ will increase the concentration of epoxide metabolite relative to the concentration of CBZ is therefore not consistent with a theory of enzyme induction and deserves further investigation.

In this report we have presented several factors that can complicate the interpretation of plasma CBZ concentrations with respect to therapeutic efficacy or toxicity of this drug. Firstly, although present knowledge of the activity of CBZ-EP in humans is not very reliable, it may contribute to the observed therapeutic or toxic effects of CBZ. The relationship between plasma concentrations of CBZ and its metabolite varies considerably from patient to patient, and depends in part on concurrently administered anticonvulsant drugs. Attempts to correlate clinical efficacy and toxicity of CBZ with drug concentrations in plasma may therefore be aided by simultaneous analysis of both CBZ and CBZ-EP. If further studies demonstrate significant anticonvulsant activity of this metabolite in humans, the therapeutic plasma or saliva concentration range should be redefined to include both compounds.

Another factor which can complicate the interpretation of total plasma concentrations of these compounds is the considerable interindividual variability in the protein binding of CBZ and CBZ-EP. The free plasma fractions of CBZ and CBZ-EP in this study ranged two- and three-fold respectively. Since only the unbound fraction of drug is considered to be therapeutically active, measurement of total plasma CBZ and CBZ-EP concentrations may not necessarily correlate with the therapeutic or toxic effects of this drug. The relatively large interindividual variation in percentage free CBZ and CBZ-EP may further imply that the reported therapeutic range of total plasma concentrations is unnecessarily large. With a large degree of variation in the protein binding, the range of total plasma drug concentrations necessary to accommodate the therapeutic range of unbound drug in plasma becomes wider.

We have shown in this study that measurements of salivary concentrations of CBZ and possibly CBZ-EP provide a clinically reliable estimation of their free, therapeutically active concentrations in plasma. As demonstrated by Troupin & Friel (1975), measurement of these compounds in saliva also provides good estimates of these drug concentrations in CSF, which may be a more accurate reflection of drug at the site of action. Saliva collection is noninvasive, painless and may allow collection of serial samples in those patients who cannot tolerate excessive blood loss, such as paediatric and geriatric patients. Based on the average S:P ratio of 0.27 in this study, and the previously established therapeutic range of total plasma CBZ concentrations of 4 to 12 µg/ml, the initial guidelines for a therapeutic range of CBZ in saliva is 1.1 to 3.2 µg/ml. Efficacy studies relating salivary CBZ concentrations to therapeutic and toxic effects will probably reveal a narrower therapeutic range for free drug in saliva. The definition of a therapeutic range for the epoxide metabolite in saliva also awaits further studies.

This work was supported in part by Grant GM-24211 from the National Institutes of General Medical Sciences, National Institutes of Health, and the Children's Hospital Rehabilitation Center, Buffalo, New York, U.S.A.

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(Received July 4, 1980)