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Original articles

Salivary free concentrations of Anti-Epileptic Drugs : an evaluation in a routine clinical setting

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Abstract

Objective : This study was aimed at correlating the salivary and serum free concentrations of anti-epileptic drugs (carbamazepine, phenytoin and sodium valproate) in a population of neurological patients in a routine clinical setting.

Method : Twenty-seven paired serum/saliva specimens from 22 patients : 10 for carbamazepine, 8 for phenytoin and 9 for sodium valproate were obtained to study these correlations. Salivary and serum free concentrations of anti-epileptic drugs, anti-epileptic drug dosing history, and associated information were collected prospectively. The salivary and serum free concentrations of the anti-epileptic drugs were simultaneously quantified using fluorescence polarization immunoassay (TDx analyzer).

Results : For both carbamazepine and phenytoin there was a strong correlation between the salivary and serum free concentrations, 0.99 and 0.98, respectively. The mean ratio of salivary to serum free carbamazepine concentration was 1.02 ± 0.11 and 0.82 ± 0.15 for phenytoin. A poor correlation between salivary and serum free concentration was observed for sodium valproate (0.70) with a mean ratio of salivary to serum free concentration of 0.48 ± 0.27.

Conclusion : Monitoring of free salivary concentrations of anti-epileptic drugs, particularly phenytoin and carbamazepine proved to be a realistic alternative in this routine clinical setting.

Key words : Adults ; carbamazepine ; epilepsy ; phenytoin ; sodium valproate ; unbound ; saliva ; serum.

Introduction

In view of their broad therapeutic spectrum and proven efficacy carbamazepine (CBZ), phenytoin (PHT) and sodium valproate (VPA) are important drugs in the treatment of a variety of seizure disorders in adults, including generalized tonic-clonic and partial seizures. Unfortunately, the use of some of these anti-epileptic drugs (AEDs) is complicated by their unique pharmacokinetic properties. At therapeutic levels, both CBZ and PHT exhibit marked intra- and interindividual as well as ethnic variability in the relationship between serum concentrations and dose, have a narrow therapeutic window and potential interactions with other AEDs with enzyme inducing capacity (e.g. phenobarbital). (Bertilsson *et al.*, 1996 ; Tomson *et al.*, 1989 ; Deleu *et al.*, 1999 ; 2001). In addition, CBZ causes autoinduction, formation of an active metabolite (CBZ-10,11-epoxide) and PHT elimination follows a saturation kinetics. VPA has a saturable binding to plasma proteins and like the other AEDs a wide interindividual variation.

Several reasons prompted us to explore the possibility of monitoring salivary concentrations of AEDs : firstly, the observation that salivary concentrations of several AEDs have proven to be valuable alternatives for the determination of their serum levels (Liu *et al.*, 1999 ; McAuliffe *et al.*, 1977) ; secondly, the potential for a significant concentration-effect relationship for the AED under consideration ; thirdly, salivary AED concentrations are assumed to reflect their free levels in tissue and may therefore have greater clinical relevance than measurements of AED concentrations in serum ; and finally, increased prevalence of human immunodeficiency virus and hepatitis infected individuals and the associated risk of accidental contamination during the manipulation of blood samples. Furthermore, saliva sampling has the advantage of being simple, not requiring any specific facilities/expertise for collection of specimens, inexpensive and noninvasive, particularly in epileptic patients with refractory seizures and epilepsy in pediatrics requiring frequent therapeutic AED monitoring. Unlike for VPA (Gorodischer *et al.*, 1997), good correlations have been reported between serum total and salivary concentrations for CBZ and PHT, with low intraindividual variability. (Bachmann *et al.*, 1983 ; Miles *et al.,* 1990 ; 1991).

The aim of this study was to assess the reliability and suitability of salivary free concentrations of CBZ, PHT and VPA, and to correlate them with their corresponding serum free concentrations, in a population of adult neurological patients taking these AEDs. Furthermore, the study explored the

feasibility of salivary AED monitoring for routine clinical purpose.

Patients and methods

Patients

Patient data were collected from routine clinical visits at the Neurology Clinic of SQUH. Twenty-two adult patients (15 male and 7 female) with neurological disorders who were taking either CBZ, PHT or VPA orally in monotherapy or in combination for at least 4 weeks were invited to participate in this prospective study. Nineteen patients suffered from epilepsy (8 had primary generalized tonic-clonic seizures ; 11 partial seizures 6 with and 5 without secondary generalization), one patient had trigeminal neuralgia and two patients were treated with AEDs for primary chronic daily headache. Their ages ranged from 16 to 75 years (28.1 ± 12.4 years (mean ± S.D.)) and body weights ranged from 45 to 105 kg (73.3 ± 18.6 kg, mean ± S.D.). The dosages, of the AED(s) averaged 8.50 mg/kg/day (range 5.41 – 14.46 mg/kg/day) for CBZ, 4.12 mg/kg/day (range 2.50 - 5.66 mg/kg/day) for PHT and 9.24 mg/kg/day (range 4.21 – 18.87 mg/kg/day) for VPA. Seventeen patients were taking AEDs in monotherapy. The patients' liver and renal functions were normal and all patients had normal albumin values. None were smokers or consumed alcohol. The patients did not take any interfering concomitant medication.

The Medical Research and Ethics Committee approved the study and verbal consent was obtained from all participating patients.

Sampling and drug assay

To ascertain that all patients attained steady-state conditions with respect to their anti-epileptic therapy they had to be on the same dose for at least 5 weeks. (Grasela *et al.*, 1983 ; Martin *et al.*, 1991, Zaccara *et al.*, 1988). Venous blood and salivary samples were obtained simultaneously at least 3 hours after the intake of the last dose of the AED(s). (Ayers *et al.*, 1977 ; Dickinson *et al.*, 1985). Two to three milliliters of serum was obtained from venous blood and stored at -80°C until assayed. Patients rinsed their mouths thoroughly and repeatedly with water, and received 5 ml of lime juice to stimulate salivary secretion. They were asked to keep the lime juice for a 2-3 minutes in the mouth and then empty the mouth and rinse it thrice with water to remove any remnants of lime juice. Minimum one milliliter of liquid clear saliva was collected by draining it into a collecting tube. The salivary samples were then frozen at -80°C pending analytic determination. Analysis was carried out in the clear supernatant of saliva (following centrifugation ; 3,000 g at room temperature for 10 min). Serum and salivary samples containing only free microsolute of each AED and their (active) metabolites were prepared by a single 10 minute ultrafiltration using the MPS Micropartition device (cut-off 30,000 MW) (No. 4010 ; Millipore, Bedford, MA, U.S.A.). Both CBZ and its metabolites filtrate at the same proportion during the ultrafiltration. Subsequently, the free concentrations of the AEDs were quantified using fluorescence polarization immunoassay using a TDx analyzer (Abbott Diagnostics, North Chicago, IL, U.S.A.). The instrument was calibrated prior to each day's run and each subject's serum and salivary samples were assayed in a single run. The reliability of the AED determinations in saliva by the fluorescence polarization immunoassay was excellent : average intra- and interassay coefficients of variation (CV) for each of the AEDs were less than 5% in serum and less than 1.5% in saliva. The average recovery over the therapeutic ranges for the serum free concentrations of CBZ, PHT and VPA was 95.5 ± 1.1%, 100.2 ± 2.9% and 100.6 ± 1.9%, respectively. The corresponding values in saliva were 100.2 ± 5.3%, 99.6 ± 1.8% and 99.2 ± 4.4%, respectively. The limits of detection for the free concentrations of the AEDs were the following : CBZ 0.2 μmol/L (0.05 μg/ml), PHT 0.08 μmol/L (0.02 μg/ml) and VPA 0. 69 μmol/L (0. 10 μg/ml).

Statistical analysis

The data are presented as mean (± S.D.) with their range. Where appropriate, linear regression analysis was used to determine correlations. A two-tailed Student's *t*-test for paired data was used to compare between two means. Statistical significance was set at $p < 0.05$.

Results

AED free concentrations were measured in 27 paired serum/saliva specimens from 22 patients : 10 for CBZ, 8 for PHT and 9 for VPA. The average salivary and serum free concentrations of CBZ, PHT and VPA, and the correlation between salivary and serum free concentration from each of the AEDs are summarized in Table 1.

The scatter plot of the salivary versus serum free concentrations of CBZ (Fig. 1 A) revealed a good linear relationship (correlation coefficient, r = 0.99, p < 0.0001). The slope of the regression analysis was equal to : y = 0.95 x + 0.23 (with y being the serum free concentration of CBZ and x the salivary free concentration of CBZ). The mean ratio of salivary to serum free concentration of CBZ was 1.02 ± 0.11 (range 0.89 to 1.27), which is close to the slope of the regression analysis for CBZ (0.95). Similar as for CBZ, Fig. 1B demonstrated a good linear relationship (correlation coefficient, r = 0.98, p < 0.0001) between the salivary and serum free

Table 1

Average salivary and serum free concentrations of CBZ, PHT and VPA, and correlation (r) between salivary and serum free concentration for each of the antiepileptic drugs. Concentrations are expressed as mean ± S.D. (range)

Anti-epileptic drug	Serum free concentration	Salivary free concentration	Correlation coefficient (r)
CBZ (n = 10)	7.5 ± 4.7 µmol/L (0.5-16.5 µmol/L)	7.6 ± 4.9 µmol/L (0.5-17.0 µmol/L)	r = 0.99 (p < 0.0001)
PHT (n = 8)	5.1 ± 3.3 µmol/L (0.9-9.0 µmol/L)	4.0 ± 2.5 µmol/L (0.7-7.2 µmol/L)	r = 0.98 (p < 0.0001)
VPA (n = 9)	30.5 ± 18.7 µmol/L (1.2-60.4 µmol/L)	14.0 ± 16.1 µmol/L (1.0-54.7 µmol/L)	r = 0.70 (p = 0.04)

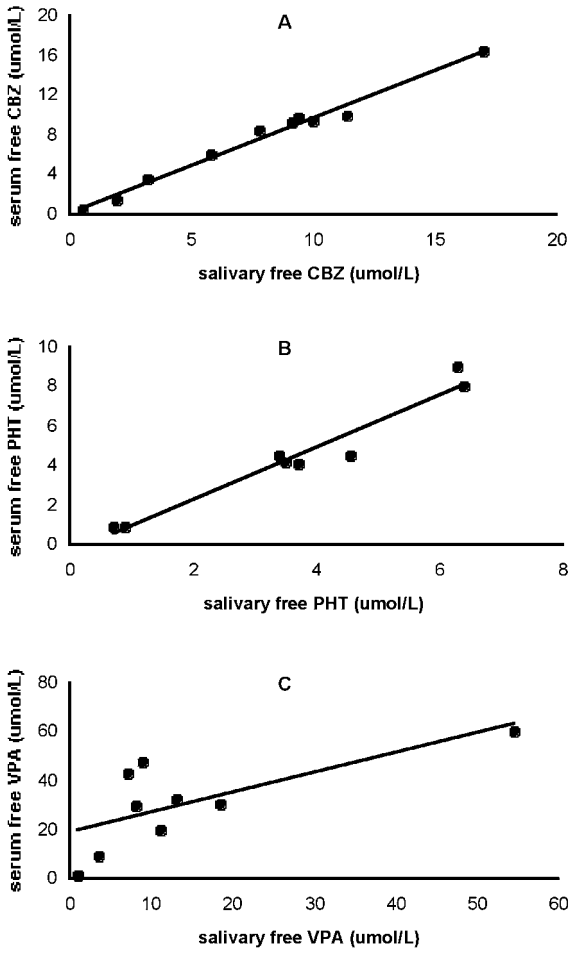

FIG. 1. — Relationship between salivary and serum free concentrations of carbamazepine (A), phenytoin (B) and sodium valproate (C) in adult patients with neurological disorders. CBZ, Carbamazepine ; PHT, phenytoin ; VPA, sodium valproate.

concentration of PHT. The slope of the regression analysis for PHT was : y = 1.33 x – 0.44 (with y being the serum free concentration of PHT and x the salivary free concentration of PHT). The mean ratio of salivary to serum free concentration of PHT was 0.82 ± 0.15 (range 0.70 to 1.01). Unlike for the CBZ and PHT, the correlation coefficient (r) between the salivary and serum free concentrations of VPA was 0.70 ($p < 0.04$) (Fig. 1C). The slope of the regression analysis for VPA equaled : y = 0.81 x – 19.09 (with y being the serum free concentration of VPA and x the salivary free concentration of VPA). The mean ratio of salivary to serum free concentration of VPA was 0. 48 ± 0.27 (range 0.17 to 0.91).

In relation to the dosage of the AED(s) taken by the patients, there was a good correlation between the dose of CBZ and VPA, and their corresponding salivary and serum free concentrations. For CBZ the dose/serum correlation coefficient (r) was 0.70 ($p = 0.02$) and the dose/saliva correlation coefficient (r) was equal to 0.68 ($p < 0.03$). For VPA, the correlation coefficients (r) for dose/serum and dose/saliva were 0.78 ($p = 0.01$) and 0.92 ($p < 0.0001$), respectively. The correlation coefficients for PHT were in the same range (dose/serum, r = 0.64 and dose/saliva, r = 0.70) but did not reach statistical significance.

Discussion

This study is unique since it is the first in which the salivary and serum free concentrations of three most commonly used AEDs (CBZ, PHT and VPA) are correlated during therapeutic monitoring in a routine clinical setting.

The conventional AEDs have a number of potentially troublesome pharmacological properties that complicate clinical management. PHT and VPA are highly bound to plasma protein (approxinately 90%) which is important to consider, because only the free drug is pharmacologically active. Many factors influence plasma protein concentration, including age, trauma, renal or hepatic disease, pregnancy, inflammatory disorders, and drug-drug interactions. Albumin concentrations generally decrease with age, resulting in higher concentrations of e.g. free PHT, whereas α_1-acid glycoprotein concentrations generally increase with age. Of the AEDs, CBZ (and its 10, 11-epoxide metabolite to a lesser extent) binds to α_1-acid glycoprotein. This binding may lead to higher total serum concentrations of CBZ in the elderly. Furthermore, all of the conventional AEDs rely primarily on hepatic metabolism. This leads to pharmacokinetic variability due to saturable metabolism (for PHT) and autoinduction (for CBZ). Because of these clinical situations, serum free concentrations of AEDs are a

useful guide in adjusting dosage, checking compliance and preventing toxicity.

Different sampling techniques have been developed for the determination and quantification of AED free concentrations in serum and saliva, including ultrafiltration, ultracentrifugation, and equilibrium dialysis. (Liu *et al.*, 1999) Unstimulated and stimulated saliva production of AEDs (CBZ and PHT) provides concentrations in the same range. Although, several methods have been used for the determination of concentrations of AEDs (Liu *et al.*, 1999), fluorescence polarization immunoassay has been reported to be one of the fastest, most precise and sensitive assay for the determination and quantification of free concentrations of AEDs in routine clinical monitoring.

The primary determinant, which governs the potential utility of saliva therapeutic drug monitoring for many drugs, is the pKa of the drug. Those AEDs, which are unionised within the salivary pH range, such as PHT (pKa = 9.2) and CBZ (pKa > 12) are theoretically minimally affected by changes in salivary pH resulting ftom the use of lime juice (citric acid). Plasma contains both the free and bound drug, while the diffusion of the drug between blood and saliva is restricted to the free drug (unbound fraction). Hence variability in the free fraction of the drug concentration can occur based on saturation of protein binding, changes in albumin concentration, changes in α_1-acid glycoprotein concentration and competitive inhibition between drugs. To avoid these potential interactions only free serum levels of AEDs are routinely measured at SQUH.

Recently, Liu and Delgado (1999) reviewed the relationship between salivary and serum concentrations of AEDs. The mean ratio of salivary to serum free CBZ concentration using different analytical techniques ranged between 1.39 to 1.44 with correlation coefficients ranging from 0.84 to 0.99. In the majority of these studies high performance liquid chromatography or gas-liquid chromatography were used for the quantification of CBZ. Only three studies used fluorescence polarization immunoassay, but in none of them serum free concentrations of CBZ were determined, and hence were unable to provide a mean ratio of salivary to serum free CBZ concentration. (Miles *et al.*, 1990 ; 1991, Rosenthal *et al.*, 1995). In the present study, the CBZ free concentrations covered the entire therapeutic range – for the treatment of seizures – of the drug in the serum (3.7 - 12.0 μmol/L or 0.87 – 2.84 mg/L) and proved to correlate significantly with the salivary free concentrations. In addition, the CBZ dose and salivary free concentrations were significantly related. Salivary total concentrations of the most important active metabolite of CBZ, CBZ-10,11-epoxide are in the range of 0.03 to 2.98 mg/L. (Liu *et al.*, 1999). In this concentration range the cross reactivity of CBZ-10,11-epoxide for the fluorescence polarization immunoassay for CBZ is in the range of 40% which indicates this metabolite contributes in the CBZ quantification.

Most studies reporting on the ratio of salivary to serum free PHT concentrations have used gas-liquid chromatography for their quantification. (Liu *et al.*, 1999). Only four studies reported on the use of fluorescence polarization immunoassay for the quantification of salivary and serum free concentrations of PHT (Bachmann *et al.*, 1983 ; Miles *et al.*, 1991 ; Cai *et al.*, 1993 ; Lifshitz *et al.*, 1990). In one of these studies, salivary free concentrations of PHT were reported 1.39 times greater than their corresponding serum free concentrations (Lifshitz *et al.*, 1990). However other studies could not confirm this observation and found ratios of salivary to serum free PHT concentrations closer to unity (0.99 to 1.11) (Bachmann *et al.*, 1983 ; Miles *et al.*, 1991 ; Cai *et al.*, 1993). In contrast with these reports, the ratio of salivary to serum free PHT concentration observed in the present study was much lower (0.85). Differences in analytical procedures including the use of salivary supernatant (Anavekar *et al.*, 1978) and the small sample size and perhaps the nature of our population (patients versus healthy volunteers) probably accounted for this. The present observation confirms the very high and strongly significant correlation between salivary and serum free concentrations of PHT. In this study, the PHT free concentrations covered the entire therapeutic range of the drug in the serum (2.8 – 9.6 μmol/L or 0.71 – 2.42 mg/L) and an excellent correlation was observed between salivary and serum free concentrations of PHT. However as could be expected from its pharmacokinetic properties, the PHT dose and salivary (and serum) free concentrations were not significantly related.

Free concentrations of VPA in saliva have been reported to be very erratic (Monaco *et al.*, 1982). The mean ratios of salivary to serum free VPA concentration range between 0 to 1.18 with correlation coefficients ranging from 0.41 to 0.43 (Van Hoeck, 1984). This poor correlation is explained by the changes in the pH gradient between serum and saliva to which this weak acidic drug (pKa = 4.9) can be subjected. Stimulation of saliva with citric acid obviously does not enhance the recovery of VPA in saliva, nor does it improve the correlation between salivary and serum free concentration of the drug (Gorodischer *et al.*, 1997).

Our results confirm the interpatient variability and difference between serum free concentration of the AED and its readily discernible and temporally coupled pharmacodynamic end-point (the clinical response) (e.g. seizure control or pain relief). (Hayes *et al.*, 1993 ; Garnett, 1995). The patient suffering from trigeminal neuralgia had low serum (and saliva) free concentrations of CBZ (1.5 and 1.9 μmol/L, respectively with a dose of 200 mg bid), but obviously the levels were sufficient to

control her neuralgic pain. Similar observation was made for the two patients treated with an AED for primary chronic daily headache. On the other hand, a patient with toxic (16.5 µmol/L) serum free concentrations of CBZ had no clinical signs of toxicity. Although the sample numbers are relative small the data indicate the presence of a linear relationship between the dosage and salivary free concentrations of CBZ and VPA. As expected, this linear relationship was not found for PHT which is most likely explained by its non-linear kinetics of the serum free drug concentration.

Conclusion

This study shows that CBZ, PHT and to a lesser extend VPA can be conveniently and accurately monitored in routine clinical practice of AEDs using lime juice-stimulated saliva and fluorescence polarization immunoassay. In view of this, salivary therapeutic drug monitoring may serve as a viable alternative to serum free concentration monitoring in routine clinical setting. From technical point of view, the use of salivary samples requires less preparation and is somehow more economical since they use up less consumables. The sample size in this study population was also too small to investigate the interaction between AEDs in those patients taking e.g. PHT and VPA concomitantly.

Further studies will indicate whether this approach is useful in clinical practice however the results in this group of patients were encouraging.

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