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A simple collection method for saliva in children: potential for home monitoring of carbamazepine therapy

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We have developed a method of monitoring plasma concentrations of antiepileptic drugs which uses salivary samples, and is suitable for home monitoring in children. To validate the method, paired plasma and saliva samples from 39 children aged between 2 and 15 years were assayed for carbamazepine and its active metabolite, carbamazepine-10,11-epoxide by h.p.l.c. The method uses a gauze-wrapped cotton ball with attached string, to absorb saliva which is then separated using a syringe and plunger. There was no adsorption of CBZ and CBZ-E and they were stable over 1 month in a domestic freezer. Plasma and salivary free CBZ and CBZ-E concentrations were highly correlated ($r^2 = 0.99$ and 0.98, respectively).

Keywords saliva carbamazepine home monitoring children

Introduction

Monitoring plasma antiepileptic concentration is an integral part of the management of childhood epilepsy. The regular blood sampling required is traumatic, fraught with technical difficulties, and affects the doctor-patient relationship. Regular monitoring is often hampered by large distances which patients have to travel. Moreover, seizures and acute alterations in the child's condition often occur at home where blood sampling is not feasible. Salivary antiepileptic concentrations have been used for non-invasive therapeutic drug monitoring. Free carbamazepine (CBZ) and carbamazepine-10,11-epoxide (CBZ-E) concentrations in saliva and plasma correlate (MacKichan *et al.*, 1981; Westernberg *et al.*, 1978). Thus, sampling saliva for measurement of drug concentrations would allow parents to collect saliva samples whenever necessary and store them in a freezer for later transport to the supervising hospital.

The aims of this study were to devise a simple, portable home method of saliva collection, to validate the relationship between CBZ + CBZ-E in saliva and simultaneously collected plasma samples, and to investigate the stability of CBZ and CBZ-E stored in a domestic freezer for a month.

This work was presented in part at the Joint Convention of the 5th International Child Neurology Congress and the 3rd Asian and Oceanian Congress of Child Neurology, Tokyo, November 1990.

Methods

A compacted cotton wool ball wrapped in gauze and securely attached to a string (Figure 1) is used to absorb saliva. The cotton ball, 1.2 cm in diameter, is placed at the front of the mouth. The end of the string is firmly held in case of accidental aspiration in a young or retarded child. Sufficient saliva is usually absorbed in a few minutes. The cotton ball is placed in the barrel of a 20 ml syringe and the saliva squeezed out, using the plunger. Samples were split into two portions, one assayed soon after collection, and the other after storage for 1 month.

Forty children, mostly outpatients, between 2 and 15 years were sampled. Simultaneous venous blood and saliva samples were obtained at least 3 h after the last carbamazepine dose to avoid contamination by residual oral drug (Dickson *et al.*, 1985). The plasma samples were ultrafiltered through Amicon Centrifree™ micropartition devices. CBZ and CBZ-E concentrations were measured in plasma and saliva by h.p.l.c. The method of Herkes *et al.* (1989) was used with the following modifications: mobile phase pH 5.1, flow rate 0.8 ml min^{-1} and an Activon 5 μ, C18, 25 cm column. The run time was 23 min. Total plasma carbamazepine was measured by fluorescence polarisation immunoassay. Each child and parent completed a questionnaire indicating their preference for blood or saliva sampling, without giving reasons for their preferences. The study protocol was approved by the Area Ethics Committee.

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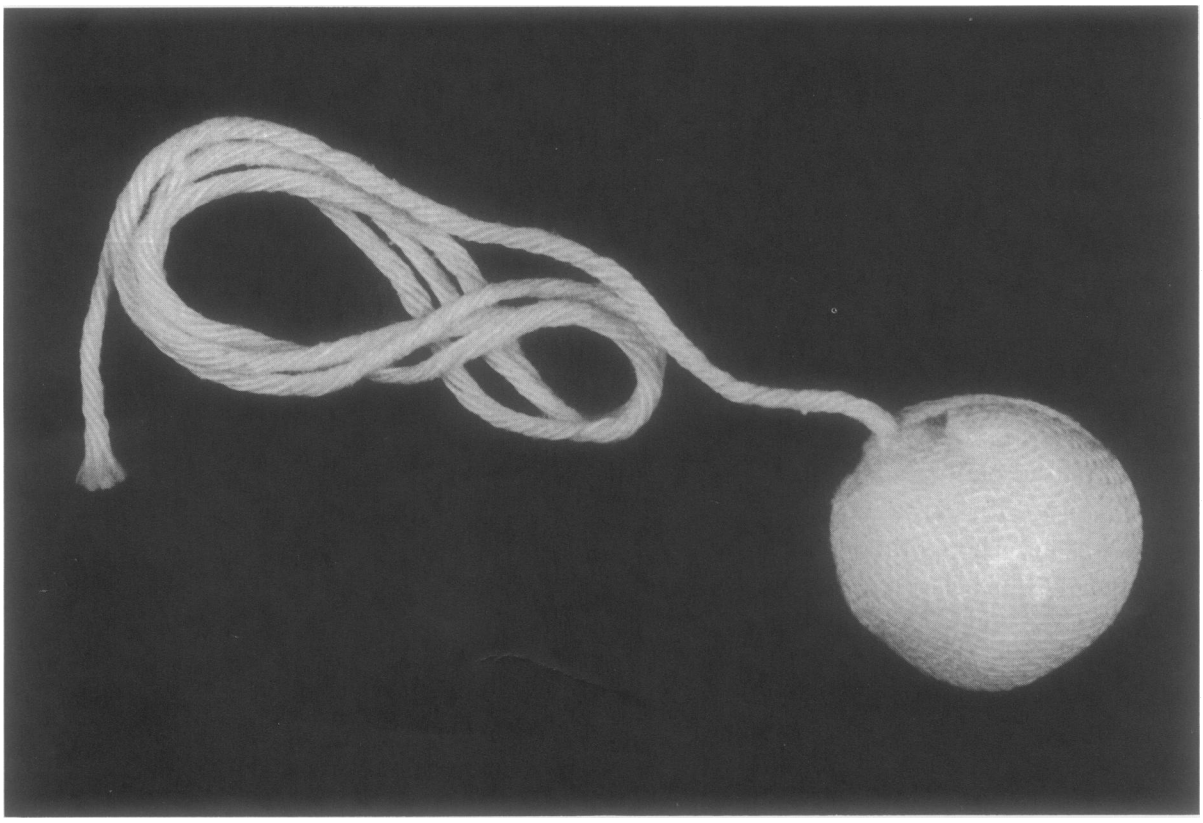

Figure 1 The saliva collection device—a cotton ball with attached string.

Aliquots of four pooled salivary solutions were prepared containing CBZ (4–17 μmol l^{-1}) and CBZ-E (2–6 μmol l^{-1}) were assayed before and after extraction from the cotton balls. Within run imprecision was determined from 10 replicate assays of low and high controls after extraction, and between run imprecision by running both controls with each run, over 30 days.

Results

Correlation coefficients (r^2) for the relationship between salivary and plasma free CBZ and CBZ-E were 0.99 and 0.98, respectively (Figure 2a,b). The ranges of CBZ and CBZ-E concentrations in saliva were 1.8–16.5 μmol l^{-1} and 0.1–3.9 μmol l^{-1}, respectively. The range of total plasma carbamazepine concentration was 18–51 μmol l^{-1}. The proportion of salivary CBZ to total plasma carbamazepine ranged from 10–50% with 95% of results falling between 10–30%. The proportion of CBZ-E to CBZ in saliva ranged from 0–110% with 85% of results being less than 30%.

Twenty-nine children and 30 parents completed the questionnaire. Not every parent agreed with their child in their preferences. Nineteen children preferred saliva sampling, nine preferred blood sampling and one was undecided. Twenty-one parents preferred saliva sampling, eight preferred blood sampling and one was undecided. Saliva was collected successfully from 39 out of 40 patients.

Sample storage over 1 month did not affect the accuracy of the method. Correlation coefficients between 'immediate' and 1 month CBZ and CBZ-E concentrations

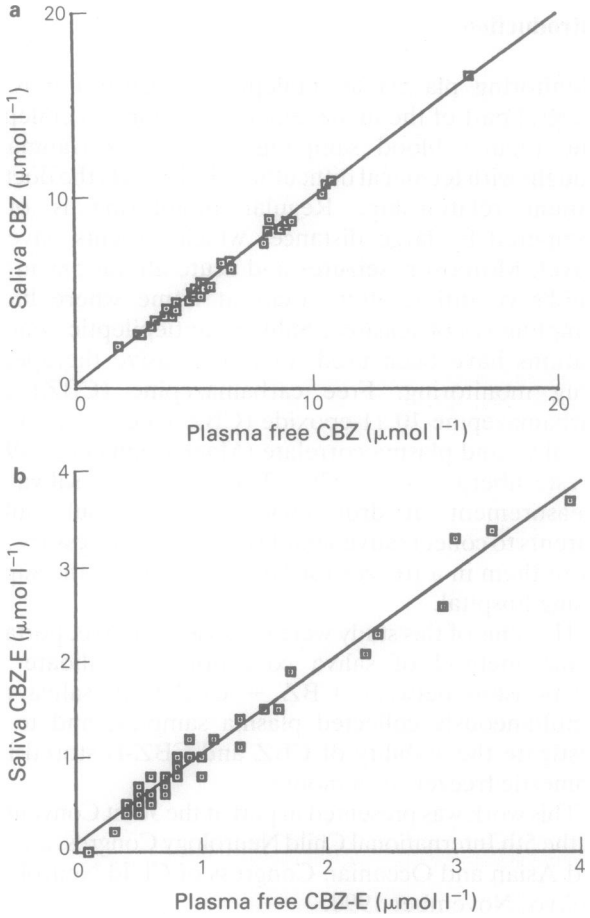

Figure 2 Relationship between salivary and plasma (a) free CBZ concentrations (y = 0.10 + 0.99x; r^2 = 0.99) and (b) free CBZ-E concentrations (y = 0.05 + 0.97x; r^2 = 0.98).

were 0.99 and 0.97, respectively. Assays on standard saliva solutions before and after extraction from the cotton ball, showed no significant differences using 1.0 ml or 1.5 ml volumes. The coefficients of variation (within run and between run, at low and high concentrations) for free CBZ and CBZ-E and for total carbamazepine ranged from 2–9%.

Discussion

As all specimens were collected at least 3 h after ingestion of carbamazepine, no contamination occurred despite the fact that no precautions were taken regarding mouth rinsing or time of food ingestion. All salivary drug concentrations were reliable predictors of free plasma drug concentrations. Stability of drug concentrations in saliva stored at +4° C (Mendez-Alvarez *et al.*, 1986) for several weeks has been established. We used a small bench-top single-door domestic refrigerator. The temperature fluctuation in its freezer was −7 to −15° C.

Various methods of saliva collection have been utilised. The simplest is dribbling into a container. This is not always possible, especially in younger children. Should multiple samples be required, dribbling may prove messy and socially unacceptable to the family. Suction devices have been popular. Most suction devices function best when attached to a source of continuous suction, which is difficult to supply in the home. Sampling saliva using cotton balls attached to a string was successful in 39 out of 40 patients. In a 2.75 year old retarded child who had very difficult venous access, saliva collection was the easier method. This method was preferred by the majority (2/3) of children and parents who answered the questionnaire. The 1/3 who preferred a venepuncture had been on chronic anticonvulsant therapy for many years and were used to having blood taken by skilled staff.

In conclusion, we have shown that accurate sampling for monitoring of salivary CBZ and CBZ-E concentrations in children at home is feasible.

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