Original Article

Journal of Epilepsy and Clinical Neurophysiology

J Epilepsy Clin Neurophysiol 2008; 14(2):55-58

Therapeutic Carbamazepine (CBZ) and Valproic acid (VPA) Monitoring in Children Using Saliva as a Biologic Fluid

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ABSTRACT

Objective: The aim of the study was to analyze retrospectively carbamazepine (CBZ) and valproic acid (VPA) salivary data collected from epileptic children during a 3-year period. **Methods:** Saliva samples stimulated by citric acid were assayed by FPIA method. One hundred and three patients (aged 1-14 years) were in CBZ or VPA monotherapy or in CBZ-VPA combined therapy. **Results:** VPA salivary levels were linearly related with daily dose, but a non-linear relationship was found for CBZ, in patients under monotherapy. VPA did not alter saliva CBZ concentration. Conversely, CBZ reduced VPA salivary levels. Non-responsive children displayed higher VPA concentrations. CBZ levels in uncontrolled patients showed non-significant difference in relation with controlled subjects even though their daily doses were higher. **Conclusion:** Citric acid stimulated saliva is reliable enough to perform therapeutic drug monitoring. Saliva drug levels in non-responsive patients would be explained according to the generalized efflux transporter overexpression hypothesis.

Keywords: Saliva concentration, carbamazepine, valproic acid, refractory epilepsy, efflux transporters.

RESUMO

Monitoramento terapêutico de carbamazepina e ácido valproico em saliva de crianças

Objetivo: O objetivo deste estudo foi avaliar retrospectivamente por 3 anos a partir de dados salivares, as terapias com carbamacepina (CBZ) e ácido valproico (VPA) em pacientes pediátricos. **Métodos:** Foram avaliadas amostras de saliva estimuladas com ácido cítrico por método FPIA em 103 pacientes (idades 1-14 anos) em monoterapia com CBZ ou VPA ou terapia combinada CBZ-VPA. **Resultados:** Níveis salivares de VPA se relacionaram linearmente com a dose diária, e a relação não linear foi encontrada em pacientes com CBZ. VPA não alterou as concentrações salivares de CBZ, porém a CBZ reduziu os níveis salivares de VPA em pacientes com terapia combinada. Pacientes refratários apresentaram altas concentrações de VPA. Os níveis de CBZ em pacientes não controlados não apresentaram diferenças significativas em relação aos pacientes controlados quando as doses diárias foram mais elevadas. **Conclusão:** Saliva estimulada com ácido cítrico é adequada para o monitoramento terapêutico. Níveis da droga na saliva em pacientes que não responderam ao tratamento pode ser explicado pelo transporte de efluxo generalizado.

Unitermos: Concentração em saliva, carbamacepina, ácido valproico, epilepsia refratária, transportadores de efluxo.

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Received Feb. 22, 2008; accepted Mar. 28, 2008.

INTRODUCTION

Carbamazepine (CBZ) and valproic acid (VPA) in mono or combined therapy are commonly selected treatments to control seizures in epilepsy.

Plasma concentrations are generally monitored as treatment follow-up having been found that CBZ concentrations show a non-linear response with dose as reported by our group.[1] A widely accepted explanation to this phenomenon is the induction the drug has over intestinal enzymes, apart from the well-known hepatic enzyme induction, both of which result in lower plasma concentrations than expected when dose is increased.

VPA total concentration in plasma shows a non-linear response with dose as well, but in this case due to plasma proteins saturation. Free drug fraction increases as plasma drug concentration rises, however free VPA level and daily dose are linearly related.[2] VPA monitoring in saliva has shown to be controversial due to saliva pH variability among individuals. A method to reduce this variability is to withdraw saliva by citric acid stimulation which causes disruption in salivary ducts equilibrium, making saliva pH less variable.3

Thirty per cent of epileptic patients cannot control seizures either using monotherapy or a combined therapy. It seems that an overexpression of proteins known as efflux pumps [ATP-Binding-Cassette (ABC) transporters] in the blood brain barrier (BBB) could provide a feasible explanation to this phenomenon.4 The presence of these transporters is recognized in different parts of the organism and is generally associated to enzymatic systems, having an important role in bioavalability and disposition of several drugs.

METHODS

A 3-year retrospective study was carried out in the Neuropediatric Department at the Children Hospital. Every Friday saliva samples were collected from children under chronic treatment either in monotherapy with CBZ or VPA, or with CBZ-VPA combined therapy. After having maintained the same daily dose for at least 2 weeks, trough saliva samples were withdrawn before morning dose, using a few crystals of citric acid placed on the tongue as a saliva flowing stimulator. After having been frozen, samples were defrosted and centrifuged and supernatant was assayed by Fluorescence Polarization Immunoassay (FPIA, TDx/Abbott Laboratories) according to manufacturer instructions, but using saliva instead of plasma. Salivary drug assay was previously validated for CBZ concentrations going from 0.5 to 4.0 mg/L and VPA levels from 1 to 8 mg/L.

Statistical Product and Service Solution (SPSS-12.0) was used for data processing. Mean, standard deviation (SD), standard error (SE), and 95% confidence interval of mean (95% CI) were calculated. Non-paired Student t-test was used for mean values comparisons, provided normal distribution of data series and similarity of variances were obtained. Linear regression between salivary drug concentration and daily dose were assessed for each anticonvulsant either whether they were administered under monotherapy or combined therapy.

Clinical status was recorded and patients were then classified as follows: patients with no seizures, patients with monthly seizures, patients with weekly seizures and patients with daily seizures.

RESULTS

A total of 103 patients presented salivary drug concentrations over their respective limit of quantification (LOQ):

- 31 patients, aged from 1 to 13 years, on monotherapy with VPA;
- 56 patients, aged from 3 to 14 years, on CBZ monotherapy;
- 16 patients, aged from 7 to 14 years, on CBZ-VPA combined therapy.

In some cases patients were monitored several times, however, data was included only when daily dose had been changed over the study period. Drug concentrations belonging to the same daily dose were averaged.

Figure 1 shows the non-linear relation between CBZ concentration and daily dose. Patients were divided into two groups: controlled patients (no seizures or monthly seizures) and uncontrolled patients (daily and weekly seizures). CBZ mean concentrations and daily doses (±95%CI) were: 1.7 (±0.1) mg/L and 16 (±2) mg/kg/day for controlled patients (N=51); 1.8 (±0.3) mg/L and 25 (±4) mg/kg/day for uncontrolled patients (N=5).

Figure 2 shows the linear relation between VPA concentration and daily dose. Patients were then divided following the same criteria as used previously for CBZ. VPA mean concentration (±95%CI) in the first group (controlled) [N=15; 1.8 (±0.5) mg/L] was significantly lower than the one obtained for the second group (uncontrolled) [(N=16) 3.9 (±0.9) mg/L], responding to an increase in dose from 21 (±5) mg/kg/day to 37 (±6) mg/kg/day, respectively.

Patients who did not control seizures went on combined therapy. Some of them improved their clinical condition, diminishing seizures frequency. Thirteen individuals displayed no seizures or 1-4 seizures a month but such episodes were catalogued as not severe, showing the following mean concentrations and daily doses (±95%CI): a) 1.8 (±0.2) mg/L and 17 (±2) mg/kg/day for CBZ, and b) 2.0 (±0.4) mg/L and 35 (±6) mg/kg/day for VPA. One patient with daily/weekly seizures was clearly under-medicated. Two patients showed higher frequency

of episodes and their anticonvulsant doses were high for both VPA (average 74 mg/kg/day) and CBZ (average 25 mg/kg/day). VPA and CBZ concentrations in saliva for these patients were on average 3.1 and 2.0 mg/L respectively.

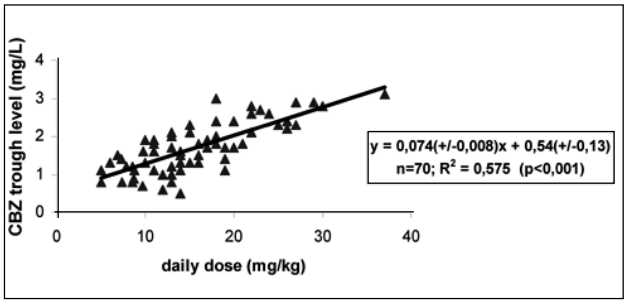

Figure 1. Saliva carbamazepine concentration versus daily dose, in 70 samples belonging to 51 responsive children under anticonvulsant monotherapy. Standard errors of the slope and the intercept are in brackets.

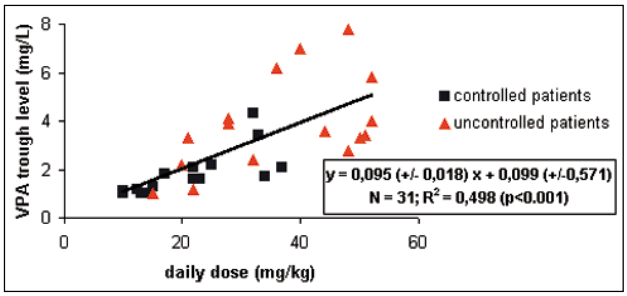

Figure 2. Saliva valproic acid concentration versus daily dose, in 31 epileptic children under anticonvulsant monotherapy. Standard errors of the slope and the intercept are in brackets. Uncontrolled patients (N=16) showed daily or weekly episodes of seizures, and controlled patients (N=15) showed no seizures or monthly seizures.

DISCUSSION

Patients on CBZ monotherapy were mainly successful in their clinical evolution. Dose adjustment in a total of 51 patients yielded 70 saliva samples. Their salivary CBZ levels (y) and daily dose (x) fitted the following straight line:

$$y = 0.074\ (\pm SE = 0.008)x + 0.540\ (\pm SE = 0.130)$$
$$R^2 = 0.572,\ n = 70,\ p<0.001$$

Even though a straight line was obtained, the intercept differed from zero ($p<0.001$). Then CBZ concentration in saliva is non-linearly related with daily dose as it was previously reported for plasma CBZ concentrations.[1] This non-linear pharmacokinetic response is explained by its autoinductive effect, the higher the administered dose is, the higher the clearance becomes.

Five patients, declared as refractory to CBZ monotherapy by physicians, showed a 31% higher dose (21 mg/kg/day) than responsive patients (16 mg/kg/day), while their salivary levels were not significantly different (1.8 and 1.7 mg/L, respectively). According to our results, unresponsive patients might have higher clearance of CBZ. Since both group ages were similar, such clearance modification seems not to be age-related. Other hypothesis will be considered in the next section.

For VPA monotherapy, dose was mostly administered every 8 hours. Dosing frequency was twice a day or four times a day in the rest of the patients. Regardless the interval of administration, all patients were monitored 9-12 hours after evening dose. For this reason several individuals presented salivary VPA levels below the LOQ, as a consequence of the use of immediate-release dosage form and VPA short half-life. Once dose was adjusted, salivary levels could be measured.

As Figure 2 shows, a significant salivary concentration-dose relationship was obtained. This fact is in agreement with the linear response free plasma VPA levels showed with daily dose (2). Even though VPA ionizes in plasma and saliva accordingly to their respective pH, our results support that stimulated saliva level is well related to its free plasma level. Then, sampling procedure assayed in this work reduced saliva pH variability sufficiently among individuals. Therefore, the use of saliva as routinary practice for VPA therapeutic monitoring might be recommended.

When seizures could not be controlled with dose increase, patients were classified as uncontrolled. In these cases, salivary VPA levels increased more than the respective daily dose. In the following section some comments will be addressed about the non-linear behavior observed in these refractory patients.

Unfortunately not all patients under CBZ-VPA combined therapy (N=16), come from both previous monotherapy groups. Since similar CBZ daily dose was kept in the group of patients with controlled seizures, it could be assessed that there was non significant difference in CBZ salivary levels between monotherapy and combined therapy with VPA. This agrees with the fact that VPA interacts with CBZ in its plasma protein binding,[5] so free plasma CBZ level as well as salivary level should not change when VPA is added to the treatment.

Conversely, VPA metabolism seems to be increased in presence of CBZ. VPA saliva concentration of 2.0 mg/L in CBZ-VPA combined therapy was lower than predicted for 35 mg/kg/day, according to monotherapy controlled patient regression. This is in agreement with previous published data (6-7). Besides, uncontrolled patients in bitherapy showed lower VPA saliva concentration (3.1 mg/L) than unresponsive individuals in monotherapy, taking into account the high daily dose received (74 mg/kg/day).

Refractory epilepsy and efflux transporter overexpression

It has been well-stated the relationship between refractory epilepsy and the overexpression of the MDR proteins in the BBB.[8-9] As reported by Lazarowski et al.,[4] overexpression generalizes to other organs as well. ABC transporters are located in several barriers such as superficial columnar epithelial cells in the lumen of the gut, biliary canalicular front of hepatocytes, epithelial cells of small biliary ductules, apical surfaces of epithelial cells of proximal tubules. It has also been reported their presence in ductal cells of salivary glands[10] and several substrates like cyclosporine A[11] and methadone[12] have shown to be preferentially transported to the luminal space.

Unsurprisingly, and according to the previous explanation, that overexpression could also be found in salivary ducts. If this was the case, the higher VPA saliva levels observed in uncontrolled patients under monotherapy might be the result of enhanced drug transfer to saliva, caused by VPA-transporter overexpression.

CBZ metabolism involves CYP3A4 enzyme acting in coordination with MDR1 efflux protein. Any kind of overexpression of the transporter would lead to higher metabolism rate due to longer interaction between the drug and the enzyme. Therefore uncontrolled patients, who could be overexpressing the transporter, would present higher presytemic and systemic clearances. Low concentrations obtained from the 5 uncontrolled patients would be in accordance to this hypothesis. Up to now, no enzyme is known to be working in coordination with transporters on VPA metabolism.

CONCLUSION

Saliva is a reliable fluid to determine CBZ and VPA levels in the routine practice, provided a standardized procedure for saliva stimulation is used.

Epileptic patients without seizures, or with low frequency of seizures, displayed linear relationship between VPA salivary level and daily dose in monotherapy. For CBZ monotherapy a non-linear relationships was found.

Saliva drug concentrations in unresponsive patients to VPA and/or CBZ treatments would be revealing an overexpression of ABC transporter in the salivary gland.

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