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Systemic and Presystemic Conversion of Carbamazepine to Carbamazepine-10,11-Epoxide During Long Term Treatment

Pietro Fagiolino*, Marta Vázquez*, Ivette Olano*, Aurora Delfino**

Department of Pharmaceutical Sciences – Chemistry School – Montevideo, Uruguay

ABSTRACT

Introduction: Carbamazepine (CBZ) undergoes biotransformation, being CYP3A4 the major cytocrome P450 (CYP) enzyme catalyzing the carbamazepine-10,11-epoxide (EPOX) formation, which is quantitatively the most important pathway in CBZ metabolism. There is evidence of dose-dependent elimination of this drug due to its autoinduction capacity. Moreover, published data showed an incomplete bioavailability of CBZ since its absorption increases when grapefruit juice was administered. Both CYP3A4 and MRP2 (located in the enterocyte) are autoinduced during long term use of CBZ. As the other enzymes involved in CBZ metabolism are negligible in the gut, presystemic biotransformation through CYP3A4 could be responsible for the bioavailability of the drug as well as EPOX formation. **Objective:** The purpose of our study was to assess the importance of presystemic formation of EPOX during the autoinduction of CBZ versus the daily administered dose. **Patients and methods:** 40 adults (average age: 28 years) and 29 children (average age: 9 years) receiving CBZ as monotherapy were included in the study. CBZ and EPOX plasma concentrations were analyzed by a previous validated HPLC method. **Results and conclusion:** The results obtained confirmed the metabolic induction after chronic administration and provided new elements to suggest a strong contribution of dose-dependent bioavailability in the non linear kinetics of CBZ.

Key words: carbamazepine, autoinduction, presystemic biotransformation, bioavailability.

RESUMO

Conversão da carbamazepina para 10,11-epóxido-carbamazepina no tratamento prolongado

Introdução: A via mais importante de metabolização da carbamazepina (CBZ) é feita pelo sistema citocromo P450 (CYP), sendo o CYP3A4 a enzima mais importante no processo de formação do 10,11-epóxido-carbamazepina (EPOX), o qual é o principal produto deste processo de biotransformação. Existe evidência da eliminação dose-dependente desta droga devido à sua capacidade de auto-indução. Trabalhos publicados mostraram uma biodisponibilidade incompleta da CBZ uma vez que sua absorção aumenta com a administração de suco de pomelo (grapefruit). O CYP3A4 e MRP2 (localizado no enterócito) são auto-induzidos durante o uso prolongado da CBZ. Uma vez que as outras enzimas envolvidas no metabolismo da CBZ são insignificantes no intestino, a biotransformação pelo CYP3A4 poderia ser responsável pela biodisponibilidade da droga, assim como pela formação de EPOX. **Objetivo:** Avaliar a importância da formação pré-sistêmica do EPOX durante a auto-indução da CBZ em comparação com a dose diária administrada. **Pacientes e métodos:** 40 adultos (idade média: 28 anos) e 29 crianças (idade média: 9 anos) em uso de CBZ em monoterapia foram incluídos no estudo. Concentrações de CBZ e EPOX no plasma foram analisadas utilizando um método de HPLC previamente validado. **Resultados e conclusões:** Os resultados obtidos confirmam a indução metabólica durante a administração crônica da CBZ e fornecem novos elementos que sugerem uma forte contribuição da biodisponibilidade dose-dependente na cinética não linear da CBZ.

Palavras chaves: carbamazepina, auto-indução, biotransformação pré-sistêmica, biodisponibilidade.

* Department of Pharmaceutical Sciences. Chemistry School. General Flores 2124. Montevideo 11800, Uruguay. Therapeutic Drug Monitoring Service. University Hospital: Dr. Manuel Quintela.
** Department of Neuropediatrics. Children's Hospital: Pereira Rossell.
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INTRODUCTION

Conversion of carbamazepine (CBZ) to carbamazepine-10,11-epoxide (EPOX) through cytocrome P450 (CYP) 3A4 as main metabolic pathway uses a mass of about 30 and 50% of the dose administered to patients during antiepileptic treatment with CBZ[1,2]. There is strong evidence of dose-dependent elimination of CBZ taking into consideration its autoinduction capacity[3].

A recent work carried out by Battino et al.[4] showed that the age of the patient and the administered dose of CBZ have a significant correlation with the apparent clearance of the drug:

a) decreased clearance in the elderly (age >65 years) compared with young adults (average age: 35 years);
b) increased clearance with increasing daily dosage for both age groups.

Oral bioavailability of CBZ during antiepileptic monotherapy is incomplete as it was shown by a study performed by some investigators[5] observing an increase of 40% of the extent of absorption when the drug was administered with grapefruit juice.

Some ingredients found in grapefruit juice such as coumarin derivatives or flavonoids may be responsible for the inhibition of the metabolizing enzyme of CBZ (CYP3A4) located in the enterocyte and/or the drug transporter protein that pumps drug out into the intestinal lumen: multiple resistance protein (MRP2). Both CYP3A4 and MRP2 suffer autoinduction by CBZ during chronic treatment[6].

As the apparent clearance is a hybrid between the real systemic clearance (CL) and the bioavailability (F), it is not clear if the cause of Bernus et al.[3] and Battino et al.[4] findings was exclusively an increased systemic clearance or if a decreasing with dose bioavailability significantly contributes to the dose-dependent kinetics of the drug as well. The results of Garg et al.[5] enable us to infer that at least 70% of the dose of CBZ is bioavailable $(0.7 = [1.4]^{-1})$. As the other enzymes involved in CBZ metabolism (CYP1A2, glucuronyl transferase) are not relevant in the intestine[7], intestinal presystemic biotransformation could be responsible either for the bioavailability of the drug as well as for the formation of the main active metabolite, EPOX.

At the University Hospital: "Dr. Manuel Quintela" and the Children's Hospital: "Pereira Rossell" a large number of patients (adults and children respectively) receiving CBZ are routinely monitored at the Therapeutic Drug Monitoring Service situated in the University Hospital through measurement of serum drug concentration. Plasma concentrations of CBZ and EPOX of chronic patients with CBZ monotherapy were analyzed in order to know the importance of presystemic formation of EPOX during CBZ autoinduction regarding the daily administered dose.

METHODS

Blood samples from patients suffering from epilepsy and stabilized with CBZ monotherapy for more than a month using the same dose and the same commercial presentation were selected for the study. As well as the routine drug concentration monitoring by fluorescence polarisation immunoassay (FPIA, TDx Abbott Laboratories), plasma samples were analyzed by high performance liquid chromatography (HPLC) for the determination of CBZ and EPOX. The HPLC technique was previously published[8].

Age, weight, daily dose, other medications and any other relevant information was obtained from the Therapeutic Drug Monitoring Request Form of our Service filled in by the treating physicians. Samples were taken before the morning dose and concentrations are expressed in mg/L. Daily dose of CBZ (D) is expressed in $mg.kg^{-1}day^{-1}$.

Mean CBZ and EPOX concentrations in plasma and D in adults and children were compared using the Student t-test for unpaired data. Dispersion in the data was evaluated by the coefficient of variation (CV = 100 × standard deviation/mean). For each age group, concentrations of CBZ, EPOX and EPOX/CBZ concentration ratios versus D graphs were plotted. An ANOVA test for regression was used to verify the significance of the regression line. The y-axis intercept was compared to zero by a Student t-test. The slopes and intercepts of the regression lines between the two groups were compared using a Student t-test.

RESULTS

The final population consisted of 40 adults (12 men and 28 women) with a mean age of 28 years (range 14-47) and 29 children (14 boys, 15 girls) with an average of 9 years (range 4-13). There were no significant differences between mean CBZ dosage for the two age groups (adults: 13.4 $mg.kg^{-1}day^{-1}$ ± CV = 49%; children: 12.8 $mg.kg^{-1}day^{-1}$ ± CV = 22%).

CBZ concentrations in adults were significantly higher (6.6 mg/L ± CV = 33%) than those found in children (4.3 mg/L ± CV = 36%) with $p < 0.001$. The same behavior was found for EPOX concentrations (adults: 1.0 mg/L ± CV = 49% and children: 0.67 mg/L ± CV = 45% with $p < 0.01$).

In the adult group significant regression lines ($p < 0.001$) were obtained for:

a) CBZ concentrations versus D;
b) EPOX concentrations versus D; and
c) EPOX/CBZ concentration ratios versus D.

The regression lines a) and c) showed intercepts that differ from zero whereas regression line b) showed a linear behavior (the intercept does not differ from zero).

So a non-linear dependency of steady-state CBZ concentrations (trough concentrations in our study) with dose was found for our adults. As an example, a CBZ concentration of 5.9 mg/L was obtained with a dose of 10 $mg.kg^{-1}day^{-1}$, while a concentration of 8.1 mg/L was the result after a dose of 20 $mg.kg^{-1}day^{-1}$.

For the children group, a significant linear regression ($p < 0.01$) was observed for EPOX/CBZ concentration ratios versus D, whereas CBZ and EPOX concentrations versus D showed the same tendency as in adults but without a statistical significance (non-linear for CBZ but a linear behavior for EPOX). Maybe, the higher interindividual variability or/and the smaller D range in this age group were responsible for this fact.

As the EPOX/CBZ-D graph in children revealed slope and intercept not different from the ones obtained in adults , all plasma data were grouped (adults plus children) to yield the following regression line and statistics:

$$EPOX/CBZ \times 100 = 0.36\ (\pm \text{ standard error} = 0.08) \times D + 10\ (\pm \text{ standard error} = 1)$$

$$n = 69,\ r = 0.476,\ F^{1}_{67} = 19.6\ (p < 0.001)$$

Figure 1 shows the correlation between EPOX/CBZ concentration ratio (metabolic ratio) versus D.

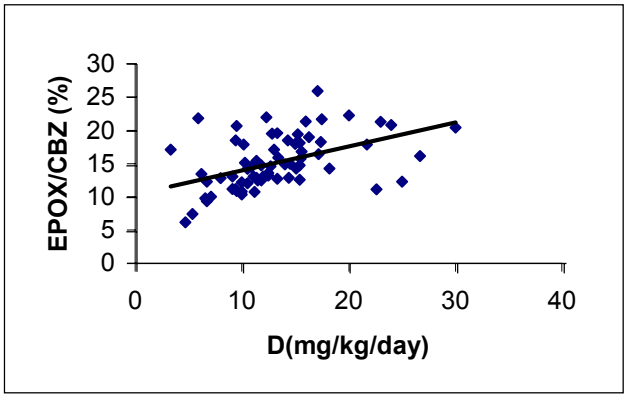

Figure 1. Relation between EPOX/CBZ ratio and CBZ dose in patients (adults and children) receiving CBZ monotherapy. The equation of the regression line was as follows: EPOX/CBZ × 100 = 0.36 (± standard error = 0.08) × D + 10 (± standard error = 1), n = 69, r = 0.476, F^{1}_{67} =19.6 ($p < 0.001$).

DISCUSSION

The following equation explains the relation between the steady-state concentration of a substance X and the administered dose (D: dosage):

$$[X]\ ss = \frac{Fx.D}{CLx}$$

being Fx the bioavailability of the administered doses (as X or as a precursor), and CLx the systemic clearance of the substance.

The results obtained confirmed the non-linear kinetics of CBZ. In other words, F_{CBZ}/CL_{CBZ} decreases with an increase in daily dose. In contrast, the linear behavior observed for the EPOX (F_{EPOX}/CL_{EPOX} = constant) would indicate that the heteroinduction effect of CBZ on EPOX hydratation[3], is compensated by an increase in EPOX bioavailability.

Some investigators[9] found that the half-life of EPOX was highly reduced with concomitant administration of phenobarbital. Based on the above consideration, the enzyme-inducing effect was mainly on the systemic clearance. A similar behavior would be expected with CBZ heteroinduction on EPOX degradation[3] and consequently the increase of EPOX bioavailability would be important in order to compensate and achieve a constant apparent clearance.

The model shown in Figure 2 summarizes in a simple way the probable absorption and disposition of CBZ and EPOX. This model suggests two ways of EPOX formation from CBZ: a) presystemic and b) systemic. If it is assumed, according to the information given in the Introduction Section, that in monotherapy $F_{CBZ} = 0.7$ and $F_{EPOX} = 0.4$ (an average of 30 and 50% of bioavailability), we can conclude that the systemic formation of EPOX represents only 10% of CBZ dose.

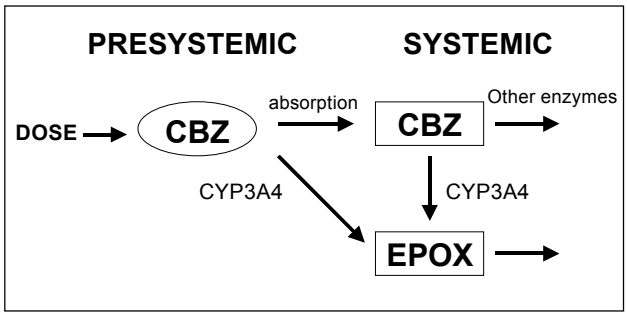

Figure 2. Model diagram to describe systemic and presystemic formation of EPOX.

Ninety percent of our metabolic ratios were obtained with dosages comprised between 6 and 23 $mg.kg^{-1}day^{-1}$. According to the experimental regression line, borderline metabolic ratios would be 12 and 18%. In other words, the metabolic ratio moves from a value to another one 50% higher while dosage increases from 6 to 23 mg $kg^{-1}day^{-1}$. Assuming that for the mean dosage (13 $mg.kg^{-1}.day^{-1}$) F_{CBZ} and F_{EPOX} were 0.7 and 0.4 respectively, it would be

interesting to deduce from the model the extent of bioavailability and systemic clearance change. In order to do this, we have to consider that autoinduction and heteroinduction operate to the same extent on all CBZ and EPOX metabolic routes. This is a reliable and possible assumption bearing in mind previous comments on drug inducers like CBZ.

To accomplish an increase of 50% in the metabolic ratio, as it was experimentally observed, iterations around $F_{CBZ} = 0.7$ were performed keeping the systemic clearance fraction of CBZ responsible for the EPOX formation and the CBZ/EPOX systemic clearance ratio constant. Hence, F_{CBZ} should have moved from 0.75 to 0.65 and consequently F_{EPOX} from 0.35 to 0.45.

Regarding these iteration results and the assumptions mentioned above, CL_{EPOX} and CL_{CBZ} would have increased 28% ($[0.45/0.35 - 1] \times 100$). So, the autoinduction of CBZ metabolism during long term monotherapy implies not only a systemic clearance increase (estimated in 28%), but also a bioavailability reduction (estimated in 13%, $[1 - 0.65/0.75] \times 100$).

Another outcome from the study was an increased apparent clearance for both CBZ and EPOX in children in comparison with the adult population. As daily CBZ dosages were similar for both adults and children as well as the metabolic ratios, it could be expected that the higher apparent clearance observed could be attributed to differences in the systemic clearance but not in the bioavailability.

This presumption is supported by children's anatomy. When compared to adults, muscle mass in children is lower in relation with liver and splanchnic region size. In other words, the relation between metabolizing organs and body mass is greater in children. We do not believe either in a higher metabolic capacity (more enzymatic activity or more enzyme content) per mass of metabolizing organ or in an altered metabolic pattern for CBZ in this age population. The latter would be more complicated to assess regarding the small age difference between both groups (averages of 9 and 28 years in children and adults respectively). The former is not evident because a higher presystemic metabolism should have displayed different metabolic ratios compared with adults.

The autoinduction and heteroinduction of CBZ does not seem to be related with the systemic concentration of the drug. No correlation was found between the metabolic ratio and CBZ plasma concentration for adults and children. So the amount of CBZ invading the enterocyte and hepatocyte after each administration would be responsible for the induction process, with no correlation with the systemic concentration but with the dose.

To sum up, our study confirms the metabolic autoinduction with chronic administration of CBZ and provides more elements to support a strong contribution of dose-dependent bioavailability, besides the systemic clearance in the non linear kinetics of CBZ.

Finally, we would like to express some brief comments related with pharmaceutical quality control and regulatory issues. Due to the incomplete bioavailability of CBZ already reported by different investigators, and inferred throughout our experimental results, it would be incorrect to continue classifying CBZ as a highly permeable drug (extent of absorption >90%) according to the biopharmaceutical classification system[10].

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Address correspondence to:
Pietro Fagiolino
Chemistry School – General Flores 2124, CC 1157
Montevideo 11800, Uruguay
E-mail: pfagioli@fq.edu.uy