Original article


Relationships between pharmacokinetic parameters of carbamazepine and therapeutic response in patients with bipolar disease

Les relations entre les paramètres pharmacocinétiques de la carbamazépine et la réponse thérapeutique chez les patients atteints de la maladie bipolaire

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Abstract. This study aimed to assess the relationship between plasma levels of carbamazepine and its active metabolite 10,11-epoxide-carbamazepine, and the therapeutic response in patients with bipolar disease. Thirteen patients were kept on a fixed individual dose of carbamazepine for 19 weeks under psychiatric care. Steady-state plasma concentrations of carbamazepine and its metabolite 10,11-epoxide-carbamazepine were measured at weeks 4, 12, and 20 by HPLC essay. Simultaneously, the psychopathologic state was assessed using the Brief Psychiatric rating scale (BPRS). Upon correlational analysis, mean BPRS scores did not correlate with the plasma levels of carbamazepine, whereas both mean plasma levels of 10,11-epoxide-carbamazepine concentrations and 10,11-epoxide-carbamazepine to plasma carbamazepine ratio were closely correlated with mean values of BPRS scores ($r = 0.80$, $p = 10^{-4}$, $r = -0.89$, $p = 10^{-3}$ respectively). Optimum therapeutic response was observed among patients who had a plasma metabolite level of 1.4 $\mu$g/mL and a plasma carbamazepine concentrations of 7 $\mu$g/mL simultaneously. These results suggest that both plasma carbamazepine and 10,11-epoxide-carbamazepine levels must be fixed to achieve optimum therapeutic response. In order to reach these conditions, inhibitor drugs (such as valproic acid) or inductor drugs (such as phenobarbital) of epoxyde-hydrolase might be coadministered with the carbamazepine in order to adapt the plasma level of 10,11-epoxide-carbamazepine.

Key words: carbamazepine, 10,11-epoxide-carbamazepine, HPLC, bipolar disorder, BPRS

Résumé. Cette étude visait à évaluer la relation entre les concentrations plasmatiques de la carbamazépine, son métabolite actif le 10,11-époxyde-carbamazépine et sa réponse thérapeutique chez les patients atteints de la maladie bipolaire. Treize patients ont été tenus individuellement à des doses fixes de la carbamazépine pendant dix-neuf semaines dans les soins psychiatriques. À l’état d’équilibre, des concentrations plasmatiques de la carbamazépine et de son métabolite le 10,11-époxyde carbamazépine ont été mesurés aux semaines 4, 12 et 20 par la méthode HPLC. En même temps, l’état psychopathologique a été évalué par l’échelle d’évaluation psychiatrique (BPRS). L’analyse de corrélation des scores moyens BPRS montre l’absence de la corrélation avec les taux plasmatiques de la carbamazépine, tandis que les concentrations plasmatisques du 10,11 époxyde carbamazépine et du rapport entre le 10,11-époxyde carbamazépine et la carbamazépine ont été étroitement corrélées avec les valeurs moyennes des scores BPRS ($r = 0.80$, $p = 10^{-4}$, $r = -0.89$, $p = 0.01$ respectivement). La réponse thérapeutique optimale a été observée chez les patients...
qui ont eu simultanément une concentration plasmatique du métabolite égale à 1,4 µg/mL et une valeur de 7 µg/mL de la carbamazépine. Ces résultats suggèrent que les concentrations plasmatiques de la carbamazépine et du 10,11-époxyde carbamazépine doivent être fixées pour obtenir une réponse thérapeutique optimale. Afin d’atteindre ces conditions, des inhibiteurs (tels que l’acide valpróïque) ou des inducteurs médicamenteux (tels que le phénobarbital) de l’époxyde hydrolase pourraient être coadministrés avec la carbamazépine afin d’adapter le niveau plasmatique du 10,11-époxyde carbamazépine.

Mots clés : carbamazépine, 10,11-époxyde-carbamazépine, HPLC, trouble bipolaire, BPRS

Carbamazepine (CBZ) (Tégrétol®) is the first drug used for bipolar disorder [1]. However, treatment with carbamazepine is associated with several side effects that complicate the use of the drug and necessitate therapeutic monitoring [2, 3]. This monitoring allows the optimization of the therapeutic dose-response efficacy and the prevention or attenuation of the risk of relapse [4, 5]. Despite the effectiveness of carbamazepine as a standard drug for bipolar treatment, important individual variations in the response to carbamazepine therapy were reported [2]. The mechanism underlying the differences in carbamazepine treatment response is not well known [6]. Some studies attribute these differences to patients’ sex, age, body weight, and physiology [7]. Other studies suggest that these variations are related to the plasma concentrations of carbamazepine and its dose [8], with the explanation that monitoring plasma carbamazepine concentrations may play a useful role in the management of patients with bipolar disorder. Moreover, it may be possible that the metabolic polymorphism of carbamazepine elimination, attributed to genetic and/or environmental origins, modifies carbamazepine plasma concentration and elimination and thus its psychotherapeutic effects.

Therefore, this study has been undertaken to explore possible correlations between clinical responses to carbamazepine and parameters such as carbamazepine and 10,11-epoxide-carbamazepine plasma concentrations, and the carbamazepine metabolic index. Our interest is to evaluate the clinical response to CBZ and its major metabolite with respect to their plasma levels.

**Materials and methods**

**Patients**

Thirteen patients with bipolar disease in the state of euthymia were recruited for this study. These patients were composed of 3 men and 10 women aged between 22 and 63 years (mean age =44.16±11.14) and were taken from the psychiatric department of the University Hospital Centre (CHU) Farhat Hached of Sousse (Tunisia). The diagnosis of bipolar disease was established using the *(Diagnostic and statistical manual of mental disorders DSM-IV)* criteria for bipolar disorder. The physical characteristics of the patients are given in table 1. All the patients in this study were treated with CBZ, and they did not take any other associated treatment.

This study was carried out in the biophysics laboratory at the Faculty of medicine. The ethics committee of the Faculty of medicine approved the study, and each patient provided informed consent.

**Clinical state assessment**

The effectiveness of treatment for the psychopathological state was determined by the Brief psychiatric rating scale (BPRS). The BPRS estimations obtained during the therapy period with CBZ were reported in our study for documenting the efficacy of treatment and are expressed in means value. A mean BPRS value is determined during 6 months of monitored treatment (180 days later). A BPRS value equal to 24 indicates a clinically stable condition.

**HPLC plasma analysis and carbamazepine, 10,11-epoxide-carbamazepine concentrations and measurement of carbamazepine metabolic ratio**

The bipolar patients recruited into the study were given carbamazepine at a dosage of 200 - 600 mg/day for 180 days. Blood samples were drawn from each patient 24 hours after the last intake of the CBZ dosage at the end of each cycle of 60 days over a period of 180 days. Blood samples were immediately centrifuged at 3000 x g for 15 minutes at 4°C, and the plasma samples collected...
Table 1. Clinical characteristics of study subjects.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>47</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>30</td>
<td>73</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>47</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>52</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>46</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>47</td>
<td>73</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>40</td>
<td>65</td>
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<tr>
<td>8</td>
<td>F</td>
<td>53</td>
<td>79</td>
</tr>
<tr>
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<td>M</td>
<td>63</td>
<td>57</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>37</td>
<td>60</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>22</td>
<td>54</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>36</td>
<td>61</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>50</td>
<td>68</td>
</tr>
</tbody>
</table>

were stored at –20°C until the time of analysis. The concentrations of CBZ and CBZ-E were assessed by the High-performance liquid chromatography (HPLC) method developed by Bhatti et al. [9], with some modifications. These modifications concern essentially the Internal Standard and the mobile phase. This mobile phase with pH 6.09 allows the separation of compounds and improves the specificity of the method.

Fifty microliters of primidone solution (5 μg/mL, IS), CBZ (10 μg/mL), and CBZ-E (10 μg/mL) were added to 0.5 mL of the plasma taken from healthy volunteers (they gave informed consent to participation in the study) in a tube. The mixture was vortexed for a few minutes and then 500 μL of sodium carbonate monohydrate was added and vortexed. After that, 7 mL of ether-diethyl-dichloromethane (2:1, V/V) was added as an extraction solvent. After 15 min of shaking the samples were centrifuged at 2000 x g for 5 min. Three milliliters of organic phase were then evaporated under dry air at 40°C. The dried analytes were reconstituted using 100 μL of mobile phase and 5 μL were injected into the HPLC. The assays were carried out using an isocratic mode with UV detection at 210 nm. The mobile phase consisted of proportions of acetonitrile, methanol, and potassium phosphate (pH6.09: 0.05;18:18:64 V/V/V) with a flow rate of 0.85 mL/min. Calibration curves were obtained by the least-squares method using linear regression. The validated method was applied to plasma samples which were taken from bipolar patients under therapy with CBZ.

Good linearity was determined from a calibration curve of the drug area ratio peak to that under the internal standard peak in the concentration ranges considered for standard solutions (2.5; 5; 6.25; 10; 12.5; 20 μg/mL for CBZ and 0.5; 1; 2; 0.25; 0.125; 5 μg/mL for CBZ-E (Table 1). The limit of quantification (LOQ) was 0.42 μg/mL for CBZ and 0.36 μg/mL for CBZ-E (Table 1). It is defined as the lowest concentration on the calibration graph.

**Linearity parameters**

The limit of detection (LOD) was 0.12 μg/mL for CBZ and 0.14 μg/mL for CBZ-E. The LOD is a concentration which gives a 3:1 signal-to-noise ratio (Table 2).

Peaks corresponding to every compound present a good separation and a good resolution. The retention time of three products is about 4.3 min for (P), 7 min for (CBZ-E) and 14 min for (CBZ).

10,11-epoxide-carbamazepine is the main metabolite of carbamazepine. The metabolic pathway from carbamazepine to 10,11-epoxide-carbamazepine is mediated mainly by the hepatic CYP3A4 enzyme.

The measurement of the concentration ratio of 10,11-epoxide-carbamazepine to carbamazepine (CBZ-E/CBZ) was used as a metabolic marker to evaluate CYP3A4 activity and therefore the elimination profile of CBZ.

**Statistical analysis**

Regression analysis was used to find correlations between the different parameters studied and the BPRS scores (health improvement of patients). Analyses of variance (ANOVAs) and t-tests were performed to test correlations between carbamazepine and 10,11-epoxide-carbamazepine plasma concentrations and carbamazepine metabolic index, as well as improvement in mean BPRS values.

Table 2. Linearity parameters of the assay.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity (y = a+bx)</th>
<th>a</th>
<th>b</th>
<th>r</th>
<th>a x LOQ (μg/mL)</th>
<th>b x LOD (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBZ</td>
<td>2.5 - 20</td>
<td>-0.229</td>
<td>1.141</td>
<td>0.997</td>
<td>0.42</td>
<td>0.12</td>
</tr>
<tr>
<td>CBZ-E</td>
<td>0.5 - 5</td>
<td>0.06</td>
<td>1.871</td>
<td>0.999</td>
<td>0.36</td>
<td>0.14</td>
</tr>
</tbody>
</table>

a: limit of quantification; b: limit of detection.
Figure 1. (A) The chromatogram of Blanc plasma spiked with standard solution (10 μg of CBZ, 10 μg of CBZ-E and 5 μg SI per mL plasma) at 210 nm. (B) A chromatogram of plasma from a patient spiked with primidone (SI: 5 μg/mL) at 210 nm.

Table 3. Carbamazepine (CBZ) dosages and concentrations, carbamazepine 10-11-epoxide (CBZ-E) concentrations, ratio of carbamazepine 10-11-epoxide (CBZ-E) to that of carbamazepine (CBZ), and BPRS mean values in bipolar patients taking CBZ.

<table>
<thead>
<tr>
<th>Patient</th>
<th>CBZ (μg/mL)</th>
<th>CBZ-E (μg/mL)</th>
<th>CBZ-E/CBZ (%)</th>
<th>D (mg/day)</th>
<th>BPRS mean values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.30</td>
<td>0.71</td>
<td>11.269</td>
<td>600</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>8.41</td>
<td>0.59</td>
<td>7.015</td>
<td>600</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>4.35</td>
<td>0.39</td>
<td>8.965</td>
<td>600</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>5.70</td>
<td>0.48</td>
<td>8.421</td>
<td>600</td>
<td>32</td>
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<tr>
<td>5</td>
<td>5.023</td>
<td>0.50</td>
<td>9.954</td>
<td>400</td>
<td>33</td>
</tr>
<tr>
<td>6</td>
<td>3.19</td>
<td>0.22</td>
<td>6.896</td>
<td>200</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>4.68</td>
<td>0.85</td>
<td>18.162</td>
<td>400</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>6.75</td>
<td>1.44</td>
<td>21.333</td>
<td>400</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>6.93</td>
<td>0.64</td>
<td>9.235</td>
<td>600</td>
<td>30</td>
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<tr>
<td>10</td>
<td>7.80</td>
<td>1.04</td>
<td>13.333</td>
<td>500</td>
<td>26</td>
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<tr>
<td>11</td>
<td>7.36</td>
<td>0.83</td>
<td>11.277</td>
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<td>29</td>
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<tr>
<td>12</td>
<td>7.53</td>
<td>0.97</td>
<td>12.881</td>
<td>600</td>
<td>30</td>
</tr>
<tr>
<td>13</td>
<td>6.97</td>
<td>0.96</td>
<td>13.773</td>
<td>400</td>
<td>27</td>
</tr>
</tbody>
</table>

Results

Association between CBZ and CBZ-E plasma concentrations, and BPRS values

The plasma concentrations of CBZ and CBZ-E were determined for a period of 180 days at the end of each 2 month cycle. The values of CBZ and CBZ-E plasma concentrations and carbamazepine dose intake for each patient as well as mean BPRS values, are given in table 3. There is large intervariability in CBZ plasma concentrations, ranging from 3.19 to 8.41 μg/mL for all patients. This wide variation was also observed for the CBZ-E plasma concentrations (0.22 to 1.44 μg/mL).

Our results show that CBZ plasma concentrations parameter, which is used as an indicator of the treatment response and dose adaptation, was not significantly correlated with the clinical efficacy given by BPRS values (r = -0.28, p =0.19) (figure 2).

Association between CBZ-E, CBZ-E/CBZ ratio and BPRS values

Figure 3 shows wide variations for all patients in CBZ-E plasma concentrations and in CBZ-E-to-CBZ ratio, which expresses CYP3A4 activity, ranging from 7.01% to 21.33%. These variations suggest wide interindividual differences in CYP3A4 activity. The CBZ-E plasma concentrations and the CBZ-E-to-CBZ plasma concentration ratio value (CBZ-E/CBZ) were calculated for each patient. Statistical analysis provides a significant association between the CBZ-E/CBZ ratio (r = 0.82, p =10^{-4}) (figure 3A), CBZ-E plasma concentrations (r=-0.78, p=10^{-4}) (figure 3B), and the BPRS values.

The metabolic ratio value increased with decreasing BPRS mean values. Thus, patients with a higher metabolic ratio value had a lower BPRS value that does not exceed the value of 24. This shows that those who have an important metabolic ratio reflect a strong enzymatic activity of
CYP3A4, and have better remission and therapeutic efficacy. This CBZ-E/CBZ ratio representing the metabolic ratio could constitute a parameter of adaptation of the prescribed dose, individual adaptation as the effective dose. In parallel, participants with a plasma CBZ-E equal to 1.4 µg/mL with BPRS near 24 are expected to respond well to treatment.

Discussion

The assessment of plasma carbamazepine concentration has been proposed as a possible predicting marker of carbamazepine efficiency in bipolar patients. However, previous studies investigating the relationship between CBZ plasma levels and its psychopathologic effects reach different conclusions. While some previous studies have pointed out that plasma carbamazepine level could be used as a predicting marker of psychotherapeutic effect of the drug in patients with bipolar disease [2, 10-12], others contradict these positive results [13-15]. Our preliminary study aimed to evaluate whether simultaneous monitoring of plasmatic CBZ and its metabolite CBZ-E concentrations could be better used to evaluate the therapeutic efficacy of the drug in Tunisian bipolar patients kept on a stable daily dose of CBZ. In this regard, our data showed that measuring the plasma CBZ concentration failed to find a significant correlation between this parameter and antipsychotic effects evaluated by the BPRS scales. On the other hand, our results are consistent with those showing no significant role of CBZ plasma levels in the prediction of therapeutic efficacy.

Therefore, the plasma concentration of carbamazepine failed to predict the therapeutic response. By contrast, our results showed a significant association between plasma 10,11-epoxide-carbamazepine concentration and clinical response to treatment, as evaluated by BPRS values. Moreover, a close significant correlation was also found between CBZ-E to CBZ ratio and the response to treatment, as evaluated by the BPRS values. Thus, our results suggest that parameters plasma 10,11-epoxide-carbamazepine level

Figure 2. Lack of relationship between BPRS values and plasma levels of carbamazepine. For example, patient (8) has a BPRS value of 24 and a CBZ plasma concentration measure of 6.75 µg/mL, whereas similar levels of plasma CBZ concentration, such as 6.30 and 6.93 µg/mL, were found in Patients (1) and (9), who had higher BPRS values of 28 and 30, respectively.

Figure 3. (A) Positive relationship between ratios of carbarnazepine-epoxide to carbamazepine (CBZ-E/CBZ) and BPRS values and (B) shows the correlation between CBZ-E plasma concentrations and BPRS values in 13 bipolar patients during chronic therapy.
and CBZ-E-to-CBZ ratio must be simultaneously considered in order to predict the therapeutic effect. Although our results should be viewed with caution due to the limited number of patients, the simultaneous increases of both plasma 10,11-epoxide-carbamazepine level and CBZ-E-to-CBZ ratio appear as a factor which contributes significantly to the increase of the therapeutic efficacy of CBZ in patients with bipolar disease. By considering a BPRS value of 24 as the optimum amelioration, our data show that this value could be reached when the plasma CBZ-E concentration was of 1.4 µg/mL and when the CBZ-E-to-CBZ ratio was 21%, which corresponds to a plasma CBZ concentration of 7 µg/mL. Thus, our results clearly demonstrate that both plasma carbamazepine and 10,11-epoxide-carbamazepine concentrations must be simultaneously fixed at 7 and 1.4 µg/mL, respectively, in order to reach the optimum therapeutic response. To the best of our knowledge, this is the first study which pointed out that both parameters must be simultaneously set to reach the optimum efficacy of CBZ. Despite the conflicting results in the literature about the plasma CBZ concentrations needed to achieve a better therapeutic response, most of these previous studies proposed different intervals for CBZ concentrations in which the mean values were situated around 7.8 µg/mL. For example, Ballenger and Post [12] have reported that the CBZ levels of 7-12 µg/mL have been recommended for use in affective disorders, while Okuma et al. [10, 11, 16] reported that 7 µg/mL of CBZ plasma concentration would be sufficient to exert antimanic and prophylactic effects in Japanese patients. Vasudev et al. [16] have found that 3-9 µg/mL of CBZ levels with an average of 6.01±2.44 µg/mL represent the therapeutic range in favorable responders. These discrepancies about the suitable plasma CBZ concentration may be explained by the intervariability of metabolic activity of CBZ, and thus, by the intervariability of plasma CBZ-E levels.

Concerning the role of the 10,11-epoxide metabolite of carbamazepine, it has been shown previously that this metabolite may be related to the degree of clinical efficacy in bipolar patients [16]. Petit et al. [17] reported a significant correlation found between 10,11-epoxide carbamazepine and the clinical response in affective disorders, which suggests that it may be responsible, to an extent, for carbamazepine’s effectiveness.

Taken together, all these studies are in agreement with our findings, which imply that both CBZ and CBZ-E are involved in the therapeutic adjustment of patients with bipolar disease, thus necessitating, the assessment of these two pharmacokinetic parameters in order to optimize the therapeutic efficacy of CBZ.

In order to reach pharmacokinetic conditions required for optimum efficacy, we propose a gradual increase of the daily administered dose of carbamazepine up to that leading to a steady-state plasma carbamazepine concentration of 7 µg/mL. We can then modulate the plasma 10,11-epoxide-carbamazepine concentration by acting on the epoxide-hydrolase activity, which is involved in the biotransformation of the 10,11-epoxide-carbamazepine to 10,11-dihydriodiol carbamazepine. It can be inhibited by numerous drugs such as valproic acid [18] which therefore increases the plasma 10,11-epoxide-carbamazepine level.

By contrast, other drugs, such as phenobarbital [19], may act as an inducer of epoxide-hydrolase activity, leading to a decrease of the plasma 10,11-epoxide-carbamazepine level. Thus, valproic acid could be coadministered with carbamazepine when the CBZ concentration is adjusted at 7 µg/mL but with a CBZ-E concentration lower than 1.4 µg/mL. In this context, there is no problem to use valproic acid because this drug has been frequently associated with CBZ in the treatment of bipolar disease[20]. In fact, it is plausible that the better response observed in some bipolar patients treated with a combination of CBZ and valproic acid could be related to the inhibition of epoxide hydrolase, which leads to an increase of the plasma CBZ-E concentration. When the CBZ concentration has been fixed at 7 µg/mL, but with CBZ-E concentration superior to 1.4 µg/mL, inductor drugs of epoxide-hydrolase could be associated with CBZ treatment. Nevertheless, to the best of our knowledge, among the drugs used in bipolar treatment, none has been confirmed to be an inducer of epoxide-hydrolase activity. In particular, phenobarbital, which is an inducer of this activity is frequently used in the treatment of epilepsy and not recognized in the treatment of bipolar disease. Thus, it would be interesting to study its effects with respect to the CBZ-E-to-CBZ ratio when it is coadministered with CBZ in epileptic patients before its eventual use in bipolar patients with plasma CBZ-E concentrations lower than 1.4 µg/mL.

**Conclusion**

These preliminary results show that plasma CBZ-E and CBZ-E-to-CBZ ratio have been successfully used to predict therapeutic efficacy among bipolar patients treated with CBZ. Thus, a new therapeutic strategy based on the simultaneous assessments of plasma CBZ and CBZ-E concentrations among bipolar patients treated with CBZ was proposed in order to adjust these parameters at values leading to the optimum efficacy.

However, due to the limited number of patients recruited in this study, these results should be viewed with caution and more data are needed before any definitive conclusion can be drawn.
Pharmacokinetic parameters of carbamazepine and therapeutic response

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Conflicts of interest: none.

References


