Carbamazepine levels in the hair of patients under long-term treatment: A preliminary study

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Abstract

Carbamazepine (CBZ) levels in the scalp hair of seventeen patients (ten male and seven female), aged from five to forty years old, receiving the drug systematically were determined after hair dissolution and solid-phase extraction procedures using both immunoassay (Abbott TDx) and gas chromatographic (GC) techniques. Carbamazepine levels in hair ranged from 13.9 to 66.3 \( \mu \text{g/g} \) (mean 26.6 \( \mu \text{g/g} \), median 20.9 \( \mu \text{g/g} \)) according to GC measurements. The immunoassay technique gave slightly higher results (mean 28.0 \( \mu \text{g/g} \), median 22.1 \( \mu \text{g/g} \)). The blood concentrations of carbamazepine, using immunoassay (Abbott TDx) technique, ranged from 2.9 to 10.7 \( \mu \text{g/ml} \) (mean 6.2 \( \mu \text{g/ml} \), median 5.7 \( \mu \text{g/ml} \)). Our data indicate the possible use of hair testing as a marker of the dosage history of patients under long-term treatment with CBZ.

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Keywords: Carbamazepine; Hair; Patients

1. Introduction

Carbamazepine (CBZ), an iminostilbene derivative, has been used for more than three decades as the drug of first choice for the treatment of trigeminal
neuralgia and is also widely used for both generalised and partial seizures, due to the rapid control of excessive cerebral electrical discharges and the low incidence of acute and chronic toxicity [1–3]. CBZ may also be useful in the acute and long-term treatment of patients suffering from affective disorders (in general, schizo-affective disorders) and as an adjuvant to neuroleptic therapy of schizophrenic psychoses [4,5]. About 75% of CBZ in plasma is protein bound (mostly to albumin) out of a total drug concentration range of 5–30 µg/ml [6]. CBZ is metabolised primarily to 10,11-epoxide, a stable and pharmacologically active compound (anticonvulsant), found in plasma at 22 to 45% of the CBZ concentration [2,7]. The free fraction of CBZ in plasma varies from 8 to 35%. Consequently, adjustment of CBZ dosage should be guided by monitoring the free level of the drug.

Effective plasma CBZ levels range from 6 to 10 µg/ml. At concentrations exceeding 12 µg/ml, dose-related effects such as unsteadiness, dizziness, double vision, lack of coordination and gastric distress are observed [8]. Aplastic anaemia, hepatitis and thrombocytopenia due to carbamazepine use have also been reported. The relationship between therapeutic effect and free levels of CBZ has not been definitely established. Free CBZ levels have been reported to vary from 0.88 to 3.8 µg/ml. Monitoring of free CBZ levels is necessary in patients with variable protein binding demonstrating low improvement in seizure control.

Serum (and also urine) analysis is effective for determining drug use only within the few days prior to sample collection. Long-term drug use may go undetected if the individual refrains from drug use for a few days before the sample collection. Hair analysis is a promising alternative to serum and urine analysis because it can provide a long term record of drug use [9].

So far, the international interest in hair testing is mainly focused upon the drugs of abuse [10]. Due to the potential for unique information that might be obtained from hair analysis, rapid progress is expected in the development of methods that offer a means of assessment of exposure to various drugs. Hair retains drug analytes for long periods of time (months to years) and analysis can provide valuable long term information on the magnitude and pattern of drug use [9,10]. Consequently, it is obvious that there will be multiple uses of hair analysis data with regard to the analyte (drugs controlled by law, psychoactive drugs, chemical substances of occupational exposure, etc.), the subject sampled (patient, prison inmate, addict, professional, arrested person, etc.) and the required information (for the physician, the police, the prosecutor, the lawyer, the subject, subject’s relatives etc.).

This is a preliminary report of our investigation to determine whether or not carbamazepine is incorporated into the hair of patients under long term treatment in measurable amounts, and whether this incorporation depends on the dosage. Such information would be valuable to physicians in order to evaluate past
medical history and also for various other medicolegal purposes. According to our literature search, such studies have not previously been performed.

2. Materials and methods

2.1. Patients and hair sampling

Hair samples from seventeen patients (ten male and seven female), aged between 5 and 40 years, and suffering mainly from epilepsy were selected for the study. The patients were receiving CBZ for long periods, ranging from two months to several years. Some of them were treated in combination with phenytoin or phenobarbital but none was treated with amitriptyline, chlorpromazine, imipramine or nortriptiline. The last four drugs were found to display cross-reactivity with CBZ using the Abbott immunoassay technique. Blood samples from these patients were taken simultaneously with the hair samples. Hair samples were cut from the head area, as close as possible to the skin of the posterior vertex in a quantity of 200 mg. Segments of equal length from those hair samples were analysed. Hairs of healthy individuals were used as a blank control.

2.2. Hair treatment and fluorescence polarisation immunoassay (FPIA) analysis

Several procedures including methods of hair dissolution (homogenisation) and solid-phase and liquid-liquid extractions after hair dissolution were studied [11,12] in order to find the optimal sample preparation procedure prior to the immunoassay (FPIA) and the gas chromatographic (GC) quantitative determination of CBZ in hair. Standard CBZ solution, obtained from the Abbott calibrator (20.0 μg/ml), was used for the quantitative determination of CBZ in the hair extracts, as shown below. The immunochemical technique (FPIA) was employed using an Abbott Analyser (TDx). Solvents and chemicals used were all of analytical grade. The blood samples, were assayed by FPIA in the Abbott TDx analyser.

According to the optimal sample preparation procedure, 100 mg of hairs was diluted in 2 ml of NaOH 2 mol/l, heated for 15 min at 80°C and vortex-mixed in a test tube. Concentrated HCl solution (37% by vol) was added and pH was adjusted to 1, the solution was heated for 20 min at 80°C and cooled at room temperature (dissolution of hair). Solid Na₂CO₃ was added, pH adjusted to 9, the solution was centrifuged for 5 min and the supernatant was transferred into a new test tube. A solid-phase extraction column of type Techelut C-18 (obtained from HPLC Technology Ltd, UK), was conditioned using 1 ml of methanol and
1 ml of distilled water. The supernatant was absorbed through the column, rinsed with 1 ml of distilled water, the column was dried for 5 min and the substance was eluted with 2 ml of dichloromethane. The solvent was evaporated, the residue was redissolved in 200 µl of saline (0.9% NaCl) and CBZ was assayed by FPIA in the Abbott TDx [11].

The analysis of hair samples from the patients was performed according to the above procedure in the same sequence with control hair fortified with standard CBZ amounts (0.5; 1.0; 2.0; 4.0 µg) using the 20 µg/ml calibrator. CBZ levels in patients’ hair were determined from the calibration curve obtained from the four standards.

The efficiency of the sample preparation procedure was studied in recovery experiments. These experiments were performed twice, adding the CBZ standard amounts before and after hair dissolution. Pure hair samples, weighting 50 to 100 mg, were spiked with standard amounts of (0.5, 1.0, 1.5 µg) of CBZ from a 20 µg/ml CBZ calibrator (i.e. procedure before hair dissolution). The recovery of the substances was determined, using the above method.

2.3. Gas-chromatographic confirmatory analysis

Hair sample analysis using GC was performed using the same preparation procedure as already described. Briefly, a new series of CBZ standards in hair and samples were treated in one sequence as stated above and the residues after dichloromethane evaporation were reconstituted in 10 µl of methanol. 1 µl of the methanolic solution was injected into the methyl-phenyl silicone SP-2250DA column of the Perkin Elmer model 8700 instrument. Conditions of GC were similar to these described in [13].

3. Results

The results of recovery experiments are shown in Table 1 (mean of four different measurements). These results yielded CV’s of less than 8%. The linear regression equations and the average recoveries for the experiments before and after hair dissolution were: a) before dissolution: \( y = 0.700x - 0.031 \) \( (r = 1.000, \ p < 0.001) \), average recovery 66.1±2.7% and b) after dissolution: \( y = 0.731x - 0.058 \) \( (r = 0.999, \ p < 0.001) \), average recovery 80.4±5.0%.

The results of the analysis of patients’ hair and blood samples, using the FPIA and GC techniques, are shown in Table 2 (mean of two different measurements after repeating the whole sequence). The statistical parameters (mean±SD and median) for the above measurements were: a) blood CBZ concentrations: 6.2±2.4 µg/ml (median: 5.7 µg/ml), b) hair CBZ concentrations by FPIA
Table 1
Results of carbamazepine (CBZ) recovery experiments

<table>
<thead>
<tr>
<th>CBZ added (µg)</th>
<th>Before dissolution</th>
<th>After dissolution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovered* (µg)</td>
<td>Percent (%) Recovery</td>
</tr>
<tr>
<td>0.5</td>
<td>0.315±0.055</td>
<td>63.0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.677±0.043</td>
<td>67.7</td>
</tr>
<tr>
<td>1.5</td>
<td>1.015±0.068</td>
<td>67.7</td>
</tr>
</tbody>
</table>

*Mean±S.D. (n = 4). There is no significant difference by t-test (p < 0.01) between each sequence of measurements.

The relationship between the hair CBZ concentrations by comparison of the fluorescence polarisation immunoassay (FPIA) and the gas chromatography (GC) technique is illustrated in Fig. 1. The corresponding equation is: \( y = 0.937x + 0.310 \) \( (r = 0.997, \ p < 0.001) \).

The relationship between the blood and hair CBZ concentrations is illustrated in Fig. 2 and the corresponding equation is: \( y = 4.348x + 1.102 \) \( (r = 0.687, \ p < 0.01) \).
Table 2
Carbamazepine (CBZ) levels in the hair and blood of patients

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Patient sex*</th>
<th>Hair type</th>
<th>CBZ dosage (mg/24 h)</th>
<th>Duration of dosage (months)</th>
<th>Blood CBZ concentrations** (µg/ml)</th>
<th>Hair CBZ concentrations** (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FPIA</td>
<td>GC</td>
</tr>
<tr>
<td>1</td>
<td>f</td>
<td>black</td>
<td>600</td>
<td>2</td>
<td>4.1</td>
<td>18.3</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>black</td>
<td>400</td>
<td>6</td>
<td>5.8</td>
<td>15.4</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>black</td>
<td>400</td>
<td>9</td>
<td>4.5</td>
<td>19.1</td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>black</td>
<td>400</td>
<td>10</td>
<td>3.9</td>
<td>17.6</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>black</td>
<td>1200</td>
<td>14</td>
<td>10.7</td>
<td>69.2</td>
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<tr>
<td>6</td>
<td>m</td>
<td>black</td>
<td>800</td>
<td>24</td>
<td>9.9</td>
<td>58.3</td>
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<tr>
<td>7</td>
<td>f</td>
<td>black</td>
<td>600</td>
<td>24</td>
<td>2.9</td>
<td>34.6</td>
</tr>
<tr>
<td>8</td>
<td>f</td>
<td>coloured</td>
<td>800</td>
<td>24</td>
<td>5.1</td>
<td>19.5</td>
</tr>
<tr>
<td>9</td>
<td>f</td>
<td>blond</td>
<td>800</td>
<td>&gt; 24</td>
<td>6.3</td>
<td>29.7</td>
</tr>
<tr>
<td>10</td>
<td>m</td>
<td>black</td>
<td>600</td>
<td>&gt; 24</td>
<td>5.7</td>
<td>32.5/45.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>head/pubic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>m</td>
<td>blond</td>
<td>600</td>
<td>&gt; 24</td>
<td>5.5</td>
<td>28.7</td>
</tr>
<tr>
<td>12</td>
<td>m</td>
<td>blond</td>
<td>600</td>
<td>&gt; 24</td>
<td>6.6</td>
<td>22.1</td>
</tr>
<tr>
<td>13</td>
<td>m</td>
<td>blond</td>
<td>600</td>
<td>&gt; 24</td>
<td>10.3</td>
<td>32.9</td>
</tr>
<tr>
<td>14</td>
<td>f</td>
<td>brown</td>
<td>600</td>
<td>&gt; 24</td>
<td>3.7</td>
<td>16.1</td>
</tr>
<tr>
<td>15</td>
<td>f</td>
<td>coloured</td>
<td>1000</td>
<td>&gt; 24</td>
<td>7.3</td>
<td>25.5</td>
</tr>
<tr>
<td>16</td>
<td>m</td>
<td>brown</td>
<td>800</td>
<td>7</td>
<td>8.1</td>
<td>19.1</td>
</tr>
<tr>
<td>17</td>
<td>m</td>
<td>black</td>
<td>200</td>
<td>6</td>
<td>4.8</td>
<td>17.6</td>
</tr>
</tbody>
</table>

*a: male, f: female.

**Mean values (n = 2).

*CBZ in combination with phenytoin or phenobarbital.
4. Discussion

Even though there is a strong criticism of the use of hair analysis results for quantitative or at best semiquantitative measurements, hair testing is a subject of growing interest not only in the field of forensic science but also in clinical pharmacology. Previous studies [14] have shown that the clinically applied antipsychotic agents, haloperidol and chlorpromazine, are deposited into hair proportionately to the given doses. Moreover, the segmental analysis of the drug along the hair shaft represents the month-dosage history, assuming a mean hair growth rate of about 1 cm per month. The factors affecting the drug concentration in hair were established to be the hair colour, the physicochemical properties of the drug, and the hair sampling location. Problems related to variable growth rate and cycle stage of hair were also found when monitoring drug levels of ofloxacin [14]. However the significance of hair drug quantitative analysis in therapeutic drug monitoring could be similarly useful as for the other medico-legal purposes.

According to our experimental studies of the standard solutions of CBZ, there are satisfactory recoveries for the procedures that include the addition of the substances before and after the hair dissolutions. This observation, in combination with the positive results from the experiments involving patients’ hair (Table 2), indicates that the relative procedures assessed could be considered satisfactory determination procedures for the reported substances.

Carbamazepine is a lipophilic substance and it would be expected to be found
in hair at high levels due to its easy transfer from blood into hair bulb cells. Immunoassay (FPIA) results for CBZ levels in hair were generally higher than corresponding GC results. This is obviously associated with the cross-reactivity of the CBZ metabolite (10,11-epoxide) affecting the FPIA results for CBZ levels. There was a significant linear regression correlation of the concentrations (µg/g) by the two techniques when samples of hair from patients treated long-term with the drug were analysed (Fig. 1).

As indicated by our preliminary results, there is no difference in CBZ hair levels of patients due to their sex. On the other hand, we remarked significantly lower value of CBZ in blond, brown and coloured type hairs in comparison to black type hairs despite similar CBZ dosage. From Table 2 it is obvious that duration of CBZ dosage very slightly affects the CBZ hair levels. On the other hand, hair CBZ levels were found to be dependent on the dosage of CBZ. Higher CBZ levels were monitored when increased doses of CBZ were administered to the patients. It is noteworthy that CBZ levels in pubic hair (43.4 µg/g, GC) of the patient (case 10, male) were determined to be much higher than in the scalp hair (30.1 µg/g, GC). A similar relationship between pubic and scalp hair morphine levels was remarked in samples taken from addicts (unpublished results).

Our results support the use of hair CBZ analysis as an index of the dosage history of the patients. We know from clinical experience that some patients try to alter their medication, reduce or even stop it, thus jeopardising the whole treatment, leading sometimes to unsatisfactory seizure control. The possibility of determining the hair levels of CBZ will therefore indicate whether or not a proper chronic use has been followed, and not only the reflection of day to day use that a blood sample provides. Hair acts as a tape recorder that continuously stores all data about the CBZ use history of the subject. There are still many issues that must be studied before hair testing for carbamazepine can be expected to give reliable information for the scientific community. For example, factors such as age, race, differences in hair growth rate, anatomic site of hair, diffusion of the drug along the hair shaft, and influences from combinations of drugs, should be further studied.

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References