Prednisone Concentrations in Human Hair

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Introduction

In 1999, France passed a law against doping, the so-called Loi Buffet. This law was designated to protect athletes against doping consequences, particularly after long-term abuse. In 2001, this law was implemented with its practical aspects. Among them, hair sampling to document chronic exposure was considered as a valid procedure.

Drug administration can be tracked by hair analysis for months or even years and thus, offers the possibility of determining long-term drug exposure (1). Corticosteroids are known to have numerous side effects. During the treatment of specific deseases, these compounds are allowed in urine for a short period of time, but in the case of doping practices, it may be of interest to evaluate chronic administration of corticosteroids. Scientific literature contains only a few reports on the identification of corticosteroids in hair (2-4), but these may be of interest to evaluate chronic administration of corticosteroids.

To evaluate the existance of such a correlation, this article reports the identification and quantitation of prednisone in human hair of patients treated with Cortancyl®.

Experimental

Hair specimens were obtained from 10 patients treated with prednisone. A hair strand was cut with scissors as close as possible to the skin in the vertex posterior region and stored at room temperature. Before extraction, hair strands were washed twice (2 min each) in 5 mL methylene chloride at room temperature. The hair section was choosen in accordance with the treatment period duration and then pulverized in a ball mill.

Prednisone was tested by our previous procedure (4) with some modifications. Briefly, 100 mg of the powdered hair was incubated in 1 mL Soerensen buffer, pH 7.6 for 16 h at 40°C, in presence of 50 ng cortisol-d₃ as internal standard. For further purification, SPE C18 Isolute extraction columns were used. Column activation was performed with 3 mL methanol (MeOH) followed by 3 mL deionized water. The incubation medium was centrifuged, and the supernatant was removed and added to the activated column, then rinsed with 1 mL acetone/deionized water (2:8, v/v), followed by 1 mL of deionized water, and finally with 1 mL of hexane. Columns were dried for 30 min, and the corticosteroids were eluted with three successive volumes of MeOH (0.5 mL each). The eluates were evaporated to dryness and resuspended in 1 mL 0.2N NaOH and re-extracted with 3 mL of diethylether. After agitation and centrifugation, the organic phase was removed and evaporated to dryness, and the dry extract was resuspended in 30 µL of MeOH.

A 2-µL volume of the extract was injected onto the column (4-µm Novapak C18 Waters, 150 x 2.0-mm i.d.) protected by a 5-µm Opti-Guard C18 fingertight guard cartridge (15 x 1.0-mm i.d.). Each 10-min chromatographic run was carried out with a binary mobile phase of acetonitrile and 2mM NH₄COOH pH 3.0 buffer, using a linear gradient (acetonitrile 20 to 60% in 4 min) generated by a 20-mL dual-syringe high-performance liquid chromatography (HPLC) pump (Applied Biosystems model 140B). The flow rate was 200 µL/min with a postcolumn split of 1:3 (flow rate infused into the ionspray: 50 µL/min).

Detection was carried out by a PerkinElmer Sciex API-100 mass spectrometer (MS). Nitrogen (purity grade 99.99%) was employed as nebulizing gas. The instrument was operated in the positive ionization mode (ionspray +4500V). Ions generated in the ion source were sampled into the mass analyzer through a 25-µm orifice held at +20V. MS data were recorded in the single ion monitoring mode. Prednisone was identified on the basis of its retention time and ion ratio (m/z 359 and 341).

Quantitative results were obtained after determination of the response factor of prednisone (m/z 359) against cortisol-d₃ (m/z 366).

Because of the lack of reference material (control positive hair samples for prednisone), validation parameters were determined on spiked hair specimens. Standard calibration curves were obtained by adding 2, 5, 25, 100, and 200 ng of prednisone to 100 mg of pulverized blank control hair (prednisone free) and 50 ng cortisol-d₃ to obtain final concentrations of 20, 50, 250, 1000, and 2000 pg/mg of hair. Extraction
recovery was determined by adding 25 ng of prednisone to 100 mg of powdered blank control hair (prednisone free) and 50 ng of cortisol-d₃ \((n = 3)\) to obtain a final concentration of 250 pg/mg. Precision was determined by adding 25 ng of prednisone to 100 mg of powdered blank control hair (prednisone free) and 50 ng of cortisol-d₃ \((n = 8)\), corresponding to a final concentration of 250 pg/mg. The limit of detection was determined by decreasing the prednisone concentration in order to obtain a signal-to-noise ratio of 2. All these validation parameters were determined after extraction of the spiked blank control hair by the established procedure, that is, HPLC separation of the analytes and MS detection.

### Results and Discussion

Under the determined analytical conditions, no interference with prednisone and the internal standard was observed by any extractable endogenous materials present in hair. The correlation coefficient of the calibration curve was 0.998 for prednisone, indicating linearity between 20 and 2000 pg/mg. Precision at 250 pg/mg (CV values) was 8% for prednisone with a relative extraction recovery of 70%. The limit of detection was 15 pg/mg for prednisone, but the limit of quantitation was fixed at 30 pg/mg.

Hair specimens were obtained from 10 patients treated with prednisone with doses ranging from 5 to 60 mg/day. Analytical results are reported in Table I. Prednisone was identified in the hair of nine patients. The concentrations ranged from 30 to 130 pg/mg. The active metabolite prednisolone was never detected, which is consistent with the fact that the major compound found in hair is the parent drug. Although the limited number of subjects precludes generalization, results are suggestive of a low incorporation rate of prednisone in human hair. For dosages ranging from 5 to 60 mg/day, prednisone concentrations were always largely under 1 ng/mg. The only negative hair sample was obtained from a woman with light-colored hair dyed black and treated with prednisone at the lowest dosage (5 mg/day). This was certainly due to the coloration of her hair, as it is well established that cosmetic treatments such as coloration, bleaching, dyeing, and waving lead to the loss of xenobiotic content of the hair (6).

The developed procedure was able to detect prednisone in the hair of patients treated with the lowest dose of prednisone, which is 5 mg/day. When the total amount of ingested prednisone was compared to the measured concentrations in hair, a low but not insignificant correlation \(R^2 = 0.578, p < 0.03\) was noticed (Figure 1). In this observation, the result obtained for patient no. 8 (103 mg of prednisone in the proximal 1-cm long hair section after a treatment period of one month) was omitted for two reasons: (1) the small amount of sample (30 mg instead of the 100 mg required), which can lead to a more approximative quantitative determination and (2) the relatively short treatment period (one month) in contrast with the 3–13-cm long hair section analyzed for the other patients where prednisone concentration reflects the mean exposure over several months (dilution due to the length of the analyzed strand).

Although the limited number of subjects \((n = 8)\) preclude generalization, results are suggestive of a dose-concentration correlation.

In the future, it would be necessary to access more treated patients and to analyze shorter

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**Table I. Analytical Results for Prednisone in the Hair of Treated Patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dosage</th>
<th>Test segment</th>
<th>Prednisone concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 mg/day</td>
<td>0 to 10 cm</td>
<td>none detected</td>
</tr>
<tr>
<td>2</td>
<td>5 mg/day</td>
<td>0 to 13 cm</td>
<td>30 pg/mg</td>
</tr>
<tr>
<td>3</td>
<td>5 mg/day</td>
<td>0 to 5 cm</td>
<td>39 pg/mg</td>
</tr>
<tr>
<td>4</td>
<td>10 mg/day</td>
<td>0 to 10 cm</td>
<td>30 pg/mg</td>
</tr>
<tr>
<td>5</td>
<td>10 mg/day</td>
<td>0 to 8 cm</td>
<td>57 pg/mg</td>
</tr>
<tr>
<td>6</td>
<td>20 to 6 mg/day</td>
<td>0 to 8 cm</td>
<td>45 pg/mg</td>
</tr>
<tr>
<td>7</td>
<td>55 to 9 mg/day</td>
<td>0 to 4 cm</td>
<td>63 pg/mg</td>
</tr>
<tr>
<td>8</td>
<td>40 to 35 mg/day</td>
<td>0 to 1 cm</td>
<td>103 pg/mg</td>
</tr>
<tr>
<td>9</td>
<td>50 to 25 mg/day</td>
<td>0 to 5 cm</td>
<td>130 pg/mg</td>
</tr>
<tr>
<td>10</td>
<td>60 to 35 mg/day</td>
<td>0 to 6 cm</td>
<td>90 pg/mg</td>
</tr>
</tbody>
</table>

* Light-colored hair dyed black.

**Figure 1.** Regression curve between total amount of ingested prednisone and prednisone concentration in hair.
hair sections corresponding to an unique dosage to confirm this observation.

References


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