First Identification of Prednisone in Human Hair by Liquid Chromatography–Ionspray Mass Spectrometry

To the Editor:

A wide range of methodologies have been investigated for the determination of corticosteroids in biological fluids, including radioimmunoassay (1), high-performance liquid chromatography (HPLC) with spectrophotometric detection (2), gas chromatography (3), and liquid or gas chromatography coupled to mass spectrometry (4,5). Blood or urine is usually employed to check drug consumption; however, the elimination of such drugs occurs within a few days. In contrast, hair is known to allow administration of a drug to be tracked for months or even years and thus offers the possibility of determining long-term drug exposure (6). In the case of corticosteroids, which are prone to numerous side effects and frequently prescribed on a long-term basis in chronic diseases, this may be of interest to assess patients’ sometimes-poor compliance with their prescribed therapy. Until now, no method was available for the determination of corticosteroids in hair samples. This paper describes a procedure based on HPLC–ionspray mass spectrometry for the identification of prednisone and its active metabolite prednisolone in human hair.

Hair samples were obtained from a 38-year-old male treated for years by Cortancyl® (prednisone, 5-mg tablets, Roussel Labs) for sarcoidosis. Hair strands were cut as close as possible to the skin, in the vertex posterior region. One-hundred milligrams was washed twice in 5 mL methylene chloride at room temperature (2 min each) then pulverized in a ball mill. Fifty milligrams 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 methylprenisolone used as internal standard. For further purification, SPE C18 Isolute extraction columns were used. Activation was operated with 3 mL methanol (MeOH) followed by 3 mL deionized water. The incubation medium was centrifugated, and the supernatant was removed and deposited on the activated column, then rinsed with 1 mL deionized water followed by 1 mL deionized water/MeOH (90:10, v/v). Columns were dried for 30 min and the corticoids eluted with three successive volumes of MeOH (0.5 mL each). The eluates were evaporated to dryness and resuspended in 30 μL 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.). Each 10-min chromatographic run was carried out with a binary mobile phase of acetonitrile/2mM NH₄COOH (pH 3.0 buffer) using a gradient (acetonitrile 30–70%) generated by a 20-μL dual-syringe 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 Perkin Elmer Sciex API-100 mass spectrometer. The instrument was operated in the positive ionization mode with a tension of +4500 V applied to the sprayer. Ions generated in the ion source were sampled into the mass analyzer by passing

Figure 1. Chromatogram in selected ion monitoring mode of detection obtained from a hair sample positive for prednisone. The determined concentration was 1.28 ng/mg.
through a 25-mm orifice held at +190 V. Mass spectral data were recorded in the single ion monitoring mode (prednisone \( m/z \) 359 and 341; prednisolone \( m/z \) 361 and 343; methylprednisolone \( m/z \) 375).

Under these analytical conditions, no interference with prednisone, prednisolone, and methylprednisolone was observed by any extractable endogenous materials present in hair (Figure 1). Hair samples tested positive for prednisone with a measured concentration of 1.28 ng/mg. The metabolite prednisolone was not detected. This is the first report of a determination of prednisone in human hair.

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References