

Detection of triamcinolone acetonide in urine and hair following pretended acupuncture treatments of hayfever

Patricia Anielski, Aniko Krumbholz, Detlef Thieme

Institute of Doping Analysis and Sports Biochemistry (IDAS Dresden), D-01731 Kreischa

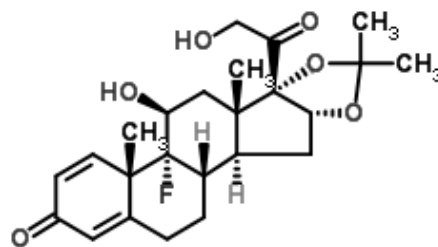
Aim: The long-term detection of synthetic glucocorticoids was requested in urine and hair samples, due to the suspicion of hidden applications in patients suffering from allergies. **Methods:** Urine was extracted with TBME following enzymatic hydrolysis. Hair samples were segmented, washed, snipped, and extracted with methanol. The hair extract was purified by SPE (Plexa, Agilent). The detection of 18 glucocorticoids in urine and 17 glucocorticoids in hair was realized by LC-MS/MS using a C18 column (Hypersil Gold, 50 x 2.1 mm, 3µm, Thermo Scientific) and a Sciex QTrap 5500 instrument. **Results:** Triamcinolone acetonide was confirmed in urine (concentrations ranging from LOD 0.4 ng/ml up to 25 ng/ml) as well as in hair samples (concentrations between LOD 0.1 pg/mg and 57 pg/mg). **Discussion:** The uptake of the synthetic glucocorticoid triamcinolone acetonide was proven for the last weeks/months before sample collection (up to one year in case of one hair sample). However, the route of application could not be differentiated. **Conclusion:** The analytical results in urine and hair substantiate the initial suspicion of a deceptive application of triamcinolone acetonide during pretended traditional acupuncture treatments.

1. Introduction

Synthetic glucocorticoids are therapeutically indicated for the treatment of inflammatory conditions and allergies, and structurally related to the endogenous glucocorticoids cortisol and cortisone. Due to serious adverse effects on endocrine, musculoskeletal, cardiovascular and immune system, gastrointestinal tract and dermis, the medical indication requiring permanent or high-dosed (parenteral) applications should be considered very carefully [1, 2, 3]. In sports, glucocorticoids belong to the class S9 according to the WADA Prohibited List [4], and are prohibited in-competition when applied by systemic routes of administration.

Case reports: Several patients reported to suffer from symptoms, which are well known side effects of a long-term, systemic treatment with glucocorticoids, e.g. suppression of the immune system, disorder of the female cycle, skin alterations, or even Cushing syndrome. Further clinical parameters tested by the attending physicians were consistent, e.g. suppression of endogenous cortisol. A previous medication with synthetic glucocorticoids was not documented, but all affected patients have been miraculously treated against allergies, psoriasis and neurodermatitis by another (naturopathic) physician using traditional acupuncture. The strong suspicion of a simultaneous injection of glucocorticoids during acupuncture treatment occurred. Similar practise has been known from multiple cases in 2013. Patients were treated by electroacupuncture against hayfever, combined with hidden injections of triamcinolone acetonide (Fig. 1).

Fig. 1. Structure of triamcinolone acetonide. In Germany, 25 preparations containing the synthetic glucocorticoid triamcinolone acetonide are available on prescription, e. g. for dermal, nasal application and for injection (intra-articular, intra-focal, sub-lesional, i.v., i.m.) [5].



The aim of our investigation was to analyse urine and hair samples of the affected persons regarding the exposure to synthetic glucocorticoids.

2. Material and Methods

2.1. Reference materials and Chemicals

Triamcinolone acetonide (Sigma-Aldrich, Schnelldorf, Germany) and d4-cortisol (CIL, Andover, USA) were used as reference materials. Stock solutions (100 µg/mL) were prepared in ethanol. Solvents were of HPLC grade (acetonitrile, methanol, ethanol, water, TBME) and obtained from Th. Geyer (Renningen, Germany). Ammonia solution (25 %, p.a.) and glacial acetic acid (p.a.) were purchased from Th. Geyer (Renningen, Germany), ammonium acetate, potassium dihydrogen phosphate and sodium hydrogen phosphate (p.a.) from Merck (Darmstadt, Germany), and ethylene glycol (p.a.) from J.T.Baker (Deventer, Netherlands). Enzyme solution (β -glucuronidase, *E. coli*) was obtained from Roche Diagnostics (Mannheim, Germany). Phosphate buffer (0.5 M, pH 6.5) was prepared by potassium dihydrogen phosphate and sodium hydrogen phosphate. HPLC mobile phases consist of a mixture of water/acetonitrile (mobile phase A: 95/5, v/v; B: 5/95, v/v) containing 2 mmol ammonium acetate and 0.1% acetic acid.

2.2. Sample preparation – Urine

The internal standard (d4-cortisol, 10 ng/mL) and 2 mL of phosphate buffer solution were added to two millilitres of urine. Enzymatic hydrolysis was realized using 40 µL of the β -glucuronidase solution, at 55 °C for 1 hour. Subsequently, liquid-liquid extraction was carried out by 4 mL of TBME. Following centrifugation, the organic phase was separated and evaporated to dryness. For LC-MS/MS measurement, samples were reconstituted by 40 µL HPLC mobile phase A. Blank urine collected from a young boy (2 years old) was also used as negative control sample. Positive control samples were prepared by adding the triamcinolone acetonide standard (calibration range 0.4 to 30 ng/mL, e.g.) to the blank urine.

2.3. Sample preparation – Hair

Hair samples were segmented according to the relevant growth period, e.g. 3 cm-segments. Before analysis, the hair segments were washed using 5 mL of methanol/water (1:1, v/v). The wash solution was separated and stored refrigerated until analysis (in case of a positive finding in hair). The hair segments were dried (vacuum compartment dryer, 80 mbar, 50 °C), snipped and weighted (50 mg if available). The internal standard cortisol-d4 (20 pg/mg) was added to the hair sample. Extraction of hair material was carried out with 2.5 mL methanol in an ultrasonic bath (at 50 °C, 4 h). Following centrifugation, the methanolic phase was separated. Before evaporation (at 60 °C, under nitrogen stream), 20 µL of ethylene glycol were added to avoid evaporation loss of the analytes. After reconstitution with 0.5 mL water, solid phase extraction was realized using BondElute Plexa cartridges (60 mg, 3 mL; Agilent Technologies, Santa Clara, USA) on an ASPEC GX 274 instrument (Gilson, Limburg, Germany). The procedure includes conditioning of the cartridges (3 mL methanol; 1 mL water), loading, washing (2 mL of a mixture of 40% methanol and 60% aqueous ammonium hydroxide solution, 2%) and the elution step (2 mL methanol/water, 90/10, v/v). After addition of 20 µL of ethylene glycol, the eluate was evaporated to dryness, and subsequently dissolved for LC-MS/MS with 40 µL HPLC mobile phase A. Head hair collected from an untreated female was used as negative control samples. Positive control samples were prepared by adding the

triamcinolone acetonide standard (calibration range 0.1 to 2.0 pg/mg, e.g.) to pulverized hair blank material.

2.4. Detection

LC-MS/MS measurement was operated on a 1200 series LC-system (Agilent Technologies, Waldbronn, Germany), coupled to a QTrap5500 mass spectrometer (AB Sciex, Darmstadt, Germany) equipped with an ESI interface. Chromatographic separation was carried out on a C18 column (Hypersil Gold, 50 x 2.1 mm, 3 μ m, Thermo Scientific, USA) by a linear gradient (mobile phase B 10 % for 1 min, 10 % to 100 % during 10 min, 100 % for 3 min). The flow-rate was 300 μ L/min, injection volume 5 μ L. The ESI source was operated in the positive ionization mode at +5500 V and a temperature of 550 °C. Following specific multiple reaction monitoring transitions were included in the method: m/z 435.0 \rightarrow 415.0, 435.0 \rightarrow 397.0, 435.0 \rightarrow 357.0, 435.0 \rightarrow 339.0 (triamcinolone acetonide); 364.0 \rightarrow 121.0 (d4-cortisol). LOD and LOQ were 0.1 pg/mg and 0.3 pg/mg, respectively.

3. Results and Discussion

Urine samples were initially screened by the laboratories procedure for doping control samples including 18 glucocorticoids. Furthermore, confirmation of triamcinolone acetonide was carried out by the method described above. Triamcinolone acetonide was identified in urine of several affected patients (concentrations ranging from LOD 0.4 ng/mL up to 25 ng/mL; results not shown). Samples were collected 2.5 to 5 weeks following the last acupuncture. The positive findings several weeks after the suspected uptake are conclusive with the depot effect of injected triamcinolone acetonide [2].

Hair samples were screened for the presence of 14 synthetic (beclomethasone, betamethasone, budesonide, ciclesonide, dexamethasone, fludrocortisone, flumethasone, flunisolide, fluocortolone, isoflupredone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide) and 3 endogenous glucocorticoids (cortisol, cortisone, corticosterone).

Head hair of several patients has been sampled up to 11 months after the last reported acupuncture. The hair analyses resulted in positive findings of triamcinolone acetonide at concentrations between LOD 0.1 pg/mg and 57 pg/mg.

Figure 2 illustrates the long-term detection of triamcinolone acetonide in hair. The hair sample originates from a female, who dated the last acupuncture nearly one year before. The analyses were processed following segmentation into 5 segments of 3 cm length each. Triamcinolone acetonide was confirmed in segments 3 (c) and 4 (d) (length 6-9 cm and 9-12 cm, respectively), covering the suspected period of uptake. The concentration was estimated between LOD (0.1 pg/mg) and LOQ (0.3 pg/mg).

4. Conclusion

The established analytical method is capable for the long-term detection of synthetic glucocorticoids. Urine and hair samples originating from several patients were tested positive on triamcinolone acetonide up to 11 months following suspected application. The analytical results in urine and hair substantiate the initial suspicion of a deceptive application of triamcinolone acetonide during acupuncture treatments. Unless the uptake of the synthetic glucocorticoid triamcinolone acetonide was proven for the last weeks/months before sample collection, the route of application could not be clarified by the analyses. Follow-up studies

should be aimed for to investigate further parameters, which are important for result interpretation (e.g., incorporation mechanism) or may limit detection capability (e.g., wash-out effect).

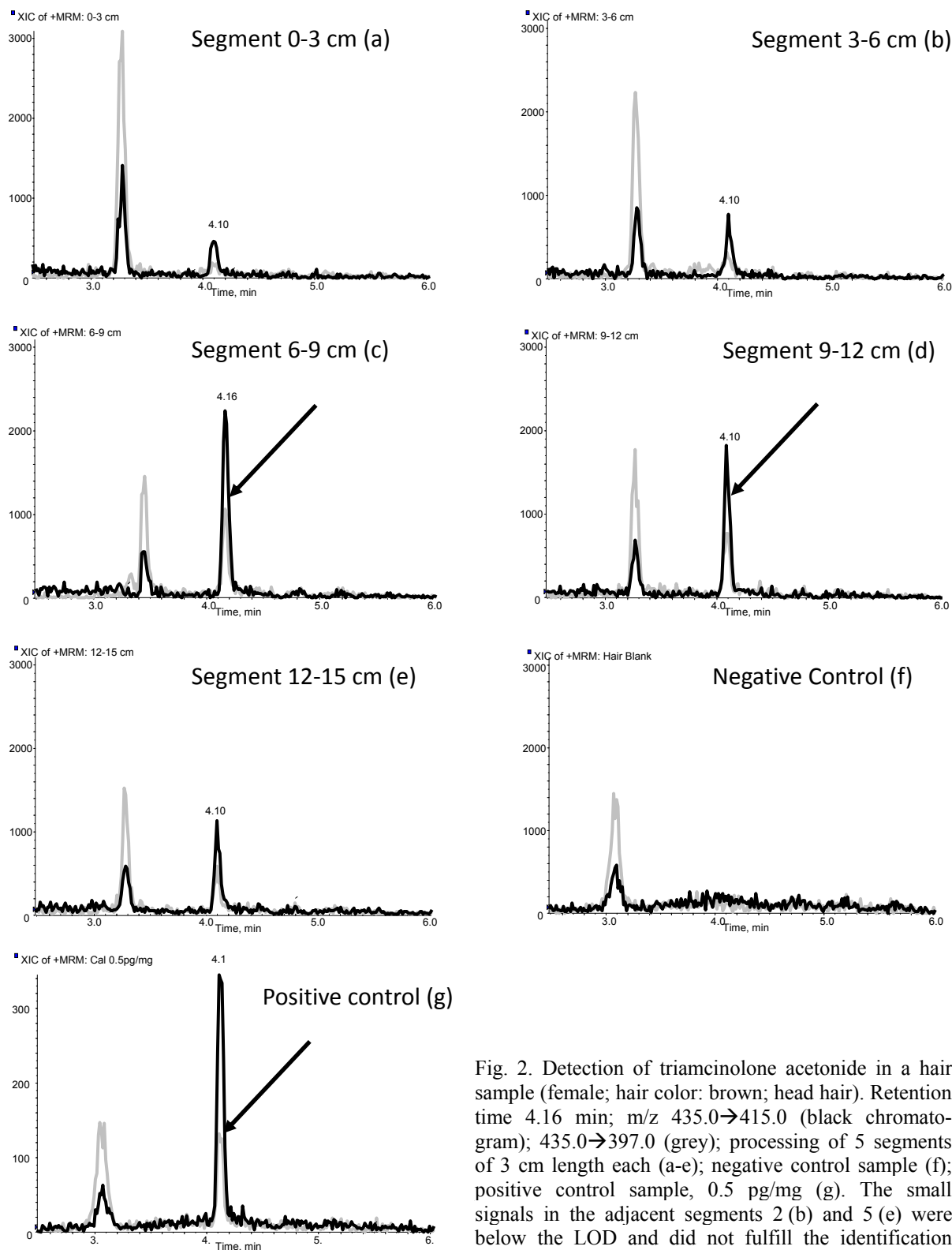


Fig. 2. Detection of triamcinolone acetonide in a hair sample (female; hair color: brown; head hair). Retention time 4.16 min; m/z 435.0 \rightarrow 415.0 (black chromatogram); 435.0 \rightarrow 397.0 (grey); processing of 5 segments of 3 cm length each (a-e); negative control sample (f); positive control sample, 0.5 pg/mg (g). The small signals in the adjacent segments 2 (b) and 5 (e) were below the LOD and did not fulfill the identification criteria.

5. References

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