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Evaluation of the reporting level to detect triamcinolone acetonide misuse in sports

Xavier Matabosch [a], Oscar J. Pozo [a], Clara Pérez-Mañá [b,c], Esther Papaseit [b,c], Josep Marcos [a,d], Jordi Segura [a,d], Rosa Ventura [a,d,*]

[a] Bioanalysis Research Group, IMIM (Institut Hospital del Mar d'Investigacions Mèdiques), Doctor Aiguader 88, 08003 Barcelona, Spain
[b] Human Pharmacology and Neurosciences Research Group, IMIM, Doctor Aiguader 88, 08003 Barcelona, Spain
[c] Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), 08193 Barcelona, Spain
[d] Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Doctor Aiguader 88, 08003 Barcelona, Spain

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ABSTRACT

Triamcinolone acetonide (TA) is prohibited in sport competitions using systemic administrations (e.g., intramuscular, IM), and it is allowed by other routes (e.g., intranasal, IN, or topical, TOP). A reporting level of 30 ng/mL is used to discriminate between forbidden and allowed administrations. We examined urinary profiles of TA metabolites after TOP, IN and IM administrations to evaluate the suitability of the current reporting level and to define the best criteria to discriminate between these administrations. TA was administered to healthy volunteers by different routes: a single IM dose ($n = 2$), IN doses for three days ($n = 6$), and TOP doses for five days followed by a single IM dose ($n = 8$). Urine samples were collected at different time intervals and they were analyzed by liquid chromatography–tandem mass spectrometry to measure TA and eight metabolites. After TOP and IN administrations, concentrations of the metabolites were significantly lower ($p < 0.05$) than after IM administrations. Concentrations of TA after IM administration were lower than 30 ng/mL for all volunteers (range 0.7–29.7 ng/mL), and they were lower than 5 ng/mL after multiple IN or TOP doses (0.1–3.6 ng/mL and 0–1.7 ng/mL, respectively). For 6β-hydroxy-TA, the main TA metabolite, greater concentrations were obtained: 10.7–469.1 ng/mL, 2.2–90.6 ng/mL and 0–57.2 ng/mL after IM, IN and TOP administrations, respectively. These results suggest that the current reporting level is not suitable to detect forbidden IM administration of TA. A lower concentration of the parent drug or the use of specific metabolites could discriminate IM from TOP or IN administrations.

1. Introduction

Triamcinolone acetonide (TA) is a potent glucocorticosteroid commonly used as anti-inflammatory drug to treat different pathologies. It can be administered by different routes (inhaled, intramuscular (IM), intranasal (IN), intraarticular and topical (TOP)), and it is used to treat many different conditions such us allergic disorders (e.g., asthma, rhinitis or atopic dermatitis), skin conditions (e.g., severe erythema multiforme and pemphigus), gastrointestinal diseases (e.g., ulcerative colitis) and rheumatic disorders (e.g., gout, psoriatic arthritis and systemic lupus erythematosus) [1]. The administration route and dosage depend on the pathology to be treated, and the patient's clinical characteristics and its response to treatment.

Glucocorticosteroids are included in the list of prohibited substances in sports because of the health risks for the athletes and evidences of ergogenic effects [2]. The use of TA and other glucocorticosteroids is prohibited in sport competitions by the World Anti-Doping Agency (WADA) when administered by oral, intravenous, IM or rectal routes, and they are allowed for therapeutic purposes using other administration routes (e.g., TOP or IN administrations) [3]. In an attempt to discriminate between legal and forbidden administrations, WADA has defined an arbitrary reporting level of 30 ng/mL for glucocorticosteroids and their metabolites [4]. Samples with concentration lower than 30 ng/mL should not be reported positive by the doping control laboratories because they are considered result of therapeutic use. However, it is known that this general reporting level is not suitable for all corticosteroids for different reasons: multiple administration routes, wide range of therapeutic doses, different

* Corresponding author at: Bioanalysis Research Group, IMIM (Institut Hospital del Mar d'Investigacions Mèdiques), Doctor Aiguader 88, 08003 Barcelona, Spain. Tel.: +34 933160471; fax: +34 933160499.
E-mail address: rventura@imim.es (R. Ventura).

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metabolic and excretion rates in urine, among others [5–7]. Therefore, strategies to distinguish between administration routes in order to discriminate therapeutic treatments from forbidden use need to be improved.

Differences in the urinary excretion of metabolites depending on the route of administration have been detected for some doping agents [8], and have been also described for other glucocorticosteroids [7,9]. Evaluation of different urinary metabolites of budesonide after forbidden oral and allowed inhaled administrations lead to the conclusion that 6β-hydroxy-budesonide is the best marker to discriminate between these administration routes compared to unchanged budesonide and to 16α-hydroxy-prednisolone, the main budesonide metabolite [7,10]. That metabolite has been recently adopted in WADA regulations to distinguish allowed and forbidden use of budesonide [4]. For methylprednisolone, a detailed study on the excretion of urinary metabolites after TOP and oral administrations showed that the use of additional metabolites improves the distinction between these administration routes compared to the use of the parent drug [11,12]. In WADA accredited laboratories the misuse of TA is reported by monitoring the parent drug as per the general reporting level of 30 ng/mL [13–15]. Unfortunately, data regarding excretion of TA and its metabolites in human urine after different administration routes is not available in the literature. Therefore, the WADA criterion has not been properly evaluated to detect TA misuse.

In a recent study, TA and eight metabolites were identified in urine after IM administration of TA using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) [16]. Structures of the metabolites are described in Fig. 1. The aim of the present study was to evaluate the urinary profiles of TA and these metabolites obtained after administration of TA by different routes (TOP, IN and IM administrations) in order to evaluate the suitability of the WADA reporting level to discriminate between allowed and forbidden administrations of TA and to define the best criteria to discriminate between these administrations.

2. Materials and methods

2.1. Chemical and reagents

Triamcinolone acetonide (TA), triamcinolone (T, M8) and ammonium formate were obtained from Sigma (St. Louis, MO, US). 6β-hydroxy-TA (6β-OH-TA, M1) and 16α-hydroxy-prednisolone-11,12,12,21,21-d_5 (16α-OH-Pred-d_5) were purchased from Toronto Research Chemicals (Toronto, Canada). Triamcinolone-6-d_1 acetonide-d_6 (TA-d_7) was obtained from CDN Isotopes (Pointe-Claire, Canada). The β-glucuronidase preparation (type *Escherichia coli* K12) was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Analytical grade di-sodium hydrogen phosphate, sodium hydrogen phosphate, potassium carbonate, ethyl acetate, acetic acid, acetonitrile and methanol (LC gradient grade), and formic acid (LC/MS grade) were obtained from Merck (Darmstadt, Germany). Milli-Q water was obtained using a Milli-Q purification system (Millipore Ibérica, Barcelona, Spain).

2.2. Sample preparation procedures

Two sample preparation procedures were applied. For the extraction of TA and neutral metabolites (M1, M2, M3, M4, M5, and M8 (Fig. 1)), two internal standards (ISTDs) (40 ng of TA-d_7, and 80 ng of 16α-OH-Pred-d_5) were added to 5 mL of urine, followed by the addition of 1.5 mL 1 M phosphate buffer pH 7. Then, β-glucuronidase from *E. coli* was added (50 μL) and hydrolysis was carried out for 1 h at 55 °C. The hydrolyzed sample was alkalinized with 400 μL of 25% potassium carbonate solution to pH 8–9 and the steroids were extracted twice with 4 mL of ethyl acetate. After shaking for 20 min and centrifugation (5 min at 1400 × *g*), the organic layers were transferred into a new tube and evaporated to dryness under a nitrogen stream in a water bath at 50 °C. The residue was reconstituted using 100 μL of a mixture of water:acetonitrile (3:1, v/v) and 10 μL were injected into the LC–MS/MS system.

For the extraction of acidic metabolites (M6 and M7), ISTD solution (250 ng TA-d_7) was added to 5 mL of urine, followed by the addition of 1 mL of 1 M potassium acetate buffer (pH 3.5). Liquid–liquid extraction was performed twice by shaking for 20 min with 4 mL of ethyl acetate. After centrifugation, the procedure was analogous to the described for the extraction of neutral metabolites.

2.3. LC–MS/MS analysis

LC–MS/MS analyses were carried out using a triple quadrupole (Xevo TQ MS) mass spectrometer provided with an orthogonal

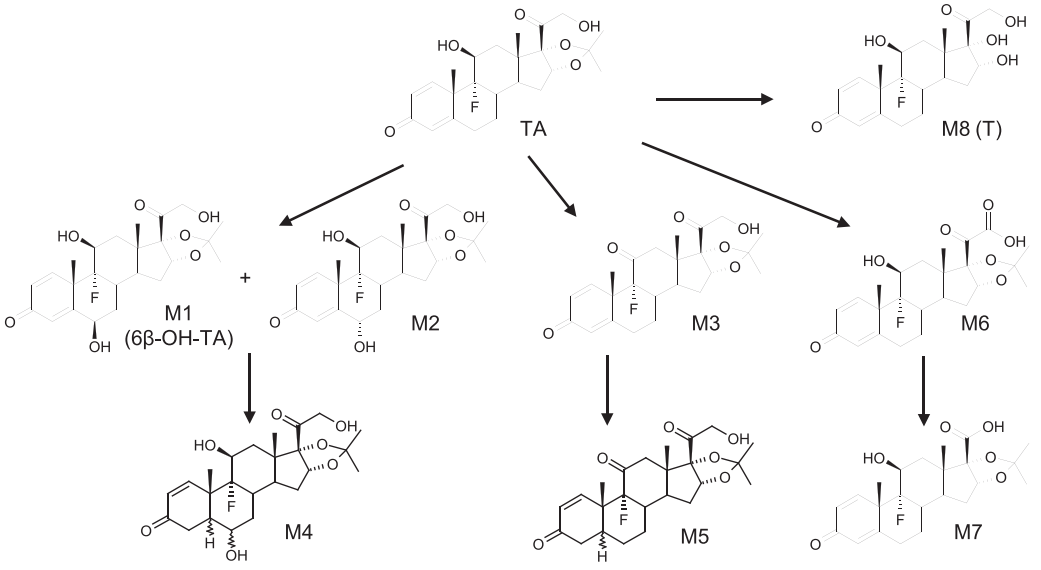

Fig. 1. Triamcinolone acetonide metabolic pathway. Triamcinolone acetonide (TA) and metabolites previously identified after IM administration.