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Methylprednisolone detection in urine following local and oral administrations

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Introduction

Glucocorticosteroids are prohibited when administered orally, rectally, intravenously or intramuscularly. In order to investigate the possibility to distinguish between permitted vs an illicit administration of these substances, we conducted two excretion studies after controlled administrations of methylprednisolone through oral and local administrations.

Mehylprednisolone urinary detection as well as the effects on the normal endogenous glucocorticosteroids (cortisol and cortisone) excretion were monitored by routine LC/MS/MS analyses after automatic SPE extraction.

Experimental

Samples obtained after controlled administrations of methylprednisolone were analyzed following the screening and confirmation procedures for methylprednisolone detection. A brief description of the method used is presented in Figure 1. Methylprednisolone, methylprednisone, 20α-hydroxymethylprednisolone (see Figure 2), cortisol and cortisone were monitored. Cortisol and cortisone were compared with population based data obtained from routine samples of the Portuguese doping control program.

Oral administration: 4 mg of methylprednisolone (Medrol®, Pfizer) were given orally to a male volunteer.

Local administration: Samples were collected from an athlete (that had a TUE accepted by the National Portuguese Antidoping Council (CNAD). 40 mg of Depo-Medrol ®, Pfizer by extra-articular infiltation) that volunteered for the study.

Results

After both administrations, concentrations higher than the reporting threshold (30 ng/mL) proposed by WADA are reached (c.a. 10 h for the oral administration and 36 h for the local one) with slightly higher peak concentration after the local administration. Methylprednisolone can be detected up to 32 h after oral administration and 80 h after local administration. The excretion profile of the 3 metabolites monitored is similar. The relative response of 20α-hydroxymethylprednisolone is in both administrations higher than methylprednisolone itself being a more sensitive marker of methylprednisolone intake.

After both administrations a deep suppression of cortisol and cortisone concentrations is observed. The lowest concentrations are below the 5% percentile of the population based data (Figures 3-4 and Table 1) reflecting the systemic effect reached in both cases.

Conclusions

After both oral and local administrations, a systemic effect is observed through the inhibition of the endogenous excretion of cortisol and cortisone. The concentrations reached in the "permitted" administration (dosage 10 times the oral administration) were higher than after an oral administration. The urinary excretion profile of methylprednisolone and its metabolites is similar in both administrations do not permitting to differentiate the route of administration.

Figure 1. Description of the method used.

3 mL of urine
↓
Add **0.3 μg of methyltestosterone (internal standard)** **1 mL of sodium acetate buffer 0.2 M pH 5.2** and **50 μL of β-glucuronidase/ arylsulfatase**
↓
Incubate the samples at 55°C for 2 h
↓
Condition the Oasis® HLB columns with **3 mL of methanol** and **3 mL of water**
↓
Load **4 mL of the samples** into the columns
↓
Wash the columns with **3 mL of sodium hydroxide 0.02M: methanol (6:4, v/v)** and **3 mL of water**
↓
Elute the samples with **3 mL of tert-butylmethyl ether: methanol (9:1, v/v)**
↓
Evaporate to dryness under nitrogen at 45°C.
↓
Reconstitute in **100 μL of mobile phase**
↓
Transfer the mixture to a 200 μL vial, and analyse the sample by LC/MS/MS

Instrument: Waters Alliance 2795 pump/ Micromass Quattro micro™
Column: Waters-XTerra MS C18 (150 mm, 2.1 mm, 5 μm)
Flow parameters
- solvent A: ACN/HCOOH 0.1% (95:5)
- solvent B: HCOOH 0.1%/ACN (95:5)
- flow rate: 0.3 mL/min
- 650

Mass spectrometric parameters
- acquisition mode: MRM
- function 1
  - time: 1-6.5 min
  - (377.3→281.3) cone 20V coll 15eV
  - (377.3→341.2) cone 20V coll 15eV
  - (377.3→359.2) cone 20V coll 15eV
- function 2
  - time: 6.5-10 min
  - (373.3→319.2) cone 20V coll 15eV
  - (373.3→337.2) cone 20V coll 15eV
  - (373.3→355.2) cone 20V coll 15eV
  - (375.3→321.2) cone 20V coll 11eV
  - (375.3→339.2) cone 20V coll 11eV
  - (375.3→357.2) cone 20V coll 11eV
- function 2
  - time: 10-16 min
  - (303.4→97.0) cone 35V coll 22eV
  - (303.4→109.0) cone 35V coll 22eV

Figure 2. LC/MS/MS analysis of a urine sample collected c.a. 12 h after the infiltration

I. 375>357; 375>339 – Methylprednisolone

II. 373>355; 373>337 – Methylprednisone

III. 377>281; 377>359 - 20α-Hydroxymethylprednisolone

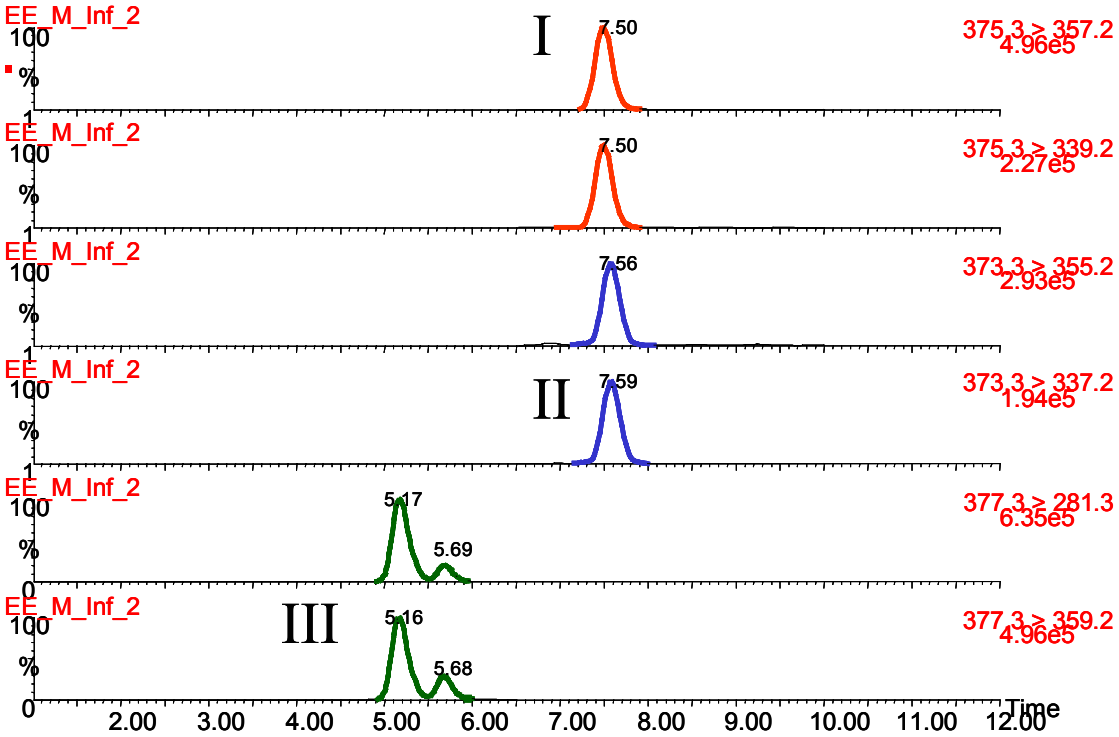


Table 1. Non-parametric data of population distribution of cortisol and cortisone in routine samples

(n=802)	5%	Median	Mean	95%
Cortisol (ng/mL)	20.1	146.8	187.0	470.8
Cortisone (ng/mL)	35.7	172.4	188.0	399.2

(5%, 95%: percentiles)

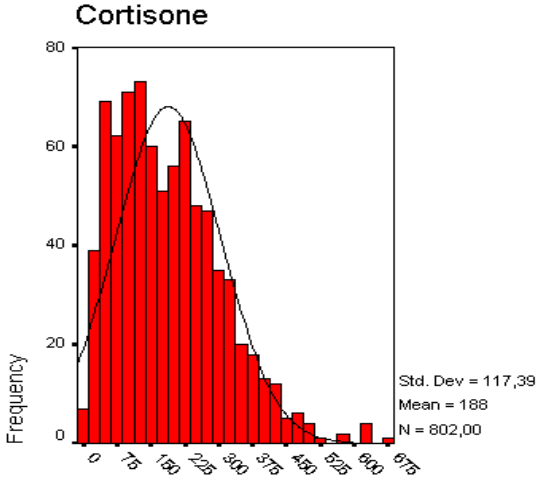
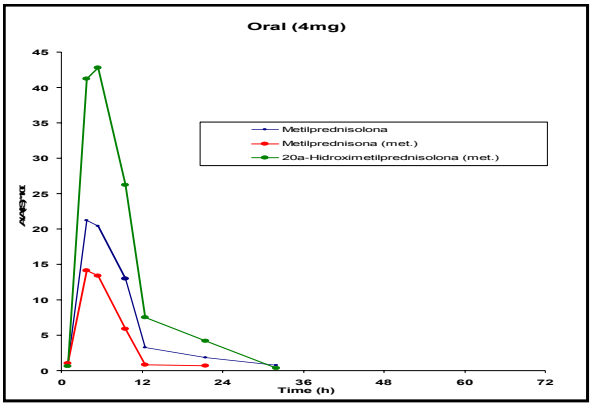


Figure 3. Relative urinary detection of methylprednisolone and metabolites after oral and local administrations



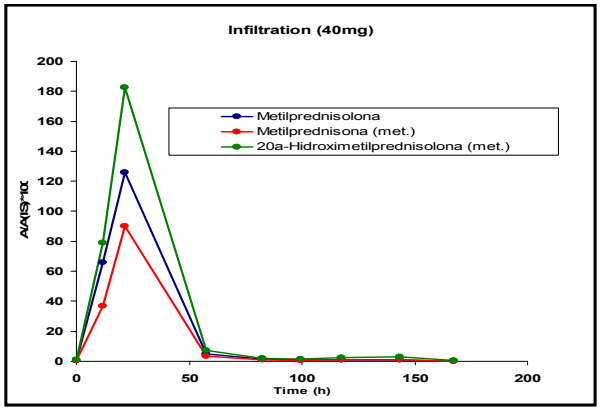


Figure 4. Urinary methylprednisolone and effect on the HPA axis after oral and local administrations