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Synthetic glucocorticosteroids administration: urinary excretion of triamcinolone acetonide

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Introduction

Synthetic glucocorticosteroids are widely used by athletes in various sports, as indicated by the annual statistics regarding the requests for TUE [1]. Glucocorticosteroids are known as very potent anti-inflammatory products. They are not only used in the treatment of chronic asthmatic symptoms, but are also currently applied in sport medicine for tendonitis, articular sprains, pain, injuries and overuse syndromes [2]. Unfortunately, their systemic use is often associated with significant side-effects, such as Cushing's syndrome.

Corticosteroids are included in the list of prohibited substances issued by World Anti-Doping Agency (WADA) [3]. Topical preparations are not prohibited, systemic use is forbidden and requires a standard TUE when medically necessary, whereas local applications and intra-articular injections are allowed under medical supervision and require an abbreviated TUE.

There is no agreement in effectiveness of corticosteroids and also the clinical guidelines are poor with respect to optimal timing, dosage and injection volume. It is also not possible to characterize the type of application (e.g. injection, inhalation, local or oral administration) related to urinary concentrations. In order to clearly evaluate and establish some criteria in relation with glucocorticosteroid administration, WADA accepted to support a research project over two years. Project partners and administered glucocorticosteroids are presented in Table 1. After acceptance of Ethical Committee, synthetic glucocorticosteroids currently administered were tested on numerous volunteers. For each compound, existing administration modes were applied (single and multiple applications) and excretion kinetics and patterns, urinary concentrations were established. Moreover, urinary free and conjugated fractions were evaluated. Analysis were performed by LC-MS^n as already published in the literature [4-7]. The first results were obtained with the application of triamcinolone

acetonide. Results for nasal and pulmonary inhalations, intramuscular, intra-articular and intradermal injections are presented.

***Table1**: Administration trial of synthetic glucocorticosteroids*

	ADMINISTRATION MODES			
SUBSTANCES	***Oral*** ***sigle and multiple***	***Injection*** ***intra-muscular*** ***single***	***Inhalation*** ***nasal / pulmonary*** ***single and multiple***	***Injection*** ***intra-and peri-articular*** ***single***
Triamcinolone ***acetonide***	_	**Paris** KENACORT RETARD (sol. 80 mg)	**Paris** NASACORT (spray 220 μg) --- **Lausanne** AZMACORT (spray 100 μg)	**Lausanne** TRIAMCORT DEPOT (sol. 40 mg)
Methyl-prednisolone	**Paris** MEDROL (tablet 32 mg)	**Paris** SOLU-MEDROL (sol. 40 mg)	_	**Lausanne** DEPOT MEDROL (sol. 80 mg)
Predinisolone	**Paris** SOLUPRED (tablet 20 mg)	**Lausanne** PREDNISOLONE STREULI (sol. 25/50 mg)	**Paris** DERINOX (spray 40 μg)	**Paris**
Dexamethasone	**Paris** DECADRON (tablet 0.5 mg)	**Lausanne** CHRONOCORTE (sol. 7 mg/ml)	**Sydney**	**Lausanne** DEXAMETHASONE HELVEPHARM (20 mg)
Betamethasone	**Lausanne** CELESTONE (tablet 0.5 mg)	**Lausanne** DIPROPHOS (sol. 7 mg/ml)	**Sydney**	**Paris**
Budesonide	**Lausanne** BUDENOFALK (capsule 3 mg)	_	**Lausanne** PULMICORT (spray 200 μg)	_
Triamcinolone	**Lausanne** KENACORT (tablet 4 mg)	**Sydney**	_	**Sydney**
Prednisone	**Sydney**	_	_	**Sydney**

Experimental

Administration of the pharmaceuticals and urine collection

All pharmaceuticals were administered at therapeutic levels under medical supervision, each to 6 volunteers. Intra-muscular, intra-dermal (KENACORT RETARD®) and intra-articular (TRIAMCORT DEPOT®) injections were applied once, whereas nasal (NASACORT®) and pulmonary (AZMACORT®) inhalations were administered both with a single dose and with multiple applications during 5 days. Urine samples were collected during 1 week or 3 months, depending on the administration mode. Samples were analyzed by both laboratories.

Urine extraction

The extraction was performed with 4 ml of urine. Internal standard (triamcinolone acetonide-d_6 for Paris and methyltestosterone for Lausanne) was added.

- Extraction of free fraction: the sample was added with 200 μl of carbonate buffer (15 % K_2CO_3, pH 9) and was passed through a SPE-C18 column. Elution of the analytes was

performed with 2 x 3 ml dichloromethane. After evaporation of the solvent, the residue was dissolved in 100 µl of mobile phase.

- Extraction of total fraction: * **Lausanne:** the sample was passed through a SPE-C18 column. Elution of the analytes was performed with 3 x 1 ml methanol. After evaporation of the solvent, the residue was dissolved in 1ml of phosphate buffer (0.2 M, pH 7) and hydrolysis was performed with 50 µl of β-glucuronidase (*E. Coli*) at 50 °C during 1 hour. After liquid extraction with TBME and evaporation of the solvent, the residue was dissolved in 100 µl of mobile phase. * **Paris:** hydrolysis of urines was performed with 50 µl of b-glucuronidase (*E. Coli*) at 55 °C during 1 hour (pH 6.5). The sample was passed through a SPE-C18 column. Elution of the analytes was performed 3 x 2 ml dichloromethane. After evaporation of the solvent, the residue was dissolved in 100 µl of mobile phase.

Analytical equipments and conditions

The urinary extracts were analyzed with a TSQ Quantum Discovery instrument from ThermoFinnigan (LC-MS^n triple quadrupole). Positive SRM mode was used (ESI).

Analytical parameters:	Mobile phase: $HCOONH_4$ 10 mM/CH_3CN (50/50, v/v)	
	Column: Zorbax RX-C_8 or Zorbax XDB-C_8 (150x1mm–5 µm)	
	Injected volume: 20 µl	
Transitions:	Triamcinolone acetonide-d_6:	441>421 and 441>403
	Methyltestosterone:	303>109.and 303>97
	Triamcinolone acetonide:	435>415.and 435>397

Results

Excretion curves were established in order to evaluate the inter-individual variability regarding excretion kinetic and pattern (Figure 1). Moreover, free fraction as well as total fraction were extracted and results were compared.

The main observations of this part of the study were:

- for nasal inhalation, the highest excreted concentration is 3 ng/ml and 14 ng/ml for free and total fractions, respectively, apart from single dose or multiple applications.

- for pulmonary inhalation, the highest excreted concentration is 6 ng/ml and 12.5 ng/ml for free and total fractions, respectively.

- for intra-dermal injection, the highest excreted concentration is 3 ng/ml and 6 ng/ml for free and total fractions, respectively.

- however, for intra-muscular injection, the highest excreted concentration is 40 ng/ml and 80 ng/ml for free and total fractions, respectively.

- Also, for intra-articular injection , the highest excreted concentration is 170 ng/ml and 380 ng/ml for free and total fractions, respectively

From the basis of the measured data, it could be assumed that in addition to inter-individual metabolic variation also the administration mode plays an important role in the concentration of free and conjugated fraction of triamcinolone acetonide. This is actually deeply investigated for all selected synthetic glucocorticosteroids.

Conclusion and perspectives

On the basis of these preliminary results, cut-off values of 10 ng/ml (free fraction) or 20 ng/ml (total fraction) are suggested as indicative thresholds. Indeed, these cut-off values associated with a detection window of about 6 days, allow the discrimination between the intramuscular/intra-articular administrations and the other applications of triamcinolone acetonide.

Analyses are being processed regarding the investigation of other synthetic glucocorticosteroids. Particular attention is paid not only on urinary concentrations but also on the metabolism of the selected substances, depending on the administration route.

Additionally, endogenous profiles will be also investigated, depending on the administered glucocorticosteroid and the application mode.

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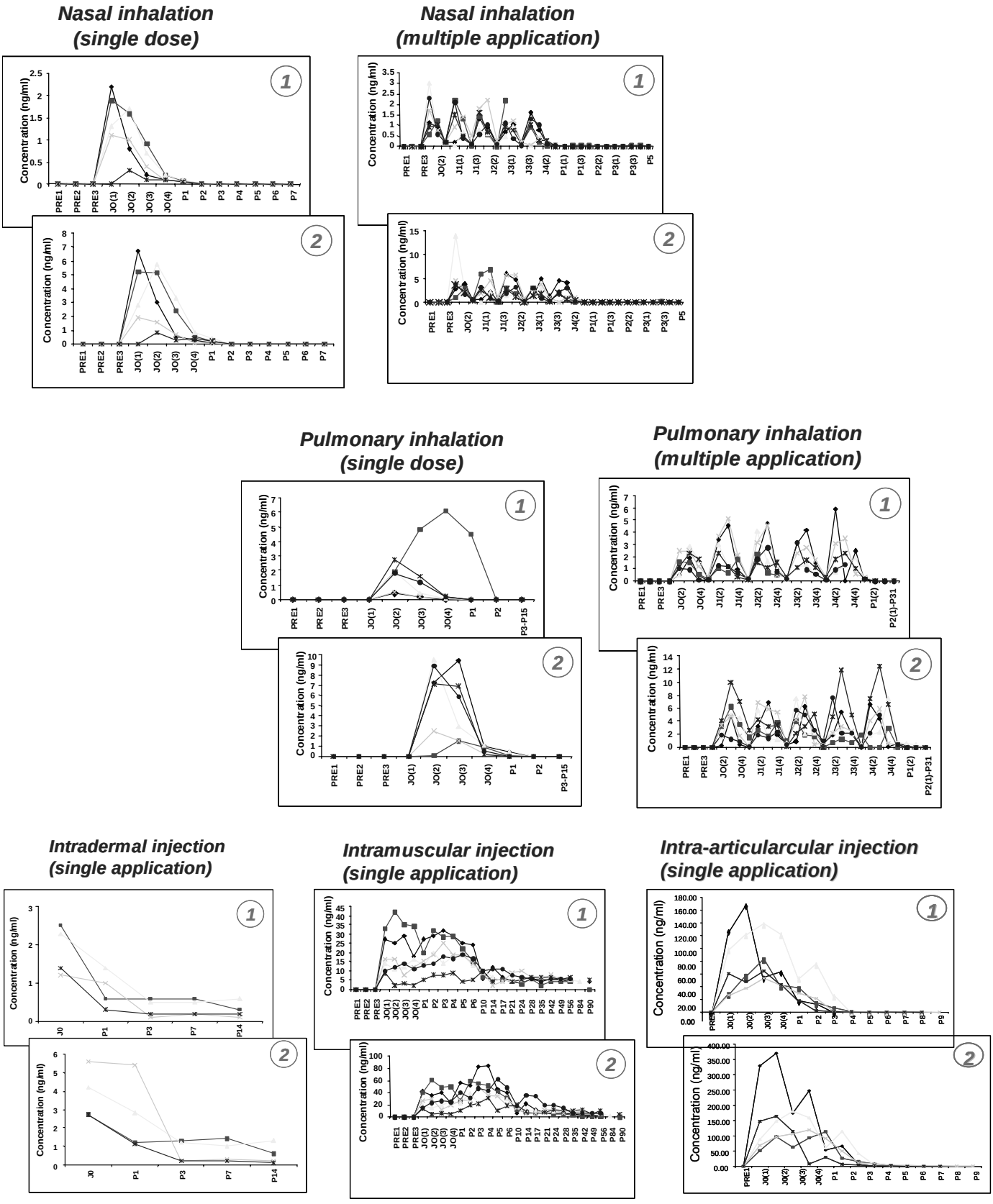

***Figure 1**: Excretion kinetics for triamcinolone acetonide [free fraction (1), total fraction (2)]*