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# Urinary Levels of Glucocorticoids Resulting from Different Routes of Administration

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## Background

The WADA Prohibited List [1] differentiates between various routes of administration of glucocorticoids. Since 2005, certain local/topical preparations are freely permitted; others require an approved Abbreviated Therapeutic Use Exemption (ATUE), whereas systemic routes of administration are prohibited. This may pose a challenge when it comes to interpretation of results, as limited information is available about the variability of urinary levels of glucocorticoids and their metabolites resulting from different routes of administration. Results from excretion studies with various glucocorticoids have previously been published by Deventer et al. [2] and by Mazzarino et al. [3]. However, previous studies do not include a direct comparison of different administration routes of the same compounds, applied to the same group of individuals.

## Experimental

### *Excretion study*

Betamethasone, prednisolone and budesonide, each in one local/topical and one systemic preparation, were administered to three groups of healthy volunteers (age 18 – 49, average 30.9). Each group consisted of eight persons, and each group received first the local/topical and later the systemic preparation of the same active compound, thereby acting as their own controls. All urine was collected for the first 36 hours, and spot urine samples were collected at 48 and 72 hours after administration. In one group receiving an intramuscular injection of betamethasone, weekly samples were collected for four weeks after administration.

| Active compound | Preparation | Route of administration | Amount of active compound |
|---|---|---|---|
| **Group 1 Betamethasone** | Betnovat "GlaxoSmithKline" | Cream | 3 mg betamethasone[1] |
| | Celeston Chronodose "Schering-Plough" | Intramuscular injection | 5.7 mg betamethasone[2] |
| **Group 2 Prednisolone** | Ultracortenol "Novartis" | Eyedrops | 0.9 mg prednisolone[3] |
| | Prednisolon "Nycomed" | Tablets | 10 mg prednisolone |
| **Group 3 Budesonide** | Pulmicort "AstraZeneca" | Inhalant powder | 0.8 mg budesonide |
| | Entocort "AstraZeneca" | Controlled-release capsules | 3 mg budesonide |

1) As valerate 2) 3 mg as phosphate and 2.7 mg as acetate 3) As acetate

### *Sample preparation and analysis*

A general sample preparation procedure and individual analytical methods were developed and validated. After addition of an internal standard (see table below), samples were extracted with *t*-butyl methyl ether at pH 9.5. After centrifugation, the organic phase was transferred to clean tubes and evaporated to dryness under nitrogen. The residue was reconstituted in 10 % aqueous acetonitrile, and the samples were analysed by LC-MS/MS on a Thermo Surveyor LC system coupled to a Thermo TSQ Quantum mass spectrometer. The column was a Thermo Betasil C18 (50x2.1 mm, 5 µm particle size), and the mobile phase was an aqueous ammonium formiate / acetonitrile gradient. The mass spectrometer was operated in positive electrospray (ESI+) ionisation mode. Individual methods were optimised for each parent compound (and metabolite where applicable), with acquisition of three Selected Reaction Monitoring (SRM) transitions for each analyte and one for the internal standard.

| Compound | Precursor ion *m/z* | Product ion(s) *m/z* | LOQ ng/mL | CV % (30 ng/mL) |
|---|---|---|---|---|
| **Betamethasone** | 393 | 279, 237, 263 | 0.2 | 4 |
| **Desoximethasone (ISTD)** | 377 | 171 | | |
| **Prednisolone** | 361 | 171, 279, 289 | 0.5 | 3 |
| **Prednisone** | 359 | 171, 265, 237 | 1.0 | 4 |
| **$D_6$-Prednisolone (ISTD)** | 367 | 270 | | |
| **Budesonide** | 431 | 226, 211, 277 | 0.1 | 11 |
| **16α-OH-prednisolone** | 377 | 323, 225, 277 | 0.2 | 6 |
| **Desonide (ISTD)** | 417 | 225 | | |

## Results

The concentration of the glucocorticoids was corrected for specific gravity (normalised to 1.020), and the average concentration within each group was plotted as a function of time. Only the concentration plots of betamethasone, prednisolone and 16α-hydroxyprednisolone

are shown below. Prednisone, the metabolite of prednisolone, showed excretion curves similar to the parent compound, but the levels were somewhat lower and reached their maximum value three hours later on average (not shown). After administration of budesonide, the concentration of the parent compound did not exceed 2 ng/mL, regardless of the route of administration (not shown).

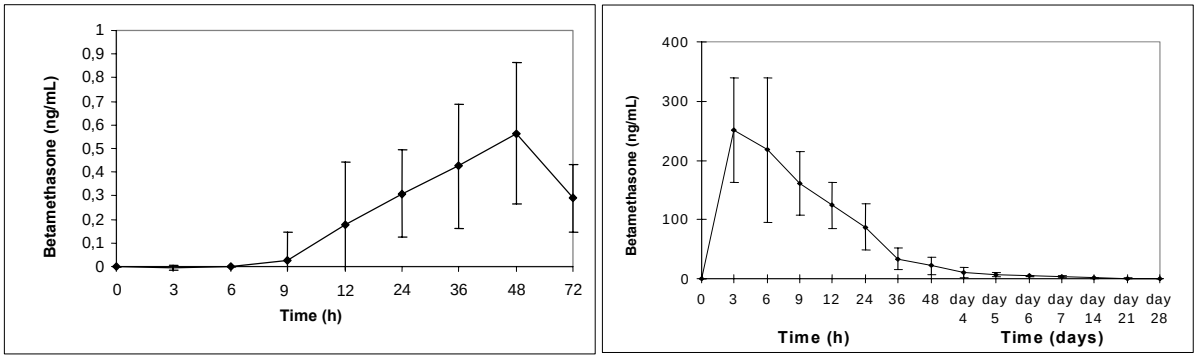


Fig. 1: Average urinary concentration of betamethasone, corrected for specific gravity, after administration of Betnovat cream (left) and Celeston intramuscular injection (right).

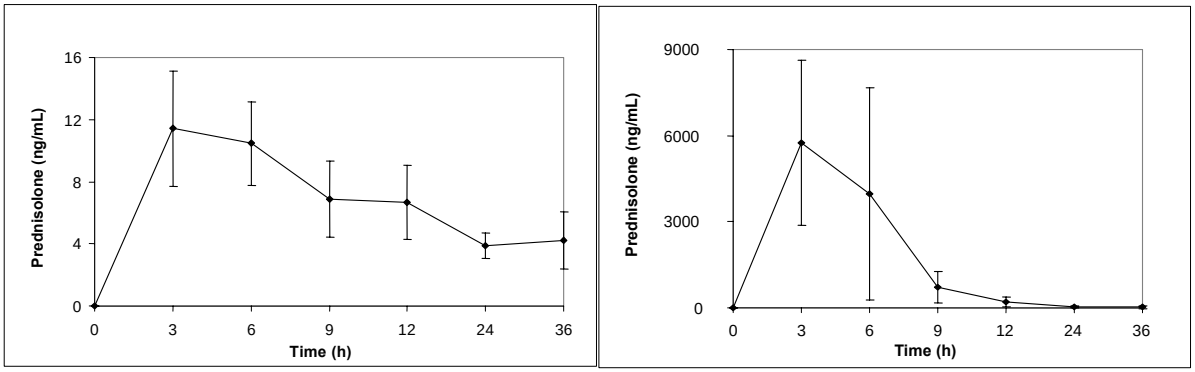


Fig. 2: Average urinary concentration of prednisolone, corrected for specific gravity, after administration of Ultracortenol eye drops (left) and Prednisolon tablets (right).

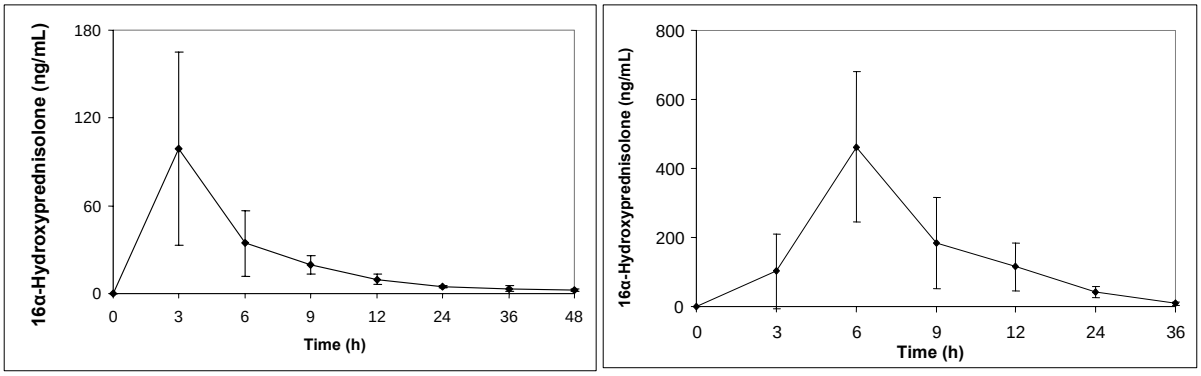


Fig. 3: Average urinary concentration of 16α-OH-prednisolone, corrected for specific gravity, after administration of budesonide in the form of Pulmicort inhalant powder (left) and Entocort capsules (right).

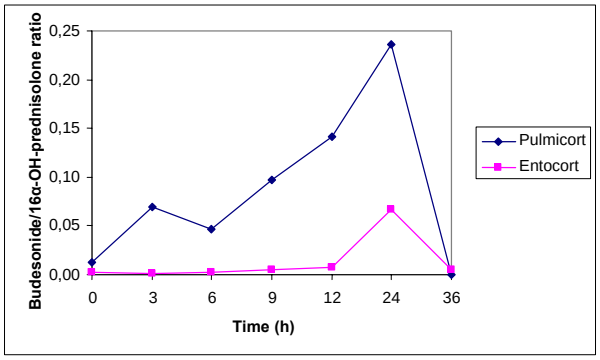


Fig. 4: Ratio of budesonide to 16α-OH-prednisolone after administration of Pulmicort inhalation powder and Entocort capsules.

## Conclusion

All three systemic preparations resulted in urinary levels exceeding the current WADA-specified reporting threshold of 30 ng/mL. With the exception of inhaled budesonide, detected in urine primarily as its 16α-hydroxyprednisolone metabolite, none of the local/topical preparations resulted in urinary levels exceeding the threshold. The findings are in agreement with the results of previous studies [2, 3]. For the preparations and dosages included in this study, the current threshold does not fully exclude administration of local/topical preparations; however, it does facilitate reporting of glucocorticoid findings resulting from all systemic routes of administration. Furthermore, there appears to be a significant difference in the urinary concentration ratio between budesonide and its metabolite, 16α-hydroxy-prednisolone, depending on whether budesonide was administered orally or by inhalation. This may be due to a greater degree of first-pass metabolism and/or degradation in the gastrointestinal tract in the case of the oral preparation, resulting in a lower ratio than was observed for the inhaled preparation.

## References

[1] The World Anti-Doping Agency. The 2007 Prohibited List. International Standard, Montreal http://www.wada-ama.org/rtecontent/document/2007_List_En.pdf (access date 10.09.2007).

[2] Deventer, K., Delbeke, F.T. (2004) Excretion Studies with Corticosteroids. In: Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U. (eds.) *Recent Advances in Doping Analysis (12)*, Köln. pp 37-44.

[3] Mazzarino, M., Braganó, M.C., Garibba, F., Rossi, F., Botrè, F. (2005) The idea of a threshold value for synthetic glucocorticoids: Urinary excretion studies following different routes of administration. In: Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U. (eds.) *Recent Advances in Doping Analysis (13)*, Köln. pp 465-468.