Monica Mazzarino, M. Cristina Braganò, Flaminia Garribba, Francesca Rossi, Francesco Botrè

The idea of a threshold value for synthetic glucocorticoids: urinary excretion studies following different routes of administration

Laboratorio Antidoping, Federazione Medico-Sportiva Italiana, Roma, Italy

INTRODUCTION

Glucocorticoids have been included in the WADA prohibited list in 2004, their screening being at present mandatory for all in-competition tests. As of now, no criteria have been fixed to discriminate the systemic (normal TUE required) from the topical (abbreviated TUE sufficient) administration of synthetic glucocorticoids. Furthermore, the 2005 WADA list allows the use of glucocorticoids in dermatological preparation [1], while the minimum required performance limit (MRPL) is fixed, in the relevant technical document for laboratories at 30 ng/ml [2]. To avoid the unnecessary confirmation (and reporting) of many analytically positive samples not corresponding to a doping offence, it is necessary to identify, possibly at the stage of the screening analysis, a way to discriminate between systemic and non-systemic administration, monitoring at the same time the urinary concentration of synthetic and natural glucocorticoids.

EXPERIMENTAL SECTION

Administration studies

Experiments have been carried out on patients undergoing treatment with corticosteroids. Five synthetic glucocorticoids were administered either locally (inhaled beclomethasone, a single dose of 2 mg; transdermal betamethasone, a single dose of 1 mg) and orally (betamethasone: a single dose of 1 mg , dexamethasone: a single dose of 0.5 mg, prednisone: a single dose of 25 mg, methylprednisolone: a single dose of 16 mg). The subjects were from both sexes (10 males + 10 females) aged 35±5. Baseline and circadian variability of the endogenous steroid profile was assessed in all subjects before and after treatment, by collecting urine samples for up to three days, every two hours (from the first

urine in the morning to the last urine in the night). The urines were prepared according to the screening procedure of conjugated anabolic steroids and analysed by GC/MS for cortisol and tetrahydrocortisol and by LC/MS for synthetic glucocorticoids. The relative concentrations of the natural (cortisol and tetrahydrocortisol) and of synthetic glucocorticoids were measured before and after administration of the selected glucocorticoid. All concentration values were corrected for a value of the specific gravity of 1.020.

RESULTS AND DISCUSSION

The urinary concentration of both cortisol and tetrahydrocortisol decreased significantly in all volunteers from four hours after the oral administration of synthetic glucocorticoids (Figure 1), while they were unaffected by the transdermal and inhalatory administration (Figure 2). The urinary concentration of synthetic glucocorticoids shows in all cases a maximum whose value is close to 30 ng/ml for long acting glucocorticoids (betamethasone and dexamethasone) and close to 80 ng/ml for intermediate acting glucocorticoids (prednisone and methylprednisolone). Betamethasone and dexamethasone can be detected up to 36-58 hours after administration, while the detection window of prednisone and methylprednisolone is narrower (24-30 hours) (Figure 3).

The urinary excretion profile of synthetic glucocorticoids after topic and inhalatory administration was very close to the LOD of the LC-MS-MS method (0.5 ng/ml for betamethasone and 5ng/ml for beclomethasone).

CONCLUSIONS

The urinary concentration of cortisol and tetrahydrocortisol significantly decreased following oral administration, the maximum value of the urinary concentration of the orally administered synthetic glucocorticoids overlapping, in all cases, the minimum value of cortisol and tetrahydrocortisol. This finding is in agreement with the well known suppression of pituitary-adrenal function observed as a consequence of systemic glucocorticoid therapy [3-5]. No effect was detected following topical administration. The peak urinary concentration was always higher than 30 ng/ml for the oral administration, but values higher than 30 ng/ml were recorded only for a limited time, this being in agreement with analogous results reported by other WADA laboratories [6]. Based on this observation, it appears that a general “threshold value” could be effective only if limited to the screening stage, and not as the final evidence of the route of administration, for which the consideration of additional parameters is mandatory.

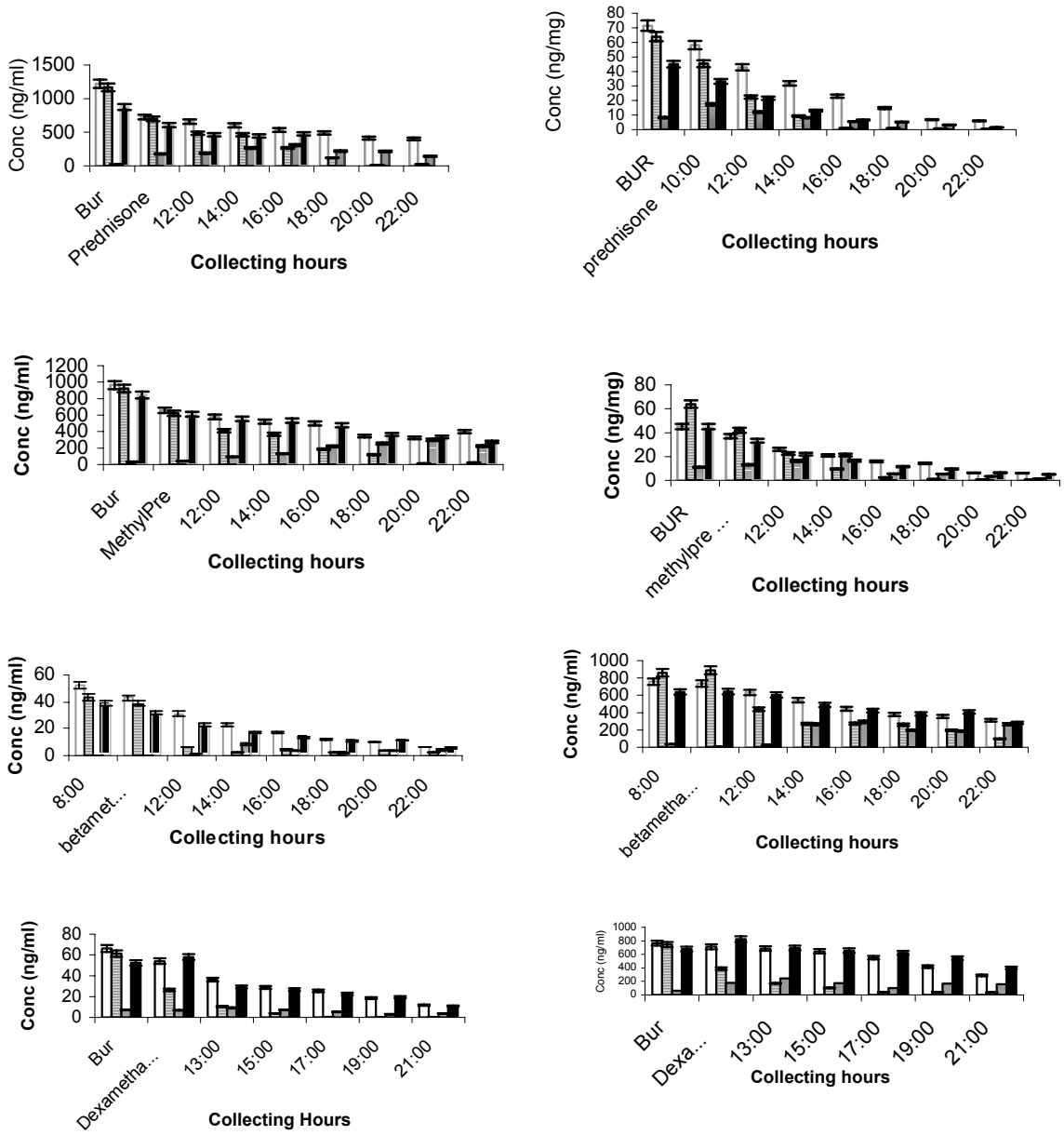

Figure 1. Circadian variability of the urinary concentration of cortisol (left) and tetrahydrocortisol (right) recorded before (open bar), during (dashed), and after 1 (grey), and 2 (black) days from the suspension of the oral administration of (from top to bottom) prednisone, methylprednisolone betamethasone and dexamethasone.

REFERENCES

1. The World Antidoping Code: The 2005 Prohibited International Standard. Available on line at http://www.wada-ama.org/rtecontent/document/list_2005.pdf.
2. WADA Technical Document TD2004MRPL. Available on line at http://www.wada-ama.org/rtecontent/document/perf_limits_2.pdf.
3. Schlaghecke, R.; Kornely, E.; Santen, R.T.; P Ridderskamp, P. *New Eng. J. Med.*, **326**: 226-230 (1992).

4. Krasner, A. S. *JAMA* **282**: 671-676 (1999).
5. Schmidt, I. L.; Lahner, H.; Mann, K., Petersenn, S. *J Clin Endocrinol Metab* **88**: 4193-4198 (2003).
6. Deventer, K.; Delbeke, F.T. In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (Eds.) *Recent Advances in Doping Analysis (12)* Sport & Buch Strauβ, Köln, 37-44 (2004).

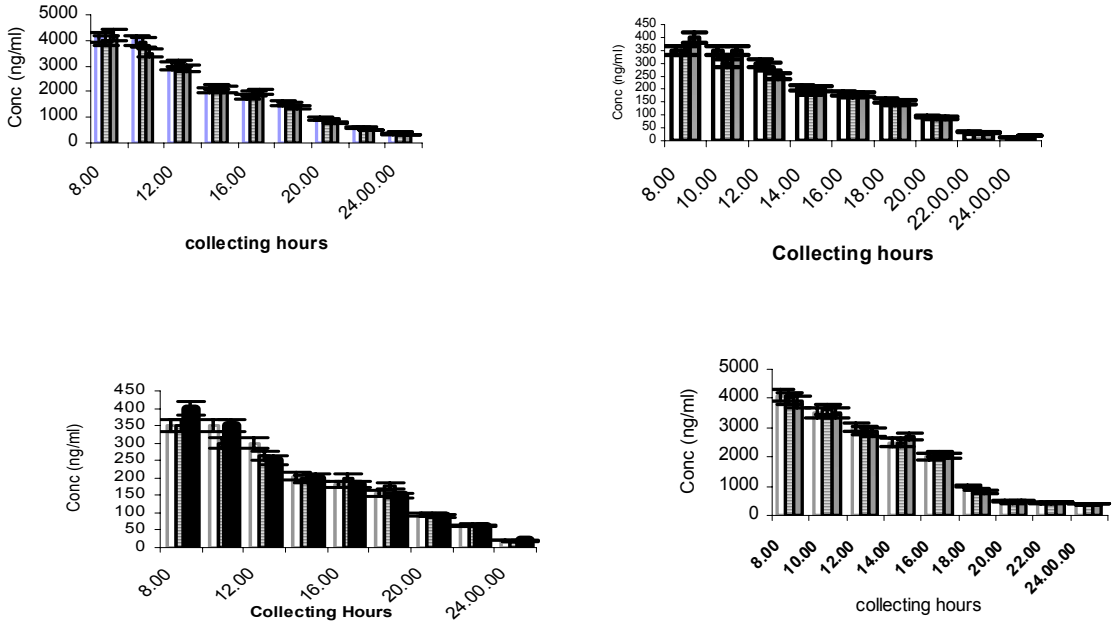

Figure 2. Circadian variability of the urinary concentration of cortisol (left) and tetrahydrocortisol (right) recorded before (open bar), during (dashed) and after 1 day (grey) from the suspension of the inhalatory administration of beclomethasone **(A)** and transdermal administration of betamethasone **(B)**.

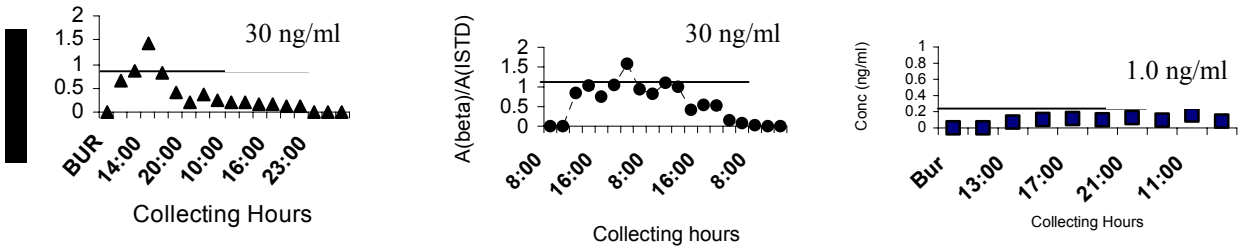

Figure 3. Urinary concentration of synthetic glucocorticoids following oral administration of prednisone (left) and betamethasone (center), and transdermal administration of betamethasone (right)