M. T. Correa Vidal, D. Martínez, R. Montes de Oca, K. Alonso Pérez, M. Granda Fraga.

Differences in urine excretion of hydrocortisone (cortisol) following two administration routes.

Antidoping Laboratory, Institute of Sports Medicine, Havana, Cuba

Introduction

Detection of glucocorticoids in doping control samples still shows serious problems in most of the cases. Its urinary concentrations are too low because they have a medium life time in urine and very short in plasma when a single dose is administrated. However, they generally show its effects on the human body for a long period of time. Another problem that presents its detection is the necessity to determine the administration route to take a decision of a doping offence. The aim of this paper was to study the differences that can be observe with intramuscular administration (illegal, TUE required) and peritendinous administration (permited with Abreviated TUE) of cortisol (hydrocortisone)[1-4].

Materials and Methods

Urine sample and drug administration: A single dose of hydrocortisone (100 mg) was administered by two different routes: intramuscular (i.m.) and peritendinous (p.t.). Sample collection was done up to three days after the beginning of the treatment. Cortisol, tetrahydrocortisol and 11β-hydroxyetiocholanolone (11-OHE) were compared with population based data obtained from routine negative samples of the Cuban Doping Control Program. Validation data of these compounds shows values for accuracy between 3,9 - 8,1%; repeatability between 5,1 - 9,6% and recovery between 82,8 – 89,5 %. The method was linear and correlation coefficient was 0,993, 0,991 and 0,992 for cortisol, tetrahidrocortisol and 11-OHE) respectivaly

Urine samples preparation: Samples were prepared following the normal screening procedure for total steroids[5].

Instrumentation: The analysis were carried out using a Hewlett-Packard 6890 gas chromatograph coupled with a 5973 quadrupole mass spectrometer detection system. Capillary column: Ultra-1 (length 17 m, inside diameter 0.20 mm and film thickness 0.11

μm); helium flow 0.9 ml/min and temperature programming of 182 °C ramped at 3.5 °C/min to 220°C, ramped 5°C/min to 235°C, ramped 40°C/min to 310°C, then 310°C held for 3 min.. Injections of 2 μL were effected at 280 °C in the split mode (split ratio 1:10) The transfer line was heated at 280°C and the ion source temperature was 230 °C. Acquisition mode: SIM.

Results

Cortisol, tetrahydrocortisol and 11β-hydroxyetiocholanolone concentrations were compared with a reference data obtained from routine negative samples from the National Antidoping Program. Both administration routes showed concentrations of these endogenous compounds greater than 95 % percentile. Table 1 and Figure 1 show these data.

Intramuscular administration shows concentrations of cortisol and tetrahydrocortisol significantly higher than p.t. administration. On the other hands, 11-OHE shows exactly the opposite (i.m administration exhibit concentrations higher than 95% percentile but still inside of individual reference range). (Figure 2)

Other significant changes on endogenous steroids profile were found for cortisone and tetrahydrocortisone excretion. These compounds increased about 5 fold its basal urine concentrations for p.t. administration. Meanwhile i.m. administration showed an increment about 3 fold for cortisone and 36 for tetrahydrocortisone.

Hydroxylated metabolites

Some 6β-hydroxylated metabolites of cortisol have been reported[6]. Variations in excretion profile of four proposed hydroxylated metabolites were studied. The area ratios of peak base for hydroxy-cortisol, hydroxy-tetrahydrocortisol, hydroxy-cortisone and hydroxy-tetrahydrocortisone to ISTD were evaluated for both administration routes. Hydroxy-cortisone excretion shows no differences for both administration routes, the remaining hydroxy-metabolites shown a significant increment by i.m. administration. Twenty-five hours after p.t. and i.m. administrations all hydroxy-metabolites go back to basal levels, except hydroxy-cortisol and hydroxy-tetrahydrocortisol for i.m. administration. (Figure 3)

Conclusion

Population-based reference ranges can be used as indirect markers in order to detect hydrocortisone consumption. Some differences between these administration routes were found. Urinary concentration of 11β-hydroxy-etiocholanolone for p.t. administration was higher to i.m. administration. On the contrary, i.m. administration shows a superior increment to p.t. administration for tetrahydrocortisol, even though both exceeding 95% percentile compare with a population-based reference range. Excretion profile of proposed hydroxy-

metabolites of cortisol, tetrahydrocortisol, cortisone and tetrahydrocortisone were described. Main variations were found for urinary concentrations of hydroxy-metabolites of cortisol and tetrahydrocortisol. They keep higher urinary concentration for i.m. administration on a period of time longer than for p.t. administration.

References

1. World Antidoping Agency. The 2006 Prohibited List. International Standard, Montreal (2006) http://www.wada-ama.org/rtecontent/document/2006_LIST.pdf (access date 23.01.2006)
2. Mazzarino, M., Rossi, F., Botré, F. (2004) Effect of the systemic vs. inhalatory administration of synthetic glucocorticoids on the urinary steroid profile. In Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U.(eds) *Recent Advances in Doping Analysis (12)*, Köln. pp 45-54.
3. Simões, S.M.S., Calçada, M., Horta, L., De la Torre, X. (2005) Methylpresdnisolone detection in urine following local and oral administration. In Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U.(eds) *Recent Advances in Doping Analysis (13)*, Köln. pp 411-414.
4. Mazzarino, M., Bragano, M.C., Garribba, F., Rossi, F., Botré, F. (2005) The idea of a threshold value for sinthetic glucocorticoids: urinary excretion study following differente routes of administration. In Schänzer, W., Geyer, H., Gotzmann, A.,Mareck, U.(eds) *Recent Advances in Doping Analysis (13)*, Köln. pp 465-468.
5. Martínez, D., Álvarez, A., Correa, M.T., Rodríguez, A., De la Torre, X., De la Torre, R. (2003) Endogenous Steroids Profiles Preliminary Evaluation for Cuban Athletes Population. In Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U.(eds) *Recent Advances in Doping Analysis (11)*, Köln. pp 335-339.
6. Schänzer, W., Gotzmann, A., Horning, S., Donike, (1994) M. Trimethylsilyl derivatisation of endogenous and synthetic corticosteroids for GC-MS identification. In *Proceeding of the 10th International Conference of Racing Analysis and Veterinans, Stockolm, Sweden*.

Table 1. Non-parametric data of population distribution for cortisol, tetrahydrocortisol and 11-OHE urine concentrations and values found in excretion study (ng/mL)

(ng/mL)	Cortisol (n=1754)	Tetrahydrocortisol (n=1638)	11-OHE (n=2784)
5% percentil	26.2	151.4	4.0
Mean	205.3	877.9	42.7
Median	153.4	685.9	21.6
95% percentil	567.8	2195.3	179.9
Maximum values concentration found in excretion study			
i.m. administration	3792.0	8202.8	425.6
p.t. administration	3329.8	2466.8	2485.4

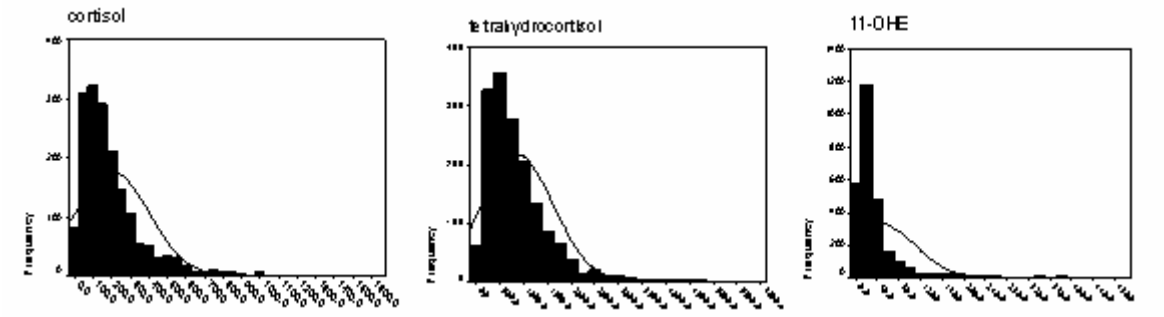

Figure 1. Non-parametric data of population distribution for cortisol, tetrahydrocortisol and 11-OHE

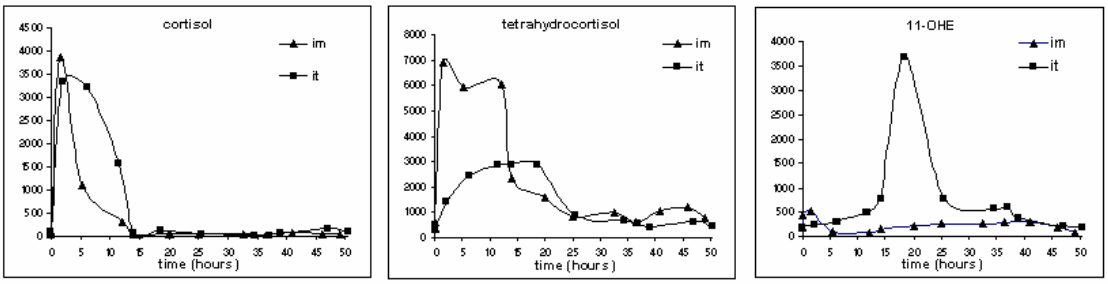

Figure 2. Excretion profile of cortisol, tetrahydrocortisol and 11-OHE in both administration routes.

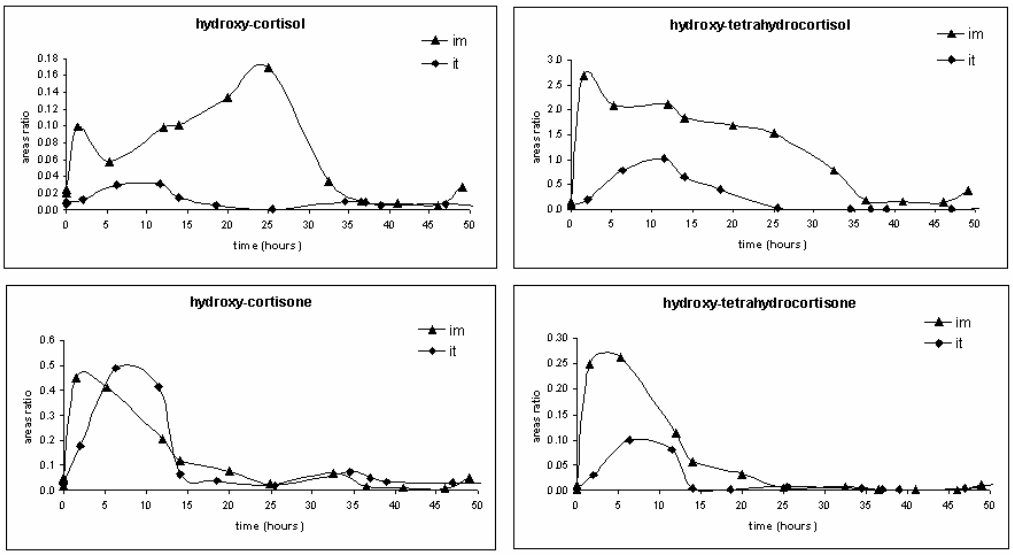

Figure 3. Excretion profile of hydroxy-metabolites of cortisol, tetrahydrocortisol, cortisone and tetrahydrocortisone for both administration routes