

The influence of hCG administration on the steroid profile

Doping Control Laboratory, Bucharest, Romania

Introduction

Human chorionic gonadotropin hormone (hCG) is included on WADA's Prohibited List, Section S2. Following some studies developed on large pools of athletes and some statistical reviews, a cut-off value of 5 mUI/mL has been recommended as doping threshold. hCG is a glycoprotein that may be used by athletes to stimulate testosterone production before competition and/or to prevent testicular atrophy during and after prolonged administration of anabolic androgens.[1-4]

This paper work shows the results obtained after intramuscular administration of hCG on three subjects and its influence on steroid profile.

Materials and method

1. Excretion study of hCG

Pregnyl -1ml containing 5.000 UI of hCG-have been injected intramuscular on 2 male subjects with ages of 28 (SB1) and 34 years (SB2) and 10000 UI of hCG have been injected intramuscular on female subject with ages of 39(SB3), in accordance with the rules of the local ethical commission. The urine samples have been collected before administration and every morning for 9 days after administration.

The samples have been stored by freezing and their pH and specific gravity have been measured before analysis.

Equipment

For the determination of β -hCG from urine samples, it has been used an automatic equipment ELECSYS 2010 from Roche. The main section of the system is the detection unit. This unit contains: a photomultiplier tube, a cell for continuous measurement, magnetic ensemble with platinum electrodes and an amplification circuit. The temperature is maintained constant at $28^{\circ}\text{C} \pm 5^{\circ}\text{C}$.

Reagents and materials

Working reagents have been purchased from Roche, and they contain:

- micro particles of streptavidine;

- anti-hCG mono-clone byotilates antibodies in phosphate buffer, with pH of 7,5;
- anti-hCG mono-clone antibodies marked with a ruthenium complex, in phosphate buffer, with pH of 6,5.

2. The analysis of steroid profile

Sample preparation

A volume of 2 ml urine has been extracted in according with the procedure for the detection of androgenic anabolic steroids - the total fraction with enzymatic hydrolysis (β -glucuronidase from *E.Coli*), followed by the extraction with tert-buthyl-methyl-ether and derivatization with MSTFA/ NH_4I /Ethantiol mixture.[5,6]

Equipment

The determination of steroid profile was performed by gas chromatography coupled with mass spectrometry method (GC/MS), using an Agilent system GC 6890N/MS 5973 Network, using a column ULTRA 1, 17m length, I.D.0,25 mm, film thickness 0,12 μm .

Reagents and materials

The reference materials for quantification of endogenous steroids were obtained by NARL – Australia and Promochem – USA.

Results and discussions

The distribution of β -hCG concentrations from the volunteer's urine is shown in Fig.1.

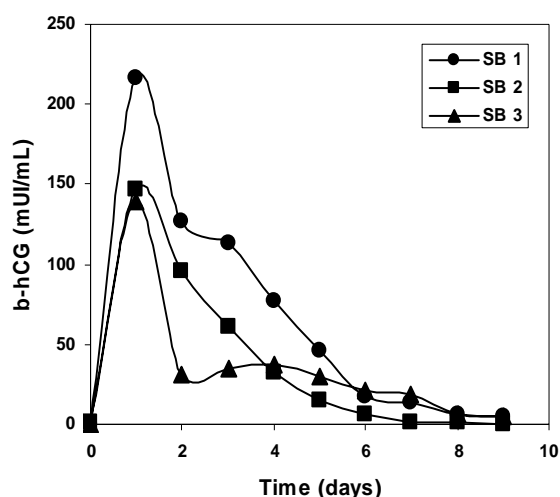


Fig.1 β -hCG excretion study

Time (days)	hCG (mUI/mL) SB 1	hCG (mUI/mL) SB 2	hCG (mUI/mL) SB 3
0	0,10	1,30	0,10
1	216,00	147,19	139,10
2	126,40	96,36	30,89
3	112,90	60,73	34,44
4	77,03	32,48	37,01
5	45,71	15,43	30,20
6	17,31	6,16	21,33
7	13,87	0,95	19,05
8	6,16	0,66	6,54
9	4,42	0,40	4,54

Tabel 1 β -hCG excretion

Excretion curves show that physiological excretion of β -hCG is similar for all the subjects. The highest levels of β -hCG concentrations, 150, 200 and 139 mUI/mL, are observed at 24 hours after administration of a single dose of Pregnyl. In the next days it can be noticed a

slow decrease, but the β -hCG concentrations stay above the upper reference limit for 6-8 days after administration (Tabel 1). Thus, on the first subject, the β -hCG values increase is much more evident and its excretion from the human body takes place slowly.

The monitoring of endogenous hormones showed high concentration values for the main parameters of the steroid profile (all values are corrected with specific gravity). At 4-5 days after administration it can be observed an increase of T, E for all subjects (Fig.2a and 2b).

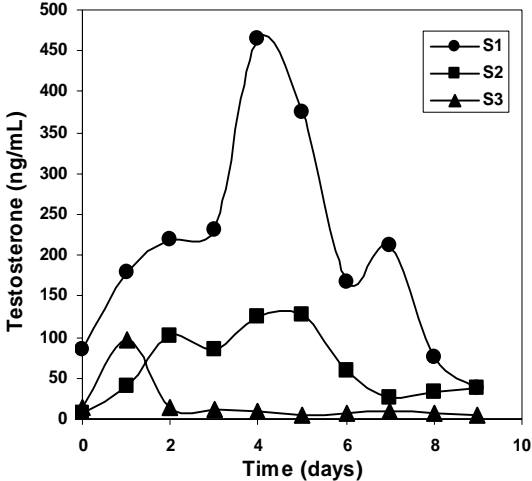


Fig.2a

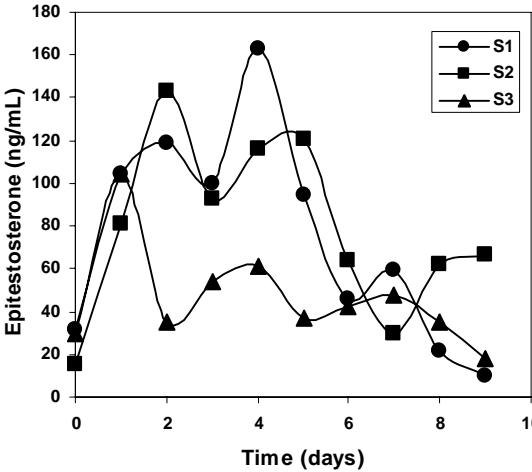


Fig.2b

Excretion curves of testosterone and epitestosterone

Although T and E value increase very much compared to the blank sample, T/E ratio remains lower than 4 (Fig 3).

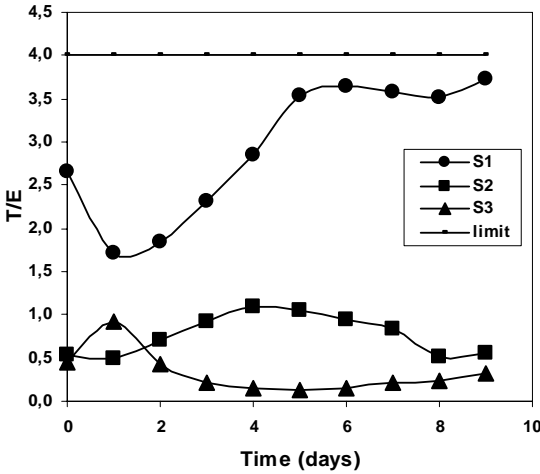


Fig.3 Evolution of T/E ratio

The different value of E and T/E ratio of the volunteers may be explained by the individual metabolism, as well as by the hormonal differences between the subjects.

Conclusions:

- The maximum value of the urinary concentration of the β -hCG after administration of Pregnyl was observed at 24 hours;
- hCG administration caused in all the volunteers the stimulation of the testosterone and epitestosterone endogenous secretion;
- T/E ratio values are below the limit although the testosterone and epitestosterone values show significant increases in comparison with the values before administration;
- We consider that in order to confirm these results, more studies on a larger number of volunteer subjects are necessary.

References:

1. Laidler, P., Cowan, D.A., Hider, R.C., Kicman, A.T. (1997) New Decisions Limits and Quality-Control Material for Detecting Human Chorionic Gonadotropin Misuse in Sports. *Clinical Chemistry* **40**, 1306-1311.
2. Stenman, U., Unkila-Kallio, L., Korhonen, J., Alfthan, H. (1997) Immunoprocudures for detecting human chorionic gonadotropin:clinical aspects and doping control, *Clinical Chemistry* **43**, 1293-1298.
3. Leinonen, A., Tahtela, R., Karjalainen, E. (1998) Detection of Human Chorionic Gonadotropin in Urine by two Different Immunoassays. In: Schaenzer, W., Geyer, H., Gotzmann, A., Mareck-Engelke, U. (eds) *Recent Advances In Doping Analysis* (6), Koeln. pp 331-335.
4. C.P.D'Angelo, G.M. Caballero, O.Teme Centurion, C.DiNardo, G.G.Cases, C.F.Ochoa, L.Chinchilla, E.Ceccarelli, M.E.Zadorecki, G.Zaccaro, M.Sol Fraguio, (2001) Steriod profile and Peptides Hormones in Urine of Argentine Athletes. In: Schaenzer, W., Geyer, H., Gotzmann, A., Mareck-Engelke, U. (eds) *Recent Advances In Doping Analysis* (9), Koeln. pp 305-309.
5. Geyer, H., Schaenzer, W., Mareck-Engelke, U., Donike, M. (1996) Factors Influencing the Steroidic Profile. In Donike, M., Geyer, H., Gotzmann, A., Mareck-Engelke, U. (eds.) *Recent Advances In Doping Analysis* (3), Koeln. pp. 95-114.
6. Vâjială, G., Mihăilescu, R., Lamor, M. (2000) Benzbromarone- a possible masking agent of androgen anabolic steroids; Identification by GC/MS and its Action upon the Steroid Profile. In: Schaenzer, W., Geyer, H., Gotzmann, A., Mareck-Engelke, U. (eds) *Recent Advances In Doping Analysis* (8), Koeln. pp. 215-220.