Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (10)

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Sport und Buch Strauß, Köln, 2002

H. M. G. PEREIRA, M. A. S. MARQUES, F. R. AQUINO NETO:
Initial Study of Incidental Contamination of Clostebol in Athletes by Sexual Intercourse
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (10). Sport und Buch Strauß, Köln, (2002) 279-283

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Initial Study of Incidental Contamination of Athletes with Clostebol by Sexual Intercourse

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Introduction

Clostebol (4-chloro-17β-hydroxy-androst-4-en-3-one) is a synthetic androgenic steroid with anabolic effects frequently used in sports to increase physical performance. The use of clostebol is prohibited by the International Olympic Committee independently of the circumstances or the means of administration[1]. The metabolism of Clostebol was previously studied by Schänzer et. al.[2] (Figure 1). Clostebol metabolites are analyzed in routine screening in doping control laboratories. Consequently, the use of medicines with clostebol is prohibited for the athletes. However, clostebol acetate is present in medicines for dermatological and gynaecological diseases. The contamination of male athletes from women treated with this medications has been used as justification of positive results found in our lab. Our goal is to appraise the possibility of detection of clostebol metabolites in men due to contamination from sexual intercourse with female partners. The possible absorption of individuals undergoing medical treatment was also evaluated.

Experimental

Experiment Design

Two healthy couples (group I) and two healthy men (group II) were involved.

In the couples experiment, 5 g of medicine (Trofodermin™, clostebol acetate 5mg/g and neomycin sulphate 5mg/g, *Searle*, São Paulo, Brazil) were applied to the women by intra-vaginal administration before sexual intercourse. After that, the medicine was washed with water from the sexual organ of the man to avoid further exposition.

The two men applied 5 g of medicine directly in the penis. After 20 minutes, the medicine was washed with water from the sexual organ of the man to avoid further exposition.

The urine of all volunteers was collected for two days.

Chemicals

Clostebol-M1 (see Fig.1) standard was kindly donated by Prof. Dr. W. Schänzer (Doping Control Laboratory Cologne). MSTFA was purchased from Chem Fabrik Karl Bucher (Waldstetten, Germany); NH_4I and 2-mercaptoethanol from Sigma (St. Louis, MO, USA) and methanol from Tedia (Fairfield, USA).

GC-MS analysis

An Agilent 6890 Series GC System equipped with a 7683 automatic sampler and an electronic pressure control and interfaced to an Agilent 5973 mass selective detector was used. The MS operating temperatures were as follows: transfer line 280°C; ion source 230°C; and quadrupole 150°C. Acquisition mode was selected ion monitoring (SIM) with a dwell times of 20 ms per ion. The electron ionisation was performed at 70eV. The GC operation conditions were: injector 280 °C; column 180 °C (initial temperature); followed by a gradient of 3.0 °C/min to 229 °C/min and 40 °C/min to a final temperature of 310 °C (held 3.0 min); total flow of He 18.4 ml/min; pressure 16.0 psi. 1 µl samples were injected in the split mode (ratio 1:10). A HP-1 fused-silica capillary column (dimethyl silicone, 17.0 m x 0.2 mm x 0.11 µm film thickness) was used.

Derivatization Conditions

The formation of TMS–Enol–TMS derivatives was achieved using MSTFA/NH_4I/2 mercaptoethanol (1000:2:6, v/w/v) reaction mixture at 60°C for 20 min.

Preparation of Stock Solutions

Stock solutions were prepared in methanol at a concentration of 1000ng/µl. These solutions were further diluted to yield appropriate working solutions for the preparation of the calibration standard. The solutions were sealed and frozen at -20°C until use. Methyltestosterone was used as internal standard (ISTD), dissolved in methanol at 1000ng/µl and diluted to 10ng/µl.

Preparations of Calibration Curve

2 mL of blank urines were spiked with a solution of 10 ng/µL clostebol-M1 to final amounts of 0.5, 1.0, 3.0, 5.0, 10.0, 20.0, 30.0 ng/mL (n=3). Methyltestosterone (ISTD; 250 ng/mL) was added to each tube. Quantification was based on peak-area ratios of selected ions of the analyte to the ISTD versus concentration.

Sample Preparation for Clostebol Metabolites Determination

Urine samples were prepared according to the confirmation procedure for conjugated steroids. The complete procedure was described by Geyer et al.[3]

Results and Discussion

Direct application of clostebol acetate in men (group II) resulted in higher clostebol-M1 amounts in urine (22 ng/ml; T_{max} = 3.5 hours, Figure 2). Contamination from sexual intercourse (men group I) resulted in amounts of clostebol-M1 near the limit of detection (between 0.9 – 3.5 ng/ml; T_{max} = 16 hours, Figure 2). The shape of the curve in the first 5 hours is not known because of the lack of samples. For the female subjects medicated (women; group I) the excretion of clostebol-M1 showed an irregular profile in the first 10 hours, reaching a maximum at 23 hours after vaginal administration and sexual intercourse (Figure 3), with amounts as high as for the self-administration of the males. The presence of clostebol-M2 (see Fig.1) was also detected in these samples (Figure 4). The ratio of clostebol-M1/M2 observed in the man of group II was 9.4 +/- 2.5 (mean +/- std). Clostebol-M1 is the long-term detected metabolite. However, it also decreases near to our detection limit 20 hours after sexual contamination for group I and 15 hours for group II. The amounts of both metabolites are lower than the values described by Debrueykere[4] after ingestion of contaminated meat and the amounts detected in routine work in our laboratory.

Conclusions

The possibility of incidental contamination from sexual intercourse was verified, meanwhile the amount of clostebol-M1 (long-term metabolite) was near the limit of detection. Since the IOC do not make any difference among circumstances or means of administration of clostebol, athletes should be warned not to use clostebol acetate based medicines as well as be aware of their partners medical treatments.

Reference

1. The OMAC 2001. Olympic Movement Anti-doping Code. Appendix A. 4.
2. Schänzer W, Donike M, *Anal Chim Acta* 1994, 275, 23-48.
3. Geyer H, Mareck-Engelke U, Schänzer W, Donike M in: *Recent Advances in Doping Analysis*. SPORT und BUCH Strauß - Köln 1994, 97-101.
4. Debruyckere G; Sagher R; Van Peteghem C: *Clin Chem* 1992, 38/9, 1869-1873.

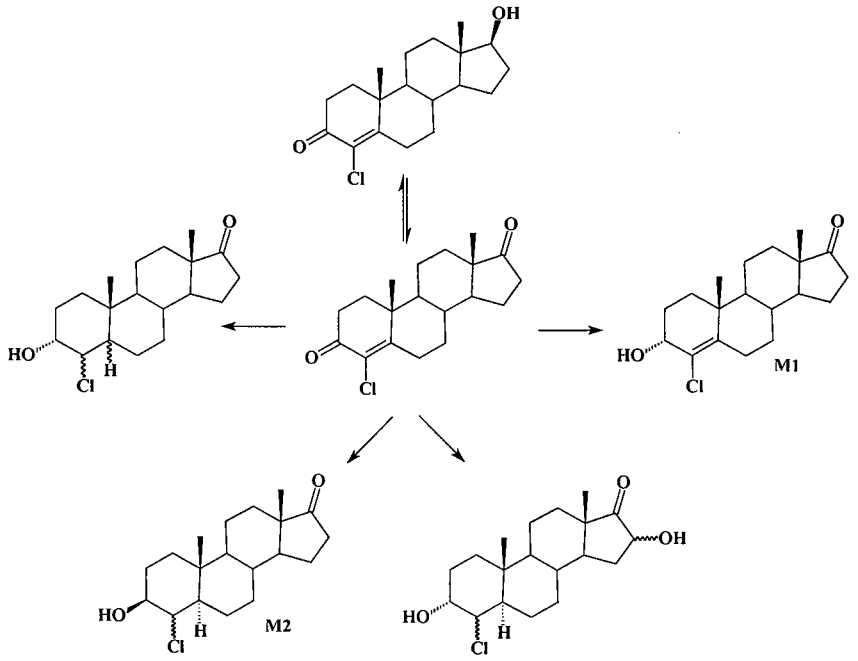

Fig. 1: Metabolism of clostebol to its main excreted metabolites in man [2].

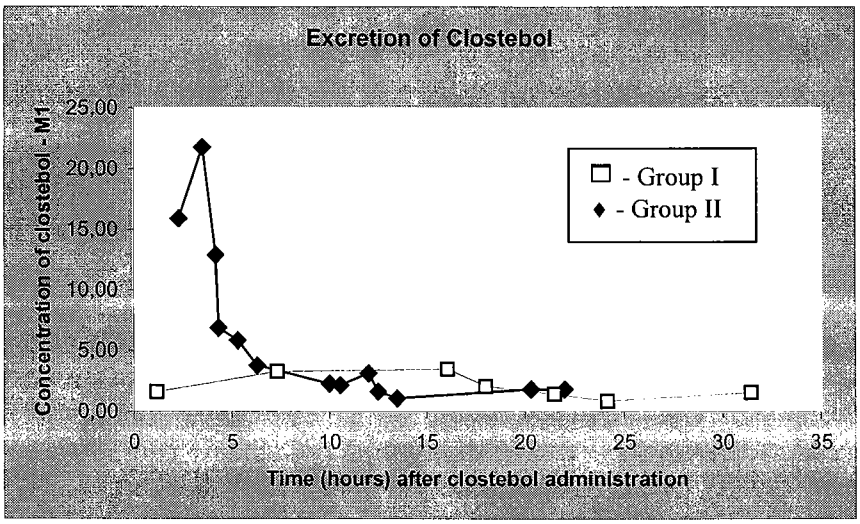

Fig. 2: Typical excretion profile of clostebol M1 in man directly exposed (group II ♦) and in "contaminated man" (group I □) .

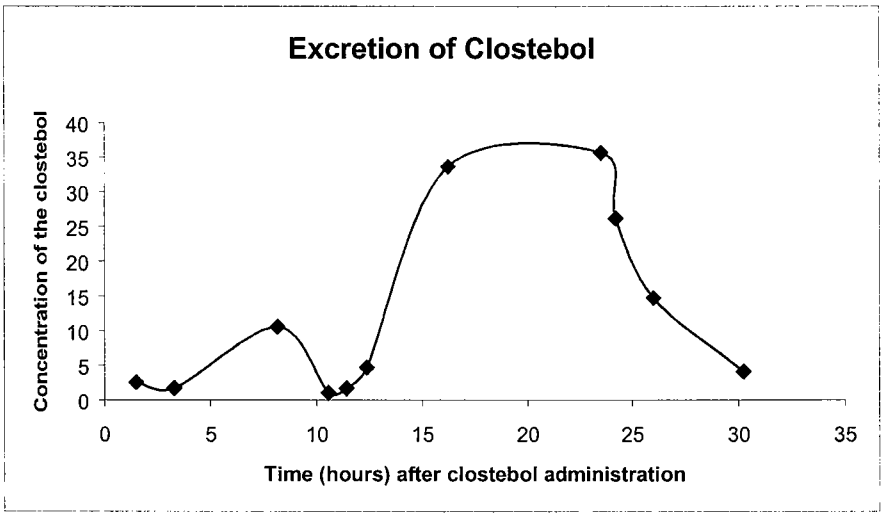

Fig. 3: Excretion of clostebol-M1 in woman (group I).

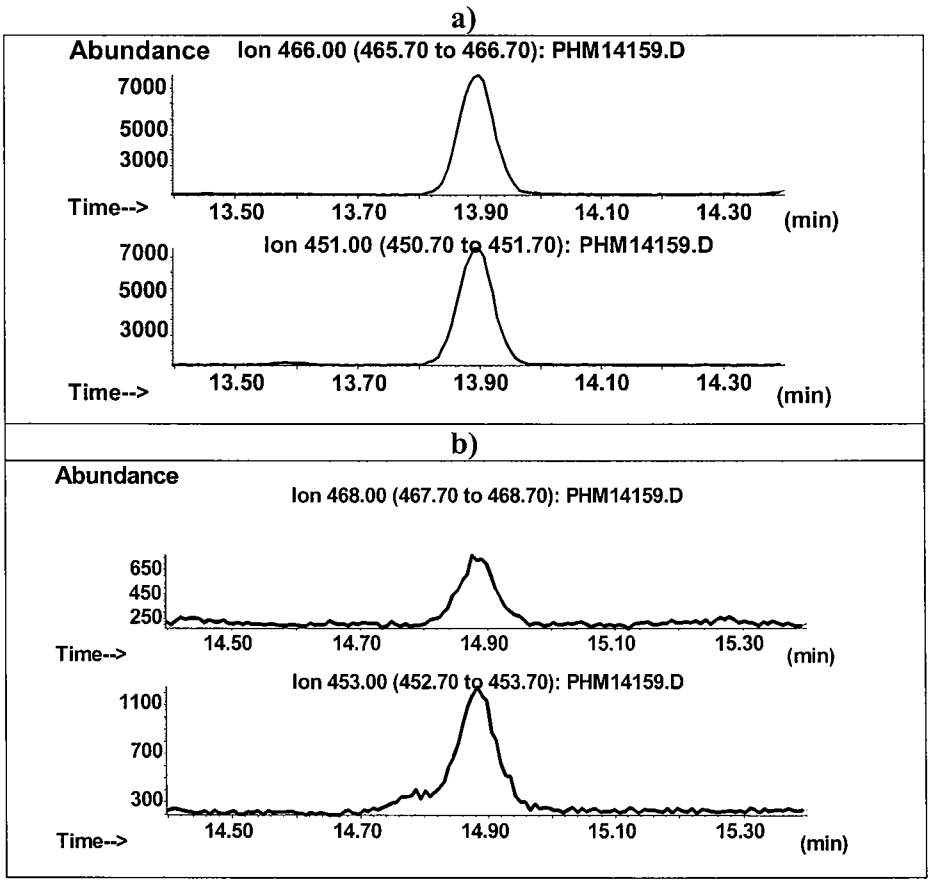

Fig. 4:SIM chromatograms of main ions of (a) clostebol-M1 and (b) clostebol-M2 metabolites. Data from man group II, 16 hours (T_{max}) after clostebol administration.