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5α-Androst-1-ene-3,17-dione: metabolism, influence on steroid profile and biological activity.

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Abstract

5α-Androst-1-ene-3,17-dione (1-AD) is listed on the World Anti-Doping Agency's prohibited list as an "exogenous anabolic-androgenic steroid" [1]. It could be marketed in dietary supplements.
An excretion study was conducted with six male volunteers, to whom were orally administered 50 mg of 1-AD in one-time application. Urine samples were collected for two weeks. The metabolites were measured as TMS-derivatives with GC/MS. The intake was detectable up to eight days based on the main metabolite 3α-hydroxy-5α-androst-1-en-17-one (1-DHA). 1-DHA was synthesized from 5α-androst-2-en-17-one via the 2,3-epoxid as an intermediate and structurally identified with GC/MS and NMR. Other identified metabolites were 17β-hydroxy-5α-androst-1-en-3-one (1-testosterone), 5α-androst-1-ene-3α,17β-diol and 5α-androst-1-ene-3β,17β-diol. Furthermore two additional metabolites, presumably 18-hydroxy-5α-androst-1-ene-3,17-dione and 19-hydroxy-5α-androst-1-ene-3,17-dione, but until now without final confirmation, as well as the parent compound, were detectable in the urine.
Ratios in the endogenous urinary steroid profile typically evaluated for doping analysis were also altered. Androsterone/etiocholanolone ratio (AND/ETIO) and 5α-/5β-androstane-3α,17β-diol ratio (Adiol/Bdiol) were increased. But there were no changes in the testosterone/epitestosterone quotient.
The parent substance 1-AD, the main metabolite 1-DHA, 1-testosterone and 3β-hydroxy-5α-androst-1-en-17-one (1-DHEA) were also tested in the yeast-androgen-screen, a test system for androgen receptor-mediated gene expression. An androgenic activity could be shown for all. 1-Testosterone was shown to be 10-times more potent than 1-AD and 100-times more potent than the equipotent substances 1-DHA and 1-DHEA.

Introduction

5α-Androst-1-ene-3,17-dione (1-AD) is listed on the World Anti-Doping Agency's prohibited list as an "exogenous anabolic-androgenic steroid", nevertheless it has been marketed in dietary supplements as a prohormone of 1-testosterone (17β-hydroxy-5α-androst-1-en-3-one).
The aim of this study was to investigate the metabolite excretion of 1-AD in urine with gas chromatography-mass spectrometry (GC-MS). Also alterations in the endogenous steroid profile were analysed. Furthermore the biological activity of 1-AD and some main metabolites was tested with a yeast-androgen-assay.

Experimental

A single oral dose of 50 mg of 1-AD was applied to six healthy young male volunteers. Urine samples were collected for two weeks. This study was approved by the Research Ethical Committee at the Sports Institute, Warsaw (date of approval 10.02.2011).

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Samples were prepared according to the routine steroid screening procedure described by Geyer et al. [2], however with separation of the unconjugated and glucuronidated fraction. After derivatisation with TMIS reagent (MSTFA/ NH_4I/ ethanethiol, 1000:2:3, v:w:v) by heating for 20 min at 60°C, the samples were measured using selected ion monitoring (SIM). An Agilent 6890N gas chromatograph coupled to an Agilent 5973 inert mass selective detector (MSD) was used with the following settings: column: Agilent Ultra-1 (polysiloxane, 17 m; 0.20 mm i.d.; 0.11 µm film thickness), carrier gas: helium, head pressure: 1bar, oven temperature program: 0min 183°C, +3°C/min, 0min 232°C, +40°C/min, 2min 310°C, injection volume: 3µL, split 1:16, injection temperature: 300°C, ionization: 70eV, EI.

As reference, 3α-hydroxy-5α-androst-1-en-17-one (1-DHA) was synthesized in a two step reaction adjusted from Comin et al. [3] (Fig. 1) and structurally confirmed by NMR and GC-MS (Fig. 2). The androgen activity of 1-AD, 1-testosterone, 1-DHA and 3β-hydroxy-5α-androst-1-en-17-one (1-DHEA) was evaluated in a yeast androgen assay in comparison to the reference DHT, a test system for androgen receptor-mediated gene expression. The detailed procedures were carried out as described by Sohoni and Sumpter [4].

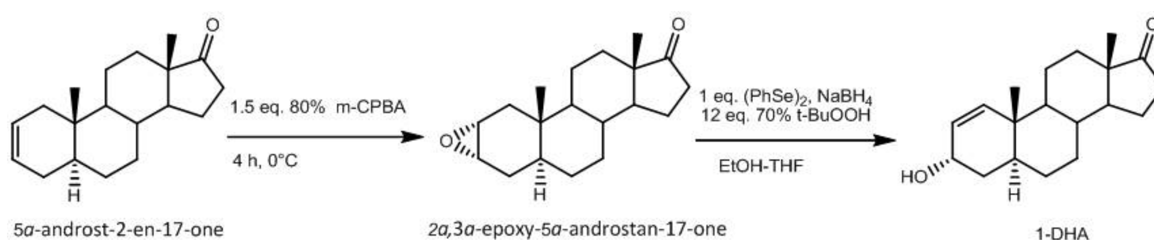

Figure 1: Synthesis of 1-DHA adjusted from Comin et al [3].

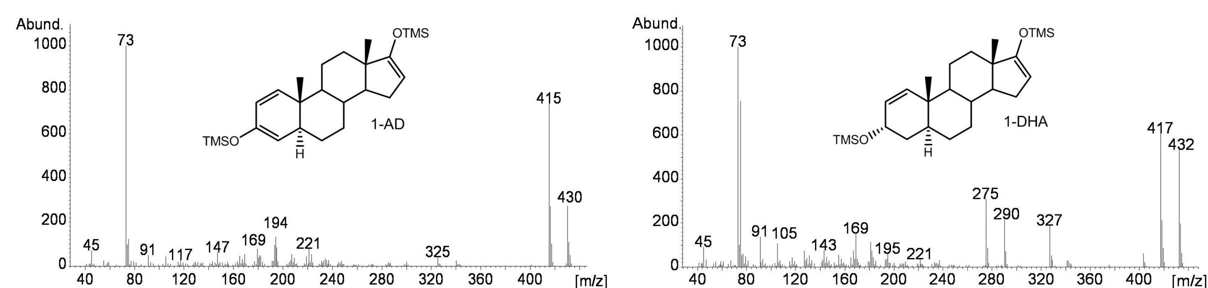

Figure 2: Mass spectra of 1-AD and 1-DHA as bis-TMS derivative.

Results and Discussion

Using SIM, the oral intake of 50 mg of 1-AD was detectable up to eight days based on the main metabolite 1-DHA in the glucuronide fraction (Fig. 3). The urine concentration maxima after about five hours were between 5.6 µg/ml and 19.7 µg/ml. This also demonstrated interindividual differences in quantitative excretion. Aside from the parent substance 1-AD, which was detectable for about three to four days in the unconjugated fraction, the metabolites were mainly excreted as glucuronides. Namely the identified metabolites in the glucuronide fraction were 1-AD, 1-testosterone, 5α-androst-1-ene-3α,17β-diol (1-Aadiol), 5α-androst-1-ene-3β,17β-diol (1-Abdiol) and two primary hydroxylated steroids, most likely 18-hydroxy-5α-androst-1-ene-3,17-dione and 19-hydroxy-5α-androst-1-ene-3,17-dione (without differentiation among each other). Retention times of their per-TMS derivatives are shown in Table 1.

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Additionally the androsterone/etiocholanolone ratio (AND/ETIO, Fig. 3) and the 5α-/5β-androstane-3α,17β-diol ratio (Adiol/Bdiol) were increased after the administration for about 12 hours. Those increased ratios are caused by an increase of the excretion of 5α-compounds, androsterone and Adiol, only. Therefore it is most likely that they occurred as metabolites of 1-AD. But this has to be confirmed in further studies.

compound	retention time [min]
1-AD	11.61
1-Testosterone	11.97
1-DHA	10.73
1-Aadiol	11.11
1-Abdiol	12.12
X-HO-1-AD	14.96
Y-HO-1-AD	16.61
Methyltestosterone	14.94

Table 1: Retention times of the parent compound 1-AD, its metabolites and the internal standard methyltestosterone.

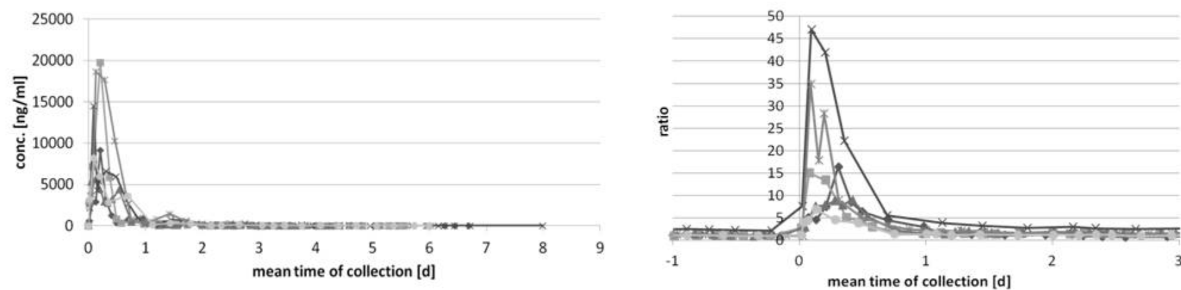

Figure 3: Excretion [ng/ml] of 1-DHA (left) and AND/ETIO ratio (right) in glucuronide fraction (6 volunteers).

In the yeast-assay an androgenic activity was shown for all substances with 1-AD (about 1/10 as active as reference DHT) and 1-testosterone (nearly equipotent as DHT) as the most active ones. The EC_{50}-values, as a parameter for the androgenic activity, were 1×10^{-9} M for 1-testosterone, 4×10^{-9} M for 1-AD, 1×10^{-8} M for 1-DHA, 6×10^{-8} M for 1-DHEA and 6×10^{-10} M for the reference DHT.

Conclusions

As 1-AD shows androgenic activity it must be classified as active hormone. The most important metabolite for doping control analysis is the main metabolite 1-DHA, which could be identified up to 8 days after the oral intake of 50 mg of 1-AD by the ratio of three diagnostic ions as recommended by the World Anti-Doping Agency (WADA) [5].

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Further additional information about this study will be available in the publication submitted to Archives of Toxicology.