Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (8)

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Sport und Buch Strauß, Köln, 2000

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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (8). Sport und Buch Strauß, Köln, (2000) 227-232

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Appearance of an Oxabolone Metabolite after Administration of OTC 19-Norsteroids – Metabolite or Artefact?

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Introduction

4-Hydroxy-norandrostenedione, the main urinary metabolite of oxabolone (4-hydroxy-nortestosterone)[1], was found in addition to the dominating nandrolone metabolites in urine samples collected after administration of "Over-the-Counter" (OTC) preparations containing 19-norsteroids. The amounts detected varied from trace levels in case of 19-norandrostenedione to reasonable metabolite concentrations after intake of 19-norandrostenediol. The poster presents the investigations and preliminary results focussed on the following questions:

- Is this finding a result of metabolism indeed or is it caused by contaminants of the OTC drugs used?
- Thus, is it possible to distinguish the application of those 19-norsteroids from the (conventional) misuse of nandrolone?

Experimental

Excretion study (medium dosage)

25 mg of 19-norandrostenedione (NorAndrostenedione, *Growth Laboratories*, a quarter of a tablet of 100 mg) were orally administered to a male volunteer (40 years old, Caucasian race, 190 cm, 98 kg) as one single dose. Urine samples were collected after 3, 7, 14, 22, 32, 44, 52, 60 and 74 hours.

Excretion studies (low dosage)

a) 1 mg of 19-norandrostenedione (NorAndrostenedione, *Growth Laboratories*, an aliquot of a tablet of 100 mg) was orally administered to a male volunteer (44 years old, Caucasian race, 176 cm, 78 kg) as one single dose. Urine samples were collected after 1.5, 4, 5.5, 10, 18 and 23 hours.

b) 2 mg of 19-norandrostenediol (source not specified, an aliquot of a capsule containing 50 mg of 19-norandrostenediol, furthermore 10 mg of progesterone and other ingredients)

were orally administered to a male volunteer (40 years old, Caucasian race, 190 cm, 98 kg) as one single dose. Urine samples were collected after 5, 10, 24 and 30 hours.

Sample Preparation

Urine samples

The sample preparation followed the normal procedure for the screening of anabolic steroids (combined fraction) with slight modifications to improve the detectability of the metabolites of interest (extraction by n-pentane/methanol *25:1 v/v*, derivatisation by MSTFA or MSTFA/NH_4I, respectively, HPLC clean-up for substance identification).

OTC drug samples

Aliquots of the preparations used were dissolved in ethanol (0.1mg/ml). In order to facilitate the identification of contaminants, these samples were fractionated by HPLC clean-up.

Results of Urine Analysis

Norandrostenedione: 25 mg study (fig. 2)

As already described [2], the steroid screening of these samples yielded dominating peaks of the nandrolone metabolites. Besides this expected finding, a small signal appeared that could be allocated to the main urinary metabolite of oxabolone *(fig. 5 and 6)*, rather at a trace level compared to norandrosterone (about 0.1%). A similar phenomenon has never been observed in former nandrolone cases since the screening for the oxabolone metabolite was introduced in 1987 in our laboratory. Only just in 1999 a "combined" nandrolone/oxabolone case came out. Taking the structural relationship between 19-norandrostenedione and the oxabolone metabolite (4-OH-norandrostenedione) into consideration, at first a hydroxylation at position 4 as a minor metabolic branch did not seem to be unlikely *(fig. 1)*.

Norandrostenedione: 1 mg study (fig. 3)

The same pattern – logically at a much lower concentration level – could be monitored in the low-dosage study modelling an intake of so called "non-declared" or "contaminated" dietary supplements containing small amounts of 19-norandrostenedione.

Norandrostenediol: 2 mg study (fig. 4)

Surprisingly, this similar study gave unexpected results: The detected amount of the oxabolone metabolite was dramatically higher (about 10% of the norandrosterone concentration) than after administration of 19-norandrostenedione. Why should an assumed 4-hydroxylation proceed much easier for 19-norandrostenediol compared to 19-norandrostenedione? Because

of the lack of a logical explanation the question arose whether this detected substance is really a metabolite or an artefact originating from contaminants of the drugs used.

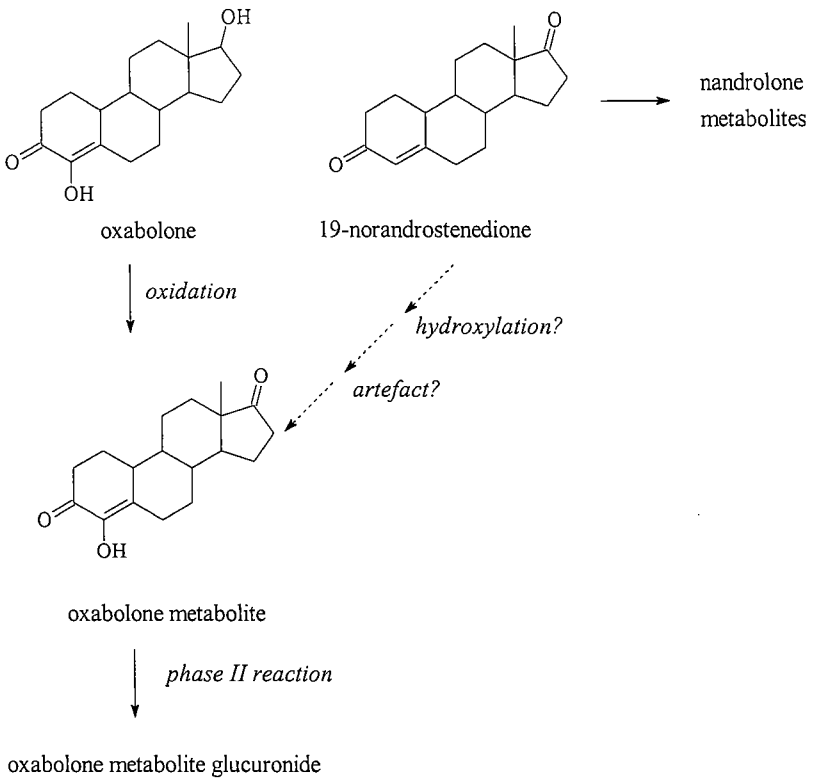

fig. 1 Structural realationship of oxabolone and 19-norandrostenedione

Results of OTC Drug Sample Analysis

The investigation for possible contaminants was focussed on two substances: the oxabolone metabolite itself and the parent oxabolone that would produce even this metabolite. Because of the expected low concentration of possible contaminants, an HPLC clean-up was used as an additional "discriminator" prior to the final identification by GC/MS-MS *(fig. 6)*. Both target analytes could be collected in different fractions.

19-Norandrostenedione

In the tablet containing 19-norandrostenedione the oxabolone metabolite was qualitatively detected at a level that could be considered as causal for the finding in urine *(fig. 6 - upper box)*, the parent oxabolone could not be found.

19-Norandrostenediol

On the other hand, only hints of traces of the parent oxabolone, that would not be enough to produce the metabolite concentration monitored in urine, could be obtained from the capsule

containing 19-norandrostenediol. In this case further investigations are necessary to discover the source for the fairly high concentrations of the oxabolone metabolite in urine.

Discussion and Conclusions

At the present state of investigation it is not possible to give a definite answer whether the observed 4-hydroxylation after administration of OTC 19-norsteroids can be clearly addressed to metabolism or contamination. The hints obtained make contaminants originating from the production of the bulk steroid precursors more likely. Following this assumption it becomes obvious that the investigations have to be extended to several brands of OTC steroids which sources should be traced back. In the same way the desired differentiation of OTC 19-norsteroids from nandrolone misuse remains uncertain, because similar contaminants, if there are any, cannot be excluded for all brands of nandrolone on the market. On the other hand, an information about a possible 4-hydroxylation in man can be rectified by a careful purification of the OTC drugs used.

References

(1) Grosse, J., unpublished results (1987)

(2) Uralets, V., Gillette, P. A., Recent Advances in Doping Analysis 6 (1999) 147-169

Acknowledgement

This work was funded by the Bundesinstitut für Sportwissenschaften, Köln, Germany (VF 0414/02)

List of figures

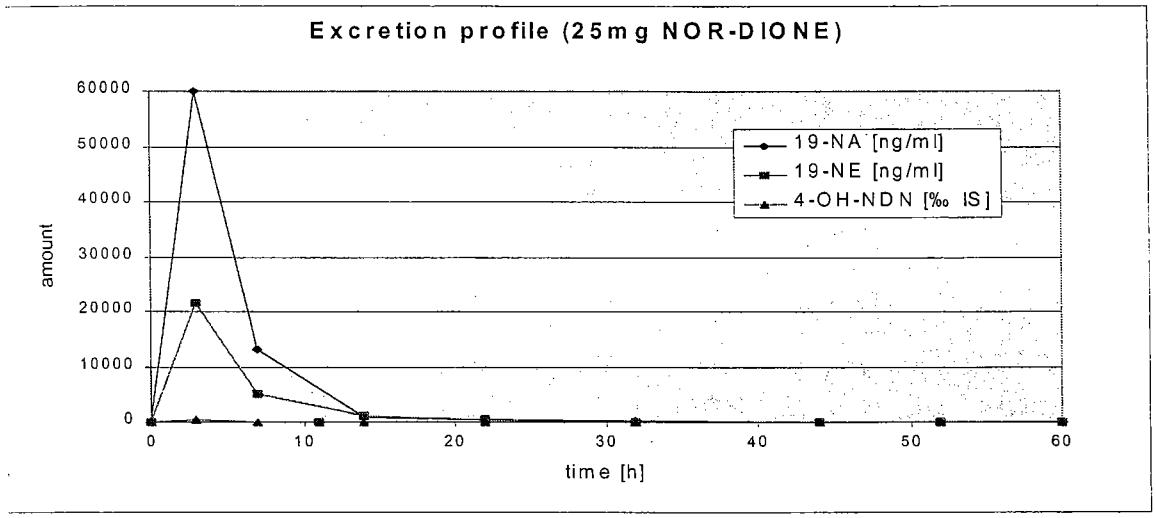

fig. 2: Excretion profile after administration of 25 mg of OTC 19-norandrostenedione

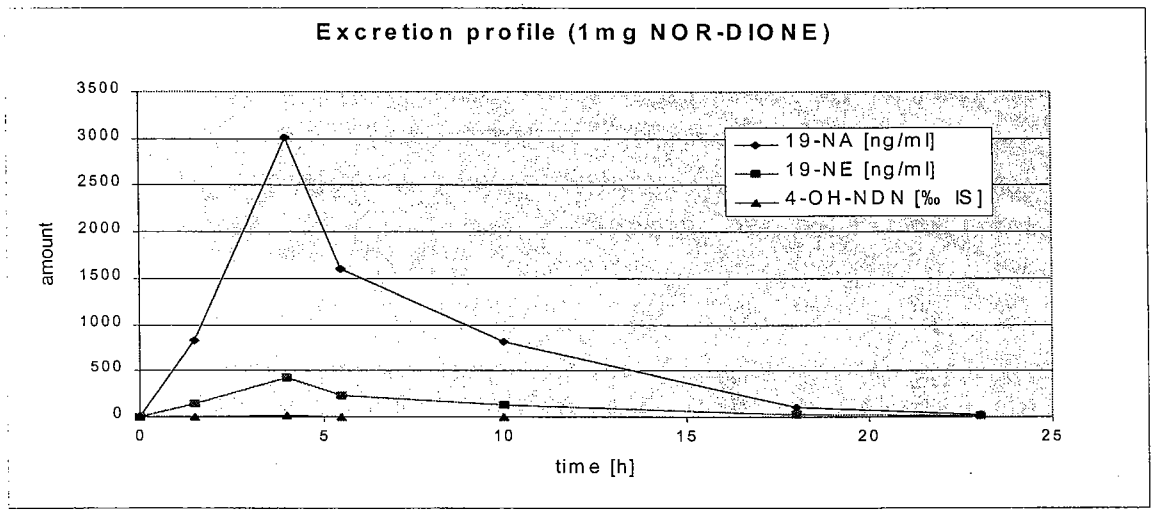

fig. 3: Excretion profile after administration of 1 mg of OTC 19-norandrostenedione

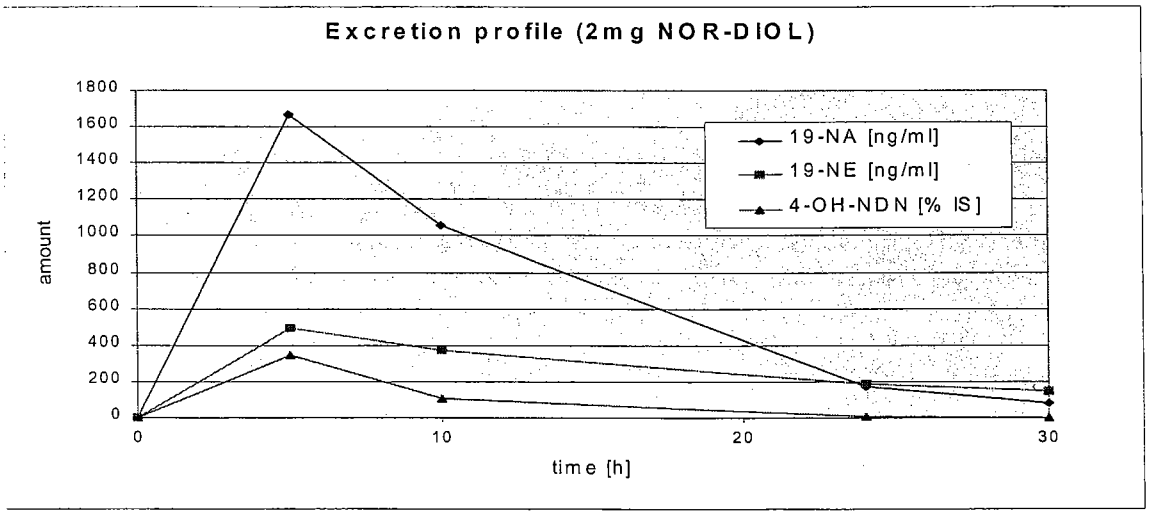

fig. 4: Excretion profile after administration of 2 mg of OTC 19-norandrostenediol

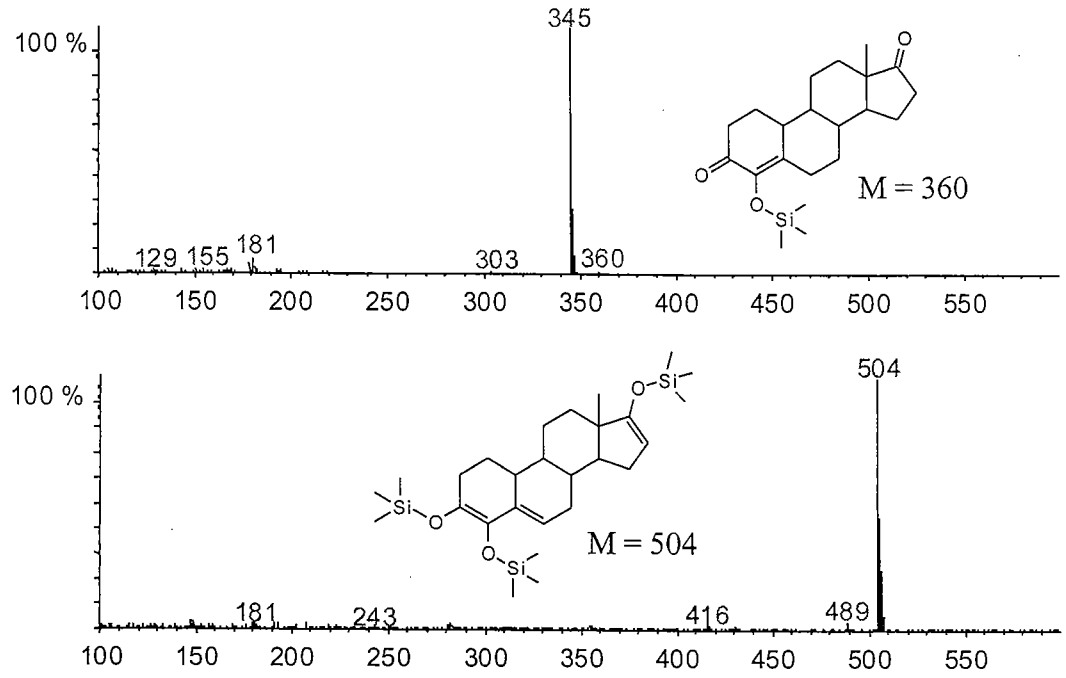

fig. 5: EI mass spectra of the oxabolone metabolite (*mono-* and *tris-TMS* derivative, resp.) obtained as contaminant of an OTC 19-norandrostenedione preparation

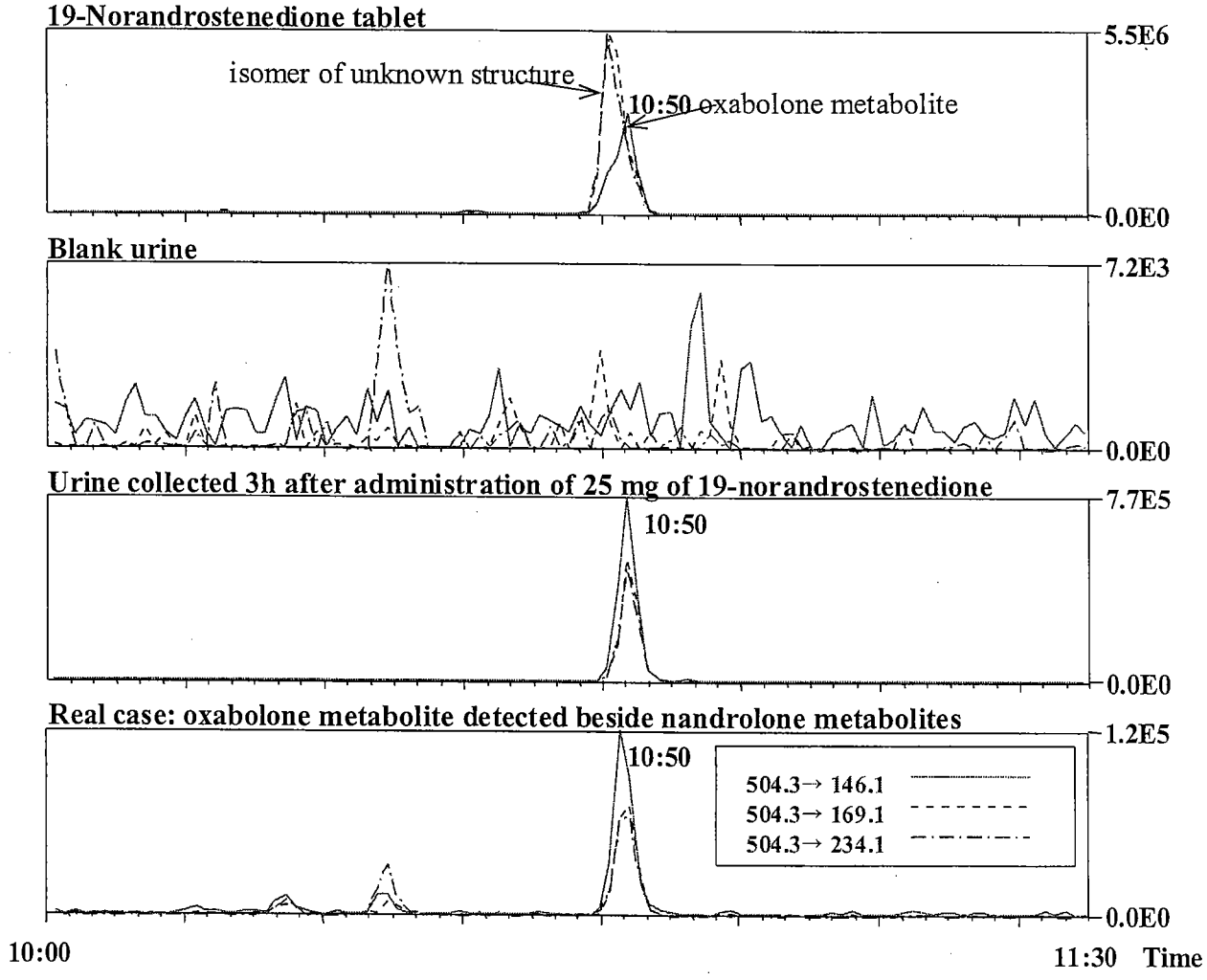

fig. 6 MS-MS identification of the oxabolone metabolite (*tris-TMS* derivative)