"Improved detection of Oral-Turinabol structure identification and elimination of metabolites and generation of reference material"

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Project Overview

As per list of the World Anti-Doping Agency (WADA) 2015 Oral-Turinabol (4-chloro-17β-hydroxy-17α-methylandrosta-1,4-dien-3-one), which was extensively misused by GDR athletes is prohibited in sports. In recent years it has been rediscovered by producers of "dietary supplements" and gained new importance in doping control (continuously increasing numbers of adverse analytical findings since 2003). Following its administration long-term metabolites with 4-chloro-17β-hydroxymethyl-17α-methyl-18-norandrost-13-ene structure have been identified. However these metabolites are not available as reference substance up to now. Even though the use of post-administration urines instead of purified reference material has been accepted in confirmatory analyses, the athletes in question may challenge the results of the anti-doping laboratories resulting in prolonged trials. The goal of the present project is to produce Oral-Turinabol long-term metabolites with 4-chloro-17β-hydroxymethyl-17α-methyl-18-norandrost-13-ene structure that cannot be synthesised via classical chemical synthesis. Therefore a joint chemical synthesis and biotechnological approach will be applied. The substrate (17,17-dimethyl-18-nor-13-ene intermediate) will be converted by single-step hydroxylation to the desired product in a whole-cell biotransformation assay using recombinant strains of fission yeasts that express the human cytochrome P450 enzymes CYP 3A4 or CYP 21 as already successfully performed for the analogue metandienone long-term metabolite by our group, that is now available in worldwide anti-doping laboratories. As already published by our group both enzymes catalyze the respective reaction in Oral-Turinabol as well. Prior to the biotechnological hydroxylation, the substrate will be chemically derived from Oral-Turinabol by Wagner-Weerwein rearrangement. Purification and subsequent NMR structure confirmation will be performed and the product distributed to the anti-doping community.

Results and Conclusions:

Anabolic androgenic steroids (AAS) are misused very frequently in sport competitions as performance enhancing agents. One of the doping compounds that has been detected with increased frequency in the last few years is dehydrochloromethyltestosterone (DHCMT, 4-chloro-17β-hydroxy-17α-methylandrosta-1,4-dien-3-one; brand name Oral Turinabol). Since the parent drug DHCMT (10) is eliminated relatively fast from the human body, efforts to detect its illicit use have concentrated on its metabolites for many years. In recent years, some metabolites with 17β-hydroxymethyl-17α-methyl-18-norandrost-13-ene structure were detected as metabolites following the administration of different 17α-methyl steroids.

The long-term DHCMT metabolites 20βOH-NorDHCMT (4-chloro-17β-hydroxymethyl-17α-methyl-18-norandrosta-1,4,13-trien-3-one) and its A-ring reduced analogue 20βOH-NorTHCMT (4ξ-chloro-17β-hydroxymethyl-17α-methyl-18-nor-5ξ-androst-13-ene-3ξ-ol) were reported earlier, however without providing reference material and assigning the stereochemistry of the latter. Very recently the stereochemistry of the latter was reported as 3α,4α,5α.

In this study we investigated the applicability of a combined chemical and biotechnological approach for the synthesis of reference material. A combination of Wagner-Meerwein rearrangement of DHCMT to NorDHCMT (4-chloro-17,17-dimethyl-18-norandrosta-1,4,13-trien-3-one) and subsequent whole-cell biotransformation with a recombinant fission yeast strain expressing human cytochrome P450 enzymes subtype 21A2 (CYP21A2) was successfully used for the synthesis of mg amounts of 20βOH-NorDHCMT.

Unexpectedly, this approach failed in the synthesis of 20βOH-NorTHCMT. It was observed that substrates with 3-hydroxy groups are neither hydroxylated on C20 by CYP21A2 nor by CYP3A4. Further confirmation of this finding was obtained by testing other 17,17-dimethyl-18-norandrost-13-ene steroids. Thus, insights into the structure-activity relationship of these enzymes were provided.

Furthermore, post administration urines of DHCMT were analyzed for the presence of 20βOH-Nor-metabolites. Both metabolites, 20βOH-NorDHCMT and 20βOH-NorTHCMT, were detected in very low abundances in the urines.