"Detection of sulfo-conjugated anabolic steroids metabolites in antidoping initial and confirmatory analysis"

Dr. Lyris, Dr. Angelis, Dr. Tsivou, Dr. Fragkaki (Doping Control Laboratory of Greece, Greece)

Project overview

Hypothesis

Anabolic androgenic steroids (AAS) are the most frequent abused substances in sports. AAS are extensively metabolized following mainly both the phase-I and phase-II reactions of testosterone. The misuse of AAS is controlled by detection of the parent AAS and /or their metabolites after enzymatic hydrolysis with glucuronidase from E. coli and extraction of their free and deliberated gluco-conjugated metabolites either with GC/MS/MS after derivatization or by LC/MS/MS. This initial screening extraction protocol ignores the sulfo-conjugated part of AAS. The rationale behind this procedure lies on the fact that the AAS are mainly glucoconjugated, where as the sulfo-conjugation happens to a smaller extent and only to specific AAS. Nevertheless, there are AAS for which the longer detected metabolite is a sulfoconjugate. Sulfo-conjugated AAS are relatively easy to be detected directly without anyhydrolysis with LC/MS/MS instrumentation. Sulfo-conjugated substances can be extracted from urine using both liquid-liquid or solid phase extraction.

Objectives

The aim of the current project was to develop a screening method that will incorporate all the known sulfo-conjugated metabolites of anabolic steroids and examine if they can be considered as long term metabolites. Furthermore, as a second step, the project would investigate the existence of not yet known sulfo-conjugated metabolites of anabolic steroids that can improve detectability and identification in either initial screening or confirmation methods.

Results and Conclusions:

Results

Anabolic steroids such as oxandrolone, madol, formebolone, methenolone, 17- methylnandrolone and mesterolone were tested for the existence of sulfo-conjugated metabolites. Metabolic samples from long-term excretion studies were tested for any sulphate metabolite and where any sulphate metabolite was found, an evaluation of its retrospectivity was performed in comparison with their free and gluco-conugated metabolites used for their monitoring in GC/HRMS analysis. In most cases where new metabolites were found, a detailed characterization of their structures based on mass spectrometry techniques was also performed. Additionally, spotted metabolic samples for oxymetholone, drostanolone, norethandrolone, danazol, clostebol, methandriol, calusterone, furazabol, fluoxymesterone, oxymesterone, boldenone, mesterolone, methandienone, methyltestosterone, oral turinabol, methenolone, and tibolone that include the known, up to that time, anabolic steroids with sulphates metabolites, as

well as other AASs with unknown sulphate metabolism were tested in order to develop a new screening method for sulphate metabolites. Samples from the above listed steroids were extracted and analyzed using a screening method based on alkaline extraction with ethylacetate and LC/QTOF analysis in a negative mode. Potential sulfate metabolites of these steroids were drawn and the molecular ions were calculated and extracted using the instrument software.

Conclusions

The investigation of sulfo-conjugated metabolites of methenolone and mesterolone led to the discovery of new metabolites of at least equal or better retrospectivity compared to the already known gluco-conjugated metabolites detected by GCMS. Furthermore, a sulfo-conjugated long-term metabolite of 17-methylnandrolone was discovered (unpublished results). The analysis of madol, formebolone and oxandrolone didn't lead to any new (sulfo-) metabolite, at least to a concentration level that would be detectable by the technology used in this study. A screening method for sulfo-conjugated metabolites was developed for several anabolic steroids based on literature data, using accurate mass measurements for the extracted ion chromatograms and leading to a number of new sulfo-conjugated metabolites. Their structures, as well as their retrospectivity for the monitoring of their parent compounds abuse for doping control purposes were not evaluated in the framework of this project.