"Evaluation of sulfate conjugated metabolites to improve detection capabilities of the misuse of anabolic androgenic steroids"

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Project overview

Anabolic androgenic steroids (AAS) are the most important group of forbidden substances detected in antidoping analyses, and improvement of the detection capabilities of their misuse is a priority in antidoping control. Studies on steroid metabolism have been traditionally performed using GC-MS methods. Phase II metabolic reactions of AAS have been normally studied by using hydrolysis with -glucuronidase enzymes, thus mainly conjugates with glucuronic acid have been systematically studied using both GC-MS and LC-MS/MS approaches. The study of sulfates has been limited by the difficulties of their efficient hydrolysis to phase I metabolites. LC-MS/MS offers the possibility to directly detect sulfate conjugates without previous hydrolysis to phase I metabolites. The application of LC-MS/MS has enabled the detection and characterization of unreported sulfate metabolites for boldenone and methyltestosterone, useful to improve detection capabilities of these AAS. Sulfated metabolites of boldenone can be used as markers of exogenous administration of boldenone. For methyltestosterone, long-term sulfated metabolites have been identified that allow for an increase between two and three times the retrospectivity of the detection. These results indicate that sulfatation is an important metabolic pathway for AAS and deserves to be comprehensively studied for all AAS using adequate technology (LC-MS/MS).

The objective of the present proposal is to continue the systematic study of sulfate conjugated metabolites of other AAS that could be used to improve detection capabilities of their misuse. A methodology based on the direct analysis of the sulfates by LC-MS/MS will be applied. Detected sulfate metabolites will be characterized and their excretion profiles will be compared with those of the steroid metabolites targeted in conventional screening procedures, in order to evaluate their interest as long-term markers of steroid misuse. Finally, a methodology addressed to the reliable detection and confirmation of new sulfate metabolites in routine antidoping analysis will be developed.

Results and Conclusions:

The objective of the study was to evaluate sulfate conjugated metabolites of different anabolic androgenic steroids (AAS). Based on the common ionization and fragmentation behaviour of steroid sulfates, precursor and neutral loss scan methods, and selected reaction monitoring methods including theoretical transitions of potential sulfate metabolites were used to

detect new sulfate metabolites in samples obtained after administration of different AAS, including 4-chloro-metnadienone, stanozolol and clostebol.

For 4-chloro-metandienone, four conjugates with sulfate were detected, and two of them (identified as isomers of 4-chloro-18-nor-17ξ-hydroxymethyl-17ξ-methylandrost-1,4,13- triene-3-one conjugated with sulfate) were detected in urine up to the last samples collected in the excretion studies available, showing for these studies the same detection window as the recently reported long-term metabolites excreted in the glucuronide fraction.

For stanozolol, eleven sulfate metabolites were detected, including 16β-hydroxy-stanozolol 16β-sulfate and other metabolites characterized based on mass spectrometric data: two isomers of 16β-hydroxy-stanozolol 16β-sulfate; two isomers of stanozolol N-sulfate; three isomers of hydroxy-stanozolol N-sulfate; 16-oxo-stanozolol N-sulfate; and 4β-hydroxy-16- oxo-stanozolol N-sulfate. Detection times up to 10 days were obtained after oral administration and, therefore, the sulfate metabolites did not improve the retrospectivity obtained with the recently described epistanozolol N-glucuronide, resulting from the initial formation of a sulfate conjugate.

For clostebol, sixteen sulfate metabolites were detected. One of them, characterized as 4ξ- chloro-5α-androst-3β-ol-17-one 3β-sulfate, was detected up to the last sample collected in the excretion studies evaluated (31 days) and, therefore, significantly improves the detection time of clostebol misuse with respect to the commonly monitored metabolites, excreted in the glucuronide fraction.

The results of the project demonstrate the importance of sulfatation as a phase II metabolic pathway of AAS and the interest to study this metabolic fraction to look for new long-term metabolites, and the need to incorporate these metabolites in the initial testing procedures for AAS in all antidoping laboratories.