

“Screening of testosterone misuse by metabolites resistant to enzymatic hydrolysis” and “Characterization, elucidation and synthesis of a potential marker for testosterone misuse”

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PROJECT REVIEW

The project has two main goals. Firstly, the project aims to evaluate the potential of 3 α -glucuronide-6 β -hydroxyandrosterone (6OH-A-3G) and 3 α -glucuronide-6 β -hydroxyetiocholanolone (6OH-Etio-3G) for the screening of endogenous anabolic androgenic steroid misuse. For this first purpose, a quantitative method validated during the project 12A13OP will be applied to several samples from excretion studies already available in our laboratory. Secondly, the project also aims to characterize, elucidate and synthesize the marker M170T298. For this purpose, a large range of analytical strategies will be applied e.g. different derivatization steps, an in-depth study of accurate mass and tandem mass spectra. Once elucidated, the marker will be synthesized and characterized by NMR. After the synthesis of the marker, it would be useful to develop a quantitative method and to check its potential for other administration routes of testosterone. It is intended to reach these goals in a follow-up project once the objectives of the present project are achieved.

RESULTS AND CONCLUSIONS:

In previous WADA funded projects (11A9RV and 12A13OP), our research group revealed that two testosterone metabolites resistant to enzymatic hydrolysis: 3 α -glucuronide-6 β -hydroxyandrosterone (6OH-A-3G) and 3 α -glucuronide-6 β -hydroxyetiocholanolone (6OH-Etio-3G) were present in urine. We characterized them, synthesized them and validated a method for their quantitation. However, their potential usefulness for doping control analysis was not fully evaluated.

On the other hand, common investigations for the screening of testosterone misuse are focused on metabolites with a predicted structure. Thus, unpredicted markers remain not evaluated. In order to cover this gap, we performed a preliminary metabolomic study which showed the presence of a marker (code M170T298) which is a potential urinary marker for testosterone administration. In order to confirm this fact, the characterization, elucidation and synthesis of this marker are required.

Thus, the project has two main goals. Firstly, the project aims to evaluate the potential of 6OH-A-3G and 6OH-Etio-3G for the screening of EAAS misuse. Secondly, the project also aims to characterize, elucidate and synthesize the marker M170T298.

Regarding the first goal, the usefulness of 6OH-A-3G and 6OH-Etio-3G was tested in three scenarios: (i) oral administration, (ii) intramuscular administration and (iii) gel administration. After oral administration of testosterone undecanoate we found that four markers containing either 6OH-A-3G or 6OH-Etio-3G presented detection windows (DWs) larger for all volunteers than those obtained by T/E and comparable to those reported by using cysteinyl metabolites. After intramuscular administration, markers containing either 6OH-A-3G or 6OH-Etio-3G provided detection windows that were similar or longer than those obtained by markers currently included in the steroid profile and clearly higher than those obtained by cysteinyl markers. Finally, the administration of testosterone gel could be screened by markers containing either 6OH-A-3G or 6OH-Etio-3G reaching similar to those obtained by markers currently included in the Athlete Biological Passport.

Regarding the second goal, during this project, we have characterized, elucidated and synthesized the marker M170T298. Our research shows that this marker is 1-cyclopentenoyl glycine (1-CPG) which is probably coming from the metabolism of the cypionic acid released after the hydrolysis of testosterone cypionate. Therefore, it can be valuable to detect the misuse of testosterone cypionate. This fact has been confirmed by analysis of samples collected after testosterone cypionate administration. The determination of 1-CPG provided the longest DWs for these samples.

PUBLICATIONS

Kotronoulas A, Gomez-Gómez A, Fabregat A, Segura J, Sheng Y, Yanyi X, Wu M, Marcos, Joglar J, Ventura V & Pozo O. Evaluation of markers out of the steroid profile for the screening of testosterone misuse. Part I: Transdermal administration. *Drug Test Anal.* 2018; 10:821-831.

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Raro M, Ibáñez M, Gil R, Fabregat A, Tudela W, Deventer K, Ventura R, Segura J, Marcos J, Kotronoulas A, Joglar J, Farré M, Sheng Y, Yanyi X, Van Eenoo P, Pitarch E, Hernández F, Sancho J, Pozo O. Untargeted Metabolomics in Doping Control: Detection of New Markers of Testosterone Misuse by Ultrahigh Performance Liquid Chromatography Coupled to High-Resolution Mass Spectrometry. *Anal. Chem.* 2015, 87, 8373–8380.