Application of the untargeted metabolomics approach to study drug metabolism for anti-doping control by metabolic prediction models (in vitro and in vivo)

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Oxandrolone is a synthetic analogue of testosterone that has high anabolic activity and can be administered orally due to the alkylation present on the 17-? carbon of the D ring. In addition, it is part of the class of doping agents with the highest number of adverse analytical results, the anabolic-androgenic steroid (AAs). This high prevalence, as well as the emergence of new structurally related substances, makes the study of metabolism an important tool for doping control. The aim of this project is to apply untargeted metabolomics approach to study metabolic prediction models (*in vitro* and *in vivo*) using the anabolic steroid oxandrolone as a reference substance to evaluate the formation of classic metabolites, long-term metabolites, and possible unknow new metabolites. Metabolomics approach based on mass spectrometry data acquisition and data analysis was employed to detect the metabolites generated after incubation of oxandrolone in the *in vitro* rat liver microsome model. Tandem mass spectra (MS2) were acquired across a variable isolation width in a mass range with all theoretical precursor ions (vDIA) after chromatographic separation. After raw data processing with MSDial software, the deconvoluted features were sent to the Global Natural Products Social Molecular Networking (GNPS) for untargeted data mining. Analysis of the molecular network made it possible to identify phase I metabolites from the enzymatic biotransformation of oxandrolone as 16-hydroxyoxandrolone and 17-hydroxymethyloxandrolone. This untargeted approach will be applied to other metabolic prediction models, such as the *in vivo* Zebrafish Water Tank (ZWT) model and the *in vitro* human hepatocyte cell line HepG2 model, both used to evaluate the formation of Long-Term Metabolites (LTM). The *in vitro* and *in vivo* models used for metabolic prediction studies, as well as the use of molecular networking (MN), prove to be potential tools for monitoring metabolites in doping control, as well as in studies to elucidate new metabolites of interest. The results generated can provide a basis for the implementation of new analysis methods aimed at monitoring metabolites of interest to anti-doping control laboratories.

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