

Project Review

“Steroid metabolism & the chimeric mouse model”

P. Van Eenoo, F. Delbeke, D. Koen, K. Deventer (DoCoLab, Ghent University, Zwijnaarde, Belgium), **G. Leroux-Roels, P. Meuleman** (CEVAC, Ghent University, Zwijnaarde, Belgium)

Anabolic steroids are amongst the most misused substances in doping control and are intensively metabolized in humans. Adequate screening for misuse of these substances therefore relies on the detection of metabolites in urine samples collected from athletes.

Most of the studies investigating the metabolism of pharmaceutically available steroids were performed in the 1980's via gas chromatography mass spectrometry (GC-MS). This research resulted in the selection of appropriate metabolites for the detection of steroid misuse. Over the years the selection of metabolites was further elaborated to include several metabolites that can result in prolonged detection times. Recent reinvestigation of metabolism via LC-MS resulted in more appropriate metabolites for this technology, than those detected via GC-MS.

Over the last decade, a high number of non-pharmaceutical steroids have been introduced on the market as “nutritional supplements” or as black market designer steroids, while most of the pharmaceutical preparations have been retracted. For both types, approval of administration by an Ethical Committee has become almost impossible. The UPA+/-SCID mouse model with humanized liver has proven to be the best appropriate model to mimic human steroid metabolism in the liver and application of this model to the metabolism of methandienone and methyltestosterone has contributed to the detection of new and long-term metabolites detectable via liquid chromatography tandem mass spectrometry (LC-MSn).

The current project would (re)investigate metabolism of several groups of steroids:

1. Substances currently listed on the prohibited list, but with limited knowledge on metabolism
2. Substances currently not explicitly listed

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Result and Conclusions

The aim of the project was to study the metabolism of different steroid compounds by means of a humanized chimeric mouse model. This uPA^{+/+}-SCID mouse model has its liver transplanted with functional human hepatocytes. In the past the chimeric mouse model has proven to be a valuable tool in elucidating the urinary steroid metabolism, especially for those steroids for which it is difficult to obtain ethical approval for human excretion studies.

In this project the metabolism of 7 steroid compounds was investigated:

- * methyl-1-testosterone
- * oxabolone
- * prostanazol
- * 6-bromo-androstenedione
- * 3 α -androstanol
- * 17-methyldrostanolone
- * 4-chloro-17-methylandrostenediol (promagnon and methylclostebol)

The metabolism of these 7 steroids was investigated by use of administration studies to the chimeric mouse model. The analyses of the mouse urine samples were performed on a combination of GC-MS and LC-MS/MS instruments. Comparing the pre- and post-administration mouse urine samples allowed us to obtain a urinary metabolic profile for all of the steroids. From all the detected metabolites, the most appropriate markers were selected based on their usefulness as target markers for steroid abuse. Those metabolites were implemented where possible in our routine screening methods for anti-doping screening, making our methods even more complete and comprehensive. Additionally, within the scope of the project, also the in vitro technique of human liver microsomes was optimized to assist this research.

In the future this promising mouse model will be used to further encourage the fight against doping by evaluating some prohormones and food supplements based on the urinary results of the chimeric mice.