PROJECT REVIEW

"Synthesis and *in vitro* metabolism studies of selective androgen receptor modulators (SARMs) currently undergoing clinical trials (IIb)"

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Anabolic agents have been one of the most frequently detected drugs in amateur and professional sport. A novel class of therapeutics presumably complementing anabolic steroids in the near future includes so-called selective androgen receptor modulators (SARMs) that have been under clinical investigations (currently IIb) for several years. Although not commercially available yet, their potential for misuse in sports is high considering the announcements of the drugs' properties. These state that without a prescribed diet or exercise regimen all subjects treated with ostarine had a dose dependent increase in total lean body mass (muscle) achieving an increase of 1.4 kg compared to placebo after three months of treatment. Treatment with ostarine also resulted in a dose dependent improvement in physical performance measured by a stair climb test achieving a clinically significant improvement in both speed and power.

Hence, doping control laboratories need to be prepared to determine these drugs and/or major metabolites in human urine. As approved drugs are currently not on-hand, promising drug candidates such as Ostarine (GTx Inc.) were chemically synthesized, purified and characterized according to published data. Subsequent *in vitro* metabolism studies will be conducted to provide insights into possible metabolic pathways of these novel compounds. These data will provide the basis for the implementation of new target analytes related to anabolic agents to doping control screening procedures.

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Results and Conclusions

The need to comprehensively screen for selective androgen receptor modulators (SARMs) in sports drug testing has become an important aspect due to the considerable anabolic properties of these drug candidates. In addition, first adverse analytical findings for the SARM Andarine were reported in 2010, demonstrating the importance of in-depth investigations concerning these compounds to ensure utmost retrospective in doping controls.

In the course of the WADA-funded project concerning the metabolism of arylpropionamide-derived SARMs, a method to detect the unchanged drug candidates S-1, S-4, S-9, S-22, S-23, S-24, and a metabolite M-11 in human urine was established and validated using liquid chromatography/tandem mass spectrometry. Using solid-phase extraction to preconcentrate the urine specimens, recoveries (85-89%), intraday and interday precisions (4.1-18.8%), and lower limits of detection (0.03-0.23 ng/mL) were determined. Specificity studies demonstrated no interfering signals at respective retention times and specific ion transitions, ion suppression/enhancement effects were not observed, and all analytes responded in a linear fashion to increasing concentrations between 5 and 80 ng/mL.

Further to the analysis of the active drugs, the phase-I and -II metabolism of S-22 and S-23 was simulated using hepatic human enzymes, and resulting metabolites were characterized by means of state-of-the-art mass spectrometric approaches employing high resolution/high accuracy Orbitrap mass spectrometry. Subsequently, the newly defined target compounds including the glucuronic acid conjugates of S-22 and S-23, their corresponding monohydroxylated and bishydroxylated analogs, as well as their B-ring depleted counterparts were implemented into an existing routine doping control procedure, which was examined for its specificity for the added substances. In order to obtain proof-of-concept data for authentic urine specimens, canine urine samples collected up to 72 h after oral administration of S-22 to dogs were analyzed using the established approach outlining the capability of the presented assay to detect the glucuronide of S-

22 as well as the B-ring-depleted metabolite (**M3**) in all samples following therapeutic (31.4 µg/kg) dosing. Finally, **M3** was chemically synthesized, characterized by nuclear magnetic resonance spectroscopy and high resolution/high accuracy mass spectrometry, and chosen as primary target for future doping control analyses.

In order to estimate the time period for uncovering an illicit administration of therapeutic dosages of SARMs, further studies after controlled oral application to humans and urine collection/analysis are required.