PROJECT REVIEW

"*Synthesis and in-vitro metabolism of sirtuins – a class of emerging drugs with misuse potential in elite sport*"

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The enzyme Sirtuin 1 (SIRT1) modulates many metabolic processes in response to low nutrient availability in the organism. It is assumed to play a major role in regulation of many cell-processes such as insulin secretion and cell death. SIRT1 activators such as SRT1720 mimic, by affecting the enzyme SIRT1, a calorie restriction and produce beneficial effects on insulin sensitivity, fatty acid oxidation in skeletal muscles, liver and brown adipose tissue, liponeogenesis in white adipose tissue and mitochondrial biogenesis.

Therefore medical research focuses on SIRT1 activators for treating metabolic diseases like Diabetes type 2, adiposity and inflammation. At present several SIRT1 activators are testing in phase-I and phase-II clinical studies.

Concerning a potential misuse in sport, some studies indicate that SIRT1 activators affect the switch of muscle fibers that enhance whole-body energy expenditure resulting in increased endurance capacity and motor skills. Therefore SIRT1 activators might pose a threat to the integrity of sport and represent potential doping substances covered by the newly established category S0 (non-approved substances) among the WADA Prohibited List. Consequently, detection methods for blood and urine concerning the intact drugs and respective metabolic products will be developed, requiring the synthesis of model sirtuin activators, their in vitro metabolism (enabling the characterization of urinary metabolites), and finally the implementation of new target analytes in routine doping controls.

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Results and conclusions:

Sirtuin-1 activators represent a class of emerging therapeutics aiming at the treatment of different diseases including type-2 diabetes, obesity, and cancer. Main effects of chosen drug candidates are attributed to mitochondrial biogenesis and transformation of muscle fibers from type-II to type-I, resulting in increased energy expenditure derived from lipids.

Several substances are in advanced clinical trials with an archetypical representative referred to as SRT-1720. Since the detailed structures of the lead drug candidates are not yet disclosed by pharmaceutical companies, four model compounds derived from SRT-1720 were chemically synthesized and characterized with physic-chemical methods including nuclear magnetic resonance spectroscopy and mass spectrometry. The reference substances were subsequently used to establish detection assays from human plasma using standard conditions and instrumentations available in doping control laboratories. Further, the model compounds were subjected to *in vitro* metabolism to generate potential target analytes to be analysed from human urine. Due to the common and conserved pharmacophore of all studied substances, comparable metabolic pathways were observed and modifications such as hydroxylation, oxidation, reduction, and eventually conjugation were identified. An assay for the analysis of sirtuin-1 activators from human urine was also established and validated, demonstrating its fitness-for-purpose in sports drug testing programs. The obtained data will facilitate and accelerate the inclusion of new drugs and drug candidates with similar nuclei into routine doping controls and the currently available compounds (e.g. SRT-1720) are already now traceable in plasma and urine using the herein developed methodologies.