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SIRT1 activators: Metabolism studies and detection in human plasma and urine

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Abstract

Sirtuins are NAD^+-depending histone deacetylases (HDACs) and catalyze the hydrolysis of the N-terminal acetylated lysine of histones. There exist seven Sirtuins (SIRT1-7) in the human organism, which differ in localization, function and substrate specificity. The best studied Sirtuin is SIRT1, which was identified as a metabolic key regulator such as the up-regulation of mitochondrial biogenesis in several tissues including skeletal muscle, brown adipose tissue and liver.
In recent years, SIRT1 activating compounds (called STACs), based on a thiazole-imidazole nucleus, were developed, aiming the treatment of metabolic disorders like obesity, high blood glucose and insulin resistance (e.g. SRT1720 and SRT2104).
SRT1720, the best studied SIRT1 activator, demonstrated in animal studies with DIO mice an enhancement of mitochondrial capacity in gastrocnemius muscle by 15%. In addition, increased muscle strength, a better locomotor behavior and a significant improvement in endurance running was recognized resulting of a switch of skeletal non-oxidative muscle fibers to oxidative muscle fibers. This metabolic effect probably results from the change in fatty acid oxidation in skeletal muscles and liver caused through a multilayer mechanism of deacetylation of transcriptional factors (e.g. FOXO1) and transcriptional co-activators (e.g. PGC-1alpha) and therefore a change in expression of genes related to metabolism. These positive metabolic effects presume SIRT1 activators to be potential doping substances for endurance sports.

In the present study the phase I and II metabolites of in-house synthesized SRT1720 and four additional SIRT1 activator models were generated employing an *in vitro* assay with human liver microsomal and S9 liver enzyme fractions. The resulting metabolic products were characterized to investigate the principle metabolism of SIRT1 activators comprising a thiazole-imidazole nucleus.
The dissociation pathways of the generated metabolites were studied with positive electrospray ionization and collision-induced dissociation on a high resolution/high accuracy mass spectrometer, LC-MS^3 measurements on a quadrupole linear ion trap mass spectrometer as well as H/D-exchange experiments. Furthermore an *in vivo* (rat) excretion study was performed yielding blood and urine samples. Additionally, a detection assay for the intact compounds in human plasma and urine for future doping control applications using a synthesized eight-fold deuterated internal standard of SRT1720 was validated.

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[3] Höppner S, Delahaut P, Schänzer W, Thevis M. (2014) Mass spectrometric studies on the in vivo metabolism and excretion of SIRT1 activating drugs in rat urine, dried blood spots, and plasma samples for doping control purposes, *J Pharma Biomed Anal*, **88**; 649-659

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