PROJECT REVIEW

"Detection of small interfering RNA (siRNA) as gene doping strategy using combined biochemical and mass spectrometric approaches."

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Small interfering RNA (siRNA) is a tool to influence and manipulate gene expression, which might be misused in sports and has therefore been prohibited aacording to established anti-doping rules. siRNA molecules bind to messenger molecules (so-called mRNA) to downregulate the synthesis of selected proteins. In general, any target gene could be downregulated on the mRNA level by applying the corresponding, complementary siRNA. Therefore, the fields to use these molecules for performance enhancement are manifold.

Due to a very short plasma halflife, siRNA molecules are modified and protected from degradation byRNAses, which complicates the prediction of a future pharmaceutical product but identifies the xenobiotic nature of such molecules and is considered a starting point for method development for doping control procedures.

The planned project includes the development of a confirmation method targeting modified siRNA and shall further provide the proof-of-principle by oral application or injection of siRNA to laboratory rodents with subsequent blood and/or urine analysis. A screening method based on the isolation of the intact siRNA strands from plasma samples followed by high resolution/ high accuracy mass spectrometry measurement was recently developed and validated and serves as basis for a confirmatory assay. For a confirmation method, tandem-mass spectra may be recorded and evaluated after optimization of the sensitivity to identify a sequence and modified nucleotides. Alternatively, modified nucleotides may be detected after degradation of the strands in plasma or urine samples. For that purpose, administration studies are of particular importance and, due to the clinical status of the substances, only preclinical studies are aimed. For the modified nucleotides, analysis is planned to be performed by LC/MS procedures.

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Results and Conclusions:

The issue of gene doping with modified genetic information that is introduced into the athletes' organism has been an emerging field in sports drug testing. Within the present project several strategies were developed in order to uncover the misuse of small interfering (si) RNA for performance enhancement. By means of siRNA as doping agent, literally every gene of interest can be temporarily silenced (knocked-down). In the present study the muscle regulator myostatin was chosen as target gene. It was shown that specific model siRNAs (designed to knock-down the myostatin messenger RNA) are detectable in rat urine after single intravenous administration at arguably therapeutic dosing for up to 24 hours. The unambiguous identification of the metabolites in urine was realized by a combination of liquid chromatography-mass spectrometry approaches and gel electrophoretic-based assays under consideration of intact metabolites as well as their hydrolysis products. The assay's performance was characterized and validated for the designed model siRNA substances, and a generic protocol and approach to uncover the misuse with to-date unknown target molecules was suggested. Here, strategies comparable to proteomics methodologies allowing for de novo sequencing were tested, which were successfully applied as long as the artificially modified nucleotides were included in the available data evaluation software. Further work will be required to expand the test to the virtually unlimited options of sequence modifications; however, the presence of a xenobiotic nucleotide or sequence of nucleotides in doping control specimens is a substantial hint towards the misuse of RNA-interfering substances.