"Detection of AICAR administration by carbon isotope ration mass spectrometry"

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Aims

Within the last few years a new class of substances emerged in the focus of sports drug testing, so called gene doping agents. One of these called AICAR (aminoimidazolecarboxyamide-ribonucleoside) has proved to enhance physical performance, even without training, in animal experiments.

In the course of clinical trials AICAR has been administered to human beings to investigate its health care properties related to type-2 diabetes. As during these studies no major side effects were reported it cannot be excluded that AICAR will be of interest for cheating athletes. Therefore, the World Anti-Doping Agency included AICAR on their list of prohibited substances in 2009 and doping control laboratories started to establish methods for detecting AICAR-abuse. Until now, urinary concentrations of AICAR have been determined and reference-based thresholds were suggested due to the fact that AICAR is an endogenously produced substance and occurs in every urine specimen. As the observed biological variability in urinary concentration was high – as also reported for other classes of substances such as steroids – these thresholds are quite high and endogenously produced AICAR can potentially be found beyond the reference limit in rare cases. In analogy to steroids, the carbon isotope ratio determination would be the method of choice to distinguish endogenous from exogenous/administered AICAR. Hence, the aims of this study are to develop and validate a method for the determination of carbon isotope ratio values of AICAR and to elucidate the endogenous $^{13}C/^{12}C$ ratios of this compound. Additionally, the $^{13}C/^{12}C$ ratios of synthesized AICAR will be determined to enable the calculation of a clear cut-off criterion between the endogenous and the exogenous AICAR.

Results and Conclusions

AICAR (5-Aminoimidazole-4-carboxamide 1β-D-ribofuranoside) is prohibited in sport according to rules established by the World Anti-Doping Agency. Doping control laboratories identify samples suspicious of AICAR abuse by measuring its urinary concentration and comparing the observed level to naturally occurring concentrations. As the inter-individual variance of urinary AICAR concentrations is large, this approach requires a complementary method to unambiguously prove the exogenous origin of AICAR. Therefore a method for the determination of carbon isotope ratios (CIR) of urinary AICAR has been developed and validated.

Concentrated urine samples were fractionated by means of liquid chromatography for analyte clean up. Derivatization of AICAR yielding the trimethylsilylated analog was necessary to enable CIR determinations by gas chromatography-combustion-isotope ratio mass spectrometry. The method was tested for its repeatability and stability over time and a linear mixing model was applied to test for possible isotopic discrimination. A reference population of n = 63 males and females was investigated to calculate appropriate reference limits to differentiate endogenous from exogenous urinary AICAR. These limits were tested by an AICAR elimination study.

The developed method fulfils all requirements for adequate sports drug testing and was found to be fit for purpose. The investigated reference population showed a larger variability in CIR of AICAR as for endogenous steroids. Nevertheless, the calculated thresholds for differences between AICAR and endogenous steroids can be applied straightforward to evaluate suspicious doping control samples with the same statistical confidence as established e.g. for testosterone misuse. These thresholds enabled the detection of a single oral AICAR administration for more than 40 h.

Determination of CIR is the method of choice to distinguish between an endogenous or exogenous source of urinary AICAR. The developed method will enable investigations into doping control samples with elevated urinary concentrations of AICAR and clearly differentiate between naturally produced/elevated and illicitly administered AICAR.

Publications

- Rapid Communications in Mass Spectrometry (2014; 28: 1194-1202)
- Presentation at the 32nd Manfred Donike Workshop in Cologne, 2014