

PROJECT REVIEW

"AICAR: Determination of AICAR-ribotide in red blood cells as long term marker for an illicit AICAR application"

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AICAR has been shown to exhibit performance enhancing effects after oral administration due to the stimulation of fat utilization and increased production of mitochondria in laboratory mice after a 4-weeks treatment with AICAR. The drug candidate has not yet received clinical approval and currently undergoes phase-II clinical trials. In urine of healthy humans considerable amounts (up to 7500 ng/mL) were present due to the natural occurrence of AICAR as a (by-) product of purine biosynthesis. Normal (log-transformed) distribution of AICAR excretion into urine was observed, if corrected to creatinine rather than to specific gravity; however, the occurrence of elevated urinary levels (> 3500 ng/mg) after an illicit administration were assumed to last for several hours only, and the detection window offers only limited options for effective doping controls.

Circulating AICAR is dedicated to enter the cells with a simultaneous biotransformation from AICAR-riboside to AICAR-ribotide (phosphorylation at position 5'). This conversion occurs in considerable extent also in erythrocytes. After exposure of erythrocytes to AICAR, the red blood cells contain a significantly elevated amount of AICAR-ribotide and this level is described to be remain stable for approximately 10 days. In the present study it is planned to investigate whether the determination of AICAR-ribotide in red blood cells can serve as potential long-term marker for an illicit AICAR administration. Riboside and ribotide concentrations in plasma and red blood cells of healthy volunteers will be determined by LC-MS/MS and quantified by means of isotope dilution mass spectrometry. Subsequently, reference values will be statistically evaluated.

Preliminary experiments have shown that in-vitro experiments (incubating AICAR reference substance in whole blood) induce immediate biotransformation of the riboside to the corresponding ribotide within the erythrocytes. After lysis the elevated ribotide levels can be determined and allow for the development of a pharmacokinetic model for in-vivo conditions.

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Results and Conclusions

AICAR (an adenosine monophosphate-activated protein kinase (AMPK) activator, 5-amino-4-imidazolecarboxamide ribonucleoside, Acadesine) belongs to the class of metabolic modulators of the WADA Prohibited List and has been banned since 2009 due to its performance-enhancing capabilities. Confiscated products from team managers and anecdotal evidence have outlined the need for adequate test methods, which have been under development from urine in particular. Main issue with this emerging drug is its natural occurrence in every individual at varying concentrations.

In the present project, the utility of erythrocytes concerning the detection of AICAR abuse was evaluated. AICAR possesses the ability to penetrate the erythrocyte membrane. After entering the red blood cell cytosol, it is converted into the corresponding phosphate, referred to as AICAR ribotide. Due to the phosphorylation, it is conserved within the erythrocyte and its concentration remains elevated for up to 10 days upon administration of therapeutic amounts of AICAR. Hence, the quantitative analysis of AICAR ribotide levels in erythrocytes was considered as a helpful tool to determine unnaturally high levels. Consequently, an assay employing a stable-isotope labelled internal standard ($^{15}\text{N}_4$ -AICAR ribotide) was developed to allow for the quantification of the target analyte from 10 μL of erythrocytes. A total of 99 blood samples (49 male and 50 female non-elite athletes) was assayed to establish a reference population-derived ‘normal’ value for AICAR ribotide, which indicates that concentrations higher than 920 ng/mL are not in agreement with naturally circulating AICAR levels. In order to simulate elevated plasma concentrations of AICAR following oral or intravenous administration, in vitro incubations were conducted at 500, 1000, and 10,000 ng/mL in agreement with literature data concerning clinical trials. A rapid uptake of AICAR into the erythrocytes and conversion to the ribotide was observed, yielding concentrations significantly higher than 920 ng/mL within 15 min. Hence, the quantitation of AICAR ribotide in red blood cells derived either from athlete biological passport samples or complementary collected specimens (such as dried blood spots, DBS) can be a viable tool to indicate and eventually prove the illicit intake of AICAR.