MANFRED DONIKE WORKSHOP
38th COLOGNE WORKSHOP
ON DOPE ANALYSIS

09.02. – 14.02.2020

PAPER PRESENTATION
POSTER PRESENTATION

ABSTRACTS

Renovato-Martins M[1,2], Dudenhoeffer-Carneiro AC[1], Du Rocher-Silva B[1], Soares F[1], Aquino Neto FR[1], Hasan M[3], Rahaman KA[3], Muresan A[3], Oh-Seung K[3], Mirotti L[1], Pereira HM[1]

Erythrocyte phenotyping of international athletes by flow Cytometry

Chemistry, Chemistry Institute, Rio de Janeiro, Brazil[1]
Department of Molecular and Celular Biology, Biology Institute, UFF, Niterói, Brazil[2]
Doping Control Center Korea, Institute of Science and Technology, Seoul, Korea[3]

Abstract

Blood transfusion is performed by cheating athletes to rapidly increase oxygen delivery to exercising muscles and enhance their performance. Homologous blood transfusion happens when blood from a different person is transfused. The method used to detect this type of doping is based on flow cytometry, by identifying variations in blood group minor antigens present on erythrocytes surface. Different authors and anti-doping laboratories use different panels of erythrocyte antigens to allow the detection of mixed populations. In total, there are more than 300 different red blood cells antigens and the detection is based on the most abundant families, normally in a total of around 8- 10 antigens. It has been reported that number of occurrences of two individuals sharing an identical phenotype in the same population of athletes is 5 times higher than the theoretical probability. This work describes the data of athletes who were analyzed by flow cytometry for the prevalence of different erythrocytes surface antigens (C, E, Fya, Fyb, Jka, Jkb and S) in a population of 360 international athletes from all 5 continents.

Rahaman KA[1,2], Muresan AR[1,2], Rafique FB[1,2], Kim KH[1], Lee KM[1], Min H[1], Kim HJ[1], Sung C[1], Son J[1], Lee J[1], Kwon OS[1,2]

Metabolites of TB-500 peptide in various i*n-vitro* enzyme systems by UHPLC-MS/MS

Doping Control Center, Korea Institute of Science and Technology, Seoul, Republic of Korea[1]

Division of Biomedical Science & Technology, KIST-School, Korea University of Sciecne and Technology, Seoul, Republic of Korea[2]

Abstract

TB-500 is a preparation of seven peptides prepared from the active site of thymosin β4. Thymosin β4 has effects on tissue regeneration, anti-inflammation, and fast repair capabilities with potential of abuse in athletes. TB-500 misuse was found in equine sports, and is known to be used illegally in athletes. In this study, we aimed to find out new metabolites of TB-500 peptide in various in-vitro enzyme systems such as human kidney microsome, rat liver microsome, rat liver cytosol, rat liver S9, human serum, and human skin S9. The metabolic reaction was conducted after prolonged incubation (22 hr) with various enzymes and the metabolites were analyzed in a full-scan mode by liquid chromatography coupled with MS/MS (Q-Exactive). As the results, human kidney microsome and rat liver microsome systems showed higher concentration of metabolites than the rest of enzymes. We found N-acetylleucine (Acetyl-Leu-OH; m/z 174) as a new metabolite. The new metabolite, N-acetylleucine (Acetyl-Leu-OH; m/z 174), was also found in all enzyme systems used. The other metabolites such as acetyl-Leu-Lys-Lys-Thr-Glu (m/z 660), acetyl-Leu-Lys (m/z 302), and acetyl-Leu-Lys-Lys (m/z 430) were detected. We suggest that human kidney microsome or rat liver microsome systems is better enzyme systems for TB-500 metabolism studies than cytosol, S9 and serum. We report for the first time N-acetylleucine as a metabolite of TB-500. Therefore, N-acetylleucine detection in serum and urine also can be a useful marker of detecting TB-500 abuse in doping.

Rafique FB[1,2], Muresan AR[1,2], Rahaman KA[1,2], Kim KH[1], Lee KM[1], Min H[1], Kim HJ[1], Sung C[1], Son J[1], Lee J[1], Kwon OS[1,2]

In vitro phase I metabolism study of diuretics clopamide by LC-MS/MS

Doping Control Center, Korea Institute of Science and Technology, Seoul, Republic of Korea[1]

Division of Biomedical Science & Technology, KIST-School, Korea University of Science and Technology, Seoul, Republic of Korea[2]

Abstract

Diuretics are often illegally used in different kind of sports discipline, and clopamide is included in the list of prohibited substances of World Anti-Doping Agency. The objective of this study was to investigate clopamide metabolites in vitro enzyme system in both negative ionization ($[M-H]^-$, m/z 344) and positive ionization ($[M+H]^+$, m/z 346) since its metabolism study was not reported yet.

In this study, the phase I metabolites were generated through an in-vitro enzyme system with rat liver microsome, and identified by using both negative and positive ionization modes of an ultra high-performance liquid chromatography/Orbitrap mass spectrometer (Q-Exactive). A full scan and *dd*-MS/MS modes were used to obtain structural information of the metabolites.

We have characterized 3 mono-hydroxylated ($[M-H+16]^-$, m/z 360) metabolites and 2 dehydrogenated metabolites ($[M-H-2]^-$, m/z 342) at collision energy of 40 eV in negative mode. In case of positive ionization, 3 mono-hydroxylated metabolites ($[M+H+16]^+$, m/z 362), and 2 dehydrogenated metabolites ($[M+H-2]^-$, m/z 344) were found at collision energy of 35 eV, based on their structures and mass spectra. These metabolites could be useful for further in vivo metabolites identification with the purpose of anti-doping analysis. The further in-vitro phase II and in-vivo studies are progressing to identify more metabolites as potential biomarkers for anti-doping analysis.