

PROJECT OVERVIEW

“a) A systems biology biomarker based approach to the detection of microdose recombinant human erythropoietin doping and b) Metabolomic profiling of recombinant erythropoietin in caucasian and east-African endurance trained athletes”

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a) The use of recombinant human erythropoietin (r-HuEpo) is prohibited by the World Anti-Doping Agency (WADA). The purpose of a research project funded by WADA in 2008 entitled “A gene-microarray based approach to the detection of recombination human erythropoietin doping in endurance athletes” was to develop new methods with improved discriminatory power relative to current detection protocols based on gene expression profiles. On the basis of the preliminary data generated by this project, blood gene expression profiles were significantly altered during r- HuEpo administration and for at least 2 weeks after the r-HuEpo administration. These preliminary data are very promising, however, it is well known that athletes are now microdosing with r-HuEpo to minimise the risk of being caught via current detection methods. Therefore, in this project, we will compare blood gene expression profiles altered using a fairly high regimen of r-HuEpo injections of 50 IU/kg body mass every two days for 4 weeks (the previously funded project) with a microdose regimen (< 40 IU/kg body mass twice a week). Blood gene expression profiles following r-HuEpo administration will be assessed using gene-microarray technology.

These results will be used to develop more specific and robust blood testing models applicable to the detection of microdose r-HuEpo doping.

b) Our research consortium has previously been funded by WADA as follows: In 2008 “A gene microarray based approach to the detection of recombinant human erythropoietin doping in endurance athletes” and a related project funded in 2010 “Application of a minimally-invasive method for RNA sampling and the addition of miRNA to the detection of recombinant human erythropoietin (r-HuEpo) use by athletes”. These recently funded projects were designed to provide the basis for the development of new detection methods with improved discriminatory capacity relative to current detection protocols using a “system biology” (i.e. gene expression and miRNA profiles assessed by the very latest gene-microarray technology) approach. Current progress with these WADA-funded projects is very encouraging and this success serves to reinforce the feasibility and need for this complex, expensive and technically demanding “systems biology” approach. Here we propose the addition of metabolomics (high resolution mass spectrometry methods) to the established research study aimed at identifying new metabolomic biomarkers that can differentiate between r-HuEpo administration and chronic altitude exposure (using samples already collected from WADA-funded studies). It is envisaged that this vital addition will help formulate new methods with improved discriminatory power relative to current detection protocols and in doing so eliminate the possibility of false-positives due to athletes living and/or training at altitude and false-negatives due to inadequate detection.

RESULTS AND CONCLUSIONS

a) Administration of recombinant human erythropoietin (rHuEpo) improves performance and hence is frequently subject to abuse by athletes. A limited detection window, a lack of sufficiently high sensitivity and specificity limit current testing. Previous WADA-funded research aimed at detecting high doses of rHuEpo doping using gene-based methods has generated promising results. The primary focus of this research is to apply this gene-based approach to the detection of microdoses of rHuEpo. The secondary aim of this research is to assess the performance effects of rHuEpo microdosing. Fourteen healthy endurance trained subjects not involved in competition during the study period participated in a 7 week randomised, double-blind, placebo-controlled crossover microdose rHuEpo regimen previously shown to increase haemoglobin mass. Maximum aerobic capacity and repeated sprint ability was assessed at baseline and during the week after the last rHuEpo/placebo injection. 18 genes were differentially expressed ten days after the first microdoses of rHuEpo. Of these 18 genes, 11 were consistently over-expressed during rHuEpo microdosing and under-expressed post administration thereby further validating the previously identified gene expression signature of rHuEpo. While no clear improvements in measures of anaerobic performance were observed following microdoses of rHuEpo, maximal aerobic capacity was significantly increased pre to post microdose rHuEpo administration by an average of 3.9%. In summary, this research provides strong evidence supporting the idea that gene-based biomarkers have real potential to improve the performance of current anti-doping methods such as the ABP for the detection of doping, not confined only to rHuEpo. In addition, this research highlights the need to improve current detection methods as rHuEpo microdosing has significant performance benefits.

b) Recombinant human erythropoietin (rHuEpo) is prohibited by the World Anti-Doping Agency (WADA) but rHuEpo remains the drug of choice for many cheating athletes wishing to evade detection using current methods. Currently, the only validated direct test for rHuEpo is isoelectric focusing, but this test is severely limited by the short detection window of approximately 36-48 hours. Recent research funded by WADA has generated exciting new transcriptomics (i.e. gene expression) data to support the proof-of-concept idea of "omics" biomarkers as the preferred next generation anti-doping approach. Metabolomics (a key "omics" technology with widespread application in biomedicine) may further enhance this potential by providing a "snapshot" of the biological state of the organism/cell. The aim of this project was to identify a robust metabolomics signature of rHuEpo using an untargeted approach in blood (plasma and serum) and urine. Plasma, serum and urine samples were analyzed using the hydrophilic interaction liquid chromatography-mass spectrometry in 20 Caucasian males following 4 weeks of rHuEpo administration (i.e. 50 IU·kg⁻¹ body mass every two days). Three blood (plasma and serum) metabolites associated with red blood cells were identified as potential markers for rHuEpo detection. Although the diagnostic value of the identified metabolites for current rHuEpo detection methods is low, a combination of replicated metabolomic markers will provide a more detailed and thorough understanding of the perturbed system(s), thereby aiding ABP experts identify and differentiate numerous doping substances and methods when reviewing athlete biological passports. The present findings should encourage further metabolomics studies and the integrated reviewing of all "omics" data generated by other WADA anti-doping studies in order to aid the development of new detection models.