PROJECT REVIEW

"Detection of autologous blood transfusion by proteomics: screening to find unique biomarkers"

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The aim of this project is to find markers for autologous blood doping, and thereby differentiate blood from doped and non-doped athletes using proteomic and metabolomic methods.

Significance

Physical performance in endurance events can be enhanced by blood boosting. Doping by EPO can today be detected while autologous blood transfusion is undetectable. This may favour the use of the latter doping method. Detecting markers of autologous blood transfusion that distinguish a doped from a non-doped athlete is therefore important.

Background

It is highly unlikely that blood can be withdrawn from the body, treated and stored without a change in any protein and/or metabolite. By using a proteomic/metabolomic approach, hundreds of proteins/metabolites can be identified at on time. Combining large amount of data with complex multivariate statistical methods enhances the possibilities to find relevant protein/metabolite markers to detect autologous blood transfusion.

Methods

DIGE and mass spectrometry (MS) will be used to detect and identify proteins. Metabolomics involves the application of nuclear magnetic resonance spectroscopy (NMR) and MS. Using proteomics and metabolomic methods in combination with chemometric data analysis to quantitatively detect perturbations in patterns of proteins and low molecular weight metabolites in biological fluids and tissues.

Results

Currently, over 1000 proteins spots and 200-300 metabolites can be detected from 100 uL of RBC. When comparing blood freshly frozen with blood frozen after 5 week of cold storage, 48 proteins were significantly altered. Using these proteins, as well as additional metabolites, it can be possible to develop a blood test by either antibodies or protein array chips. The most suited method can be decided only when the proteins and metabolites have been identified. In addition, extensive testing using the current proteomic and metabolimic approaches as well as the chosen test method must be done to in order to select optimal settings.

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Results and Discussion

Autologous blood doping, in where a portion of an athlete's RBCs are harvested and stored to later be reintroduced to the same athlete short before a competition, cannot be detected by conventional doping tests. A proteomic approach was used to screen for makers of red blood cell (RBC) membrane proteins, altered by storage.

As hypothesised, we could detect an altered protein pattern when comparing fresh and stored RBCs, regardless if the RBCs were stored for 6 weeks at +4º C or -80º C. After storage at +4º C, 58 protein spots were significantly altered ($p<0.05$) (38 increased and 20 decreased) and after storage at -80º C we detected 71 altered protein spots (70 were increased and 1 decreased). We observed some spots which were similarly increased in both +4º C and -80º C experiment, and also some with opposite change.

It is concluded that proteomics is a good screening method to find markers in RBC:s which is sensitive for cold/freeze storage. The changed proteins could be used as markers for both homologous and autologous blood doping using other methods, such as anti-body based methods and protein array. As no current method exists for detection of autologous blood doping, our results may guide further research to develop a more clinically applicable method.