PROJECT REVIEW

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

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Aim/Significance

- Differentiate blood from transfused and non-transfused individuals.
- Correlate changes in protein pattern to Hb, physical performance and VO2max

Finding markers of autologous blood doping is important in order to maintain the fundamental aspect of sports: fair play.

Background/Hypothesis

Physical performance can be enhanced by blood boosting. Doping using the hormone EPO and homologous blood (non-self) can today be detected while autologous (self) blood transfusion is undetectable. Red blood cells (RBC) can be stored for up to 5 weeks in +4º C and for several years in -80º C. It is highly unlikely that blood can be withdrawn from the body, treated and stored without change in any protein.

Methods

By using proteomic methods, thousands of proteins can be separated and identified. In combination with multivariate statistical methods protein markers to detect autologous blood transfusion can be found. Separation of proteins is done by 2D DIGE and protein identification done by mass spectrometry. We can quantitatively detect changes in protein patterns, thereby separate blood from doped and un-doped individuals.

Results

A 10% difference in protein abundance can be detected (95% CI). Over 2300 proteins protein spots can be separated from 100 uL of RBC. Fresh blood was compared with blood stored for 5 weeks in -80º C. 48 proteins were altered, including enzymes (e.g. catalase) stress (e.g. HSP 71) and structural (e.g. actin) proteins. Ongoing experiments have detected ~80 proteins changed by storage in +4º C for 5 weeks.

Study design

After blood donation (10 subjects) and storage for 4 weeks at 4º C, RBC will be reinfused. Blood samples will be taken from the subjects several times before and after donation and reinfusion. Control samples will be taken from a matched group. Haemoglobin, physical performance and VO2max will be measured on 7 occasions.

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Results and Conclusions

The specific aims of this study were:

I. Differentiate blood from transfused and non-transfused individuals.

II. Correlate changes in protein pattern to Hb, physical performance and VO_{2max}

We have investigated the possibilities to use proteomics as a tool to screen the human Red blood cell (RBC) membrane proteome for novel and unique biomarkers useful for development of future diagnostic point-of-care tests. A comparison between fresh and freeze-stored (-80° C) RBC’s were performed using the 2D DIGE technique. From findings in freeze-stored blood, 20 candidate proteins were identified.

A blood transfusion study was subsequently performed where 10 subjects underwent an autologous blood transfusion (2 x 450 mL donated whole was blood and 2 x 300 mL washed RBC’s re-infused) after 16 week freeze-storage of the RBC’s. Blood samples were drawn at 13 time points for hematological and proteomic analyses and physical performance testing done 9 times.

Forty eight hours after blood transfusion, Hb increased by 5%, physical performance (Running time to exhaustion) was increased by 15% and VO_{2max} by 16%. Only a weak correlation (R^2 = 0.33) was seen between Running time and VO_{2max}.

Blood samples taken from the subjects as well as from the transfusion bags were analyzed by proteomic and standard clinical methods. There is a clear separation of blood taken from a freeze stored bag and fresh venous blood. Different protein profiles between blood taken before and after a transfusion can be visualized. Some of these results were confirmed by Western blot.

Because no method is today available to directly detect an autologous blood transfusion, we believe that our method under development will provide a solution in a near future, and the current work-plan is to have a prototype (alpha-version) ready for testing within 18-24 months, pending funding and the speed of technical advancements.