"Test for blood transfusion (autologous/homologous) based on observed changes of erythrocyte membrane protome"

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Project Summary

Detection of autologous blood transfusion is probably currently the greatest challenge in the doping control. For the reasons of convenience, for its considerable security and the difficulty of detecting it, autologous transfusion has become the method chosen by many athletes to illegally increase their oxygen delivery capacity. This project aims is to study the proteome changes of erythrocytes during storage, prior to (re) infusion of the blood to the same athlete.

For the project were selected proteins associated with the erythrocyte membrane, because they are easily available and have been well characterized in previous studies with methodologies such as 2D electrophoresis, DIGE, liquid chromatography coupled with mass spectrometry, and other combinations of the above.

The studies made so far can be classified broadly as protein-centered or peptide-centered. In the first case, the proteomics approach is based on the separation of membrane proteins from the sample by 2D electrophoresis; in the second case, the most powerful among those described is the isobaric tagging (iTRAQ), which allows quantitative change detection in multiple samples.

The results of our preliminary studies, as well as those available from other authors, identified proteins which can be grouped as follows:
• cytoskeletal proteins,
• transmembrane proteins
• other proteins related to our research findings by other researchers.

To continue our study, we consider efforts in two directions.
• First, to validate the results obtained, confirm the same with the largest number of subjects,
• Second, to verify by independent technical changes detected in the membrane by proteomic methods, we use Western blot and / or flow cytometry.

Ultimately, the goal is to develop a reliable test for the analysis of doping by autologous transfusion, using appropriate markers from membrane proteins or the cytoskeleton of erythrocytes, whose levels vary due to storage of blood in standard transfusion conditions.

Results and Conclusions

Autologous blood transfusion doping (ABT) is defined as the transfusion of

stored red blood cells (RBCs) from the same individual. The World Anti-Doping Agency (WADA) includes ABT in the List of Prohibited Substances but no official method exists yet to directly detect it.

In this project the focus was directed towards quantification of erythrocyte membrane protein changes after transfusion. Previously described data showed that some proteins translocate from cytosol to the membrane during erythrocyte storage under standard blood banking conditions. Based on this data, we have selected a panel of proteins to be further characterized. The list included peroxiredoxin-2, glyceraldehyde-3-phosphate dehydrogenase, catalase, tropomodulin-1, sorcin and annexin-7. Using immunoblotting we have observed that in general these proteins were already present in fresh blood samples and their levels increased on the membrane of the erythrocyte during in storage. Additionally, when stored RBC concentrates were mixed in vitro with fresh blood simulating a transfusion procedure, an increase of the marker proteins in the mixtures was observed. However, when blood from recently transfused patients was analyzed, there was no a clear trend in the increment or decrement of the marker proteins. On the other hand, we have observed that the training for endurance disciplines as compared to non-endurance disciplines (both in aquatic sports) seem to influence the basal concentrations of some of the marker proteins, especially those linked with the oxidation protection of RBC.

Based on all the above considerations, it is doubtful whether the changes in marker RBC membrane proteins by translocation from cytosol to membrane may afford useful methodology for detecting blood transfusion in antidoping control by analyzing blood collected from a transfused athlete. However, the analysis of a transmembrane protein, glycophorin A, unraveled modifications that modify protein molecular weight during blood bag preparation or storage conditions, suggesting that characteristic changes on erythrocyte surface are occurring. Therefore, the characterization of the time sequence line of these modifications produced on integral membrane proteins could represent a useful approach to detect autologous blood transfusion.