"Impact of cryopreservation on red blood cell function- New strategies to detect autologous blood doping"

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PROJECT REVIEW

Autologous blood doping is applied in order to increase red blood cell (RBC) mass and thus oxygen transport capacity of the blood and to improve exercise performance.The International Olympic Committee (IOC) banned blood boosting after the 1984 Olympics and indirect markers for blood doping, including total haemoglobin mass measurements, or to test for the excretion of metabolites of bag plasticizers in the urine have been developed.

The Athlete Biological Passport was introduced a couple of years ago and consists of a longitudinal monitoring of biologic measures to identify patterns suspicious of doping and evaluation of such abnormal patterns by a panel of experts. Parameters included are the hemoglobin concentration and the reticulocyte percentage but the latest incidents (e.g. doping charge Claudia Pechstein) proved that the interpretation of hematological parameters are difficult because of wide inter-individual differences. Thus, at present no reliable method exists to detect autologous blood doping. We have previously investigated how blood storage affects RBC deformability, which is an indispensable characteristic of RBC to ensure the oxygen supply to the tissues, and found that during hypothermic storage of liquid blood units RBC deformability decreases with increasing storage duration. This was associated with cell senescence and reduced nitric oxide bioavailability. Nowadays blood bags are cryopreserved for later re-infusion and thus the aim of the study is to investigate the impacts of cryopreservation on RBC deformability and associated nitric oxide signaling pathways. We hypothesize an impact of cryopreservation on these parameters. Thus, the investigation of RBC function and related cellular pathways seems to be a promising attempt in order to detect autologous blood doping.

Results and Conclusions:

Structure and function of red blood cells (RBC) may be affected during cryopreservation (freezing, storage, thawing of RBC) and identification of respective cell markers might aid to hint towards reinfusion of stored blood. In vitro and in vivo tests with cryopreserved RBC were conducted to investigate the effects of cryopreservation on parameters of RBC aging, integrity, (nitric oxide) metabolism, deformation and viscosity. Results revealed that cryopreservation does not affect basal blood parameters or cell metabolism but reduces cell aging which was reported to be a major problem in liquid preserved blood samples stored at 4°C. Overall ability of RBC to deform was reduced after cryopreservation but sensitivity to shear stress was increased which might be related to remaining freezing medium in the cells that might affect the membranous structure of the RBC. Cryopreservation

also alters osmotic stability of RBC further indicating that the membrane and/or the membrane-cytoskeleton interaction is affected by cryopreservation. Finally, viscosity of RBC was reduced which again, hints towards an effect of freezing medium on the viscoelastic properties of RBC. Nitric oxide metabolism, which accounts – at least in part –for RBC deformation process, was not affected by cryopreservation.

Reported effects were less measurable in vivo compared to in vitro suggesting that blood volume that was reinfused to respective donors in vivo was too little to detect any measurable changes in the tested parameters and which might not precisely reflect doping praxis. In conclusion, the data of the present study were the first to investigate changes of cryopreservation on RBC structure and function in more detail. Cryopreservation affects RBC structure leading to functional changes which needs further investigation to assess its effects in vivo.