Title: Assessment of mitochondrial respiratory function in cryopreserved platelets

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Abstract: Platelets (PLTs) are potentially powerful models for the diagnosis of mitochondrial dysfunction, offering a minimally invasive approach in comparison to tissue biopsies. However, rapid isolation of PLTs and respiratory measurements are required to avoid post-blood harvesting stress and cellular activation followed by metabolic alterations. As an alternative, cryopreservation is a promising strategy to preserve mitochondrial function in PLTs and its development has become an interesting approach to preserve blood cells for long periods of time. Despite of PLTs sensitivity to temperatures below 18 ºC [1], cryopreservation of PLTs has been used as a successful method for the study of inflammatory properties [2,3], however the impact over mitochondrial function remains unknown. The objective of our study was to optimize the cryopreservation of human PLTs for the measurement of mitochondrial respiration.

Human blood samples were collected from healthy human volunteers via venous puncture. PLTs were isolated following Sumbalova *et al*. protocol [4]. Different cryopreservation conditions were evaluated: PLTs were resuspended in autologous plasma with DMSO (first assays: 5% DMSO in the presence and absence of 10 mM EGTA; second assays: 10% DMSO in the presence 10 mM EGTA). Samples were stored at -80 °C for one, two and four weeks. After defrosted, PLT mitochondrial function was measured by High-Resolution Respirometry using the Oroboros O2k-FluoRespirometer by adding various combinations of substrates, inhibitors and uncouplers (SUIT-protocol-003 D018).

During the first cryopreservation conditions tested, cold storage stress was detected as an overall decrease in mitochondrial respiration, without differences in the 1- to 4-weeks cryopreservation groups. Flux control ratios (*FCR*) of cryopreserved and freshly isolated PLTs were preserved without qualitative changes. Cell viability of cryopreserved PLTs decreased by 20% based on calculated respirometry viability index. Our results show that after 1-week of cryopreservation in autologous plasma with 10% DMSO and 10 mM EGTA, PLTs respiration was 26% decreased only on electron transfer (ET)-capacity of intact PLTs and S-linked ET-capacity in permeabilized PLTs in comparison to freshly isolated PLTs. However, the differences in ET-capacity observed with cryopreservation in intact PLTs disappear after cell viability corrections.

These results illustrate that cryopreservation still imposes a substantial damage on PLTs. Modifications, such as DMSO concentration and cell density during cryopreservation, will be investigated in order to improve the cryopreservation method preserving cell viability and mitochondrial respiratory function.

References

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