The mitochondrial membrane potential: an old assay revisited

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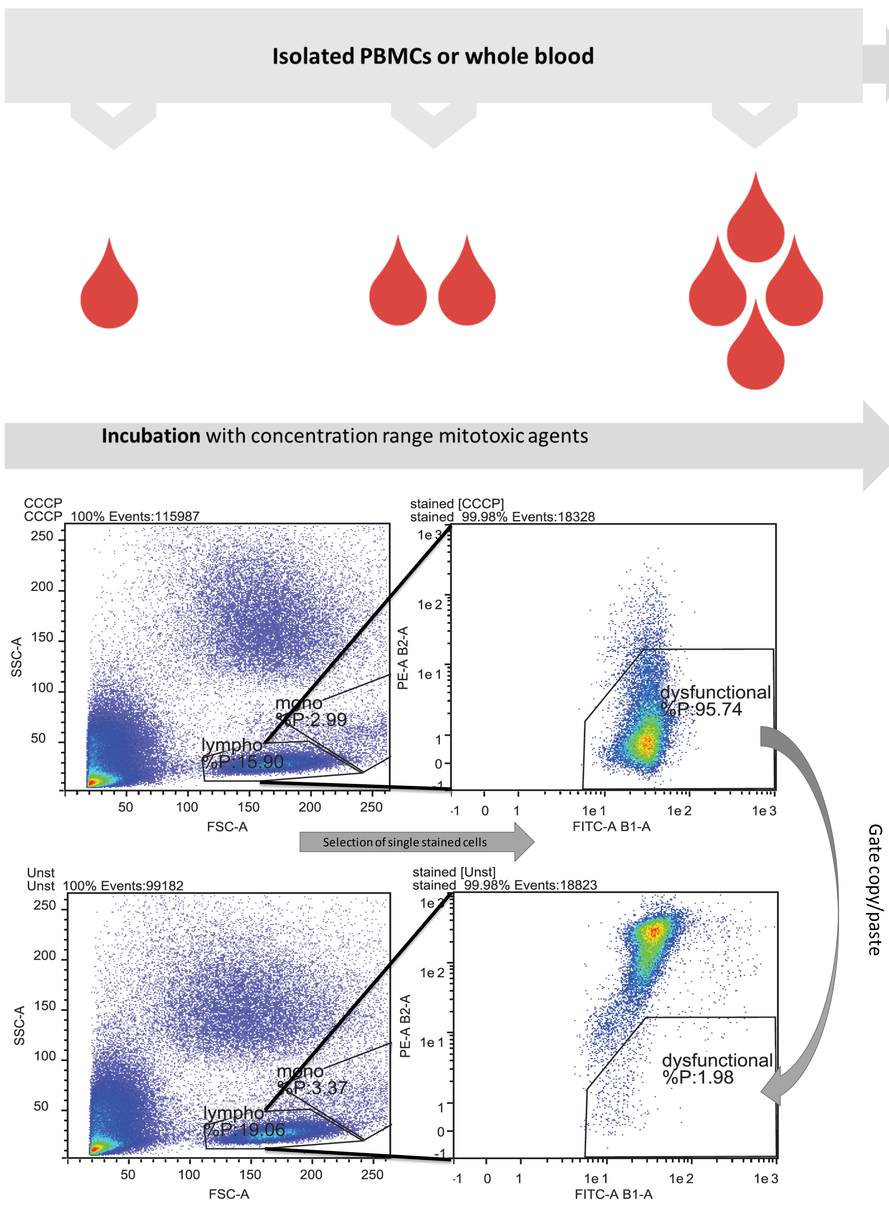
Introduction

- Dysfunctional mitochondria and resulting oxidative stress, may play a causal role in different pathophysiological conditions.
- Mitochondrial dysfunction can be quantified by flow cytometric assessment of the mitochondrial membrane potential (MMP) using the JC-1 dye.
- This technique is being used for more than 20 years in fundamental research, but rarely applied in primary human cells, as we do show here.
- Using fresh primary human material, makes this assay valuable for clinical trials.

AIM

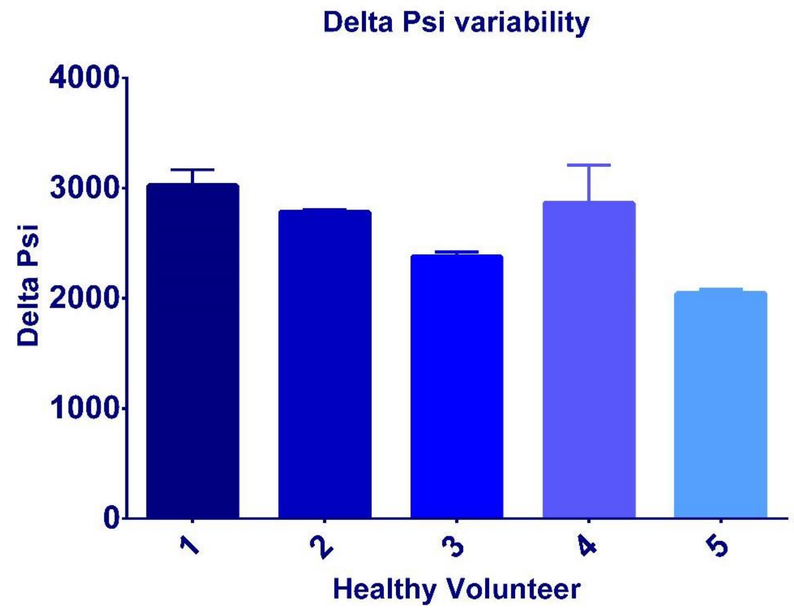
- To study the performance of JC-1 based MMP measurement.
- To study the effects of mitotoxic agents on MMP.
- To relate JC-1 based MMP to apoptosis and cell death markers AnnexinV, Propidium Iodide.

Methods

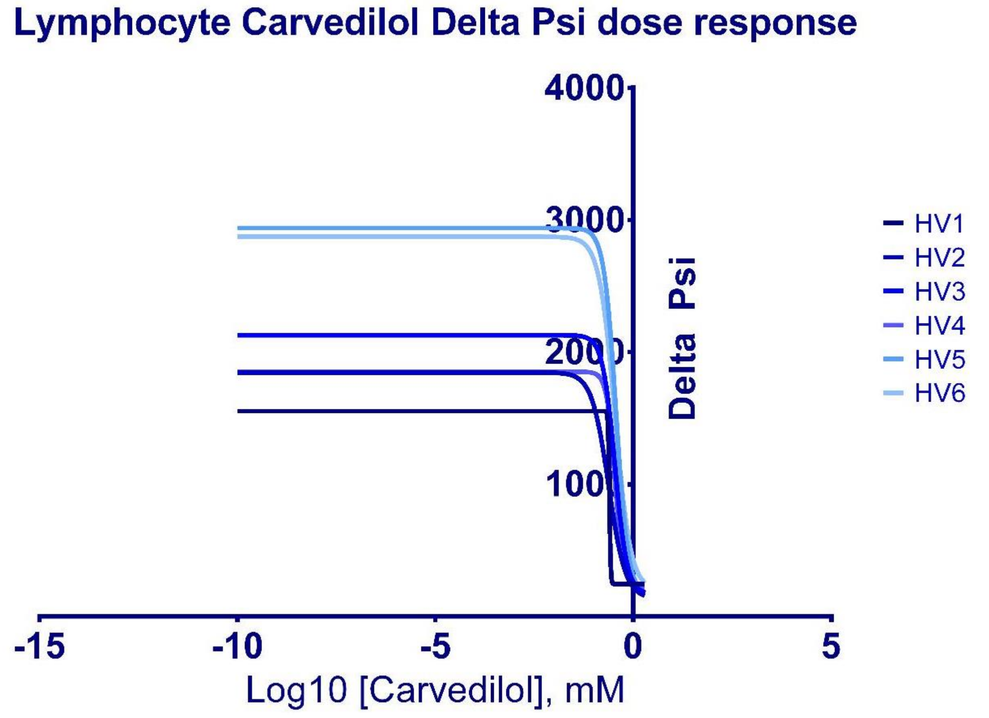


Results

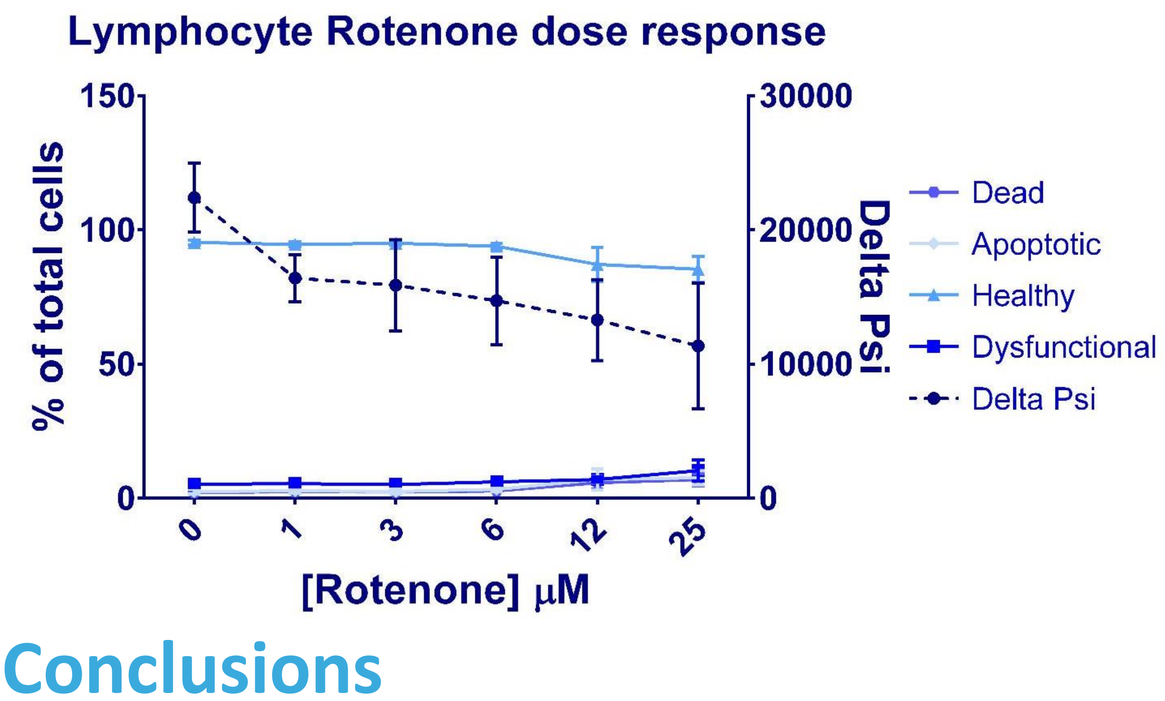
- Assay variability for basal MMP (Delta Psi) was low (average CV for triplicate measurements: 7%).
- Inter-individual variability (Delta Psi) was limited (CV: 15%, 5 donors).



- MMP varied significantly between monocytes, lymphocytes and neutrophils (Delta Psi (CV) of 9358 (54%), 22185 (25%) and 3612 (32%) ΔΨ, respectively, data not shown).
- IC50 for Carvedilol and Verapamil was 0.33mM and 0.46mM, respectively (CVs: 15-20%, 6 donors).



- Rotenone induces an MMP collapse (delta Psi) in the absence of apoptosis or cell death (AnnexinV and Propidium Iodide; figure below).



Conclusions

- MMP (basal and induced collapse), assessed in fresh human specimens, may serve as valuable readout measure for future clinical trials studying mitochondrial function in health and disease, or the effect of (pharmacological) intervention on mitochondrial function.
- Rotenone is the preferred mitotoxic agent to induce MMP collapse, and will be studied in greater detail (combination with mitoprotective agents, and also focusing on oxidative stress measures).

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