ROS production under pathological condition at tissue normoxia: do the cells still survive?

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The aim of the project was to investigate the oxygen dependence of H_2O production on permeabilized rat liver hepatoma cells lines and on crude and pure mitochondria isolated from this cell line. We were also interested in how the H_2O_2 production affected under low oxygen levels in the presence of inhibitor of the mitochondrial electron transfer system (ETS) or a redox cycler, called menadione or vitamin K. Moreover, the aim was to reinvestigate the theory of the ROS-induced ROS release at different oxygen concentration.

We have measured simultaneously the mitochondrial respiration and H_2O_2 production using High Resolution FluoRespirometer on permeabilized McA7 cells treated with Rotenone, Menadione and Antimycin for 2 hours. In order to reinvestigate the theory of the ROS-induced ROS release, the cells were treated with 50 µM menadione for 2,4 and 6 hours with menadione. The cell viability was measured with trypan blue before each experiment.

Last year we have seen that there is a linear relationship between the H_2O_2 flux and oxygen concentration on permeabilized cells and on mitochondria isolated from the cell line. The cells treated with rotenone and menadione for 2 hours showed the similar trend regarding the H_2O_2 production: at low oxygen concentration the H_2O_2 flux was lower compared to those at high oxygen levels. The menadione treated cells for 2, 4 and 6 hours did not show elevated ROS production over time; the highest rate of H_2O_2 generation was observed after the 2-hour treatment. However, after 6 hours the respiration stared to decrease, without a decrease in the cell viability. We can exclude that the decline in the respiration is related to elevated H_2O_2 generation. It might be possible that at the beginning of the treatment increased H_2O_2 production caused damage in the mitochondria, which resulted in the decline of the respiration and later the cell viability. But the mitochondrial dysfunction cannot be explained by the ROS-induced ROS release over time. In disagreement with the literature (Kavcic et al 2017; Loor et al 2010), menadione did not induce elevated ROS formation over time. As Teixeira and his co-workers revealed, menadione can disrupt mitochondrial function via elevated ROS formation (Teixeira et al 2018). In order to address this issue, on one hand we need to test the acute effect of menadione on the McA cells, on the other hand the mitochondrial ATP production, mitochondrial membrane potential, lipid peroxidation and the aconitase activity supposed to be measured after acute addition of menadione and after 2, 4 and 6 hour-treatment.

Literature

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