

# **Doxorubicin-induced metabolic alterations in H9C2 cells and effects of preincubation with dexrazoxane, a cardioprotective agent**

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## **Introduction**

Currently, doxorubicin treatment in oncology is limited by its irreversible cardiotoxicity whose gravity depends on cumulated dose and can range from subclinical myopathy to patient's death. The main toxicological mechanism is the production of reactive oxygen species (ROS), leading to an oxidative stress impairing mitochondrial function and membrane integrity. It results in cardiomyocytes death either by apoptosis or necrosis, clinically expressed by a progressive heart failure. Nowadays, the main strategy to deal with this cardiotoxicity is the co-administration of dexrazoxane, a cardioprotective agent that acts by reducing ROS production through iron chelation. However, this strategy shows limited efficacy and there is a need for new cardioprotective tools. The goal of this research is to study doxorubicin-induced metabolic alterations and the protective role of dexrazoxane in order to highlight possible new targets to counteract doxorubicin cardiotoxicity.

## **Material and methods**

Rat cardiomyoblasts H9C2 (2-1) (ECACC 88092904) were cultured in DMEM according to ECACC guidelines. For all exposure procedures, cells were first seeded with a density of 30.000 cells/cm<sup>2</sup> and were kept growing during 48 hours before any exposure. For oxidative stress and metabolomic investigations, cells were randomly assigned into 4 groups : a control group (CTR), a 0,3 µM doxorubicin-exposed group (DOX), a 3 µM dexrazoxane-exposed group (DEX) and a group pre-incubated with 3 µM of dexrazoxane during 30 min before 0,3 µM doxorubicin exposure (DEX+DOX). Oxidative stress was assessed after 2 hours of exposure with a quantitative fluorimetric DCFH-DA assay of reactive oxygen species (ROS) production and a <sup>1</sup>H-NMR-based metabolomic study was carried out after 24 hours of exposure on both culture media and extracted intracellular polar metabolites. Acquired <sup>1</sup>H-NMR spectra were baseline and phase-corrected and TSP-calibrated. Spectral area from 0,08 to 10 ppm was subdivided into integrated sub-regions of 0,04 ppm wide. The water peak was deleted and each subregion integral was normalized to spectrum total area. Multivariate data analysis were performed on data and discriminant metabolites were identified using several databasis.

## **Results**

Results of DCFH-DA assay describe a clear increased production of ROS when H9C2 cells are exposed to doxorubicin. This production is proportional to doxorubicin concentration in a linear way. Dexrazoxane preincubation with a dose 10 times higher than doxorubicin dose can reduce significantly the ROS production.

The metabolomic study highlighted some metabolic alterations due to doxorubicin exposure : a switch from mitochondrial aerobic energy metabolism to cytosolic anaerobic metabolism (increase of lactate, phosphocreatine and glutamine production and secretion, decrease of UDP-glucose level), a cell response to oxidative stress by an increased intracellular taurine level, modification of amino acids metabolism. The metabolomic study also highlighted metabolic effects of dexrazoxane pre-incubation : recovery of mitochondrial aerobic

metabolism (decrease in lactate, glutamine levels and increase in UDP-glucose level), activation of choline metabolism (phosphocholine, glycerophosphocholine and serine increased levels) for biological membranes synthesis, activation of creatine phosphorylation and an increased secretion of succinate. Interestingly, choline metabolism activation may be linked to cell survival and growth pathways. Concomitantly, succinate secretion may promote proliferation pathways by simulating GPR91 receptor. Choline metabolism and GPR91 could be potential targets for improving cardioprotection during doxorubicin exposure and are therefore attractive for further investigations.

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