Contribution of *in vitro* ^{1}H-NMR metabonomics for studying doxorubicin-induced cardiotoxicity and protective effects of dexrazoxane

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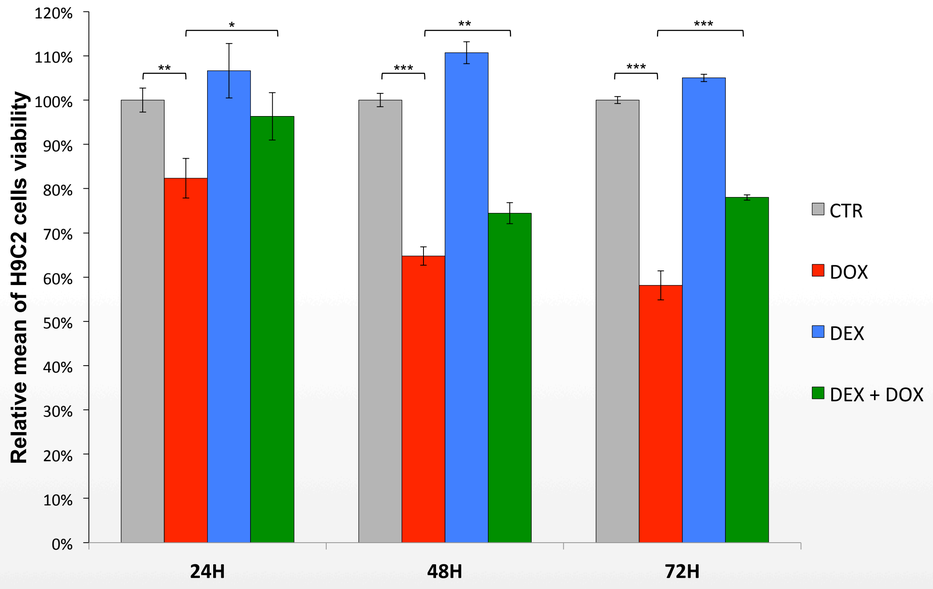
Currently, doxorubicin anti-cancer treatment is limited by its irreversible cardiotoxicity which depends on cumulated dose and can range from subclinical myopathy to patient's death *(1,2)*. The main toxicological mechanism is the production of reactive oxygen species (ROS), leading to an oxidative stress causing apoptosis or necrosis in cardiomyocytes, clinically expressed by a progressive heart failure *(3)*. Nowadays, the main strategy to deal with this cardiotoxicity is the co-administration of dexrazoxane, a cardioprotective agent reducing ROS production through iron chelation. However, this strategy shows limited efficacy and there is a need for new cardioprotective strategies *(1)*. The goal of this research is to studying doxorubicin-induced metabolic alterations and the protective role of dexrazoxane. This strategy should help in highlighting possible new targets to counteract doxorubicin cardiotoxicity.

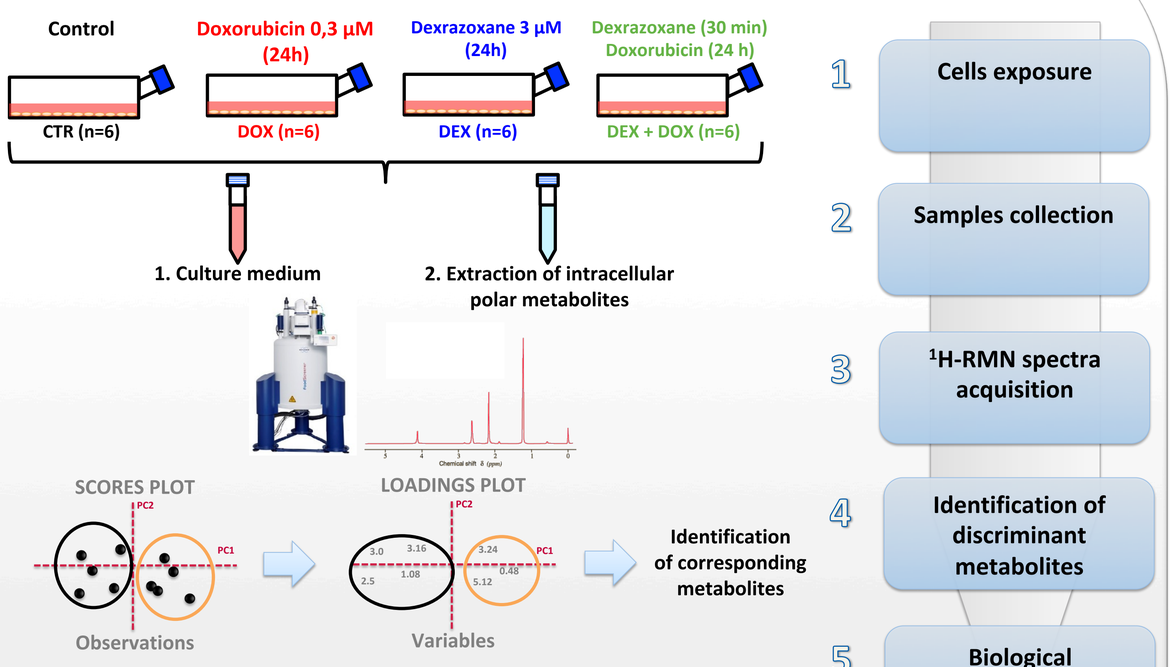
1. Cellular viability assessment



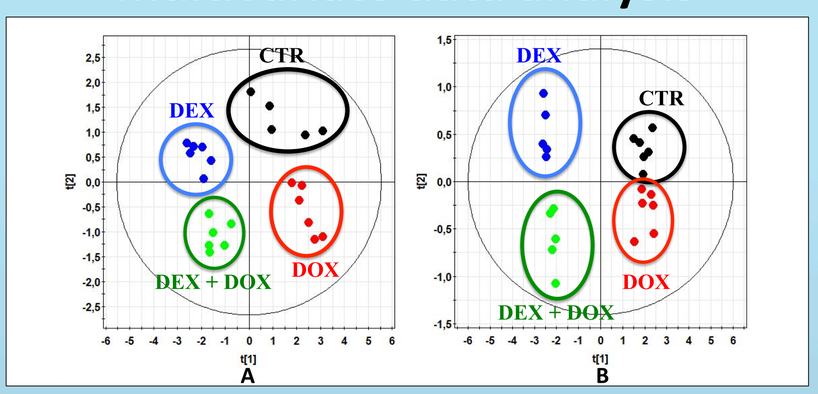
*Relative mean of H9C2 cells viability measured 24, 48 and 72 hours after a single exposure to doxorubicin, dexrazoxane, dexrazoxane + doxorubicin. ANOVA one way : * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001*

H9C2 cells viability was measured 24, 48 and 72 hours after a single exposure to doxorubicin (0,3 µM), dexrazoxane (3 µM) and dexrazoxane + doxorubicin, by crystal violet assay. Results highlighted that dexrazoxane is able to protect partially against doxorubicin-induced cell death.

2. Metabonomic study design



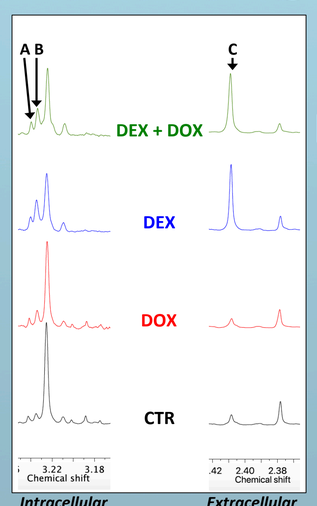
Multivariate data analysis



***A** : Scores plot of PLS-DA performed on cellular extracts. Parameters : R^2_{cum} = 0,59 Q^2_{cum} = 0,54, Hotteling's T2 = 0,95, P-value (CV-ANOVA) < 0,05. **B** : Scores plot of PLS-DA performed on culture media. Parameters : R^2_{cum} = 0,59 Q^2_{cum} = 0,52, Hotteling's T2 = 0,95, P-value (CV-ANOVA) < 0,05. **Legend** : CTR group (•), DOX group (•), DEX group (•), DEX+DOX group (•).*

H9C2 cells were seeded in 175 cm^2 flasks and kept growing during 48 hours. Cells were randomly separated into 4 groups : control cells, cells exposed to 0,3 µM doxorubicin, cells exposed to 3 µM dexrazoxane and cells exposed to dexrazoxane 30 min. before doxorubicin exposure. A ^{1}H-NMR based metabonomic study was carried out on both intracellular and extracellular compartments.

Potential targets for cardioprotection



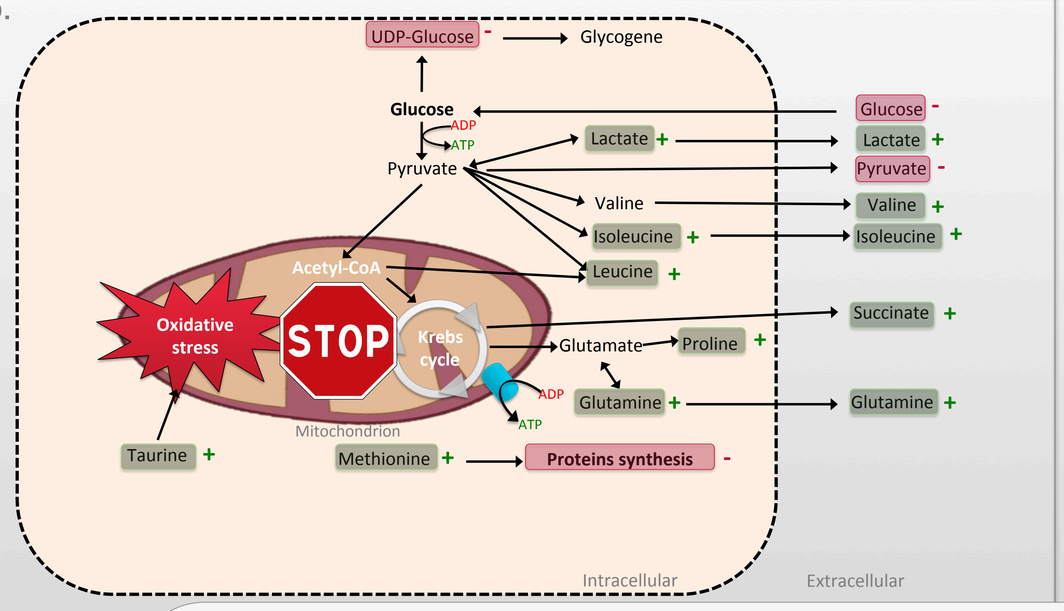
***A :** Glycerophosphocholine **B :** Phosphocholine **C :** Succinate*

Choline metabolism enhanced & Succinate secretion enhanced

Possible implication of PI3K/AkT pathway and GPR91 succinate receptor for cellular survival promotion

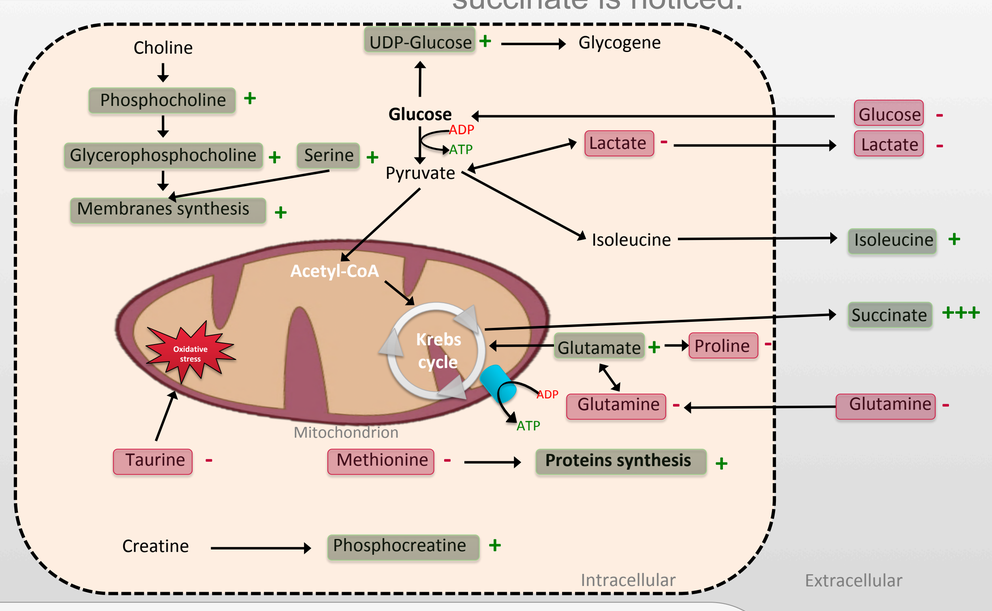
3. Metabolic effects of doxorubicin

Results of the metabonomic study suggest an oxidative stress-induced impairment of the Krebs cycle, resulting in a metabolic switch to anaerobic conditions with glycolysis as main ATP production pathway : Pyruvate is metabolized mainly into lactate and amino acids. A decrease of proteins synthesis and an increase of anti-oxidant defenses are suggested too.



4. Metabolic effects of pre-incubation with dexrazoxane

Dexrazoxane reduces the doxorubicin-induced oxidative stress, leading to a recovery of mitochondrial metabolism with a decrease of lactate production and an increase of proteins synthesis. Choline metabolism is highly stimulated for membranes synthesis and an important secretion of succinate is noticed.



References

1. Chung W-B, Youn H-J. Pathophysiology and preventive strategies of anthracycline-induced cardiotoxicity. The Korean Journal of Internal Medicine. 2016 Jul 1;31(4):625–33.
2. Groarke JD, Nohria A. Anthracycline cardiotoxicity: a new paradigm for an old classic. Circulation. 2015 Jun 2;131(22):1946–9.
3. Damiani RM, Moura DJ, Viau CM, Caceres RA, Henriques JAP, Saffi J. Pathways of cardiac toxicity: comparison between chemotherapeutic drugs doxorubicin and mitoxantrone. Archives of Toxicology. 2016 Jun 25.