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Disclosing the effect of doxorubicin and mitoxantrone on cardiac mitochondrial proteome: an *in vivo* approach using a murine model

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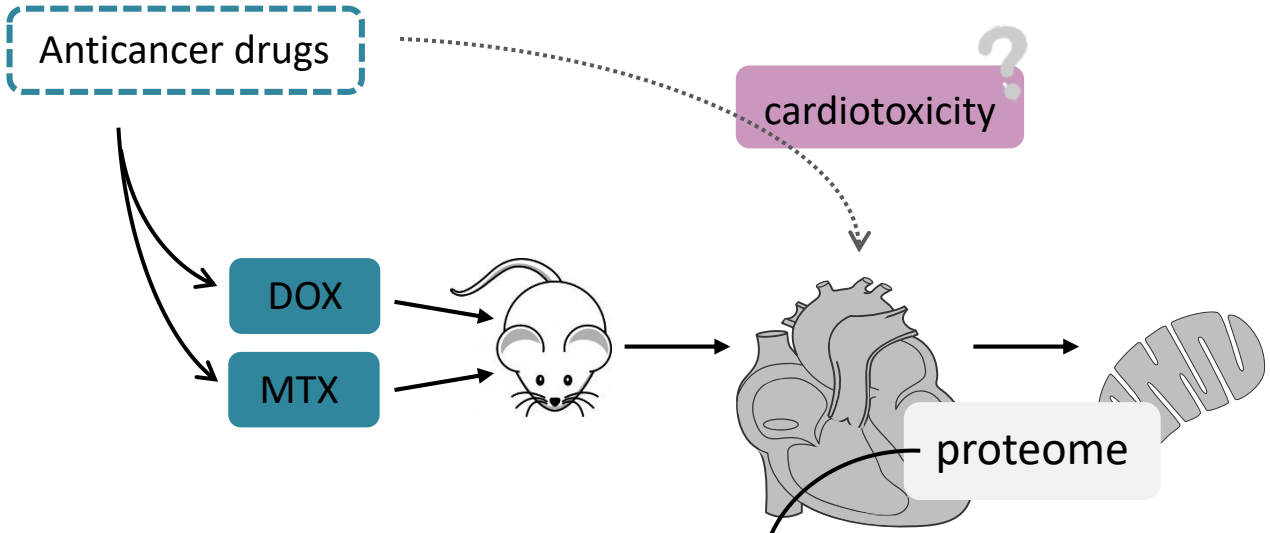
Disclosing the effect of doxorubicin and mitoxantrone on cardiac mitochondrial proteome: an *in vivo* approach using a murine model



DOX and MTX induced:

- Decreased mitochondrial density in cardiac muscle;
- Metabolic adaptations more evident in the heart of DOX animals.

❖ MTX seemes to have a higher impact on the remodeling of mitochondrial proteome.

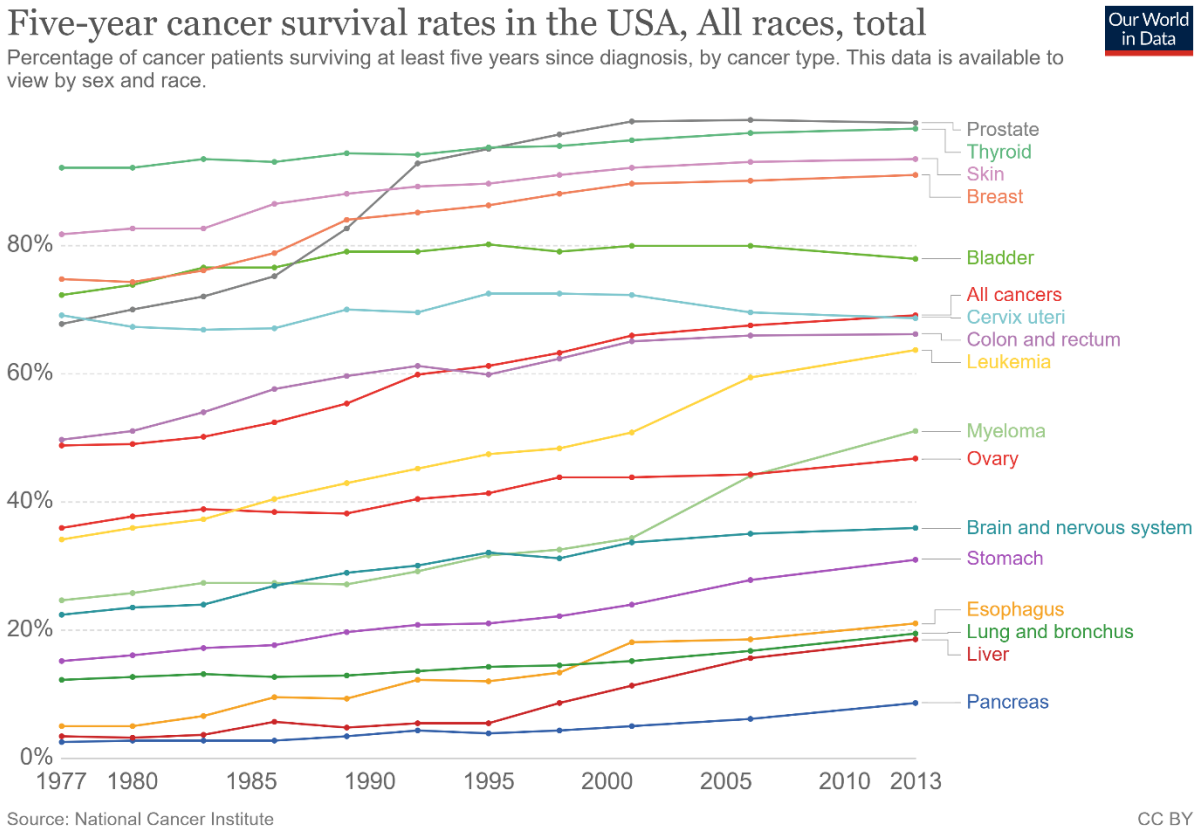
Abstract

The number of cancer survivors has increased considerably due to the current therapies. Nevertheless, the cardiac side effects in these patients are still a concern. Our goal was to study the effects of doxorubicin (DOX) and mitoxantrone (MTX) on the molecular mechanisms harbored in the heart of male mice. Six intraperitoneal administrations were given to the animals. DOX- and MTX-treated animals received a total cumulative dose of 9 and 6 mg/kg, respectively. Whole cardiac tissue and corresponding enriched mitochondrial fractions were analyzed by immunoblot and enzymatic techniques. Additionally, enriched mitochondrial fractions were studied by mass spectrometry-based proteomics. From this analysis 693 different proteins were identified, assigned to the biological processes “small molecule metabolic process”, “oxidation-reduction process” and “carboxylic acid metabolic process”. The distribution analysis of the mitochondrial proteome data showed clustering among the conditions. Indeed, MTX treatment presented less similarities with control. Moreover, DOX and MTX promoted a decrease on mitochondrial density. Metabolic adaptations were noticed, more evident for DOX. Concomitantly, metabolic adaptations were noticed, more evident in the heart of DOX treated mice. Indeed, increased GAPDH-to-ATP and ETFDH-to-ATP ratios were observed. Thus, more than differences in cardiac mitochondrial proteome, these drugs seem to decrease this organelle density.

Keywords: anticancer drugs; cardiotoxicity; proteomics; mitochondria

Introduction

In the last decades, the number of cancer survivors has increased considerably due to the huge efficacy of **anticancer therapies**, namely earlier detection and improved treatment.



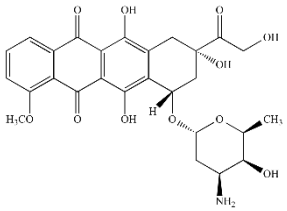
Roser M, *et al. Cancer*. 2019; *OurWorldInData.org*

Introduction

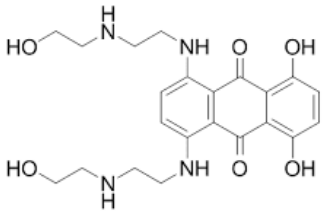
Among the several **anticancer therapies**, **chemotherapy** is the most frequently used.

Chemotherapeutic agents, such as:

doxorubicin (**DOX**)



mitoxantrone (**MTX**)



widely used to treat breast cancer, infantile solid tumors, sarcomas and lymphomas

Roser M, *et al. Cancer*. 2019; *OurWorldInData.org*

Colombo A, *et al*. *Curr Treat Options Cardiovasc Med.* 2014;*16(6):313*

Introduction

However, the **chemotherapeutic agents** affect non-cancer cells leading to adverse side effects, such as fatigue, alopecia, **cardiotoxicity** and neurotoxicity.

symptoms

arrythmias

electrocardiographic changes

heart failure

Reported outcomes:

DOX

- ↑ reactive oxygen species (ROS);
- ↑ oxidative stress;
- ↕ calcium and iron homeostasis;
- ↓ energy levels;
- ↑ apoptosis.

MTX

- ↓ energy levels;
- ↑ oxidative stress;
- ↕ calcium homeostasis.

Hrynchak I, *et al. Drug Metab Rev*. 2017;*49(2):158–96*

Colombo A, *et al*. *Curr Treat Options Cardiovasc Med.* 2014;*16(6):313*

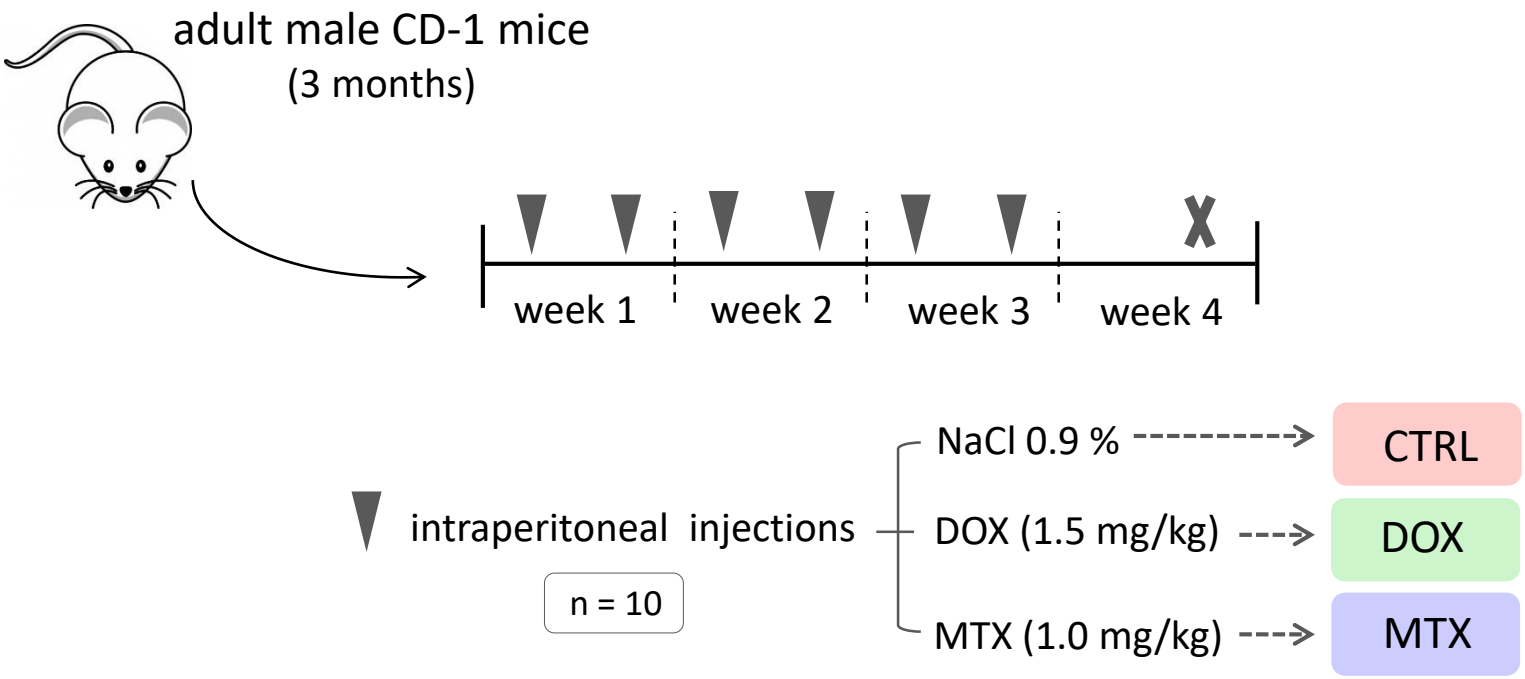
Introduction

Both DOX and MTX seem to affect **cardiac mitochondrial dynamics**, although the exact mechanism of action is still unclear.

Our goal was to study the effects of DOX and MTX on the cardiac mitochondrial proteome remodeling of adult male CD-1 mice.

McGowan JV, *et al*. *Cardiovasc Drugs Ther.* 2017;*31(1):63–75*

Methods

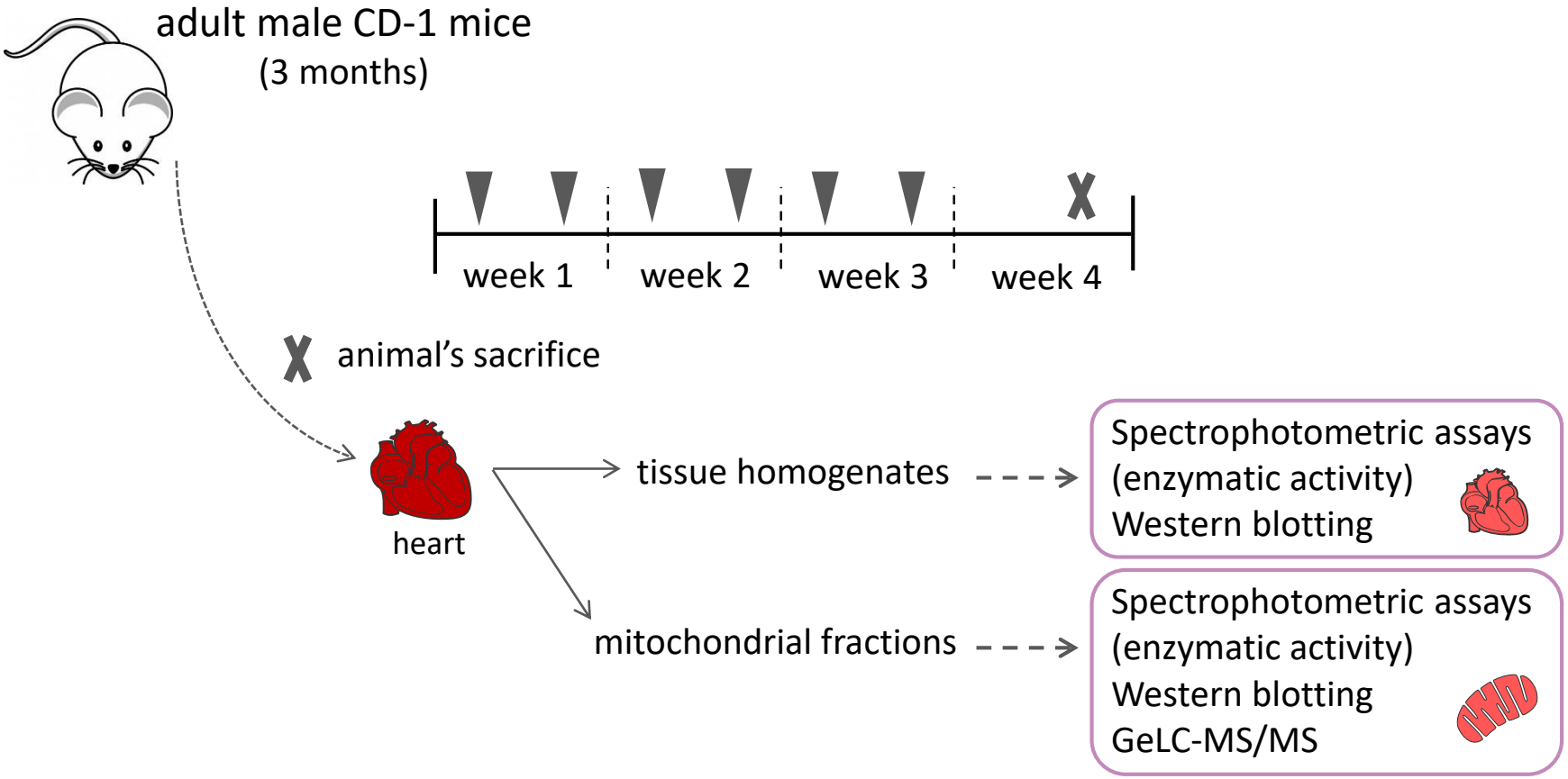


Total cumulative dose of:

- 9 mg/kg for DOX animals
- 6 mg/kg for MTX animals

Animal welfare was assessed daily during the entire experimental period.
The experiments were performed with the approval of the Portuguese National Authority for Animal Health (reference number 0421/000/000/2016) and of the ORBEA of ICBAS-UP (project number 140/2015).

Methods



Statistical analysis was performed with GraphPad Prism (version 6.0.1).
Experimental groups were compared using ordinary one-way ANOVA followed by Turkey's multiple comparisons test ($p < 0.05$).

GeLC-MS/MS: combines one dimensional SDS-PAGE with liquid chromatography-tandem mass spectrometry

Results and discussion

Effect of DOX and MTX on morphometric parameters

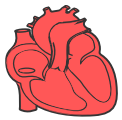
	DOX	MTX
Whole body weight (g)	⟷	⟷
Heart mass (g)	⟷	⟷
Heart mass-to-whole body weight (mg/g)	⟷	⟷
Heart mitochondrial isolation yield (mtDNA-to-tDNA)	⟷	⟷

Results are presented as significantly increased (⬆), decreased (⬇) or no significantly different (⟷) related to the control group.

DOX and MTX administration did not induce significant differences on morphometric parameters compared to control mice.

mtDNA: mitochondrial deoxyribonucleic acid; tDNA: total deoxyribonucleic acid

Results and discussion



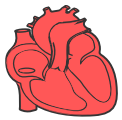
Effect of DOX and MTX on mitochondrial biogenesis

	DOX	MTX
CS activity ($nmol \cdot mg^{-1} \cdot min^{-1}$)	↓	↓
PGC-1alpha (arbitrary units of optical density)	↔	↔
PGC-1alpha/CS activity	↔	↔

Results are presented as significantly increased (↑), decreased (↓) or no significantly different (↔) related to the control group.

CS: citrate synthase; PGC-1alpha: peroxisome proliferator-activated receptor γ coactivator 1 alpha

Results and discussion



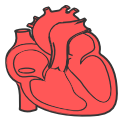
Effect of DOX and MTX on metabolism

	DOX	MTX
ATP-B (arbitrary units of optical density)	↔	↔
GAPDH (arbitrary units of optical density)	↔	↔
GAPDH/ATP-B	↑	↔
ETFDH (arbitrary units of optical density)	↔	↔
ETFDH/ATP-B	↑	↔

Results are presented as significantly increased (↑), decreased (↓) or no significantly different (↔) related to the control group.

ATP-B: ATP synthase beta; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; ETFDH: electron transfer flavoprotein dehydrogenase

Results and discussion



Effect of DOX and MTX on mitochondrial biogenesis and metabolism

DOX and MTX administration promoted decreased mitochondrial density compared to control mice;

DOX administration led to increased GAPDH/ATP-B and ETFDH/ATP-B ratios compared to control mice.

Results and discussion

Effect of DOX and MTX on cardiac mitochondria proteome remodeling

	DOX	MTX
CS activity (nmol·mg^{-1}·min^{-1})	⟷	⟷
ATP synthase activity (µmol·mg^{-1}·min^{-1})	⟷	⟷
ATP-B (arbitrary units of optical density)	⟷	⟷
MnSOD (arbitrary units of optical density)	⟷	↓

Results are presented as significantly increased (↑), decreased (↓) or no significantly different (⟷) related to the control group.

MTX administration induce decrease on the antioxidant enzyme MnSOD compared to control mice.

CS: citrate synthase; ATP-B: ATP synthase beta; MnSOD: manganese superoxide dismutase

Results and discussion

Effect of DOX and MTX on cardiac mitochondria proteome remodeling

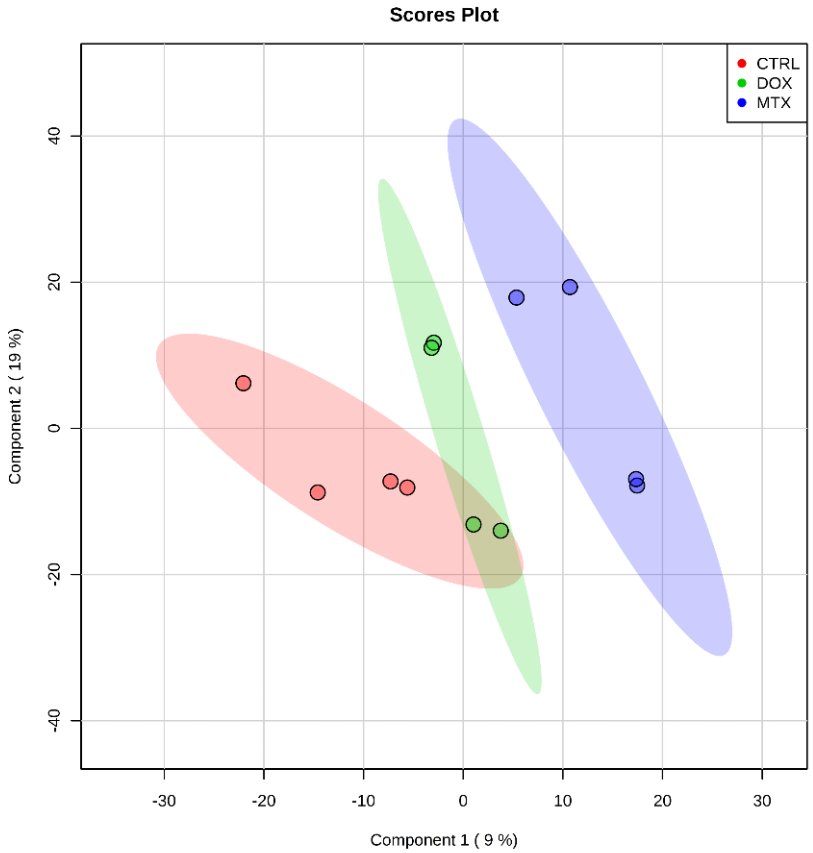
GeLC-MS/MS analysis resulted in the identification of **693 different proteins**, assigned to the biological processes “small molecule metabolic process”, “oxidation-reduction process” and “carboxylic acid metabolic process” according to string*.

Distribution analysis

* Szklarczyk D, *et al*. *Nucleic Acids Res*. 2015;43(D1):D447–52

Results and discussion

Effect of DOX and MTX on cardiac mitochondria proteome remodeling



Distribution analysis*

Clustering among the groups;

MTX administration presented less similarities with control mice than DOX administration.

* Based on partial least squares-discriminant analysis (PLS-DA) of free available MetaboAnalyst 4.0 software (http://www.metaboanalyst.ca)

Conclusions

- ✓ DOX and MTX therapy induced:
  - Decreased mitochondrial density in cardiac muscle;
  - Metabolic adaptations more evident in the heart of DOX animals.
- ✓ MTX seems to have a higher impact on the remodeling of mitochondrial proteome.

Acknowledgments

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