Disclosing the cardiotoxic effects of the anticancer agents doxorubicin and mitoxantrone on cardiac metabolism, homeostasis and autophagy using CD-1 mice

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Introduction

The number of cancer survivors, in the last decades, has increased considerably due to the current anticancer therapies. **Doxorubicin (DOX)** and **mitoxantrone (MTX)** are classical chemotherapeutic agents, that inhibit topoisomerase II, widely used to treat solid tumors, leukemia and lymphomas. MTX has also been used for the treatment of multiple sclerosis. Despite the therapeutical success of these agents, both have been associated to severe cardiac adverse side effects such as arrythmias, electrocardiographic changes and even heart failure[1,2]. However, the molecular mechanisms involved appear to be divergent[3] and need to be further investigated.

Therefore, our goal was to study the molecular mechanisms underlying the cardiotoxicity of DOX and MTX, focusing on **cardiac muscle metabolism, homeostasis and autophagy** and using adult male CD-1 mice.

Methodology

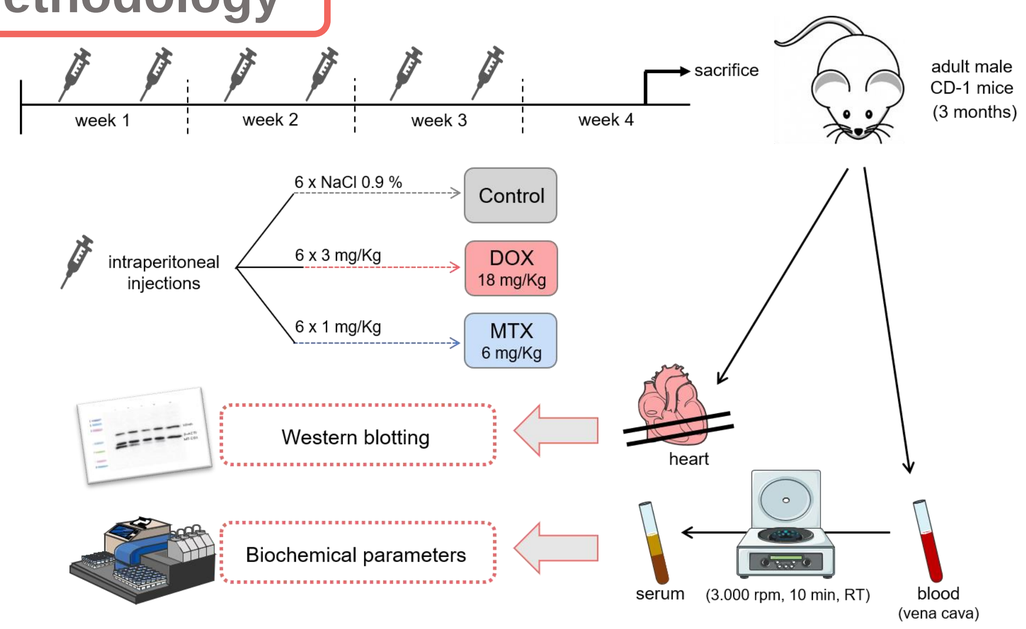

Figure 1 - Schematic representation of the methodology used. Animal welfare was assessed daily during the entire experimental period. The experiments were performed with the approval of the Portuguese National Authority for Animal Health (DGAV; reference no. 0421/000/000/2016) and the local Committee Responsible for Animal Welfares (ORBEA of ICBAS-UP; project no. 140/2015).

Results

DOX decreased serum total protein and glucose concentration, and increased aminotransferases activity

Table 1 - Biochemical parameters measured on serum using an AutoAnalyzer (Prestige 24i, Cormay PZ, Diamond Diagnostics, Holliston, MA, USA). Values are expressed as mean ± SD (n = 6-9 for control and MTX, n = 4-5 for DOX). The groups were compared using ordinary one-way ANOVA followed by Tukey's multiple comparisons test ($p<0.05$), using GraphPad Prism (version 6.0.1). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs control; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ vs DOX. **ALAT**: alanine aminotransferase, **ASAT**: aspartate aminotransferase

	Control	DOX	MTX
Total protein (g/L)	47.79 ± 2.61	41.42 ± 1.89*	43.35 ± 4.53
Glucose (mg/dL)	357.9 ± 39.74	221.3 ± 44.88**	323.7 ± 61.84#
ALAT (U/L)	52.36 ± 16.70	82.90 ± 13.40*	34.88 ± 19.39###
ASAT (U/L)	24.60 ± 5.74	46.95 ± 11.53***	25.20 ± 8.02##

- **DOX** decreased **serum total protein** and **glucose concentration**, while increased **ALAT** and **ASAT** activity compared to **control**.

DOX decreased ATPB, ETFDH, PFKM and GADPH expression, while MTX only decreased the GAPDH

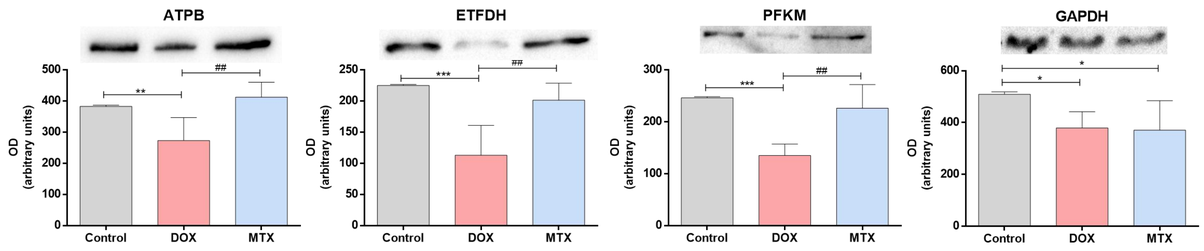

Figure 2 - Cardiac muscle metabolism proteins measured on cardiac homogenates using Western blot. Representative image of the Western blot obtained is presented. Values are expressed as mean ± SD (n = 4-6). The groups were compared using ordinary one-way ANOVA followed by Tukey's multiple comparisons test ($p<0.05$), using GraphPad Prism (version 6.0.1). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs control; ## $p<0.01$ vs DOX. **ATPB**: ATP synthase subunit β, **ETFDH**: electron transfer flavoprotein ubiquinone-oxidoreductase, **PFKM**: phosphofructokinase, **GAPDH**: glyceraldehyde 3-phosphate dehydrogenase

- **DOX** decreased **ATPB**, **ETFDH** and **PFKM** expression compared to **control**;
- Both **DOX** and **MTX** decreased **GAPDH** expression compared to **control**.

Neither DOX nor MTX caused major changes on cardiac homeostasis

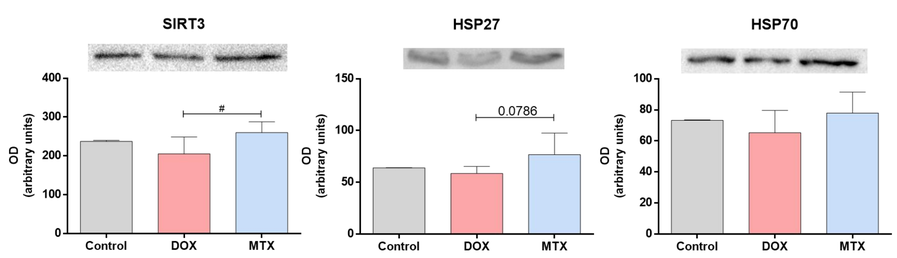

Figure 3 - Homeostasis proteins measured on cardiac homogenates using Western blot. Representative image of the Western blot obtained is presented. Values are expressed as mean ± SD (n = 4-6). The groups were compared using ordinary one-way ANOVA followed by Tukey's multiple comparisons test ($p<0.05$), using GraphPad Prism (version 6.0.1). # $p<0.05$ vs DOX. **SIRT3**: NAD-dependent deacetylase sirtuin-3, **HSP27**: heat shock protein 27, **HSP70**: heat shock protein 70

- Neither **DOX** nor **MTX** induce changes on the expression of **SIRT3**, **HSP27** or **HSP70** proteins compared to **control**.

Both DOX and MTX affected cardiac autophagy

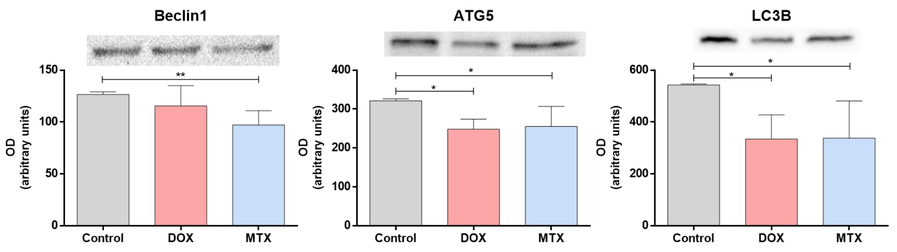

Figure 4 - Autophagy proteins measured on cardiac homogenates using Western blot. Representative image of the Western blot obtained is presented. Values are expressed as mean ± SD (n = 4-6). The groups were compared using ordinary one-way ANOVA followed by Tukey's multiple comparisons test ($p<0.05$), using GraphPad Prism (version 6.0.1). * $p<0.05$, ** $p<0.01$ vs control. **ATG5**: autophagy protein 5, **LC3B**: microtubule-associated protein light chain 3

- **MTX** decreased **Beclin1** expression compared to **control**;
- No differences on **Beclin1** expression were promoted by **DOX**;
- Both **DOX** and **MTX** decreased **ATG5** and **LC3B** expression compared to **control**.

Conclusions

The results indicate that:

- DOX induced a general cardiac metabolic adaptation towards the downregulation of glycolysis and fatty acids oxidation;
- Cardiac homeostasis was not impacted neither by DOX or MTX, however both drugs appear to contribute differently to this process;
- Cardiac autophagy seems more affected by MTX, although both drugs decreased autophagic proteins.

References

[1] Colombo, A. *et al. Cardiac Complications of Chemotherapy: Role of Biomarkers*. Curr. Treat. Options Cardiovasc. Med. 16, 313 (2014). [2] Hrynchak, I. *et al*. *The importance of drug metabolites synthesis: the case-study of cardiotoxic anticancer drugs*. Drug Metab. Rev. 49, 158–196 (2017). [3] McGowan, J. V. *et al*. *Anthracycline Chemotherapy and Cardiotoxicity*. Cardiovasc. Drugs Ther. 31, 63–75 (2017).

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