Mitoxantrone disrupts cardiac metabolism along with proteolysis and regeneration in aged mice

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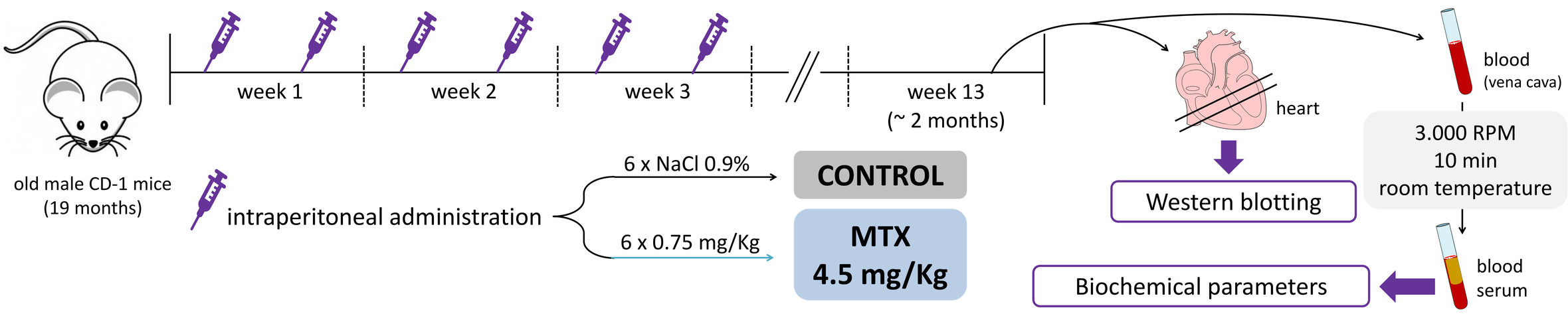
INTRODUCTION

Mitoxantrone (**MTX**) is a chemotherapeutic agent used to treat solid tumors and hematological malignancies, as well as multiple sclerosis. Nevertheless, MTX has been associated with serious adverse side effects, such as cardiotoxicity. Considering that aging is a known risk factor for the development of cardiovascular diseases and cancer, our study aimed to assess the molecular effects of MTX on cardiac muscle of aged mice.

METHODOLOGY



Animal experiments were performed with the approval of the national competent authorities - General Directorate of Food and Veterinary Medicine (DGAV, reference nº 0421/000/000/2016) and of the ORBEA of ICBAS-UP (project nº 140/2015). Mice welfare was monitored daily. The experimental groups were statistical compared using two-sided t-test (p<0.05) of GraphPad Prism (version 6.0.1). Results are presented as significantly increased (↑), decreased (↓) or no significantly different (↔) related to the CONTROL group: * p<0.05; ** p<0.01.

RESULTS & DISCUSSION

MTX decreased whole-body weight

Morphometric parameters measured on the day of sacrifice. Results (n = 5-8) represent weight or length.

Whole-body weight (g)	↓*
Heart weight (g)	↔
Tibial length (cm)	↔
Heart weight-to-whole body weight (mg/g)	↔
Heart weight-to-tibial length (g/cm)	↔

MTX increased serum glucose concentration

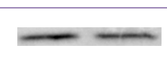
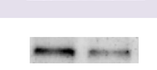
Biochemical parameters measured on **serum** using an AutoAnalyzer (Prestige 24i, Cormay PZ, Diamond Diagnostics, Holliston, MA, USA). Values (n = 5-8) represent concentration or activity.

Total protein(g/L)	↔	Cholesterol (mg/dL)	↔
Albumin (g/L)	↔	ALAT (U/L)	↔
Glucose (mg/dL)	↑**	CK-MB (U/L)	↔

ALAT: alanine aminotransferase, **CK-MB**: creatine kinase-MB

MTX decreased cardiac glycolysis, autophagy, proteolysis and regeneration

Proteins measured on **cardiac muscle homogenates** using **Western blotting**. Representative image of the Western blot obtained is presented. Results (n = 6-7) represent optical density.

GLUT4		↓*	Beclin1		↔
PFKM		↓**	ATG5		↓*
ETFDH		↔	LC3B		↔
ATPB		↔	Atrogin		↓*
PGC-1α		↔	SCFR		↔
Tfam		↔	CITED4		↔
Mitofusin1		↔	CEBPβ		↓**

GLUT4: glucose transporter GLUT4, **PFKM**: phosphofructokinase, **ETFDH**: electron transfer flavoprotein ubiquinone-oxidoreductase, **ATPB**: ATP synthase subunit β, **PGC-1α**: peroxisome proliferator-activated receptor γ coactivator 1 α, **TFam**: mitochondrial transcription factor A, **ATG5**: autophagy protein 5, **LC3B**: microtubule-associated protein light chain 3, **SCFR**: mast/stem cell growth factor receptor Kit, **CITED4**: Cbp/p300-interacting transactivator 4, **CEBPβ**: CCAAT/enhancer-binding protein β

- Old mice administered with MTX showed decreased whole-body weight, suggestive of molecular changes.
- MTX-treated mice are less dependent on glycolysis as cardiac energetic pathway, retrieved by the decreased content of GLUT4 and PFKM on cardiac muscle, despite the increased circulating glucose concentration.
- Moreover, MTX influenced cardiac autophagy, proteolysis, and regeneration, indicated by the decreased content of ATG5, Atrogin, and CEBPβ, respectively.
- Future studies should focus on these unexplored pathways as they may explain the cardiotoxicity induced by MTX.

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