Unveiling the role of inflammation in DOX-induced cardiotoxicity: an *in vivo* study

Ana Reis-Mendes[1*], Ana Isabel Padrão[2], Margarida Duarte-Araújo[3,4], José Alberto Duarte[2,5], Salomé Gonçalves-Monteiro[6,7], Fernando Remião[1], Félix Carvalho[1], Emília Sousa[8,9], Maria Lourdes Bastos[1], and Vera Marisa Costa[1*]

[1]UCIBIO, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; [2]Research Center in Physical Activity, Health and Leisure (CIAFEL), Laboratory for Integrative and Translational Research in Population Health (ITR), Faculty of Sport, University of Porto, Porto, Portugal; [3]LAQV/REQUIMTE, University of Porto, Porto, Portugal; [4]Department of Immuno-Physiology and Pharmacology, Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal; [5]IINFACTS - Institute of Research and Advanced Training in Health Sciences and Technologies, Department of Pharmaceutical Sciences, University Institute of Health Sciences (IUCS), Paredes, Portugal; [6]LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; [7]MOREHealth, Outcomes Research Lab, Portuguese Institute of Oncology at Porto Francisco Gentil (IPO Porto), Porto, Portugal; [8]Laboratory of Organic and Pharmaceutical Chemistry, Chemistry Department, Faculty of Pharmacy, University of Porto, Porto, Portugal; [9]CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal. **E-mail: afreis.mendes@gmail.com, veramcosta@ff.up.pt**

INTRODUCTION

Doxorubicin (DOX) is a topoisomerase II inhibitor commonly used in the treatment of several types of cancer[1]. However, despite its efficacy, DOX can potentially cause fatal adverse effects, like cardiotoxicity[1]. This work aimed to assess the role of inflammation on DOX-induced cardiotoxicity in infant and adult populations.

EXPERIMENTAL PROTOCOL

Two differently aged (infant or adult) CD-1 male mice were administered with biweekly with DOX for 3 weeks until reaching a therapeutic relevant **cumulative dose (18.0 mg/kg).** The animals' welfare was assessed daily, and mice were euthanized one week after the last injection. Hearts were removed, weighed, and processed for histological and immunohistochemistry analysis or for immunoblotting analysis. The experiments were performed with the approval of the Portuguese National Authority for Animal Health (General Directory of Veterinary Medicine) (reference number 0421/000/000/2016).

RESULTS

Histopathology findings showed that DOX caused cardiotoxicity in both populations

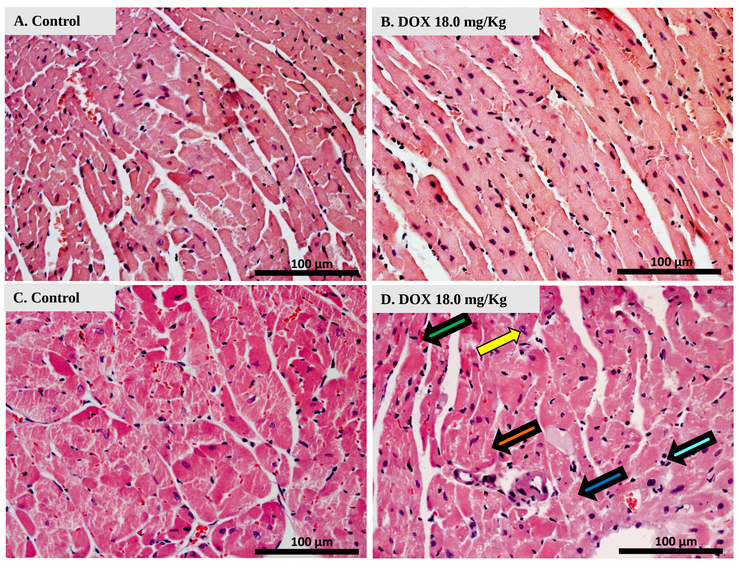

Figure 1. Cardiac histopathology evaluation done by light microscopy from DOX-treated adult and infant animals and respective controls, as assessed by haematoxylin and eosin staining [A-D]. Light micrograph from: [A] infant mice controls; [B] infant mice given a cumulative dose of 18.0 mg/kg DOX; [C] control adult mice; [D] adult mice given a cumulative dose of 18.0 mg/kg DOX. The adult DOX-treated group presents large and uncondensed nucleus (yellow arrow), vacuolization (orange arrow), inflammatory infiltration (cyan arrow) and vascular congestion (green arrow) are shown. Necrotic zones (blue arrow) are evident. Scale bar = 100 µm, n = 3. Images taken at 40×.

Increase in fibrotic cardiac tissue was only observed in DOX-treated adults

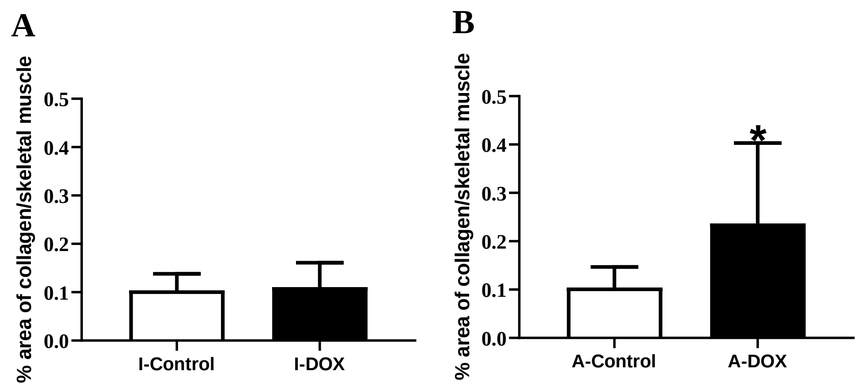

Figure 2. Semi-quantitative analysis of fibrosis in the heart of the 18.0 mg/kg DOX-treated and respective controls groups, in [A] infant and [B] adult animals. Results of Sirius red staining, given in area of collagen/skeletal muscle, are presented as mean ± SD and were obtained from 3 animals from each treatment group. Statistical comparisons were made using the Mann-Whitney test: *p < 0.05, DOX vs. control.

In DOX-treated infant mice protein carbonylation increased, while in DOX-treated adult mice those levels decreased

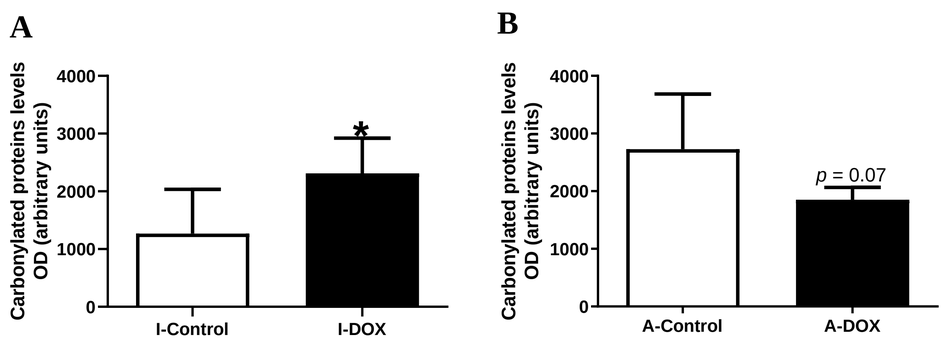

Figure 3. Protein carbonylation cardiac content evaluated by slot blot in [A] infant mice and [B] adult mice exposed to a cumulative dose of 18.0 mg/kg DOX. Values are expressed as mean ± SD and were obtained from six/seven animals from each treatment group. Statistical comparisons were made using the t-test: *p < 0.05, DOX 18.0 mg/kg vs. control. OD: optic density.

Macrophage M1 marker increased in DOX-treated infant and adults

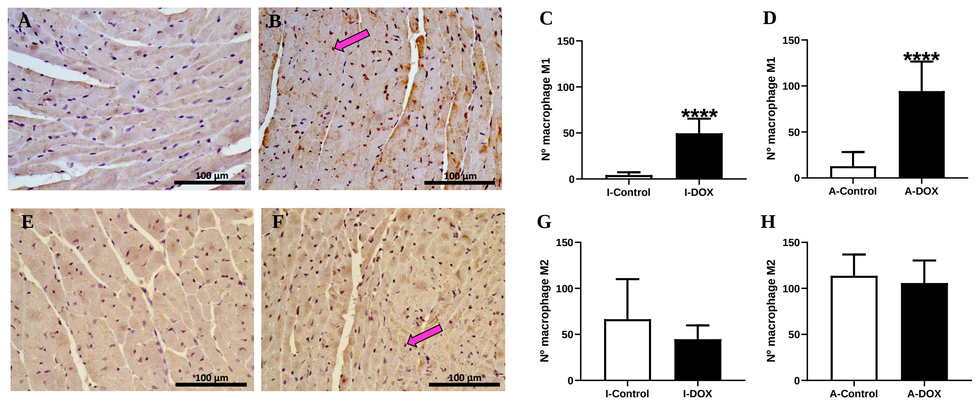

Figure 4. Representative photomicrographs of **immunohistochemistry from DOX-administered adult and respective controls, by CD68 (a marker for macrophage M1) [A–B] and by CD206 (a marker for macrophage M2) [E–F] detection in the heart.** Number of cells staining positive for activated [C-D] macrophages marked as M1 and [G-H] macrophage marked as M2 in the heart of DOX-treated and controls groups, in [C and G] infant and [D and H] adult animals. Results were expressed as mean ± SD. Results were obtained from 3 animals from each treatment group. Statistical comparisons were made using the t-test for macrophage M2 in infant group and the Mann-Whitney test for all other analyses: ****p<0.001, DOX vs. Control. Scale bar = 100 µm, n = 3. Images taken at 40×.

Expression of nuclear factor kappa B (NF-κB) p65 subunit only increased in DOX-treated adult animals

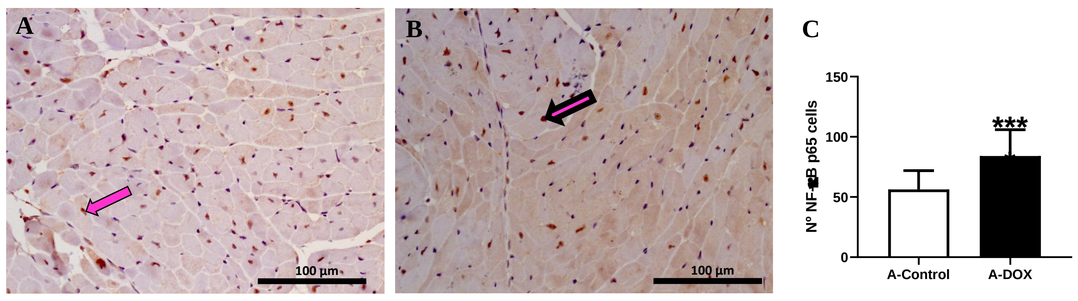

Figure 5. Representative photomicrographs of **immunohistochemistry from DOX-administered adult and respective controls, by NF-κB** [A–B] detection in the heart. [A] Light micrograph from the control; [B] Light micrograph from adult mice given DOX. Number of cells staining positive for activated NF-κB in the heart of DOX-treated and control groups, in [C] adult animals. Results were expressed as mean ± SD. Results were obtained from 3 animals from each treatment group. Statistical comparisons were made using the Mann-Whitney: ****p<0.001, DOX vs. Control. Scale bar = 100 µm, n = 3. Images taken at 40×.

Myeloperoxidase and IL-6 expression significantly decreased, NF-κB p65 expression significantly increased and a tendency for increased TNFR2 was observed in adult mice treated with DOX

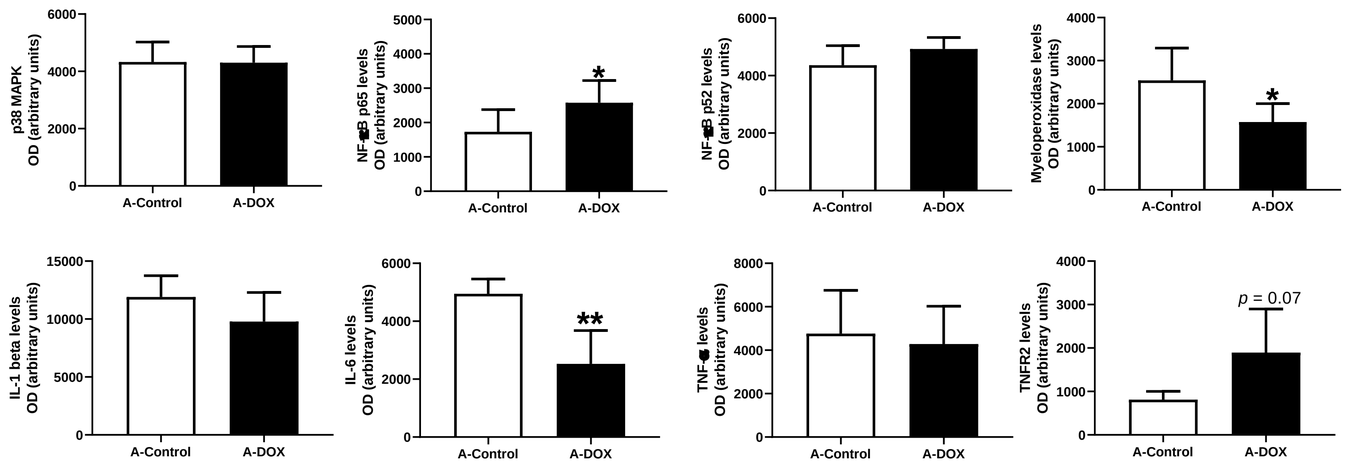

Figure 6. p38 MAP Kinase (p38 MAPK) (40 kDa), nuclear kappa B nuclear transcription factor (NF-κB) p65 (60 kDa), NF-κB p52 (50 kDa), myeloperoxidase (48 kDa), Interleukin-1 beta (IL-1 beta) (35 kDa), interleukin-6 (IL-6) (24 kDa), tumour necrosis factor-α (TNF-α) (25 kDa), type 2 TNF receptor (TNFR2) (75 kDa) expression evaluated by western blotting, from infant DOX-administered and control mice (data not shown), and adult mice exposed to a cumulative dose of 18.0 mg/kg DOX (A-DOX) and respective controls (A-Control). Values are expressed as mean ± SD and were obtained from 6 (infant) and 6-7 (adult) animals from each treatment group. Statistical comparisons were made using the t-test: *p < 0.05, DOX vs. control. OD: optic density.

Conclusions

In conclusion, adult mice seemed to be more prone to DOX-induced toxicity. Inflammatory markers underwent variable modulations, but NF-κB p65 pathway activation is consistent with the observed toxicity and deserves further analysis.

References [1] Reis-Mendes AF, Sousa E, de Lourdes Bastos M, Costa VM. The Role of the Metabolism of Anticancer Drugs in Their Induced-Cardiotoxicity. *Curr Drug Metab* 2015; **17**(1): 75-90.

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