COMUNICAÇÃO ORAL 4

Molecular alterations underlying Doxorubicin's Chronic Cardiotoxicity in a mouse model

Sofia Reis Brandão[1,2,3*], Ana Reis-Mendes[1,2], Margarida Duarte-Araújo[4], Maria João Neuparth[5,6], Félix Carvalho[1,2], Rita Ferreira[3], Vera Marisa Costa[1,2]

[1]Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal;

[2]UCIBIO-Applied Molecular Biosciences Unit, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal;

[3]LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal;

[4]Department of Immuno-Physiology and Pharmacology, Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal;

[5]Research Centre in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sports, University of Porto, Porto, Portugal;

[6]TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU CRL, 4585-116 Gandra, Portugal.

✉ sofiarbrandao@ua.pt

Doi: https://doi.org/10.51126/revsalus.v4iSup.250

Resumo

Introduction: In the last decades, the number of cancer survivors has increased considerably due to the current anticancer therapies. Doxorubicin (DOX) is a chemotherapeutic agent widely used to treat solid tumors and hematological malignancies. Nevertheless, it has been associated with severe cardiac adverse effects, either in the short or the long term by yet not clear mechanisms [1,2]. **Objectives:** Our aim was to study the short- and long-term effects of DOX on molecular mechanisms associated with cardiac metabolism, homeostasis, autophagy, and mitochondrial biogenesis using adult mice. **Material and Methods:** Adult male CD-1 mice received 6 intraperitoneal injections of 1.5 mg/kg DOX (DOX groups) or saline [control groups (CTRL)] for three weeks, biweekly, as this dosing scheme is pharmacologically relevant. Mice welfare was monitored daily. 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). Animals were sacrificed one week (CTRL1 and DOX1) or 5 months (CTRL2 and DOX2) after the last injection, where the blood and the heart were collected. Biochemical parameters were determined on serum using an autoanalyzer. Heart proteins' expression and activity were assessed by Western blot and enzymatic techniques, respectively. Statistical analysis was performed using one-way ANOVA followed by the Tukey's multiple comparisons test. **Results:** DOX2 animals had increased serum albumin levels and decreased cholesterol levels compared to CTRL2, while DOX1 animals showed a trend towards decreased albumin levels with their respective CTRL1. Regarding the heart, DOX2 animals showed a trend towards increased expression of the phosphorylated form of AMP-activated protein kinase (pAMPK) and heat shock protein 27 (HSP27) compared to CTRL2. The expression of Beclin1, autophagy protein 5 (ATG5) and microtubule-associated protein 3 light chain (LC3B) was decreased in DOX2 animals compared to CTRL2. Moreover, DOX2 animals showed decreased activity of citrate synthase (CS) and expression of mitochondrial transcription factor A (TFam). **Conclusions:** The longest time point of sacrifice (DOX2) seems to reveal a greater impact on the assessed parameters, being that these results strengthen the importance of monitoring patients after anticancer therapy has ended.

Keywords: doxorubicin; long-term cardiotoxicity; autophagy; mitochondrial density.

Keypoints / learning objectives:

- Cardiac remodeling occurred at long-term after doxorubicin exposure;
- Doxorubicin treatment evaluated at long-term led to larger molecular effects than short-term;
- The main cardiac molecular pathways affected were autophagy and mitochondrial biogenesis.

References:

[1] Colombo A, Sandri MT, Salvatici M, Cipolla CM, Cardinale D. Cardiac Complications of Chemotherapy: Role of Biomarkers. Curr Treat Options Cardiovasc Med 16: 313-8, 2014.

[2] Brandão SR, Reis-Mendes A, Domingues P, Duarte JD, Bastos ML, Carvalho F, Ferreira R, Costa VM. Exploring the aging effect of the anticancer drugs doxorubicin and mitoxantrone on cardiac mitochondrial proteome using a murine model. Toxicology 459: 152852, 2021.

Acknowledgments: This work was financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the projects UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. SRB and

ARM acknowledge FCT and European Social Fund for theirs grants (SFRH/BD/138202/2018, SFRH/BD/129359/2017). VMC acknowledges her grant (SFRH/BHD/110001/2015), received by Portuguese national funds through FCT, I.P., under the Norma Transitória DL57/2016/CP1334/CT0006.

COMUNICAÇÃO ORAL 5

A preliminary study on five exhumed bodies and their burial graves from the Cemetery of Prado do Repouso (Porto, Portugal)

Angela Silva-Bessa[1,2,3*], Shari L. Forbes[4], Ricardo Jorge Dinis-Oliveira[2,3,5], Áurea Madureira-Carvalho[3,6], Maria Teresa Ferreira[1]

[1]University of Coimbra, Centre for Functional Ecology, Laboratory of Forensic Anthropology, Department of Life Sciences, Coimbra, Portugal.

[2]UCIBIO-REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.

[3]TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, 4585-116, Gandra, Portugal.

[4]Department of Chemistry, Biochemistry and Physics, University of Québec at Trois-Rivières, Quebec, Canada.

[5]Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal.

[6]LAQV-REQUIMTE, Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Porto, Portugal.

**✉angela.bessa@cespu.pt*

Doi: https://doi.org/10.51126/revsalus.v4iSup.251

Resumo

Introduction: The Cemetery of Prado do Repouso (Porto, Portugal) was first opened to the public in 1839 after the prohibition of burials inside churches [1]. As decreed by Portuguese law [2], it is prohibited to open any type of burial place without a warrant within three years after the inhumation. Still, this timeframe has been proving insufficient for skeletonization to take place in Portuguese cemeteries. If a body is not fully decomposed, the inhumation will continue for successive periods of two years until complete decomposition is achieved [2]. **Objectives:** The aim of this preliminary study is to characterise five exhumed bodies and their burial graves with the perspective of understanding the rate of decomposition. **Material and Methods:** Five female cadavers were exhumed at the Cemetery of Prado do Repouso with the purpose of cremation or relocation to an ossuary as requested by the families of the deceased. Age-at-death ranged from 64 to 78 years-old, while postmortem interval fluctuated between 7 and 11 years. Three individuals were fully skeletonised; one individual was partially mummified and partially skeletonised; and one individual was simultaneously mummified, putrid, and skeletonised. Soil was sampled from the surface of the grave, on top of the superior coffin board, and under the lower coffin board in three body regions: head, pelvis, and feet. Soil samples were characterised according to their pH, electrical conductivity, colour, humidity content, bulk density, and organic matter content. **Results:** With the exception of the moisture content that ranged between 5.60% and 24.74% (mean = 13.48%; SD = 5.71), the obtained values did not show high discrepancies between and within the burials of skeletonised and preserved individuals. pH values slightly varied from 4.26 to 6.28 (mean = 5.70; SD = 0.52) while organic matter content oscillated between 3.23% and 5.52% (mean = 4.44%; SD = 0.58). **Conclusions:** Given that soil, as an extrinsic factor, show no substantial differences between graves, it is expected that intrinsic factors may have contributed to the different stages of decomposition. Hair and nails were sampled from all five individuals during the exhumations, and elemental analysis by ICP-MS and toxicological analysis by GC-MS will be conducted.

References:

[1] Decreto de 21 de setembro de 1835. Secretaria d'Estado dos Negócios do Reino: Diário do Governo.

[2] Decreto-Lei nº 411/98 de 30 de dezembro de 1998. Ministério da Saúde: Diário da República, p. 7251-7257.