The neurotoxicity of doxorubicin in the brain of adult CD-1 mice

Ana Dias-Carvalho[1*], Mariana Ferreira[1,2], Ana Olívia Fernandes[1,3], Ana Reis-Mendes[1], Margarida Duarte-Araújo[4,5], Rita Ferreira[2], Félix Carvalho[1], João Paulo Capela[1,6], Susana I. Sá [3,7], and Vera Marisa Costa[1]

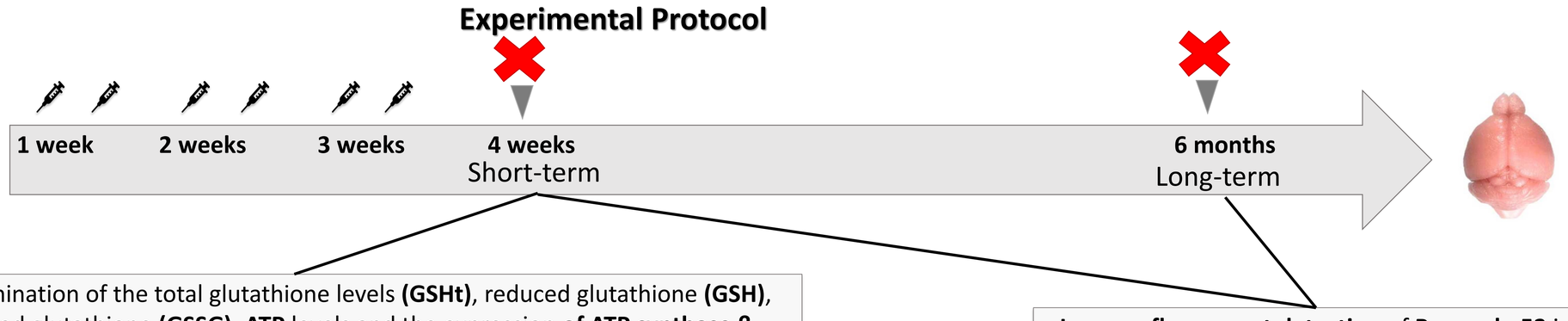
[1]UCIBIO, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; [2]LAQV/REQUIMTE, Chemistry Department, University of Aveiro, Aveiro, Portugal; [3]Faculty of Medicine, Department of Biomedicine, Unit of Anatomy, University of Porto, Porto, Portugal [4]LAQV/REQUIMTE, University of Porto, Porto, Portugal; [5]Department of Imuno-Physiology and Pharmacology, Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal; [6]FP-ENAS (Unidade de Investigação UFP em Energia, Ambiente e Saúde), CEBIMED (Centro de Estudos em Biomedicina), Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Porto, Portugal; [7]Faculty of Medicine, Center for Health Technology and Services Research (CINTESIS), University of Porto, Porto, Portugal

*email: arcdc97@gmail.com

Introduction

Despite its success in cancer-treatment, **chemotherapy** targets healthy tissues, which leads to toxicity and long-term health problems (1). On recent years, the toxicity of chemotherapy on the brain as been more studied since cancer survivors reported decline in cognitive functions. The term "**chemobrain**" has come to summon the cognitive deficit effects of chemotherapy in the long term (2). **Chemobrain affects 17% to 34% of chemotherapy-treated patients** (2). **Doxorubicin (DOX)** is a widely used chemotherapeutic agent with a broad spectrum of activity against neoplastic cells (3). **Chemobrain** is now a well-recognised secondary effect of chemotherapy with unknown underlying mechanisms. Our work aimed to evaluate the **neurotoxicity of a clinically relevant cumulative dose of DOX** in the brain of adult mice, mainly focusing on the long-term neurotoxic effects.

Experimental Protocol



Determination of the total glutathione levels **(GSHt)**, reduced glutathione **(GSH)**, oxidized glutathione **(GSSG)**, **ATP** levels and the expression **of ATP synthase β**, **manganese superoxide dismutase (MnSOD)**, **endothelial nitric oxide synthase (eNOS)**, **nuclear factor kappa B subunit p50 (NF-κB) and glycogen synthase kinase-3 (GSK-3)** were determined through western blot.

Stereological analysis of **volume and glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes in the hippocampal** formation regions.

Immunofluorescent detection of **Bax and p53** in the **prefrontal cortex (PFC)** and **hippocampal formation.**

- sacrifice

Results

Table 1 – Results of the brain determinations in the short-term group of DOX treated animals.

Short-term			
GSHt	No changes	ATP synthase β	No changes
GSSG		MnSOD	
GSH		eNOS	
GSH/GSSG		NF-κB	
ATP		GSK-3	
Hipocampal formation volume			

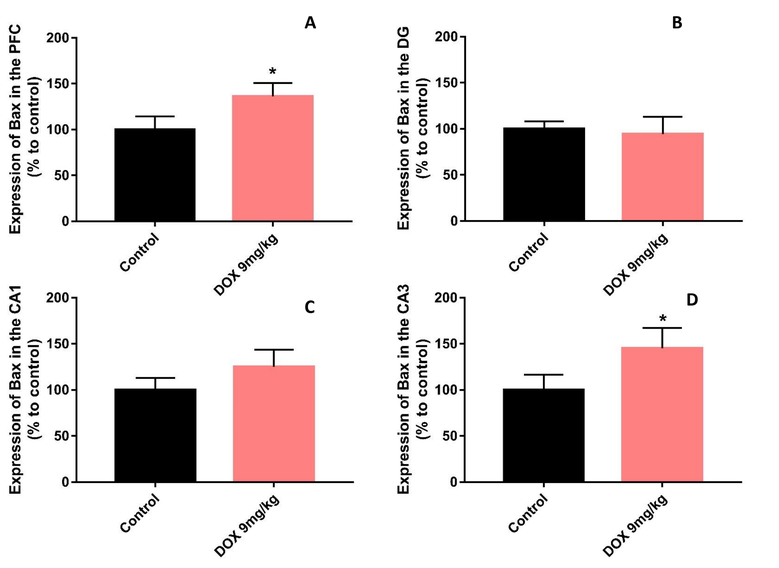


Figure 1 – **Bax expression in the PFC (A), and hippocampal formation regions DG (B), CA3 (C) and CA1 (D)** in adult mice brain exposed to a total cumulative dose of 9 mg/kg of DOX and sacrificed after one week. Data, as % of mean control, are represented as mean ± SD from 4 animals in each group. Statistical comparisons were made using a t-test analysis with Welch's correction (*p<0.05 vs control).

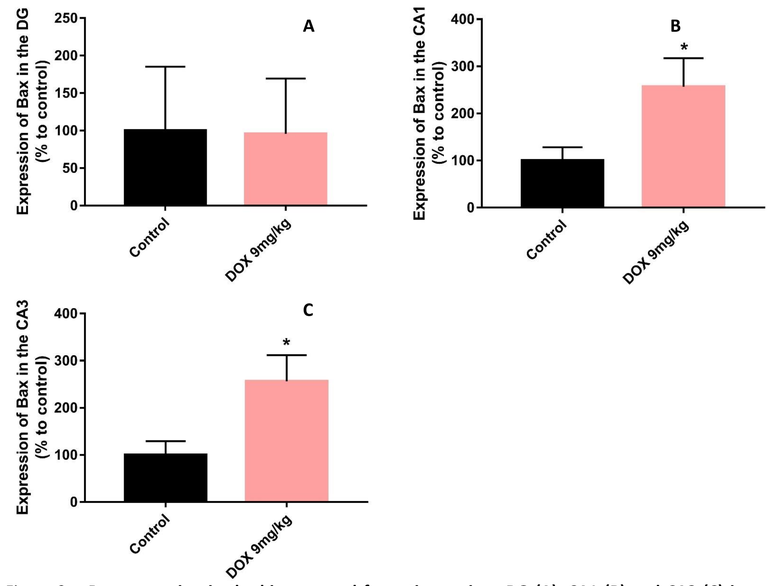


Figure 2 – **Bax expression in the hippocampal formation regions DG (A), CA1 (B) and CA3 (C) in adult mice brain** of the **long-term group** exposed to a total cumulative dose of 9 mg/kg of DOX. Data, as % of mean control, are represented as mean ± SD from 4 animals in each group. Statistical comparisons were made using a t-test analysis with Welch's correction (*p<0.05 vs control).

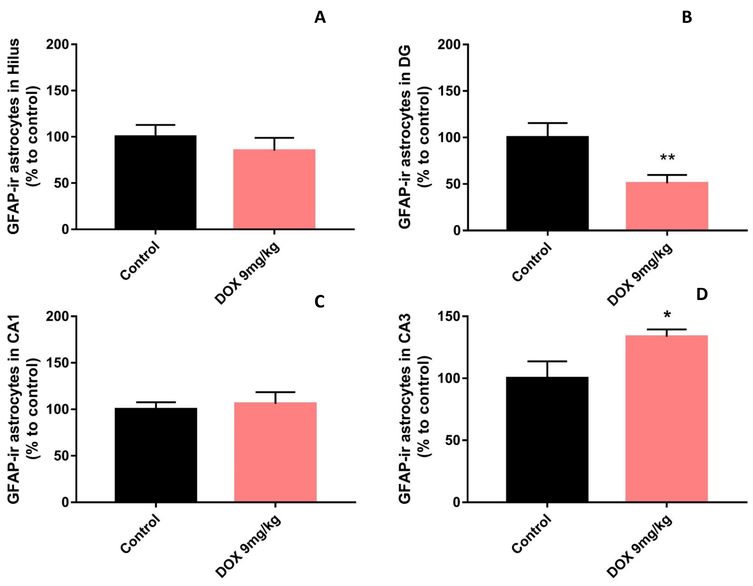


Figure 3 – **GFAP-immunoreactive astrocytes (GFAP-ir astrocytes) in the hippocampal formation regions Hilus (A), DG (B), CA3 (C) and CA1 (D)** in adult mice brain exposed to a total cumulative dose of 9 mg/kg DOX one week after last dose. Data, as % of mean control, are represented as mean ± SD from 4 animals in each group. Statistical comparisons were made using a t-test analysis with Welch's correction (*p<0.05, **p<0.01 vs control).

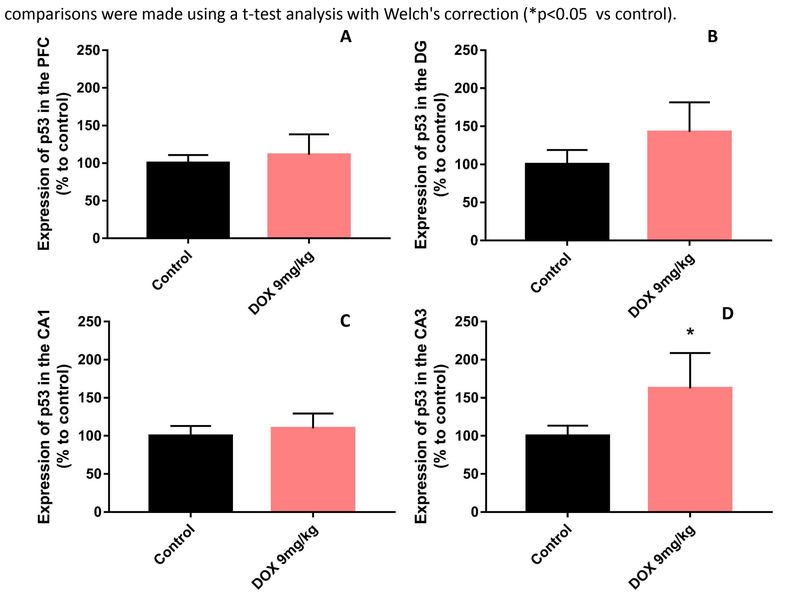


Figure 4 – **p53 expression in the PFC (A), and hippocampal formation regions DG (B), CA3 (C) and CA1 (D)** in adult mice brain exposed to a total cumulative dose of **9 mg/kg of DOX after one week**. Data, as % of mean control, are represented as mean ± SD from 4 animals in each group. Statistical comparisons were made using a t-test analysis with Welch's correction (*p<0.05 vs control).

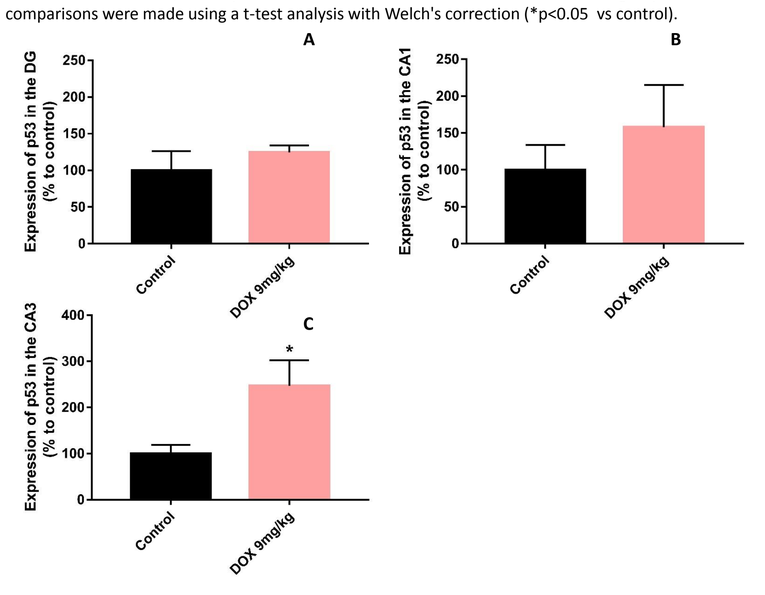


Figure 5 – **p53 expression in the hippocampal formation regions DG (A), CA1 (B) and CA3 (C)** in adult mice brain of the **long-term** group exposed to a total cumulative dose of 9 mg/kg of DOX. Data, as % of mean control, are represented as mean ± SD from 4 animals in each group. Statistical comparisons were made using a t-test analysis with Welch's correction (*p<0.05 vs control).

Discussion and conclusions

Considering the glutathione determinations, ATP levels and western blot determinations, no meaningful changes were observed in the short-term evaluation **in whole brain extract**. The dose of 9 mg/kg DOX also did not altered the volume of the hippocampal formation, however it caused alterations in the **total number of estimated GFAP-immunoreactive astrocytes, mainly increased number in the dentate gyrus and increased number in the CA3 region**, indicating a possible process of astrogliosis in the latter region mentioned. Regarding the **apoptotic markers**, in the **short-term study, Bax increased in the PFC and CA3 region of DOX-9** whereas in the **long-term Bax expression increased in the CA1 and CA3 regions and p53 increased in the dentate gyrus and CA3 region**. In summary, DOX significantly increased apoptotic markers (Bax and p53) in the adult mice brain and those changes persisted even 6 months after treatment. These changes could be involved in the cognitive impairments detected in treated patients.

References:

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