STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 69, 2, 2024 (p. 7-22)
doi:10.24193/subbbiol.2024.2.01

Assessment of circulating biomarkers in a rat model of doxorubicin-induced cardiotoxicity

Emilia Anca[1], Francesca Sabău[1], Andreea Vădan[1], Mădălina Marinescu[2], Emilia Licărete[2], Corina Roşioru[2], Anca Daniela Stoica[2], Camelia Dobre[2]✉, and Manuela Banciu[1,2]

[1]Doctoral School of Integrative Biology, Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania; [2]Faculty of Biology and Geology and Center of Systems Biology, Biodiversity and Bioresources, Babeş-Bolyai University, Cluj-Napoca, Romania
✉***Corresponding author, E-mail: camelia.dobre@ubbcluj.ro.***

Article history: Received 07 November 2024; Revised 04 December 2024; Accepted 04 December 2024; Available online 10 December 2024

Abstract. The number of cancer survivors is increasing as cancer therapies become more and more effective. As a consequence, cardio-oncology is nowadays more shifted towards detecting and treating conditions such as CTOX, which refers to heart damage as a result of cancer treatment. Currently, a standardized way of evaluating and monitoring CTOX does not exist, and patients undergo nonspecific and lengthy tests, so they are often diagnosed when heart damage is irreversible. Thus, we assessed a panel of circulating biomarkers that can be used to monitor time-dependent changes in Wistar rats treated with conventional or liposomal DOX. After validation this panel might be applied in clinics to enhance accuracy of screening patients undergoing DOX-based therapy approaches.

Keywords: CTRCD, CTOX, DOX, doxorubicin, circulating biomarkers

Introduction

Heart disease and cancer are the top leading causes of death from non-communicable diseases worldwide (Dattani *et al.,* 2023). As the number of people affected by cancer increases, so are the efforts towards finding efficient treatments. This urges the need of finding solutions to boost the wellbeing of cancer survivors, especially as the global population is aging. In older cancer survivors, the effects of cancer and cancer treatment have a long-term negative impact (Schmidt *et al.*, 2022).

Cancer patients and survivors are undergoing many screenings aimed at monitoring the course of disease, effectiveness of treatment, or early spotting of relapses, and not enough attention is paid towards screening for potential health problems that arise from the cancer treatment itself. To address this issue, specific screenings for diseases that occur following cancer treatment can be conducted simultaneously with regular check-ups through biomarker panels.

Circulating biomarkers are an easily accessible, minimally invasive, and cost-effective way of patient screening (Ahmad *et al.*, 2023). One of the diseases that could benefit from this type of screening is cancer treatment-related cardiac dysfunction (CTRCD), that arises from various treatments, such as radiotherapy, cytotoxic chemotherapy, and targeted therapy (Bloom *et al.*, 2016). CTRCD manifests as cardiotoxicity (CTOX), which is one of the most important side effects of many cytotoxic anti-cancer agents. The most known type of therapy known to have this effect is anthracyclines (Cardinale *et al.*, 2020). CTOX presents as heart failure, hypertension, and decrease in left ventricle ejection fraction (LVEF), among other cardiovascular effects *(*Perez *et al.*, 2019).

Anthracyclines, a class of cytotoxic antibiotics extracted from *Streptomyces* bacteria, are used in the treatment of various solid tumors and hematologic malignancies (Morelli *et al.*, 2022). The most used anthracycline is doxorubicin (DOX), available in its conventional form or encapsulated in different types of liposomes (Rivankar, 2014).

Anthracyclines cause CTOX in a dose-dependent manner, and can occur at any point during treatment and up to several years post-treatment, when it could be exacerbated by other pre-existing cardiovascular conditions. Generally, CTOX arising during treatment or up to one year after the completion of treatment is considered acute, whereas CTOX diagnosed after this period is chronic. Acute CTOX can be reversed by cardioprotective strategies or dose reductions. Chronic, or late-onset CTOX is hard to diagnose, and is often irreversible (Cardinale *et al.*, 2020).

CTOX is currently diagnosed through imaging studies and by assessing heart disease-related blood biomarkers, and both means of diagnosis have some important downsides. Imaging tests require high expertise, are costly, often cannot be repeated due to risks associated with exposure to radiation, and in the case of pediatric patients, require sedation. Moreover, subclinical signs of CTOX cannot be detected through these procedures. The blood biomarkers used for the evaluation of CTOX, such as troponins and natriuretic peptides, were selected for their meaning in general cardiovascular disease assessments, using cut-off values that are not specific to CTOX, due to lack of standardization for this disease.

The present study aims at evaluating a panel of biomarkers in a rat model of DOX-induced CTOX, during and after treatment with conventional DOX, and only for the duration of treatment for a PEGylated liposomal formulation of DOX. The biomarkers were selected based on their association with oxidative stress produced by DOX administration and their specificity to heart-related conditions. Through the weekly assessments of our biomarker set, we focused on identifying periods at which biomarkers are more likely to show significant changes.

Ultimately, the overall goal of our research is to inform future human studies on the key time-points at which biomarkers should be evaluated in order to close the knowledge gap between which biomarkers work and what how we can use them to quicky diagnose patients and when cardioprotective strategies should be implemented.

Materials and methods

Animals, treatment and sample collection

The study was conducted in accordance with the requirements of the European Directive 2010/63/EU. The protocol was approved by the Scientific Council of the Babeș-Bolyai University of Cluj-Napoca under the reference number 14.172/02.11.2021. In total, 40 adult male Wistar rats were used, that were maintained in appropriate hygienic conditions with constant temperature and humidity, and were gently handled at the Laboratory Animal Facility (Zoobase) at the Babeș-Bolyai University in Cluj-Napoca. Rats were housed in standard cages, had an average weight of 250 g at the beginning of the experiment and were given free access to standard food and water, on a 12-hour light/dark cycle.

The DOX group (n=20) received weekly tail vein injections of 3.75 mg/kg body weight of doxorubicin hydrochloride (European Pharmacopeia Reference Standard, D2975000, Merck) dissolved in vehicle (0.9% sodium chloride, B.Braun) in order to reach a cumulative dose of 15 mg/kg over the course of 4 weeks. The same vehicle was administered to the Control group (n=20).

The LCL-DOX (n=5) group received DOX encapsulated in long-circulating liposomes, prepared using the method described by (Sesarman *et al.*, 2018), in the same cumulative dose and administration protocol as the DOX group. To serve as a control group, LCL-PBS rats (n=5) received PBS only, encapsulated in the same type of liposomes, using the same volume of the compound.

The i.v delivery of the therapeutic agents was chosen over intraperitoneal injection administration in order to limit pain and inflammation which could affect animal welfare.

Venous blood from the tail vein was collected weekly before treatment administration, in heparin-coated tubes. After centrifugation, plasma was stored at -20 degrees Celsius until analysis. At the end of the 4-week course of treatment, for the DOX and Control groups, half of each group (n=10) was sacrificed, and the remainder of the individuals were maintained without any treatment for an additional 4 weeks. The LCL-DOX and LCL-PBS groups were only kept in the experiment until the end of treatment. All rats were sacrificed by exsanguination under anesthesia.

Biochemical assays

Plasma Galectin-3 (Gal-3) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) levels were assayed using ELISA kits purchased from EIAab (Cat. no. E0498r and E0485r, respectively). Plasma calcium (1-413-0200), iron (1-418-0150), total cholesterol (1-023-0200) and triglyceride (1-053-0200) levels were evaluated using a BIOELAB ES-100C analyzer using reagent kits purchased from SwissFarm.

Statistical analysis

Student's t test was used for all analyses which were performed using GraphPad Prism 9.3.0 software (Boston, MA). Outliers were identified using the ROUT method (Q = 5%) and were removed from the analyses. Statistical significance was considered at p-values <0.05 such as follows: ns ($p>0.05$; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$, ****, $p<0.0001$).

Results

Calcium

In our experiment, plasma calcium concentration in the DOX group recorded an overall increasing trend compared to the Control group. The highest difference between these groups was recorded on week 2 (7.92% increase), after the first dose of treatment. At 4 weeks post-treatment, plasma calcium concentration was lower in the DOX group, and it was the only time-point comparison that

reached statistical significance (3.82% decrease, P-value of 0.0079). Compared to baseline, calcium concentration was 5.46% (P-value of 0.0173) lower at the end of the untreated period than at the beginning of the experiment (Fig. 1A.).

The liposome-treated groups had a similar trend, with a statistically significant increase of 26.68% (P-value of 26.68) after the first administered dose, followed by a slight decrease (4.39% and 2.34% at weeks 2 and 3, respectively). However, post-treatment, LCL-DOX plasma calcium values were 11.22 higher (P <0.0001) compared to LCL-PBS. Post-treatment, the LCL-DOX group calcium concentration was 4.77% higher than at the pre-treatment time-point, although not statistically significant (Fig. 1B.). The concentration of plasma calcium was 24.14% lower in the LCL-DOX group compared to DOX at the end of the treatment (P <0.0001) (Fig. 1C).

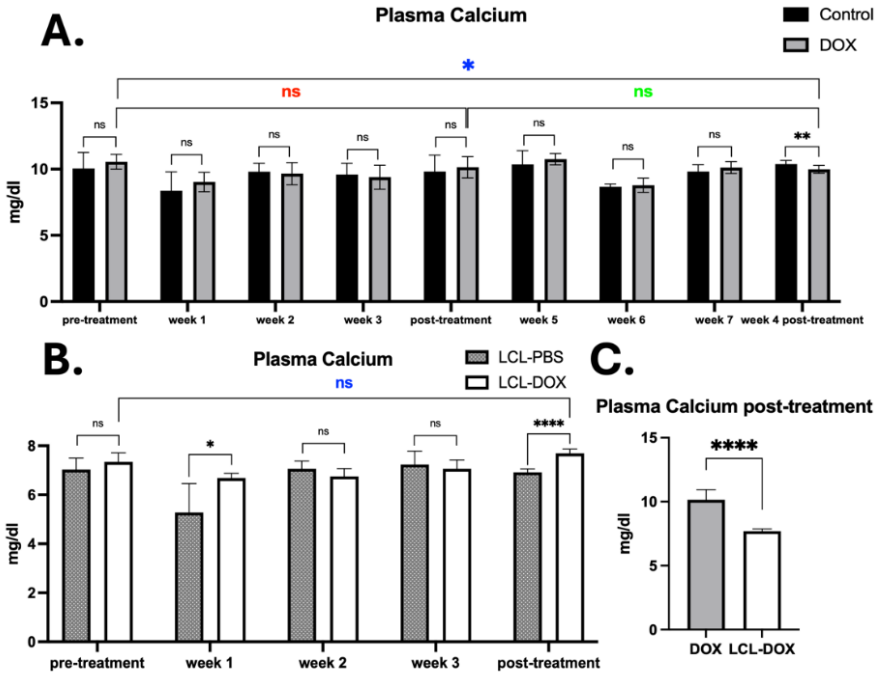

Figure 1. Plasma calcium concentration. A. weekly values recorded for the Control (vehicle treated) and DOX (DOX treated with a cumulative dose of 15 mg/kg divided into 4 weekly i.v injections) groups. B. weekly values recorded for LCL-PBS (vehicle treated) and LCL-DOX (liposomal DOX, with the same therapeutic protocol) groups. C. comparison between DOX and LCL-DOX treated groups at the end of the treatment. Data represent mean ± standard deviation (SD) for each group of animals in their corresponding week, pre-, during or post-treatment. Statistical significance was considered as follows: p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001, ****, p<0.0001.

Cholesterol

Weekly recordings of the plasma cholesterol values showed a general upward trend that is highly visible in the post-treatment period (Fig. 3). Between the DOX and Control groups, the most statistically significant difference was recorded post-treatment, with a 27.43% increase (P-value <0.0001) for the DOX-treated group. Compared to baseline values, cholesterol concentration was 38.95% higher post-treatment (P <0.0001) and 103.66% higher at week 4 post-treatment (P-value of 0.0005, Fig. 2A.).

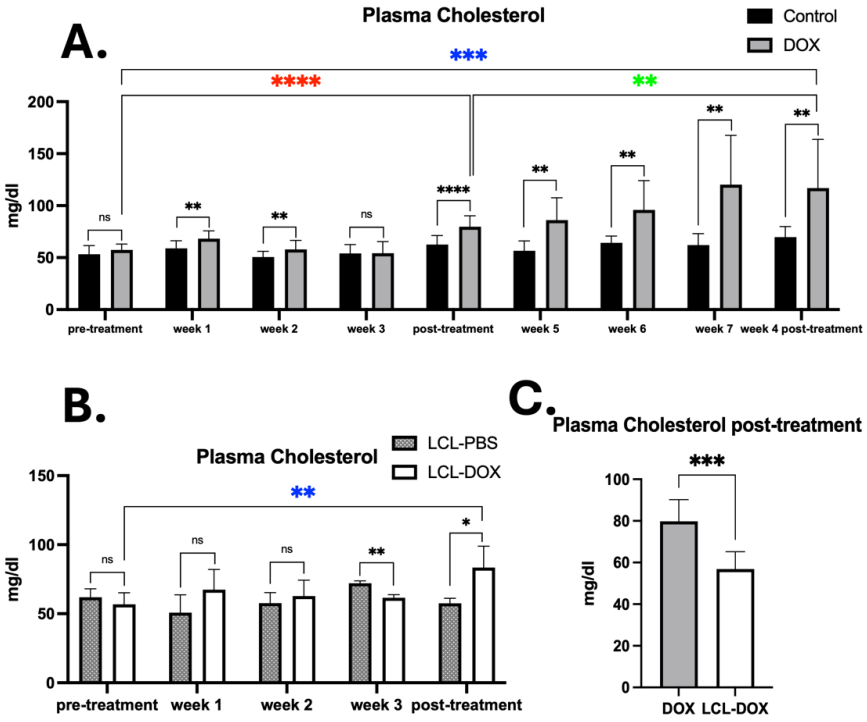

Figure 2. Plasma cholesterol concentration. A. weekly values recorded for the Control (vehicle treated) and DOX (DOX treated with a cumulative dose of 15 mg/kg divided into 4 weekly i.v injections) groups. B. weekly values recorded for LCL-PBS (vehicle treated) and LCL-DOX (liposomal DOX, with the same therapeutic protocol) groups. C. comparison between DOX and LCL-DOX treated groups at the end of the treatment. Data represent mean ± standard deviation (SD) for each group of animals in their corresponding week, pre-, during or post-treatment. Statistical significance was considered as follows: p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001, ****, p<0.0001.

Regarding cholesterol values in the liposome-treated groups, the same increasing trend in concentration was observed for LCL-DOX, with a peak value achieved post-treatment (44.77%, P-value of 0.0146). However, at week 3, LCL-DOX cholesterol concentration was 14.51% lower compared to LCL-PBS (P value of 0.0146, Fig. 2B.).

On the comparison between the two formulations of DOX administered, the liposomal form of DOX decreased cholesterol values with 29.45% (P-value of 0.0003, Fig. 2C).

Triglycerides

For the DOX group, plasma triglyceride concentration was higher starting with week 3 compared to Control, and increased in the post-treatment period with a peak difference of 105.29% (P-value of 0.0013) 3 weeks after the last dose. Compared to pre-treatment, the concentration of triglycerides was 111.33% higher (P-value of 0.0072) at the end of the post-treatment period (Fig. 3A.).

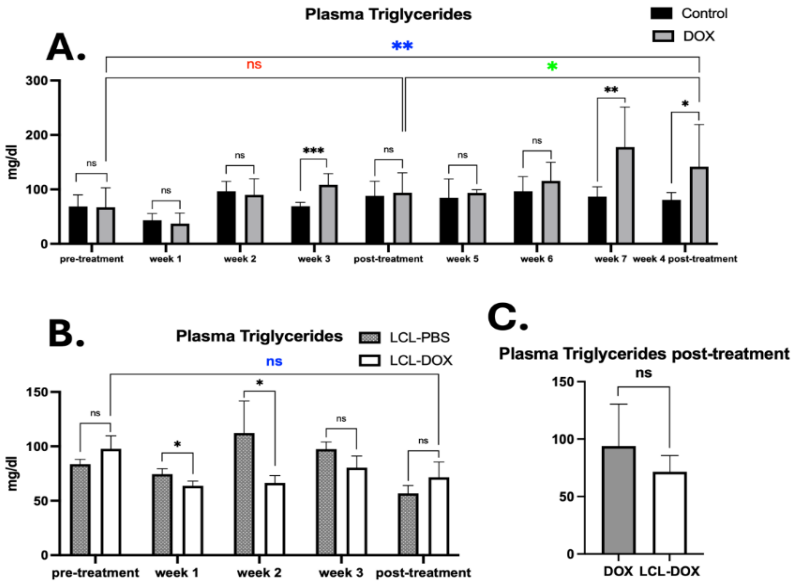

Figure 3. Plasma triglycerides concentration. A. weekly values recorded for the Control (vehicle treated) and DOX (DOX treated with a cumulative dose of 15 mg/kg divided into 4 weekly i.v injections) groups. B. weekly values recorded for LCL-PBS (vehicle treated) and LCL-DOX (liposomal DOX, with the same therapeutic protocol) groups. C. comparison between DOX and LCL-DOX treated groups at the end of the treatment. Data represent mean ± standard deviation (SD) for each group of animals in their corresponding week, pre-, during or post-treatment. Statistical significance was considered as follows: p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001, ****, p<0.0001.

Liposome-treated groups followed the opposite trend, as LCL-DOX triglyceride values were lower than LCL-PBS during treatment and increased by 25.56% post-treatment. The most statistically significant difference was observed at week 2 (40.77% decrease and P-value of 0.0198, Fig. 3B.). Although not statistically significant, triglyceride values were 23.81% lower for LCL-DOX compared to the DOX group at the end of treatment (Fig. 3C.).

Iron

In the DOX and Control groups, plasma iron values showed a significant degree of variability over the course of the experiment. The most notable differences between these two groups were observed in the first week (65.82% higher values for DOX, P-value of 0.0047), followed by a sharp decline in week 3 (19.86% lower, P-value of 0.0404) during treatment. In the post-treatment week, peak values were identified where DOX group plasma iron concentration was 62.04% higher compared to Control (P-value of 0.0134). Compared to pre-treatment, iron concentration was lower at both post-treatment time-points (Fig. 4). This assay was not performed for the LCL-PBS and LCL-DOX groups.

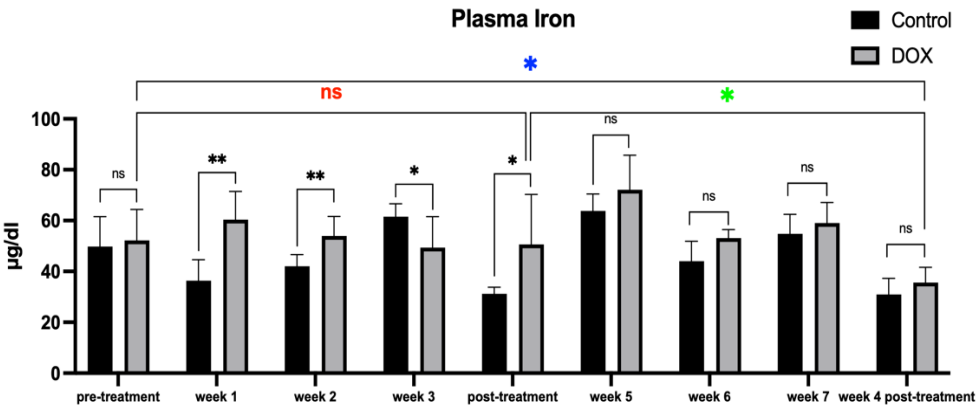

Figure 4. Plasma iron concentration. Weekly values recorded for the Control (vehicle treated) and DOX (DOX treated with a cumulative dose of 15 mg/kg divided into 4 weekly i.v injections) groups. Data represent mean ± standard deviation (SD) for each group of animals in their corresponding week, pre-, during or post-treatment. Statistical significance was considered as follows: p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001, ****, p<0.0001.

Galectin-3

In our experiment, free DOX treatment did not generate any statistically significant differences regarding plasma Gal-3 concentration in the comparison between DOX and Control groups. However, following the first two weeks of treatment, an increase in Gal-3 levels of 31.13% and 21.81% were observed. Similarly, in the last two weeks of the post-treatment period, DOX Gal-3 values increased by 25.2 and 38.56%. Compared to baseline, week 4 post-treatment values for the DOX group were almost 38% higher (Fig. 5A.).

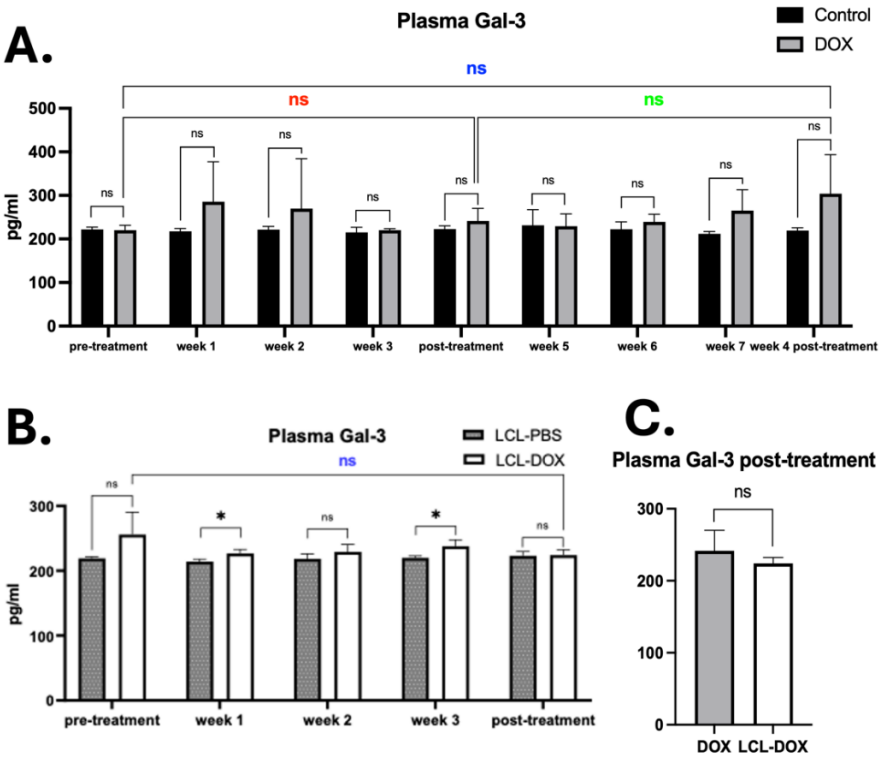

Figure 5. Plasma Gal-3 concentration. A. weekly values recorded for the Control (vehicle treated) and DOX (DOX treated with a cumulative dose of 15 mg/kg divided into 4 weekly i.v injections) groups. B. weekly values recorded for LCL-PBS (vehicle treated) and LCL-DOX (liposomal DOX, with the same therapeutic protocol) groups. C. comparison between DOX and LCL-DOX treated groups at the end of the treatment. Data represent mean ± standard deviation (SD) for each group of animals in their corresponding week, pre-, during or post-treatment. Statistical significance was considered as follows: p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001, ****, p<0.0001.

As opposed to the trend seen for the DOX and Control groups, liposomal DOX treatment led to an increase in Gal-3 plasma concentration that is most noticeable at weeks 1 and 3 (5.84% and 8.19%, respectively), with a decrease of 12.47% post-treatment compared to baseline (Fig. 5B.). No significant difference was observed between the two forms of DOX treatment (Fig. 5C.).

N-terminal prohormone of brain natriuretic peptide

For the classic administration of DOX, NT-proBNP concentration was overall higher in the DOX group compared to Control. However, values for both groups slowly declined until the post-treatment week, and continued to increase in the following weeks. The only statistically significant difference between the DOX group is seen in the post-treatment week (48.36%, P-value of 0.0081) compared to baseline (Fig. 6A.).

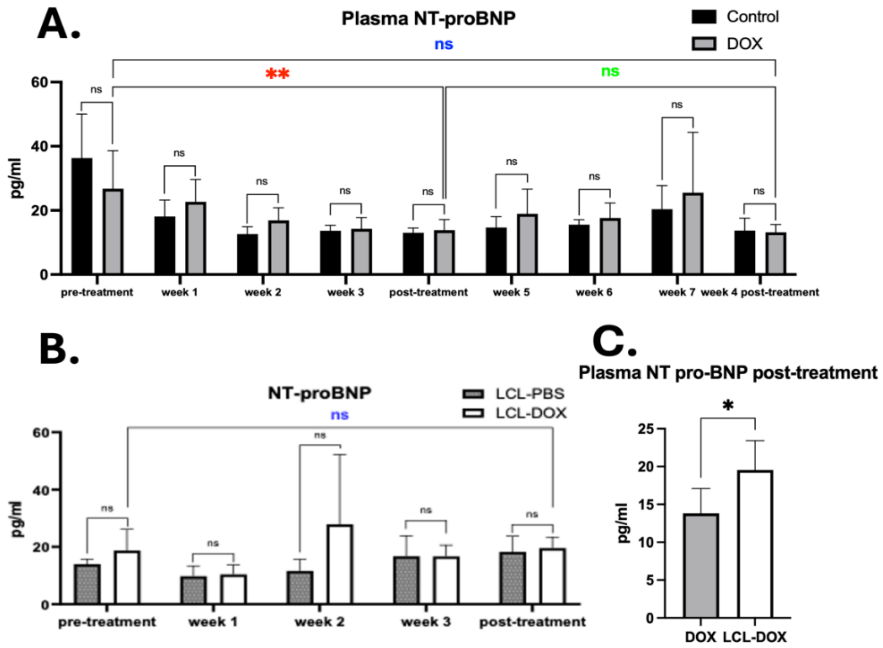

Figure 6. Plasma NT-proBNP concentration. A. weekly values recorded for the Control (vehicle treated) and DOX (DOX treated with a cumulative dose of 15 mg/kg divided into 4 weekly i.v injections) groups. B. weekly values recorded for LCL-PBS (vehicle treated) and LCL-DOX (liposomal DOX, with the same therapeutic protocol) groups. C. comparison between DOX and LCL-DOX treated groups at the end of the treatment. Data represent mean ± standard deviation (SD) for each group of animals in their corresponding week, pre-, during or post-treatment. Statistical significance was considered as follows: p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001, ****, p<0.0001.

The liposomal form of DOX followed the same trend, with weekly values higher in the treated groups, apart from week 3, when values were similar for both groups. The most significant difference was recorded on week 2, when LCL-DOX NT-proBNP concentration was 139.76% higher than LCL-PBS (Fig. 6B.).

Liposomal administration of DOX led to a 41.43% increase in plasma NT-proBNP concentration compared to free DOX (Fig. 6C.).

Discussion

DOX is a highly potent chemotherapeutic agent, and along with its high efficacy in treating cancer, the long-term use of this compound should also be taken into account when choosing a DOX-based therapeutic regimen. This is especially important in the case of pediatric patients and in cancers with high survival rates, where post-treatment quality of life should also be considered.

One of the many ways DOX stimulates tumor cell apoptosis is through disruption of calcium homeostasis. This is mainly done by inhibiting mechanisms responsible for calcium reuptake, thus producing an increase in calcium concentration and the overproduction of ROS. This mechanism, although beneficial for treating cancer, is one of the contributing factors to the occurrence of CTOX (Shinlapawittayatorn *et al.*, 2022). Calcium homeostasis is of utmost importance for cardiac cells, due to its role in contractile function. Moreover, ROS are an important threat for these cells, due to their high vulnerability to oxidative stress (Pagan *et al.*, 2022).

In our experiment, free DOX administration did not lead to any significant changes in plasma calcium concentration, while liposomal DOX exhibited increases in calcium concentration during the first and last week of treatment, which shows that this formulation has a bigger impact on hypercalcemia even though weekly mean values were overall lower in both liposomal treatment groups. This result suggests a possible ameliorative effect of liposomal formulations on calcium dysregulation induced by DOX.

Cholesterol metabolism is severely impaired by both cancer and its therapeutic strategies, which often leads to other comorbidities besides CTRCD, thus increasing disease burden especially in older, individuals.

Belger *et al.* (2024) reviewed recent findings regarding risk factors for the development of DOX-induced CTOX and concluded that despite the limited data available regarding dyslipidemia as a risk factor for CTOX, current studies show that hyperlipidemia as a result of DOX treatment is strongly associated with cardiovascular events. Due to the lack of studies investigating long-term effects of anthracycline-based regimens on lipid metabolism, the duration of lipid dysregulation following treatment is unclear (Bhatnagar *et al.*, 2022).

Our results show that free DOX administration leads to hyperlipidemia that worsens in the post-treatment period. Total cholesterol values increased more post-treatment, and were doubled at the end of the experiment compared to baseline values and were 0.5-times higher than at the end of treatment.

Due to the small number of animals that were administered the liposomal formulations, the effect of this type of treatment was only investigated for the treatment period. Despite this limitation, the results in the LCL-DOX group at the post-treatment time-point are similar to those seen for the free DOX group. With these results, we can conclude that both therapies had the same effect on total cholesterol during treatment.

Triglyceride levels followed the same pattern as cholesterol in the post-treatment period, although differences between DOX and Control groups were actually lower. For LCL-DOX rats, a decrease in concentration was only observed at week 3.

The link between disrupted lipid metabolism and the development of heart disease is well-demonstrated, and DOX therapy has been proven as a contributing factor to hyperlipidemia in both cancer patients (Sharma *et al.*, 2016) and animal models (Abdulkareem Aljumaily *et al.*, 2021). In rats, one of the proposed mechanisms for the lipid metabolism alteration is through the inhibition of adipogenesis by the downregulation of the peroxisome proliferator activated receptor gamma (PPARγ) (Arunachalam *et al.*, 2013). Dyslipidemia is known to increase oxidative stress, especially in the heart, but due to the lack of long-term thorough studies focused on this side effect of DOX, its role in CTOX is inconclusive. In a study conducted by Simões *et al.* (2021), where breast cancer patients were followed for 1 year, it was shown that high triglycerides could offer a cardioprotection in some patients.

Another mechanism by which anthracyclines, and implicitly DOX contribute to CTOX development is by interfering with iron metabolism. This class of medications increase iron overload and contribute to ROS production (Huang *et al.*, 2022), which could exacerbate CTOX through oxidative stress (Kumfu *et al.*, 2022).

In our experiment, free DOX treated rats had increased plasma iron concentration, which seemed to slowly return to baseline, suggesting the contribution of excess iron on CTOX development could be temporary.

Gal-3 is a novel biomarker with prognostic value for heart failure and other cardiovascular diseases (Vucic *et al.*, 2023; Jiang *et al.*, 2021), but its purpose in CTOX prediction or monitoring is not yet clear. In the CECCY trial (NCT01724450), Gal-3 was not able to predict CTOX development (de Barros Wanderley *et al.*, 2022). In other human studies, this biomarker was successfully used for the prediction of treatment effectiveness (Shafiq *et al.*, 2020; Niang *et al.*, 2022). To our knowledge, this biomarker has not been evaluated

in animal models of CTOX thus, we sought to identify if Gal-3 could be included in a battery of biomarkers that could serve as a tool for the identification of CTOX.

In the free DOX group, Gal-3 spikes were observed at the start and before the end of treatment, and at the last analyzed time-point DOX Gal-3 levels were clearly higher than the ones for the Control group. For the liposomal DOX formulation, we observed a similar trend, except that the difference between LCL-DOX and LCL-PBS weekly values is smaller. This may be due to the less damaging side effects of liposomal DOX formulations (Franco *et al.*, 2018).

NT-proBNP is currently one of the few biomarkers recommended by the European Society of Cardiology for the screening of patients undergoing anthracycline-based therapies (McDonagh *et al.*, 2024). Multiple studies investigating its potential biomarker role have been conducted, but the results are inconclusive. NT-proBNP showed predictive power for the detection of subclinical CTOX in childhood cancer survivors (Demissei *et al.*, 2020), patients undergoing anthracycline-based therapies followed for 6 months post-treatment (Bisoc *et al.*, 2020), or followed during the course of treatment (Dong *et al.*, 2022). In smaller studies, an association between CTOX and NT-proBNP elevation could not be established (Posch *et al.*, 2022; Ruggiero *et al.*, 2013). Due to conflicting results between studies, as in the case of Gal-3, we chose to include NT-proBNP in our battery of biomarkers.

Similarly to Gal-3, NT-proBNP levels seemed to normalize after two weeks of treatment, and then gradually increased in the post-treatment period in the free DOX group. In the case of liposomal DOX, a similar trend was observed, but due to the absence of data in the post-treatment period for this group, we cannot compare the effects of the two formulations during the 4 weeks after the end of treatment.

Taken together, our results show that the most notable changes in biomarker levels occur at the beginning of treatment and close to the completion of the treatment. In order to correctly establish a set of biomarkers for prediction and diagnostic purposes, further studies should focus on establishing cut-off values for each biomarker that showed predicting/diagnosing power.

Conclusions

In this article, we sought to evaluate several biomarkers during the course of DOX treatment, and investigate whether or not a liposomal DOX formulation could influence these biomarkers differently. Our results showed that some of the CTOX-inducing effects of DOX administration are temporary, as iron levels slowly decrease after treatment. Free DOX administration induced changes in blood lipid levels that worsen post-treatment, so the search for cardioprotective strategies

based on lipid lowering compounds should focus on the post-treatment side effects of DOX administration. Lastly, a liposomal formulation of DOX could potentially alleviate sudden changes in these biomarkers, but close attention should be paid to the hypercalcemia produced by this formulation.

References

Abdulkareem Aljumaily, S. A., Demir, M., Elbe, H., Yigitturk, G., Bicer, Y. & Altinoz, E. (2021). Antioxidant, anti-inflammatory, and anti-apoptotic effects of crocin against doxorubicin-induced myocardial toxicity in rats. *Environ Sci Pollut R*, *28*(46), 65802-65813. https://doi.org/10.1007/s11356-021-15409-w

Ahmad, A., Imran, M. & Ahsan, H. (2023). Biomarkers as biomedical bioindicators: approaches and techniques for the detection, analysis, and validation of novel biomarkers of diseases. *Pharmaceutics 15*(6), 1630. https://doi.org/10.3390/pharmaceutics15061630

Arunachalam, S., Tirupathi Pichiah, P. B. & Achiraman, S. (2013). Doxorubicin treatment inhibits PPARγ and may induce lipotoxicity by mimicking a type 2 diabetes-like condition in rodent models. *FEBS Lett*, *587*(2), 105-10. https://doi.org/10.1016/j.febslet.2012.11.019

Belger, C., Abrahams, C., Imamdin, A. & Lecour, S. (2024). Doxorubicin-induced cardiotoxicity and risk factors. *Int J Cardiol Heart Vasc*, *50*, 101332. https://doi.org/10.1016/j.ijcha.2023.101332

Bhatnagar, R., Dixit, N. M., Yang, E. H. & Sallam, T. (2022). Cancer therapy's impact on lipid metabolism: mechanisms and future avenues. In *Front Cardiovasc Med*, *9*, 925816. https://doi.org/10.3389/fcvm.2022.925816

Bisoc, A., Ciurescu, D., Rădoi, M., Tântu, M. M., Rogozea, L., Sweidan, A. J. & Bota, D. A. (2020). elevations in high-sensitive cardiac troponin t and n-terminal prohormone brain natriuretic peptide levels in the serum can predict the development of anthracycline-induced cardiomyopathy. *Am J Ther*, *27*(2), e142-e150. https://doi.org/10.1097/MJT.0000000000000930

Bloom, M. W., Hamo, C. E., Cardinale, D., Ky, B., Nohria, A., Baer, L., Skopicki, H., Lenihan, D. J., Gheorghiade, M., Lyon, A. R. & Butler, J. (2016). Cancer therapy-related cardiac dysfunction and heart failure: part 1: definitions, pathophysiology, risk factors, and imaging. *Circ Heart Fail*, *9*(1), e002661. https://doi.org/10.1161/CIRCHEARTFAILURE.115.002661

Cardinale, D., Iacopo, F. & Cipolla, C. M. (2020). cardiotoxicity of anthracyclines. In *Front Cardiovasc Med*, *7*, 26. https://doi.org/10.3389/fcvm.2020.00026

Dattani, S., Spooner, F., Ritchie, H. & Roser, M. causes of death. *Our World in Data*. (2023) [Accessed 28 August 2024] https://ourworldindata.org/causes-of-death

de Barros Wanderley, M. R., Ávila, M. S., Fernandes-Silva, M. M., das Dores Cruz, F., Brandão, S. M. G., Rigaud, V. O. C., Hajjar, L. A., Filho, R. K., Cunha-Neto, E., Bocchi, E. A. & Ayub-Ferreira, S. M. (2022). Plasma biomarkers reflecting high oxidative stress in the prediction of myocardial injury due to anthracycline chemotherapy and the effect of carvedilol: insights from the CECCY Trial. *Oncotarget*, *13*(1). https://doi.org/10.18632/ONCOTARGET.28182

Demissei, B. G., Hubbard, R. A., Zhang, L., Smith, A. M., Sheline, K., McDonald, C., Narayan, V., Domchek, S. M., DeMichele, A., Shah, P., Clark, A. S., Fox, K., Matro, J., Bradbury, A. R., Knollman, H., Getz, K. D., Armenian, S. H., Januzzi, J. L., Tang, W. H. W., … Ky, B. (2020). Changes in cardiovascular biomarkers with breast cancer therapy and associations with cardiac dysfunction. *J Am Heart Assoc*, *9*(2), 214-223. https://doi.org/10.1161/JAHA.119.014708

Dong, Y., Wu, Q. & Hu, C. (2022). Early Predictive Value of NT-proBNP Combined With Echocardiography in anthracyclines induced cardiotoxicity. *Front Surg*, *9*, 898172. https://doi.org/10.3389/fsurg.2022.898172

Franco, Y. L., Vaidya, T. R. & Ait-Oudhia, S. (2018). Anticancer and cardio-protective effects of liposomal doxorubicin in the treatment of breast cancer. *Breast Cancer: Target (Dove Med Press)*, *10*, 131–141. https://doi.org/10.2147/BCTT.S170239

Huang, J., Wu, R., Chen, L., Yang, Z., Yan, D. & Li, M. (2022). Understanding anthracycline cardiotoxicity from mitochondrial aspect. *Front Pharmacol*, *13*, 811406. https://doi.org/10.3389/fphar.2022.811406

Kumfu, S., Chattipakorn, S. C. & Chattipakorn, N. (2022). Iron overload cardiomyopathy: using the latest evidence to inform future applications. *Exp Biol Med 247*(7). https://doi.org/10.1177/15353702221076397

McDonagh, T. A., Metra, M., Adamo, M., Gardner, R. S., Baumbach, A., Böhm, M., Burri, H., Butler, J., Čelutkienė, J., Chioncel, O., Cleland, J. G. F., Crespo-Leiro, M. G., Farmakis, D., Gilard, M., Heymans, S., Hoes, A. W., Jaarsma, T., Jankowska, E. A., Lainscak, M., … Skibelund, A. K. (2024). 2023 Focused update of the 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: developed by the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail*, *26*(1), 811406. https://doi.org/10.1002/ejhf.3024

Morelli, M. B., Bongiovanni, C., Da Pra, S., Miano, C., Sacchi, F., Lauriola, M. & D'Uva, G. (2022). Cardiotoxicity of anticancer drugs: molecular mechanisms and strategies for cardioprotection. *Front Cardiovasc Med*, *9*, 847012. https://doi.org/10.3389/fcvm.2022.847012

Niang, D. G. M., Ka, S., Hendricks, J., Diouf, D., Gaba, F. M., Diouf, A., Diop, M., Mbow, M., Faye, B., Diallo, R. N., Niang, M. D. S., Deme, A., Mbengue, B. & Dieye, A. (2022). Profile of plasma galectin-3 concentrations, inflammatory cytokines levels and lymphocytes status in breast cancer under chemotherapy. *Open J Immunol*, *12*(01). https://doi.org/10.4236/oji.2022.121001

Pagan, L. U., Gomes, M. J., Gatto, M., Mota, G. A. F., Okoshi, K. & Okoshi, M. P. (2022). The role of oxidative stress in the aging heart. *Antioxidants*, 11(2), 336. https://doi.org/10.3390/antiox11020336

Perez, I. E., Taveras Alam, S., Hernandez, G. A. & Sancassani, R. (2019). Cancer therapy-related cardiac dysfunction: an overview for the clinician. *Clin Med Insights Cardiol*, *13*, 1179546819866445. https://doi.org/10.1177/1179546819866445

Posch, F., Niedrist, T., Glantschnig, T., Firla, S., Moik, F., Kolesnik, E., Wallner, M., Verheyen, N., Jost, P. J., Zirlik, A., Pichler, M., Balic, M. & Rainer, P. P. (2022). Left ventricular ejection fraction and cardiac biomarkers for dynamic prediction of cardiotoxicity in early breast cancer. *Front Cardiovasc Med*, *9*, 933428. https://doi.org/10.3389/fcvm.2022.933428

Rivankar, S. (2014). An overview of doxorubicin formulations in cancer therapy. In *J Cancer Res Ther*, *10*(4), 853-858. https://doi.org/10.4103/0973-1482.139267

Ruggiero, A., De Rosa, G., Rizzo, D., Leo, A., Maurizi, P., De Nisco, A., Vendittelli, F., Zuppi, C., Mordente, A. & Riccardi, R. (2013). Myocardial performance index and biochemical markers for early detection of doxorubicin-induced cardiotoxicity in children with acute lymphoblastic leukaemia. *Int J Clin Oncol*, *18*(5), 927-933. https://doi.org/10.1007/s10147-012-0458-9

Schmidt, M. E., Goldschmidt, S., Hermann, S. & Steindorf, K. (2022). Late effects, long-term problems and unmet needs of cancer survivors. *Int J Cancer*, *151*(8), 1280-1290. https://doi.org/10.1002/ijc.34152

Sesarman, A., Tefas, L., Sylvester, B., Licarete, E., Rauca, V., Luput, L., Patras, L., Banciu, M. & Porfire, A. (2018). Anti-angiogenic and anti-inflammatory effects of long-circulating liposomes co-encapsulating curcumin and doxorubicin on C26 murine colon cancer cells. *Pharmacol Rep*, *70*(2), 331-339. https://doi.org/10.1016/j.pharep.2017.10.004

Shafiq, A., Moore, J., Suleman, A., Faiz, S., Farooq, O., Arshad, A., Tehseen, M., Zafar, A., Ali, S. H., Din, N. U., Loya, A., Siddiqui, N. & Rehman, F. K. (2020). Elevated soluble galectin-3 as a marker of chemotherapy efficacy in breast cancer patients: a prospective study. *Int J Breast Cancer*, *2020*, 4824813. https://doi.org/10.1155/2020/4824813

Sharma, M., Tuaine, J., McLaren, B., Waters, D. L., Black, K., Jones, L. M. & McCormick, S. P. A. (2016). Chemotherapy agents alter plasma lipids in breast cancer patients and show differential effects on lipid metabolism genes in liver cells. *PLoS ONE*, *11*(1), e0148049. https://doi.org/10.1371/journal.pone.0148049

Shinlapawittayatorn, K., Chattipakorn, S. C. & Chattipakorn, N. (2022). The effects of doxorubicin on cardiac calcium homeostasis and contractile function. *J Cardiol*, *80*(2), 125-132. https://doi.org/10.1016/j.jjcc.2022.01.001

Simões, R., Silva, L. M., de Oliveira, A. N., Alves, M. T., Pestana, R. M. C., de Souza, I. D. P., Oliveira, H. H. M., Soares, C. E., Sabino, A. de P. & Gomes, K. B. (2021). identification of clinical and laboratory variables associated with cardiotoxicity events due to doxorubicin in breast cancer patients: a 1-year follow-up study. *Cardiovasc Toxicol*, *21*(2), 106-114. https://doi.org/10.1007/s12012-020-09600-7

Vucic, R. M., Andrejic, O. M., Stokanovic, D., Stoimenov, T. J., McClements, L., Nikolic, V. N., Sreckovic, M., Veselinovic, M., Aleksandric, S., Popadic, V., Zdravkovic, M. & Pavlovic, M. (2023). Galectin-3 as a prognostic biomarker in patients with first acute myocardial infarction without heart failure. *Diagnostics*, *13*(21), 3348. https://doi.org/10.3390/diagnostics13213348