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Benefits of Regular Physical Activity on Doxorubicin-induced Kidney Toxicity

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Daniela Filipa Cardoso

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To my father

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Resumo

A doxorrubicina (DOX), antraciclina usada na quimioterapia no tratamento de diversos cancros, encontra-se associada a vários efeitos colaterais, nomeadamente, à toxicidade induzida nos mais diversos tecidos em doentes oncológicos e/ou sobreviventes. O dano renal, o aumento da espessura da cápsula de Bowman (ECB) e a deposição de colágeno nos rins, são alguns dos efeitos adversos associados à diminuição da função renal. No entanto, a atividade física regular poderá ser utilizada como um recurso não farmacológico e terapêutico para a diminuição desses efeitos, tanto em humanos como em animais. Alguns estudos têm vindo demonstrar os benefícios da prática regular de exercício físico na diminuição da toxicidade renal em ratos saudáveis, nomeadamente nos parâmetros referidos anteriormente, no dano renal, na deposição de colagénio e na espessura da capsula de Bowman. O objetivo deste trabalho foi verificar a toxicidade e os mecanismos subjacentes à DOX; e se a prática de exercício físico poderia ser capaz de reverter a toxicidade renal induzida pela prolongada administração da DOX, imitando um protocolo de quimioterapia, em ratos saudáveis. Trinta e quatro ratos Wistar machos foram divididos aleatoriamente em 2 clusters: 1) tratado com DOX (DOX, n=17) semanalmente com uma injeção intraperitoneal (i.p) de 2 mg/kg de DOX durante 7 semanas e 2) tratado com solução salina estéril (SSS, n=17) com a administração semanal de uma injeção i.p de veiculo durante 7 semanas. Duas semanas após a última injeção, 5 animais de cada grupo (SSSG, n=5; DOXG, n=5) foram sacrificados e os restantes divididos em subgrupos: sedentários (DOXsedG, n=6; SSSsedG, n=6) e ativos (DOXactG, n=6; SSSactG, n=6). Ambos os subgrupos foram colocados individualmente numa jaula durante 2 meses. A jaula dos animais ativos estava equipada com uma roda de aço. No final do protocolo, os animais foram sacrificados e os rins foram analisados histologicamente. Os resultados relevaram que os rins dos animais tratados com DOX obtiveram maiores níveis de dano, de deposição de colagénio e um aumento da ECB a curto prazo quando comparado com o SSSG group ($p<.05$). Por outro lado, a longo prazo nos animais do DOXsedG apenas se verificou

maior dano e um aumento da ECB relativamente ao DOXG group ($p<.05$). Enquanto que, no grupo ativo com DOX, o dano renal, a deposição de colagénio e a ECB diminuíram relativamente ao grupo DOXsedG ($p<.05$). Em suma, a corrida voluntária de forma regular, parece melhorar e atenuar os efeitos colaterais renais em ratos saudáveis após um tratamento quimiotático prolongado.

Palavras-chave: Atividade física, Cancro, Doxorrubicina, Rim, Toxicidade, Dano renal, Espessura da cápsula de Bowman, Colagénio

Abstract

Doxorubicin (DOX), an anthracycline used in cancer treatments, has a lot of side-effects, such us the capacity for induce high levels of toxicity on tissues and organs of patients and cancer survivors. Renal damage, increasing collagen deposition and the thickness of Bowman's capsule (TBC) are some side-effects which occur after a chemotactic treatment, and most of them are connected with renal dysfunction and failure. However, it is well documented that regular physical activity is important to reduce the side-effects, and could be used as a therapy improving renal structure and function on rats and humans. Several studies have verified the benefit effects of regular exercise practice on the reduction of kidney toxicity in healthy rats, decreasing their levels of damage, collagen content and also decrease the thickness of bowman's capsule. The aim of this study was to understand the mechanisms underlying DOX-induce kidney toxicity and verify if physical exercise practice could be used to improve and revert these side-effects caused by a prolonged DOX administration on healthy rats. Thirty-four male Wistar rats were randomly divided into 2 clusters: 1) one treated with doxorubicin (DOX, n=17), which received weekly an intraperitoneal (i.p.) injection of 2 mg/kg of DOX for 7 weeks, and 2) the other treated with sterile saline solution (SSS, n=17) that received i.p. injections of vehicle during 7 weeks. Two weeks after the last injection, five animals from each group (SSSG, n=5; DOXG, n=5) were euthanized while the remaining rats were subsequently divided into sedentary (DOXsedG, n=6; SSSsedG, n=6) and active subgroups (DOXactG, n=6; SSSactG, n=6). Both groups were placed individually in cages during 2 months, however the cages of active animals were equipped with a run wheel for voluntary running. At the end of the protocol, animals were euthanized and kidneys were histologically examined. The results revealed higher levels of renal damage, collagen deposition and an increase of TBC at short-term, on DOXG animals comparatively with SSSG group ($p<.05$). While, at long-term it was observed higher levels of damage and an increase of TBC ($p<.05$). However, the collagen content remain similar between DOXG and DOXsedG groups. On active group, compared with DOXsedG, a decreased of renal damage, collagen content and

the TBC ($p<.05$) was observed. These results allow concluding that regular voluntary running, applied after a prolonged DOX administration, is effective to attenuate the harmful effects of this drug in kidneys.

List of Abbreviations

ANT - Anthracycline

DOX - Doxorubicin

DOXactG - Group Doxorubicin Active

DOXG - Group Doxorubicin

DOXsedG - Group Doxorubicin Sedentary

ECB - Espessura da Cápsula de Bowman

i.p - Intraperitoneal

LM - Light Microscope

MMP - Matrix Metalloproteins

NT - Nephrotoxicity

ROS - Reactive Oxygen Spices

RPE - Regular Physical Exercise

SSSG – Group Sterile Saline Solution

SSSactG - Group Sterile Saline Solution Active

SSSsedG - Group Sterile Saline Solution Sedentary

TBC - Thickness of Bowman's Capsule

TGF-β - Transforming Growth Factor Beta

1. General introduction

Cancer is a large group of diseases which is characterized by an uncontrollable proliferation and differentiation of cells process in any part of the body; then these abnormal cells can disseminating all over the body, creating metastasis (Stevinson et al., 2016; World Health Organization, 2015). The prevalence of cancer has been growing and in 2012 there were 14.1 million new cases of cancer and 8.2 million deaths (Cancer Research UK, 2014). In the next two decades, it is expected a 70% growth in cancer cases (World Health Organization, 2015). More precisely in 2025, 19.3 million new cases *per* year are expected (Centers for disease control and prevention, 2016). In children, to 15 years old, the incidence of cancer varies between 0.5% and 4.6% of all types of cancer and the incidence rate varies between 50 to 200 million worldwide (IARC World Cancer Report, 2014). These numbers point out to the undeniable importance of studying, not only prevention but also detection and treatment. Indeed, advances in early detection and in cancer treatments led to a substantial increase in the number of oncological survivors over the last 20 years (Vijayvergia & Denlinger, 2015). In addition, around 60% of individuals with cancer, live more than 5 years after the diagnosis of their disease (Buffart & May, 2014), which further underlies the importance of studying mechanisms through which life quality can be increased.

Despite the availability of a series of cancer treatments (World Health Organization, 2003), the majority of patients also do chemotherapy (Schmitz et al., 2010). This method is used since 1943 (World Health Organization, 2003). One of the drugs administered during chemotherapy is DOX which is an ANT antibiotic (Chabner et al., 2001). ANT have been used in the treatment of malignant neoplasms such us leukemia and solid tumors (Lipshultz et al., 2014). The treatment using DOX was established in 1960 (Arcamone et al., 1969) and since then it is considered one of the most efficient methods for treating oncological diseases (Moylan, 2015). As a case in point, it is estimated that more than 50% of childhood survivors had received treatments with ANT's (Sterba et

al., 2013) and after the introduction of DOX in treatments the 5-year survivor rate among children increased approximately to 80% (Ward et al., 2014).

However, in spite of its efficiency in treating cancer itself, DOX has as important side-effects. In other words, the problem is not the treatment itself but the toxic side-effects that it can cause (Ayla et al., 2011). The present research is especially interested in this aspect of DOX treatments. We will therefore now focus on the main side-effects associated with cancer treatments involving DOX. One of the most commonly known side-effects of DOX is the induction of high levels of cardiotoxicity both in adults and children (Franco & Lipshultz, 2015; Lustberg & Zareba, 2016) that have led to a limited clinical use of this drug (Carvalho et al., 2009). Depending on the dosage, DOX can induce a progressive cardiac damage manifesting itself in decreased ejection fraction of the left ventricle, heart failure (Scott et al., 2011) and myocardial infarction (Franco & Lipshultz, 2015). DOX is also known for causing pulmonary, testicular and hematological toxicity, among other damages (Ayla et al., 2011). Importantly for our purpose, DOX has been reported to cause a series of damaging effects at the kidney level and this is going to be the focus of the present research.

Firstly, by increasing nephrotoxicity, DOX is known to create glomerular capillary permeability and tubular atrophy (Ayla et al., 2011; El-Sheikh et al., 2012; Jadhav et al., 2013; Mustafa et al., 2015). Moreover, DOX is responsible for the triggering of an oxidative stress on rat kidneys (Mokni et al., 2016) and can also increase the glomerular volume and induce renal edema (Peng et al., 2012a). Furthermore, the use of this drug has been associated with renal dysfunction. Indeed, histological analyses show glomerular and tubule-interstitial damage (Elsherbiny & El-Sherbiny, 2014). Among animals treated with DOX, there was a glomeruli distortion, vascular congestion, tubules focal atrophy necrosis and exfoliation and the filtration space obliterated disappeared (Su et al., 2015). The percentage of collagen increased in the kidneys of rats treated with DOX compared with rats that have not received this drug (Egger et al., 2015). This deposition happens in the renal interstitial tissue and in the renal cortex (Peng et al., 2012a; Peng et al., 2012b). Of special relevance for the goal of the present research, the renal injuries found in rats treated with DOX are extremely similar

to the ones found in humans with chronic kidney disease, and this mainly because of the development of primary focal segmental glomerulosclerosis (Lee & Harris, 2011).

Given the (unfortunate) large list of side-effects of DOX treatments, we aim at looking at factors that might help to minimize the detrimental impact of this drug on the body, and specifically on the kidney functions. Among these factors, we can find physical activity. Nowadays it is unquestionable that regular physical exercise (RPE) is paramount in decreasing the risk of obesity, cardiovascular diseases, premature mortality and morbidity related to chronic diseases (Kummer et al., 2013; West et al., 2014). Importantly, the psychological and physical benefits of RPE have been extended to cancer survivors (Kummer et al., 2013; Wang et al., 2015). There is evidence that physical exercise could be an option to buffer the side-effects felt by the cancer survivors (Scott et al., 2011). Specifically, and importantly for the goals of the present research, the benefits of physical exercise are well documented on the acute and chronic cardiotoxicity effects caused by DOX described above (Hayward et al., 2012; Lien et al., 2015). There is also some evidence showing that endurance training can reduce DOX cardiotoxicity in individuals with cancer (Hydock et al., 2012). In addition, doing RPE can help in the process of cell survival, proliferation and growth in chronic kidney disease cases induced by DOX (Peng et al., 2012b). Furthermore, 60 minutes of swimming seems to restore the renal edema status and to improve collagen deposition after a singles injection of DOX (Peng et al., 2012a). Relatedly, aerobic exercise has improved the nitrite and serum urea levels in renal structure decreased damage in the kidney tissue and increased the activation of the oxidant system among patients being treated with Cisplatin, (Zeynali et al., 2015). Cisplatin is considered a drug belonging to the platinum classes, even if it is not an ANT like DOX, cisplatin is one of the drugs used in chemotherapy treatments, which one of the most important side effects is nephrotoxicity (such as DOX) (Hanigan & Devarajan, 2003).

Goals of the present thesis

The main goals of the present study are: 1) to better understand the mechanisms underlying the side-effects induced by DOX and; 2) to examine the role of regular voluntary running as a non-pharmacological therapy in minimizing the side-effects on kidney structure of animals exposed to chemotactic treatments involving the administration of DOX. Given the lack of research addressing the role of physical activity in buffering the detrimental effects of DOX therapy, our first goal is going to be addressed by a more in-depth review of the literature. This review will focus on the one hand, on the role of physical activity in cancer patients and in animal models treated with drugs similar to DOX and, on the other hand on the specific effects of DOX on cancer patients' kidneys as well as in rats. This approach will allow us to have a clearer picture of the state of the art of research on both domains and therefore to elaborate a more informed hypothesis for the experimental study that will follow. In this study, the main goal is to advance knowledge concerning the specific toxic effects of DOX on the kidney structure and understand the benefits of voluntary running on rats treated with DOX.

Lastly, in the current study is expected an increase of renal damage, collagen deposition and the TBC on kidney structure of sedentary rats treated with DOX, even at short and long-term. However, it is supposed that regular voluntary running will ameliorate kidney function and structure decreasing their damage, collagen deposition and the TBC.

1.1. Structure of the dissertation

This dissertation is according to the Scandinavian Model and it is divided into four sections:

Section 1: This chapter has the general introduction, the main goals, also the importance of this study and the structure of the dissertation.

Section 2: This is composed of a state of the art about "Potential mechanisms of kidney toxicity induced by doxorubicin and the benefit effects of regular exercise as a therapy in the renal toxicity".

Section 3: This chapter is an experimental study: <u>"Favorable effects of regular voluntary running on kidney toxicity induced by doxorubicin in Wistar rats"</u>.

Section 4: In this chapter are presented the main conclusions of this dissertation.

Section 5: Lastly, in this section are present the references.

2. __State of the art__

Among other treatments, chemotherapy is one of the most used treatment in a wide oncological diseases cases (Schmitz et al., 2010). This type of treatment could be composed of a variety of chemotactic agents, which are classified into several classes: antimetabolites, alkylating agents, platinum derivates, anthracyclines and like agents, and natural alkaloids (World Health Organization, 2003).

Anthracyclines (ANTs) are considered antineoplastic antibiotics introduced in clinical practice (Simunek et al., 2009), which increased the success of treatments on cancer survivors (Štěrba et al., 2013). Doxorubicin (DOX), commercially known as Adriamycin, is an antineoplastic drug belonging to the ANT's group, used in combination with other drugs in chemotherapy for cancer treatment in both adults and children (Cancer Research UK, 2015; Injac & Strukelj, 2008; Lustberg & Zareba, 2016; Saffi et al., 2010). DOX *($C_{27}H_{29}NO_{11}$)* is composed by a quinone and hydroquinone, a carbonyl group at C-13 and a C-14 hydroxyl group, also an amino-sugar daunosamine attached by a glycosidic bond to the C-7 on the tetracyclic ring (Štěrba et al., 2013). In 1960, DOX was isolated from cultures of *S. peucetius var. caesius.*, a species of actinobacteria (Simunek et al., 2009; Štěrba et al., 2013) and is the hydroxylated congener of daunorubicin (daunomycin) (Gewirtz, 1999). Since then, is considered one of the most efficient drugs in the treatment of oncological diseases (Moylan, 2015; Štěrba et al., 2013). Over the years, DOX has been mainly used to treat a variety of cancers such as leukemia's, lymphomas (Hodgkin and Non-Hodgkin), multiple myeloma, sarcoma, as well as ovarian, thyroid, breast, lung, gastric, and pediatric cancers (Das et al., 2016; Shi et al., 2011).

2.1. Pharmacokinetics and pharmacodynamics of doxorubicin

Pharmacokinetic of DOX diverge among humans and animals (Lee & Harris, 2011). In humans, DOX is predominantly metabolized in the liver to the major metabolite, doxorubicinol, having a quick distribution and a slow elimination. Approximately 40-50% of DOX is eliminated in seven days mostly through the bile and around 4-5% is excreted in the urine in five days (Lee & Harris, 2011; Robert & Gianni, 1993). On the other hand, in rats DOX has a slowly excreted by the urine and bile, which promotes the drug deposition in different tissues inclusively on the kidneys making them susceptible to injury and toxicity (Lee & Harris, 2011; Yesair et al., 1972).

The main therapeutic actions of DOX is to achieve the cancer cells and to inhibit the topoisomerase II, which is an importance enzyme for nucleic acids' replication and transcription, avoiding DNA and RNA synthesis (Chen & Liu, 1994). Moreover, DOX has also another mechanism of action, which is the increased oxidative stress with further damage to DNA, proteins and cellular membranes (Injac & Strukelj, 2008; Shi et al., 2011; Thorn et al., 2011). This drug induces high levels of toxicity however the mechanisms of DOX-induce toxicity are not fully described and is also poorly understood (El-Moselhy & El-Sheikh, 2014; Gurel et al., 2015). There are some speculations about this process, with several studies suggesting three physiological mechanisms underlying DOX-induce toxicity: oxidative stress, inflammatory activity and apoptosis (Korga et al., 2012; Park et al., 2012; Zhang et al., 2009). However, the most acceptable mechanism still is the oxidative stress (El-Moselhy & El-Sheikh, 2014).

Focus on oxidative stress mechanism, DOX is oxidized in an unstable metabolite, semiquinone, which is converted again in DOX (Injac & Strukelj, 2008; Thorn et al., 2011). Due to this process, there is an increase of production of reactive oxygen species (ROS) with oxidative stress and mitochondrial dysfunction, which damage DNA, proteins, and membranes, and promotes ATP depletion. All these features contribute to the occurrence of necrosis in different cell types, affecting diverse organs (Shi et al., 2011). The oxidative stress and damage are also promoted by the increased activation of mitochondrial Matrix Metalloproteins

(MMP) (Taskin et al., 2014). By this mechanism, DOX is considered an inducer of toxicity in neoplastic and non-neoplastic cells, as in heart, liver, lungs, testis and kidneys cells (Thorn et al., 2011; Yasuda et al., 2010). In the literature, several studies report a possible oxidative injury and an increased oxidative stress in kidneys after DOX administration. After 24 hours of DOX administration, it was observed a decrease of glutathione concentrations, an antioxidant which protects the cells from cytotoxic damage, and an increase of lipid peroxidation after 4 hours after the drug administration (El-Sheikh et al., 2012). According to El-Moselhy and El-Sheikh (2014), the decreased of glutathione content and the increased of lipid peroxidation products, after DOX administration, are markers of oxidative stress, that can induce toxicity on kidney structure. Oxidative stress with oxidative damage of cell components could trigger the apoptotic pathways of cell death (Thorn et al., 2011). All this data indicate that inflammation, oxidative stress, and cell apoptosis are common processes described in DOX toxicity studies, suggesting that they are the main reasons for DOX-induced toxicity.

2.2. DOX-induce side-effects

Despite its effectiveness in fighting cancer, therapeutic doses of DOX have many side-effects, which can develop during or shortly after treatment or even some decades later (Moylan, 2015). Once the cancer survivors' quality of life tends to decrease due to the DOX-induced toxicity in normal cells (Prylutska et al., 2015), its clinical use is limited by the doses (Marques-Aleixo et al., 2016). Indeed, this antibiotic has been associated to a number of acute and chronic side-effects (Shi et al., 2011).

Among other, nauseas, vomiting, alopecia, neutropenia, arrhythmias, myelosuppression, loss of hearing, mucositis, decrease of blood cell count, diarrhea (Injac & Strukelj, 2008), cardiomyopathy, heart failure (Kouzi & Uddin, 2016), and cardiac dysfunction (Dursun et al., 2011) could be acute side-effects that can occur during or within 2-3 days after DOX administration. However, mostly of the time, the effects on patients are asymptomatic (Shi et al., 2011).

One the other hand, the long-term side-effects are most commonly reported in the literature and trends to prevail in time (Kavazis et al., 2016; Polegato et al., 2015). The long-term side-effects could develop within weeks or months after a repetitive and prolonged DOX administration (De Beer et al., 2001; Injac & Strukelj, 2008). This drug is known for their toxicity in a variety of organs such as heart, liver, lung, kidney, blood cells, and testis (Injac & Strukelj, 2008). However, a major chronic side-effect caused by this drug is the cardiotoxicity that has been well studied and documented (Moylan, 2015; Scott et al., 2011). It is known that this cardiac toxicity is dose-dependent (Kremer et al., 2001).

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2.2.1. Side-effects on kidneys

Though, beyond the cardiac toxicity, there are other DOX side-effects that have been reported in the literature, such as those resulting from the drug toxicity in the kidneys (Hassan et al., 2014). The evidence of kidney toxicity is poorly described and understood (Yasuda et al., 2010), however, the studies about this side-effect have been increasing.

In rats, DOX is responsible for inducing nephropathy, heavy proteinuria, associated with swelling and vacuolization of epithelial cells. In addition, tubular dilatation was also report, as a consequence of kidney damage induced by DOX (Injac & Strukelj, 2008). According to Ayla et al. (2011), the kidney damage was visible in proximal tubules with a presence of vacuolization in endothelial cell cytoplasm, cellular damage and capillary dilatation. The damage on cellular membranes, organelles, and genetic material, with lipid peroxidation and protein oxidation, were also reported. Furthermore, degenerative changes and vacuolization of the endothelial cells with an increased thickness and disorganization of glomerular capillary basement membranes were also observed. After 8 weeks of DOX administration, a severe inflammatory infiltration by neutrophil granulocytes, lymphocytes, and macrophages was described (Szalay et al., 2015).

DOX is one of the drugs responsible for the occurrence of nephrotic syndrome (Park et al., 2014), resulting from the damage and apoptosis of podocytes (Tao

et al., 2014), leading to proteinuria and glomerulosclerosis development (Min et al., 2016) with further renal failure (Karanovic et al., 2016). Podocytes are an important element of the glomerular filtration barrier, so their loss can lead to a progressive kidney disease (Zhong et al., 2016). Furthermore, the proteinuria is nearly associated with dysfunction of glomerular endothelial cells (Jeansson et al., 2009) and the increased permeability of glomerular filtration membrane, which is composed of vascular endothelial cells, podocytes, and the glomerular basement membrane (Wang et al., 2016). Consequently, this drug decreases plasma albumin and the total levels of protein content, increases blood urea nitrogen and plasma creatine levels (Lee & Harris, 2011). In rats, the oxidative stress on kidney structure is associated with a reduction of glutathione concentrations and lower activity of glutathione reductase (Saenko Iu et al., 2005). Moreover, the collagen deposition in kidney tissue is also a side-effect of this drug. The interstitial fibrosis area is higher in rats treated with DOX when compared with rats that did not receive the same treatment (Park et al., 2014). Of note that, renal fibrosis is a common manifestation of many chronic kidney diseases that could result in renal failure (Liu, 2006). In parallel, some of these effects occur also in humans, such as the glomerular damage is similar to human focal segmental glomerulosclerosis. So, it is important to better understand the mechanisms of DOX toxicity in humans for improving their quality of life after DOX treatment.

2.3. The benefits of physical exercise practice before, during and after DOX treatment

Nowadays, the effectiveness of RPE practiced before, during and/or after cancer treatments is well documented (Courneya & Friedenreich, 2007) assuming higher importance on improving cancer therapy effects (Yu & Jones, 2016). Exercise is considered as a non-pharmacological protection and therapy that prevent and attenuated many side-effects causing by a prolonged DOX administration and

has a lot of benefits when it is done before, during or even after DOX treatment (Scott et al., 2011).

Several studies investigated the potential of regular exercise practice in cancer prevention and concluded that there is an association between the levels of physical activity and the decreased risk of having some types of cancer (Brown et al., 2012). Moreover, aerobic exercise practice before DOX treatment also has protective effects in the development of drug side-effects (Scott et al., 2011). Acute exercise 24 hours before the DOX administration has a cardioprotective effects observed on end-systolic pressure, left ventricular developed pressure and higher maximal rate of ventricular pressure (Wonders et al., 2008). This evidence shows the protection of exercise prior treatment against cardiac dysfunction induced by DOX, which might be explained by a decreased rate of ROS formation.

During cancer treatment, the RPE practice provides many physiological and psychological benefit effects (Brown et al., 2012). Low-intensity exercise training during chronic DOX treatment works as a protector against cardiac toxicity and dysfunction, probably by enhancing antioxidant defenses and inhibition of cardiomyocytes apoptosis (Chicco et al., 2006). Indeed, endurance training seems to improve myocardial tolerance to DOX (Ascensao et al., 2005a).

Regular exercise practice seems to be also important for cancer survivors and exercise could have a therapeutic role instead of a protective one. In fact, the benefits of exercise on heart therapy after DOX treatment are well documented. After DOX treatment, aerobic exercise seems to attenuate left ventricular dysfunction (Scott et al., 2011).

To our knowledge, there are only a few studies relating the effects of exercise on normal kidneys in laboratory animals treated with DOX. In rats, which was injected with a single dose of DOX (8.5 mg/kg), it was verified that endurance exercise restored the glomerular size and attenuated the collagen deposition after running 60 minutes 3 times per week for 13 weeks. A normalization of TGF-beta, PDGF-BB, p-PDGFR, p-PI3K and p-AKT expressions were also observed (Peng et al., 2012b). According to Peng et al. (2012a), sixty minutes of swimming seems to have better effects at the renal edema status, collagen levels,

decreasing their levels and in the prevention of fibrosis of the glomerular mesangium. Moreover, exercise training has also the potential to increase the activity of antioxidant system in renal cells (Zeynali et al., 2015), which could decrease the oxidative stress, with further attenuation of cellular damage and cell death and collagen deposition. According to Chen et al. (2013), on DOX-induced kidney chronic disease, a treadmill exercise for 11 weeks, 30 or 60 minutes 3 times per week, attenuated renal cells' apoptosis. It might be speculated that if the exercise would be done in an early stage of kidney disease it would be possible to better control the disease progress.

3. Experimental Article

Favorable effects of regular voluntary running on kidney toxicity induced by doxorubicin in Wistar rats

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Abstract

This study aimed to verify the effectiveness of regular exercise on the cellular damage and collagen deposition in rat kidney induced by a prolonged doxorubicin (DOX) administration, mimicking a chemotherapy protocol. Thirty-four male Wistar rats were randomly divided into 2 clusters: 1) one treated with DOX (n=17), receiving weekly an i.p. injection of 2 mg/kg for 7 weeks and 2) the other treated with sterile saline solution (SSS, n=17) that received i.p. injections of vehicle for 7 weeks. Two weeks after the last injection, five animals from each cluster (SSSG, n=5; DOXG, n=5) were euthanized while the remaining rats were subsequently divided into sedentary (DOXsedG, n=6; SSSsedG, n=6) and active subgroups (DOXactG, n=6; SSSactG, n=6). Active animals were placed individually in cages with a run wheel for voluntary running during 2 months, whereas sedentary animals were housed individually in conventional cages, with movements restricted to the cage space. At the end of the protocol, animals were euthanized and kidneys were histologically examined. Comparing to SSSG, kidneys from DOXG revealed higher levels of damage, collagen content, and increased bowman's capsule thickness ($p<.05$). The levels of damage and thickness of bowman's capsule increased on DOXsedG comparing to DOXG ($p<.05$). Comparatively to DOXsedG, the DOXactG presented an overall improvement in kidney structure ($p<.05$), with decreased collagen content and thickness of bowman's capsules. The results allow concluding that voluntary running, applied after a prolonged DOX administration, attenuated the long-term harmful effects on kidney structure induced by a DOX treatment mimicking a chemotherapy protocol.

Keywords: Physical exercise; Renal structure; Tissue damage; Anthracycline; Collagen deposition; Nephrotoxicity.

Introduction

Doxorubicin (DOX) is an anthracycline antibiotic that since 1960 has been used to treat many types of cancers such as leukemia, lymphomas, carcinomas, and other solid tumors (Chen et al., 2016; Das et al., 2016; Gu et al., 2015; Mousavi et al., 2016; Moylan, 2015), turning out to be one of the most efficient drugs to treat adult and pediatric cancers (Lustberg & Zareba, 2016; Mousavi et al., 2016; Moylan, 2015). Mitochondria are the main target of this drug's toxic effects, both in neoplastic and normal cells, with a consequent decrease of energy production and respiratory efficiency, favoring the occurrence of cellular death (Taskin et al., 2014). Consequently, beyond the expected toxic effects on neoplastic cells, high and repeated doses of DOX can induce non-desirable side effects on patients, both in a short and in a long-term in cancer survivors (Wu et al., 2017). Indeed, harmful side effects of DOX have been reported for different organs as heart, liver, and kidneys (Kumral et al., 2016), which might be explained, among other factors, by their content of mitochondria-rich cells. The nephrotoxicity induced by DOX is expressed in animals studies by the existence of tubular necrosis (Yilmaz et al., 2006) and glomerular atrophy with increased permeability (Kumral et al., 2016). Glomerular vacuolization, glomerulosclerosis, tubules dilatation with cellular atrophy (Chmielewska et al., 2015), and a mild leukocyte infiltrates (Kumral et al., 2015) in parallel with the existence of interstitial damage (Elsherbiny & El-Sherbiny, 2014), are also described in rats at short and long-term after DOX administration. Regarding this commitment of interstitial space, several animal studies showed that the percentage of collagen deposition increased in the kidneys (Egger et al., 2015; Szalay et al., 2015), predominantly affecting the renal cortex (Peng et al., 2012b) after DOX treatment.

Nowadays, physical exercise is regarded as a nonpharmacological therapy for cancer diseases (Scott et al., 2011), which can carry many benefits for patients, during and after their treatments (Wang et al., 2015). For instance, both in humans and animal models, the effectiveness of RPE against DOX-induced cardiac injury has been well documented (Kouzi & Uddin, 2016). Similarly, to the cardioprotection induced by physical exercise, it would be interesting to observe

if RPE could also have similar protective effects on kidneys, especially after a prolonged DOX administration mimicking the chemotherapy treatment. To our knowledge, just three single studies using animal models showed a favorable effect of RPE on renal toxicity induced by DOX, but only after a single dose administration of this drug (Chen et al., 2013; Peng et al., 2012a; Peng et al., 2012b). Indeed, 60 minutes of swimming was effective to restore the renal edema status and attenuate the collagen deposition in kidneys, after a single dose (7,5 mg/Kg) of DOX (Peng et al., 2012a). In the same line, daily physical exercise ameliorated the renal cells apoptosis after a single dose (8,5 mg/Kg) of DOX (Chen et al., 2013). Besides, sixty minutes of treadmill exercise improved nephropathy induced by a single injection of DOX (8,5 mg/Kg), restoring the glomerular size and decreasing the collagen content (Peng et al., 2012b). Based on these advantageous results of the RPE obtained with protocols with a single dose of DOX, it might be expected that the exercise applied after repeated and prolonged administration of the drug, mimicking a chemotherapy treatment, could be equally beneficial at short and long-term, for the structure of kidneys. In this sense, the aim of this study was to characterize the harmful histological repercussions of a prolonged protocol of DOX administration, for 7 weeks, and to verify the effectiveness of voluntary running, applied after treatment, to revert/attenuate the tissue damage and collagen deposition in Wistar rat kidneys.

Material and Methods

Sample and experimental design

Thirty-four male Wistar rats from the Charles River Laboratories, 8 weeks old (body weight of 266g ±15,57g) were used in the present study.
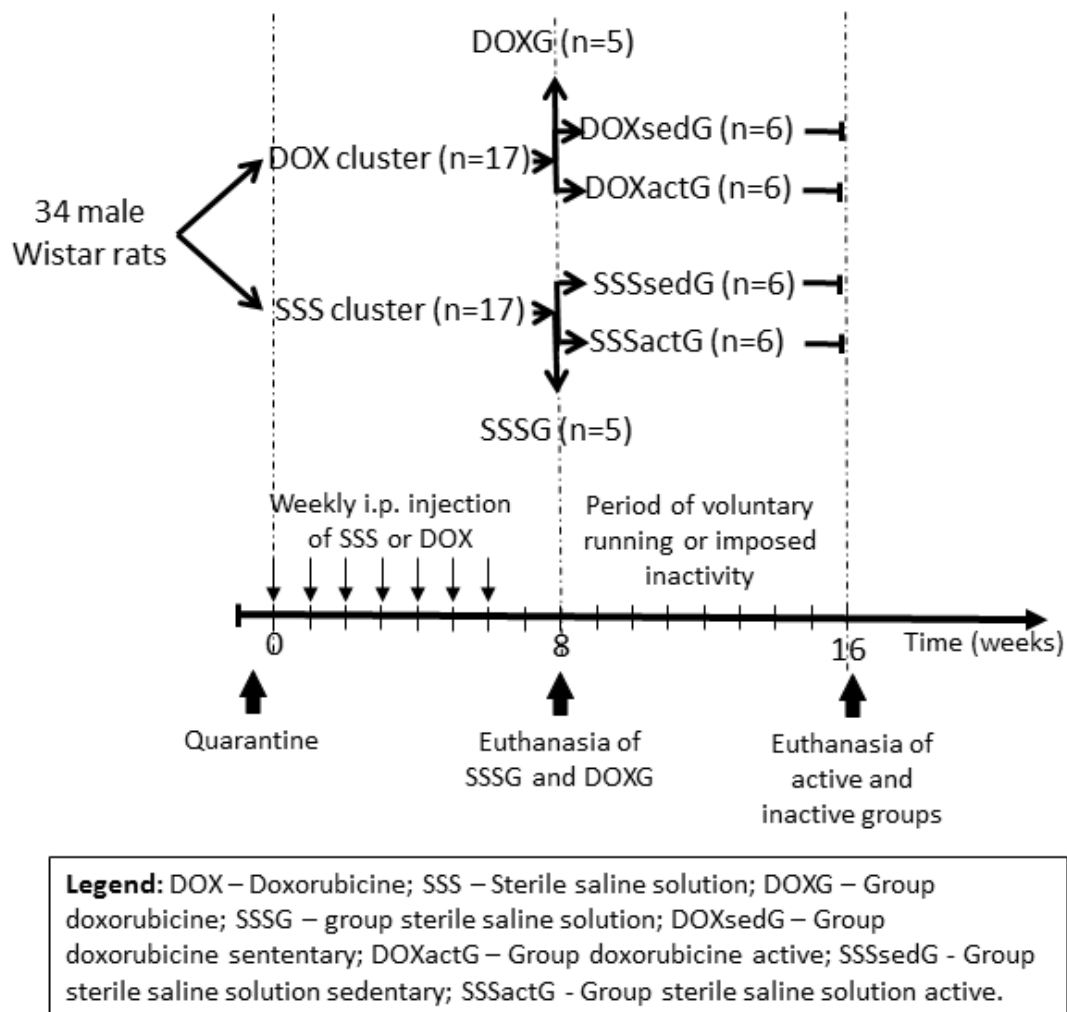

Figure 1: Experimental design.

The body weight of each animal was monitored regularly throughout the experimental period, and all interventions were conducted in accordance with the recommendations of the National Institute of Health (NIH) *Guide for Care and*

Use of Laboratory Animals. Animals, with free access to rodents' food and water, were kept individually under controlled conditions, with a temperature of 22±1°C and 50% of humidity, exposed to an inverted cycle of 12h/12h light/dark.

After 1 week of quarantine, animals were randomly divided into two clusters: 1) the DOX animals (n=17), which received weekly an i.p. injection of doxorubicin (2 mg/kg diluted in 0.5 ml sterile saline; D1515 Sigma-Aldrich Co. LLC) for 7 weeks (with a cumulative dose of 14 mg/kg), and 2) the SSS animals (n=17), which received weekly an i.p. injection of sterile saline solution (0.5 ml) for 7 weeks.

Two weeks after the last injection, 5 animals were euthanized in each cluster, composing the DOXG and SSSG groups for assessment of short-term DOX effects. The remaining animals from the initial clusters were subsequently divided into sedentary (DOXsedG, n=6; SSSsedG, n=6) and active subgroups (DOXactG, n=6; SSSactG n=6). Animals from active groups were individually placed in cages equipped with a running wheel allowing for voluntary running during two months, whereas the activity of sedentary animals was restricted to normal ambulation within their cage space (floor area of 800 cm^2 approximately, Tecniplast, Buguggiate, Italy). The daily running distance (km) was monitored in SSSactG and DOXactG. At the end of the two months period, all animals were euthanized.

Euthanasia and renal sample processing

Animals were anesthetized with an i.p. injection of Xylazine (20mg/kg) and Ketamine (80mg/kg), weighed, and further euthanized by exsanguination. After laparotomy, both kidneys from each animal were excised, quickly washed in saline buffer, superficially dried, and weighed. The kidneys' relative body weight was calculated based on the formula: kidneys' weight x100%/body weight.

From each animal, small pieces of 5 mm^3 from both kidneys were fixed with 4% paraformaldehyde (0.1M PBS, pH of 7.2-7.4, with 2.5% w / v sucrose, and 0.1% glutaraldehyde) during 24 hours at 4ºC. These samples were then washed in PBS (0.1M, pH of 7.2-7.4) and dehydrated through graded ethanol solutions, cleared

in xylene and mounted in paraffin according to routine histological protocols. Sections of 5 µm were cut from paraffin blocks on a microtome (Leica Microsystems, Model RM2125) with a disposable stainless steel blade (Leica Microsystems, Model 819), floated onto warm water (42–44 ºC), and mounted on silane-coated slides (Sigma, S4651-72EA). After dewaxing with xylene and rehydration with graded alcohol, slides were stained with hematoxylin/eosin or with Picrosirius Red, as described elsewhere (Moreira-Gonçalves et al., 2015), and examined in a light microscope (LM) (Carl Zeiss Imager A1 Axio) with a magnification of 400x.

Morphological evaluation

Semi-quantification of kidney damage: for each group, more than 30 tissue sections were analyzed in a blind fashion to evaluate the severity of the following parameters: i) cellular degeneration, ii) interstitial inflammatory cell infiltration, iii) necrotic zones, and iv) alteration of tissue organization (Ascensao et al., 2005b). The severity of cellular degeneration was scored according to the number of cells showing any alterations (dilatation, vacuolization, pyknotic nuclei, and eosinophilic cells) within the complete LM visual field: grade 0 = no change from normal; grade 1 = a limited number of isolated cells (until 5% of the total cell number); grade 2 = groups of cells (5–30% of cell total number); and grade 3 = diffuse cell damage (30% of total cell number). The Inflammatory activity was graded into: grade 0 = no cellular infiltration; grade 1 = mild leukocyte infiltration (1 to 3 cells per visual field); grade 2 = moderate infiltration (4 to 6 leukocytes per visual field); and grade 3 = heavy infiltration by neutrophils. The necrotic level was assessed as: grade 0 = no necrosis; grade 1 = dispersed necrotic foci; grade 2 = confluent necrotic areas; and grade 3 = massive areas of necrosis. The severity of tissue disorganization was scored according to the percentage of the affected tissue: score 0 = normal structure; score 1 = less than one-third of tissue; score 2 = more than one-third and less than two-thirds; score 3 = more of two-thirds of tissue. Since the total score was calculated by the sum of each score

parameter, the highest possible score for each section analyzed was 12 and the lowest was 0.

Assessment of fibrous tissue accumulation: All 204 images from all groups stained with Picrosirius Red were analyzed with Image Pro Plus 6.0 software (Media Cybernectics, Inc). The area occupied by collagen (stained red) within the kidney tissue was quantified for each visual field and expresses in % following the methodology described elsewhere (Moreira-Gonçalves et al., 2015).

Assessment of Bowman's capsule thickness: To measure the thickness of the glomerular capsule, the Image J Software 1.50i (National Institute of Health, USA) was used with a microscale. In each animal, more than 68 Bowman's Capsules were measured on the slides stained with Picrosirius Red. The thickness of capsules was measured in two randomly selected sites, and all values (expressed in µm) obtained were averaged for each slide.

Statistical analysis

The Kolmogorov-Smirnov test allowed investigating within-group normality for the variables. Shapiro-Wilk test was only performed to analyze the normality of the body weight, kidney weight, and relative kidney weight variables. The variables with a normal distribution (values of a total of collagen content, bowman's capsule thickness, body weight, kidney weight and relative kidney weight) were treated with parametric tests, using multifactorial ANOVA (DOX x Time x Exercise). The non-parametric Kruskal-Wallis test was used in variables without normal distribution (Semi-quantification of kidney damage). Results of all parametric data are presented as mean ± standard deviation (SD) and of non-parametric data are presented as median (interquartile range). IBM SPSS Statistics 23.0 was used and differences were considered significant with a $p < .05$.

Results

Sample characterization and voluntary running performed

Table 1 depicts the animals' body weight at sacrifice as well as the kidneys' absolute and relative body weights. The total running distance performed by SSSactG and DOXactG groups is also shown in table 1.

The repeated administration of DOX affected, in a short-term (DOXG vs. SSSG), the normal growth of the animals, shown by the lower body weight of DOXG ($p<.05$), without affecting the absolute and relative kidneys weight ($p>.05$). However, although the absence of significant differences between DOXG and SSSG, an apparent trend to increase kidney volume on DOXG was observed ($p=0.113$).

In long-term, the administration of DOX also promoted higher body weight in sedentary animals (DOXG vs. DOXsedG, $p<.05$) without affecting the kidneys absolute and relative weight.

The voluntary running distance was not different between DOXactG and SSSactG ($p>.05$), suggesting that the ability to perform physical work was not compromised by DOX treatment.

Kidney damage

Data in Table 2 shows the kidney histological alterations, illustrating the toxic effects of DOX at short (SSSG vs. DOXG) and long-term (DOXG vs. DOXsedG). The favorable effects of voluntary running on DOX-induced long-term toxicity are also shown in Table 2 (DOXactG vs. DOXsedG).

Kidneys from animals of SSSG, SSSsedG, and SSSactG showed, in general, a preserved structure, although the presence of some minor and punctual alterations (Fig.1) more notorious in SSSsed animals ($p<.05$ SSSG vs. SSSsedG), which were not attenuated by voluntary running ($p>.05$ SSSsedG vs. SSSactG).

The short-term DOX toxicity was expressed by signals of cellular degeneration (p<.05 DOXG vs SSSG), mainly detected by cytoplasmic vacuolization, eosinophilic cells and pyknotic nuclei, especially affecting the proximal tubules (Fig.1). In these convoluted tubules, the presence of necrotic areas with leukocyte infiltration were also frequent in DOXG (p<.05 vs. SSSG). In DOXG, the loss of tissue organization was notorious by an increased level of vascular congestion, by an apparent general changing of the glomerular structure, and by the presence of interstitial edema (Fig.1), characterized by the increased space between nephron convoluted tubules (p<.05 DOXG vs SSSG). Many cellular debris were visible within the proximal and distal convoluted tubules. The total score of tissue damage was higher in DOXG group (p<.05 DOXG vs SSSG).

At the long-term point of view, as shown in Table 2, the level of necrotic areas, tissue disorganization and the total score of tissue damage increased in DOXsedG comparatively to DOXG (p<.05). In DOXsedG, the existence of fibrinoid material and cellular debris inside dilated convoluted tubules (Fig.1), and a great amount of conjunctive tissue proliferation in the interstitial space were histological characteristics commonly observed.

Although presenting similar kidney structural alterations as DOXsedG (Figs. 2 & 3), the levels of cellular degeneration, necrosis, inflammation, tissue disorganization, and total score of tissue damage were significantly reduced in DOXactG (Table 2), suggesting that voluntary running attenuated the long-term kidney damage induced by DOX treatment (p<.05 DOXsedG vs DOXactG).

Collagen deposition and Bowman's capsule thickness

Figure 4 depicts the percentage of total collagen content in all studied groups. DOX-induced an increase of the percentage of collagen content, at short-term (p<.05 DOXG vs SSSG), without significant variations at long-term (p>.05 DOXG vs DOXsedG). Of note that voluntary running attenuated the collagen deposition on DOXactG (p<.05 DOXactG vs. DOXsedG). One the other hand, the percentage of collagen content was higher on DOXactG comparing to SSSactG

(p<.05), suggesting that voluntary running does not entirely repair/revert the collagen deposition promoted by DOX.

Figure 5 represents the bowman's capsule thickness in all groups. A significant increase in this parameter was observed at short ($p<.05$ SSSG vs DOXG) and long-term ($p<.05$ DOXG vs DOXsedG) after DOX treatment. It must be highlighted that voluntary running decreased the TBC in animals treated with DOX ($p<.05$ DOXsedG vs DOXactG).

Discussion

The obtained results clearly show the harmful effects of DOX administration mimicking a chemotherapy treatment on kidney structure, expressed by histological alterations after two weeks the last injection of DOX, with a progressive aggravation over time and with enhancement of collagen content. Of note that these injuries are very similar to those already described in humans after DOX treatment (Turnberg et al., 2006), suggesting analogous pathophysiological mechanisms, among which might be oxidative stress (Abo-Salem et al., 2009). If this was the picture in sedentary animals, in active animals the structural alterations were partially attenuated.

Short-term DOX toxic effects

Regarding the control animals, treated with SSS, they showed, in general, kidneys with a preserved morphological structure, although the existence of some punctual and disperse alterations, mainly expressed by cellular vacuolization in proximal tubules, which can be explained by the functional wear and/or even by the histology technical procedures. In opposition, animals treated with DOX increased their body weight and revealed an altered kidney structure with signals of cellular degeneration, necrosis, inflammation, with a general increase of collagen deposition and the TBC.
At short-term, the damage induced by DOX mainly affected the cells of proximal convoluted tubules, which might be explained, among other factors, by their richness in mitochondrial content, the main target organelle of this drug (Su et al., 2015). In these proximal tubular cells, it is described that DOX activate caspase-3 through the mitochondrial pathway, triggering apoptosis (Su et al., 2015). Of note that these histological abnormalities are in accordance with the already described in the literature, with several works highlighting the glomerular and tubular damage induced by DOX, with degenerative changes and vacuolization on cytoplasm of proximal tubules' cells, but also affecting glomeruli (Ayla et al., 2011; Sadek et al., 2016; Zickri et al., 2012). The renal injury might initially be

triggered by an enhanced mitochondrial reactive oxygen species production (Abo-Salem et al., 2009), followed by an inflammatory response with infiltrating inflammatory cells induced by DOX (Liu et al., 2016; Sadek et al., 2016). One the other hand, in several cortical regions, a local tubular dilatation was mainly observed in distal tubules, with their lumen fulfilled with hyaline material and cellular debris, suggesting the occurrence of tubular obstruction probably motivated by protein precipitation and deposition of debris derived from upstream cellular necrosis affecting proximal tubules. Even after two weeks the last DOX injection, a slight increase of collagen content was already observed in interstitial space, affecting kidney cortex and medullary areas. This extensive fibrosis might be due to the DOX-induced enhanced production of transforming growth factor beta (TGF-β) (Ren et al., 2016), a cytokine mainly produced by renal tubular epithelial cells and interstitial cells (Li et al., 2006), which plays a central role in the progression of the tubular epithelial-mesenchymal transition in renal fibrosis-inducing extracellular matrix deposition (Ren et al., 2016). The occurrence of a continuous inflammatory tissue reaction, favoring the movement of proteins from the vascular space to the interstitium with the formation of a fibrin network, may also contribute, directly or indirectly, to the observed general renal fibrosis (Liu, 2011). Massive fibrosis avoids the integral reconstruction and functionally of tissue and organs. Of note that, inflammation and fibrosis are critical roles of renal diseases (Pohlers et al., 2009). In addition, DOX was also responsible for increasing the TBC comparatively with SSSG, may be by the general activation of collagen production or due to the high mechanic tension on the glomeruli.

Voluntary running attenuates long-term DOX toxic effects

At long-term, the body weight of animals increased on DOXsedG group, even without affecting the work capacity as expressed by the absence of significant differences in running distance between SSactG and DOXactG. The damage induced by DOX on DOXsedG was more evident and aggravated comparatively to DOXG. After two months of last DOX administration, a more accentuated and dispersed tubular dilatation, with lumen fulfilled with hyaline material and cellular

debris were found. This alteration was also described in recent studies, however, only a single injection of DOX was applied in rat models (El-Moselhy & El-Sheikh, 2014; Sadek et al., 2016). The aggravation with time of the lesions initially found in DOXG suggests the existence of an active pathophysiological process that does not finish with the end of DOX treatment. Moreover, the signals of an active inflammatory reaction observed in DOXsedG reinforce this suggestion. Nevertheless, the levels of collagen content remained similar to those observed in DOXG. Although kidney functionality was not assessed in our study, it is known that large amounts of collagen content are associated with renal dysfunction and failure (Ren et al., 2016; Sadek et al., 2016). Even without long-term aggravation of fibrosis, the TBC on sedentary group increased comparing to DOXG group, highlighting the long-term renal toxicity induced by DOX. Once the collagen content did not increase at long-term, the speculation of general activation of conjunctive tissue production maybe is not the most acceptable theory. While, in DOXsedG group high deposition and an increased of the debris inside the tubules was visible, which could explain the higher tension on glomeruli. Debris deposition may clog the tubules preventing the blood circulation and rise the thension, as result of this tension the TBC increased.

In the current study, the active group treated with DOX, compared with sedentary group, showed reduced levels of cellular degeneration, inflammatory activity, necrosis and tissue disorganization; moreover, DOXsedG evidenced a decreased collagen deposition and bowman's capsule thickness. However, a total reversing and restoring of kidney normal structure was not verified. Voluntary running attenuated kidney damage on DOXactG, probably due to the benefits of exercise on the immune system (Miyagi et al., 2014), improving cell defenses. Therefore, this immune system protection through the exercise, reduce the apoptotic cells levels. In addition to the last point, the enhancement of vascularization and the existence of lower levels of cytokines after RPE practice could explain, at least in part, the decrease the inflammatory response observed in these animals. Moreover, the decrease of cytokines levels also allows to regulate the antioxidant defense system (Zeynali et al., 2015) not compromising the renal function. Among the other cytokines, exercise also normalized the

expression of TGF-β (Peng et al., 2012b) reducing the inflammatory process suggesting a lower collagen deposition on kidneys. The reduction of Bowman's capsule thickness observed in DOXactG can be explained by a lower glomerular filtration tension and also by the lower collagen deposition. After RPE practice, all these physiological benefits on kidneys, allow to prevent or/and reduce the toxic effects induced by this drug. However, the mechanisms of the exercise protection on kidney toxicity induced by DOX are poorly understood. In humans, is possible to verify the same structural changes after a prolonger DOX administration (Peng et al., 2012b; Sadek et al., 2016). Fortunately, exercise could improve kidney function in humans by improving metabolic factors (Moinuddin & Leehey, 2008).

In conclusion, DOX has a severe toxic side effects even short or long-term. Although, two months of voluntary running could be considered as favorable to ameliorate this side effects induced by DOX. Based on these results, the prescription of RPE for cancer survivors must be considered to attenuate the side effects of the treatments. However, more studies are needed to explain the mechanisms of DOX-induce nephrotoxicity as well as the mechanisms of protection promoted by regular exercise.

Table 1: Values (presented as mean±standard deviation) of body weight and kidney absolute and relative weight from all groups, and total running distance performed by active groups.

	Sterile Saline Solution			Doxorubicin		
	SSSG	SSSsedG	SSSactG	DOXG	DOXsedG	DOXactG
Body Weight (g)	386.4 ±29.70 [a]	434.7 ±57.46	422.7 ±29.70	312.8 ±58.32 [b]	393.8 ±74.62	363.2 ±46.61
Kidney Weight (g)	2.1 ±0.68	2.3±0.20	2.3±0.40	2.0±0.34	2.4±0.55	2.5±0.82
Relative Kidney Weight (%)	0.5±0.16	0.5±0.08	0.5±0.07	0.7±0.22	0.6±0.08	0.7±0.17
Total running distance (Km)	-	-	23.9±6.99	-	-	30.5±19.84

Notes: SSSG- Group Sterile saline solution; SSSsedG- Group Sterile saline solution sedentary; SSSactG- Group Sterile saline solution active; DOXG- Group Doxorubicin; DOXsedG- Group Doxorubicin sedentary; DOXactG- Group Doxorubicin active.

[a] $p < 0.05$ vs DOXG;

[b] $p < 0.05$ vs DOXsedG

Table 2: Values (presented as a Median (Interquartile Range)) of histologic alterations used to assess kidney damage in all groups.

	SSSG	DOXG	SSSsedG	DOXsedG	SSSactG	DOXactG
Cellular Degeneration	0(1)	3(1) [a]	1(1) [b]	3(1)	0(1) [d]	1(1) [b]
Necrosis	0(0)	2(1) [a]	1(1) [b]	3(1) [c]	0(1) [d]	1(1) [b]
Inflammatory Activity	0(0)	2.50(1) [a]	1(1) [a b]	3(1)	0(1) [d]	1(1) [b]
Tissue Disorganization	0(1)	2(0) [a]	1(1) [b]	3(0) [c]	0(1) [d]	1(1) [b]
Total Score	1(1)	10(2) [a]	2(0) [a b]	11(3) [c]	2(1) [d]	5(1) [b]

Notes: SSSG- Sterile saline solution group; DOXG- Doxorubicin group; SSSsedG- Sterile saline solution sedentary group; DOXsedG- Doxorubicin sedentary group; SSSactG- Sterile saline solution active group; DOXactG- Doxorubicin active group.

[a] $p < .05$ vs. SSSG

[b] $p < .05$ vs. DOXsedG

[c] $p < .05$ vs. DOXG

[d] $p < .05$ vs. DOXactG

Figure 2: Representative photomicrographs stained with hematoxylin and eosin of kidneys from group sterile saline solution (SSSG), group sterile saline solution sedentary (SSSsedG), group sterile saline solution active (SSSactG), group doxorubicin (DOXG), group doxorubicin sedentary (DOXsedG), and group doxorubicin active (DOXactG). On DOXG a large amount of necrotic cells was verified; interstitial edema was observed on DOXsedG and DOXactG; and tubular dilatation with hyaline deposition on DOXsedG.
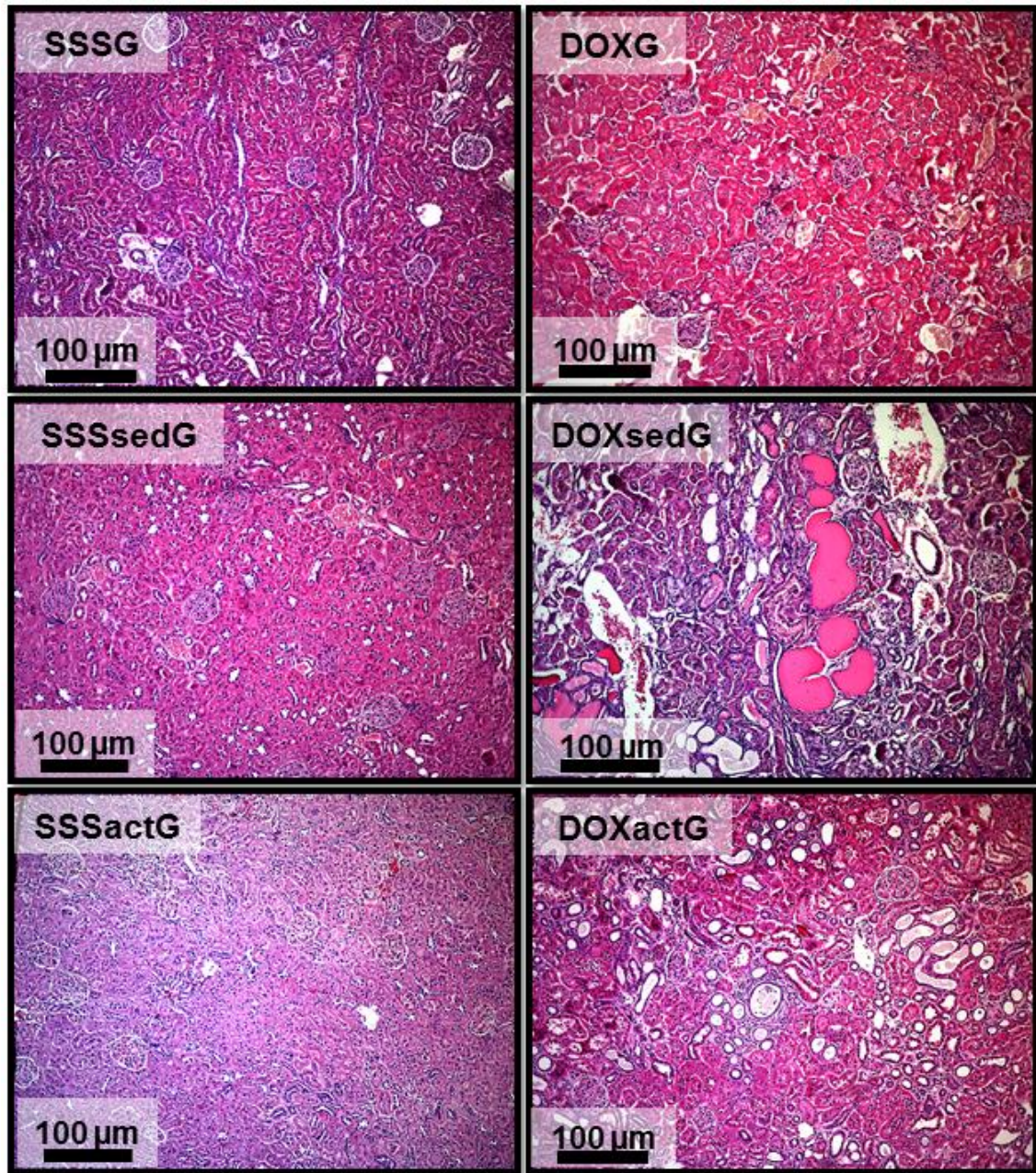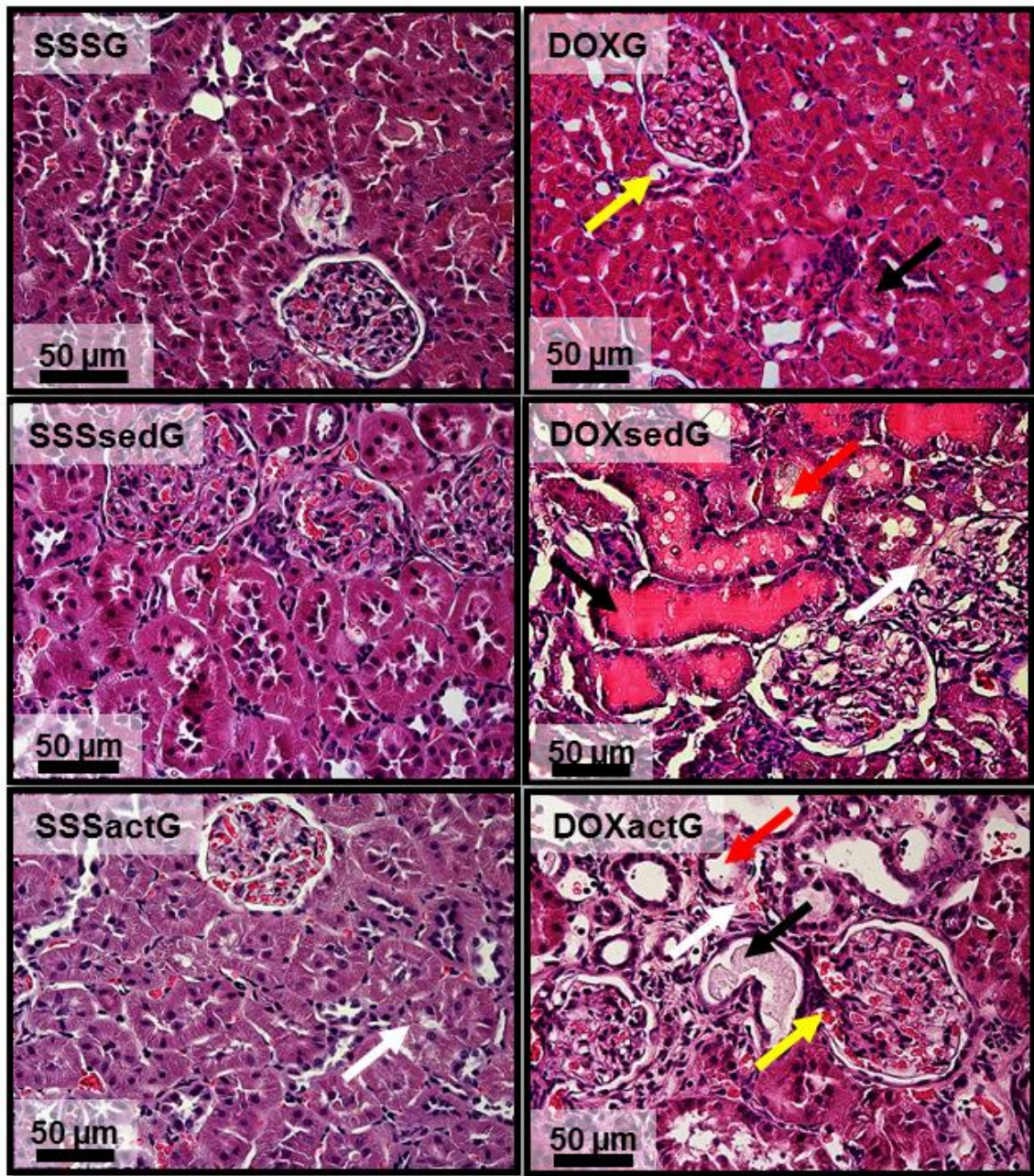
Figure 3: Representative photomicrographs stained with hematoxylin and eosin of kidneys from group sterile saline solution (SSSG), group sterile saline solution sedentary (SSSsedG), sterile group saline solution active (SSSactG), group doxorubicin (DOXG), group doxorubicin sedentary (DOXsedG), and group doxorubicin active (DOXactG). On DOXG was verified the existence of edema (Yellow arrow) and vacuolated cells (Black arrow). A deposition of hyaline (Black arrow), a distal tubular dilatation (Red arrow) and conjunctive tissue (White arrow) was observed on DOXsedG. In DOXactG the hyaline content in tubules (Black arrow) was less than the last group, while were also found tubular dilatation (Red arrow). It is noted the conjunctive tissue around the tubules (White arrow) and vascular congestion (Yellow arrow).
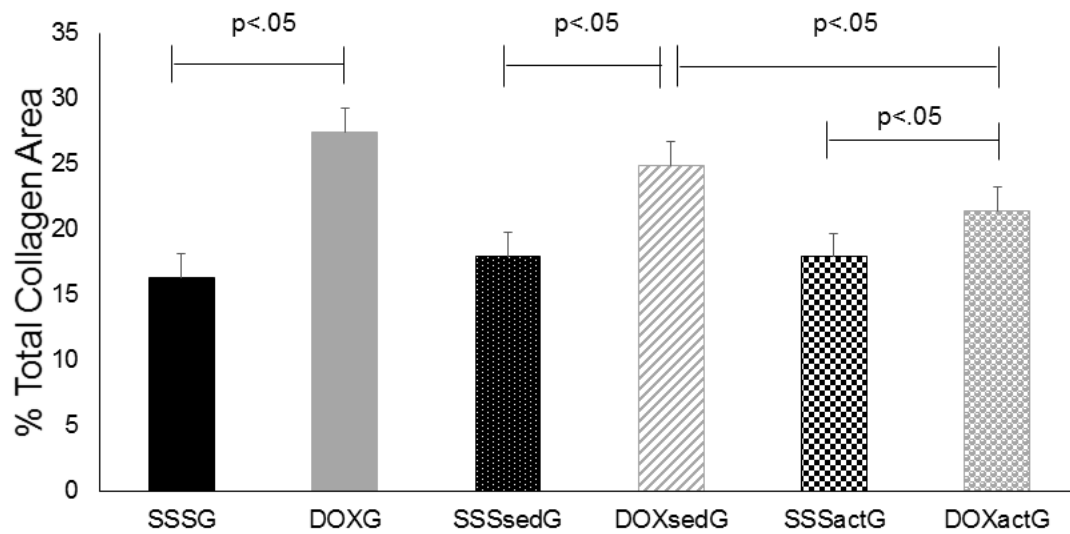
Figure 4: Values (presented as mean±standard deviation) of the percentage of total collagen deposition in kidney area in group sterile saline solution (SSSG), group sterile saline solution sedentary (SSSsedG), group sterile saline solution active (SSSactG), group doxorubicin (DOXG), group doxorubicin sedentary (DOXsedG), and group doxorubicin active (DOXactG).

Figure 5: Values (presented by mean standard deviation) of thickness of the Bowman's capsules of the kidneys in group sterile saline solution (SSSG), group sterile saline solution sedentary (SSSsedG), group sterile saline solution active (SSSactG), group doxorubicin (DOXG), group doxorubicin sedentary (DOXsedG), and group doxorubicin active (DOXactG).
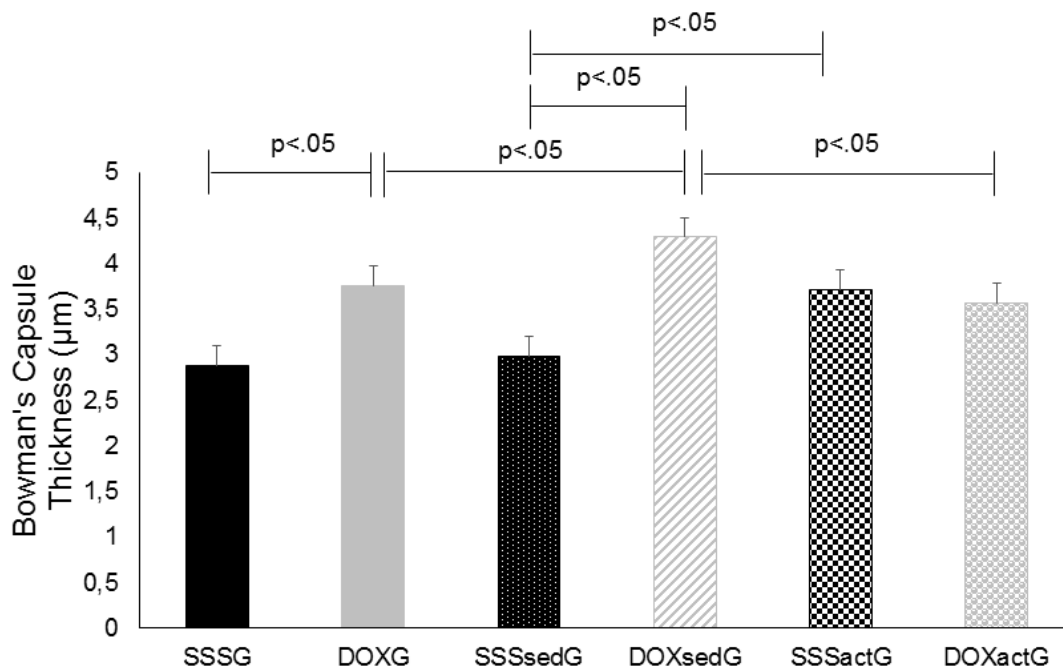
4. Main Conclusions

Assuming the proposed goals on the first chapter, at short-term DOX was responsible for induced:

- Renal damage
- An Increased collagen deposition
- An increase of TBC

While, at long-term:

- Increased renal damage
- Increased the TBC

One the other hand, regular voluntary running was responsible for attenuated the side-effects induced by DOX:

- Ameliorated kidney damage
- Decreased collagen content
- Decreased the TBC

The results are in line with our previews hypotheses. DOX-induced side-effects such as renal damage, increase collagen deposition and the TBC at short-term and in long-term only aggravated renal damage and increased the Bowman's capsule thickness. However, regular voluntary running could be used as a therapy to attenuate the harmful effects caused by a prolonged DOX administration, improving renal structure and function.

Despite these results, as a suggestion for future studies, more studies are needed to understand the mechanisms of DOX-induced kidney toxicity and which physiological mechanisms are underlying of physical exercise to attenuate the renal toxicity induced by DOX.

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