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Physical exercise contributes to cisplatin-induced nephrotoxicity protection with decreased CD4+ T cells activation

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ABSTRACT

Cisplatin is a chemotherapy used to treat different types of cancer, such as testicular, bladder and head and neck. Physical exercise has been shown to improve cancer therapy and recently, it was demonstrated to be able to diminish side effects such as acute kidney injury (AKI), a common side effect in cisplatin treatment. In both cases, the modulation of inflammatory cytokines seems to be one of the mechanisms, but little is known about the immune cells in this process. Here, we investigated the role of CD4 + T cells in the AKI protection by physical exercise. We subjected C57Bl6 mice to long-term physical exercise (EX) before cisplatin treatment. Sedentary groups were used as control (CT). We confirmed that physical exercise decreased AKI by evaluating creatinine and Kim-1 levels, in the serum and kidney respectively. Analyzing the organs weight, we noticed a decrease in sedentary (CIS) and exercised (CIS-EX) cisplatin treated groups. Epididymal and brown adipose tissue weight were decreased in cisplatin treated subjects in comparison to untreated groups, as well as liver and spleen. We then investigated the profile of CD4 + T cells in the spleen and we observed increased levels of Tregs and CD4+CD25+ cells in CIS group, while CIS-EX presented similar amounts as control groups. Analyzing the kidney lymph nodes, we noticed a decrease of CD4+ cells in both CIS and CIS-EX group. However, a more activated phenotype (CD69+ and CD25+) was observed in CIS groups in comparison to CIS-EX group, as well as the presence of Tregs. We then investigated the production of cytokines by these cells and no difference among the groups was observed in cytokines production in splenic CD4 + T cells. However, a clear increase in TNF and IL-10 production was observed in CD4 + T cells from lymph nodes, while CIS-EX group presented similar levels as the control groups. We confirmed that physical exercise was able to diminish cisplatin-induced AKI with concomitant decrease in CD4 + T cell activation.

1. Introduction

Chemotherapy is still one of themain and most efficient strategies in cancer treatment. Cisplatin is a small-molecule that binds to DNA inducing DNA damage (Siddik, 2003). It has been used as therapy in testicular, ovarian, bladder, head and neck cancer and also in lymphomas, sarcomas and multiple myelomas (Pabla and Dong, 2008). Although broadly used, one of most common side effects is kidney toxicity [2]. Cisplatin-induced acute kidney injury (AKI) involves inflammatory and immune response (Ozkok et al., 2016). T cells are the main players of adaptive immune system, and there are two major groups: T helper (CD4+) and cytotoxic (CD8+) cells. These cells are

Abbreviations: CT, sedentary control group; EX, exercised control group; CIS, cisplatin treated sedentary group; CIS-EX, cisplatin treated exercised group; AKI, acute kidney injury

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known to promote AKI (Kinsey and Okusa, 2014) and they are responsible for orchestrating the inflammatory response mediated by innate immune cells such as neutrophils (Zwacka et al., 1997). Renal cells injured by cisplatin produce IL-33 that attracts and activates CD4 + T cells to the kidney (Akcay et al., 2011). T cell immunoglobulin mucin 1 (Tim-1, also known as Kim-1) modulates T cell response, but it is also associated with damaged proximal tubules in the kidney. Nozaki et al., used anti-Kim-1 to diminish cisplatin-induced AKI, and using Rag knockout mice they have proved that the protection observed in anti-Kim-1 treatment was dependent on T cells (Nozaki et al., 2011). Physical exercise was shown to prevent cancer development (Friedenreich and Orenstein, 2002) and diminish side effects such as cachexia (Batista et al., 2013) and nephrotoxicity (Miyagi et al., 2014; Zeynali et al., 2015). It has been associated with anti-inflammatory response (Ford, 2002), however, the effects of physical exercise on T cells are not completely understood. It was shown that exercise increased CD4 + T cells of a subtype known as Treg, which is a CD4 + T helper cell with the ability to suppress immune response (Handzlik et al., 2013), while in patients with breast cancer treated with chemotherapy, exercise was reported to induce CD4 + CD69 + T cells, suggesting an increase in T cell activation. Although T cells act upon cisplatin-induced AKI, little is known about its modulation under physical exercise. We have previously shown that aerobic exercise was able to diminish cisplatin-induced cachexia and AKI, by inducing HO-1 and modulating IL-6 expression (Miyagi et al., 2014). Here, we investigated whether T cells could also be involved in aerobic exercise protection against cisplatin-induced AKI.

2. Objective

Our aim was to investigate whether physical exercise could protect against renal dysfunction by modulating CD4 T cells.

3. Methods

3.1. Animals

We used C57Bl6 6–8 week old male mice provided by UNIFESP-CEDEME. The animals were kept in individual cages in a 12/12 h cycle of light at 25 °C with food and water *ad libitum*. Protocols were approved by The Ethics Committee of the Institute of Biomedical Sciences of the University of Sao Paulo (number 046 over sheet 102 of the book 02, on April 20th, 2011).

3.2. Physical exercise

Aerobic exercise was performed on a treadmill training protocol described previously by Miyagi et al. (2014). Briefly, animals were subjected to pre-training for 3 days to select mice that were likely to exercise. Animals were divided in the following groups: sedentary control group (CT, n = 9), exercised group (EX, n = 10), cisplatin sedentary group (CIS, n = 8) and cisplatin exercised group (CIS-EX, n = 11). Animals (EX and CIS-EX) followed a training protocol for 5 weeks (Fig. 1 and Table 1). Kidney injury was induced by single i.p. dose of cisplatin (20 mg/kg – "Citoplax" Bergamo©). After 96 h of drug administration, mice were anesthetized with Ketamine-Xylazine i.p. and immediately sacrificed, organs were collected, weighed, frozen in liquid nitrogen and stored at -80 °C for subsequent measurements (Fig. 1 and Table 1).

3.3. Kidney injury

AKI was accessed by serum creatinine levels using colorimetric assay (Labtest, Brazil) and read with a Synergy microplate reader (BioTek, USA). Kidney damage was also evaluated by the expression of the molecule Kim-1 in the kidney. RNA was isolated from kidney by using TRIzol Reagent (LifeTechnologies, USA) according to the manufacturer's protocol. We synthetized double-strand cDNA with SuperScript III RT (Life Technologies) and analyzed Kim-1 (also known as *havcr1* – Taqman Mm00506686, Themo) expression with qPCR. Inflammatory T helper cytokines were also evaluated by qPCR, such as IFN gamma (Taqman Mm00801778, Thermo) and IL-17 (Taqman Mm00439619, Thermo). We used the ABI Prism 7300 sequence detection system (Thermo). The expression of housekeeping *hprt* (Taqman Mm00446968, Thermo) gene was used to normalize the samples RNA expression.

3.4. Cell phenotype

Spleens and kidney draining lymph nodes were collected in PBS and they were individually smashed using 70μm cell strainers. Splenocytes were treated with 4 ml of lysis buffer (NH4Cl 155 mM, NaHCO3 10 mM and EDTA 0.1 mM, pH 7.2) per spleen at room temperature for 2 min. After this step, the same procedure was applied to lymph node cells and splenocytes. Cells were washed and stained with anti-CD4-Pacific Blue, anti-CD25-PECy7 and anti-CD69-PercP. Cells were fixed with Cytofix/Cytoperm (BD biosciences) and permeabilized with Perm wash (BD biosciences) and stained with anti-Foxp3-FITC, anti-TNF-APC and anti-IL-10-PE (Biolegend and BD biosciences). The acquisition of the samples was performed using FACSCanto II (BD biosciences) and analysis with FlowJo software.

3.5. Statistical analysis

We employed one-way analysis of variance (ANOVA) for the statistical analysis and significance was evaluated with the Bonferroni's post-test. Data were plotted with GraphPad Prism 5 software.

4. Results

4.1. Physical exercise diminished cisplatin-induced AKI

Animals were subjected to aerobic physical exercise (EX and CIS-EX) and after six-weeks training, cisplatin was injected in sedentary (CIS) and exercised groups (CIS-EX). Sedentary group without cisplatin injection was used as control (CT). Histological analysis CT and EX groups revealed no evidence of inflammation with discrete peritubular capillary congestion (Fig. 2A, B), while in CIS and CIS-EX group we observed signs of renal tubulopathy such as larger and/or pleomorphic nuclei alterations, but with not clear difference between CIS ans CIS-EX

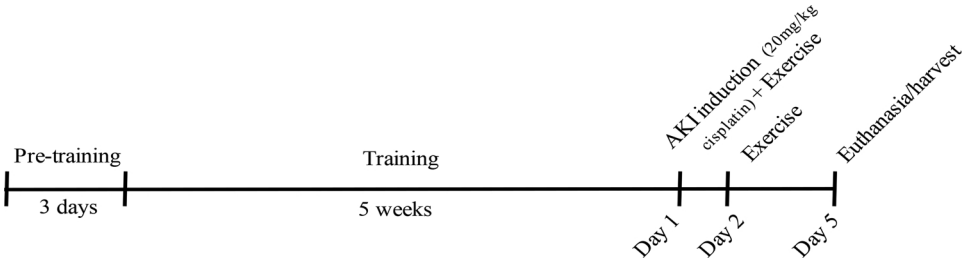

Fig. 1. Training protocol. Mice were submitted through adaptation for 3 days (at a speed of 15 m/min or 20 m/min for 30 or 35 min). After that, mice trained for 5 weeks (according to Table 1), then, at day 1, we induced AKI with 20 mg/kg of cisplatin and mice trained for 60 min (at a speed of 10 m/min). At day 2, mice trained for 60 min at the same speed as the previous day. Mice were euthanized and material was harvest at day 5.