How to cite: Sagrillo LM, Zorzi VN, Royes LFF, Fighara MR, Bonadiman BSR, Cattani MFMR, *et al.* Physical exercise protects dynamic balance and motor coordination of rats treated with vincristine. Rev Bras Fisiol Exerc 2020;19(5):336-349. https://doi.org/10.33233/rbfex.v19i5.4220

RBFEx
ISSN Online: 2675-1372
ISSN Printed: 1677-8510

Brazilian Journal of Exercise Physiology

Original Article

Physical exercise protects dynamic balance and motor coordination of rats treated with vincristine

O exercício físico protege o equilíbrio dinâmico e coordenação motora de ratos tratados com vincristina

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ABSTRACT

Introduction: Physical exercise has been shown to be an important modulator of the antioxidant system and neuroprotective in several diseases and treatments that affect the central nervous system.
Aim: to evaluate the effect of physical exercise in dynamic balance, motor coordination, exploratory locomotor activity and in the oxidative and immunological balance of rats treated with vincristine (VCR).
Methods: 40 adult rats were divided into two groups: exercise group (6 weeks of swimming, 1h/day, 5 days/week, with overload of 5% of body weight) and sedentary group. After training, rats were treated with 0.5mg/kg of vincristine sulfate for two weeks or with the same dose of 0.9% NaCl. The behavioral tests were conducted 1 and 7 days after each dose of VCR. On day 15 we carried out the biochemical analyzes of the cerebellum.
Results: The physical exercise was able to protect against the loss of dynamic balance and motor coordination and, had effect per se in the exploratory locomotor activity, and neutralize oxidative stress, damage DNA and immune damage caused by VCR up to 15 days after the end of the training protocol.
Conclusion: we observed that previous physical training protects of the damage motor induced by vincristine.

Key-words: Exercise, Oxidative Stress, Neuroprotection, Cerebellum.

RESUMO

Introdução: O exercício físico tem se mostrado um importante modulador do sistema antioxidante e neuroprotetor em diversas doenças e tratamentos que afetam o sistema nervoso central.
Objetivo: avaliar o efeito do exercício físico no equilíbrio, coordenação motora, atividade locomotora exploratória e no balanço oxidativo e imunológico de ratos tratados com Vincristina.
Métodos: 40 ratos adultos foram randomizados em dois grandes grupos: grupo exercício (6 semanas de natação, 1h/dia, 5 dias/semana, com sobrecarga de 5% do peso corporal) e grupo sedentário. Após o protocolo de treinamento, os ratos foram tratados com uma dose semanal de 0,5mg/kg de sulfato de Vincristina (2 semanas) ou com a mesma dosagem de NaCl a 0,9%. Os testes comportamentais foram realizados 1 e 7 dias após cada dose de Vincristina. No dia 15 foram realizadas as análises bioquímicas do cerebelo.
Resultados: O exercício físico foi capaz de proteger contra a perda de equilíbrio e coordenação motora e teve efeito per se na atividade locomotora exploratória. Neutralizou o estresse oxidativo e reduziu o dano imunológico e de DNA causados pela Vincristina até 15 dias após o término do protocolo de treinamento.
Conclusão: observou-se que o treinamento físico prévio protege do dano motor induzido pela Vincristina.

Palavras-chave: Exercício Físico, Estresse Oxidativo, Neuroproteção, Cerebelo.

Received on: June 23, 2020; Accepted on: August 4; 2020.

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Introduction

Cancer is a disease that causes more death in developed countries [1]. Due to the population growth and aging, the incidence rate is projected to increase worldwide, particularly in countries less developed, where 82% of the world population lives [2].

In addition to being a high-cost annual investment, cancer treatment is considered one of the most challenging problems in medicine. Antitumor drugs most often have a lower therapeutic index which means the therapeutic dose very close to the toxic dose [3]. vincristine (VCR) is an anti-mitotic function chemotherapy has become extensively incorporated into multi-agent chemotherapy regimens for a vast number of malignancies including acute lymphoblastic leukemia, lymphomas, sarcomas, neuroblastoma, and kidney, liver, lung, brain and breast tumors amongst others. It is the most commonly used in pediatric patients, despite its great performance, frequently has dose-limiting neurotoxicity which can be devastating [4]. The side effects can be changes in balance through vestibular damage due to aggression to the eighth cranial nerve, dizziness, nystagmus, vertigo [5] and peripheral neuropathy [4].

Although the molecular mechanisms of neurotoxicity induced by chemotherapy have not yet been fully elucidated, studies have shown an increased reactive oxygen species (ROS) production and increased lipid peroxidation in the brain tissue of rats exposed to VCR. Thus indicating an association between chemotherapy and increased oxidative stress, which may lead to a reduction of the viability of the neurons [6,7]. In this sense, therapies that positively modulate the antioxidant system may be important tools in the treatment of various diseases and toxicities that affect nerve system.

There is evidence of the general health benefits of regular physical exercise, both in healthy people and in various diseases [8], which vary with the duration and intensity of the exercise, as well as with age, previous health status, and gender practitioner [9]. Physical exercise is able to modulate the immune system, increasing the number of natural killer cells and circulating lymphocytes, thus decreasing the body's chances of contracting infections [10]. Under these conditions, the immune system would be better qualified to fight cancer.

Studies in experimental models have shown that physical exercise also positively modulates the antioxidant system, increasing the content and/or activity of the enzymes superoxide dismutase, catalase and glutathione peroxidase (SOD, CAT and GPx) in both muscle and brain of rats [11,12], as well as, improves the survival rate of Purkinje neurons by decreasing the expression of reactive astrocytes [13]. Ozbeily *et al.* [14] have demonstrated the potential protective role of swimming exercise (chronic effect), that reduced stress-induced brain oxidative damage, as well as, improved levels of anxiety and cognitive functions. However, is not completely understood the effects of previous physical exercise on the protection of cerebellar damage after chemotherapy with VCR. Thus, the objective of this study was to test the hypothesis that physical exercise changes dynamic balance and motor coordination, exploratory locomotor activity, and the oxidative and immunological balance of rats treated with VCR.

Methods

The manuscript was written in accordance with the ARRIVE guidelines.

Animals and ethics statement

To perform this study, we used male Wistar rats provided by the Central Biothery of the Federal University of Santa Maria. The rats were young adults and weighed between 270 and 300g. They were kept in a light-dark cycle of 12 hours at a temperature of 22 ± 1ºC, with food and water ad libitum. All protocols were submitted to the evaluation by the Ethics Committee of the Federal University of Santa Maria (090/2014). And the number of animals used was as minimal as possible to provide consistent effects of our results.

Experimental design

For this study were designed two steps protocols: 1) Physical exercise: the animals were divided into two large groups: exercise (n = 20), submitted to a 6-week swimming protocol, and sedentary (n = 20). 2) Drug therapy: the animals were divided in four studies groups: exercise + VCR; exercise + saline; sedentary + VCR; sedentary + saline. On the first day after the end of step 1 protocol (day 0), in each large group, the rats were treated intraperitoneally with Vincristine (VCR; n = 10) or with saline solution (Saline; n = 10) at a dose of 0.5mg/kg. This treatment was repeated after seven days. On days 1 (24 hours after the first dose of VCR), 7 (seven days after the first dose), 8 (24 hours after the second dose) and 15 (seven days after the second dose) the animals were submitted to evaluation tests exploratory locomotor behavior and body balance. At day 15, the animals were euthanized and cerebellar and blood tissues were collected for biochemical analysis (Figure 1).

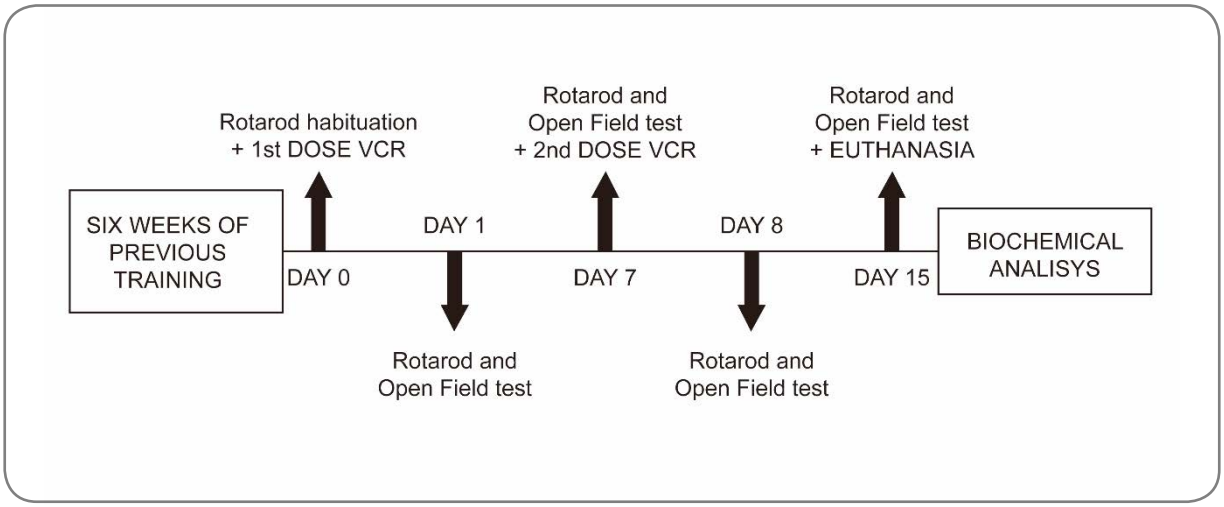


Figure 1 - Experimental design.

Physical exercise protocol

The protocol performed here was based on Gobatto *et al.* [15] and Souza *et al.* [16] studies. In the six-week period, the rats selected for the exercise group were submitted to swimming training, performed on a circular plastic drum (diameter, 120cm, depth, 90cm) filled with water maintained at a temperature of 32 ± 2ºC. The training consisted of daily swimming for 60 minutes, five days a week, for six weeks, between 9:00 and 11:00 am. The first week was an adaptation period to swimming without weights. After that, the rats were submitted to swimming training with a workload

(5% of body weight) to improve resistance. The sedentary group of rats were placed in a separate tank with little water (5cm deep) at 32ºC ± 2ºC, 5 days/week, with no extra load. When completed the six-week protocol, both groups received either VCR or saline treatment, according to randomization.

Drug treatment

Vincristine sulfate was dissolved in saline solution (0.9% NaCl) and administered at two doses of 0.5mg/kg (calculated daily body weight), with a one-week interval between them, generating a cumulative dose of 1.0mg/kg. The control group received the same saline solution dosage alone. For the administration of chemotherapy, was chosen the intraperitoneal route, since the kinetics of the drug is much similar the intravenous route, used in humans [17].

Behavior tests

Rotarod test

In the rotarod test (Harvard Apparatus, Holliston, MA, USA) the rats were individually placed on the top of the rotating rod (diameter 4cm), so that forward locomotion was necessary to avoid a fall. The animals were tested using a constant speed of 18rpm for a maximum time of 5 minutes. The latency for the fall was automatically recorded by a magnetic plate contained in the apparatus itself. In the first two days after the end of swimming protocol, to each rat was allowed to become familiar with the device at a constant speed of 18rpm [18]. The test was performed on days 1, 7, 8, and 15 and included an initial test, with no drug treatment, for baseline assessment (day 0).

Exploratory locomotor activity

To evaluate the exploratory and spontaneous locomotor activity of rats, we used the open field test as described by Shabani *et al.* [18] and modified in this study. Briefly, the animals were placed in a square area (56cm wide x 56cm long x 20cm high), with subdivisions on the floor indicating small quadrants that defined the central and peripheral areas. The locomotion, in the field, was tracked over a 4-min period and, recorded using a high-resolution monochrome camera. The results were stored and analyzed with Ethovision Software (v.8). Total distance traveled (mm) and number of crossings (n) were calculated for later comparison between groups [18]. The open field test evaluation was performed after the drug administration on days 1, 7, 8 and 15, to observe whether behavioral changes among groups were time-dependent.

Ex vivo assays

At day 15, seven days after the last treatment, the animals were euthanized by decapitation. Blood tissue was collected and, the cerebellum was exposed by removal of the parietal and occipital bones and frozen at -80°C for structure preservation and, subsequent biochemical analyses.

Dihidroclorofluorsceína (DCFH) measure

The potential generation of ROS through the treatments was monitored using a fluorescent agent, 2,7'-dichlorofluorescein (DCF). This assay was based on the following chemical assumption: dichlorofluorescein diacetate (DCFH-DA) is capable of diffusing through cell membranes [19]. Once within the cells this molecule

is deacetylated by the action of the intracellular esterase enzymes forming dihydrochlorofluorescein (DCFH) a non-fluorescent product. DCFH in the presence of ROS is oxidized (preferably peroxides, hydroperoxides and NO) to a highly fluorescent dichlorofluorescein (DCF). Thus, the higher absorbance detected, which means the higher fluorescence, the greater the occurrence of oxidizing compounds. The DCFH results were expressed in percentage of the control.

Free DNA by fluorimeter - Picogreen

For DNA fragmentation analysis, was used the picogreen method. This technique quantifies the DNA released in the medium due to cellular apoptosis to detect cytotoxicity. It may also be used as a measure of genotoxicity when exposing a pure DNA molecule to a particular compound. To this technique, blood plasma was used and the procedures following out the protocol described by Ha *et al.* [20]. Briefly, in 1.5mL microtube were added 80µL of 1X TE buffer, 10µL of the sample and 10µL of the diluted picogreen reagent (1:10), and homogenized 10 times. Samples were incubated at room temperature for 5 to 30 minutes. Then, samples were read in the fluorimeter at wavelengths: 480nm excitation, 520nm emission. The control group samples contained a known dsDNA. The assay was performed in triplicate. The interpretation of the values obtained is given in such a way that, the higher the fluorescence value, the freer DNA there is in the medium, indicating cell death. The results were expressed in percentage of the control.

Blood analysis

Hemogram was performed in all groups at the end of treatment to observe the main effects of Vincristine and swimming training in the immune system. The values of platelets, total leukocytes, and blood hematocrit were considered. This analysis was performed at the Veterinary Hospital of the Federal University of Santa Maria, by the professionals responsible for the on-site examination, with the observation of the researcher responsible for this study.

Activity of enzyme Catalase (CAT)

CAT activity was analyzed spectrophotometrically by the method described by Aebi [21], which involves monitoring the disappearance of H_2O_2 in the presence of the enzyme at the wavelength of 240nm. After treatment of the cerebellar cells, an aliquot of tissue was added in 50mM Potassium Phosphate buffer, pH 7.0. The enzymatic reaction was initiated by addition of H_2O_2. The results were expressed as units/mg protein.

Activity of enzyme Superoxide Dismutase (SOD)

To verify SOD activity was performed an assay described by Misra and Fridovich [22], where one part of the cerebellum sample was properly homogenized in a volume of 40 (w/v) with Tris-HCl 10mM (pH 7.4). SOD was expressed as units/mg protein.

Statistical analysis

Statistical analysis was performed using the SAS System for Windows (Statistical Analysis System), versão 9.2 (SAS Institute Inc, 2002-2008, Cary, NC, USA). For biochemical variables comparison among the four groups the analysis of variance for repeated measurements (ANOVA) was used, followed by Bonferroni test for nonparametric data. To compare the behavioral variables between the four groups and be-

tween the various evaluations, the analysis of variance for repeated measures (ANOVA) was used, followed by the Tukey test for comparison between groups, and the contrast profile test for comparison between evaluations. With the variables, were transformed into ranks due to absence of normal distribution. For the correlation analysis between the behavioral and biochemical data, was performed the Spearman correlation test, in which the delta of the values found for each rat was used as an average of each day of evaluation, and compared with the biochemical results of the same animal. The closer to 1 is the Spearman correlation coefficient (represented by the letter r), the higher the correlation. The significance level adopted for the statistical tests was 5%, that is, $p < 0.05$.

Results

The sample consisted of ten rats in each experimental group. The results demonstrated a beneficial effect of previous physical exercise on protection against loss of body balance and motor coordination, induced by the administration of Vincristine, through modulation in the antioxidant and immune system.

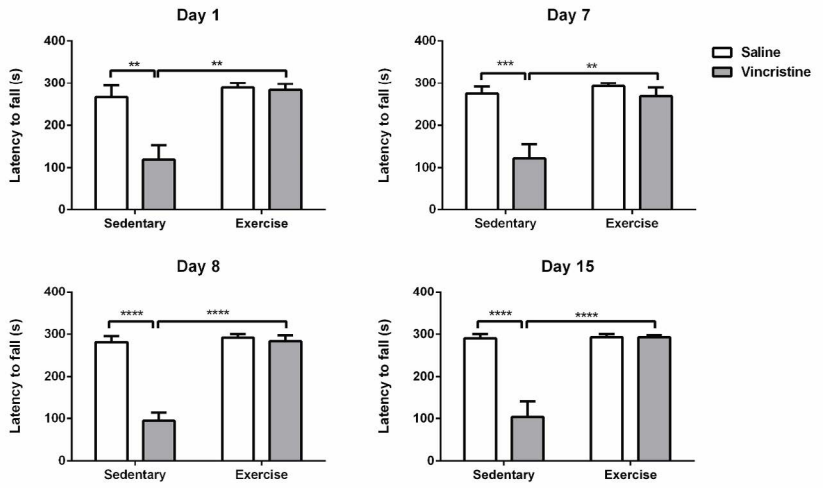
Behavioral tests

Protective effect of physical exercise against the damage in dynamic balance and motor coordination, induced by treatment with VCR, in Rotarod test

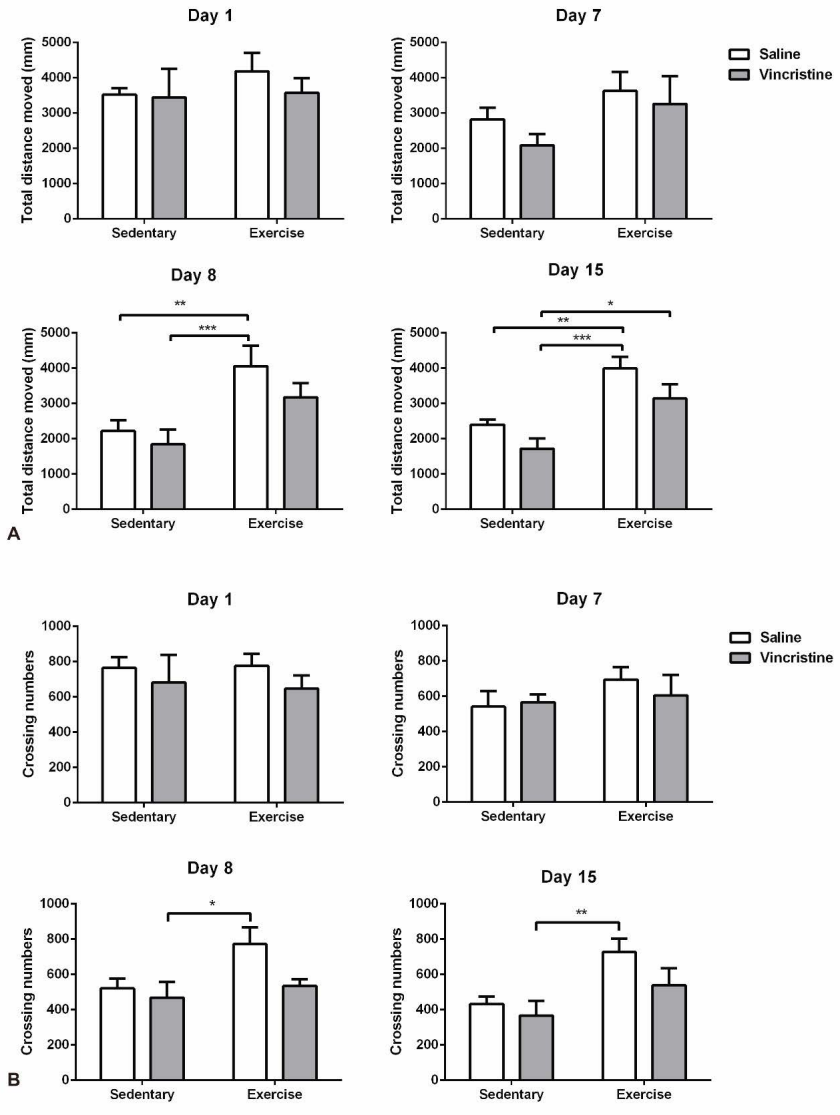
Physical training could minimize the damage caused by the VCR, having the trained rats performed better in the balance test when compared to the sedentary rats. The protective effect of physical training was maintained from the first (day 1, $F(1,23) = 8.299$, $p = 0.0084$), to the last day of evaluation (day 15, $F(1,23) = 21,37$; $p = 0,0001$). As shown in figure 2, the treatment with VCR affected the balance of the sedentary rats, which remained in the rotarod shorter periods when compared to the untreated rats. The damage lasted on all valuation days (day 1, $F(1,23) = 9,758$; $p = 0,0048$ to day 15, $F(1,23) = 21,37$; $p = 0,0001$. Figure 2).

Open field analysis

The results in the open field test indicate that the VCR did not cause significant effects on the analyzed parameters, except for the rearing on the first day of evaluation. It can be observed, however, that the trained animals maintained the exploratory locomotor activity longer than the sedentary animals. The significant difference were demonstrating in the distance traveled on day 8 ($F(1,20) = 12,97$, $p = 0.0018$) and 15 ($F(1.20) = 23.84$, $p < 0.0001$. Figure 3A) and in the number of crosses on the same days (day 8, $F(1,24) = 5,155$, $p = 0.0324$ and day 15, $F(1.24) = 8.487$, $p = 0.0076$. Figure 3B). On the last day of the evaluation, there was an even greater decrease in the distance covered by sedentary animals treated with VCR ($F(1.20) = 6.226$, $p = 0.0215$), when comparing to the trained and treated animals.



Repeated measures ANOVA test. Values obtained by averages. **p<0.01; ***p<0.001; ****p<0.0001.
Figure 2 - Effect of VCR on the body balance of trained and sedentary rats, evaluated in the rotarod at pre-treatment (day 0), one and seven days after the first dose (days 1 and 7) and one and seven days after the second dose of the drug (days 8 and 15).



A. Total distance moved (mm), and **B.** Crossing numbers (n). Repeated measures ANOVA test. *p<0.05; **p<0.01; ***p<0.001.
Figure 3 - Open field test was performed on the first and seventh days after the first dose (days 1 and 7) and on the first and seventh days after a second dose of VCR (days 8 and 15).

Ex vivo assays

Protective effect of physical exercise against oxidative stress and genotoxicity caused by VCR treatment.

The DCFH analysis showed that VCR treatment led to an increase in ROS levels in the cerebellum of sedentary rats ($F(1.20) = 6.729$, $p = 0.0174$) when compared to the control group. Previous physical training could minimize the toxic effects of VCR exposure, demonstrating lower oxidative damage ($F(1.20) = 9.272$, $p = 0.0064$) in trained animals when compared to the sedentary animals (Figure 4A). A strong correlation between the DCFH and delta of rotarod (mean of the evaluation days: 0, 1, 7 and 15), showed that higher the oxidative stress generated by drug toxicity, the greater the imbalance of the animals in the rotarod test ($p = 0.002$, $r = -0.74272$. Figure 4B).

VCR-treated rats presented higher DNA damage ($F(1.24) = 19.87$, $p = 0.0002$) compared to untreated, demonstrating the genotoxic potential of this drug. Physical training was able to protect against this damage ($F(1,24) = 7,650$, $p = 0.0107$. Figure 4C). The moderate correlation between picogreen and delta of rotarod showed that higher the genotoxicity generated by drug, the greater the imbalance of the animals in the rotarod test ($p=0.0037$, $r=-0.61801$. Figure 4D).

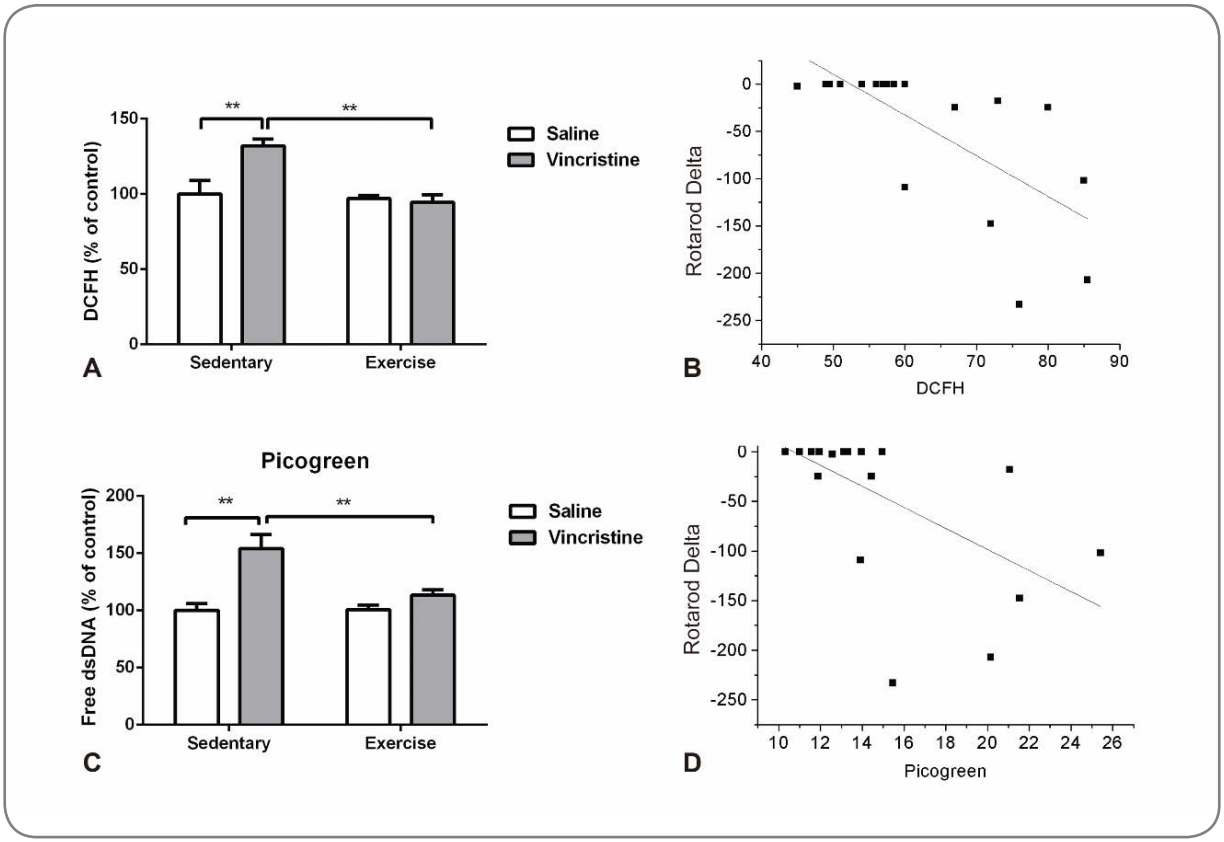


Figure 4 - **A.** Analysis of oxidative stress by increasing the peroxyl group in DCFH. Result expressed by the percentage of control; **B.** Correlation analysis between the rotarod tests (delta) and DFCH: $p < 0.01$; $r = -0.74272$; **C.** Analysis of DNA fragmentation by the picogreen method. Result expressed as a percentage of the control; and **D.** Correlation analysis between the rotarod tests (delta) and picogreen: $p < 0.01$; $r = -0.61801$. p = P-value and r = Spearman's correlation coefficient. $^{**}p<0.01$; $^{***}p<0.001$.

Protective effect of physical exercise against the immunological damage caused by VCR treatment

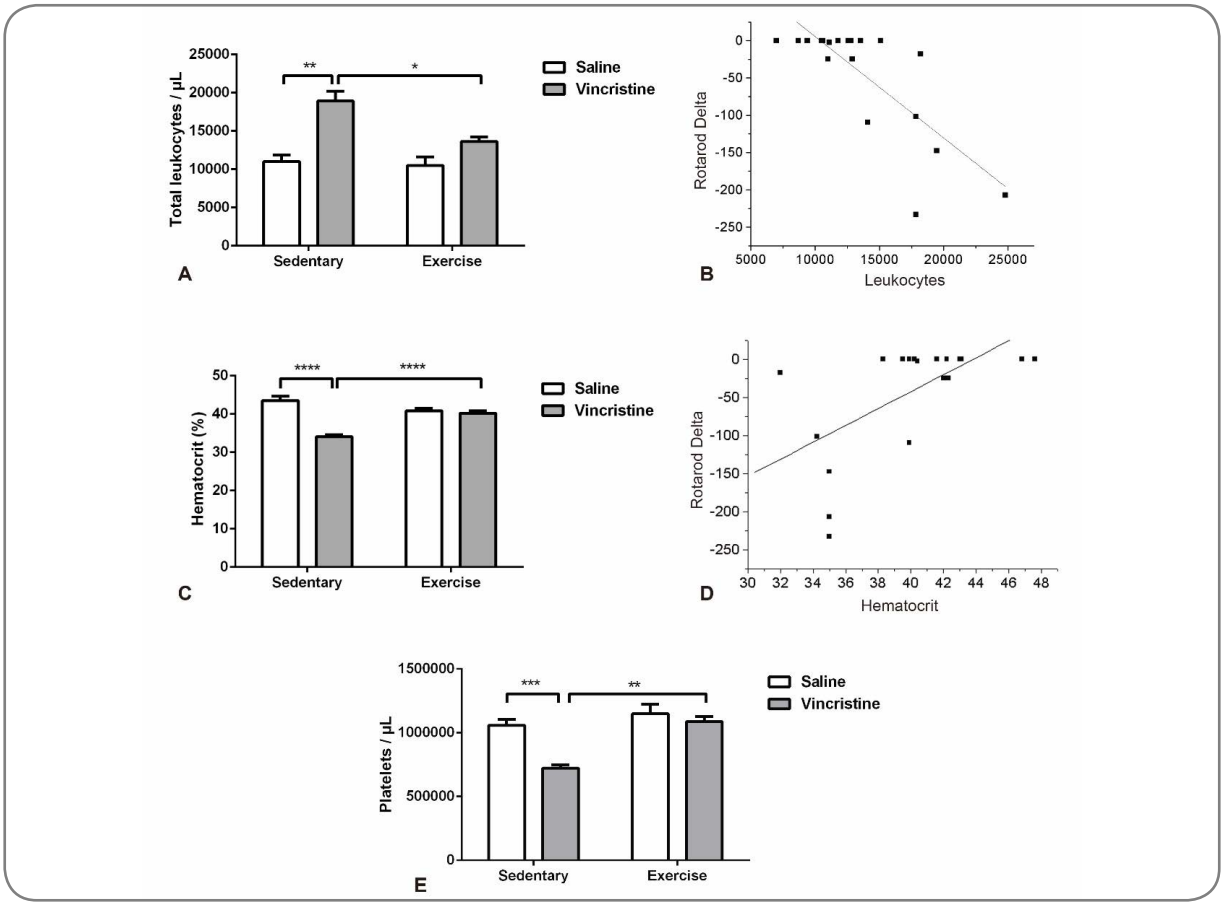
In hematological analyses, in view of the fact that the reference value for the total leukocyte count are 7,300 - 12,660/μL, was observed that VCR treatment caused leukocytosis, that is, an increase in the number of leukocytes in sedentary rats ($F(1,20) = 30.05$, $p<0.0001$), indicating greater general toxic effect in sedentary rats treated with VCR when compared to animals of the control group. Physical training significantly reduced this toxic effect ($F(1,20) = 5,602$, $p = 0.0281$, Figure 5A). This data showed a negative correlation with the rotarod test ($p < 0.0001$; $r=-0.77183$. Figure 5B), indicating that the higher the overall toxic effect (leukocytosis), the lower the ability to maintain the balance in the test. In addition, it presented a moderate and strong correlation, respectively, with the oxidative analyzes of DCFH ($p = 0.0075$; $r = 0.5783$) and picogreen ($p = 0.0002$; $r = 0.73268$), indicating that the same toxicity effect caused by VCR treatment in the cerebellum, was also capable of causing general immunological damage.

It was also observed that VCR treatment reduced hematocrit levels (represented by % red blood cells, reference value between 41.1 and 51.1%) in sedentary rats ($F(1.16)=51,17$; $p < 0.0001$) when compared to those who did not receive treatment, which means the red blood cell deficit in the organism and may be indicative of anemia. The previous physical training minimized this damage ($F(1,16) = 38.72$, $p < 0.0001$, Figure 5C). There was a moderate correlation between hematocrit and DCFH analysis ($p = 0.0062$; $r =-0.58997$. Figure 5D), indicating that the higher the oxidative stress, the lower the levels of red blood cells in the blood and, consequently, the greater the chance of anemia.

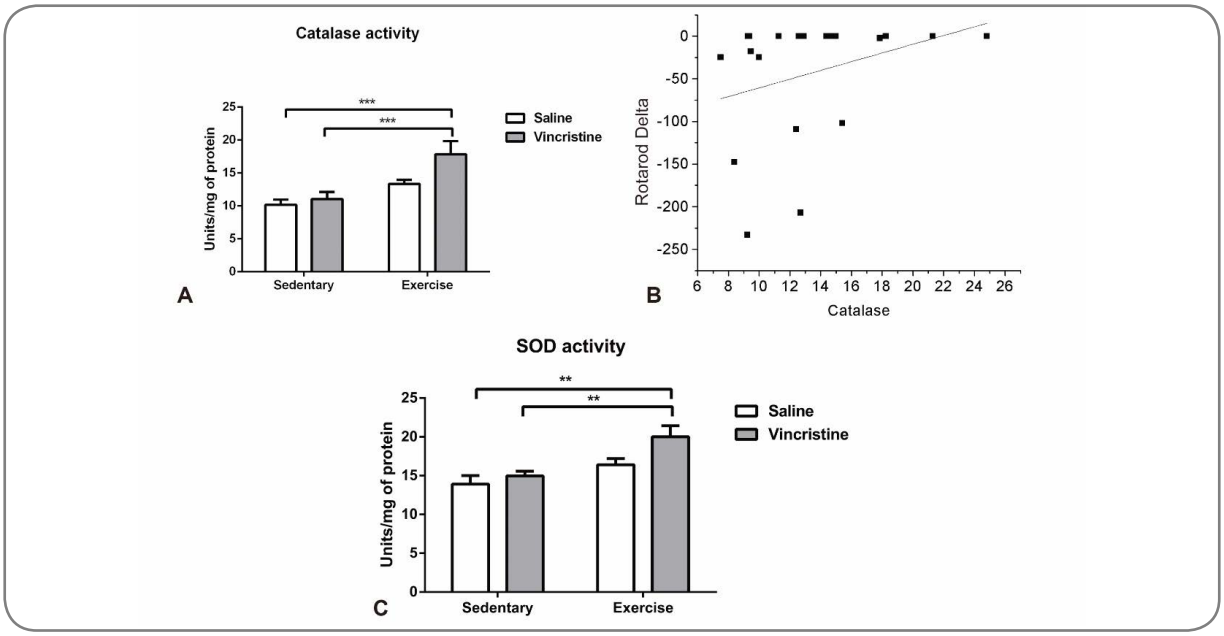
In addition, considering the reference values for platelet counts of 840,000 to 1,240,000/μL, it can be observed that VCR caused significant decrease in platelet count in sedentary rats ($F(1,17) = 16.14$, $p = 0.0009$) compared of the control group, suggesting that this drug has toxic effects on the immune system. The physical exercise was able to protect the damage caused by the drug since it differed significantly from the sedentary group ($F(1,17) = 7.727$, $p = 0.0128$. Figure 5E), up to 15 days after the training protocol, in all these analyses.

Protective effect of the previous physical exercise in improvement in the antioxidant system

Previous physical training increased the antioxidant activity of CAT and SOD enzymes in the animals treated with VCR ($F(1,20) = 16,13$; $p = 0.0007$ and $F(1,5) = 12,34$, $p = 0.0031$) compared to sedentary animals, also treated with the drug (Figure 6A and 6C). There was a positive correlation between CAT activity and the rotarod tests ($p = 0.0500$; $r = 0.43640$. Figure 6B), which showed that the improvement in the animals antioxidant system due to physical training protected of the imbalance in the VCR treated rats.



A. Total leukocytes - values expressed in µL; **B.** Correlation analysis between the rotarod tests and total leukocyte: p <0.0001; r = -0.77183; **C.** Hematocrit - values expressed in %; **D.** Correlation analysis between the rotarod tests and hematocrit: p <0.01; r = -0,58997; and **E.** Platelets - values expressed in µL. P = P-value and r = Spearman's correlation coefficient. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.
Figure 5 - Blood count analysis.



A. Activity of catalase enzyme (CAT), demonstrated in units/mg of protein, and **B.** Spearman correlation analysis between the tests of rotarod and the activity of the enzyme CAT: p < 0.05; r = 0,43640; **C.** Activity of superoxide dismutase enzyme (SOD), demonstrated in units/mg of protein. p = P-value and r = Spearman's correlation coefficient. **p < 0.01; ***p < 0.001.
Figure 6 - Analysis of antioxidant enzymes.

Discussion

The present study was conducted with the aim of verifying if the physical exercise changes the dynamic balance and motor coordination, exploratory locomotor activity and oxidative and immunological balance of rats treated with Vincristine (VCR). To our knowledge, this is the first study that evaluates the effects of physical exercise in preventing the toxicity induced by VCR.

The effective antitumor dose and the time of treatment with VCR are still not well established in the literature. VCR may be given to pediatric patients weighing less than 10kg (body surface area <1m^2) at 0.05-0.065mg/kg weekly, and in children weighing more than 10kg (body surface area ≥ 1m^2) a bolus injection dose. The vinca alkaloids of 1.5–2.0mg/m^2 may be given weekly. For adults the common dose is 1.4mg/m^2 weekly [23]. In addition, there is little research related to the toxicity of VCR to the cerebellum. The current research was based on the dosage described by Shabani *et al.* [18] in which rats' cerebellum functions were profoundly affected by exposure to VCR and, were observed cognitive, behavioral and motor function deficits in the cumulative dose of 1.0mg/kg. In this study, the possible decay of balance and motor behavior caused by cerebellar damage was not evaluated, nor were biochemical analysis performed to observe the causes of the findings. In present study, it was decided to analyze the blood and cerebellar structure on the seventh day after the last dose because immunosuppression and oxidative stress are higher after the seventh day of VCR application [24,25].

It is well known that peripheral neurotoxicity and other motor changes induced by VCR has provoked an important deficit of dynamic balance and motor coordination [26, 27], however, the cerebellar damage induced by the drug may corroborate for this finding. The rotarod test, used as a reference for cerebellar damage [28], demonstrated that the latency for the first drop in the device was lower in the treated animals compared to the untreated ones with VCR. The latency time is the main measure obtained in the rotational bar and reflects the product of four major factors involved in the rodent's motor function: 1) dynamic equilibrium; 2) motor coordination; 3) motor planning; 4) motor learning; and (v) general and neuromuscular physical conditions [29]. In the present study, DCFH and picogreen analysis showed that this imbalance may be correlated to increased cerebellar oxidative, immune and DNA damage caused by the drug administration.

It was observed that physical training protected against damage in balance and motor coordination induced by the drug administration up to 15 days after the end of the training protocol. Protection might be explained by a positive modulation of the antioxidant system due to an increasing of SOD and CAT enzymes activities, which demonstrates that in some way the animals that performed previous physical training have the best-prepared organism to combat the increase of the oxidative stress caused by the VCR. This result agrees with some evidence that suggest that adaptive responses to moderate exercise involve an increase in antioxidant defenses and reduction in basal oxidant production [16,30]. Besides that, animals treated with VCR showed DNA damage. Antitumor drugs can cause DNA damage, and the vinca alkaloids such as VCR induce cytotoxicity through interaction with tubulin. Biochemical and biological effects on microtubules include competition for the intracellular transport of amino acids, inhibition of purine, synthesis of RNA and DNA proteins and lipid rupture of the cell membrane [31]. In this study, physical training acted as a genoprotective, in DNA protect and repair [32], minimizing the deleterious effects of the drug.

According to the literature, physical exercise improves cognitive function and improves motor dysfunction in rats [33]. In present study, exploratory locomotor activity of animals was demonstrated through software tracking in open field test that showed that the physical training was able to increase the total distance covered and the number of crosses when compared to the group of sedentary animals. Exercise was also able to keep the total leukocyte, hematocrit and platelet levels of the animals treated with VCR within reference values and it was observed with this, one better condition of the rat to keep dynamic balance and motor coordination in the rotarod test too. Therefore, physical training proved to be determinant in keeping a good physical and immune status during VCR treatment. Myelosuppresion can be associated with any vinca alkaloid as a result of prolonged treatment, unintentional high-dose treatment, or in highly susceptible patients [28]. This condition is a common side effect among patients who undergo chemotherapy [34] and it directly influences the life quality of people with cancer. In the studies carried out by Valenti [35] and Stephenson [36] with cancer survivors, was observed that those who exercised before diagnosis achieved a better life quality than patients who do not exercise.

As a limitation, this is an experimental study and therefore the findings cannot be extended to clinical practice. However, from the positive results found, we encourage future research that uses moderate physical exercise in humans in order to better understand the possible ways of protecting dynamic balance and motor coordination in individuals who will be treated with chemotherapy.

Conclusion

The most important finding of the study shows that moderate regular aerobic exercise, such as a swimming, protects the dynamic balance and motor coordination damage induced by the systemic administration of vincristine. This benefit is related to the positive effect of exercise as a modulator of the cerebellar antioxidant system, which increases the activity of CAT and SOD enzymes and reduces reactive oxygen species and DNA damage. In addition, physical exercise was able to keep the levels of leukocytes, platelets and hematocrit within normal limits in rats treated with the drug and, by itself, maintained the exploratory locomotor activity of the analyzed rats. The study also shows that, even 15 days after training, the beneficial effects of exercise can still be observed.

Academic link
This article represents part of the master's dissertation by Luiza Minato Sagrillo, supervised by professor Dr. Aron Ferreira da Silveira in the Postgraduate Program in Human Communication Disorders, at the Federal University of Santa Maria, Rio Grande do Sul, Brazil.

Potential conflict of interest
No conflicts of interest with potential potential for this article have been reported.

Financing source
There were no external sources of funding for this study.

Authors' contributions
Conception and design of the research: Sagrillo LM. **Getting data:** Sagrillo LM, Zorzi VN, Fighera MR, Bonadiman BSR. **Analysis and interpretation of data:** Sagrillo LM, Zorzi VN, Royes LFF. **Statistical analysis:** Sagrillo LM, Zorzi VN. **Obtaining financing:** not applicable. **Writing of the manuscript:** Sagrillo LM, Cattani MFMR, Bonadiman BSR. **Critical review of the manuscript for important intellectual content:** Royes LFF, Silveira AF.

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