Evaluation of stress and anxiety in mice with colorectal cancer submitted to physical exercise

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ABSTRACT
Purpose:To evaluate the effect of physical exercise on the behavior of rodents with colorectal cancer induced by using the elevated cross maze. Methods:Forty male hairless mice induced colorectal cancer were used, divided into five groups: G1) submitted to pre- and post-induction swimming; G2) pre- and post-induction stairway; G3) post-induction swimming; G4) post-induction stairway; G5) sedentary. At the end of the 14th week, the animals were subjected to the cross maze test.Results: The mean dwell time in the open arm for G1 was 4.17±6.50; G2 37.52±40.7; G3 85.84±42.5; G4 32.92±23.17; and G5 4.09±4.43. In the closed arm, they were 264±23.43 in G1, 187.60±47.73 in G2, 147.50±40.03 in G3, 182.00±40.40 in G4, and in G5 235.36±14.28. In the center, G1 got 31.86±20.18, G2 74.85± 28.37, G3 66.69±19.53, G4 60.55±10.46, and G5 60.55±23.65. Conclusion: Aerobic exercise for seven weeks after tumor induction had less impact on animal behavior. On the other hand, it significantly increased the stress level of the animals when applied for 14 weeks before and after tumor induction.

Keywords: Models, Animals, Neoplasms Associated with Colitis, Elevated cross maze test, Exercise.

1 INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in men and the second most common in women. In 2018, there were about 1.9 million new cases worldwide1. The therapeutic management of RCC is usually invasive and aggressive, involving surgery, radio and chemotherapy. Among the adverse effects of treatment is reduced quality of life, associated with stress, anxiety and depression, so the literature highlights a prevalence of depression ranging from 1.6 to 57% and a prevalence of anxiety ranging from 1 to 47.2 % in patients with RCC2,3.

Evidence such as Benatti and Pedersen4shows the effect of exercise as anti-inflammatory on various tissues and organs. Chronic inflammation is considered one of the main mechanisms to promote and accelerate the development neurodegenerative diseases, as well as neoplasms. This process mainly involves the continuous activity of various cytokines. The skeletal muscle can function as an endocrine organ due to its production of growth hormones and cytokines known as myokines, which are induced by an exercise stimulus, such as the brain-derived neurotrophic factor protein (BDNF) producing actions in the brain, such as in neuroplasticity function, besides presenting antidepressant effect, generating improvement in the cognitive system. According to a study by Sartorie et al.5, voluntary physical activity increases the levels of mature BDNF in the hippocampus of mice, supporting the effect of exercise on antidepressant effects.

The literature points to the existence of a relationship between stress, progression, appearance of tumors, and the appearance of metastases, due to the triggering of neuroendocrine processes and deregulation of the immune system6. Furthermore, the literature points to an inverse relationship between physical activity and cancer risk7. Physical exercise reduces the risk of mortality from RCC by 38%. The effects of physical exercise on mood improvement, anxiety, stress, and depression reduction, as well as certain advantages on cognition have also been described6,7.

Although the literature shows the capacity of physical exercise to improve well-being and reduce the risk of RCC, there are still few studies on the relationship between physical exercise acting as a possible factor in improving the adverse effects derived from RCC, such as anxiety, stress, and depression. This issue becomes relevant since such behavioral factors affect neuroendocrine processes and the immune system and may contribute to the development/aggravation of the disease. Therefore, this study aimed to evaluate the effect of physical exercise on the behavior of rodents with RCC induced by using the elevated plus maze test, a method validated to investigate anxiety-like behavioral aspects in rodents8.

2 METHODS

The study was developed in the Laboratory of Experimental Carcinogenesis of the Graduate Program in Health and Development of the Midwestern Region of the Federal University of Mato Grosso do Sul (UFMS), municipality of Campo Grande (MS), Brazil. All steps and procedures were performed according to the ethical principles established by the National Council for the Control of Animal Experimentation (CONCEA) and filed with the UFMS Ethics Committee on the Use of Animals No. 1,091/2019.

Thirty-nine endogamous male HRS/J mice, known as bald mice, were used as an experimental model of infection. The animals were obtained from the central vivarium of the Center for Biological and Health Sciences (CCBS) at UFMS.

Mice were 4-5 weeks old and had an initial weight of 25 g. The animals were kept in collective cages (dimension 40 × 35 × 17 cm), containing four animals/cage. The cages were housed on the same shelf at a height of 1 meter from the floor and exposed to light in the same way as all cages, at a temperature of approximately 25°C, with a 12-hour light/dark cycle, receiving standard feed (Nuvital® CR1) and water AD Libitum. They were acclimated to laboratory conditions for 14 days prior to the experiment. The animals were randomly divided into five groups:

- G1: animals submitted to swimming (aerobic exercise) before and after tumor induction (n = 8);
- G2: animals submitted to stair training (resistance exercise) before and after tumor induction (n = 8);
- G3: animals submitted to swimming (aerobic exercise) after tumor induction (n = 8);
- G4: animals submitted to stair training (resistance exercise) after tumor induction (n = 8);
- G5: animals induced cancer and kept sedentary (n = 7).

Physical exercise protocols

The exercise protocols were performed on alternate days.

Aerobic exercise

For this activity, swimming was chosen. The animals were adapted six days before the beginning of the protocols and performed the exercises gradually.

It was performed in a 29 L tank of heated water (31±2 °C) with a height of 27.6 cm and width of 33 cm. The animals swam in groups of four mice. The duration of the swimming sessions was increased gradually, starting at 10 minutes and increasing by 5 minutes every three weeks9.

Resistance exercises

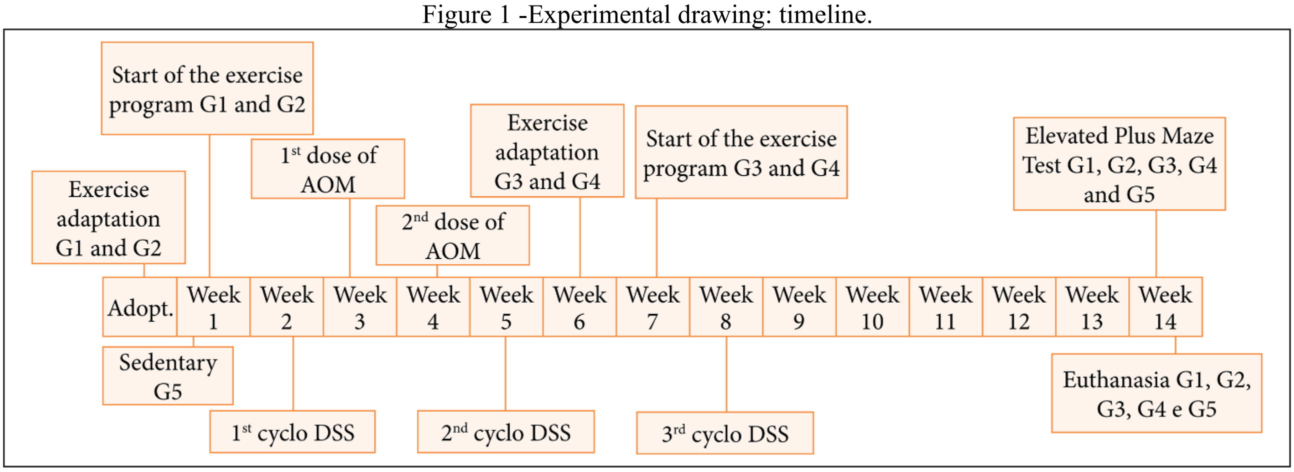
The model of resistance training on vertical ladders was used, which consists in the animals climbing with weights tied on their tails until they reach a compartment at the top of the ladder with a total height of 110 cm, with steps made of stainless steel bar, the distance between steps: 1 cm, inclination 80º. There were ten repetitions of climbing to the top of the ladder with a 2 minute rest between climbs. They started with an overload of 10% of body weight, G2 finished with 50% of body weight, and G4 finished with 50% of body weight in the 14th week10.

Induction of colitis and colorectal cancer

For the induction of colitis, mice were given water containing dextran sulfate sodium (DSS) 2.5% (MP Biomedicals, Santa Ana, CA, USA) for three consecutive seven-day cycles, interspersed with two weeks of water, in order to induce intestinal inflammation (Fig. 1)11.

For cancer induction, all animals received two intraperitoneal (right lower quadrant of the abdomen) injections of azoximethane (AOM - Sigma-Aldrich Laboratory), a total dose of 20 mg/kg, divided into two weeks, 10 mg/kg per week of AOM[11].

Figure 1 -Experimental drawing: timeline.



AOM: azoxymethane; DSS: dextran sulfate sodium.

Cancer Induction Assessment

The presence of tumor in the colon was evaluated by means of fluorescent optical imaging in the near infrared (NIR) and X-ray imaging using the In-Vivo Xtreme/Bruker II system belonging to the Laboratory for Studies in Experimental Models of Disease, after euthanasia for the presence of polyps and histological changes present in the distal colon.

To detect fluorescent imaging of the tumor, the animals received a dose of 0.334 mg/kg of the fluorescent biomarker IR-780 iodide Dye (Sigma Aldrich) intraperitoneally. After 12 hours, the animals were anesthetized in a 2% isoflurane chamber and transferred to the In-Vivo Xtreme/Bruker II system and kept anesthetized by means of isoflurane cones attached to the animal's head. Fluorescent NIR images were captured using a 760 nm filter for excitation and 830 nm for emission. Fluorescent and X-ray images were captured simultaneously (and overlapped) for perfect anatomical localization.

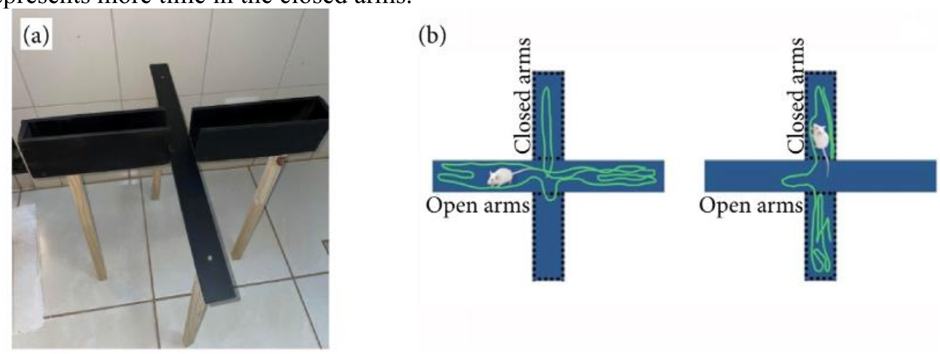
Raised cross maze test: equipment setup

Our maze was made of wood with four arms, cross-shaped, two open arms without walls and two closed by 10 cm high walls; 25 cm long, 5 cm wide, and a 5 × 5 cm central platform raised 50 cm off the ground (Fig. 2a).

Each mouse was placed in the center of the platform, with its head facing one of the open arms; the dwell time in the elevated cross maze was 5 min[12]. Sessions were recorded by a camcorder and analyzed by recording behavior using the X-PloRat 3.0[13] program.

The behavior was recorded as to the length of stay and entry into the open and closed arms. The activity of the mice in the open arms reflects a conflict between the animal's preference for the protected area (closed arms) and its innate motivation to explore a new environment. Increased dwell time and entries into the open arm signals are interpreted as decreased anxiety-like behavior (Fig. 2b)8.

Figure 2 - (a) Image of the elevated cross maze used to test mice; (b) typical mouse behavior at low (left) and high (right) anxiety levels. The green trace demonstrates the movement of the animal. The left represents more time in the open arms, while the right represents more time in the closed arms.



Statistical Analysis

The results of the elevated cross maze test, expressed as a mean±standard deviation, were submitted to the non-parametric Kruskal-Wallis test with Dunn's post-test comparing the exercise of the experimental groups (G1, G2, G3, and G4) with the sedentary control group (G5). Pvalues less than 5% ($p<0.05$) were considered significant.

3 RESULTS

Confirmation of tumor development

By means of fluorescence evaluation in the NIR, performed on the last day of the experiment, it was possible to observe fluorescent marking in all groups analyzed, indicating tumor development in the colon of the animals (Fig. 3). These findings were confirmed after euthanasia by polyps and histological changes (such as aberrant crypts, adenomas, and low and high grade dysplasia) in hematoxylin and eosin in the distal region of the colon (Fig. 4).

Figure 3 - Images captured by the In-Vivo Xtreme/Bruker II system. (a) Animals overlaid by fluorescence emitted from the colon (NC: negative control); (b) section photo of colon overlaid by fluorescence emitted from polyps; (c) section photo of distal colon.

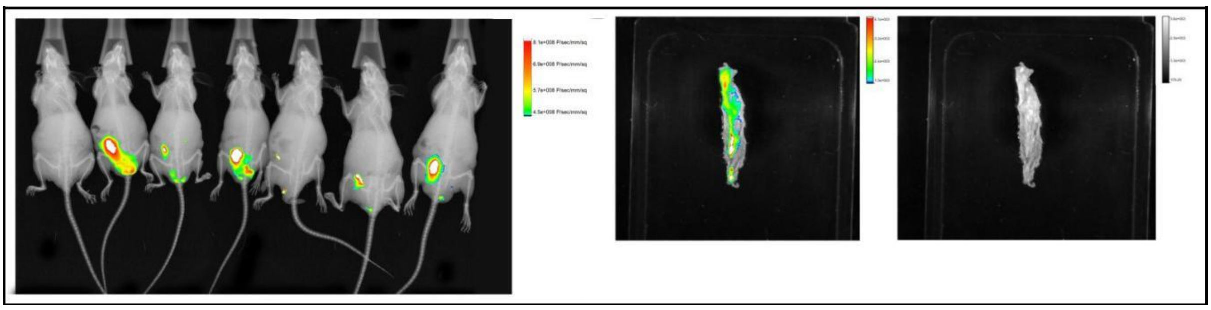
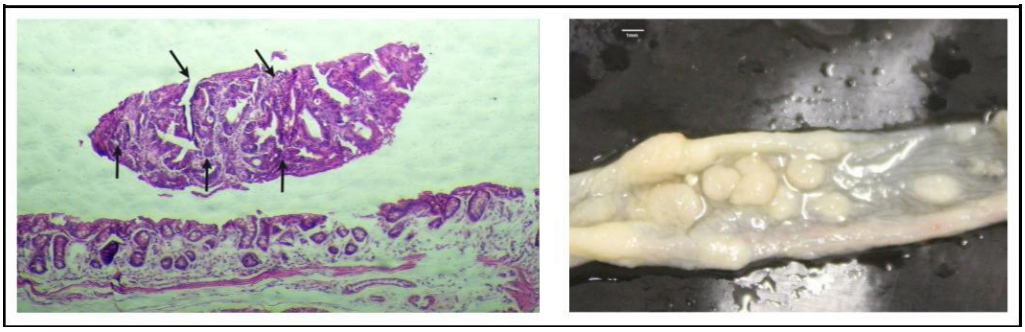


Figure 4 - (a) Histological changes, arrows indicating colonic adenoma; (b) polyps in the distal region of the colon.



Raised cross maze test

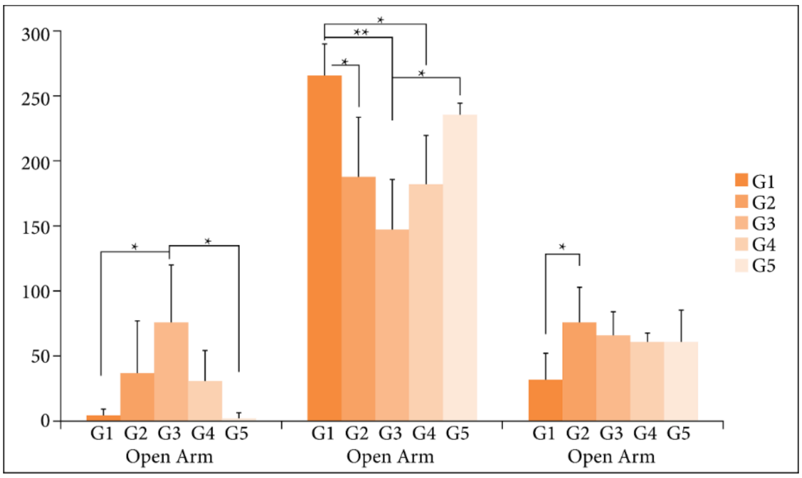
The results of the comparison between the exercise and sedentary experimental groups regarding the absolute time in seconds spent in the open, closed and central arms in the elevated cross maze test are described below and illustrated in Fig. 5.

The mean time (mean±standard deviation) in the open arm for G1 was 4.171±6.50; G2 37.52±40.7; G3 85.84± 42.5; G4 32.92±23.17; and G5 4.09±4.43. There was a significant difference in the open arms between G1 and G3 ($p<0.05$) and also between G3 and G5 ($p<0.05$).

In the closed arm, the time in G1 was 264±23.43; G2 187.60±47.73; G3 147.50±40.03; G4 182.00±40.40; and G5 235.36 ±14.28. We identified a highly significant difference between G1 and G3 ($p<0.0001$) and a significant difference ($p<0.05$) between G1 and G2; G1 and G4; and G3 and G5, with G5 remaining longer in the closed arm.

The mean time in the center for G1 was 31.86±20.18; G2 74.85±28.37; G3 66.69±19.53; G4 60.55±10.46; and G5 60.55±23.65. There was no significant difference between the experimental exercise groups and the sedentary control group with respect to center. However, there was a significant difference between the G1 and G2 groups ($p<0.05$).

Figure 5 -Dwell time in the open, closed, and central arms during the elevated cross maze test for each group of animals submitted to the study.



G1: aerobic group before and after tumor induction; G2: resistance exercise group before and after tumor induction; G3: aerobic exercise group after tumor induction; G4: resistance exercise group after tumor induction; G5: sedentary group; *significant difference ($p<0.05$); **very significant difference ($p<0.0001$).

4 DISCUSSION

Regarding the results of the groups in the open arms, G1 (aerobic, pre- and post-induction) and G3 (aerobic, post-induction), although some groups underwent the same type of exercise, showed different results. G1 had a significantly shorter mean time spent on the open arms than G3. This fact evidenced a behavior contrary to what was expected, which was the reduction of anxiety/stress behaviors in both groups, not only in one.

A possible determinant of this result in our study would be the triggering of a defensive response and risk assessment behavior in animals chronically exposed to exercise14, since G1 had a 14-week physical activity schedule compared to the seven weeks in G3. Considering that both groups were under the same conditions in which the test was performed, differing only as to the physical activity schedule, it can be highlighted that swimming may determine different responses on rodent behavior according to the exposure period15.

Another factor to be considered is that the swimming activity for mice is a forced test in which the animal seeks escape rather than spontaneous swimming, triggering an increase in plasma corticosterone catecholamines and glucose response. However, the literature points to the forced swim test on a short schedule to reduce anxiety in these animals, in protocols ranging from one repetition to 15-day swimming exercise protocols16-18.

Exposure to stressful stimuli triggers an adaptive physiological response that results in anxiety and stress behavior. This response involves different areas of the nervous system: the amygdala, the hypothalamic-pituitary-adrenal axis, and the locus coeruleus. If an organism cannot adapt to the stress response, it becomes, instead of an adaptive physiological response, a harmful pathological response19,20.

Physical exercise, especially aerobic activities, has antidepressant and anxiolytic effects and protects against the deleterious consequences of stress. The positive effects of physical exercise are due to the reduced activation of central areas previously discussed through neuroendocrine signals such as BDNF19,21.

In our data, G3 showed a significant difference ($p < 0.5$) when compared to G5 (sedentary). This result corroborates the effect of physical exercise in controlling anxiety in rodents already described. On the other hand, the group of sedentary animals remained exposed only to stressors, such as those derived from colitis and neoplastic evolution in the colon.

DSS-induced colitis in rodents increases plasma levels of corticosteroids and proinflammatory cytokines, such as elevated interleukin-6 and GRO-alpha in the brain. Consequently, this response determines signals in the central nervous system, triggering a reflex response to stress and anxiety-like behavior, which is worsened by weight loss and progressive debilitation due to neoplasia22. Therefore, swimming activity positively affected anxiety-like behavior in our study. However, the time of exposure to the activity may have been a limiting factor, because, given the same conditions between G1 and G3, differing only on the time of exposure to the activity; only G3 showed a significant difference compared to the control (sedentary group).

G2 (pre and post-induction) and G4 (post-induction), representing the stair climbing modality, showed no significant differences with the other groups regarding changes in anxiety-like behavior. In the graphical analysis, we can see a similarity between the two groups, with only small differences between the dwell time in each compartment of the elevated cross maze (ECL). However, when analyzing G2 and G3, comparing them with G5 (sedentary group), there was a difference regarding the time spent in the open arms, although there was no statistically significant difference. It should be noted that the exercise of climbing a vertical ladder requires manipulation of the animal, such as placing it on the ladder, in addition to a gradual load, which may be a factor that interfered with our results,14,23,24.

Although the CCR tumor induction model used in our study is similar to that used in studies found in the literature, no analysis was found that used DSS as part of tumor induction and evaluated the anxious type pattern in colorectal cancer animals undergoing exercise physical examination.

The hypothesis of this study was directed at the effects of exercise in animals with AOM- and DSS-induced CRC. Nevertheless, future studies with animals, not induced by CRC, are needed. Thus, the isolated effect of exercise in healthy animals can be individualized and analyzed between groups with CRC/sedentary, CRC/physical activity, healthy/sedentary, and healthy/physical activity.

5 CONCLUSIONS

Aerobic exercise for seven weeks after tumor induction had less impact on animal behavior. On the other hand, it significantly increased the stress level of the animals when applied for 14 weeks before and after tumor induction.

The pre- and post-induction or post-induction resistance exercise groups showed no difference in behavior between them. Yet, there was a slight improvement in the reduction of stress/anxiety type behavior compared to the control (sedentary group).

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