Original Article

Effect of resisted training on survival of c57bl / 6 mice with cachexia associated with cutaneous melanoma

Efeito do treinamento resistido na sobrevida de camundongos c57bl/6 com caquexia associada ao melanoma cutâneo

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ABSTRACT | OBJECTIVE: The objective of this study was to verify the effect of resistance training on the survival of C57BL / 6 mice with cachexia associated with cutaneous melanoma. **MATERIAL AND METHODS:** The sample consisted of 64 (female C57BL/6 mice, aged between 10 and 12 weeks, with approximately 50 ± 5 grams of body weight. The mice were randomly distributed into four groups: i. control mice, with tumor induction, physically inactive (Control, n = 16); ii. mice submitted to daily resistance training only before tumor induction (Training 1, n = 16); iii. Mice submitted to daily resistance training before and after tumor induction (Training 2, n = 16); iv. Mice submitted to daily resistance training after tumor induction and presented a cachectic condition (Training 3, n = 16). In the resistance training procedure (RT) with shock, a 110 cm high, 18 cm wide, 2 cm between the steps, and 80 degrees inclination ladder was used. In the procedure for shock resistance training (TR), a ladder 110 cm high, 18 cm wide, 2 cm between the rungs, and 80 degrees of inclination was used. The resisted exercise is based on the climbing of the mice. On the exit platform, an electric shock was applied as a stimulus to climb the stairs. In this step, the shock was applied to the four legs of the animal with an electric voltage of 20 volts at a frequency of 45 Hertz during six series of eight repetitions, each with ninety-seconds intervals between the series. The mice groups underwent follow-up for no more than 15 days after diagnosis of cachexia to compare overall cancer-related survival between the study groups. Kaplan-Meier survival curves were estimated for each event, and the curves of the different groups were compared using the Log-rank test. The proposed survival time was 25 days after inoculation. **RESULTS:** The results presented in this study showed no significant difference (p <0.05) between the training proposals. **CONCLUSION:** There was no difference in animals' survival with cachexia associated with the syngeneic melanoma skin tumor model with either resistance exercise or sedentary intervention.

KEYWORDS: Resistance Training. Cancer. Cancer-Related Cachexia. Cutaneous Singular Melanoma Model. Survival.

RESUMO | OBJETIVO: O objetivo desse estudo foi verificar o efeito do treinamento resistido na sobrevida de camundongos C57BL/6 com caquexia associada ao melanoma cutâneo. **MATERIAL E MÉTODOS:** A amostra foi constituída por 64 (camundongos C57BL/6 fêmeas, com idade entre 10 e 12 semanas, com cerca de 50 ± 5 gramas de peso corporal. Os camundongos foram distribuídos aleatoriamente em quatros grupos: i. camundongos controle, com indução de tumor, inativos fisicamente (Controle, n = 16); ii. camundongos submetidos ao treinamento resistido diário somente antes da indução tumoral (Treino 1, n = 16); iii. camundongos submetidos ao treinamento resistido diário antes e após da indução tumoral (Treino 2, n = 16); iv. camundongos submetidos ao treinamento resistido diário após a indução tumoral e apresentado quadro caquético (Treino 3, n = 16). No procedimento para treinamento resistido (TR) com choque, foi utilizada uma escada com 110 cm de altura, 18 cm de largura, 2 cm entre os degraus e 80 graus de inclinação. No procedimento para TR com choque, foi utilizada uma escada 110 cm de altura, 18 cm de largura, 2 cm entre os degraus e 80 graus de inclinação. O exercício resistido baseia-se na subida dos camundongos. Na plataforma de saída, aplicava-se um choque elétrico como estímulo para subir as escadas, nesta etapa era aplicada o choque nas quatro patas do animal com uma tensão elétrica de 20 volts a uma frequência de 45 Hertz durante seis séries de oito repetições, cada uma com noventa segundos de intervalo entre as séries. Os grupos de camundongos foram submetidos a acompanhamento por no máximo 15 dias após o diagnóstico da caquexia a fim de comparar a sobrevida geral relacionada ao câncer entre os grupos de estudo. As curvas de sobrevivência de Kaplan-Meier foram estimadas para cada evento e as curvas dos diferentes grupos foram comparadas usando o teste de Log-rank. O tempo de sobrevida proposta foi de 25 dias após inoculação. **RESULTADOS:** Os resultados apresentados nesse estudo mostraram que não houve diferença significativa (p <0,05) entre as propostas de treino. **CONCLUSÃO:** Não houve diferença na sobrevida de animais com caquexia associada ao modelo tumoral singênico de melanoma cutâneo com intervenção de exercício resistido ou sedentários.

PALAVRAS-CHAVE: Treinamento Resistido. Câncer. Caquexia Relacionada ao Câncer. Modelo Melanoma Cutâneo Singênico. Sobrevida.

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Introduction

Melanoma is a malignant neoplasm that originates in melanocytes, skin cells that produce a pigment called melanin.[1] It starts with a small pigmented cutaneous tumor on normal skin, most often in areas exposed to the sun, but almost half of the cases occur from pre-existing melanocyte nevi.[2]

Melanoma is the most aggressive skin cancer, often showing rapid growth and metastatic spread. Melanoma is classified in four ways due to its clinical and histological characteristics: nodular melanoma, malignant melanoma lentigo, sacral and superficial extensive melanoma.[3] According to Araújo et al.[4], malignant cutaneous melanoma constitutes about 5% of malignant cutaneous tumors, presenting high incidence and high lethality, and responsible for the vast majority of skin cancer deaths. Melanoma survival has gradually increased in recent years.[4]

Cachexia is associated with cancer's adverse effects, reducing physical function, tolerance to antineoplastic treatment, and decreased survival.[5] It is a specific and debilitating type of malnutrition in patients with advanced malignant tumors concerning 20% of deaths in cancer patients.[6] According to Duval et al.[5], one of the challenges of cachexia in cancer is the control of weight loss, mainly related to significant metabolic changes, anorexia, and reduced intake of calories, lipids, and proteins. It is important to identify early its triggering factors to aid and preserve these functions[7], considering the challenges of progressive weight loss with cancer-associated cachexia.

Adaptations caused by physical exercise have an impact on the prevention of several diseases, including cancer.[2] Activity and physical exercise bring numerous benefits, and for the individual[8], according to Santos et al.[9], training, in general, can induce positive changes both in the synthesis pathway and in protein degradation. Both pathways have related to the loss of muscle tissue in cancer patients. Azevedo et al.[8] suggest that people who practice physical exercise have the greatest possible strength in relation to their functions in various activities, increasing the quality of life as they age, consequently increasing life expectancy. Among the various training possibilities, RT is presented as a positive proposal in treating cachexia associated with cancer, and RT causes neuromuscular and hypertrophic adaptations that can contribute to the improvement of this pathological situation.[10]

Adding the benefits of physical exercise in life expectancy with cachexia associated with cancer, it is observed that the possible responses can lead to improved survival in this complex situation.[11]Thus, the present study aims to verify the effect of resistance training on the survival of C57BL / 6 mice with cachexia associated with cutaneous melanoma.

Materials and methods

Sample characterization and ethical care

The study is characterized as experimental, analytical, prospective, and with a quantitative approach. This study was approved by the Ethics Committee on Animal Experimentation and Welfare at the State University of Montes Claros (Unimontes) and had a favorable opinion for execution. The sample arbitrarily consisted of 64 (female C57BL / 6 mice, aged between 10 and 12 weeks, with about 50 ± 5 grams of body weight.

Experimental draw

The mice were randomly assigned to four groups: i. control mice, with tumor induction, physically inactive (Group 1, n = 16); ii. mice submitted to daily resistance training only before tumor induction (Group 2, n = 16); iii. mice submitted to daily resistance training before and after tumor induction (Group 3, n = 16); iv. mice subjected to daily resistance training only after diagnostic diagnosis (Group 4, n = 16).

Diagnosis of cachexia

The tenth day was established for the diagnosis of cachexia after tumor induction for all animals. The choice of arbitrary was based on the current scientific literature on the use of this standardization.[9]

Tumor induction

All C57Bl / 6 mice were immobilized and submitted to the dorsal region's trichotomy, close to the neck, to induce the tumor.[9] Soon after, the absence of painful reflex sensitivity was verified.[9] After asepsis with iodized alcohol, the animals were inoculated with 5x105 cells of murine melanoma B16-F10, resuspended in 50μL, in the dorsal subcutaneous region, close to the base of the neck (flank).[9] The inoculation of this quantity of viable cells in the subcutaneous region can complete a mitotic cycle in 24 hours and enlarge the tumor within 3-4 days.[9,12]

Resistance training instruments and procedures

The RT procedure with shock was a ladder 110 cm high, 18 cm wide, 2 cm between the steps, and 80 degrees of inclination. The resisted exercise is based on the climb of the mice. On the exit platform, an electric shock was applied as a stimulus to climb the stairs. In this stage, the shock was applied to the four legs of the animal with an electrical voltage of 20 volts at a frequency of 45 Hertz during six sets of eight repetitions, each with a ninety-second intervals between sets.[13] In the proposal of group 2, there were 15 sessions of RE. In the proposal of group 3, there were 40 sessions of RE; in the postponement of group 4, there were 15 sessions of RE. The session lasted 25 minutes.

Survival analysis

The mice's groups were followed up for a maximum of 15 days after the diagnosis of cachexia to compare the overall survival related to cancer between the study groups. However, all mice were sacrificed as soon as they had started to die, exhibiting severe impairment of bodily functions or behavior due to extensive necrosis, ulceration, and growth of the tumor mass.

Euthanasia occurred due to cervical dislocation. At the first moment, the animals were anesthetized with ketamine (75 mg/kg) / xylazine (5 mg/kg) respectively.[14,15] The collected tissues were weighed using an analytical precision digital scale (A. Cientifica EEQ9003E).[13,14]

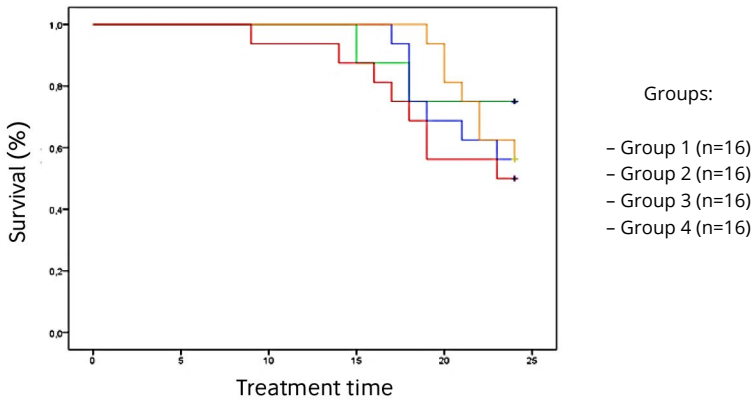
Data processing

The data collected were analyzed using SPSS (Statistical Package for the Social Sciences) version 20.0. The confidence level adopted in all analyzes was set at 95% ($p < 0.05$). The Kaplan-Meier survival curves were estimated for each event, and the curves of the different groups were compared using the Log-rank test.

Results

The results presented in this study showed that there was no significant difference between the training proposals. The proposed survival time was 25 days after inoculation.

Figure 1. The survival analysis of the C57BL / 6 mice showed no significant difference between the proposed treatments



Discussion

Cachexia in cancer is a debilitating consequence of disease progression, characterized by significant weight loss through catabolism of skeletal muscle and adipose tissue.[10] The literature points out that those who practice regular physical activity have a better quality of life and life expectancy than those who are less active.[16]

Due to its anti-inflammatory effect, TR is effective in neutralizing muscle catabolism, increasing protein synthesis, and reducing protein breakdown, thus successfully improving muscle strength, physical function, and quality of life in patients with cachexia unrelated to cancer.[10] The TR regulates cancer survivors' immune function, playing an anti-inflammatory role in the tissues and reducing the concentration of the pro-inflammatory cytokines' TNF-α and IL-617. Thus, when implementing appropriate exercise interventions, it may be possible to reverse protein catabolism while increasing protein synthesis and lean body mass, neutralizing cachexia.[10] Thus, physical exercise in general acts in the prevention of cancer incorporated as part of the standard practice in cancer care, seen as an adjunct therapy that helps neutralize the adverse effects of cancer and its treatment.[17,18]

RT is safe during and after cancer treatments and results in physical functioning improvements, quality of life, and cancer-related fatigue in various groups of cancer survivors.[19] The proposal of resistance training for rodents is presented as a methodological alternative for studies, as it presents similar results with humans.[13] However, the model proposed in this study of cachexia associated with cancer was very aggressive, such a situation may have been a limiting factor, as survival of animals was different when compared to group 1 (control), so experimental studies with other methodological models of physical exercise with C57BL / 6 mice should be encouraged to elucidate the gaps left here.

Conclusions

In this context, we conclude that there was no difference in the survival of animals with cachexia associated with the syngeneic tumor model of cutaneous melanoma with resistance exercise intervention when compared with the control group.

Author contributions

Lopes JVN was responsible for writing the article, statistical design. Soares LVR was responsible for data collection and animal management. Soares ER was responsible for animal management. Alves MR was responsible for the statistical design and laboratory analysis. Rodrigues VD was responsible for the general orientation and coordination of the research.

Competing interests

No financial, legal, or political conflicts involving third parties (government, companies and private foundations, etc.) have been declared for any aspect of the submitted work (including, but not limited to, grants and funding, participation in advisory council, study design, preparation manuscript, statistical analysis, etc.).

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