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13. ABSTRACT (Maximum 200 Words) The purpose of this study is to examine the effects of exercise training on tumor response to anthracycline-based chemotherapy. Eighty athymic Fischer 344 mice will be purchased at 21 days and at 26 days of age, MDA-MB-231 carcinoma cells (3×10^6 cells prepared from donor tumors) will be subcutaneously implanted into the right flank of all animals. At 40 days of age, animals will be randomly assigned to one of four groups: (i) exercise alone (n=20), (ii) doxorubicin alone (n=20), (iii) exercise plus doxorubicin (n=20), and (iv) control (n=20). Animals assigned to doxorubicin alone and exercise plus doxorubicin will receive intravenous injections of doxorubicin at 5mg/kg every 7 days. The exercise group will be progressively trained to run at 22m/min at 0% grade for 45 minutes 5 days/week for 8 weeks. Forty eight hours after the final exercise session, all experimental animals will be sacrificed via carbon dioxide anesthetization and cervical dislocation. The primary tumor will be surgically removed, weighed and histologically processed. The primary outcome will be tumor volume measured in two dimensions. Secondary outcomes will be tumor growth delay calculated as the number of days for each individual animal tumor to reach 1000 mm ³ compared with the control group.				
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INTRODUCTION

In recent years a burgeoning number of reports have emerged examining the role of exercise as an adjunct therapeutic intervention for breast cancer patients during adjuvant chemotherapy[3]. Based on these reports it seems reasonable to accept exercise as a safe and beneficial adjunct therapeutic intervention during conventional breast cancer chemotherapy. However, studies have focused exclusively on exercise as a therapeutic modality to attenuate treatment-associated symptoms with the ultimate goal of enhancing or maintaining quality of life[3]. An essential but unaddressed pre-requisite to this line of investigation is to determine whether concurrent exercise training influences the antineoplastic effects of chemotherapy[2]. Exercise training may dramatically influence the metabolic and hormonal extracellular milieu responsible for tumor progression and/or chemotherapeutic resistance. Moreover, additional exercise training adaptations such as improved peripheral blood flow[5] and nitric oxide endothelial-mediated vasodilation may improve the delivery of anthracycline-based chemotherapy. Overall, these adaptations favor an environment that reduces hormonal and metabolic substrates available for tumorigenesis resulting in reduced cellular proliferation, malignant transformation and potentially increased therapy sensitivity and tolerability. However, no study to date has examined the effects of exercise training on the tumor response to anthracycline-based chemotherapy. As such, the purpose of this study is to examine the effects of exercise training on tumor response to anthracycline-based chemotherapy in mice bearing the MDA-MB-231 human mammary carcinoma cell line.

BODY

The following section describes the research accomplishments achieved to date associated with each tasks outlined in the approved statement of work.

Task 1: Obtain Ethical Approval for Study (Months 1-2)

The ethics application was originally submitted to the Cross Cancer Institute's Animal Research Ethics Board on June 27, 2003. Unfortunately, we experienced a number of problems in

attempting to secure ethical approval for this study. Although the project had received external funding from the U.S. Department of Defense Breast Cancer Research Program, the committee also sent the proposal for external merit review. In totality the whole process took approximately five months and ethical approval was finally awarded in October 2003. The process of obtaining Cross Cancer Institute ethical approval was far more complicated than we originally anticipated and significantly delayed the initiation of the project.

Task 2: Pilot Testing of Breast Carcinoma Cell Lines and Anthracycline-Based Chemotherapy (Months 2-4)

After finally obtaining ethical approval we initiated the first of two pilot studies investigating the growth rate of breast carcinoma cell line MDA-MB 231. To confirm the tumorigenicity of MDA-MB 231 human breast carcinomas under our laboratory conditions we initiated a pilot study using 18 athymic female mice. **Cells were harvested in the exponential growth phase and were stained with Trypan Blue and the number of viable cells was counted using a hemacytometer.** Mice were then subcutaneously implanted with 1×10^6 , 2×10^6 , and 3×10^6 cells in the right flank of 6 mice per group ($n=18$ in total) anesthetized with Isoflurane. Mice were then observed to determine the number of animals to develop palpable tumors at 30 days and determine the average tumor volume. **Tumor volume was measured in two dimensions with microcalipers using the following equation: $V = L \times W \times 0.5W$ where W is the width of the xenograft and L is the length as suggested by Bandyopadhyay et al. [1], Qin et al. [12] and Trial et al. [13].** If less than 4 animals have palpable tumors at 30 days, the number of cells was to be increased and the experiment repeated using 6 athymic female mice. **Unexpectedly, breast carcinoma cell line dosages of 1×10^6 , 2×10^6 , and 3×10^6 cells generally failed to produce palpable tumors at 30 days in athymic female mice. After consultation with my team and other investigators at our institution (Cross Cancer Institute) with prior experience with the breast carcinoma cell line MDA-MB 231, we concluded that we were satisfied with the viability of the cells and therefore opted to increase the cell line dosage**

before locating an alternative source of cells. As such, we repeated the experiment, however this time injecting mice subcutaneously with 5×10^6 cells. This cell line dosage produced palpable tumors by 30 days in all mice. Therefore, we concluded that these tumor cells were viable and this dosage was used for subsequent experiments.

Following pilot study one, we initiated pilot study two to determine an appropriate dose and schedule of Adriamycin in athymic female mice bearing MDA-MB 231 human breast carcinoma cell line (5×10^6). In previous reports, athymic female mice bearing the MDA-MB 231 breast carcinoma cell line have been treated with various doses and schedules of Adriamycin (e.g., 5mg/kg every four days and 5mg/kg every 7 days intravenously in a lateral tail vein and 5mg/kg every 5 days representing the highest tolerable doses and reflecting current clinical therapy guidelines). Although these protocols were effective at significantly reducing tumor volume in MDA-MB 231 breast carcinoma bearing mice, animals also experienced weight loss (approaching 10% of initial weight by 2 weeks) indicating that maximum tolerated dosage was met or exceeded. Because exercise may also produce weight loss, we performed a second pilot study with athymic mice bearing the MDA-MB 231 human breast carcinoma cell line to determine an appropriate dose and schedule of Adriamycin to produce a 5-7.5% weight loss. Twelve experimental animals were purchased at 21 days of age and allowed to acclimatize for 10 days prior to the commencement of pilot study two. At 31 days of age, MDA-MB-231 carcinoma cells (5×10^6 cells prepared from donor tumors) were subcutaneously implanted into the right flank of all animals. By 38 days all experimental animals had palpable tumors and were subsequently randomly assigned to receive the following doses: 3mg/kg (Group 1), 4 mg/kg (Group 2), 5mg/kg (Group 3), and 6mg/kg (Group 4) every 7 days for 8 weeks[6]. Injection sites were rotated to minimize local tissue irritation and injury. Adriamycin timing was consistent for all animals. The results of pilot study 2 are provided below.

Mean Tumor Volumes per Group

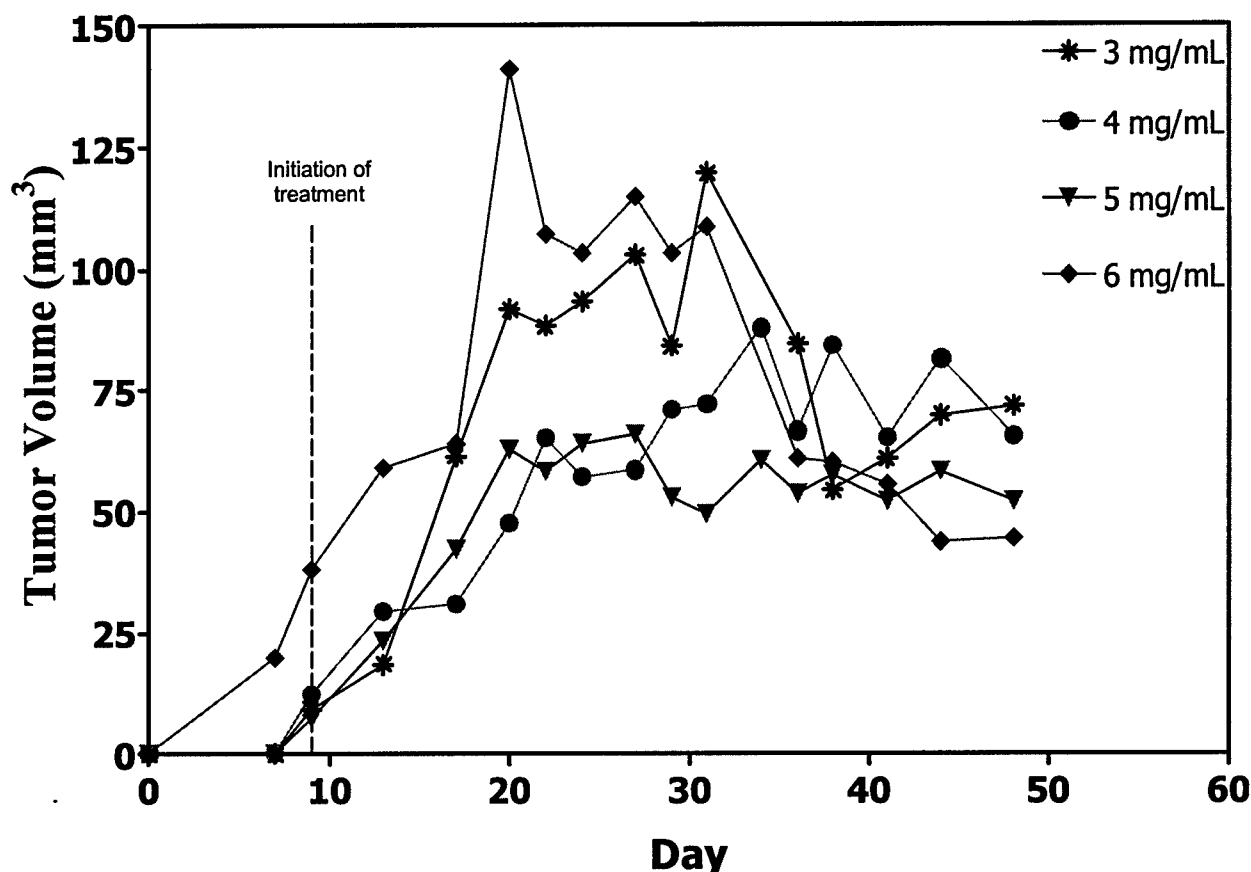


Figure 1. Determining the appropriate dose and schedule of Adriamycin in athymic female mice bearing MDA-MB 231 human breast carcinoma cell line (5×10^6). Twelve mice were randomly assigned into four groups of three. ★ 3 mg/kg every 7 days, ● 4 mg/kg every 7 days, ▼ 5 mg/kg every 7 days ■ 6 mg/kg every 7 days. Tumor growth after initiation of Adriamycin treatment. By Day 7 mice in all groups had palpable tumors. Tumor volumes rapidly increase in all groups until approximately day 20 onwards when Adriamycin administration begins to check the tumor growth rate in all groups. Close observation of the tumor growth rates reveals that Adriamycin dosage in Group 1 (3 mg/kg) provided adequate tumor growth until approximately Day 39 where tumor volumes rapidly increase, suggesting tumors had become resistant to this level of Adriamycin dosage. Group 4 (6 mg/kg) also provides adequate tumor growth control, however from approximately Day 27 onwards tumor volumes continually decrease, suggesting that these tumors may eventually become immeasurable. Finally, Groups 2 (4 mg/kg) and 3 (5 mg/kg) both provide adequate tumor growth control. Mice were sacrificed when tumor volume reached 1100mm³ as required by institutional guidelines. Statistical analysis of the mean tumor growth rates were not performed because we were interested in which drug dosage provided controllable tumor growth control across the entire study rather than which drug dosage produced the lowest tumor volume at the end of the study.

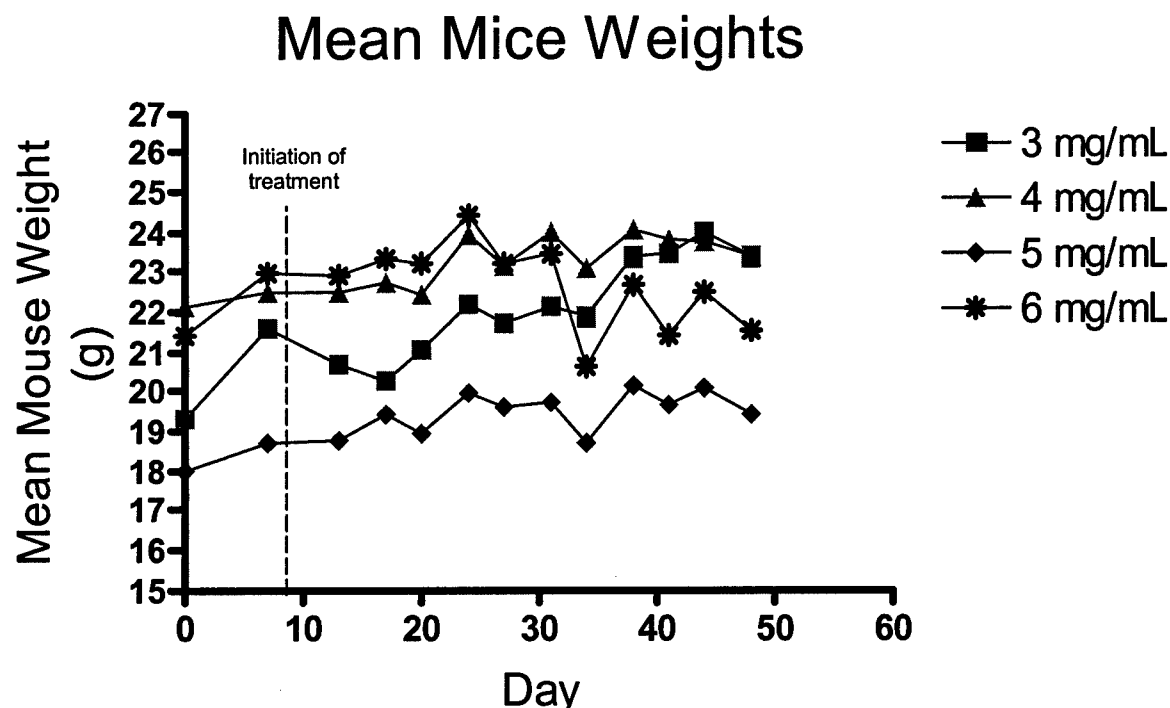


Figure 2. Determining the appropriate dose and schedule of Adriamycin in athymic female mice bearing MDA-MB 231 human breast carcinoma cell line (5×10^6). Twelve mice were randomly assigned into four groups of three. ★ 3 mg/kg every 7 days, ● 4 mg/kg every 7 days, ▼ 5 mg/kg every 7 days ■ 6 mg/kg every 7 days. Mean mice weights after initiation of Adriamycin treatment. By Day 7 mice in all groups had palpable tumors. Close observation indicates minimal mean mice body weight loss in all groups over the entire course of the experiment, except in Group 4 (6 mg/kg) where mean mice body weight rapidly decrease from approximately Day 30 onwards suggesting that that maximum tolerated dose has been reached or exceeded.

Taking into consideration the mean tumor volumes (Figure 1) and the mean mice body weights per group (Figure 2) we concluded that that 4mg/kg every 7 days (Group 2) was the most appropriate dose of Adriamycin. In Group 1 (3mg/kg), the drug dosage was well tolerated however this dosage did not provide adequate controllable tumor cell growth. Similarly, in Group 4 (5 mg/kg) the drug dosage was also well tolerated, however from approximately Day 27 onwards tumor volumes continually decreased and we were concerned that these tumors may eventually become immeasurable. In addition, 4 mg/kg was providing adequate tumor growth control, so subjecting mice to higher doses of

Adriamycin (5 mg/kg) and possibly higher toxicity to achieve similar tumor control seemed inane. Finally, in Group 4 (6 mg/kg) the drug dosage was not well tolerated, mice lost weight from approximately Day 30 onwards suggesting that maximum tolerated dose has been reached or exceeded. Again, similar to Group 3, from approximately Day 19 onwards, tumor volumes continually decreased and we were again concerned that these tumors may eventually become immeasurable. Based on this evidence, 4 mg/kg was selected as the appropriate dosage of Adriamycin. Importantly, several previous reports examining the pharmacokinetic profile of Adriamycin in athymic mice [4, 7, 9, 14], have demonstrated that similar dosing schedules produced tumor growth delay and statistically significant decrease in mean tumor volume with acceptable toxicity. Furthermore, the pharmacokinetic profile of doxorubicin in athymic mice has been shown to mimic clinical pharmacokinetics and pharmacodynamics of anticancer drugs in the clinical setting [9].

In the interest of using the lowest number of mice possible, we did not include a positive control group in the second pilot study. We realize that without an untreated control it is difficult to conclude that the different dosages of Adriamycin have any effect on tumor growth. However, it appears that all doses of Adriamycin produced some form of controllable tumor growth (see Figure 1). Our observation that the tumor volume growth curves in pilot study one were higher with the same breast carcinoma cell line dosage over the same number of days in comparison to pilot study two supports this notion. In addition, the main experiment does include a positive control group therefore we will be able to definitely conclude from this on-going experiment if 4 mg/kg of Adriamycin every 7 days does influence tumor growth.

Task 3: Confirm Testing/Intervention Protocol (Months 4-5)*a. Clarify exercise training, breast carcinoma cell line administration, and drug dosage protocol*

Following the completion of the pilot studies (as described) we wanted to confirm all experimental procedures before initiating the final protocol. The results of pilot study one indicated that the breast carcinoma cell line dosage of 5×10^6 cells produced palpable tumors by 30 days and was appropriate for the study. The results of pilot study two indicated that 4mg/kg of doxorubicin every 7 days produced controllable tumor growth without significant weight loss and was also deemed appropriate for the main study. Regarding the exercise training protocol, animals will be trained on a treadmill with 6 chambers, allowing 6 animals to be trained each session. To ensure that the physical and social environments are similar, a second, treadmill will be used as a sham-exercise for the non-exercising groups as suggested by Thompson⁷⁵. Control animals will be placed on a stationary treadmill for 45 minutes for 5 days/wk at 0 m/min at 0% grade for eight weeks. As such, each animal will receive the same handling and treadmill containment as the experimental groups with the exception of exercise training. The exercise groups will be progressively trained to run at 22m/min at 0% grade for 45 minutes 5 days/week for 8 weeks. The animals will be continuously monitored for the entire duration of exercise. This training intensity corresponds to approximately 70-75% of maximal oxygen uptake[10, 15]. Exercise training will begin at 10m/min, 0% grade, for 10 minutes for 5 days/week in weeks 1 and 2 of the 8-week exercise training protocol and will be systematically increased until the desired training protocol is achieved. A puff of air will be used to encourage animals to exercise, an electric shock will not be used as negative reinforcement. In situations where mice are unable to adhere to the designated exercise protocol, treadmill speed will be reduced 3m/min until a minimum speed of 13m/min is reached. If mice are unable to exercise at 13m/min, exercise training will be terminated. This exercise training protocol was adapted from previous research examining the effects of exercise training on immune response and

antioxidant expression in untrained rats[8, 10] and is reflective of current national exercise guidelines for cardiovascular health in adults[11]

b. Clarify necropsy and histological procedures

Forty-eight hours following the final exercise training session, all experimental animals will be sacrificed via carbon dioxide. The primary tumor will be surgically removed, weighed and histologically processed. The lungs will be removed and examined visually as well as microscopically for the formation of tumor cell colonies. Additional tissues such as a skeletal muscle, the heart and the liver and blood will also be removed at necropsy. These tissues will be individually placed in i) a labeled vial and flash frozen with liquid nitrogen, and ii) formalin fixed for future analysis. The animals will also be sacrificed by CO₂ and the tumors dissected and processed as above if any of the following conditions are met:

- if a tumor is beginning to corrode through the skin
- if a tumor is inhibiting the natural movement of the animal
- if a tumor is causing irritation to the animal
- if a tumor measures 1.0cm x 1.0cm or greater
- if feet develop abrasions
- if mice are injured

All procedures will be performed at the Cross Cancer Institute under the direction of John Mackey. Histopathology of necropsy specimens will be performed by Dr. Chiu, University of Alberta Hospital, Department of Pathology. Suspected infection-associated animal mortalities will be thoroughly investigated via a complete post-mortem dissection in Health Services Laboratory Animal Services (HSLAS) at the University of Alberta. Specifically, the necropsy form will be completed and Drs. Nation and Uwiera (HSLAS pathologists) will be immediately contacted and de-briefed regarding the animal mortality. Drs. Nation and Uwiera will then perform a complete dissection of the animal including a bacterial culture on the gastrointestinal

contents to identify any abnormal bacteria and a complete analysis of the animals' food so comparison bacteria analyses can be performed.

- c. *Ensure all necessary equipment (e.g., drugs, cell lines, etc) is purchased and/or operational*

All equipment and supplies have been purchased and are operational

Task 4: Data Collection (Months 5-9)

At the time of this report we are ready to initiate main data collection. We have been granted a one-year extension of our award and anticipate study completion by November 2004.

KEY RESEARCH ACCOMPLISHMENTS

- At the time of this report we have not yet initiated our main collection, therefore we no key research accomplishments at this time.

REPORTABLE OUTCOMES

- Based on the expected outcomes of this trial we have recently submitted a grant to the US Department of Defense Breast Cancer Research Program Multidisciplinary Postdoctoral Fellowship Award to translate this research into the clinic. The aim of the submitted proposal is to extend our forthcoming preclinical data in a phase II randomized trial examining the effects of exercise training on tumor vascularity and response in women receiving neoadjuvant chemotherapy for primary breast cancer.
- This award has provided an employment opportunity for a laboratory technician at the Cross Cancer Institute, Edmonton, Canada. In addition, this award has also allowed the technician to attend several training courses on mouse handling and necropsy.
- This award has also provided a unique training experience for an undergraduate student from the University of Alberta. This student has also attended several training courses supported by this award.

CONCLUSIONS

Again, given that at the time of this report we are about to initiate the main data collection, it is difficult to summarize the results in light of the importance and implications of the completed research. However, given the recent explosion of interest in the potential role of exercise as a supportive therapy for cancer patients during chemotherapy it is essential to investigate if exercise training influences the efficacy of chemotherapy in animals bearing breast cancer. Chemotherapy is accepted as an effective treatment to improve survival in breast cancer patients. Therefore, identifying complementary treatment approaches to optimize the effectiveness of this treatment are of obvious importance.

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APPENDICES

Not applicable