Effects of Exercise Training on Antitumor Efficacy of Doxorubicin in MDA-MB-231 Breast Cancer Xenografts

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Abstract

Purpose: Exercise is becoming readily accepted as a beneficial adjunct therapy to maintain or enhance quality of life in breast cancer patients undergoing adjuvant chemotherapy. An essential precursor to these studies is to investigate whether exercise modulates the antitumor efficacy of chemotherapeutic agents.

Experimental Design: Athymic female mice were transplanted with MDA-MB-231 breast xenografts and randomly assigned to one of four groups (n = 21 per group): (a) control, (b) exercise-only, (c) doxorubicin-only, or (d) exercise + doxorubicin. Exercise groups performed progressive treadmill running up to 18 m/min at 0% grade for 45 minutes, 5 d/wk for 8 weeks.

Results: Tumor growth delay was significantly longer in the doxorubicin-only and exercise + doxorubicin groups compared with the control (median 42 versus 25 days, P = 0.0082; 36 versus 25 days, P = 0.029, respectively) and exercise-only groups (median 42 versus 25 days, P = 0.029; 36 versus 25 days, P = 0.080, respectively). There was no significant difference between the doxorubicin-only and exercise + doxorubicin groups (median 42 versus 36 days, P = 0.33), suggesting that moderate intensity exercise does not significantly influence doxorubicin-induced tumor growth delay.

Conclusion: These studies are essential to fully understand the safety and application of exercise as a supportive intervention in cancer control.

Several recent randomized trials have examined the role of exercise as a supportive intervention for breast cancer patients undergoing conventional adjuvant chemotherapy (1–3). Results of these trials have provided preliminary evidence that exercise training is a feasible and supportive intervention that may attenuate a broad range of deleterious symptoms (e.g., functional decline, fatigue, nausea) associated with cytotoxic therapy, leading to clinically relevant improvements in the patients' quality of life (1–3). Although the importance of quality of life as a clinical end-point is clear, a critical and previously unexplored corollary to this line of investigation is whether exercise training influences the anticancer effects of conventional cytotoxic therapy. The potential interaction between exercise and chemotherapy efficacy is biologically plausible. Exercise is a potent pleiotropic intervention that influences a wide spectrum of biological processes that could potentially modulate the cytotoxicity of chemotherapeutic agents. Indeed, prior preclinical studies have reported both an inhibitory (4–8) and augmentary (9–11) effect of endurance exercise training on mammary tumor growth and progression, although others have reported no association (12). To our knowledge, however, no study has examined the potential interaction between exercise and concurrent administration of chemotherapy.

Materials and Methods

MDA-MB-231 breast carcinoma cells (American Type Culture Collection, Rockville, MD; prepared from donor animals at 5 × 10⁶) were s.c. implanted into the right flank of 92 female athymic nude mice (Harlan Teklad, Madison, WI) aged 3 to 4 weeks. Animals in which tumors failed to grow were excluded from the study (n = 8). All animals were fed a modified basal diet (Harlan Teklad) with 40% of calories from fat to reflect a typical North American diet (13) and water ad libitum. The diet was freshly prepared weekly to prevent the fat from becoming rancid.

Following tumor establishment (14 days, tumor volumes ~ 300 mm³), mice were stratified by body weight and tumor volume and randomly assigned to one of four groups (n = 21 per group): (a) control group, (b) exercise-only, (c) doxorubicin-only, or (d) exercise + doxorubicin. Doxorubicin (Adriamycin hydrochloride, Sigma-Aldrich Canada, Ltd., Oakville, ON) was given via weekly i.v. lateral tail vein injections at 4 mg/kg for 8 weeks. Injection sites were rotated to minimize local tissue irritation and injury. Exercise groups performed progressive...
treadmill running up to 18 m/min at 0% grade for 45 minutes, 5 d/wk for 8 weeks. Exercise training began at 10 m/min, 0% grade, for 10 minutes for 5 d/wk on weeks 1 and 2, and was systematically increased until the desired exercise protocol was achieved. This training intensity corresponds to ~70% to 75% of murine maximal oxygen uptake (14). Electrical stimulation was not used to encourage the animals to run. To ensure similar physical and social environments, a second treadmill was used as a sham exercise for the nonexercising groups (15). Tumor volume and body weight were measured twice weekly and animals were monitored continuously for the entire duration of exercise. Animal care was approved and in accordance with the Institutional Animal Care and Use Guidelines at the Cross Cancer Institute, Edmonton, Canada.

Mice were sacrificed when tumor volume reached 1,100 mm$^3$ as required by institutional guidelines, or 48 hours following the final exercise training session. The primary end point was tumor growth delay, calculated as the number of days for each individual tumor to reach 1,100 mm$^3$. Tumor growth delay survival curves were analyzed using the Cox model for pairwise comparisons and relative risk estimates generated with 95% confidence intervals (CI). The log-rank test was used for the overall group comparison. Changes in body weight were analyzed using independent-samples t tests. Two-tailed tests were used for the analysis with a $P < 0.05$ considered significant.

### Results and Discussion

Tumor growth delay and between-group comparisons are presented in Table 1. Tumor growth delay was significantly prolonged in the doxorubicin-only and exercise + doxorubicin groups compared with the exercise-only and control groups. There was no significant difference between doxorubicin-only and exercise + doxorubicin groups or exercise-only and control groups. At 45 days, Kaplan-Meier estimates indicated a 35% survival rate (95% CI, 17-54%) for the doxorubicin-only group compared with 20% (95% CI, 7-33%) in the exercise + doxorubicin group, 16% (95% CI, 2-31%) in the exercise-only group, and 0% in the control group (Fig. 1). Body weight did not significantly change over the course of the experiment in any group (Fig. 2). All mice achieved the designated exercise protocol.

The American Cancer Society recently published guidelines recommending that all cancer patients be encouraged to exercise during chemotherapy. These recommendations are primarily based on the promising preliminary evidence of the effects of exercise on maintaining or enhancing quality of life (16). As such, studies investigating the potential interaction between exercise and chemotherapy efficacy are essential to the interpretation and acceptance of exercise as a modifier of quality of life. As expected, groups that received doxorubicin had significantly prolonged tumor growth delay than groups who did not receive cytotoxic therapy. However, the key finding of this investigation was that moderate-intensity treadmill running did not significantly modulate doxorubicin-induced tumor growth delay in MDA-MB-231 breast carcinoma xenografts.

A considerable number of exercise trials and preclinical studies in oncology have provided indirect evidence of the potential biological mechanisms that may underlie the complex and multifaceted interaction between exercise, the tumor, and the antineoplastic effects of anthracycline-based chemotherapy. These biological mechanisms include, but are not limited to, exercise-modulated changes in hormonal (17) and metabolic profile (18), nitric oxide–mediated peripheral blood flow (19, 20), angiogenesis (21, 22), endogenous antioxidant expression (23), and pharmacokinetic profile of agents (24). However, the present findings provide preliminary evidence that an exercise training program reflective of national exercise guidelines for cancer prevention and cardiovascular health (25) in humans may not sufficiently perturb these pathways to interact with the anticancer effects of doxorubicin on breast carcinoma xenografts.

Several prior preclinical reports have shown an inhibitory effect of exercise training on spontaneous and chemically induced tumor growth, metastatic progression and microvessel density without concurrent chemotherapy (4, 7, 8, 26, 27). However, almost all of these studies have examined the effects of exhaustive treadmill running at ~80% to 90% of maximal oxygen consumption (VO$_{2\text{max}}$). These previous findings in combination with the present results suggest that exercise training needs to be done at a high intensity to activate tumor-modulating pathways (>70% VO$_{2\text{max}}$; ref. 26). Support for this notion is provided by the fact that the tumor growth curves for the exercise-only and control groups were essentially identical in the present study (see Fig. 1). Of course, from a clinical perspective, it is unlikely that many breast cancer patients undergoing cytotoxic therapy will be able or willing to exercise at 80% to 90% VO$_{2\text{max}}$. Thus, further research is required to investigate if a dose-response relationship exists between exercise and cancer progression during concurrent antineoplastic therapy.

### Table 1. Tumor growth delay of athymic female mice implanted with MDA-MB-231 breast carcinoma xenografts after treatment with doxorubicin (4 mg/kg every 7 days), exercise training (18 m/min, 0% grade, 45 minutes, 5 d/wk for 8 weeks), doxorubicin plus exercise, or no intervention control

<table>
<thead>
<tr>
<th>Group</th>
<th>Median tumor growth delay (d)</th>
<th>Relative risk (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exercise</td>
<td></td>
</tr>
<tr>
<td>No doxorubicin</td>
<td>25</td>
<td>25</td>
<td>0.85 (0.41-1.7)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>42</td>
<td>36</td>
<td>1.44 (0.69-3.0)</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>0.38 (0.15-0.76)</td>
<td>0.54 (0.27-1.1)</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.0084</td>
<td>0.080</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Tumor growth delay calculated as the number of days for each individual tumor to reach 1,100 mm$^3$. Tumor growth delay survival curves were analyzed using the Cox model for pairwise comparisons and relative risk estimates generated with 95% CI.
Obviously, several important differences exist between our mouse model of breast cancer and patients with breast cancer in the clinical setting. First, mice in the present study were only a few weeks post-weaning and thus, immature. Second, hormonal and immune profile of the groups is not equivalent. This may be particularly important given the potential biological interaction between exercise, immune/hormonal profile, and breast cancer prognosis (28). Finally, our breast cancer cell line was implanted s.c. rather than at the relevant orthotopic site (i.e., mammary pad). Thus, caution must be taken when extrapolating the present results to women undergoing cytotoxic therapy for early-stage or metastatic breast cancer.

To summarize, the present results suggest that exercise training does not significantly modulate the antitumor efficacy of doxorubicin in breast cancer xenografts. Further studies investigating the effects of exercise on the cytotoxic effects of...
chemotherapy and other conventional agents in the clinical setting are warranted. Such studies are essential to fully understand the safety and application of exercise as a supportive intervention in cancer control.

References

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