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Brandon Jones

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Creatine Supplementation Does Not Alter Proliferation or Doxorubicin Sensitivity of Mammary Carcinoma Cells

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Abstract

Objectives: Doxorubicin (DOX) is an effective chemotherapy drug used to treat breast cancer, but is limited by detrimental side effects such as cardiotoxicity and skeletal muscle atrophy. Supplementation with creatine has been shown to protect cardiac and skeletal muscle cells from the cytotoxic effects of DOX. However, a concern with dietary creatine supplementation in cancer patients is the possible protection of cancer cells from therapeutic DOX toxicity. Thus, we investigated the effects of in vitro creatine supplementation on proliferation and survival of DOX-treated MAM-BII, rat mammary carcinoma cells. Methods: MAM-BII cells were seeded in triplicate at 15,000 cells/well in a 96-well microplate for an eight day incubation in one of six treatments: no treatment, 5 mM DOX, 2mM creatine, 2mM creatine + 5 mM DOX, 20mM creatine, 20mM creatine + 5 mM DOX. Media and treatments were refreshed every 48 hours. An MTT assay was run on day 8 to assess remaining cell number in each well. The results represent an average from three time separated experiments, with differences between treatments identified using a two-way ANOVA (IBM SPSS 27) and a significance threshold of 0.05.

Results

Mammary carcinoma cells (MAM-BII) were grown in minimum essential media supplemented with 10% fetal bovine essence (FBE) and 1% penicillin/streptomycin. MAM-BII cells were plated in triplicate at 15,000 cells/well in a 96 well microplate for an eight day incubation in one of six treatments: No treatment (Control), 5 mM DOX, 2mM creatine, 2mM creatine + 5 mM DOX, 20mM creatine, 20mM creatine + 5 mM DOX. To ensure adequate substrate concentrations, the treatment media was refreshed every 48 hours.

On day eight of the incubation, an MTT assay was run to examine cellular proliferation and analyzed using a spectrophotometer at 595 nm. Cells were selected on the final incubation day as a visual verification of the MTT assay. The results are a summary of the three, time separated incubations. The main effects and interactions between DOX and creatine were identified using a two-way ANOVA (IBM SPSS 27) with a significance set at 0.05.

Table 1: MAM-BII Incubation Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>2mM Cr</th>
<th>5mM DOX</th>
<th>5mM DOX + 2mM Cr</th>
<th>20mM Cr</th>
<th>20mM Cr + 5mM DOX</th>
<th>5mM DOX + 20mM Cr</th>
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<tbody>
<tr>
<td>No DOX</td>
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<tr>
<td>5 mM DOX</td>
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<tr>
<td>2mM Cr</td>
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<tr>
<td>2mM Cr + 5 mM DOX</td>
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<tr>
<td>20mM Cr</td>
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<tr>
<td>20mM Cr + 5 mM DOX</td>
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<tr>
<td>5mM DOX + 20mM Cr</td>
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</table>

Figure 1. MTT assay. When compared to Control, the MTT assay of MAM-BII cells treated with 5mM DOX revealed a significant main effect of DOX, consistent with impaired proliferation (P < 0.001). MAM-BII cells incubated in low creatine (2mM) and high creatine (20mM) had no significant main effect on proliferation (P = 0.771). The cells treated with 5mM DOX and creatine exhibited no interaction between creatine and DOX (P = 0.974).

Figure 2. Cell images of each treatment on the final day (day 8) of the incubation. Provided as a visual verification of the MTT assay.

References


Summary and Conclusion

The 5mM concentration of DOX was observed to be cytotoxic to MAM-BII cells over the eight day incubation used in this study. The cytotoxicity was demonstrated with an MTT assay (Figure 1) and verified with cell visualization (Figure 2). This low concentration of DOX (5mM) required an extended period of time (8 days) to decrease cell number significantly and was selected to give creative sufficient time to potentially reduce the cytotoxic effects of DOX.

Neither the low physiological dose of creatine (2mM) nor the high dose of creatine (20mM) had a significant main effect on the mammary carcinoma proliferation in-vitro.

A secondary outcome was to investigate the effect of creatine on MAM-BII cell sensitivity to DOX. Creatine did not have a significant interaction with DOX cytotoxicity for the 2mM. The 2mM creatine concentration had a significant effect on DOX in mammary carcinoma cells.

This research suggests that the use of dietary creatine in breast cancer patients receiving DOX will not impact the anticancer effects of DOX. Future research is needed to investigate the effect of creatine supplementation and DOX sensitivity on a variety of cancer cells.

Acknowledgement

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