

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 MAT-BIII, rat mammary carcinoma cells. **Methods:** MAT-BIII 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 nM DOX, 2mM creatine, 2mM creatine + 5 nM DOX, 20mM creatine, 20mM creatine + 5 nM 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:** As expected, the number of MAT-BIII cells was significantly reduced ($P < .001$) after DOX treatment, consistent with impaired proliferation or cytotoxicity. Neither low nor high-dose creatine exposure altered the number of cells, or the suppressive effects of DOX as no interaction between creatine and DOX was identified ($P > 0.05$). **Conclusion:** DOX decreased proliferation of MAT-BIII mammary carcinoma cells, indicating this cell line is sensitive to the effects of this chemotherapy drug. There was no indication that creatine influenced proliferation or DOX sensitivity of the mammary carcinoma cells. The absence of an effect in this experiment suggests that creatine could be supplemented to alleviate the adverse side effects of DOX treatment in human breast cancer patients. Future research aims to elucidate further the possible interactions between DOX and creatine in cancer cell metabolism. **Support/Funding Information:** This research was supported by the Graduate Student Association of the University of Northern Colorado.

Introduction

Creatine monohydrate is a popular dietary supplement that has traditionally been used to enhance athletic performance. Supplementing the diet with creatine increases lean body mass, reduces muscular fatigue, and improves sprint performance (1, 2). More recently, creatine has gained attention as a possible clinical supplement in several disorders including cardiovascular disease, muscular dystrophy, and cancer (3-5). The role of creatine in cancer is particularly interesting because of its potential to attenuate skeletal muscle wasting and the cardiotoxic effects of chemotherapy treatments.

Creatine supplementation research has primarily focused on the bioenergetic benefit of increased phosphocreatine concentrations promoting enhanced ATP synthesis; however, creatine can also help regulate glycolysis, decrease cytosolic acidosis, or scavenge reactive oxygen species (6, 7). These protective properties support the potential use of creatine as an approach to mitigate the negative side effects commonly observed in cancer patients undergoing chemotherapy treatments. Doxorubicin (DOX) is a commonly used chemotherapy agent, but its use is limited by its toxic effects on cardiac and skeletal muscle. Creatine supplementation has been shown to attenuate DOX-induced skeletal muscle dysfunction and improve cardiac cell survival (8, 9); however, the effect of creatine supplementation on cancer cell proliferation and sensitivity to DOX is still unclear.

Cancer progression is known to alter the phosphocreatine system (10). Therefore, the interaction between cancer and the phosphocreatine system is a potential concern for creatine supplementation during chemotherapy treatment. Interestingly, *in-vivo* studies have demonstrated an ability of creatine supplementation to decrease tumor growth (11, 12). Alternative *in-vitro* research, however, indicates that overexpression of the ubiquitin mitochondrial creatine kinase enzyme enhances cellular proliferation and inhibits apoptosis in mouse and human breast cancers (13, 14). The phosphocreatine system has also been shown to significantly impact the sensitivity of cancer cells to DOX. For example, decreasing the expression of cytosolic ubiquitin creatine kinase in leukemia and ovarian cancer cell lines displayed an increased sensitivity to DOX (15, 16). The current project aims to further explore the effect of creatine supplementation on cell proliferation and DOX sensitivity in mammary carcinoma cells.

Methods

Groups	
Control (No treatment)	5nM DOX (5nM Doxorubicin)
2mM Cr (2mM Creatine)	2mM Cr + 5nM DOX (2mM Creatine + 5nM Doxorubicin)
20mM Cr (20mM Creatine)	20mM Cr + 5nM DOX (20mM Creatine + 5nM Doxorubicin)

Table 1: MAT-BIII Incubation Groups

Mammary carcinoma cells (MAT-BIII) were grown in minimum essential media supplemented with 10% fetal bovine essence (FBE) and 1% penicillin/streptomycin. MAT-BIII 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 nM DOX, 2mM creatine, 2mM creatine + 5 nM DOX, 20mM creatine, 20mM creatine + 5 nM 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. Cell images were taken 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 interaction between DOX and creatine were identified using a two-way ANOVA (IBM SPSS 27) with a significance set at $\alpha = 0.05$.

Results

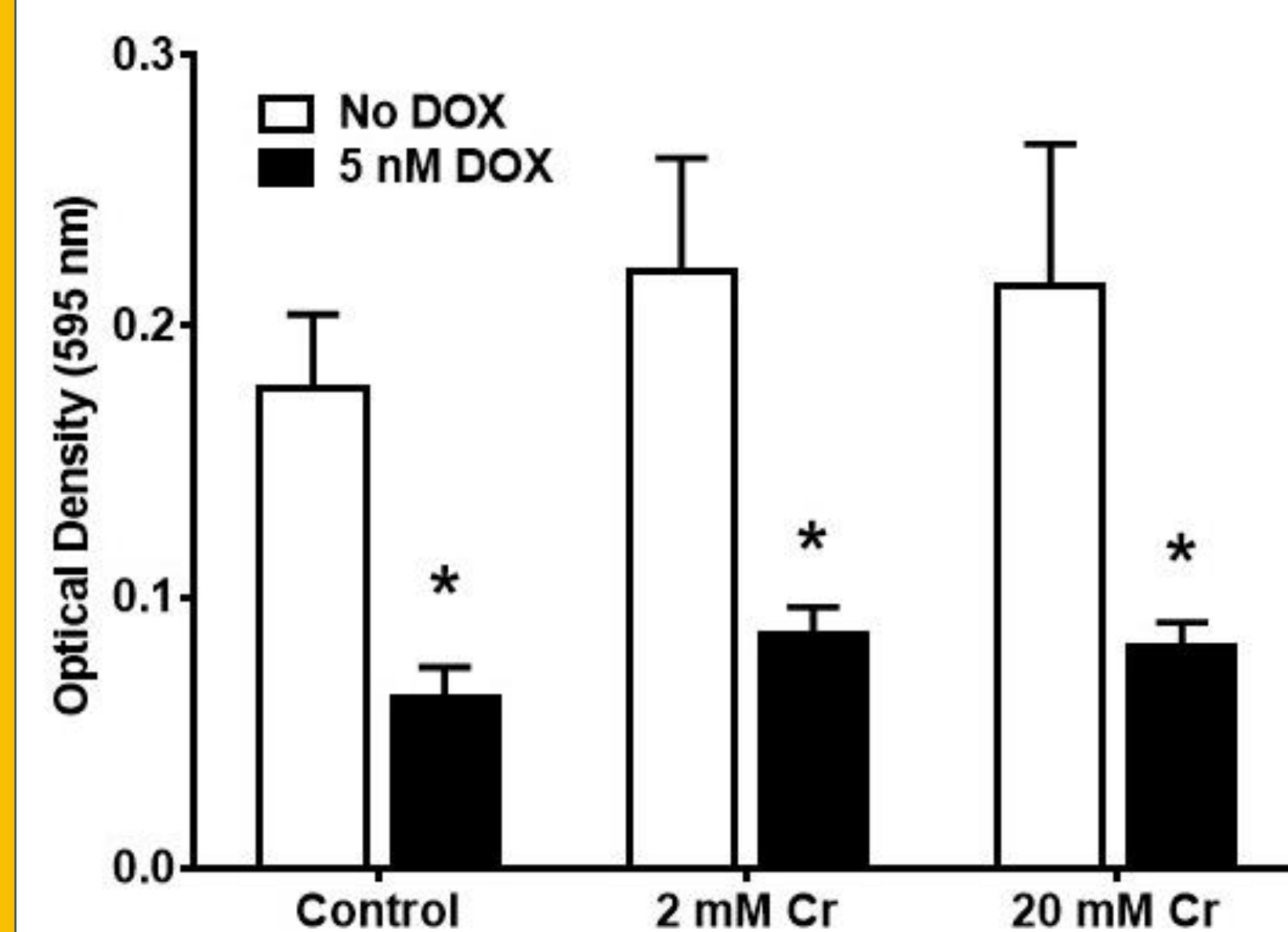


Figure 1: MAT-BIII Doxorubicin (Dox) and Creatine MTT Assay

Figure 1. MTT assay. When compared to Control, the MTT assay of MAT-BIII cells treated with 5nM DOX revealed a significant main effect of DOX, consistent with impaired proliferation ($P < .001$). MAT-BIII cells incubated in low creatine (2mM) and high creatine (20mM) had no significant main effect on proliferation ($P = 0.771$).

The cells treated with 5nM 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.

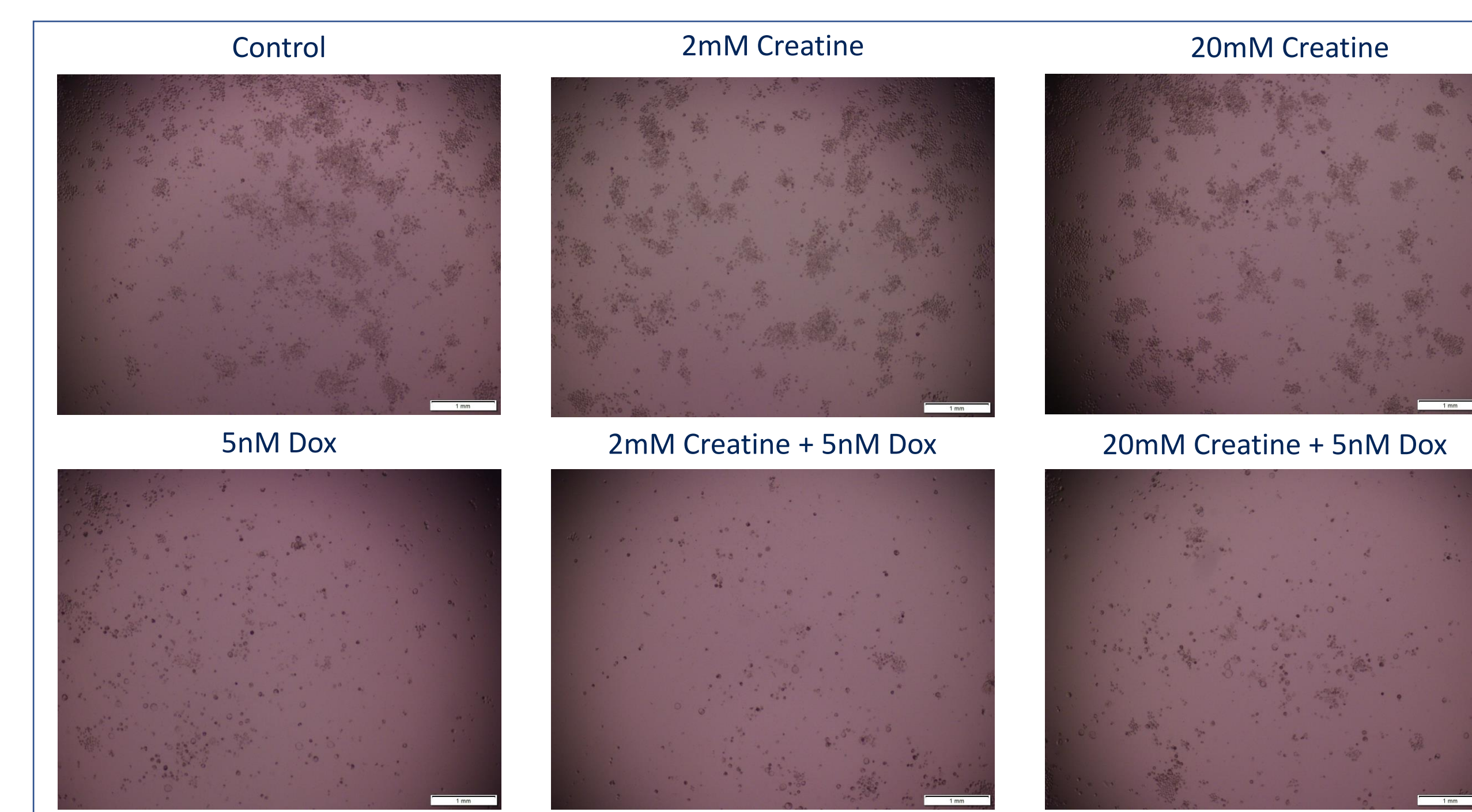


Figure 2: MAT-BIII Doxorubicin (Dox) and Creatine Incubation Pictures

Summary and Conclusion

The 5nM concentration of DOX was observed to be cytotoxic to MAT-BIII 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 (5nM) required an extended period of time (8 days) to decrease cell number significantly and was selected to give creatine 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. This supports the hypothesis that creatine would not affect mammary carcinoma proliferation *in-vitro*.

A secondary outcome was to investigate the effect of creatine on MAT-BIII cell sensitivity to DOX. Creatine did not have a significant interaction with DOX cytotoxicity. Neither the 2mM nor the 20mM 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.

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Acknowledgment

This research was supported by the Graduate Student Association of the University of Northern Colorado.