CAHD 2020

CYTOTOXIC AND ANTI-PROLIFERATIVE EFFECTS OF FUCOSTEROL, ALONE AND IN COMBINATION WITH DOXORUBICIN, IN 2D AND 3D CULTURES OF TRIPLE-NEGATIVE BREAST CANCER CELLS

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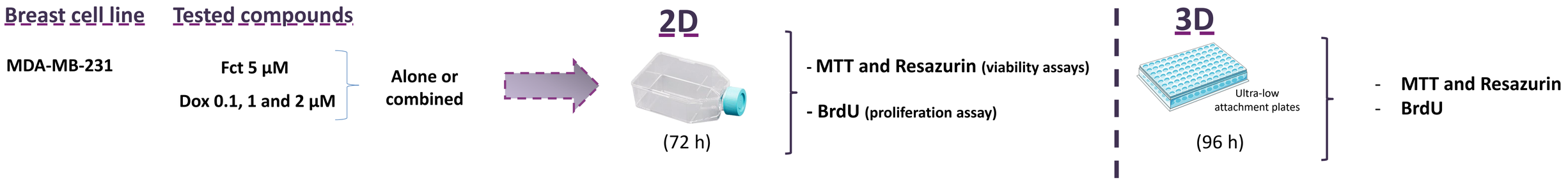
INTRODUCTION

- Triple-negative breast cancer (TNBC) has the poorest BC prognosis, being chemotherapy the mainstream treatment [1].
- Recent in vitro studies revealed a potential synergistic effect of selected natural compounds in combinatorial therapy with anti-cancer drugs [2] such as doxorubicin (Dox) which is frequently used for TNBC [3].
- Because of its antioxidant [4] and antitumor effects [5], the brown seaweed phytosterol Fucosterol (Fct) is one of these promising compounds.
- **Aim:** Using a TNBC cell line (MDA-MB-231), we aimed to test the effects of Fct alone and in combination with Dox on cell viability and proliferation, in monolayer and three-dimensional (3D) cultures.

MATERIAL AND METHODS



Statistical Analysis: Descriptive and inferential statistics were performed using GraphPad Prism 6.0 software (GraphPad Software, La Jolla, CA, USA). The results are expressed as mean ± standard error of mean (SEM), relative to negative control of three independent experiments (three duplicates per replica). Significant differences ($p < 0.05$) were assessed by one-way ANOVAs, followed by the post-hoc Holm-Šídák multiple comparison test, whenever the ANOVA disclosed significant results for the tested effects. The normality and homogeneity of variance were confirmed by the Shapiro-Wilk test and the Levene test, respectively.

RESULTS

Viability assays

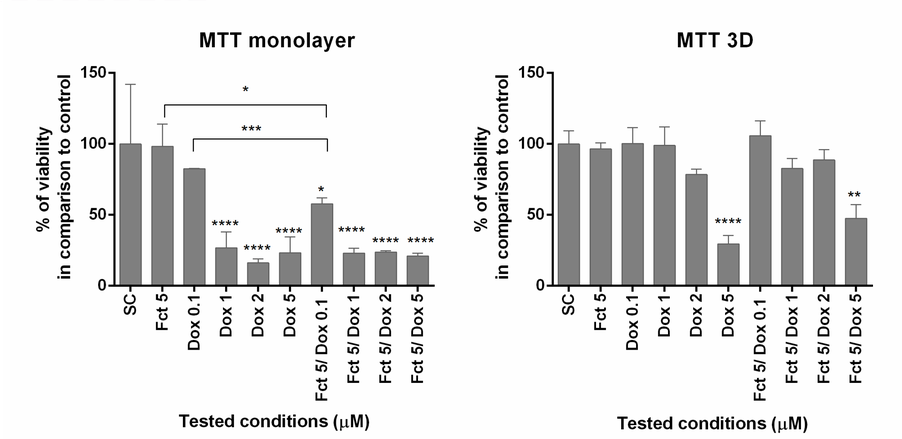


Figure 1. Effect of fucosterol (Fct) at 5 µM alone and in combination with Doxorubicin (Dox) at 0.1, 1 and 2 µM, on cell viability in monolayer (A) and 3D (B) assessed by MTT assay. Cells treated with 0.1% DMSO (SC) and Dox 5 µM were included as negative and positive controls, respectively. The results were expressed as the percentage of cell viability relative to SC (* $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$).

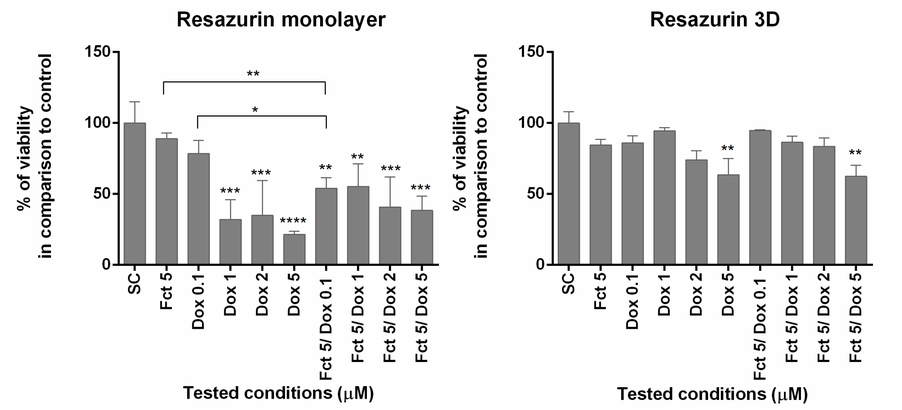


Figure 2. Effect of fucosterol (Fct) at 5 µM alone and in combination with doxorubicin (Dox) at 0.1, 1 and 2 µM, on cell viability in monolayer (A) and 3D (B) assessed by resazurin assay. Cells treated with 0.1% DMSO (SC) and Dox 5 µM were included as negative and positive controls, respectively The results were expressed as the percentage of cell viability relative to SC (* $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

The effects on cell viability assessed by MTT and resazurin very were similar

Fct alone did not have cytotoxic effects on the MDA-MB-231

In monolayer, both assays Dox (≥ 1 µM) induced cytotoxic effects in all tested conditions (alone and in combination)

In monolayer, the combination of Fct 5 µM with Dox 0.1 µM statistically differed from SC, and from the exposure of each compound alone

Proliferation assay

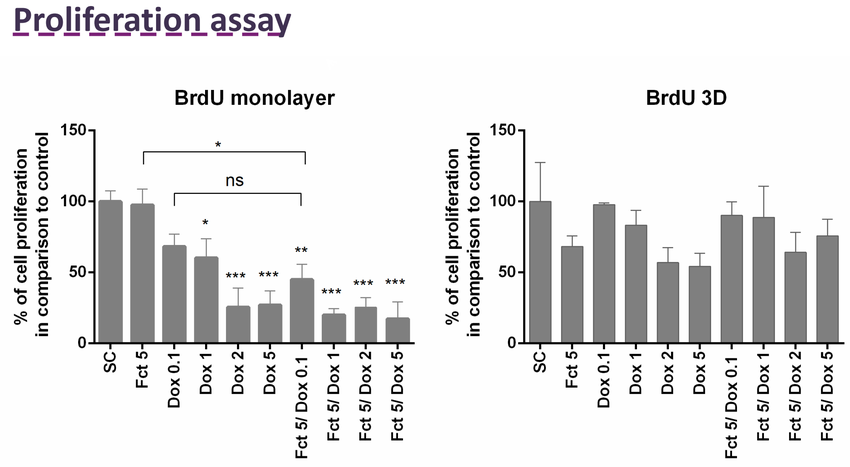


Figure 3. Effect of fucosterol (Fct) at 5 µM alone and in combination with doxorubicin (Dox) at 0.1, 1 and 2 µM, on cell proliferation in monolayer and 3D, assessed by BrdU assay. Cells treated with 0.1% DMSO (SC) and Dox 5 µM were included as negative and positive controls, respectively. The results were expressed as the percentage of cell viability relative to SC (* $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

Fct alone did not affect cell proliferation on the MDA-MB-231

In monolayer, Dox (≥ 1 µM) had anti-proliferative effects on MDA-MB-231

In monolayer, Dox 0.1 µM alone did not affect cell proliferation, but in combination with Fcol 5 µM, it reduced significatively the % of cell proliferation in relation to SC

In 3D culture:
- **cell viability only differed from the control at Dox 5 µM**
- **none of the tested conditions revealed effects on cell proliferation**

CONCLUSIONS

- **Under certain conditions, Fct seem to have potential to increase effects of Dox.**
- **Our results corroborate other studies reporting more resistance of cancer cells to treatments when in 3D culture.**
- **In this case, more resistance to the cytotoxic activity of Dox and its combination with fucosterol, reinforcing the importance of using these more complex models for drug screening.**

FUTURE PERSPECTIVES

- **Fct in low concentration seemed to have potentiated the Dox action. This is a very interesting result that needs to be further explored.**
- **Of particular importance would be to study the mechanisms related to the interplay between Fct and Dox, namely using a pathway-focused gene expression analysis and additional cell-based assays. As to the latter, and because Fct may change the levels of reactive oxygen species (ROS) [6].**

REFERENCES

[1] Dai, X., T. Li, Z. Bai, Y. Yang, X"Breast cancer intrinsic subtype classification, clinical use and future trends." American Journal Cancer 10: 2029-2943.ISSN:2156-6976/ajcr0014158.
[2] Lopes-Costa, E.; Abreu, M.; Gargiulo, D.; Rocha, E.; Ramos, A. A., Anticancer effects of seaweed compounds fucoxanthin and phloroglucinol, alone and in combination with 5-fluorouracil in colon cells. J Toxicol Environ Health A 2017, 80, (13-15), 776-787.10.1080/15287394.2017.1357297[3] [3] Lin, S.-R.; Chang, C.-H.; Hsu, C.-F.; Tsai, M.-J.; Cheng, H.; Leong, M. K.; Sung, P.-J.; Chen, J.-C.; Weng, C.-F., Natural compounds as potential adjuvants to cancer therapy: Preclinical evidence. Br J Pharmacol 2020, 177, (6), 1409-1423.10.1111/bph.14816
[4] Lee, S.; Lee, Y. S.; Jung, S. H.; Kang, S. S.; Shin, K. H., Anti-oxidant activities of fucosterol from the marine algaePelvetia siliquosa. Arch Pharm Res 2003, 26, (9), 719-722.10.1007/BF02976680
[5] Bae, H.; Lee, J.-Y.; Song, G.; Lim, W., Fucosterol suppresses the progression of human ovarian cancer by inducing mitochondrial dysfunction and endoplasmic reticulum stress. Marine drugs 2020, 18, (5), 261
[6]Khanavi, M.; Gheidarloo, R.; Sadati, N.; Ardekani, M. R.; Nabavi, S.; Tavajohi, S.; Ostad, S., Cytotoxicity of fucosterol containing fraction of marine algae against breast and colon carcinoma cell line. Pharmacogn Mag 2012, 8, (29), 60-64.10.4103/0973-1296.93327

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