GnRH ANALOGUE CONJUGATED WITH MITOXANTRONE: A NOVEL ANTIPROLIFERATIVE AGENT IN BREAST CANCER CELLS

Michail Deiktakis[1], Eleni Kantidenou[1], Aikaterini Kalantidou[1], Georgia Biniari[2], Christos Markatos[3], Vlasios Karageorgos[3], Eirini Dermitzaki[1] Theodore Tselios[2], George Liapakis[3], Maria Venihaki[1]

1 Department of Clinical Chemistry, School of Medicine, University of Crete, Heraklion, Greece
2 Department of Chemistry, University of Patras, 26504 Rion, Greece
3 Department of Pharmacology, School of Medicine, University of Crete, Heraklion, Greece

BACKGROUND

Breast cancer is one of the most common type of cancer in women. Even though many new treatments have been developed, the incidence of this disease is increasing every year [1].
The gonadotropin releasing hormone GnRH is a decapeptide that coordinates the release of Luteinizing Hormone and Follicle Stimulating Hormone, in order to regulate sex steroids and gametes. Moreover, GnRH interacts with the GnRH receptor (GnRH-R). Importantly, GnRH-R is highly expressed in various types of cancer cells, such as breast cancer cells. According to studies, GnRH analogues have antiproliferative effect in breast cancer cells, through their interaction with the GnRH-R [2].

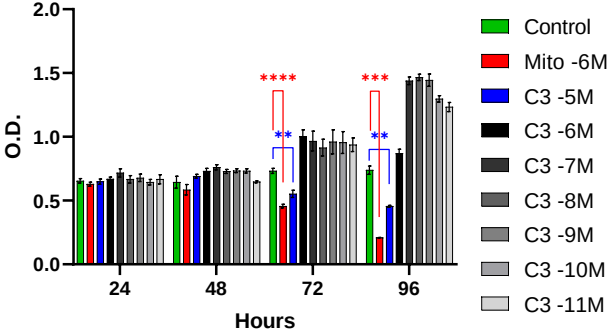
AIM

The aim of this study was to develop novel GnRH analogues with increased antiproliferative actions, by conjugating them with the cytotoxic agent, mitoxantrone, thus creating the analogue Con7. Con3 is expected to release mitoxantrone into cancer cells after its binding to GnRH-R and as a result internalize the GnRH-R/Con7 complex.

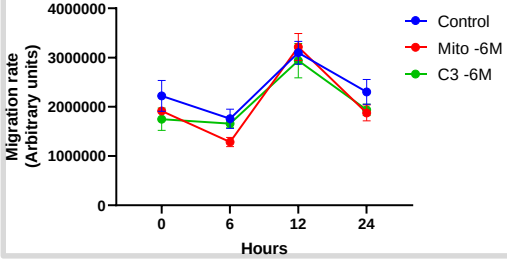
METHODS

To create the Con3 analogue we chemically modified the GnRH analogue, leuprolide and conjugated it with mitoxantrone. The human breast cancer cell line MDA-MB-231 was used in order to examine the antiproliferative properties of Con7. Specifically, we incubated the breast cancer cells with Con7 at different concentrations for 1-4 days and measured the viability rate with the MTT assay. Furthermore, we investigated the effect of Con3 in cell migration rate using the scratch assay. The statistical analysis of values was perfomed using the GraphPad Prism software. Two-Way ANOVA was performed for the comparison of the effect of Con7 among groups and values are expressed as mean ± SEM.

RESULTS



Antiproliferative effect of **Con3** incubated with **MDA-MB-231** cells at different concentrations measured with **MTT** assay at 4 time points. Data from 2 independent experiments with at least 5 replications/ group.



Cell migration rate in MDA-MB-231 cells incubated with Con3 and measured by scratch assay. Data from 1 experiment with at least 3 replications/ group.

The novel GnRH analogue Con3 inhibited the proliferation of MDA-MB-231 cells in a time-dependent manner (1-4 days). Con3 showed a statistically significant antiproliferative effect at day 3 and 4 and was able to reduce the proliferation rate in 10^{-5} M.
Con3 did not affect cell migration rate at any time point

CONCLUSION

Proliferation of MDA-MB-231 cells was inhibited by Con3 in a time-dependent manner, reaching maximum inhibitory effect at day 4. Moreover, the inhibitory effect of Con7 was comparable to that of unconjugated mitoxantrone, a known anticancer drug. Finally, Con3 did not increase cell migration rate in cancer cells.

REFERENCES

[1]: Ghaly, H., & Varamini, P. (2021). New drug delivery strategies targeting the GnRH receptor in breast and other cancers. *Endocrine-related cancer*, *28*(11), R251–R269. https://doi.org/10.1530/ERC-20-0442

[2]: Maggi, R., Cariboni, A. M., Marelli, M. M., Moretti, R. M., Andrè V., , Marzagalli, M., & Limonta, P. (2016). GnRH and GnRH receptors in the pathophysiology of the human female reproductive system. *Human reproduction update*, *22*(3), 358–381. https://doi.org/10.1093/humupd/dmv059

DISCLOSURES

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH - CREATE - INNOVATE (project code: T2ΕΔΚ 02056).Authors declare no further conflicts of interest.

Contact information

Maria Venihaki, Department of Clinical Chemistry, School of Medicine, University of Crete, Herakleion, Greece

venycham@uoc.gr