NOVEL GnRH ANALOGUES WITH ANTIPROLIFERATIVE PROPERTIES

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INTRODUCTION

The gonadotropin-releasing hormone (GnRH) is a hypothalamic 10-amino acid peptide that regulates the function of the reproductive system, by controlling the secretion of the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) from pituitary. GnRH exerts its biological actions through its interaction with the GnRH receptor (GnRH-R). In addition to healthy cells, histopathological analysis of many tumors, including endometrial ones, has revealed the expression of the GnRH-R. Previous studies have shown that GnRH analogues exert cytotoxic effects on several cancer cells, through their interaction with the GnRH-R expressed in these cells.

AIM

In this study, we developed GnRH analogues conjugated with the cytotoxic agent, mitoxantrone (Con 3, Con7) or without mitoxantrone (Con-P1, Con-P2). We determined their antiproliferative activity, in the Ishikawa endometrial cancer cells, which express the GnRH-R. We hypothesized that binding of GnRH analogues to their receptor results in the internalization of the GnRH-R/GnRH conjuagate complex and the release of mitoxantrone in the cytoplasm, which subsequently exerts its cytotoxic effects.
The release of mitoxantrone from GnRH conjugates is anticipated to be achieved by the thioredoxin reductase system. This system is expressed in cancer cells, and it breaks the conjugates con3 and con7 into their free peptide and the cytotoxic drug mitoxantrone.

METHODS

To create the con3 and con7, we chemically modified the GnRH analogue, leuprolide, and conjugated it with mitoxantrone. In order to examine the antiproliferative effects of con3 and con7, we used the MTT assay. The MTT assay is a colorimetric assay, which determines cell metabolic activity, an indicator of cell proliferation. We incubated the endometrial cancer cell line, Ishikawa, with con3 and con7 at different concentrations for 1-4 days, in order to determine their antiproliferative activity. We also incubated Ishikawa with con-p2, con-p1, leuprolide and mitoxantrone to compare the results with the con3 and the con7.

RESULTS

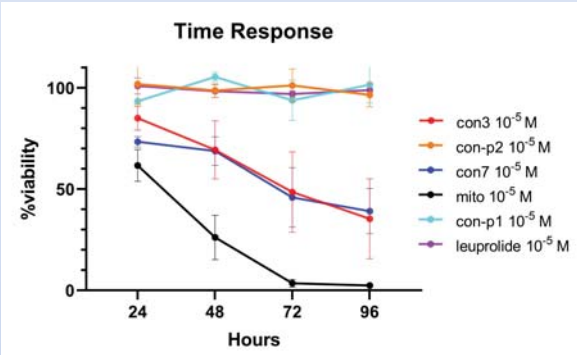

***Figure 1**: Proliferation of Ishikawa cells at different , treated with con3, con7, con-p2, con-p1 or mitoxantrone at a standard concentration (10^{-5}M).*

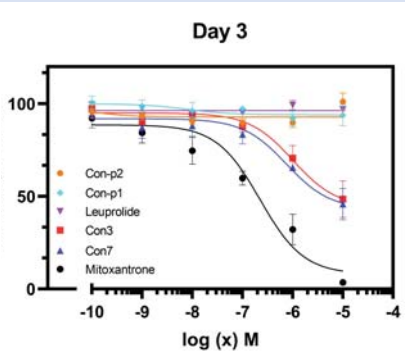

***Figure 2**: Antiproliferative effects of increasing concentrations of con3, con7, leuprolide, con-p1, con-p2 and mitoxantrone at day 3.*

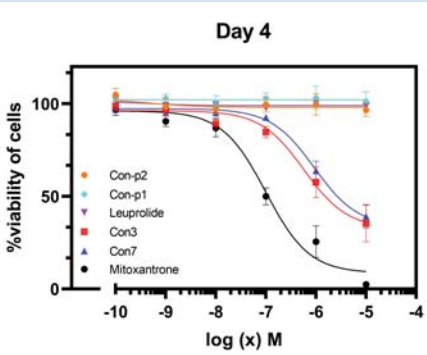

***Figure 3**: Antiproliferative effects of increasing concentrations of con3, con7, leuprolide, con-p1, con-p2 and mitoxantrone at day 4.*

We tested the ability of all compounds tested to inhibit the proliferation of endometrial cancer cells, Ishikawa (n≥3). The results have shown that the proliferation of Ishikawa cells was inhibited by con3 and con7 in a dose-dependent and time-dependent manner (1-4 days). The antiproliferative potency of con3 after exposure of Ishikawa to the conjugate for 2,3 and 4 days, was 0,98 μM, 1 μM, and 0,58 μM, respectively. The antiproliferative potency of con7 after exposure of Ishikawa to the conjugate for 2,3 and 4 days, was 0,73 μM, 0,75 μM, and 0,93 μM, respectively. In marked contrast, leuprolide, con-p2 and con-p1 had no effect on the proliferation of these cells (n≥3).

CONCLUSIONS

- Proliferation of Ishikawa cells was inhibited by the GnRH analogue conjugated with mitoxandrone, con3 or con7, in a time and dose-dependent manner.
- Proliferation of Ishikawa cells was not affected by the mitoxantrone-free peptides (con-p2 and con-p3) or leuprolide.
- This study will put the basis for the development of novel cytotoxic agents specifically targeting GnRH-R expressing tumors

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