A novel mitoxantrone-GnRH analogue conjugate with antitumor effects

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BACKGROUND

The gonadotropin releasing hormone (GnRH) plays a key role in the function of the reproductive system through its interaction with its receptor (GnRH-R) as it regulates the circadian secretion of follicle stimulating hormone (FSH) & luteneizing hormone (LH), which in turn affect steroidal sex hormone production[1]. However, GnRH seems to have a direct action on certain cancer types as GnRH-R is also highly expressed in various types of breast and genital tumor cells, including the ovarian ones[2]. Previous studies have shown that GnRH analogues exert antitumor effects on ovarian and other cancer cells, through their interaction with the GnRH-R expressed in these cells.

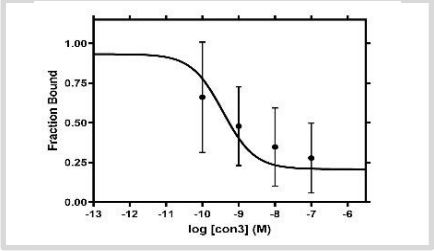
AIM

In this study we aimed to develop novel GnRH analogues with antitumor properties, by conjugating them with the cytotoxic agent, mitoxantrone, thus creating the conjugate Con3. Con3 is anticipated to release mitoxantrone into cancer cells after its binding to GnRH-R and subsequent internalization of the GnRH-R/Con3 complex. Con3 is expected to release mitoxantrone into cancer cells using the thioredoxin reductase system , expressed in these cells[3], which decomposes the conjugate into a free peptide and the cytotoxic drug mitoxantrone .

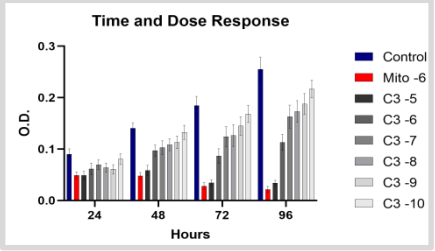
METHODS

To create the con3 conjugate we chemically modified the GnRH analogue, leuprolide and conjugated it with mitoxantrone. Evaluation of the pharmacological properties of the con3 was performed in competition radioligand binding studies using [^{125}I]-DTyr^{6} -His^{5} -GnRH as radioligand and membrane homogenates from HEK 293 cells stably expressing the GnRH-R, as previously described[4]. The apparent binding affinities (IC50 values) were obtained by fitting the binding data to a one-site competition model. Further assays to examine the antiproliferative effects of con3 were performed by incubating the ovarian cancer cell line, SK-OV-3, with con3 at different concentrations for 1-4 days and using the MTT assay.

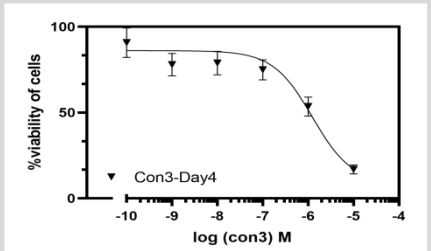
RESULTS



Radioligand binding assay using increasing concentrations of con3.



MTT assay: Antiproliferative effects of increasing concentrations of con3 at different days



MTT assay: Antiproliferative effects of increasing concentrations of con3 at day 4

Con3 binds to GnRH-R in a dose-response manner, with the high affinity of 0.06 nM, thus allowing us to characterize its antiproliferative actions. This was accomplished by testing its ability to inhibit the proliferation of ovarian cancer SK-OV-3 cells. The results have shown that the proliferation of SK-OV-3 cells was inhibited by Con3 in a time-dependent manner (1-4 days).

Interestingly, Con3 inhibited the proliferation of SK-OV-3 cells in a dose-dependent manner. The antiproliferative potency of con3 after exposure of SK-OV-3 to peptide for 2,3 and 4 days, was 1.4 μM, 0.85 μM, 0.97 μM and 1,1 μM, respectively.

CONCLUSION

- Con3 binds to GnRH-R with high affinity (0.06 nM)
- Proliferation of SK-OV-3 cells was inhibited by Con3 in a time and dose dependent manner.
- Con3 could set the basis in developing novel cytotoxic agents specifically targeting GnRH-R expressing tumors

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DISCLOSURES

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