Pt-Mal-LHRH, a newly synthesized compound attenuating breast cancer tumor growth and metastasis by targeting overexpression of the LHRH receptor

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Abstract

In the United States one in eight women will be afflicted with breast cancer. Triple negative (4T1) breast cancers account for 15% of all breast cancers and are significantly more aggressive than other subtypes. Treatment options for triple negative breast cancer are limited due to the cancers being unresponsive to hormonal therapy. Our recent work revolves around developing a novel chemotherapeutic agent that will direct therapy selectively to 4T1 cancer cells while decreasing systemic distribution.

Others have reported an upregulation of the luteinizing hormone-releasing hormone (LHRH) receptor on 4T1 cells. We have designed and synthesized a Pt-Mal-LHRH compound that uses the LHRH peptide to selectively target and deliver Platinum intracellularly to the 4T1 cells. Platinum is used in chemotherapy in common compounds such as cisplatin and carboplatin. Both cisplatin and carboplatin are effective chemotherapeutic agents, however, they are not cancer specific and elicit debilitating side effects.

To address, whether Pt-Mal-LHRH is more selective and efficacious than carboplatin we have designed in-vitro experiments to compare other chemotherapeutic agents. First, we verified that Pt-Mal-LHRH reduces 4T1 proliferation through an MTT assay. We found that Pt-Mal-LHRH reduces 4T1 viability compared to carboplatin. Next, we found through flow cytometry that Pt-Mal-LHRH increases 4T1 apoptosis compared to carboplatin. In addition, we found a 20-fold increase in cellular uptake of Pt-Mal-LHRH compared to carboplatin. Taken together we have found that Pt-Mal-LHRH increases 4T1 cell death through increased cellular uptake. Our future directions will decipher the molecular mechanism of action including DNA binding rates and adduct formation.

Introduction

For 2015, there are an estimated 1,658,370 new cancer cases diagnosed in the United States, of which 231,840 are attributed to breast cancer with 40,290 deaths. The individual survival rate and prognosis depends on the type of cancer, in which, highly aggressive and invasive carcinomas promote the most mortality. In women, breast cancer is the forefront leading cause of all cancer among women (41%) followed by uterine corpus (8%), and colon and rectum cancer (8%). Additionally, in the United States, breast cancer afflicts 1 in 8 women during their lifetime, this high prevalence provides evidence for the need to foster new therapeutic interventional research. Despite recent advances in the first-line treatment of breast cancer, many patients eventually relapse, their tumors become chemoresistant, and the patients subsequently die of the disease. One of the major problems in cancer chemotherapy is the deleterious side effects of anticancer drugs designed to destroy rapidly dividing cells, including those found in healthy tissues. Due to these severe side effects, doctors often resort to dose reduction, treatment delay or discontinuance of therapy. To combat this problem, a new approach to chemotherapy intervention was devised by synthesizing Pt-Mal-LHRH, a Carboplatin analog which selectively targets cancer cells.

Pt-Mal-LHRH Reduces Breast Cancer Cell Viability

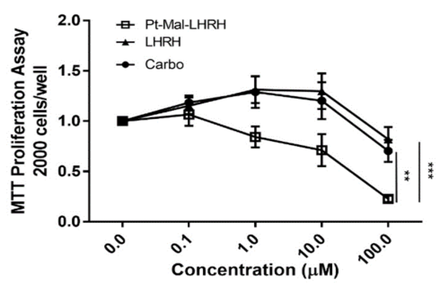

Figure 1: Pt-Mal-LHRH attenuates breast cancer cell viability. 4TI breast cancer cells were treated with Pt-Mal-LHRH, LHRH, or Carboplatin (Carbo) from a range of 0.1μM to 100μM. Viability rates were analyzed by a MTT assay after a treatment incubation of 72 hours. **, p>0.01 ***. p>0.001, ****, p>0.0001

Pt-Mal-LHRH is More Selective Towards Cancer Cells

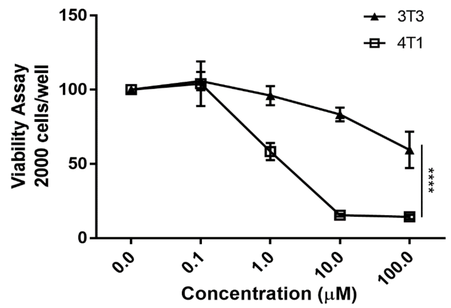

Figure 2: Pt-Mal-LHRH attenuates 4T1 breast cancer cell viability compared to normal mammary cells (3T3). 4T1 and 3T3 cells were treated with Pt-Mal-LHRH from a range of 0.1μM to 100μM. Viability rates were analyzed by a MTT assay after 72 hours of treatment. (n=3) ****,p>0.0001

Pt-Mal-LHRH has Greater Cellular Uptake Than Carboplatin

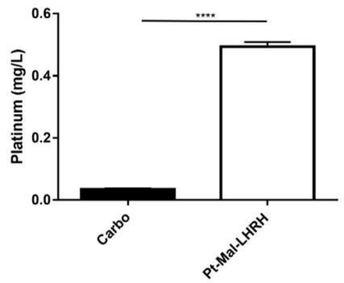

Figure 3: Cellular uptake of Pt-Mal-LHRH is significantly greater in cancer cells. To measure drug uptake 1x106 cells were treated with Platinum-LHRH or Carboplatin (100μM) for 24 hours. Cells were collected and metal (platinum) concentration mg/L was measured using ICP-MS. (n=3) ****, p>0.0001

DNA Guanine Adduct Formation with Platinum

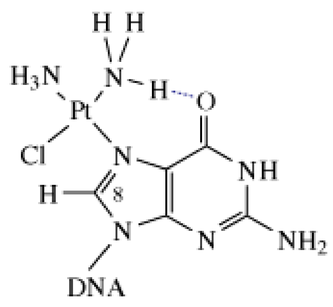

Figure 4: Future Experiment. A kinetic profile will be determined for the action of Pt-Mal-LHRH and compared to the kinetic profile of Carboplatin for similarities to the binding of Guanine residues and subsequent DNA cross-link formation and destruction, over a period of time.

Pt-Mal-LHRH Flow Cytometry

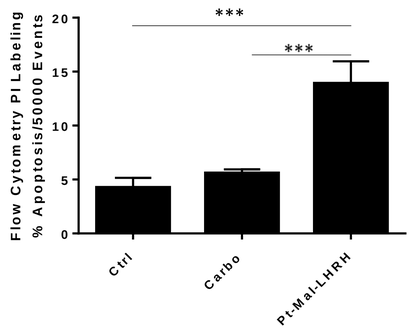

Figure 5: Pt-Mal-LHRH was confirmed to be cytotoxic by flow cytometry using a propidium iodide stain. Pt-Mal-LHRH displayed a 20-fold increase in 4T1 cellular uptake compared to carboplatin. There was a decrease (p<0.0001) in 4T1 cell viability compared to 3T3 normal fibroblast cells

Pt-Mal-LHRH Reduces Tumor Size *In-Vivo*

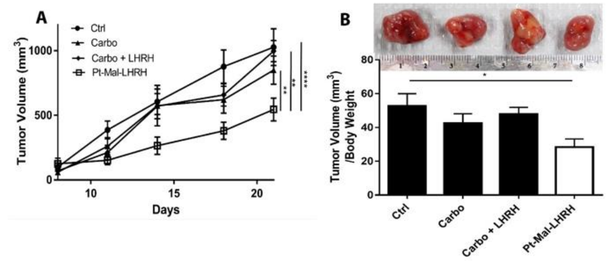

Figure 6: Breast cancer tumor growth is attenuated by Platinum-LHRH treatment. Female BALB/c mice were implanted with 4T1 cells (1x106) in the right second mammary fat pad. Tumors were grown for 7 days, distributed into treatment groups, and treated with Carboplatin (Carbo), Carboplatin and LHRH, or Platinum-LHRH (5mg/kg/wk) by intraperitoneal injection for 2 wks. Tumor volume was measured over the 2 wk injection period **(A)** along with end tumor weight **(B)**.

Summary

Platinum based chemotherapy remains a current standard of cancer treatment, especially in aggressive late stage breast carcinomas. However, due to increased resistance to Cisplatin and Carboplatin, newer strategies of drug intervention are needed. As a result, Pt-Mal-LHRH was synthesized to alleviate the need for more efficacious chemotherapy.

Our current data suggests;

- Pt-Mal-LHRH attenuates breast cancer cell viability.
- Pt-Mal-LHRH is more selective to cancer cells, resulting in greater uptake of drug compared to normal mammary cells.
- Cancer cell migration is attenuated by Pt-Mal-LHRH
- Pt-Mal-LHRH reduces tumor size as well as attenuates lung tumor colonization.

Future Direction

- Determine therapeutic profile of Pt-Mal-LHRH, including toxic and lethal dose indexes.
- Further evaluate Pt-Mal-LHRH's ability to attenuate metastasis.

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