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An Investigation to Study the Role of Novel Rhenium Compounds on Endometrial Uterine Cancer Cell Lines

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Abstract

Endometrial cancer of the uterus is highly maslignant with an increase rate of morbidity and mortality in both childbearing age and postmenopausal women. Rhenium compounds have been shown to have therapeutic properties against various cancers both *in vitro* cell lines and *in vivo* animal models. In this *in vitro* study, we investigated the effects of a novel group of Rhenium ligands on a uterine cancer cell line. Our initial results showed that these compounds are cytotoxic, induces apoptosis and prevents tubulin polymerization in these uterine cancer cell lines, we also found these novel Rhenium compounds to be noncytotoxic to healthy human blood lymphocyte cells, thus proving their safety and efficacy in future translational studies.

Keywords

Rhenium Compounds; Uterine Cancer; Apoptosis; Tubulin; lymphocytes

INTRODUCTION

Despite ongoing research, cancer remains a deadly disease that impacts millions of patients. Platinum based treatment options have been effective in the past, but there are limitations to these compounds [1]. and certain cancers are becoming resistant to treatment with platinum-based compounds. To help overcome the limitations and resistance to platinum-based therapies, alternatives to platinum have been studied in recent years [2]. Rhenium-based compounds are an appealing alternative to platinum-based compounds. These rhenium-based compounds are typically less toxic to healthy tissue than platinum-based compounds, while also providing anti-cancer properties [3].

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Endometrial cancer is the most commonly diagnosed gynecologic cancer. About 50,000 American women are diagnosed with the disease every year [4]. Endometrial cancer is also the most common form of uterine cancer, so it is frequently referred to as uterine cancer [5]. Cisplatin and other platinum compounds are effective in Endometrial cancer treatment but toxicity of these drugs to the systemic organs calls for searching safer effective alternative chemotherapeutic agents [6].

In this study, rhenium-based compounds were synthesized and tested against an endometrial cancer cell line as well as a healthy lymphocyte cell line for toxicity studies. Data was collected to observe toxicity and anti-cancer properties; inducing apoptosis and interactions with tubulin.

MATERIALS AND METHODS

Rhenium compounds were synthesized with different ligands according to the procedure described in our earlier study [5] and named PR series for the following drugs:

- PR-1: Tricarbonylperrhenato(bpy)rhenium(I)
- PR-2: Tricarbonylperrhenato(Phen)rhenium(I)
- PR-3: Tricarbonylperrhenato(5-MePhen)rhenium(I)
- PR-4: Tricarbonylperrhenato(neocuproin)rhenium(I)
- PR-5: Tricarbonylperrhenato(5,6-Me₂Phen)rhenium(I)
- PR-6: Tricarbonylperrhenato(bathophen)rhenium(I)
- PR-7: Tricarbonylperrhenato(bathocuproine)rhenium(I)
- PR-8: Tricarbonylperrhenato(4,7-Me₂Phen)rhenium(I)
- PR-9: Tricarbonylperrhenato(3,4,7,8-Me₄Phen)rhenium(I)

An additional highly effective Rhenium compound was also synthesized named as

- CH-2: Tricarbonylchloro(bpy)rhenium(I)

All the drugs were dissolved in DMSO at different concentrations which was also used as a vehicular control.

Cell Culture

HTB112 endometrial cancer cell lines were obtained from ATCC (USA) and cultured in McCoy's medium supplemented with FBS, Penicillin and Streptomycin and maintained at 37 C in 5% CO₂ incubator.

The healthy lymphocyte cell lines were obtained from Coriell Institute (USA) and cultured in RPMI medium supplemented with FBS, Penicillin and Streptomycin and maintained at 37 C in 5% CO₂ incubator.

LDH Assay

Lactate Dehydrogenase assay was done by using a LDH assay kit from Abcam Corporation (USA) following standard protocol using a microplate reader at 405nm to study cytotoxicity of the PR series 1–9 Rhenium compounds at 2 micromolar dose with DMSO as vehicular control.

MTT Assay

MTT assay reagents were purchased from Fisher Scientific Corporation (USA) and the assay was performed on CH2-treated healthy human lymphocytes incubated for 48 hours at various concentrations. The results were determined using a standard plate reader.

Apoptosis Assay

Annexin V-APC Assay Kit from Abcam Corporation (USA) employs an APC-conjugated Annexin V as a probe for phosphatidylserine detection on the outer membrane of apoptotic cells; After treating the HTB112 cell lines with the CH2 compound at 2 micromolar dose with DMSO vehicular control for 48 hours, the following steps were carried out. First, we collected $1-5 \times 10^5$ cells by centrifugation. Next the cells were resuspended in 500 μ L of 1X binding buffer, 5 μ L of annexin V-FITC was added and subsequently Incubated at room temperature for 5 min in the dark. Photographs were finally taken under a fluorescence microscope.

Tubulin Assay

Tubulin Tracker Deep Red provides deep-red/far- red fluorescence when bound to polymerized tubulin in living cells. Tubulin Tracker Deep Red is based on Docetaxel conjugated with a bright, photostable deep-red fluorophore. Docetaxel belongs to the family of cytoskeletal drugs that target tubulin. Tubulin Tracker Deep Red absorbs and emits optimally at 652 nm and 669 nm, respectively and can be visualized with standard Cy5 filter settings using almost any fluorescent imaging instrument. After 48 hours of incubation, CH2 treated HTB112 cells-at 2 μ M dose and DMSO control- were stained by Tubulin tracker red dye purchased from Fisher Scientific Corporation (USA) and photographed under fluorescence microscope.

RESULTS

We observed effective cytotoxicity of the PR series compounds on uterine cancer cell lines at a minimal dose of 2 μ m at 48 hours incubation (Figure 1), however, the ongoing COVID-19 Pandemic caused the lack of chemical supplies and we continued our research work to find out the effectiveness of our other Rhenium ligand compound, CH2. We initially tested the toxic effects of this Rhenium compound on a healthy human Lymphocyte cell line by MTT assay. Our results showed that at 2 μ m dose, the drug did not induce significant cell death to these cells supporting the safety of this compound (Figure 2). Next, an apoptosis assay with Annexin V assay kit was carried out and it was found that CH2 compound induces apoptosis of the HTB112 endometrial cancer cell lines (Figure 3). In order to further investigate the precise mechanism of cell death induced by this drug, a Tubulin tracker assay

was performed and CH2 Rhenium compound was found to prevent tubulin aggregation which could be a possible cause of cell death (Figure 4).

DISCUSSION

Endometrial cancer has a very high incidence among women of child bearing age due to hormonal influence, it causes a high rate of mortality and morbidity [12–14]. Though Cisplatin and Carboplatin are effective, they produce highly toxic adverse effects. This study was aimed at searching for an alternative drug in the form of different Rhenium ligands. Earlier *in vitro* and *vivo* studies, including some from our lab, have reported that Rhenium compounds show anticancer properties against many types of neoplasms [2,6,11]. The Rhenium compounds used in this study were named as the PR series and CH2 compound. Although, the PR series compounds showed effective anticancer properties against uterine cancer cells, as evidenced by our LDH assays, the unavailability of reagents due to current pandemic compelled us to continue with the CH2 Rhenium compound. The CH2 Rhenium ligand not only demonstrated anticancer efficacy, as evidenced by the apoptosis and anti tubulin assay, but also showed lack of toxicity to the healthy lymphocyte cells even at a very high dose, unlike the platinum based anticancer drugs which are extremely toxic to blood cells causing anemia and susceptibility to infections as their side effects. This is the first study to reveal that the Rhenium compound, CH2, is capable of preventing tubulin polymerization playing a possible role in blocking cell division and proliferation, hopefully will be investigated on other Rhenium ligands in the future. We also found that the nature of cell death initiated by these Rhenium compounds was through the apoptotic pathway instead of necrosis. Further studies to investigate the role of these novel Rhenium compounds in other types of cancers and to study tubulin polymerization preventing properties of other Rhenium ligands may prove helpful in transferring them from bench to bedside as an effective chemotherapeutic agent.

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LDH of HTB112 cells after exposure to PR series for 48 hours

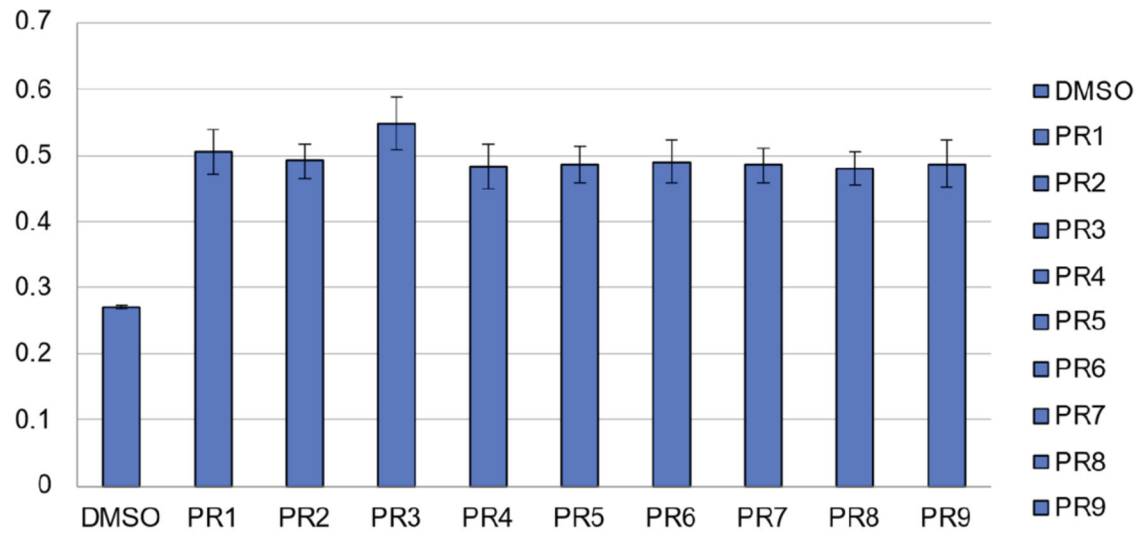


Figure 1:

Data showing the cytotoxic effects of the novel PR series compounds at a dose of 2 μ M on the uterine cancer cell lines exposed for 48 hours, DMSO was used as vehicular control for each experiment.

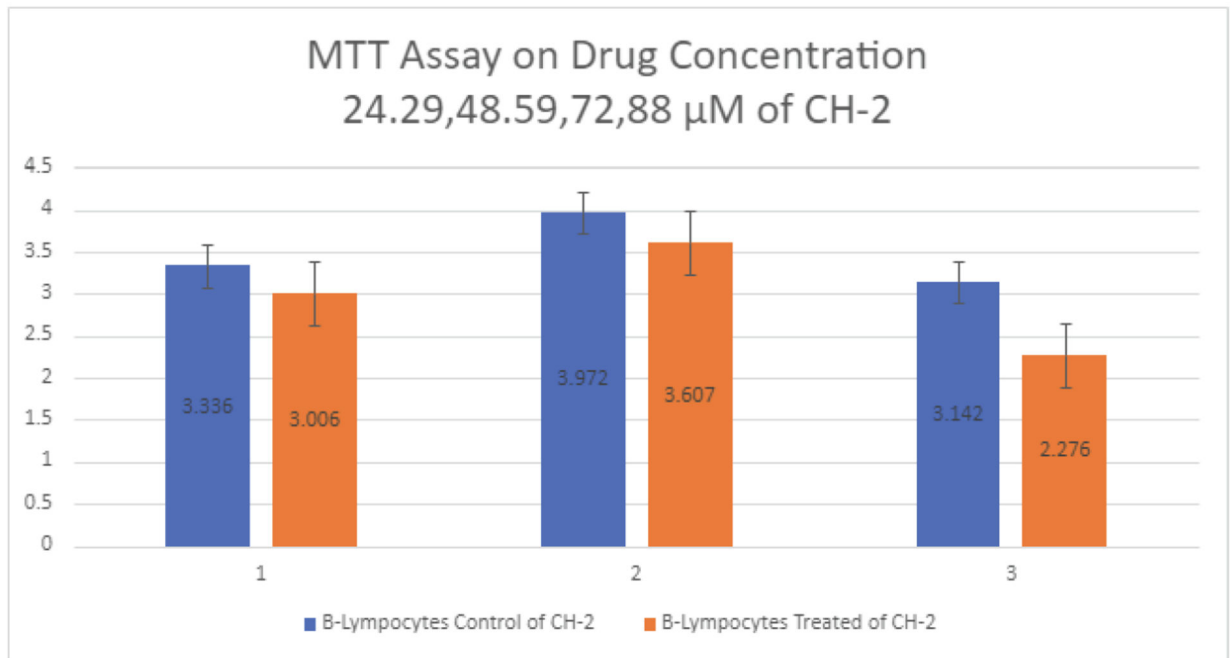
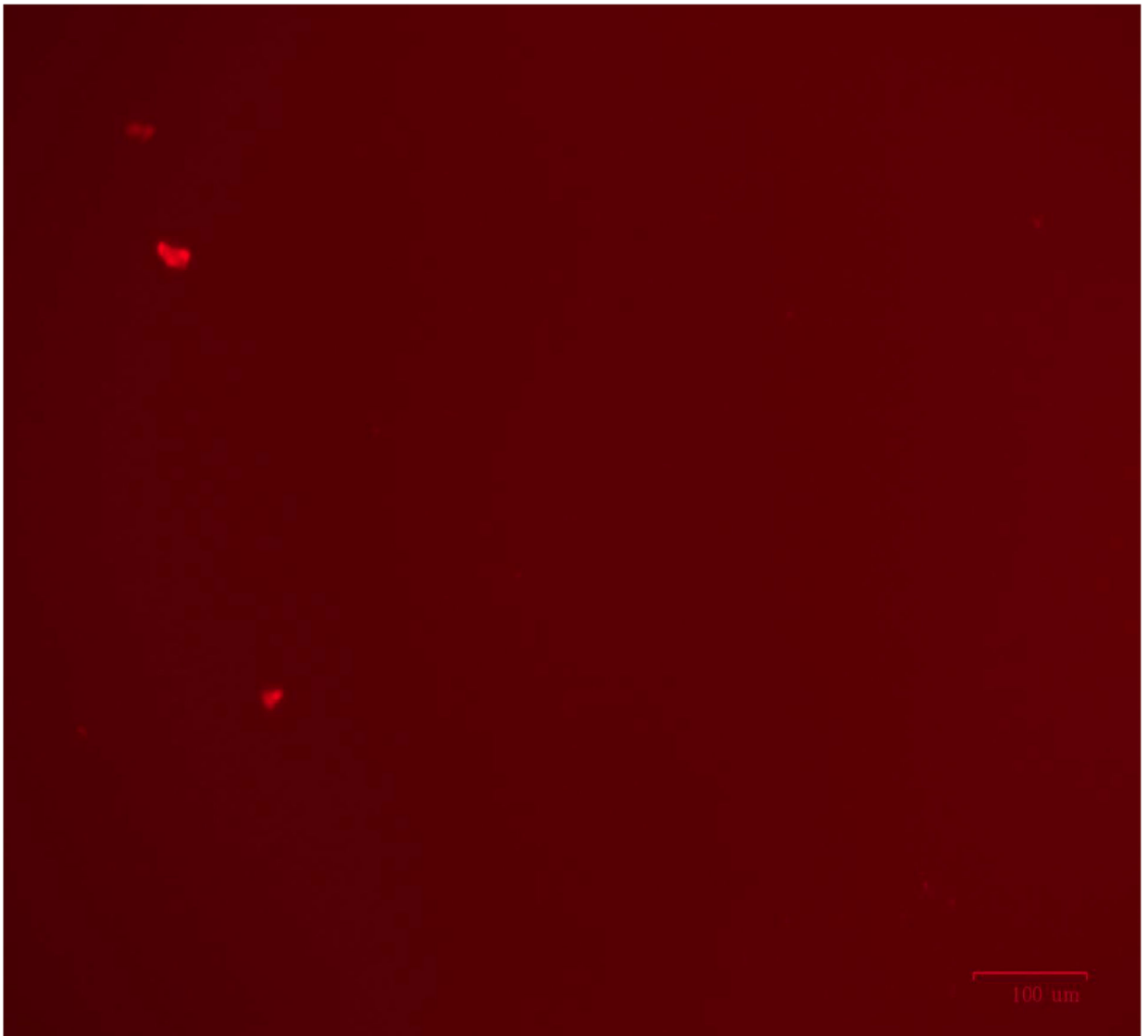


Figure 2:

MTT studies on B-lymphocytes showed that CH-2 has low toxicity to human lymphocyte cells. By adding different concentrations of CH2 along with vehicular control DMSO to cells for 48 hours, there was little difference in cell death observed in the treated compared to the control.



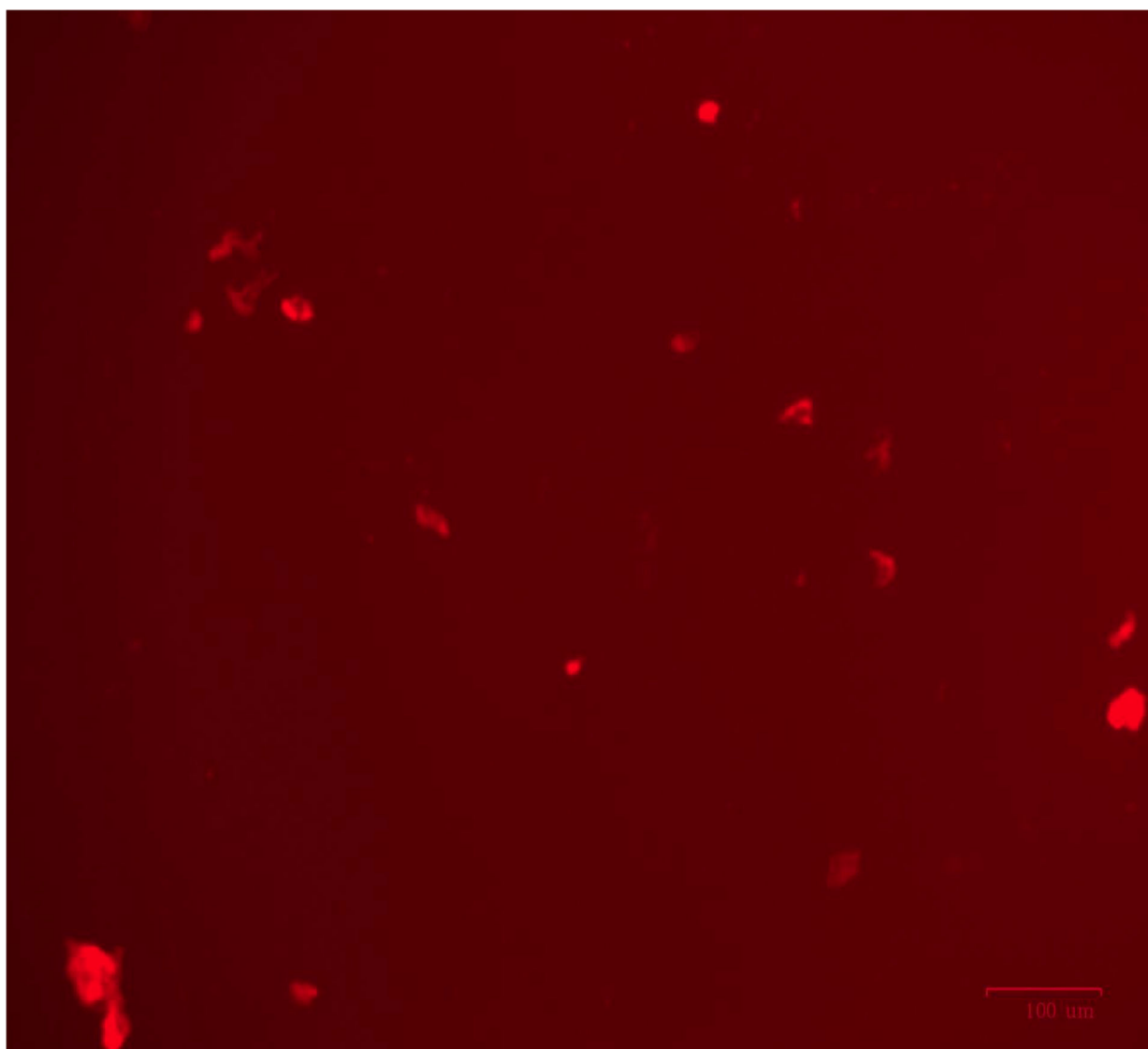


Figure 3:
a and **b:** By using Tubulin Tracker Deep Red to measure tubulin in cells, changes in tubulin were measured after exposure to CH2 compound. More tubulin was observed in cells exposed to the CH2 than in control cells exposed only to DMSO. This suggests that the CH series of rhenium-based compounds may provide their anti-cancer properties by targeting tubulin.



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Figure 4:

- a.** Annexin V apoptosis detection of Vehicular control DMSO treated Uterine cancer cells.
b. Using the annexin V- FITC apoptosis detection kit, an increase in apoptosis was detected in Uterine cancer cells exposed to CH-2 Figure **4b** in comparison to DMSO vehicular control for 48 hours.