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PENTAMETHYLCARBOXYLATE RUTHENOCENE BASED ANTITUMOUR AGENT

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Background: Unlike iron ruthenium is not an essential element for life. However, the behavior of ruthenium compounds in biological systems and, in particular their use as antitumour agents has attracted much attention recently. This study aims to determine antitumour properties against human tumour cell line HeLa of pentamethylcarboxylate ruthenocene. **Methods:** This is an in vitro study by applying an experimental within post only control group design. Cisplatin, a clinical used medicine was applied for control. The human tumour cell line Hela was used for the test. The cells were cultured at 37^0C in 5% CO_2/air in Roswell Park Memorial Institute Medium 1640 and seeded overnight in 96 well microtitre plates. The penthamethylcarboxylate ruthenocene was dissolved in 1,2 dimethoxy ethane and diluted with culture media. The plates were assays by measuring optical density in the range of 490-655 nm. The D_{37} values were then calculated. **Results:** The pentamethylcarboxylate ruthenocene compound tested was found to be more cytotoxic than cisplatin (with D_{37} = 422 nM compare to D_{37} = 705 nM for cisplatin). **Conclusions:** The compound tested, pentamethylcarboxylate ruthenocene was found more potent as an antitumor compare to cisplatin a clinical used antitumor for curing testicular carcinoma.

Keywords: iron; essential; element; cell; potent

INTRODUCTION

Therapeutic success of *cisplatinum* (*cis*-diamminedichloroplatinum(II)) and some second generation platinum complexes bearing organic ligands has paved the way for future research into the development of new metal-based antitumor agents including organo-ruthenium compounds. Since the discovery of *cis*-platinum, many transition metal complexes have been synthesized and assayed for antineoplastic activity. In recent years, ruthenium-based molecules have emerged as promising antitumor and anti-metastatic agents with potential uses in platinum-resistant tumors or as alternatives to platinum. Ruthenium compounds theoretically possess unique biochemical features allowing them to accumulate preferentially in neoplastic tissues and to convert to their active state only after entering tumor cells. Intriguingly, some ruthenium agents show significant activity against cancer metastases but have minimal effects on primary tumors.[1]

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Behaviuor of ruthenium compounds in biological system and, in particular, their use as antitumour agents has attracted much attention recently. The triad metals in the ruthenium group (Fe, Ru, and Os) posses interesting chemistry due to their ability to form many different complexes. Ruthenium, which is similar to iron, has an ability to form a large variety of coordination complexes with many inorganic or organic ligands including biomolecules.[2] This metalmay also mimic Fe in some of its metabolic processes. *Bis*(η^5-cyclopentadienyl ruthenium derivatives (ruthenocene) are potential candidates as new antitumor agents.[3,4]

Cancer, a highly heterogeneous disease was characterized by continuous uncontrolled growth and expansion of abnormal cells. In general, in tumor cells the signaling pathways regulating cellular processes, as cell growth and division and cell-to-cell communication result strongly altered. Furthermore, the cancer cells accumulate repeated mutations that provide a selective growth advantage over other cells. In addition, some cancer cells become invasive and then metastasize. This characteristic together with the genetic and phenotypic heterogeneity of the tumor cells makes cancer disease particularly difficult to treat and eradicate.[5]

The antitumour tested was carried out following the most frequently used method for describing toxicity in *in vitro* test. Based on this test inhibitory doses or inhibitory concentration was then determined. The inhibitory dose, ID_n, indicates the dose at which a tumour development is stopped. An index (subscript) is used to indicate the percentage of tumour cells destroyed, which in this study we used ID_{37}. Thus, ID_{37} reveals the dose for which 37% of the tumour cells are destroyed.

In this study, penthamethylcarboxylate ruthenocene was tested for its potency for curing cervical carcinoma.

MATERIALS AND METHODS

Penthamethylcarboxylate ruthenocene was prepared by Dr. Michel Williams. The compound was then tested as an anti cervical carcinoma, HeLa. The cell line HeLa was used for the *in vitro* assays. The cells were cultured at 37^0C in 5% CO_2/air in Roswell Park Memorial Institute Medium 1640 (referred to from now as culture medium) containing 1mM pyruvate, 50 µM nicotinamide, 100 IU/mL penicillin, 100 µg/mL streptomycin, 3 mM HEPES and 5% fetal calf serum. Cells were seeded in 96 well microtitre plates, 2000 cells per 100 µL culture medium per 6 mm microtitre well. The cells were allowed to attach overnight.

The penthamethylcarboxylate ruthenocene was dissolved in 1,2-dimethoxy ethane and diluted with culture medium to appropriate concentrations. Dilution were such that the final concentration of 1,2-dimethoxy ethane was in each case below 5% and did not interfere with the results. After overnight incubation of the cell line, the drug candidate was plated out in triplicate. The plates were incubated for ca. 2.5 hours, then, the supernatant was decanted and replaced with no more than two drops of fresh culture medium (using a Pasteur pippet). The plates were incubated for ca. 2.5 d, the control by this time was nearly confluent. After 2.5 d incubation, 10 µL of MTS/PMS solution was added to the culture wells. Then, the plates were placed in an incubator and, after 45 min the amount of reduced formazen produced was assayed by measuring the optical density at dual wave lengths in the range 490-655 nm using a Bio-Rad 3350 plate reader. The ID_{37} values were calculated from the graph of percent log surviving cell versus concentration of the drugs.

RESULTS

ID_{37} value data of penthamethylcarboxylate ruthenocene tested for anti cervical carcinoma, cell lines, HeLa was listed in Table 1. The ID_{37} of this compound was obtained from the graph of log % survival cell versus drug dose as indicated by Figure 1.

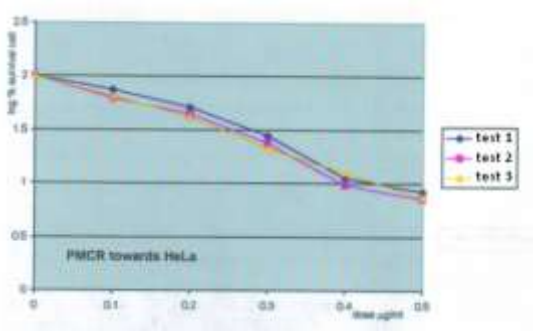

Figure 1
Graph of Log % Survival Cell Vs Drug Dose for penthamethylcarboxylate ruthenocene

Table 1
Inhibition Dose, ID_{37} obtained for penthamethylcarboxylate ruthenocene against HeLa

Test	ID_{37} penthamethylcarboxylate ruthenocene		Cisplatin (nM)
	µg/mL	(nM)	
1	0.19		
2	0.22		
3	0.24		
Mean	0.22±0.003	422	705

DISCUSSIONS

In this study we obtained that the compound of penthamethylcarboxylate ruthenocene (Figure 2) is potent drug candidate for curing cervical carcinoma, Hela cell line. As can be seen from Table 1, ID_{37} for this compound was 422 nM compare to 705 nM for cisplatin against HeLa cell line.

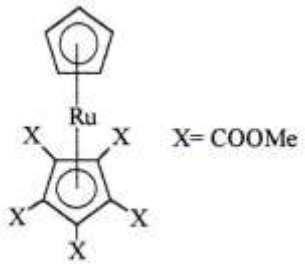

Figure 2
penthamethylcarboxylate ruthenocene

The antitumour properties of metallocenes were draw back since the finding of Dombrowski et al (1986) and Kopf-Maier and Kopf (1988).[6,7] There are three reason that Ruthenium potent to develop for medicinal use. These are (1) slow ligand exchange kinetics, (2) multiple accessible oxidation states, and (3) the ability to mimic iron in binding to certain biologic molecules.[8] The last properties, Ruthenium can mimic iron, that makes Ruthenium has similar properties to Iron in binding to serum transferrin and albumin.[9] These proteins solubilize and transport iron in plasma. Since rapidly dividing cells (including cancer cells) have a greater

requirement for iron, this results in up-regulation of the number of transferring receptors on the cell surface, resulting in sequestration of more circulating iron-loaded transferrin. To this end, in vivo studies have shown that there is a 2- to 12-fold increase in ruthenium concentration in cancer cells compared to healthy cells, depending on the cell type.[10] Since ruthenium preferentially targets cancer cells, its systemic toxicity, at least in theory, is expected to be reduced. Moreover, it has been shown that ruthenium is transported into cells by both transferrin-dependent and transferrin-independent mechanisms.[11] Transferrin-mediated ruthenium uptake is more efficient when transferrin is saturated with iron to a physiologic degree. As in cancer cell there is always an increase in the demand for iron, the transferrin receptors are thus over expressed and therefore the delivery of the ruthenium drugs can be enhanced.

CONCLUSION

Penthamethylcarboxylate ruthenocene was found more potent than cisplatin *in vitro* to inhibit growth of HeLa cell lines. This properties was probably due to Ruthenium mimic Iron, therefore, increase of iron demand during cancer will enhance delivery of ruthenium drug.

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