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ANTITUMOR ACTIVITY OF RU-PARA-CYMENE-μ-O-DICHLORIDE AGAINST DALTON'S LYMPHOMA *IN VIVO* AND *IN VITRO*

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ABSTRACT

Ruthenium complexes are an object of great attention in the field of medicinal chemistry because of their selective antimetastatic properties and low systemic toxicity in comparison to cisplatin, the widely used antitumor drug. Ruthenium-para-cymene-μ-O-dichloride, a newly synthesised Ruthenium compound was used against Dalton's lymphoma in vivo and in vitro to examine the cytotoxicity of the drug. The percentage survival of mice bearing Dalton's lymphoma after treatment with different concentrations of Ruthenium-para-cymene-μ-O-dichloride was found to increase significantly along with increase in the life span of tumor bearing mice. The percentage survival and percentage increase in the life span of tumor bearing mice were found to be dose dependant. Similarly percentage survival of lymphoma cells in vitro was found to be dose dependent.

KEY WORDS

Ruthenium-para-cymene-μ-O-dichloride, Dalton's lymphoma, cytotoxicity, antimetastatic

INTRODUCTION

Transition metal based compounds constitute a discrete class of chemotherapeutics widely used in the clinic as antitumor and antiviral agents. Most established antitumor metallodrug routinely used in the clinical therapies is Cisplatin. Cisplatin is currently being used for the treatment of testicular, ovarian, head, neck and germ cell tumors [1, 2]. However, its optimal use is prevented by its dose limiting nephrotoxicity. In the recent past, new platinum compounds, carboplatin and oxaliplatin have been introduced, but their use is limited due to very narrow therapeutic index. Further, drug resistance and side effects have limited their clinical utility [3, 4]. These limitations have prompted the search for more effective and less toxic metal based antitumor agents. Ruthenium complexes have attracted much interest as alternative drug to cisplatin in cancer chemotherapy. Ruthenium complexes have similar ligand exchange kinetics to those of platinum(II) complexes and different oxidation states are accessible under different physiological conditions [5,6]. It is commonly accepted that Ru(III) compounds are rather inert while Ru(II) are much more reactive towards their biological targets. Activation of relatively inert Ru(III) compounds compared to Ru(II) is supposed to occur in the tumor masses much easily compared to healthy tissue [7]. The Ru(III) compounds may act as prodrugs, which are reduced to its active Ru(II) form by glutathione, ascorbate and single electron transfer protein. The altered metabolism, microbial infection, higher level of glutathione and lower pH, altogether provide a reductive environment in the cancerous tissue, which help in promoting the antitumor activity of Ru compounds [8]. A few Ruthenium complexes with amine, heterocyclic and sulphoxide ligands have been reported to exhibit in vivo antitumor properties [7, 9-14].

While a number of ruthenium compounds exhibiting antitumor properties have been developed [15-18], the prototype compound RAPTA-C remains the most effective anticancer compounds of this series that has been extensively used in experimental studies against a number of murine cell lines [19]. Like RAPTA-C, another widely studied ruthenium analog NAMI-A has

been reported to be effective against Lewis Lung Carcinoma B16 and mammary carcinoma [13, 20]. Recent studies using combined arene derivatives have shown a rapid inhibition of DNA synthesis in human Mc 300 melanoma cells while protein synthesis was inhibited only later suggesting arene-ruthenium DNA interactions on the initial cytotoxic process. These complexes have shown dual synergistic effect with properties of both the arene-ruthenium chemotherapeutics and the porphyrine photosensitizer [21].

Although a number of ruthenium compounds have been synthesized but a few proved to possess antitumor activity against a limited number of cell lines. Present study was aimed to access the antitumor activity of a newly synthesized ruthenium compound, Ruthenium-para-cymene-µ-O-dichloride against Dalton's lymphoma in mice both in vivo and in vitro.

MATERIALS NAD METHOD

C_3H/He strain of mice was maintained in the laboratory as per the norms of Institutional Ethical Committee. Both male and female mice, 8-10 weeks old, weighing 20-22g were used in all sets of experiments. Animals were kept in polypropylene cages and were fed on commercial diet (Goldmohar, Lipton, India) and tap water ad libitum. Mice were kept under standard condition (especially pathogen free, temperature ranging from 22-23ºC and relative humidity 65-70%). Transplantable ascites Dalton's lymphoma was obtained from Chittaranjan National Cancer Research Centre, Kolkata, India and maintained in the laboratory by regular serial transplantations by injecting $2x10^7$ cells/mice in PBS after a regular interval of 10 days. Ruthenium compound, Ruthenium-para-cymene-µ-O-dichloride was a gift from Prof. O. K. Medhi, Department of Chemistry, Gauhati University, Guwahati, India. Other chemicals like DMEM, ADM, FCS and DFBS were purchased form Hi-media, Mumbai, India.

Tumor Growth Pattern: Animals were selected randomly and divided into experimental and control groups of 10 mice each according to randomized block design. To study the effect of ruthenium compound on tumor growth inhibition in vivo, animals were transplanted with $2x10^7$ cells/mice i.p.. The day of tumor transplantation was recorded as day 0. On day 4 post tumor transplantation, mice bearing palpable tumor were treated i.p. with single injection of 9, 15, 25 or 35 mg/kg of Ruthenium-para-cymene-µ-O-dichloride. The mean survival time and % increase in life span of tumor bearing mice was recorded. Percentage increase in the life span (% ILS) was calculated by formula % ILS= [(T-C)/C] x 100; Where T is the mean survival time of the experimental animals and C is the mean survival time of control animals.

Cytotoxicity assay in vitro: For cytotoxicity assay in vitro, Dalton's lymphoma cells were plated at high density ($8x10^7$ cells/dish) at time 0 in DMEM containing 10% FCS, 10mM $NaHCO_3$, 0.3% glutamine, antibiotics and different concentrations of ruthenium-para-cymene-µ-O-dichloride for a fixed treatment duration of 1 hour. Control dishes were treated with equal amount of PBS used as a solvent for the compound. At the end of the drug treatment, the medium containing drug was aspirated off, cells were washed with isotonic PBS, resuspended in ADM with DFCS and incubated for 72 hrs at 37º C. After incubation cells were trypsinized and viable cells were counted by Trypan Blue Exclusion test. Each assay was repeated thrice and the results were analyzed by Student's *t* test.

RESULTS AND DISCUSSIONS

In the present studies a newly synthesized ruthenium compound, Ruthenium-para-cymene-µ-O-dichloride was tested against murine Dalton's lymphoma in mice. Percentage survival of mice bearing tumor after treatment with this compound is shown in **Fig.1**. A direct correlation was observed between the dose of the compound administered and increase in the % survival of mice. All the control animals without any treatment failed to survive beyond 10 days post tumor transplantation. When the tumor bearing mice were treated with 9 mg/kg of the compound, 100% of animals survived up to 13 days of post tumor transplantation and the maximum survival period was found to be 30 days. When the dose of ruthenium compound was increased to 15 mg/kg, the maximum survival time increased to 35 days. Further increase of

concentration to 25 and 35 mg/kg resulted in the maximum survival time to 40 days.

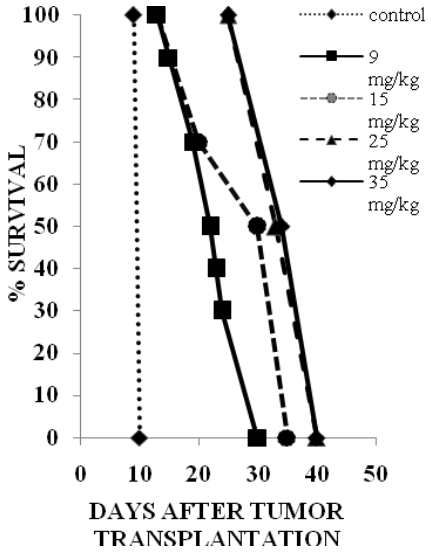

Fig 1: Survival of Mice with Dalton's lymphoma after treatment with different concentrations o Ruthenium-para-cymene-µ-O-dichloride

The % increase in the life span of tumor bearing mice after treatment with different concentrations of the compound was found to be dose dependant (**Fig. 2**). The % increase in life span was found to be 94% after treatment with 9 mg/kg Ruthenium-para-cymene-µ-O-dichloride which increased to 130% when the dose was increased to 15 mg/kg. Similarly, the higher doses (25 and 35 mg/kg) further increased the life span of tumor bearing mice to 165% and 195% respectively. This shows that mice treated with different doses of compound resulted in maximum survival time of 40 days with maximum increase in the life span of 195% without showing any tumor free survivor.

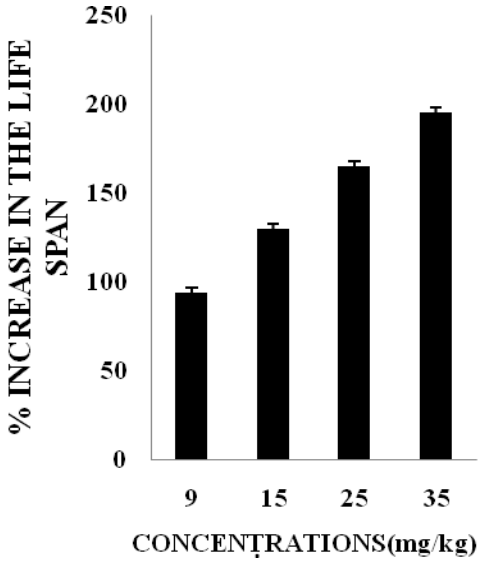

Fig 2: % Increase in the life span of tumor bearing mice after treating with different concentrations of Ruthenium-para-cymene-µ- µ- O-dichloride

The present investigation has shown the effects of Ruthenium-para-cymene-µ-O-dichloride at the cellular level through a study of its lethal activity in an in vitro system. Survival of Dalton's lymphoma cells treated in vitro with different concentrations of the compound for fixed time duration of 1 hr is shown in the **Figure 3**. There exists an inverse linear correlation between the concentration of Ruthenium-para-cymene-µ-O-dichloride in the medium and % survival of lymphoma cells. IC_{50} of the compound was found to be 27 µg/ml (**Fig.3**). Tumor cells exhibited 80-90% survival when incubated with 5-15 µg/ml Ruthenium-para-cymene-µ-O-dichloride for 1 hr, which decreased to 35% when the concentration was increased to 50 µg/ml.

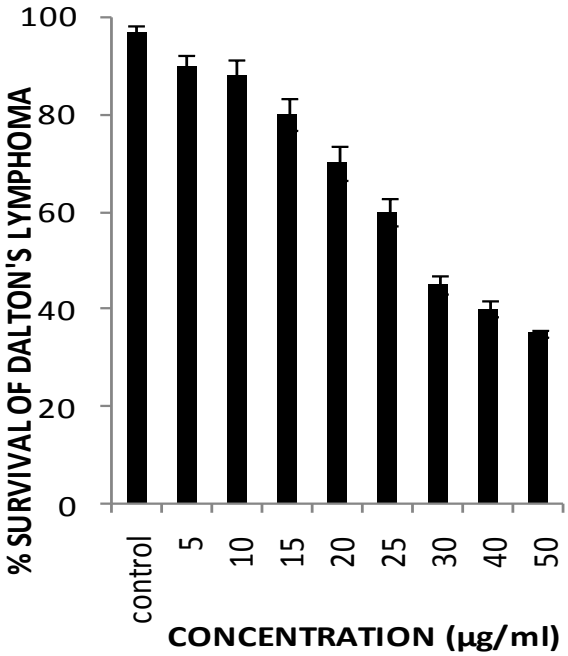

Fig.3. % Survival of Dalton's lymphoma cells after treatment with different concentrations of Ruthenium-para-cymene-µ-O-dichloride *in vitro*

The transition metal based drugs are important class of chemotherapeutic agents widely used in cancer chemotherapy. One of the most widely used metallic drugs is cisplatin but its use is limited due to its nephrotoxicity [22, 23]. Among the transition metal compounds, ruthenium appears to be a likely candidate in near future even though its chemistry differs from platinum. The most significance differences are ruthenium's octahedral chemistry and greater propensity to undergo redox reactions. The hypoxic environment of many tumors may favour the reduction of ruthenium (III) compounds to ruthenium (II) compounds species, which binds rapidly. Ruthenium complexes are presently receiving great attention in the fields of biological, pharmaceutical and medicinal chemistry as antitumor agents.

Most of the studies reporting ruthenium compounds as antitumor agents have been carried out in vitro and only a few in vivo. Like the present study, ruthenium-DMSO complexes have been reported to increase the life span of tumor bearing mice in vivo. These compounds have also been reported to be effective against several murine models including a cisplatin resistant P-388 leukemia [24]. Chatterjee et al. (2008) studied the effect of RAPTA-C induced apoptosis in EAC cells isolated from peritoneal cavity of tumor bearing mice and reported that the apoptosis was dose dependent. In the present studies, the effect of Ruthenium-para-cymene-µ-O-

dichloride has also been found to be dose dependent. 9 mg/kg of this compound was able to enhance 94% increase in the life span of tumor bearing mice whereas 35 mg/kg enhanced the survival to almost 200%. The antitumor action of ruthenium (II) complexes might be the consequences of direct DNA binding and damage [25]. Ruthenium compounds apper to penetrate reasonably well within the tumor cells, binding effectively to DNA and proteins [26]. A ruthenium (III) complex having significant cytotoxic properties has been reported to bind firmly to DNA and also modify its structural conformation [27]. However, dose dependent regression of Dalton's lymphoma in vivo by a ruthenium (II) complex containing CNEB has been reported to be via decline in lactate dehydrogenase including mitochondrial dysfunction-apoptosis pathway without any toxicity to the normal tissues [28]. In contrast to the view that DNA is the main target for ruthenium drugs other authors have claimed DNA independent mechanisms, such as inhibition of metalloproteinases, interference with the adhesion processes, and scavenging of nitric oxide are responsible for the antitumor and antimetastatic activity of these compounds.

Results obtained in the present in vitro study with Dalton's lymphoma cells are expressions of cell killing assay and not the growth inhibition assay. It was found that cytotoxicity of Ruthenium-para-cymene-µ-O-dichloride depends upon its concentration in the culture media. Tumor cells treated with increasing concentrations of Ruthenium-para-cymene-µ-O-dichloride decreased cell survival which may be probably due to increase in Ru-DNA adduct formation. Earlier a significant correlation has been reported between the percentage survival of Dalton's lymphoma cells and DNA platination. It has been reported earlier that tumor cells treated with increasing concentration of cisplatin declined the cell survival and increased DNA platination [29]. Studies using combined arene derivatives of ruthenium also have shown a rapid inhibition of DNA synthesis in human Me300 melanoma cells [21].

CONCLUSIONS

Present study exhibited the dose dependent efficacy of Ruthenium-para-cymene-µ-O-dichloride against murine Dalton's lymphoma both in vivo and in vitro. This efficacy might be due to Ru-DNA adduct formation.

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