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An Innovation in the Cancer Therapy by the Interaction of Docetaxel to the Demethylpodophyllotoxin site of Tubulin

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ABSTRACT: Docetaxel is a chemotherapy medication used to treat a vast variety of cancer. It includes breast cancer, lung cancer, brain cancer etc. This treatment is administered to the patient through injection in veins. Optical ultracentrifuge studies of Demethylpodophyllotoxin-treated tubulin show a small reduction in 25-S to 30-S peaks at 0°C. In electron microscopic studies the ring structure of tubulin is seen at 0° but disappears if the temperature of tubulin incubated with Demethylpodophyllotoxin is raised to body temperature. Docetaxel inhibits the binding of Demethylpodophyllotoxin to tubulin more effectively and economically competes with podophyllotoxin, that inhibits colchicine binding to tubulin.

KEY WORDS: Tubulin, Docetaxel, Demethylpodophyllotoxin, tubulin, interaction, polymerization, inhibitors, competitive mechanism.

1. INTRODUCTION

Efficacy of Demethylpodophyllotoxin with tubulin receptor can be increased so many fold by the combination of docetaxel that shows significant antitumour activity in vivo against leukemia in mice and in vitro against cell derived from a human carcinoma of the nasopharynx^[1]. I have examined this tumour inhibitor for its effects on cell division in the eggs of marine organisms. We found docetaxel-Demethylpodophyllotoxin combination prevented the formation of spindle more effectively which was formed prior to 1st cleavage. This suggested docetaxel-demethylpodophyllotoxin combination exerts its antimitotic activity more effectively through its effect on spindle microtubules, in comparison to other spindle poisons such as colchicines, podophyllotoxin etc^[2]. To test this, I have worked with isolated brain tubulin which can be a substitute for mitotic tubulin in isolated spindles, also supported by some researchers like Sakai et al^[3] and Cande et al.^[4]. After showing that Demethylpodophyllotoxin inhibited in vitro assembly of brain tubulin, I also examined its site of action on the tubulin molecule. Podophyllotoxin, which has some competitive mechanism^[5], further studies by Wilson et al.^[6] have revealed that the Demethylpodophyllotoxin binding site on tubulin may also be the binding site for other antimitotic drugs. In this report I have found that Docetaxel-Demethylpodophyllotoxin combination inhibited the binding of other drugs to tubulin more effectively by a competitive mechanism. Thus this technique becomes more effective in cancer therapy.

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II.MATERIALS

Demethylpodophyllotoxin, ethylene glycol, bis- (β -aminoethyl ether)-N,N'-tetraacetic acid, piperazine-N,N'-bis(2-ethane sulphonic acid), and sephadex G-25-80 were obtained from Sigma Chemical Co. St. Louis, Mo. Demethylpodophyllotoxin, steganol, and podophyllotoxin were isolated in the laboratory of Dr. S. Morris Kupchan, Department of Chemistry, University of Virginia. Docetaxel is a white powder and is the active ingredient available in 20mg and 80mg Taxotere single dose vials of concentrated anhydrous docetaxel is polysorbate 80ml/20mg Taxotere is distributed in a blister carton containing one single-dose vial of taxotere (docetaxel) preparation in 0.5mL sterile pyrogen-free anhydrous polysorbate 80, and a single dose taxotere solvent vial containing 1.5ml 13% ethanol in saline medium to be combined and diluted in a 250mL infusion bag containing 0.9% sodium chloride or 5% glucose for administration. 80 mg Taxotere is supplied identically but with 2.0 mL polysorbate 80 and 6.0 mL 13% ethanol in saline. The docetaxel and solvent vials are combined to give a solution of 10 mg/mL and the required dose is

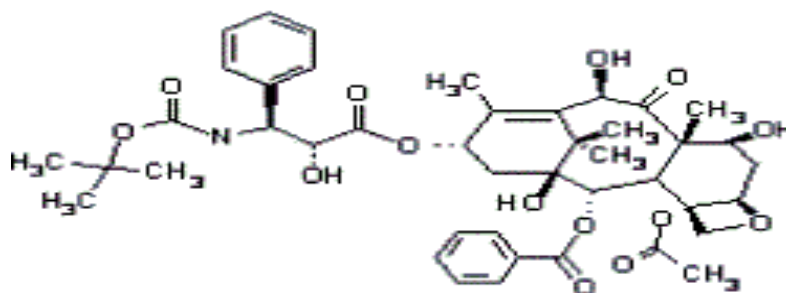


Fig.1- represents the structure of Docetaxel in which bold bonds are showing attacking sites of it.

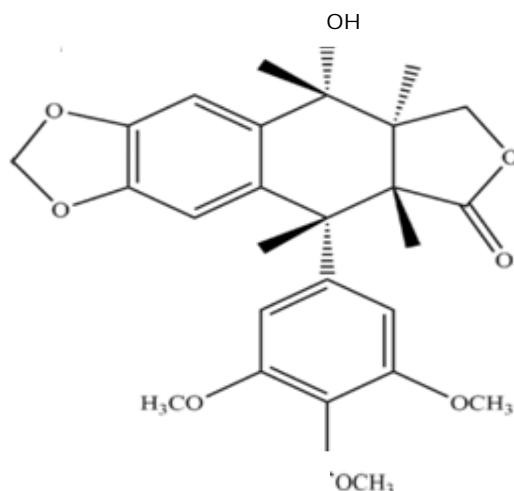


Fig.2- shows the structure of Demethylpodophyllotoxin in which bold bonds are showing highly attacking sites of Docetaxel

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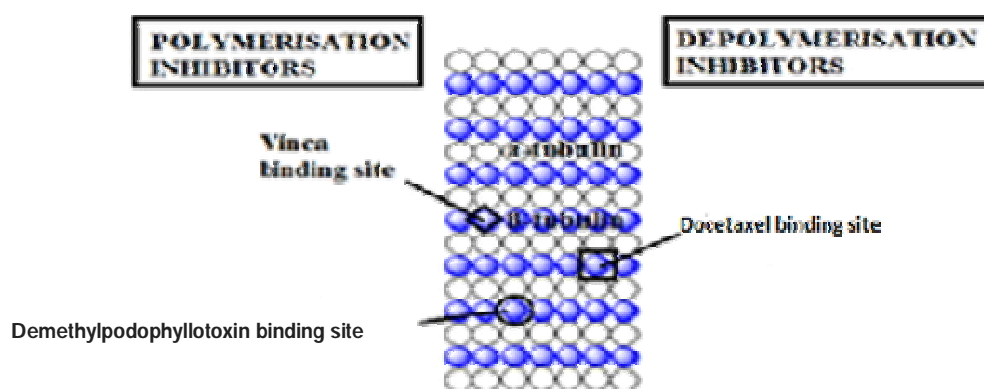


Fig.3- Represents interaction of antimitotic natural products with tubulin receptor

drawn from this solution. Vials have an overfill to compensate for liquid loss during preparation, foaming, adhesion to vial walls and the dead volume. 20 mg vials may be stored for 24 months below 25°C away from light and 80 mg vials for 26 months in the same conditions. Recently Sanofi has got approval for one-vial formulation with this one-vial formulation, the preparation of the infusion solution is simplified by eliminating the first dilution step. The two-vial and one-vial formulation contain the same drug substance docetaxel trihydrate, and same excipients (ethanol, polysorbate 80 and citric acid). The one-vial formulation is administered as an aqueous intravenous solution that contain the same drug substance in the same concentration as already approved through two-vial formulation the same grade, quality, and quantity of polysorbate 80 are present in the infusion solution of both formulation. The only difference between these two formulation is the quantity of ethanol.

III.METHODS

Docetaxal can be produced from the eggs of marine organisms like sea otter, wolf eels, trigger fish etc through KCl injection or electrical stimulation^[7] by the use of Zeiss polarizing phase microscope. Purification of tubulin of mammalian brain can be done within 2 hours of slaughter after the removal of blood vessels and meninges and by the addition of 1mL of 100 mM piperazine-N,N'-bis(ethane-2-sulphonic acid) containing 1mM ethylene glycol bis(β-amino ethyl ether)-N,N'-tetraacetic acid and 50*10⁻³ M CaCl₂ (pH 6.8) was added per gram of brain. The sample was then homogenized in a glass homogenizer and then centrifuged for 30 min at 4-8°C and supernatant was made 4mm in glycerol and 1mm in GTP to initiate microtubule assembly^[8]. Tubulin was polymerized to form microtubules at body temperature for 30 min. Microtubules were collected by centrifugation 23-28°C. Further tubulin purification was obtained by repeated polymerization and depolymerisation in the absence of glycerol with centrifugation.

*The protein concentration of samples was determined by the method of Lowry et al ^[9] with bovine serum albumin as the standard.

*The polymerization of tubulin was followed by an increase in turbidity at 37° after addition of 1mm GTP ^[10]. Turbidity measurements were performed at 350 nm in a Beckman K25 recording spectrophotometer.

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Vol. 8, Issue 6, June 2019

*To test for possible reversal of Demethylpodophyllotoxin binding to tubulin, a centrifuge desalting technique was used^[11].

*The polymerization of tubulin was checked with cross-polarizer (Arthur H. Thomas Co., Philadelphia, Pa). Birefringence was observed when microtubules were formed. Formed microtubules were always seen on examination of birefringent samples in the electron microscope. This technique was therefore used for rapid examination of tubulin polymerization.

*Electron microscopic techniques were also applied for this interaction and their impact on the affected body organs.

IV.RESULT

After fertilization process, different concentrations of docetaxel and several other derivatives were added to the eggs. Cleavage of eggs was completely inhibited by 3×10^{-7} M concentration of Demethylpodophyllotoxin. As a result, the final size of mitotic apparatus was reduced as compared to that of extra grown mitotic apparatus. The minimum drug concentrations of different inhibitors required to inhibit the cleavage of eggs of echinoderms are given in the following table_

Table-1 shows minimum required concentration of Inhibitors

Inhibitors	Minimum Concentration(M)
1. Docetaxel	6×10^{-8}
2. Demethylpodophyllotoxin	4×10^{-7}
3. β - Peltatin	2×10^{-8}
4. Combretastatin-A4	2.5×10^{-6}
5. Centaureidin	3×10^{-8}
6. Rotenone	3.5×10^{-5}

Predicted activity of inhibitor α 1/Molar concentration

Using Demethylpodophyllotoxin as our controlled antitubulin agent with docetaxel, we obtained resistance against the growth of three human cancer cell lines. As an inhibition of tubulin assembly, docetaxel was found to be quite active. Using docetaxel-Demethylpodophyllotoxin association at the place of colchicines, I have found a relatively strong effect on the reaction. Treatment with docetaxel increases the survival time in people affected with different types of cancer. Conjunctive treatment of docetaxel with Demethylpodophyllotoxin has been showed to lead improved survival rate as well as improved quality of life and reduction to pain as compared with other treatments. Enhanced effects of radiation therapy when combined with docetaxel has been observed over mice. Docetaxel has also been found to have cellular uptake and is retained longer intracellularly than other substitutes.

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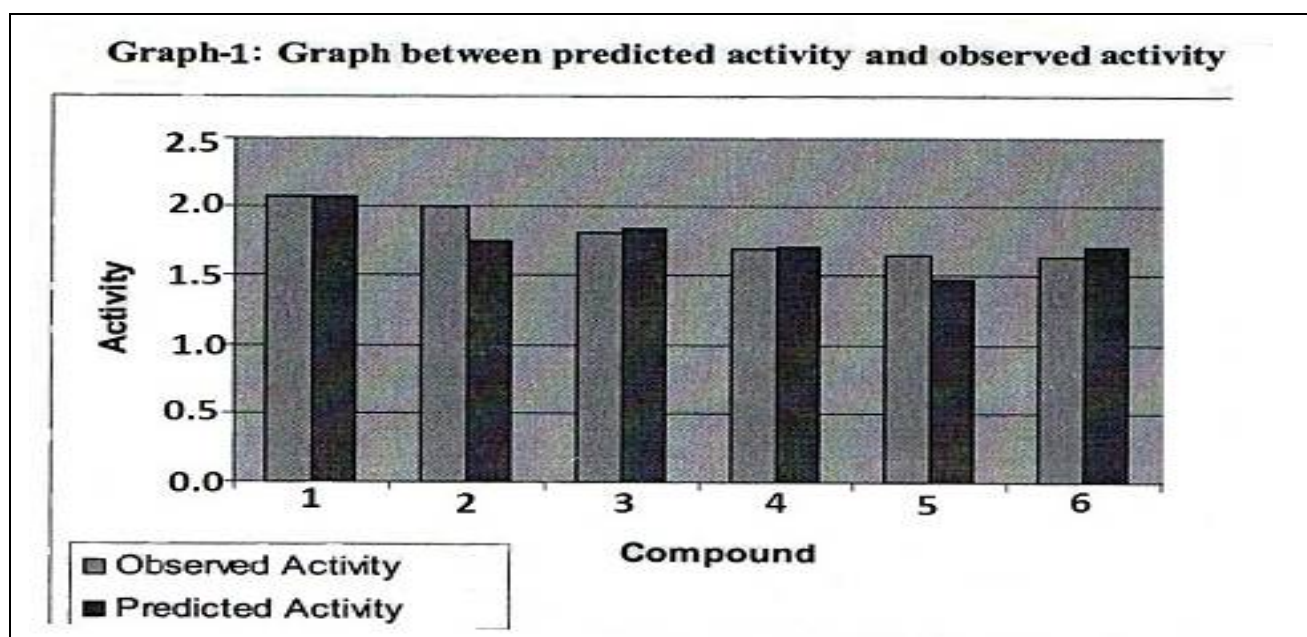
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Vol. 8, Issue 6, June 2019

Table-2 shows values of predicted activities of Inhibitors

S.No.	Compound	Predicted Activity
1-	Docetaxel	2.090
2-	Demethylpodophyllotoxin	1.850
3-	β - Peltatin	1.830
4-	Combretastatin	1.715
5-	Centaureidin	1.467
6-	Rotenone	1.715



Thus treatment with Docetaxel-Demethylpodophyllotoxin drug is found to be more effective even in its small doses leading to fewer and less severe adverse effects^[12].

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Vol. 8, Issue 6, June 2019

V.DISCUSSION

We have compared the effects of Demethylpodophyllotoxin with other anti-mitotic natural products like colchicines and found that Demethylpodophyllotoxin in place of colchicines proved more effectively with the combination of docetaxel in the treatment of cancer cells. Podophyllotoxin shows similarity in binding colchicines with Demethylpodophyllotoxin with increased efficacy. We have shown that Demethylpodophyllotoxin inhibits the polymerization of brain tubulin in vitro with complete inhibition occurring if there are 3 tubulin promoters for each Demethylpodophyllotoxin molecule (at a tubulin concentration of about 2×10^{-5} M). In addition, the binding appears to be partially reversible, since at least 70% of the tubulin polymerizes after separation of tubulin from Demethylpodophyllotoxin-docetaxel assembly which is observed on short sephadex columns.

In analyzing further the effects of these drugs on tubulin, we have used the optical ultracentrifuge and electron microscope. Analysis of solutions of polymerizable brain tubulin is complicated because such solutions exhibit 2 or 3 peaks when examined in the optical ultracentrifuge. At pH 6.7, tubulin isolated without glycerol shows a 6 S peaks and 30 S or 36 S peaks (the exact S value depends upon the components of the mixture and the method of depolymerization)^[13,14]. When examined in electron microscope, such solution exhibit small 70-A particles and a series of double ring structure of about 500 Å⁰ in diameter^[15,16]. As the pH is raised^[17,18,19], another peak appears in the optical ultracentrifuge, which shows an extrapolated S value of 20; simultaneously, the size of the 30 S component decreases the latter disappears at about at pH 7.2^[20-25]. These changes are reversible; the pattern for a given pH is also shifted systematically^[26-33] if the tubulin is prepared in glycerol by the technique of Shelanski et al. In the electron microscope the 20 S component appears to consist of single rings although under some conditions these appear to collapse and yield amorphous large granules.

VI.CONCLUSION

We thank Dr. Morris Kupchan for supply of the compounds used in the study and for encouragement in this work. We dedicate this work to his memory, with great sadness and at his untimely death. We also thank Nettie Ivy for technical assistance and for a critical reading of the manuscript and Charles Mitchell for his help in sedimentation experiments and also CDRI team, Lucknow.

Antimitotic drugs such as the taxane docetaxel and the vinca alkaloid vintafolide, show a promising result in taxane and anthracycline-resistant cancers. However, the toxicity of these drugs as well as acquired drug resistance allows for opportunity to develop agents with increased tolerability and specificity. My investigation has opened the pathways of cancer cell resistance to antimitotic drugs will result in subsequent identification of novel biomarkers for future chemotherapy possessing increased efficacy.

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Vol. 8, Issue 6, June 2019

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Vol. 8, Issue 6, June 2019

BIOGRAPHY



Dr. Satyendra Singh was born in District- Balrampur, U.P., India on 11th July, 1977. He is an assistant professor of Chemistry in S.V.N.P.G. College Kalan, Sultanpur, U.P., India. He has received his degree of Class X in 1993, Intermediate in 1995, B.Sc (Chemistry & Botany) in 1998, M.Sc degree with Chemistry in 2001 and his Ph.D. in Chemistry (2011) from Dr. R.L. Avadh University, Faizabad. Apart from all these necessary courses, he has successfully achieved the professional courses also like Post Graduate Diploma in Computer Application from Dr. R.L. Avadh University, Faizabad in 2002 and B.Ed. in 2006 from Rajarshi Tandon Open University, Allahabad. Maintaining an active research laboratory with Dr. R.K. Singh (Former Principal & H.O.D. of Chemistry, M.L.K. (P.G.) college, Balrampur) as collaborator, Dr. Satyendra Singh's research interest focus the study on inhibitor- receptor interaction of estrogen and anti mitotic natural products. Dr. Satyendra Singh co-authored a dozen of research publications in international & national journals. He has also attended 5 international and 7 national seminars in applied sciences on recent topics. Not only the research pub. & seminars, Dr. Singh had also become a valuable part of international & national scientific workshops in Lucknow & Allahabad respectively. Dr. Satyendra Singh allows himself as a teacher to share his passion for chemistry to the students of various institutions up to postgraduate level. The most satisfying aspect of his work is helping to shape the lives and minds of students in an appropriate field. He continuously works as a source of inspiration and facilitates student learning and understanding of chemistry through guided inquiry, direct instructions, investigations, problem solving and group discussion methods etc. He is also responsible for class preparation, class-room management as well as developing and grading assessments, and meeting with the parents to discuss about their ward's progress. He is giving his most of the time to each & every student of the society through his valuable suggestions to develop a strong nation. Besides he is also running a Science Channel on You Tube by the name of 'Life Capsule' to enable the modern students to learn at digital & online platform in today's scenario.