A PROFILE OF PACLITAXEL

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ABSTRACT

Treatment of cancer is a challenge to physicians, and it requires multiple treatments. But the role of paclitaxel cannot be neglected in cancer treatment. It is the most promising anti-tumour agent. As the drug has poor water solubility, number of alternative controlled release formulations has been developed having variety of dose regimens to overrule the limitations of cremophor based formulation. More advanced Drug delivery systems includes Nanoparticles, Microemulsion, Liposomes, Oncogel, Microspheres, Cyclodextrin complex, etc. Also the drug has flexible structural aspects hence lots of modifications are possible in the structure.

Keywords: paclitaxel; cremophor; nanoparticles; formulations.

INTRODUCTION

Cancer in general terminology is group of diseases caused by abnormal and unrestricted growth of cells. It has high morbidity and mortality, being the second most cause of all death after cardiovascular diseases. Treatment of ‘gulma’ [cancer] by using herbs was described in the first surgical treatise from India, Sushruta Samhita, as far back as 2500 BC, and Ayurveda also described treatment of cancer with certain plants¹. Eber Papyrus described the same in 1500 BC². Since then, numbers of natural products with diverse chemical structure have been isolated for anti-cancer agents out of which paclitaxel are one of the novel broad spectrum drug.

Paclitaxel, tax-11-en-9-one, 5β,20-epoxy-1,2α,4,7β,10β,13α,hexahydroxy-4,10-diacetate-2-benzoate-13-α-phenylhippurate, a poly-oxygenated naturally occurring diterpene alkaloid, was first isolated by Wall and Wani from the bark of Taxus brevifolia. Paclitaxel is one of the broadest spectrum anticancer agent approved by the Food and Drug Administration FDA for the treatment of advanced ovarian cancer³-⁵. Today, it is considered as one of the most important chemotherapeutic drugs in cancer chemotherapy for clinical treatment of cancer of lungs, head, neck, bladder, AIDS related Kaposi’s sarcoma, and endometrial cancers etc⁶-¹². Paclitaxel is found in the bark of yew trees [Taxus] which grows extremely slowly & having very low yield¹³. So alternate routes have been investigated for the production of paclitaxel which include production in plant suspension, biotechnical method, fungal resources, total synthesis and semi synthesis. Paclitaxel exerts its action by binding microtubules and causes kinetic suppression (Stabilization) of microtubule dynamics¹⁴. The paclitaxel arrest the cell cycle at mitotic phase & causes the cytotoxicity. Paclitaxel is hydrophobic in nature due to which suitable vehicle is required for delivery of paclitaxel.

CHEMISTRY OF PACLITAXEL

Paclitaxel has the empirical formula C₄₇H₅₁NO₁₄ and a molecular weight of 853.9. It consists of a taxane nucleus to which an uncommon four-membered oxetane ring is linked to C₄ and C₅ and an ester is attached at C₁₃.

STRUCTURE ACTIVITY RELATIONSHIP

Structure activity relationship (SAR) investigations of taxanes have been carried out for seeking higher activity towards tumors and less toxicity towards normal tissues. General structure of paclitaxel includes Toxoid ring system with A, B, C, D rings. It is known that certain modifications at certain positions in the molecule results in great differences in activity. The modification of paclitaxel can be divided into two parts:
A) MODIFICATIONS OF SKELETON

The skeleton of paclitaxel includes the A, B, C and D rings in the diterpene part, which has eight oxygenated positions at positions 1, 2, 4, 5, 7, 9, 10, and 13.

<table>
<thead>
<tr>
<th>MODIFICATION</th>
<th>STRUCTURAL OUTCOME</th>
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<tbody>
<tr>
<td>Modifications at C₁ Position</td>
<td><img src="image1" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>A series of analogues without C₁-OH were prepared for studying microtubule stabilization and cytotoxicity. Position is not important for the activity of paclitaxel.</td>
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</tbody>
</table>

| Modifications at C₂ Position | ![Chemical Structure](image2) |
| SAR studies at the C₂ position showed that both the nature and stereochemistry of the 2-benzoyl group are of great importance for activity. 2-Debenzoyl-2-tigloyl paclitaxel (4), the first natural analogue of paclitaxel with a modified ester group at C₂, retained microtubule-binding activity, but displayed decreased cytotoxicity as compared to paclitaxel and 2-debenzoyl-2-senecioyldocetaxel analogue. These results suggest that C₂ benzoyloxy is essential for activity.|

| Modifications at C₄ Position | ![Chemical Structure](image3) |
| 4-deacetylpaclitaxel, 4-deacetoxy paclitaxel, and 4-ether, ester, carbonate and carbamate derivatives. 4-Deacetylpaclitaxel (5) and 10-acetyl-4-deacetyldocetaxel (6) were inactive in the microtubule assembly assay. Compound (5) also showed no cytotoxicity towards several tumor cell lines. These results illustrate the importance of the 4-acetyl moiety for microtubule binding. Chordia et al. reported two 4-deacetoxy paclitaxel derivatives (7 and 8), which were significantly less active than paclitaxel in microtubule assembly and cytotoxicity assays. This result again indicates that an ester substituent at C₄ is essential for the biological activity. |

| Oxetane Moiety at C₅-C₆ Position | ![Chemical Structure](image4) |
| The presence of the oxetane ring was shown to be essential for the bioactivity of paclitaxel. In order to clarify the role of the oxetane oxygen atom, several groups synthesized paclitaxel analogues with nitrogen, sulfur and other heteroatoms. Compounds with nitrogen were inactive in cytotoxicity assays. These results suggested a specific interaction of the heteroatom with the protein. |
The compounds (10) and (11) were synthesized and evaluated for their *in vitro* cytotoxicity towards the human colon cancer cell line. Aminopaclitaxel (11) was significantly less active than paclitaxel, but the azido analog (10) was 2- to 3-fold more cytotoxic than paclitaxel\(^{22}\).

### Modifications at C\(_6\) Position

The derivatization of the C\(_6\) hydroxyl or change of its stereochemistry has no significant effect on anticancer activity of the molecule. Although Esterification at C\(_7\) resulted in loss of *in vitro* microtubule assembly activity, but not cytotoxicity. These observations suggested that esters at C\(_7\), which tend to improve water solubility, might serve as useful prodrugs of paclitaxel\(^{23}\). Hence, the C\(_6\) position was frequently modified to act as prodrugs for increasing the water-solubility of paclitaxel.

### Modification at C\(_7\) Position

Studies on the modification at C\(_9\) imply that the functional group at C\(_9\) may be one effective factor of the tubulin binding site in addition to two further key tubulin binding regions at the C\(_13\) ester side chain and the oxetane ring of paclitaxel. Klein reduced the C\(_9\) carbonyl group to a hydroxyl group and obtained compound (13), whose cytotoxicity was higher than that of paclitaxel\(^{24}\).

### Modifications at C\(_9\) Position

Previous studies on naturally occurring taxanes indicated that acetylation of the C\(_9\) hydroxyl group is not essential for the anti-tumor activity. Modifications at C\(_9\) position do not decrease the activity of analogues. Both 10-deacylcephalomannine (14) and 10-deacetylpaclitaxel (15) obtained from *Taxus wallichiana* showed considerable cytotoxicity and also affected microtubule disassembly\(^{25}\).
Paclitaxel contains C₁₃ side chain, which is essential for cytotoxicity of paclitaxel. Modification at C₂´ and C₃´ position are of having significant effect on antitumor activity.

<table>
<thead>
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<th>MODIFICATION</th>
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<tr>
<td>Modifications at the C₁₃ Side Chain</td>
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<tr>
<td>This side chain is essentially required in taxol for anticancer activity. The C₂´ hydroxyl is important for activity. When this hydroxyl is protected, activity is reduced to a great extent.</td>
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<td>C₃´ aryl group is critical, and is required for better activity. While amide’s aryl group may be replaced by similar aryl or alkyl groups. On replacement with methyl group, activity is reduced 19-fold.</td>
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<td>Stereochemistry at C₂´ and C₃´ has a dramatic effect on activity. The (2´S, 3´S) isomer is significantly less active than natural (2´R, 3´S) isomer, but the (2´S, 3´S) and (2´R, 3´R) isomers shows comparable activity with the natural isomer.</td>
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</table>

N-Acyl group required but many different N-acyl groups give improved activity. Group may be changed to alkenyl or substituted phenyl. Some groups give improved activity. Removal of 10-acetate or deacetoxylation to form the 10-deacetoxy derivative donot make major changes in activity. Reduction of C-9 carbonyl group to -OH group increases activity slightly. Esterification epimerization or removal does not cause significant loss of activity. Contraction of the C-ring to a 5-membered ring reduces activity. Opening of the oxetane ring gives inactive products, substitution of S for O reduces activity. Removal of acetate reduces activity. Replacement by other groups can increase activity. Acylxoyl group essential, certain alkenyl and substituted aromatic groups give improved activity.

Hydroxyl group helpful but not essential.
MECHANISM OF ACTION
Paclitaxel kills the cancerous cell by cytotoxicity and apoptosis. Paclitaxel exhibits a unique mechanism of action; it binds to microtubule and causes kinetic suppression (stabilization) of microtubule dynamics. Microtubules are actually cylindrical structures made up of proteins (mainly tubulin) that are involved in various cellular functions such as movement, ingestion of food, controlling the shape of cells, sensory transduction and spindle formation during cell division. In normal case, the tubulin polymerizes to microtubule and again microtubulin converts into tubulin. This whole routine process exists in equilibrium state. But Paclitaxel mainly binds to microtubules, rather than to tubulin dimers. The binding site for paclitaxel is the N-terminal 31 amino acids of the β-subunit of tubulin in the microtubule, unlike the binding sites of colchicine, vinblastine and podophyllotoxin for GTP. The microtubules formed due to paclitaxel action are not only very stable but are also dysfunctional. The cancerous cells lack a checkpoint to detect the absence of spindle and attempt to continue the cell cycle leads to cell death.

1. Normal case
   Tubulin $\xrightarrow{\text{Microtubulin}}$ Microtubulin bundles
   (Polymer) $\xrightarrow{\text{Normal cell cycle}}$

2. In case of Taxol
   Tubulin $\xrightarrow{\text{Microtubulin}}$ Stable bundles of microtubulin
   (monomer) (Polymer) Size=22A°
   $\xrightarrow{\text{Defective cell cycle, new cells without spindles; instant cell death}}$

Paclitaxel kills cancerous cells through the induction of apoptosis by p53-independent pathways.

PHYSICAL PROPERTIES AND PHARMACOKINETICS
Paclitaxel is white to off-white crystalline powder. It is highly lipophilic, insoluble in water and melts at around 216-217 °C. The generally accepted dose is 200–250 mg m⁻² and is given as 3 and 24 h infusion. Pharmacokinetics of paclitaxel shows wide variability. Terminal half-life was found to be in the range of 1.3–8.6 h (mean 5 h) and the steady-state volume of distribution was found to be ~87.1 m⁻². The drug undergoes an extensive P-450 mediated hepatic metabolism and less than 10% drug in the unchanged form is excreted in the urine. Most of the drug is eliminated in feces. More than 90% of the drug binds rapidly and extensively to plasma proteins. The highest concentration of the paclitaxel following a 6-h infusion in rats was found to be in lung, liver, kidney and spleen and was essentially excluded from brain and testes.

PACLITAXEL DOSE AND DRAWBACKS OF THE FORMULATIONS
Paclitaxel has a low therapeutic index, and the therapeutic response is always associated with toxic side-effects. It should be only used when the potential benefits of paclitaxel therapy outweigh the possible risks.

In the early development of paclitaxel, a high incidence of acute hypersensitivity reaction characterized by respiratory distress, hypotension, angioedema, generalized urticaria and rash were observed. It is generally felt that the vehicle Cremophore EL (Polyoxyethylated castor oil vehicle and dehydrated alcohol) contributes significantly to the hypersensitivity reactions, leading to peripheral neurotoxicity, neutropenia, etc. An additional problem linked to the CrEL solvent is the leaching of plasticizers from PVC bags and infusion sets used routinely in clinical practice. Consequently CrEL formulation need to be prepared and administered in either glass bottles or non-PVC infusion systems with inline filtration. This leads to the need of search of alternative formulations of paclitaxel. The maximum tolerated dose (MTD) of paclitaxel administered by a 3-h infusion to patients with solid tumors was found to be 225–240 mg m⁻² without any hypersensitivity reactions but resulted in hypotension. A summary of Therapeutic Efficacy and Toxicities is presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1: Summary of Therapeutic Efficacy and Toxicities</th>
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<tbody>
<tr>
<td>a) Tumors responding to paclitaxel</td>
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<td>b) Dose limiting toxic effects</td>
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<td>c) Different systems</td>
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PACLITAXEL

PRODUCTION OF PACLITAXEL

Natural Resources
It is difficult to obtain sufficient quantities of the compound from its natural sources. Paclitaxel constitutes only 0.01-0.03% of the dry weight of the bark of the Pacific yew tree. In addition, several other taxane including 10-DAXP, 10-DAB III, and cephalomannine have been obtained from the needles, which can be used for semi-synthetic production of paclitaxel. At present, the culture of seedlings and the growth of yew trees in plantations have been widely considered as the most feasible method to obtain paclitaxel and its precursors.

Biotechnological Approaches
These include plant tissue cultures, cell suspension cultures, hairy root cultures, recombinant microorganisms and the induction of paclitaxel biosynthesis in cell culture systems. Especially Taxus cell cultures have been considered as a promising means for paclitaxel production.

Fungal Resources
In 1993, Stierle et al. were the first to report a paclitaxel producing endophytic fungus, Taxomyces andreanae, which was isolated from yew trees. Although the yield of paclitaxel was only as low as 24-50 ng/L, the greatest problem of using fungal fermentation for paclitaxel production represents very poor and unstable yields.

Total Synthesis
Total synthesis of paclitaxel is a challenge, because of four complicated rings (A, B, C rings and the oxetane ring) and 11 chiral centres in the molecule. Nicolaou and Holton describe two different schemes (Scheme 1 & 2) for total synthesis of paclitaxel from compound A (2-Acetyl 3-methyl-but-2-enoic acid ethyl ester) and compound B (4-Methyl-1-(1,2,2 trimethyl-cyclopentyl)-6-oxa bicycle[3.1.0]hexane) respectively as precursor.

FORMULATION OF PACLITAXEL
Paclitaxel was earlier formulated in a vehicle composed of 1:1 blend of Cremophor EL (polyethoxylated castor oil) and ethanol which is diluted with 5-20-fold in normal saline or dextrose solution (5%) for administration. Taxol, the most popular formulation of Paclitaxel has serious drawbacks including:
- Cremophor EL contributes serious allergic reactions.
- Leaching of plasticizers from PVC bags and infusion sets.
- Increase systemic exposure to paclitaxel.
- Lack of specificity
- Poor solubility
- Low bio distribution

To overcome these drawbacks several novel drug delivery systems are formulated for Paclitaxel as shown in Table 2.
Table 2: Formulations of Paclitaxel with their advantages

<table>
<thead>
<tr>
<th>FORMULATION</th>
<th>DESCRIPTION</th>
<th>GASTRO INTAKE</th>
<th>THERAPEUTIC USE</th>
<th>EXPERIMENTAL STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nabo Paclitaxel (BB)-1 (bimane)</td>
<td>Paclitaxel protein bound particles and direct delivery to tumor cells</td>
<td>Preformed aggregates in tumor cells and strong tumor lesion swelling</td>
<td>Breast cancer</td>
<td>Phase I: evaluation of activity, MTB and biomarker activity. Dose range: (1-15) mg/m²/week for 3 weeks, 6 weeks, or 12 weeks.</td>
</tr>
<tr>
<td>Paclitaxel-loaded PEG-nanoparticles</td>
<td>Paclitaxel is bound to polyethylene glycol (PEG) and encapsulated in nanocapsules.</td>
<td>Polyethylene glycol (PEG) is biocompatible and biodegradable.</td>
<td>Lung cancer</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>Surgically implanted PLGA-embedded microspheres (PLGA-ELS)</td>
<td>Poly (D,L-lactide-co-glycolide) microspheres were loaded with paclitaxel by dissolving both drugs in triacetin and spray drying.</td>
<td>Safety studies showed that the nanocapsules delivered paclitaxel to lung metastases in mice.</td>
<td>Lung cancer</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>Paclitaxel monohydrate nanoparticles</td>
<td>Monohydrate prepared using the spray-particle blank method.</td>
<td>Paclitaxel hygroscopy reduces its shelf life and affects its solubility.</td>
<td>Lung cancer</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
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<td>Paclitaxel-loaded PEG-nanoparticles</td>
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</tr>
<tr>
<td>Information containing PLGA</td>
<td>Information prepared by self-replicating drug delivery system (SRLD)</td>
<td>Improves the half-life of plasmid DNA and transfects cells in vivo.</td>
<td>Lung cancer</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>Pacfix</td>
<td>Micelle-forming system developed by Sandoz Pharmaceuticals</td>
<td>Effects can be observed locally and are sustained.</td>
<td>Lung cancer</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>Intravenous hydroxyethyl starch (130-140 kDa)</td>
<td>Protective delivery of Paclitaxel, normoglycemia.</td>
<td>Rapid elimination that could be administered after brief or short duration.</td>
<td>Lung cancer</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>DSG Pacfix</td>
<td>Dose-specific and adjustable Paclitaxel in an injectable formulation.</td>
<td>Function as a solvent for a hydrophobic drug.</td>
<td>Metastatic malignant tumors</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>Liposomal paclitaxel (Pluon)</td>
<td>Liposomes of paclitaxel, inulin, and polyethylene oxide.</td>
<td>Targeting receptor-mediated drug delivery.</td>
<td>Brain tumors</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>Paclitaxel-cyclofed th [complexes]</td>
<td>Drug complexed in a suitable drug delivery system.</td>
<td>Increase tumor binding and redistribution of drug.</td>
<td>Peptide cancer chemotherapy</td>
<td>Hardness efficiency was checked using FIC analysis.</td>
</tr>
<tr>
<td>PLGA-embedded Paclitaxel</td>
<td>PLGA-embedded Paclitaxel in a suitable drug delivery system.</td>
<td>Good biocompatibility, good solubility of drug, and minimal side effect profile.</td>
<td>Adjuncts</td>
<td>Cell death was performed on immobilized Paclitaxel.</td>
</tr>
<tr>
<td>Oncopel</td>
<td>Oncopel formulation of Paclitaxel drug.</td>
<td>Improvement of drug delivery and tumor lesion swelling.</td>
<td>Superbly tolerated by patients undergoing chemotherapy.</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>Imulmae-implantable dispensing system</td>
<td>Imulmae-implantable dispensing system for Paclitaxel (PLGA-PTL) microspheres.</td>
<td>Improved placement of PLGA-PTL microspheres into the tumor lesion.</td>
<td>Lung cancer</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>Magnetic nanoparticle embedded Paclitaxel</td>
<td>Magnetic nanoparticle embedded Paclitaxel.</td>
<td>Surface interactions provide a magnetic field for drug targeting.</td>
<td>Magnetic nanoparticle embedded Paclitaxel.</td>
<td>Cell death was due to hypoxia, small cell lung cancer, and therapy.</td>
</tr>
<tr>
<td>Chioma-implanted Paclitaxel</td>
<td>Chioma-implanted Paclitaxel.</td>
<td>Selective chemical concentration and distribution of drug in tumor lesion.</td>
<td>Multiple tumors</td>
<td>Characterization studies were done and CICR was measured.</td>
</tr>
</tbody>
</table>
Paclitaxel is one of the most important and broadest spectrum anticancer drugs approved by FDA for the treatment of cancer. This review provides a complete description of paclitaxel, its Synthesis, SAR, and Mechanism of action, Doses, Production and Formulations with special emphasis on its Novel drug delivery system. Also it highlights how these Paclitaxel formulations is an effective tool in the therapy of cancer.

ABBREVIATIONS USED
EL : Ethoxylated
CrEl : Cremophore ethoxylated
PLGA : Poly (lactic-co-glycolic acid)
DPPC : Dipalmitoyl phosphatidylcholine
DMPC : Dimyristoyl phosphatidylglycerol
DSPE : Distearoyl phosphatidylethanolamine
PEG : Polyethylene glycol
MPEG : Methoxy polyethylene glycol
CR : Cremophor
PTX : Paclitaxel
SPAnNa : Self-doped poly[aniline-cosodium N-(1-one-butyric acid) aniline]

REFERENCES


