Taxus – The Panacea for Cancer Treatment

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ABSTRACT:
Cancer has been major challenge for healthcare sector for a long time. The use of chemotherapy and radiation therapy has its obvious side effects. The scientists have been looking to natural remedies for the treatment of cancer. There have been significant discoveries in this direction such as Vinblastine the plant alkaloids. Recent discovery of Taxol a diterpenoid obtained form Taxus species has opened new vistas in the treatment of cancer and can be considered a major break through. This article is an attempt to overview the various aspects such as biological & geographical sources, active principles, mechanism of action, extraction, isolation, estimation and traditional uses of Taxol.

INTRODUCTION
Just few years ago, the chemical known as Taxol made headlines as a break through treatment for ovarian cancer.

Taxol is a diterpenoid belonging to taxane group of compounds present in Genus-Taxus,Family Taxaceae

Some of its important species are
Taxus baccata-English/European yew
Taxus cuspidate-Japanese yew
Taxus brevifolia – Pacific yew
Taxus Canadensis – Canadian/American yew

Taxus is a green tree with linear leaves. It is mainly found in Himalayas in India at a height of 6000to11000 feet.

Yield of Taxol obtained from bark is 0.007%to0.01%.

Other names for Taxus are Chinwood, Fish poison, Arrow poison and Mitotic spindle poison.

Taxol and its derivatives offer hope in cancer where other treatments like surgery and radiotherapy have failed. It can also be given as adjuvant to other cancer therapies.

The biggest problem is that the drug is very difficult to obtain. Researchers have to extract the substance from the bark of the trunk, in the process they have to kill the plant which is even more frustrating, the yews grows very slowly and plants yield very little bark.

One hundred years old tree gives 3Kg of bark form which only 300 mg of taxol can be obtained, 15000ib. dried bark gives one kg of taxol and due to its complex structure it is not economically feasible to synthesize taxol. Artificially Endophtic fungus of Pacific yew, Taxomyces andreanae, when
cultured on an artificial medium, produces Taxol and Baccatin-III. Quantities produced are very less but free from contamination.

**Morphology**

Taxus is an evergreen tree with linear leaves that are dark green in color on upper surface and yellow green on lower surface. It has a berry like fruits composed of stony seeds nearly surrounded by thick, fleshy cup-shaped aril. Plant is characterized by needle shaped leaves. Male flowers have 6-14 anthers, shaped like shield. Female apparatus has ovule wrapped in red aril that turns fleshy when ripe.

**CHEMICAL CONSTITUENTS**

Different constituents reported in leaves, barks and stems are Saccharides, Polysaccharides, Cyclitols, Fatty acids, Sterols, Bisflavonoids (Sciadophtysin, kayaflavone), Proanthocyanidins, Lignin and cyanogenic glycosides.

Most important constituents are Deterrence with a taxane nucleus including Taxasines, Taxagifin, Baccatin III and its derivative.

Alkaloid Taxine is a complex mixture of Taxine A & B and their derivatives.

Most important taxanes reported are Taxol (0.007-0.001%); 10-deacetyltaxol (0.022%), Cephalomanine (0.031%?) and 10-deacetylcephalomanine.

10- Deacetylbaccatin is renewable starting material for taxol

**Acidic constituents of Taxus baccata**

The acidic constituents found in T. baccata

By GC-Ms analysis are

- Benzolic
- Salicylic
- Cinnamic
- M-Hydroxy
- Benzoic
- O-Hydroxy
- Phenyl acetic
- P-Hydroxy
- Phenylacetic
- Pyrocatechuic
- Vanillic
- Gentisic
- r-Reorcylic
- Other constituents in trace amounts are Tannin, Ephedrine, resin and lignin.

**CHEMISTRY OF TAXOL**

- Taxol is a complex Diterpenoid with Taxane ring system and 4 membered Oxetane ring.
- An ester side chain at position 13 of the taxane ring is essential for the cytotoxic activity of the drug.
- Presence of hydroxyl group at position 2 of ester side chain enhances the activity.
SOLUBILITY OF TAXOL

The drawback of Taxol is its insolubility in water, which makes its administration complex and difficult. Currently doctors use Cremophor El and Polyethoxylated castor oil as a liquid medium for IV administration of Taxol. But polyethoxylated castor oil may sometimes induce hypersensitivity reaction, which is prevented by premedication with corticoids, antihistamines and H2-receptor antagonists.

Important derivatives of taxane are Paclitoxel & Docetoxel. These are mainly used as anticancer drugs. Paclitoxel has been isolated and purified by DABUR (India) Ltd. The company has conducted clinical trials through International clinical Research organization (CROS) so as to augment the acceptability of its product in the International market.

METABOLISM

Taxol is eliminated via hepatic metabolism. It is contraindicated in liver dysfunction.

Traditional Uses

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As tea in hydrophobia, viper bites, heart ailments, abortificients stomachache, Bronchitis, skin cancer, scurvy, rheumatism, oxytocic,. Management of labor pain, antithromboetic etc.

OTHER USES

✓ It is mostly used as a drug of choice in ovarian, breast and lung cancer (non small cells).
✓ Anti-epileptic
✓ Abortifient
✓ Treatment of tapeworm infestation
✓ In tonsillitis
✓ Some more uses are carminative, tranquilizer, cardiotonic, antipyretic, expectorant, antispasmodic & antiseptic.

SIDE EFFECTS

✓ Immunosuppressant
✓ Deadening of patient’s sensory nerves
✓ Nausea and alopecia
✓ Hypersensitivity TOXICITY
✓ Cardiac depression stopping the heart in diastole
✓ Circulatory failure

ANTICANCER ACTION OF TAXOL

Taxol targets microtubules produced by α & β tubuline (dimeric proteins) Microtubules are responsible for the formation of mitotic spindle, which is necessary for cell division α and β tubuline polymerize to form microtubules in presence of Microtubule Associated Protein (MAP) and Guanosine Tri Phosphate (GTP). Taxol brings about polymerization of microtubules in absence of MAP&GTP. Due to this, microtubules formation is much enhanced which causes detrimental effects on dividing cells, which leads to blockage of cell cycle. Eventually abnormal clusters are formed and microtubules get distributed in cytoplasm, which is non – functional.

Taxol also inhibits cell migration thus preventing spread of metastatic cancer cells. Taxol derivatives like paclitxotel mainly act at the G-2/M- phase junction. Docetoxel is primarily active in S-phase of mitosis. These compounds are selectively toxic to proliferating cells because non-dividing cells don’t require mitotic spindle and thus remain unaffected by Taxol.
SCHEMATIC REPRESENTATION DEPICTING
MECHANISM OF ACTION OF TAXOL

Simple precursor CO2.NH3. GLYSINE

PURINE SYNTHESIS     PYRIMIDINE SYNTHESIS

RIBONUCLEOTIDE

DEOXYRIBONUCLEOTIDE

DNA

RNA

PROTEINS [α tubulin & β tubulin]

Ploymerization
In absence of MAP and
GTP by TAXOL

MICROTUBULES

Microtubules are necessary for formation of mitotic spindle and cell division.
EXTRACTION OF TAXOL

Plant material +3 part of hexane
Extracted for 24 hours at room temp

Plant material extracted with
CH2Cl2: Methanol (1:1) for
24 hours at Rome Temperature
Filter

Hexane extract discarded

Organic extract evaporated to
Dryness at 35°C under reduced
pressure

Residue dissolved in minimum
Amount of methanol.

Partitioned between Methanol: water (1:1) in
order to achieve better separation

Organic layer evaporated to dryness
At room temperature at 35°C

Residue wash
Aqueous layer discarded

TAXOL
ESTIMATION OF TAXANES

a) ELISA METHOD

An Enzyme Linked Immuno Assay System (ELISA) has been developed for the determination of Taxane in bark extract and other biological samples. The immunoassay uses indirect competitive inhibition, which can detect taxol, cephalomannine and baccatin III at varying levels of sensitivity.

The relative cross reactivity with other taxanes comprises the ability to detect high taxol producers, but this advantage may be eliminated with the introduction of monoclonal antibody kits for specific taxanes.

b) HPLC METHOD

HPLC analysis is performed on column packed with phenyl bonded silicagel and phase separated in the reverse phase mode using Methanol: Water: Methyl cyanide (20:67:13) as solvent system.

Sample volume injected is 5 μl.

Sample is prepared by dissolving dried CH2CL2 and partitioned in fraction using CH3OH at concentration of 25 mg/ml (125 μg/inj)

Complete baseline separation between cephalomannine, 10-deacetyltaxol and taxol is achieved with this method.

Alternatively curosil column can be used with 12.4 mM sodium acetate pH 4.5: Acetonitrile (52.%, 47%) at ml/min flow rate.

A slight modification of elution condition (12.4 mM sodium acetate, pH 5.0: Acetonitrile (55:45) was necessary to give baseline separation between 10-deacetyl-baccatin III and baccatin III.

The Isocratic HPLC method permits rapid analysis of large number of crude extract with excellent resolution of taxanes.

C) COLUMN CHROMATOGRAPHY

Uses mobile phase-methanol: Water (68:32) at 1 ml/min, detection at 230nm.

CONCLUSION

The news of taxols unusual method of attacking cancerous cells has excited the cancer research community. Cancer tends to become resistant to treatment over the time period and because taxol kills tumor cells in a novel fashion, it offers hop to patients whose disease is not responding to current therapy.

It appears that taxol has promising future in the treatment of cancer. Realizing full potential of taxol and taxane compounds efforts are underway to devise methods for synthesis and semisynthesis to fulfil the requirements in a very short period of time.

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