REVIEW ARTICLE

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Camptothecine - A Novel Anticancer Agent from Tissue Cultures of *Nothapodytes foetida*

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Camptothecines have a broad spectrum of antitumor activity both *in vitro* and *in vivo* and various clinical properties. *Nothapodytes foetida* has much higher contents of camptothecine (0.3% dry weight) and its analogs than all other botanical sources of camptothecines. This article indicates the need of systematic studies on cell cultures of *Nothapodytes foetida* to enhance the production of camptothecine and its analogs by employing various strategies.

There is an urgent need to develop new cancer chemotherapeutic agents with activity against the disease-types still resistant to current therapies and to overcome the development of multidrug resistance, which is increasingly observed in the treatment of many tumors[1]. The plant kingdom is one of the attractive sources of novel antitumor compounds. The National Cancer Institute (NCI) of United States of America has conducted an intensive screening program since 1955 and has identified various potent compounds from higher plants. These antitumor compounds include, camptothecine and its derivatives, maytansine, tripdiolide, homoharringtonine, bruceantin, ellipticine, thalicarpine, indicine-N-oxide, baccharin, vinblastine, vincristine, taxol and podophyllotoxin derivatives including etoposide[2].

Camptothecine (CPT) is a pyrrolo quinolone alkaloid first isolated from 'Xi Shu' *(Camptotheca acuminata* Decaisne), a Chinese deciduous tree of the family Nyssaceae. Wall *et al.*[3] isolated CPT from bark extracts of *C. acuminata*. Camptothecines have exhibited a broad spectrum of antitumor activity both *in vitro* and *in vivo*. 10-hydroxy camptothecine (HCPT), which is more potent but less toxic than CPT[4,5], is so far one of the promising derivatives of camptothecine. SmithKline Beecham Pharmaceuticals discovered topotecan (Hycamtin®, TPT, an analogue of HCPT) which has been approved by the FDA for Refractory ovarian cancer. Daiichi Pharmaceuticals Co. Ltd., Japan, discovered irinotecan (CPT-11, an analogue of HCPT), which is approved in Japan for the treatment of lung, cervical and refractory ovarian cancers, squamous cell carcinoma, non-Hodgkin's lymphoma and colorectal, stomach and breast cancers[6]. 9-Amino-20(R, S) camptothecine (9-AC) is found to be effective in human colon cancer[7], which has been approved by the FDA.

CPT inhibits the replication of RNA viruses (retroviruses) such as human immunodeficiency virus (HIV), Equine infectious anaemia virus (EIAV), and Moloney murine leukaemia virus (Mo-MuLV) that cause many severe diseases in humans and animals including AIDS[8-12]. CPT possesses activity against trypanosomes and *Leishmania*[13]. 10-methoxy camptothecine is found more potent than CPT as an inhibitor of Herpes simplex virus[14]. Psoriasis vulgaris can effectively be treated with CPT-DMSO (dimethyl sulfoxide) solution[15].

CPT and its analogs inhibit topoisomerase I catalytic activity, they stabilize the DNA/protein complex by forming topoisomerase I-DNA adduct so that the normal, rapid process of strand division, disentangling and rejoining is arrested at mid-stage[16,17]. It was found that CPT and TPT inhibit HIV-1 LTR activity induced by viral transactivator (Tat) and cytokinesis[10,18] (fig. I). Interaction of topoisomerase I with advancing replication forks results in DNA double-strand breaks. CPT, which is unable to bind to free topoisomerase I, binds to the topoisomerase I-DNA adduct and thus inhibit DNA synthesis.

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Nothapodytes foetida:

Camptothecine is reported in plants viz, *Camptotheca acuminata*[7] (Nyssaceae), *N. foetida*[19] (Iccacinaceae), *Merrilliodendron megacarpum* (Iccacinaceae[20]), *Ophirrohiza mungos*[14] (Rubiaceae), *Ophirrohiza pumila*[21] (Rubiaceae), *Ervatamia heyneana*[20] (Apocynaceae) and *Mostuea brunonis*[22] *(Loganiaceae). N. foetida* has much higher contents of CPT (0.3%dwt), 9-MCPT and analogs than all other botanical sources of camptothecines[23]. CPT is distributed in all parts (stem, stem bark, leaf, root, root bark and fruit) at all stages of *N. foetida.*

N. foetida (Wight) Sleumer, formerly *Mappia foetida* Meirs is a small tree of the family Iccacinaceae. It is distributed in Assam, South India, Ceylon, Burma, Thailand, Combodge, Lan Yu II (SE. of Formosa, Botel, Tobago, Kolyo syo), Ryu-Kyu Is. (Yaeyama group), W. Sumatra and Philippines. The genus *N. foetida* includes *N. obtusifolia* (within china), *N. montana* (within Thailand, NE. Sumatra, W. Java, W. Sumbawa) and *N. pittosporoides* (within China, Indochina)[23,24].

Chemistry:

Camptothecine ($C_{20}H_{16}O_4N_2$) is a pyrrolo-(3,4-b)-quinoline alkaloid, that is, 4(S)-4-ethyl-4-hydroxy-1H-pyrano-(3',4':6,7)-indolizino-(1,2-b)-qunoline-3,14(4H,12H)-dione (fig. 2). It is an yellow compound that reacts readily with bases to give water-soluble salts. As would be expected from its aromatic pentacyclic structure, CPT is high melting point, with characteristic ultraviolet and fluorescence spectra. Wall *et al.*[25] reported that 9-AC is found more potent than CPT. The chemical constituents present in *N foetida*[26] are camptothecine, 10-hydroxy camptothecine (HCPT), methoxy camptothecine (9-MCPT), 20-O-acetyl camptothecine, foetidin I, mappicine, 9-methoxy mappicine, mappicine-20-O-β-D-gentiobioside, mappicine-20-O-β-D-glucopyranoside, 9-methoxy mappicine-20O-O-β-D-gentiobioside, 17-hydroxy mappicine-20O-O-β-D-glucopyranoside, nothopodytines A (mappicine ketone), nothodytines B (9-methoxy mappicine ketone), 22-hydroxycuminate, pumiloside, daucosterol, sitost-4-en-3-one, β-sitosterol, stigmost-4-en-3-one, 3-β-hydroxystigmost-5-en-7-one, stigmost-5-en-3β-7α-diol, thiamine and uracil. It also contains trigonelline (Proteid), scopoletine (coumarin), lupeol(triterpene), 4-hydroxy benzaldehyde, lignin, phenyl propanoid and lipids.

Biosynthesis:

Camptothecine, being a quinoline alkaloid, is biogenetically linked to the group of indole alkaloids[27]. Indole alkaloids are biosynthesised from a combination of indolic and monoterpenoid units. Tryptophan, tryptamine, mevalonate, geraniol, loganin, secologanin, vincoside and isovincoside (strictosidine) have been established as precursors of indole alkaloids. The biosynthetic pathway of camptothecine is depicted in fig. 3.

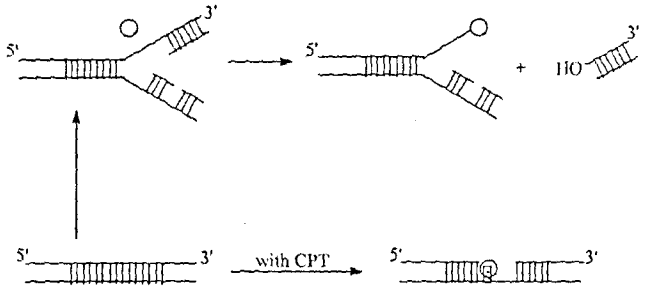

Fig. 1: Mechanism of the inhibition of topoisomerase - I by CPT.

○ Topoisomerase I ⊡ CPT.

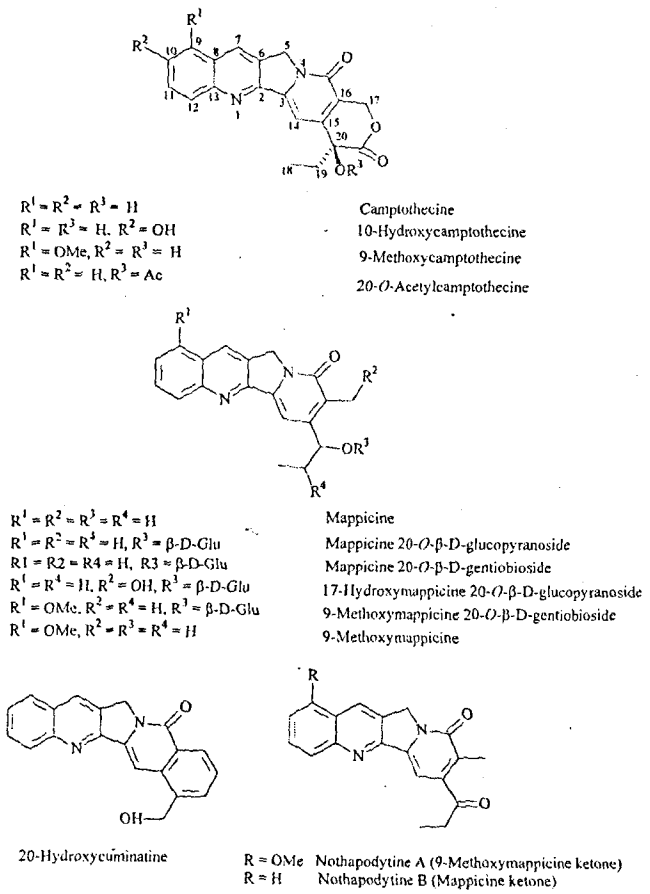

Fig. 2: Chemical structures of Camptothecine and its analogs from *Nothapodytes Foetida.*

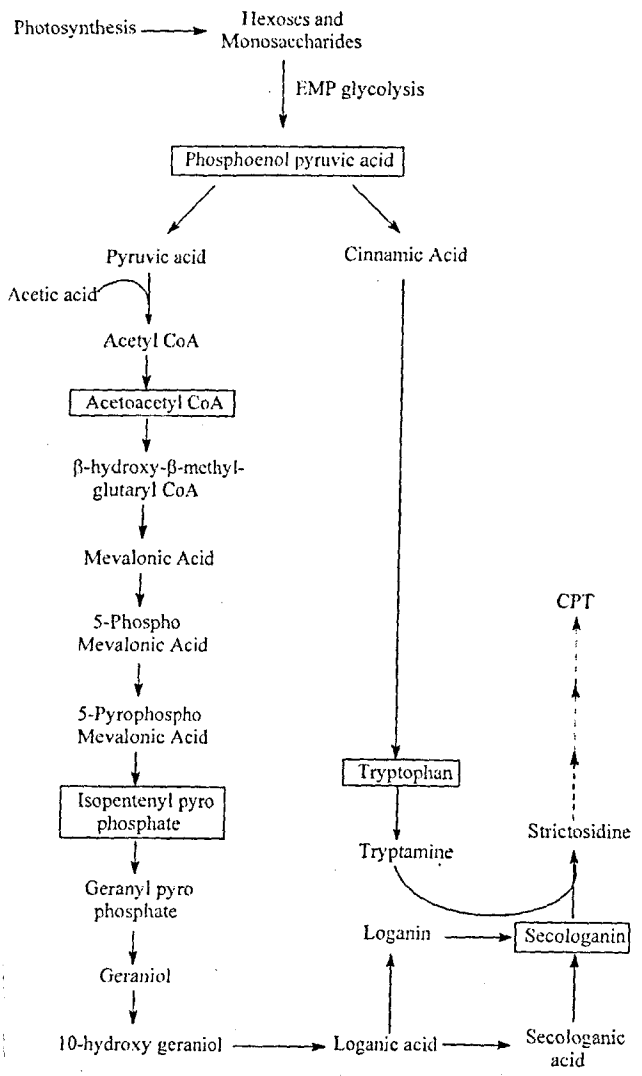

Fig. 3: Biosynthesis of CPT.

--> indicates that it involves several steps in the formation of CPT from strictosidine.

Tissue culture:

CPT and its analogs are obtained from *C. accuminata, O. mungos, E. heyneana and N foetida.* 9-MCPT is only isolated from *N. foetida* and not from other plants. 9-MCPT is precursor for the semisynthesis of 9-amino CPT. No viable commercial method of synthesis, low yields from intact plants, poor seed germination, high market price coupled with various biological activities has encouraged intense research for alternative methods for the production of camptothecine and its analogs from *N. foetida.* Plant cell culture has emerged as a viable route for production of paclitaxel (Taxol) and provides a model for production of anticancer agents from woody plants[28] and may be applicable to CPT.

Roja and Heble[29] reported that the unorganized callus can be developed from the immature embryos of *N. foetida* on Murashige and Skoog (MS) medium supplemented with (1 mg/l) benzyl adenine (BA) and (2 mg/l) 2,4-dichlorophenoxy acetic acid (2,4-D). The differentiated plantlet cultures showed slightly higher amounts of 9-MCPT (0.0007% dwt) than the undifferentiated callus cultures (0.0001% dwt). Veeresham and Shuler[30] demonstrated the initiation of callus (after 4 w of incubation at 25±2° in dark) from excised embryos of *N. foetida* on MS medium supplemented with picloram (2 mg/l) and 3% (w/v) sucrose. The cultures produced CPT (0.00095% dwt) and traces of 9-MCPT. Satheeshkumar and Seeni[31] reported the regeneration of single shoot (0.8-1.2 cm) in each culture after 6 w from the isolated nodes (1.0-1.2 cm) and shoot tips (1.0-1.5 cm) of *N. foetida* cultured in MS medium containing varying concentrations of thidiazuron (TDZ), BA and combinations of 6-γ-γ-(Dimethylallyl amino)-purine (2iP) and gibberellic acid (GA_3). Thengane *et al.*[32] reported the regeneration of adventitious shoots from different seedling explants of *N. foetida* in MS medium supplemented with various concentrations of TDZ. However, there is no report on production of CPT and its analogs from leaf initiated callus cultures as well as cell cultures of *N. foetida.*

Extraction:

Wall *et al.*[3] reported a hot hexane-heptane extraction of dried plant material followed by an extraction with ethanol. The concentrate of the ethanol extraction was partitioned between water and chloroform. Silica gel chromatography of the methanol insoluble material from the chloroform extract, followed by crystallization from ethanol-acetonitrile gave CPT as pale yellow needles. Van Hengel *et al.*[33] described a method in which, an amount of freeze-dried cells were ground in a mortar and sonicated in methanol. Water and dichloromethane were added for extraction. After centrifugation the dichloromethane phase, which was proved to contain the camptothecine, was recovered and evaporated to dryness and the residue was dissolved in methanol. Veeresham and Shuler[30] reported a simple procedure in which the callus was freeze-dried, extracted with methanol and kept overnight in a deep freezer. After thawing, the methanolic layer was separated and the process was repeated for complete extraction. The combined methanolic extract was evaporated to dryness, water was added to the residue and extracted with chloroform. The chloroform ex-

tract was evaporated to dryness and dissolved in methanol and subjected for TLC and HPLC analysis.

Thin layer chromatography (TLC):

The alkaloids were monitored for purity by TLC on silica gel plates developed with chloroform-methanol[29] (24:1), chloroform-ethanol[30] (24:1) and chloroform-ethanol-ethylacetate-hexane[33] (20:9.5:65.2:5.3). R' values of CPT and 9-MCPT were 0.5 and 0.6-0.67 respectively. CPT gives intense blue fluorescence whereas 9-MCPT gives violet fluorescence under UV light of 254 and 366 nm wavelength.[33] HCPT gives pink fluorescence.[34]

High pressure liquid chromatography (HPLC):

Van Hengel *et al.*[33] described a HPLC method of analysis using Waters Model 510, RP column Chromopak Lichro sorb RP C-18, 100 mm and UV absorbance detector (ABI Analytical Kratos Division, Model Spectroflow 757) for the detection of camptothecine at 256 nm. The flow rate was 1 ml/min and the mobile phase used was acetonitrile-water (25:75). Roja and Heble[29] reported a HPLC analysis using Waters Associates (Model ALC/GPC 244) with C-18 Novopak column (15 cmX3.9 mm) and acetonitrile-water (1:3) solvent system with a flow rate of 1 ml/min and the UV detector set at 254 nm. Wiedenfed *et al.*[35] reported a HPLC method using Liquisorb RP C-18, 25 cm, 10 μm, gradient elution (0.04 M $H3PO_4$):acetonitrile 9:1 upto 1:1, 10 min, flow rate 3.5 ml/min, UV detection at 254 nm. Puri *et al.*[36] demonstrated an HPLC analysis using RP C-18 Novopak Column (15 cmx3.9 mm) with eluent, 60% aqueous methanol, flow rate 1 ml/min and detection at 256 nm for the quantitative analysis of CPT and its analogs. Veeresham and Shuler[30] described a linear gradient HPLC analysis with Pump A:water with 0.1% trifluoroacetic acid and Pump B: recovered acetonitrile (86%, 14% water) with 0.1% trifluoroacetic acid. The analysis was carried out on Waters delta pak C18 column (15 cmx4.6 mm) with 4 μ with guard column and at a flow rate of 0.85 ml/min. The wave lengths for CPT and 9-MCPT are 253 nm and 267 nm respectively. *N. foetida* is a potential source of CPT and its analogs. Due to its several clinical properties, it has been over exploited. Nonavailability of commercial method of synthesis, necessiated immediate attention for multiplication of *N. foetida* and search for alternative methods of production for CPT and its analogs from *N. foetida.* Plant tissue culture offers advantages over conventional methods for production of secondary metabolites and multiplication of woody plants.

In conclusion, there is a need to under take systematic study on cell suspension cultures to enhance the production of therapeutically important CPT and its analogs by employing a variety of elicitors, precursors, genetic transformation and metabolic engineering of biosynthetic pathway of CPT and its analogs for higher productivity through cell cultures before the commercialization of the technique.

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