ISSN 2278 - 540X EISSN 2278 - 5396

DRUG DISCOVERY

# Current panorama on Camptothecin in Cancer treatment

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Received 12 September; accepted 16 October; published online 01 November; printed 16 November 2012

## ABSTRACT

This paper is a review on Camptothecin and its solubility, QSAR; release effects while combining with various polymers, DNA damage, Programmed cell death and MMR effect in cells. Camptothecin (CPT) is an effective chemotherapeutic agent for treatment of patients with cancer. The mechanisms underlying CPT-mediated responses in cancer cells are not fully understood. MicroRNA (miRNA) play important roles in tumorigenesis and drug sensitivity. However, the interaction between camptothecin and miRNA has not been previously explored. To determine the inhibitory nature of sublethal doses of camptothecin (CPT) and topotecan (TPT) treatments on normal human endothelial cells in vitro, as well as the in vivo antiangiogenic activity as compared to another antiangiogenic compound, TNP-470 and to a nonspecific cytotoxic agent, cisplatin. Loss of a functional mismatch repair (MMR) system in colorectal cancer (CRC) cells is associated with microsatellite instability and increased sensitivity to topoisomerase inhibitors.

**Key Words:** CPT- Camptothecin, MMR- Mis Match Repair, QSAR- Quantitative Structural Activity Releationship.

## 1. INTRODUCTION

In 1966 Monroe Wall wrote of his work at the Eastern Regional Research Laboratory: “The joint effort of chemists and botanists proved to be a good model for future programs. Indeed, it firmly established the fact that the close cooperation between chemists and botanists was required for a successful natural products program.” Monroe Wall was born in Newark, New Jersey, in 1916 and earned his undergraduate and graduate degrees from Rutgers University. In 1941 he joined the USDA’s ERRL in Philadelphia where he worked on potential agricultural alternatives for products, such as rubber, critical to the war effort. After the war, he became involved in plant screening, and in 1957 a chance visit from Jonathan Hartwell of the Cancer Chemotherapy National Service Center changed the course of Wall’s career. It was Hartwell who convinced Wall to send NCI one thousand ethanolic plant extracts for antitumor activity testing. A year later Wall learned that one of the plants demonstrated potent activity: Camptotheca acuminata. But the USDA was not interested in anticancer drug research, so Wall’s ambition to identify the active component in C. acuminata had to be put on hold until July 1960 when the Research Triangle Institute recruited him. According to Mansukh Wani, an organic chemist born in Bombay, India, who Wall lure d to RTI in 1962, the Institute was “nothing but four ‘walls.’ It was not until the fifth ‘Wall’ arrived that the chemistry programs, in the form of the Natural Products Laboratory, started moving.”

Wani describes Wall as “a go-getter” and “a very dedicated scientist.” About Wall, Wani adds, “persistence was his greatest virtue, but patience was not.” Wall and Wani collaborated for forty years, a collaboration that began with research into the nature of the compounds responsible for the antitumor activity in C. acuminata. By 1963 RTI had an approximately 20 kg sample of the wood and bark of C. acuminata available, and Wall and Wani and colleagues at RTI began what they describe as ‘bioactivity-directed fractionation.’ In this the crude plant extract is purified in an iterative manner. At each stage the ‘fractions’ are tested for bioactivity. Those fractions showing the most potent activity are carried on to the next stage of purification. The process is repeated many times until the compound(s) responsible for the bioactivity observed with the crude extract are isolated. In the 1960s this was a difficult and slow process requiring great skill and intuition on the part of the researchers using equipment that can now be found only in museums. By the time Wall and Wani began fractionating their samples it was known that C. acuminata was very active in the L1210 mouse leukemia assay, which was unusual since most plants did not exhibit such activity. It was this activity that had aroused the interest of NCI. All of the fractions of the extract were analyzed both by the in vivo L1210 mouse life prolongation assay and by the KB in vitro cytotoxicity assay. The pure compound isolated as a result of the fractionation was given the name camptothecin and it was shown to be the agent that was not only very active against L1210 leukemia but also against P388 leukemia cells.

RTI scientists Keith Palmer and Harold Taylor did the original isolation of camptothecin, and Ed Cook worked on determining i ts structure. At that point, Wall asked Wani, who he described as having “the knack to work on small amounts of material,” to prepare a camptothecin derivative for single X-ray crystallography. The derivative was sent to Andrew McPhail and George Sim at the University of Illinois who reported back within a few weeks the tentative structure of camptothecin, which is unusual although it is related to the indole alkaloids. The research on the isolation and structure of camptothecin was published in 1966 in the Journal of the American Chemical Society, the first paper Wall, Wani, and colleagues published on a natural product with anticancer potential. Wani describes the isolation of camptothecin as “the most exciting scientific event in my life.” Because camptothecin showed such promising antitumor activity, the NCI decided to proceed with clinical trials. But camptothecin is not soluble in water (which makes delivery of a potential drug difficult) so the trials were conducted with a water-soluble sodium salt that could be formulated for intravenous delivery. In the trials, some patients with gastrointestinal tumors responded to treatment for a short time.

## 2. INDICATION

- Ovarian Cancer,
- Colon rectal Cancer.
- Lung Cancer



Balasubramanian J,
Current panorama on Camptothecin in Cancer treatment,
Drug discovery, 2012, 2(5), 16-18,

## 3. MECHANISM OF ACTION

Despite some encouraging successes, the use of camptothecin as an anticancer agent languished for almost fifteen years until its unique mode of action for killing tumor cells was determined. Camptothecin traps an important cellular enzyme, topoisomerase I, in complexes with DNA. This prevents cancer cell DNA replication and results in the death of the cancer cell.

## 4. CAMPTOTHECIN AND QSAR DETAILS

An intact lactone ring of camptothecins is a structural requirement for their anticancer activity. Propionate esters of camptothecin (CPT) and 9-nitrocamptothecin (9NC), CZ48 and CZ112, respectively, have been synthesized as derivatives resistant to lactone hydrolysis and are chemotherapeutically active. In this study, we have examined the mechanism of action of CZ48 and CZ112 and their distribution, metabolism, and toxicity. CZ112 incubated in human plasma retained its lactone structure longer than 9NC (t1/2: 10.5 and < 1 hr for CZ112 and 9NC, respectively). This resistance to lactone hydrolysis was also observed in mouse plasma or albumin solutions. Neither CZ48 nor CZ112 inhibit topoisomerase I and thus are prodrugs dependent on hydrolysis to CPT or 9NC, respectively. Rates of hydrolysis of CZ48 to CPT are higher by homogenates of mouse liver, spleen, lung, and kidney than by plasma. Rates of hydrolysis by tumor cells in culture vary and were higher by breast cancer and melanoma cells than by colon cancer cells. On the basis of these and other data, it is proposed that CZ48 and CZ112 may act as anticancer agents by resisting hydrolysis to camptothecins while in circulation. Hydrolysis in tissues may release intact lactone in target tissues

## 5. CAMPTOTHECIN AND ITS SOLUBILITY

The solubility of camptothecin (CPT), a highly potent antineoplastic agent, as a function of different concentrations of cyclodextrins (alpha-cyclodextrin, alpha-CD; beta-cyclodextrin, beta-CD; and gamma-cyclodextrin, gamma-CD; hydroxypropyl-beta-cyclodextrin, HP-beta-CD; and randomly substituted dimethyl-beta-cyclodextrin, RDM-beta-CD, and dimethyl-gamma-cyclodextrin, RDM-beta-CD) in 0.02 N HCl solution at 25 degrees C was investigated. The results showed a linear increase in the solubility of CPT with increasing concentration of CDs. The apparent stability constants (K(c)) for the CPT complexes with alpha-CD, beta-CD, gamma-CD, HP-beta-CD, RDM-beta-CD, and RDM-gamma-CD were 188, 266, 73, 160, 910, and 40.6 M(-1), respectively, suggesting that RDM-beta-CD afforded the most stable complex. At a 25% w/v concentration of RDM-beta-CD, the solubility of CPT was 228.45 +/- 8.45 microg/ml, about 171 times higher than that in 0.02 N HCl. The stability of CPT in pH 7.4 buffer at 25 degrees C also increased linearly with an increase in the concentration of RDM-beta-CD.

## 6. CAMPTOTHECIN AND ITS SOLUBLE ANALOUGE

The camptothecins are a new class of chemotherapeutic agents which have a novel mechanism of action targeting the nuclear enzyme topoisomerase I. Knowledge of the structure-activity relationships of the parent compound camptothecin has led to the development of effective soluble analogues with manageable toxicities. Broad anti-tumour activity shown in preclinical studies has been confirmed in phase I/II studies for irinotecan and topotecan. Two other derivatives, 9-aminocamptothecin and GI 147211C, are undergoing phase I and early phase II evaluation. Although camptothecin is a plant extract, it and most of its derivatives are not affected by the classic P-gpMDR1 mechanism of resistance which may allow the development of novel combination chemotherapeutic regimens. Important areas of future endeavour will include the development of rational combination regimens and the pursuit of randomised trials. Based on single agent data, colorectal cancer and non-small-cell lung cancer should be the focus for future irinotecan studies. Small-cell lung cancer and ovarian carcinoma are logical tumour types to pursue with topotecan. Both 9-aminocamptothecin and GI 147211C are too early in their clinical evaluation to make recommendations about their future roles. Finally, the unfolding story of camptothecin analogue development will give important insights into the predictive value of preclinical observations on relative efficacy, schedule dependency, combination strategies and resistance mechanisms which have helped determine the strategies for clinical evaluation of these agents.

## 7. CAMPTOTHECIN AND ITS RELEASE EFFECTS

Camptothecin loaded solid lipid nanoparticles (CA-SLN) coated with poloxamer 188 were produced by high pressure homogenization. The CA-SLN were characterized by transmission electron microscopy and electrophoretic mobility measurement. In vitro release characteristics of camptothecin from CA-SLN were studied at different pH media. The concentration of camptothecin in organs was determined using reversed-phase high-performance liquid chromatography with a fluorescence detector after oral administration of CA-SLN and a camptothecin control solution (CA-SOL).The results indicate SLN could be a promising sustained release and targeting system for camptothecin or other lipophilic antitumor drugs after oral administration.

## 8. CAMPTOTHECIN AND ITS PRODRUG

7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (irinotecan, CPT-11) is a camptothecin prodrug that is metabolized by carboxylesterases (CE) to the active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), a topoisomerase I inhibitor. CPT-11 has shown encouraging antitumor activity against a broad spectrum of tumor types in early clinical trials, but hematopoietic and gastrointestinal toxicity limit its administration. To increase the therapeutic index of CPT-11 and to develop other prodrug analogues for enzyme/prodrug gene therapy applications, our laboratories propose to develop camptothecin prodrugs that will be activated by specific CEs. Specific analogues might then be predicted to be activated, for example, predominantly by human liver CE(hCE1), by human intestinal CE (hiCE), or in gene therapy approaches using a rabbit liver CE (rCE). This study describes a molecular modeling approach to relate the structure of rCE-activated camptothecin prodrugs with their biological activation. Comparative molecular field analysis, comparative molecular similarity index analysis, and docking studies were used to predict the biological activity of a 4-benzylpiperazine derivative of CPT-11 [7-ethyl-10-[4-(1-benzyl)-1-piperazino]carbonyloxycamptothecin (BP-CPT)] in U373MG glioma cell lines transfected with plasmids encoding rCE or hiCE. BP-CPT has been reported to be activated more efficiently than CPT-11 by a rat serum esterase activity; however, three-dimensional quantitative structure-activity relationship studies predicted that rCE would activate BP-CPT less efficiently than CPT-11. This was confirmed by both growth inhibition experiments and kinetic studies. The method is being used to design camptothecin prodrugs predicted to be activated by specific CEs.

## 9. CAMPTOTHECIN AND PEG

An improved synthesis of the hindered PEG-camptothecin diester transport form has been achieved using the Mukaiyama reagent. We have also assessed the effect of changing the electronic configuration of the (d-position of PEG-camptothecin transport forms on the rates of hydrolysis of the pro-moiety, and attempted to correlate these differences to efficacy in two animal models. In addition to the simple substitution of N for O, other synthetic modifications of these atoms were accomplished by employing heterobifunctional linker groups. The half lives by disappearance (rates of hydrolysis) of the transport forms in buffer and rat plasma were determined. It was established that anchimeric assistance to hydrolytic breakdown of the pro-moiety occurs in a predictable manner for some of these compounds. Results for the new derivatives in a P388 murine leukemic model and HT-29 human colorectal xenograft study are also presented. The use of a glycine linker group was found to provide similar efficacy in rodent models to that of simple camptothecin 20-PEG ester, and displayed enhanced pharmacokinetics.

## 10. CAMPTOTHECIN AND DNA DAMAGE

Replication-mediated DNA damage by camptothecin induces phosphorylation of RPA by DNA-dependent protein kinase and dissociates RPA:DNA-PK complexes.Replication protein A (RPA) is a DNA single-strand binding protein essential for DNA replication, recombination and repair. In human cells treated with the topoisomerase inhibitors camptothecin or etoposide (VP-16), we find that RPA2, the middle-sized subunit of RPA, becomes rapidly phosphorylated. This response appears to be due to DNA-dependent protein kinase (DNA-PK) and to be independent of p53 or the ataxia telangiectasia mutated (ATM) protein. RPA2 phosphorylation in response to camptothecin required ongoing DNA replication. Camptothecin itself partially inhibited DNA



Balasubramanian J,
Current panorama on Camptothecin in Cancer treatment,
Drug discovery, 2012, 2(5), 16-18,

synthesis, and this inhibition followed the same kinetics as DNA-PK activation and RPA2 phosphorylation. DNA-PK activation and RPA2 phosphorylation were prevented by the cell-cycle checkpoint abrogator 7-hydroxystaurosporine (UCN-01), which markedly potentiates camptothecin cytotoxicity. The DNA-PK catalytic subunit (DNA-PKcs) was found to bind RPA which was replaced by the Ku autoantigen upon camptothecin treatment. DNA-PKcs interacted directly with RPA1 *in vitro*. We propose that the encounter of a replication fork with a topoisomerase-DNA cleavage complex could lead to a juxtaposition of replication fork-associated RPA and DNA double-strand end-associated DNA-PK, leading to RPA2 phosphorylation which may signal the presence of DNA damage to an S-phase checkpoint mechanism.

## 11. CAMPTOTHECIN AND HEMATOPOIETIC PROGENITORS

20(S)-Camptothecin (CAM), topotecan (TPT, active ingredient in Hycamtin) and 9-amino-20(S)-camptothecin (9AC) are topoisomerase I inhibitors that cause similar dose-limiting toxicities to rapidly renewing tissues, such as hematopoietic tissues, in humans, mice, and dogs. However, dose-limiting toxicity occurs at tenfold lower doses in humans than in mice. The purpose of the current study was to determine whether hematopoietic progenitors of the myeloid lineage from humans, mice, and dogs exhibit the differential sensitivity to these compounds that is evident in vivo.

## 12. CAMPTOTHECIN AND PCD

The parasites of the order kinetoplastidae including Leishmania spp. emerge from most ancient phylogenic branches of unicellular eukaryotic lineages. In their life cycle, topoisomerase I plays a significant role in carrying out vital cellular processes. Camptothecin (CPT), an inhibitor of DNA topoisomerase I, induces programmed cell death (PCD) both in the amastigotes and promastigotes form of L. donovani parasites. CPT-induced cellular dysfunction in L. donovani promastigotes is characterized by several cytoplasmic and nuclear features of apoptosis. CPT inhibits cellular respiration that results in mitochondrial hyperpolarization taking place by oligomycin-sensitive F0-F1 ATPase-like protein in leishmanial cells. During the early phase of activation, there is an increase in reactive oxygen species (ROS) inside cells, which causes subsequent elevation in the level of lipid peroxidation and decrease in reducing equivalents like GSH.

## 13. CAMPTOTHECON AND p53

Various DNA-targeting agents may initiate p53-dependent as well as p53-independent response and subsequent apoptosis via alternative cellular systems which include for instance p73, caspase-2 or Bcl-2 family proteins. The scope of involvement of individual molecules in this process and the mechanisms governing their potential interplay are still not entirely understood, in particular in highly aggressive cancers such as in malignant melanoma. In this work we investigated the role and involvement of both p53-dependent and -independent mechanisms in selected melanoma cell lines with differing status of p53 using a model DNA topoisomerase I inhibitor camptothecin (CPT).

## 14. Camptothecin and UCN

Derivatives of camptothecins, topoisomerase I inhibitors and 7-hydroxystaurosporine (UCN-01), a protein kinase C (PKC) inhibitor and cell cycle checkpoint abrogator, are promising anticancer drugs. We characterized the apoptotic response to camptothecin and UCN-01 for the 8 human breast carcinoma cell lines (MCF-7, MCF-7/ADR, T47D, HS578T, BT549, MDA-N, MDA MB231, MDA435) from the National Cancer Institute (NCI) Anticancer Drug Screen. MCF-7 and T47D cells exhibited marked resistance to apoptosis, whereas MCF-7/ADR (NCI/ADR-RES) and HS578T cells exhibited the most pronounced apoptotic response. Apoptotic response was not correlated with growth inhibition measured by sulforhodamine B (SRB) assay, indicating that apoptosis is not the only mechanism of drug-induced cell death. Measurements of topoisomerase I levels and cleavage complexes and of PKC isoforms demonstrated that primary target inhibition was not correlated with apoptotic response. Several key apoptotic pathways were evaluated.

## 15. CAMPTOTHECIN AND MMR

Loss of a functional mismatch repair (MMR) system in colorectal cancer (CRC) cells is associated with microsatellite instability and increased sensitivity to topoisomerase inhibitors. In this study, we have investigated whether a defect in double-strand break (DSB) repair by non-homologous end-joining (NHEJ) could explain why MMR-deficient CRC cells are hypersensitive to camptothecin (CPT), a topoisomerase I inhibitor. To evaluate the efficiency and the fidelity of DSB repair, we have transiently transfected plasmids containing cohesive or non-complementary ends in cells with various MMR defects. We have observed that the repair efficiency of DSB with cohesive and non-complementary ends is comparable in all cell lines. In contrast to the MMR-proficient cell line HT29, the MMR-deficient cell lines were highly accurate in repairing DSB with cohesive ends, but this characteristic could not be directly assigned to the primary MMR deficiency.

## 16. CAMTOTHECIN AND SCLEROSIS

The main manifestation of systemic sclerosis (SSc) is the overproduction of extracellular matrix, predominantly type I collagen. This study was undertaken to evaluate the effects of noncytotoxic doses of the topoisomerase I inhibitor camptothecin (CPT) on collagen production in the activated dermal fibroblasts from patients with SSc and healthy donors. The fibroblasts were cultured in the presence or absence of CPT. Production of collagenous proteins by fibroblasts was determined in cell and matrix layers by ELISA and in conditioned media by [$^3$H]proline incorporation, gel electrophoresis, and autoradiography. Expression of α2(I) collagen (COL1A2) mRNA was measured by northern blot, and the activity of COL1A2 promoter was determined by a chloramphenicol acetyltransferase assay. CPT ($10^{-7}$ M) decreased the deposition of type I collagen by 68%, of type III by 38%, and of type VI by 21% in SSC fibroblasts and to a lesser degree in healthy controls. Similarly, CPT ($10^{-8}$ M to $10^{-6}$ M) significantly inhibited secretion of newly synthesized collagenous proteins into conditioned media by 50%. CPT ($10^{-8}$ M to $10^{-6}$ M) caused a significant dose-dependent inhibition of COL1A2 mRNA levels and COL1A2 promoter activity, both by as much as 60%. The inhibitory effect of CPT on collagen production by fibroblasts from patients with SSc suggests that topoisomerase I inhibitors may be effective in limiting fibrosis in such patients.

## 17. SUMMARY

The camptothecins are a new class of chemotherapeutic agents which have a novel mechanism of action targeting the nuclear enzyme topoisomerase I. Knowledge of the structure-activity relationships of the parent compound camptothecin has led to the development of effective soluble analogues with manageable toxicities. Broad anti-tumour activity shown in preclinical studies has been confirmed in phase I/II studies for irinotecan and topotecan. Two other derivatives, 9-aminocamptothecin and GI 147211C, are undergoing phase I and early phase II evaluation. Although camptothecin is a plant extract, it and most of its derivatives are not affected by the classic P-gpMDR1 mechanism of resistance which may allow the development of novel combination chemotherapeutic regimens. Important areas of future endeavour will include the development of rational combination regimens and the pursuit of randomised trials. Based on single agent data, colorectal cancer and non-small-cell lung cancer should be the focus for future irinotecan studies. Small-cell lung cancer and ovarian carcinoma are logical tumour types to pursue with topotecan. Both 9-aminocamptothecin and GI 147211C are too early in their clinical evaluation to make recommendations about their future roles. Finally, the unfolding story of camptothecin analogue development will give important insights into the predictive value of preclinical observations on relative efficacy, schedule dependency; combination strategies and resistance mechanisms which have helped determine the strategies for clinical evaluation of these agents.



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