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IMPROVEMENT AND REPLACEMENT OF CAMPTOTHECINS IN CANCER THERAPY DUE TO THE DECREASE IN THEIR SELECTIVITY AND EFFICIENCY

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THE DECREASE IN THEIR SELECTIVITY AND EFFICIENCY

Abstract: DNA replication, transcription, and chromosome condensation require enzymes that can regulate the topological changes occurring in DNA following these processes. Topoisomerases are such enzymes. These catalyze the cleavage of single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA), the passage of DNA through the resulting break, and the rejoining of the broken phosphodiester backbone. Chemical agents able to interfere with these enzymes present in the cancer cells have outstanding therapeutic efficacy in human cancer. Camptothecin derivatives (acting on type IB topoisomerases) have been shown to stimulate DNA cleavage by topoisomerases leading to cell death by apoptosis, but they are not always specific to tumor cells; they are also toxic and there's much resistance to them. That is why right now there is considerable interest in improving the selectivity of these agents by making them sequence specific. Another way to improve the therapeutic efficacy of the drugs is inactivation of NF-κB molecule which is a negative regulator of apoptosis in cancer cells. There is also considerable interest in developing new non-camptothecin drugs that would also inhibit topoisomerases I but in a more efficient and less toxic manner. In summary, the topoisomerase inhibitors are effective anti-cancer agents to some extent, but the current research focuses on improving their efficacy and selectivity and possibly replacing them altogether with better novel topoisomerase I poisons.

Colorectal and ovarian cancers are two of the most common cancers within the population of the United States. Ovarian cancer is one that begins in the cells that constitute the ovaries, including the epithelial cells, germ cells, and the sex cord-stromal cells. This type of cancer accounts for four percent of all cancers among women and ranks fifth as a cause of their deaths from cancer. The American Cancer Society statistics for ovarian cancer estimate that there will be 25,400 new cases and 14,300 deaths in 2004 [4]. Colorectal cancer, on the other hand, includes cancers of both the large intestine (colon) and the rectum. This is the second-leading cause of cancer related deaths in the United States. Only lung cancer claims more lives. Each year, more than 135,000 Americans are diagnosed with colorectal cancer and 56,000 die from it[2].

Over the last several years the structures of topoisomerases, which are very abundant in cancer cells, have finally come to light due to new, innovating techniques. These enzymes can

change the topological state of DNA by breaking and rejoining DNA strands. Topoisomerases are involved in all aspects of DNA structure and metabolism. As a result of this essential task, topoisomerases are necessary for the viability of all organisms and they have become targets for topoisomerase inhibitors, which have become widely used in recent times due their efficacy as antimicrobial and antitumor agents [1].

Camptothecin topoisomerase I inhibitors form a relatively new class of anticancer drugs. They are the only family of topoisomerase I inhibitors in clinical use for the treatment of mainly ovarian and colorectal cancers [3]. The parent compound camptothecin (CPT) was discovered forty years ago. It is extracted from the oriental tree *Camptotheca acuminate* (Nyssaceae family). It consists of five rings (A through E), the fifth being a lactone ring. In the first years after the discovery, the drug was highly unmanageable due its severe and unpredictable hematological and non-hematological effects. It was responsible for myelosuppression, diarrhea, nausea, vomiting and hemorrhagic cystitis in patients treated with it. Later, it was discovered that these toxic effects were caused by the low water solubility of the drug. During 1980s the interest in CPTs increased because its mechanism of action was better understood. Various CPT derivatives have been developed with modifications of rings A and B. This increased their water solubility and they have been approved to treat colorectal and ovarian cancers [8]. The two discussed here are irinotecan (or CPT-11) and topotecan. However, their toxicity and the tumor cells' increasing resistance to these poisons causes the current clinical experiments to focus on improving their efficacy for now but ultimately to develop novel non-camptothecin drugs that provide better results and have less adverse effects on patients [13].

Topoisomerases are excellent targets for drug inhibitors so it is necessary that their reaction mechanism is known. A property of DNA in all known organisms is the DNA

supercoiling, which is the bending and the twisting of the helical DNA. Cellular DNA is coiled in the form of a double helix, in which both strands of the DNA coil around and axis. The further coiling of that axis upon itself produces DNA supercoiling. Within the cell's nucleus DNA is strained to maintain its supercoiled state. In almost every instance, the strain is a result of an underwinding of the double helix – there are fewer helical turns than expected. This is a deviation from the most stable DNA form and the only way to accommodate this strain is for the DNA to supercoil. A property of DNA that topoisomerases can change is the linking number, which can be defined as the number of times one strand of DNA crosses the other. Negative supercoiling (induced by underwinding of the double helix) reduces the linking number of the relaxed DNA strand. Positive supercoiling on the other hand, which is induced by overwinding, increases the linking number [3]. While cells stably maintain their genomic DNA with negative supercoiling, essential cellular activities such as transcription and replication introduce additional supercoils and topological distortions in DNA. Since DNA supercoiling and topology occupy a central position in cellular regulation, the control of DNA supercoiling is an important pharmaceutical target. All topoisomerases reduce the supercoiling and change the linking number by cleaving DNA through the formation of a transient covalent phosphotyrosine enzyme/DNA complex. Topoisomerases are divided into type I and type II enzymes, which respectively introduce single-stranded and double-stranded breaks in DNA [10]. Eukaryotic cells have both type I and type II topoisomerases. This research paper will focus on the type IB topoisomerases which are the targets of camptothecins and generally relax DNA by removing negative supercoils (increasing linking number). The mechanism and structure of type I eukaryotic topoisomerases is similar to that found in prokaryotes [3].

Topoisomerase type I is a protein of the metaphase chromosome scaffold-like structure. Certain regions of DNA are associated with the nuclear scaffold. The regions of DNA associated with the scaffold are separated by loops of DNA with 20,000 to 100,000 base pairs. The scaffold itself contains several proteins and one of those is topoisomerase I. The enzyme is extremely important in the assembly of chromatin so it is abundant in rapidly dividing cells such as cancer cells. Thus, any inhibitors of this enzyme will kill these cells. In the interphase, topoisomerase I is bound to the nuclear matrix and so is the DNA replication machinery. This is a very important fact that allows the topoisomerase inhibitors to cause lethal lesions in DNA. The nuclear matrix is the network of proteins that provides a structural framework for organizing chromatin and facilitating transcription and replication [3]. Included in type I topoisomerases are two subfamilies: type IA and type IB. Type IA topoisomerases include eukaryotic topoisomerase IIIα and IIIβ while type IB topoisomerases include the eukaryotic topoisomerase I [1]. The type IB topoisomerases are encoded by the TOP1 gene [11] and are all monomers of mostly 80-100 kDa in size. The eukaryotic type IB topoisomerases have a catalytically active fragment of the human enzyme bound to DNA. The human enzyme contains several substructures, which are termed core subdomains I, II, III, the linker domain, and the C-terminal domain. The enzyme grasps DNA in a 'C' clamp and the core subdomains I and II form the upper half of the clamp. Subdomain III forms the lower half of the clamp. The linker domain lies between subdomain III and the C-terminal domain. The core subdomains and the C-terminal domains are the ones that make most contact with the DNA strand. Then the DNA is further contacted by two loops from subdomains I and III. Thus, the DNA is entirely enclosed by the protein. The active site tyrosine (Tyr-723) is on the α-helix of the C-terminal domain, on the lower half of the clamp [1].

This enzyme preferentially binds to double-stranded DNA [11]. The DNA is trapped between the upper and lower clamps. If the enzyme were likened to a hand, then the DNA is grasped between the fingers and the palm (Figure 1). The linker domain looks like a thumb pointing away from the palm. Once DNA is trapped by the enzyme it is positioned for cleavage. Once one strand is cleaved (referred to as the G-strand) then the grip on it loosens allowing the second strand of DNA (referred to as the T-strand) to rotate about the phosphodiester bond opposite the cleavage point, reducing the negative supercoil. The rest of the G-strand molecule, however, remains anchored to the enzyme by the phosphotyrosine bond formed by the active site tyrosine with the 5' end of the cleaved G-strand. ATP is not necessary for this step because there is enough energy stored in the supercoiled duplex for this rotation to take place. The linker is positively charged so it helps to attenuate the rotation of DNA by subtly interacting with the strand as it rotates. After the rotation, as the negative supercoil is reduced, the cleaved strand is religated [1].

Recently, there have been two camptothecin derivatives registered for cancer treatment: irinotecan (CPT-11) for the treatment of colorectal cancer and topotecan (hycamtin) for the treatment of ovarian cancer. These analogs inhibit the religation reaction of the type I topoisomerases, especially of the type IB enzymes as these are the ones that preferentially deal with double stranded DNA [8]. Camptothecins form a ternary complex: topI +DNA +drug [13]. Normally, topI-DNA covalent complexes are freely reversible, since the tyrosine-G-strand complex is reversible and the energy trapped in the phosphotyrosine bonds can be used to religate the G-strand [1]. Upon removal of the enzyme, the DNA returns to its original, undamaged state [8]. For the camptothecin derivatives to work and kill the tumor cells the cells need to replicate. Camptothecins convert the topI-DNA complex into a lethal lesion by making

the complex an irreversible double strand break (Figure 2). This occurs when the cleavage complex frozen by camptothecin collides with a replication fork, which unwinds DNA for replication. The collision causes the fork to break but the lethal break in DNA occurs. Thus, the religation of the G-strand does not occur and the break endures [5]. The higher the levels of topoisomerase I, the higher the frequency of the complexes, and the higher the number of lethal DNA lesions. The irreversible double strand break leads to a frozen cell cycle at the S/G_2 phase, activation of apoptosis (programmed cell death [9]) pathways, and finally to cell death. Cells undergoing S-phase of the cell cycle are found to be much more sensitive to camptothecins than those with G_1 or G_2/M phases. In colon, prostate, ovary and esophagus tumors, elevated topoisomerase I levels have been found, leading to the use of camptothecins in treatment of mainly these forms of cancer [8].

Recent experiments have suggested that CPT-11 and topotecan can also inhibit angiogenesis, and this contributes to their anti-tumor activity [8]. Angiogenesis is the process of formation of new capillaries around the tumor and the invasion of stroma of surrounding tissue by the existing blood vessels. It also includes the development of inter- and intra-cellular spaces to form lumina. In normal tissues, the process of angiogenesis is tightly controlled. It is a delicate balance of factors that promote or inhibit this process. When tumor cells acquire angiogenic properties this balance is lost. The process is much less inhibited than normally [6]. This neovascularization has been correlated with increasing invasion and metastases of various human tumors. Experiments in mice show, however, that by increasing the CPT-11 dosage the area of angiogenesis in tumors decreases, following a negative curve. At a dose of 210 mg/kg a significant reduction of vessel formation has occurred [8].

Compared with topotecan, CPT-11 shows a higher antitumor activity in mice when given separately and at low dose and the reason for this is the lactone ring of the CPT-11. One of the most obvious indications of further clinical development of CPT-11 is colorectal cancer, since this type of a tumor is commonly not very sensitive to cytotoxic agents. In addition to the activity of CPT-11 as a single agent in colorectal cancer (overall response ~14%, see Table 1), studies also indicate significant antitumor activity in non-small cell lung cancer, small cell lung cancer, gastric cancer, malignant lymphoma, acute leukemia, cervical cancer and pancreatic cancer. The activity of single agent topotecan has been described in several tumor types, using various means of administration, including ovarian cancer (overall response rate 35 to 38 %, Table 1), small-cell lung cancer, non-small cell lung cancer, breast cancer, myelodysplastic syndrome, and chronic myelomonocytic leukemia [8].

Table 1. Response Rates of CPT-11 and Topotecan as Single Agents. Table adapted from [8].

Tumor type	CPT-11 response %	Topotecan response %
Colon	13 (OR), 15 (OR)	
Gastric	18-43 (OR)	
Pancreatic	9-19 (OR)	
Small cell lung cancer	16-47 (OR)	39 (OR), 24 (OR)
Non-small cell lung cancer	16-34 (OR)	13 (OR)
Head/neck	21 (OR)	13 (OR)
Cervix	24 (OR)	
Ovarian		35-38 (OR)
Breast		10 (OR)
Chronic myelomonocytic leukemia		27 (CR)
Myelodysplastic syndrome		43 (CR)

Abbreviations: OR - overall response; CR - complete response.

Irinotecan is a unique camptothecin derivative. This drug has clear antitumor activities in a variety of human cancers. Unlike most camptothecins, CPT-11 is a prodrug and requires bioactivation by tissue carboxylesterase (CES2) for form active metabolite SN-38 (Figure 3). The antitumor activity of SN-38 is at least 100-fold greater than that of irinotecan, but it has a

short plasma half-life. Once in the plasma, irinotecan can be metabolized by cytochrome P450 3A enzymes to form NCP (7-ethyl 10-(4-amino-1-piperidino) carbonyloxycamptothecin and APC (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin]. Although these two compounds are not directly linked to antitumor activity, their formation reduces the amount of irinotecan able to be activated into SN-38. In aqueous solutions irinotecan, APC and NCP undergo a pH-dependant reversible inter-conversion between the ring-closed lactone form and the ring-opened carboxylate form. Only the lactone form is believed to cross the cell membrane and act as the topoisomerase I poison. The carboxylate form is not active. The SN-38 circulates in the cell. But is eventually deactivated by uridine diphosphate glucuronosyltransferases (UGTs) and forms its glucuronide, SN 38G. Irinotecan and SN-38 are eventually effluxed out of the cell by transporters like P-glycoprotein (Pgb), breast cancer resistant protein (BCRP), and multidrug resistance associated proteins (MRP1 & MRP2) [7].

For some years now irinotecan has been the main drug used in treatment of colon and small cell lung cancers. In addition, it has appreciable activity against a variety of adult and pediatric central nervous system tumor xenografts, including high-grade glioma and ependymoma [6]. In clinical studies various schedules for administering the drugs were tested. In Europe, for CPT-11, the highest planned and actual dose intensities were reached when the drug was administered once every three weeks. In the USA and Japan, the highest dose intensities were reached with the once a week schedule. When it comes to topotecan the general consensus around the world is 1.5 mg/m^2/day, intravenously delivered over 30 minutes for 5 consecutive days every three weeks. The oral administration of topotecan is currently not registered [8]. The most common administering schedule for CPT-11 drug is 100-150 mg/m^2 over 30 to 90 minute infusion every 4 to 6 weeks, 200 to 350 mg/m^2 over 30 or 90 minutes every

3 or 4 weeks, and 100-150 mg/m^2 over 90 minutes weekly infusion. Generally, lower doses given over prolonged periods of time are more effective due to a greater production of the active SN-38 metabolite [7].

Even though the camptothecin derivatives presently used in treatment of mainly colorectal and ovarian cancers have had much success, they have two major limitations: 1) at physiological pH they are in equilibrium with their inactive carboxylate form; and 2) the camptothecin-trapped cleavage complexes reverse within minutes after drug removal, which imposes long and repeated infusions for cancer treatment. Recently, there also have been many cases of resistance to these drugs attributed to various reasons [13]. Topoisomerase I levels in tumor cells are frequently reduced when these cells are continuously exposed to topI inhibitors, i.e. camptothecins. There have also been reported point mutations of the topoisomerase I gene and reduced drug accumulation caused by overexpression of transporters that efflux the drugs out of the cell. These include the MDR-1, P-glycoprotein, BCRP and other transporters. In addition, increased SN-38 inactivation by UGTs leads to reduced drug levels of the active compound and increases the resistance to camptothecins. Chemoresistance might also be caused by the activation of nuclear factor-κB (NF-κB), a transcription factor, and its activation is caused by administration of camptothecins. The activation of NF-κB leads to suppression of cell death normally caused by the topoisomerase I poisons. CPT administrations also activate another important chemical that adds to the resistance against these drugs: cyclin dependent kinase inhibitor (CD-K1). This molecule arrests the cell in the G_1 or G_2 phase of the cell cycle. As mentioned earlier, mainly the S-phase tumor cells are sensitive to camptothecins; thus, the arrest caused by CD-K1 leads to increased resistance against the drugs [8].

Another problem that camptothecins pose is their toxicity. For irinotecan life-threatening diarrhea is the main toxicity associated with repeated dosing, although neutropenia (abnormal decrease in the number of neutrophils in the blood [9]) has also been reported in several clinical trials. The diarrhea is unpredictable and the incidence of severe diarrhea ranges from 11 to 36.4% in colorectal cancer patients. The severity of diarrhea is thought to be caused mainly by the extent of glucuronidation of SN-38. Since SN 38G has a longer half-life than SN-38 and is more abundant than SN-38, there is sufficient time for it to accumulate in large intestinal epithelial cells where the enteric bacteria deconjugate it causing local damage and inducing diarrhea [7]. However, the once every three-weeks schedule seems to suggest a lower non-hematological toxicity. Still, though, cumulative behavior for leucopenia (a decrease in the number of white blood cells in the blood [9]) and delayed diarrhea has been reported. CPT-11 related diarrhea may be divided in an acute and a delayed form. The acute form appears during or within several hours after infusion. Delayed diarrhea is severe and unpredictable, present at all dose levels and increasing in intensity and frequency at higher dose levels. The exact mechanism of this type of diarrhea is not known, but it has been suggested that an abnormal intestinal ion transport may be responsible. This is probably related to altered epithelial intestinal cells. The average time of onset of delayed diarrhea is two to seven days. Sometimes, taste perversions, elevated hepatic transaminases or fatigue may occur. Alopecia (or baldness [9]) is common, especially at higher dose levels. In CPT-11 treated lung cancer patients, pneumonitis has been found much more frequently than in patients with other tumors. At all dose-levels of CPT-11, gastrointestinal cramps, nausea, vomiting, asthenia and diaphoresis can be observed. At higher dose levels salivation, anorexia, visual accommodation disturbances and lacrimation (abnormal secretion of tears [9]) were seen. For topotecan, the dose-limiting

toxicities are neutropenia and thrombocytopenia (an abnormal decrease in the number of platelets in the blood [9]). Severe thrombocytopenia is also a rare side effect after CPT-11 therapy. After topotecan or CPT-11 administration anemia is frequently observed, but rarely is clinically significant. At higher dose-levels of topotecan mucositis and late diarrhea may become dose-limiting toxicities. However, the non-hematological side effects for topotecan are rare, in contrast to CPT-11 [8].

Due to resistance to and the adverse effects of the drugs new approaches are being developed to improve the current camptothecin derivatives. One way to improve the therapeutic potential of topoisomerase I poisons is by inhibiting NF-κB. Normally, complexes of NF-κB reside in an inactive state within the cytoplasm bound to the inhibitory IκB protein. Following stimulus from the cell, activation of IκB kinases leads to phosphorylation of the inhibitory IκB protein, which is then immediately degradated by the 26S proteasome. Thus, the NF-κB units are free to bind to nuclear DNA and activate transcription of specific genes that inhibit apoptosis. However, this process also occurs after exposure to topoisomerase inhibitors, like the camptothecins, which increases tumor cells' resistance to these drugs. To ensure that the IκB proteins are degraded by 26S proteasomes, they are modified in the cell by the addition of multiple monomers of ubiquitin – a polypeptide consisting of 76 amino acids. However, if proteasome inhibitor, such as MG-132 or PS-341, is used on tumor cells, it does not come to the degradation of IκB and the NF-κB is thus not activated. But the proteasome inhibitors cannot be used before the treatment with a camptothecin, because then the apoptosis is actually even lower than when only camptothecin is used – cell survival is enhanced. Studies suggest that this is due to the fact that many other factors, besides NF-κB, actually cause the cell to undergo apoptosis. Apoptosis induced by topoisomerase inhibitors is actually NF-κB independent. It seems that the

proteasome inhibition process functions as a downstream effector of apoptosis following DNA damage caused by camptothecins. Camptothecin treatment of non-small cell lung cancer cells followed by proteasome inhibitor treatment significantly increases the population of apoptotic cells (sub-G_1 population). The apoptosis occurs either in late S-G_2 phase or possibly when exiting from mitosis, resulting in cells with sub-G_1 DNA content. SN-38 treatment followed by PS-341 treatment leads to tumor cells undergoing apoptosis at a rate approximately 2 fold higher than with SN-38 alone (Figure 4). Thus, the therapeutic potential of camptothecins can be improved and resistance to these drugs can be lowered by the post treatment with proteasome inhibitors [12].

Another way to improve the efficacy and especially the selectivity of camptothecins is with the use of sequence-specific ligands. Sequence specific modulation of gene expression can be achieved by oligonucleotides that bind to DNA by the triplex formation. These have been used as sequence-specific ligands to guide recombination or DNA cleavage in various experiments. When bound to specific chemical agents, triplex-forming oligonucleotides (TFOs) can deliver the agent to a specific sequence on the DNA for modification by the chemical. The oligonucleotide acts as a carrier molecule and its specific binding in the major groove of the double helix anchors the chemical agent at the triplex site specific for the TFO used. Thus, the use of topoisomerase I poisons with the TFOs offers a more efficient system to induce a sequence-selective DNA damage by cellular enzymes [11].

Normally, topoisomerase I enzymes have a low degree of site selectivity – it has been suggested that they mostly recognize DNA structural features. Camptothecins, however, have a two base sequence that they select for to stabilize the lethal cleavage: T↓G (the arrow denotes the site of cleavage by the enzyme). Two main approaches have been explored to enhance the

efficacy and selectivity of topoisomerase I inhibitors, i.e. camptothecins: 1) the delivery of these agents specifically to tumor cells by using antibodies or peptides or liposomes or viruses; and 2) their targeting to specific genes by attaching them to sequence-specific TFOs in order to induce a sequence-specific DNA damage. The newest strategy is to target DNA cleavage by these drugs to specific sites on the genome upon their linkage to a sequence-specific DNA ligand. The TFOs bear the camptothecin at their 3' end and stimulate the DNA cleavage by the enzyme only on the 3'-side of the triplex where the drug is anchored, and decrease it at the other characteristic sites of the drug. Binding of the TFO to this DNA site brings the poison in such a position that it inhibits the religation of the topI cleavage complex provided that the topI cleavage site is located at the appropriate position with respect to the triple-helical structure. In the future, the ability of TFO-conjugates to target topoisomerase cleavage to specific genes could provide a new model for the development of more specific chemotherapeutic agents and sequence-selective DNA damaging agents. If, for example, it is known that a certain type of cancer has a specific sequence activated which is dormant in healthy cells than the TFO-conjugate can be made to bind to that sequence and cleave it, causing lethal lesions in these tumor cells [11].

At this time, clinical experiments are being performed on a new line of topI inhibitors, those of non-camptothecin origin, because camptothecins' efficacy continues to decrease as more resistance to them develops. Polycyclic aromatics represent the most promising topI inhibitors for clinical development as anticancer agents. Indolocarbazoles are the most advanced class of non-camptothecin polycyclic drugs in terms of chemotype, clinical development and structure-activity. Unlike camptothecins, indolocarbazoles intercalate into DNA and bind to triplex DNA. They also inhibit the religation step of the topoisomerase I process, but the drugs are more potent. They stabilize cleavage complexes at sites bearing T, A, or C at the 3'-end of the cleaved

DNA and G on the 5'-side of the cleavage. There is still some resistance to indolocarbazoles (the drugs share a common cellular efflux system with camptothecins) but it is lower than that for camptothecins, because these drugs may target other cellular pathways besides topI. There are various kinds of indolocarbazoles. NB-506 is one that shows remarkable activity against experimental human cancer xenografts, including colon and lung cancers, while having a low toxicity profile. It is effective in regressing tightly staged tumors when dosed consecutively for 10 days. J-107088, another indolocarbazole, shows potent activity against lung cancer and prostate cancer. It also may be active in childhood and adult malignant brain tumors. Compared with CPT-11 and topotecan, it clears less rapidly out of the system, which puts it as a future rational choice for CNS tumors, particularly in patients treated with anticonvulsants, which cause glucuronidation (or inactivation) of CPT-11 and topotecan [13].

Another line of non-camptothecin drugs are the minor groove binding topI inhibitors. Of these, benzimidazoles and ecteinascidin 743 deserve most attention. Benzimidazoles bind to AT-rich sequences of the minor groove of DNA, causing widening of the minor groove. They trap topI complexes and induce them to cause lethal cleavages in the minor groove. Camptothecin-resistant human lymphoblast cells are only 4-5-fold cross-resistant to these drugs. Ecteinascidin 743 is a potent topI inhibitor from the Caribbean tunicate ("sea squirt"). It shows remarkable activity in soft tissue sarcomas, and solid tumors including ovarian cancers. The drug traps topI-DNA cleavage complexes *in vitro* and in cancer cells but it seems to act by a unique mechanism of action: poisoning of transcription-coupled nucleotide excision repair [13].

Topoisomerase I has been proven to be an effective target in a wide range of malignancies, and substantial clinical activity has been seen with the use of camptothecins. The many biochemical and pharmacological investigations provide a clear and almost complete

mechanism of action of this class of agents. Our understanding of topI biochemistry and its importance will enable us to improve the current camptothecins in use and develop new topI inhibitors that are more efficient and less toxic and might possibly replace the current camptothecins on the market. However, continued successful development of topoisomerase I-targeting drugs requires further investigation and more clinical research. There are still some obscurities even about the structure and mechanism of action of camptothecins, due their relatively new status in clinical treatments. Future pharmacogenetic studies will provide us more knowledge on these issues. This should finally result in individualized dosing of topI inhibitors that gives an adequate and less toxic therapy for the individual cancer patient. In addition, combination studies with other anticancer agents with different cellular targets are also ongoing and might eventually lead to higher response rates when compared to camptothecin treatment alone [8].

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