Preclinical Studies of Water-insoluble Camptothecin Congeners: Cytotoxicity, Development of Resistance, and Combination Treatments

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Abstract
Water-insoluble camptothecin (CPT) congeners are rapidly establishing themselves as promising anticancer drugs. In vitro, they have exhibited: (a) insensitivity to elevated levels of P-glycoprotein that confers multidrug resistance; (b) selective killing of malignant cells traversing the S-phase of the cell cycle, while leaving viable normal cells, which are arrested at the S-G2 boundary or continue to divide; (c) no cross-resistance with several other anticancer drugs; and (d) potentiation or enhancement of cytotoxicity when appropriately used in combination with tumor necrosis factor, ionizing radiation, and hyperthermia. In addition, development of cell resistance to water-insoluble CPT congeners in vitro is accompanied by increased sensitivity to other anticancer drugs. Furthermore, water-insoluble CPT congeners have exhibited an unprecedented activity against a wide variety of human tumors xenografted in nude mice by inhibiting growth and inducing regression of carcinomas of the lung, breast, ovary, colon, stomach, pancreas, and prostate, as well as malignant melanoma, lymphoma, and leukemia. More importantly, oral administration of the water-insoluble CPT congeners in clinical studies with cancer patients makes other route(s) of administration unnecessary.

Introduction
CPT is an indole alkaloid produced by the Oriental tree Camptotheca acuminata. CPT can be extracted from various parts of the plant, i.e., bark, wood, leaves, roots, and fruit, but the highest levels are found in young leaves (1). The antitumor activity of CPT extracts was first reported in 1966 (2), and it was subsequently shown to be associated with the ability of CPT to inhibit DNA and RNA synthesis in cultured mammalian cells (3-9). It was also reported that removal of the drug from the cell culture restores RNA and DNA synthesis (4, 5), and this led to the suggestion that CPT directs its action at cells progressing through the S-phase of the cell cycle (7). In clinical studies conducted by the National Cancer Institute in the late 1960s, CPT was converted and used in its highly water-soluble sodium salt, CPT-Na+, which demonstrated minimal antitumor activity and several unpredictable toxicities, including myelosuppression, hemorrhagic cystitis, diarrhea, nausea, vomiting, and dermatitis (10-13). These results led to discontinuation of clinical studies, and interest in CPT diminished. Following the disappointing reports on clinical studies with CPT-Na+, it was demonstrated that the CPT-Na+ molecule is produced by opening the lactone ring of the parent CPT molecule, and the resulting product is practically devoid of antitumor activity while it acquires toxicity (reviewed in Ref. 14).

The interest in CPT was renewed in the late 1980s-early 1990s following reports that the enzyme topo I is the major cellular target of CPT congeners (15-19); topo I is synthesized at high levels in advanced stages of human colon adenocarcinoma but not in normal tissues (20); and several CPT congeners demonstrate an unprecedented effectiveness against several human tumors grown as xenografts in immunodeficient nude mice (21, 22). The structures of various CPT congeners determine their activity (18, 19). The carboxylate (i.e., open E ring) form is much less active than the lactone (i.e., closed E ring) form of CPT, both in vivo and in cell cultures (Fig. 1; Refs. 18 and 23), and the semisynthetic and water-soluble CPT derivatives, Irino- tectan (previously known as CPT-11) and Topotecan, demonstrate antitumor activity in preclinical studies (24, 25). In addition, recent studies of CPT integrity in human plasma and blood have demonstrated that human serum albumin binds to the carboxylate form with a 200-fold higher affinity than the CPT lactone, and this results in rapid and complete conversion of the lactone to the carboxylate form (26). On the other hand, CPT lactone binds to the erythrocyte membrane, and this enhances its stability (27). Therefore, albumin, erythrocytes, and perhaps other blood components are important for stabilization of the CPT lactone (28).

The water-soluble Irino- tectan and Topotecan have been studied more extensively than water-insoluble derivatives of CPT, and studies dealing with Irino- tectan and Topotecan have been reviewed recently (29-33). However, there has been an increased interest in water-insoluble CPT derivatives, particularly after it was shown that members of this group demonstrate high antitumor activity against human cancer cells grown in culture and as xenografts in animal models (20, 22, 34-39). In addition, the inconvenience linked to the insolubility of several CPT congeners has been circumvented by p.o. administration of CPT and 9NC enclosed in gelatin capsules (40). Finally, the hallmark and common property of all water-soluble and -insoluble CPT derivatives is that their action is directed against topo I, but beyond this similarity, the two groups, i.e., water-soluble and -insoluble derivatives, differ in several aspects, including...
Preclinical Studies of Water-insoluble CPT Congeners

Interference of CPT with Topo I Activity

Topo I is a monomeric enzyme of Mr 100,000 and the major target of CPT (15) and its active derivatives. Topo I and another nuclear enzyme, topo II, modulate the topological state of chromatin DNA by introducing transient DNA breaks and removing excessive supercoils. Therefore, topo I and topo II are important for a number of vital cellular processes, including DNA replication and recombination, RNA transcription, and chromosomal decondensation (for recent reviews on the biology, biochemistry, and molecular biology of topoisomerases, see Refs. 48–50). In general, topo I induces breaks on one strand, whereas topo II induces breaks on both strands of supercoiled DNA, and thus, covalent complexes of topo-DNA fragments termed cleavable complexes are generated. This allows for the removal of supercoils, and it is followed by an enzymatic DNA reunion (48–50).

CPT binds to the cleavable complex subsequent to the DNA cleavage step, thus inhibiting the reunion process. Formation of the topo I-DNA-CPT complex has been demonstrated both in a cell-free system and in cultured mammalian cells (15–19). Although CPT binds to the topo I-DNA complex, there is no detectable binding of this drug to isolated topo I or purified DNA (17). CPT has to be in the S configuration to bind to the topo I-DNA complex and preferentially stabilizes topo I-mediated cleavage of T-G linkages (52, 53) following formation of the covalent bond between a tyrosine residue of the enzyme and the 3'-phosphate group of thymidine (54). Although the nature of the CPT-binding site remains unknown, interaction of CPT with the topo I-DNA complex seems to be necessary for the cytotoxic effects of this agent. Available evidence supports a model in which prolonged CPT-induced stabilization of the topo I-DNA complex is associated with conversion of the single-strand breaks to irreversible double-strand breaks (55), followed by a characteristic internucleosomal degradation of chromatin DNA and, ultimately, cell death. For CPT to inhibit the breakage-reunion mechanism effectively, it is required that the target cells are in the replicative phase, i.e., S-phase, of the cell cycle and that the cell contains active topo I (56–58). Furthermore, topo I-mediated DNA breaks may be necessary, but not sufficient, for cell death, because an endonuclease activity is also necessary to induce internucleosomal DNA cleavage while the cleavable complex is stabilized by CPT (59). Also, several other cellular parameters and/or events may regulate the CPT-induced cytotoxicity (see below).

CPT-induced Cytostasis or Cytotoxicity

In early in vivo studies, large differences were observed in survival times of mice with different leukemia types treated with CPT (60), and this could not be explained. Later in in vitro studies, it was observed that cell sensitivity as well as the cell cycle effects of CPT were different between the lymphocytic L1210 and MOLT-4 cells and the myelogenous HL-60 and KG-1 leukemia cell lines (61). Reportedly, both mouse L1210 and human MOLT-4 cells exhibit low sensitivity to CPT and accumulate at the G2 phase of the cell cycle, with appearance of hyperdiploid cells in cultures subjected to prolonged treatments of low drug concentrations, whereas CPT-treated HL-60 and
KG-1 cells selectively died when progressing through S and G2 phases (61). It was suggested that the differential response of the leukemia cells to CPT depends on cell type-specific factors that modulate the cell cycle phase sensitivity to CPT (61). CPT-treated leukemia cells that exhibit DNA degradation at the S-phase die by programmed cell death or apoptosis (62–65). Furthermore, recent studies have demonstrated that certain human leukemia cells treated with the CPT congener 9NC die by apoptosis while traversing through the S-phase of the cell cycle, whereas other 9NC-treated leukemia cells accumulate in the G2 phase and resist death (34). The differential responses of the 9NC-treated cells in vitro have been correlated with the tumorigenic ability of these cells in vivo; that is, in the presence of 9NC, transformed breast, ovarian, prostate, and lung cells that do not induce tumors in nude mice accumulate in the G2 phase, whereas cells that induce tumors in nude mice die by apoptosis when they traverse the S-phase (35–37). These studies have also generated two interesting observations from clinical viewpoints: (a) lower 9NC concentrations applied for long periods of treatment are more effective than higher concentrations applied for short periods of treatment in inducing apoptosis (37); and (b) once 9NC initiates the process of apoptosis in tumorigenic cells, these cells are irrevocably committed to death and continue to die, even after removal of the drug from the culture (35). It is also of interest that 9NC treatment of nontumorigenic cells results in a fraction of nondividing hypodiploid cells (35, 37), in agreement with observations reported on various leukemia cell lines treated with CPT (61). It is not yet understood why CPT and its derivatives are cytostatic for nontumorigenic and cytotoxic for tumorigenic cells, but several possibilities exist. One possibility is that nontumorigenic and tumorigenic cells exhibit different rates of process and/or efficiency of repair of the CPT-induced DNA breaks, and this may correlate with the selective cytotoxicity of CPT (66–68). In general, the repair mechanism can correct CPT-induced and topo I-mediated DNA damage, but the damage remains unaltered or is partially repaired in cells with deficient DNA repair mechanisms, as is the case with DNA repair-deficient cell lines from patients with ataxia telangiectasia and Cockayne’s syndrome (69, 70). Also, poly(adenosine diphosphoribose) polymerase synthesis may be important for CPT-induced cell killing, because poly(adenosine diphosphoribose) polymerase-deficient Chinese hamster ovary cells are resistant to the topo II-directed drug etoposide but hypersensitive to CPT (71). Finally, cell cycle controls may also correlate with the CPT-induced cytostasis or cytotoxicity. Lack of arrest in G1 or G2 may not provide the cell with the time required for DNA repair and may result in damage overload that cannot be repaired. In this regard, it has been shown that CPT-induced arrest of cells in G2 involves the protein kinase cdc2-cyclin B (72), which is required for G2-M phase transition. Like other agents that damage DNA followed by apoptosis, CPT induces a transient and unscheduled activation of cdc2-cyclin B during the period that follows DNA damage but precedes apoptosis (73).

Hence, deregulation of cyclins, cell cycle-regulated kinases and phosphatases, p53 mutations, and expression of specific genes such as c-myc and bcl-2 may correlate with drug-induced cytostasis or cytotoxicity (66, 74, 75). Nevertheless, this selective cytotoxicity of CPT and its derivatives against tumorigenic cells in vitro and in vivo is a property of these drugs not exhibited by other known anticancer drugs. It seems, however, that although the presence of topo I is required, it is not sufficient for induction of cytotoxicity by CPT in cycling cells (68).

Resistance to CPT

Drug resistance can be intrinsic or acquired. In most cases, the basis for the intrinsic resistance is undefined and probably involves a combination of factors and parameters such as kinetic factors, intrinsic biochemical factors, location of cells within the solid tumor mass, and others. In the case of acquired drug resistance, a population of cancer cells initially sensitive to the drug becomes drug resistant. In general, acquired resistance is the result of selection of a subpopulation of resistant cells in a tumor and subsequent enrichment in the presence of the anticancer drug that kills sensitive cells while leaving insensitive cells unaffected. The various mechanisms of resistance to anticancer drugs reported in the literature have been described (76, 77). With regard to CPT, some mammalian cell lines selected for resistance to CPT exhibit decreased levels of topo I, which, in turn, results in a decreased number of DNA single-strand breaks compared with wild-type cells (78–80). Decreases in topo I levels have also been associated with rearrangement and hypermethylation of the topo I gene (81), as well as the presence of mutations in the topo I gene (82–86). The proposed hypotheses with regard to acquisition of CPT resistance by cells have been studied extensively by monitoring topo I activity and synthesis in a series of human leukemia U-937 sublines with increasing resistance to 9NC (87). Comparison of wild-type cells and cells with low 9NC resistance showed that: (a) the cells have similar proliferations rate in vitro; (b) express similar levels of topo I mRNA; synthesize proteins of 100, 75, and 67 kd immunoreactive with the antibody to topo I; (c) demonstrate similar levels of topo I catalytic activity in the absence of 9NC, whereas in the presence of 9NC, topo I activity from the resistant cells is approximately 10-fold more resistant than from wild-type cells; (d) differ in the extent of topo I methylation, with the resistant topo I being hypermethylated; (e) differ in the nucleotide sequence of topo I cDNAs at position 361, resulting in the presence of serine or phenylalanine in topo I of wild-type or resistant cells, respectively; and (f) both induce tumors following xenografting in nude mice (86, 87). Further increases in 9NC resistance of the cells are accompanied by decreases in the proliferation rate, the appearance of morphological and functional features that correlate with granulocytic maturation, decreased synthesis of topo I, increased synthesis of topo II, and the inability to induce tumors when xenografted in nude mice (87). Like CPT, acquisition of 9NC resistance does not correlate with the presence of P-glycoprotein (81) that confers cell resistance to structurally unrelated drugs (reviewed in Refs. 88 and 89). Overexpression of P-glycoprotein has been associated with drug resistance in several proliferative diseases of the hematopoietic cells (reviewed in Ref. 90). Finally, 9NC resistance is a stable feature of the U-937 cells, because they continue to

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4 P. Pantazis, unpublished results.
exhibit resistance after they are transferred and propagated in 9NC-free media for a prolonged period (87).

The observation that decreases in topo I synthesis or activity in 9NC-resistant cells are accompanied by increases in topo II synthesis (82, 84, 87) may have clinical implications, i.e., it may lead to the development of effective combination chemotherapy for cancer patients (see "Combination Chemotherapy").

Combination Treatments

In general, an anticancer drug is rarely used alone but usually in combination with another agent(s) based on a rationale developed in a preclinical cell culture, animal studies, or earlier clinical studies. Recent reports have indicated that preclinical investigations have been initiated on cancer therapy using water-insoluble CPT congeners in various combination treatments, including combination chemotherapy and combined modality. These studies are reviewed below.

Combination Chemotherapy. The improved curative efficacy of drug combinations, compared with single agents, has been demonstrated in animal models and by the higher response rates to curing cancer patients, in cases in which cures are possible. The accepted guidelines generally used in selecting drugs for use in combination chemotherapy have been described briefly (91, 92). Accordingly, combination chemotherapy of CPT with other anticancer drug(s) will be successful if the drugs used: (a) have nonoverlapping toxicities and/or generate relevant toxicities at different times after treatment; (b) target different cellular mechanisms; and (c) do not display cross-resistance.

An early study showed that cotreatment of V79 Chinese hamster lung fibroblasts with CPT and a topo II-directed drug, amsacrine or etoposide, resulted in reduced cell killing compared with treatment with amsacrine or etoposide alone (62). Subsequently, a similar antagonism was reported between CPT and topo II-directed drugs amsacrine, etoposide, and daunorubicin, when used simultaneously to treat human leukemia cells in vitro, whereas CPT or the topo II-directed drug alone exhibited higher cytotoxicity assessed by colony formation in soft agar (93). Consequently, it was suggested that simultaneous treatment with CPT and a topo II-directed drug would not benefit the cancer patient (93). These observations were confirmed with similar in vitro studies on human colon carcinoma cells treated simultaneously with CPT and etoposide (94), but other studies have shown that amsacrine, doxorubicin, and etoposide have additive effects on the cytotoxicity of CPT in an in vitro model of concanavalin A-stimulated human splenocytes (95). No explanation has been reported for the differences in these results, but one possibility is a variable sensitivity to the drugs by the cells used in each study. Furthermore, sequential treatment of colon carcinoma cells with CPT and etoposide not only circumvented antagonism between the two drugs, but also their cytotoxic effects became additive if the two treatments were separated by 6 h or more (94). Therefore, it seemed that sequential drug treatment was important for successful combination chemotherapy involving CPT. To investigate this further, human leukemia U-937 cells were subjected to combination chemotherapy using 9NC and various other anticancer drugs, including the topo II-directed drugs amsacrine and etoposide, the Vinca alkaloid vincristine, and the antimetabolite methotrexate. Drug-induced cytotoxicity was determined by rates of cell proliferation, changes in cell morphology, and relative quantitation of apoptotic cells by flow cytometry. In one study, the U-937 cells were treated with 9NC until they developed resistance to this drug, then the cells were treated with the second drug (87, 96, 97). The 9NC-resistant cells displayed much higher sensitivity than the parental cells to treatments with amsacrine, etoposide, and daunorubicin (96, 97). In another study, the U-937 cells were initially treated with etoposide until they developed resistance to this drug. These cells were more sensitive than the parental cells to low concentrations of 9NC treatment. Taken together, these studies on 9NC- and etoposide-resistant U-937 cells have led to the hypothesis that cells and tumors that develop resistance to drugs directed against one topoisomerase concomitantly develop sensitivity to drugs directed against the other topoisomerase. Pertinent to these results is the suggestion that there is a compensatory interaction between the two DNA-cleaving enzymes, topo I and topo II (98–100). Apparently, such a compensatory relationship could have important practical consequences, because a switch of dependence from one topo to the other topo would allow further treatment with the appropriate antitopoisomerase drug. However, to maximize the effectiveness of the alternating combination chemotherapy, the cells or tumors must be treated extensively with one drug to ensure absolute dependence on one topo. In addition, the 9NC-resistant but not the etoposide-resistant cells exhibit higher sensitivity than the parental cells to treatment with vincristine (97). Studies of increased sensitivity to Vinca alkaloids by 9NC-resistant cells may also lead to development of a clinical protocol of a more effective combination chemotherapy.

Finally, several studies have shown that selection of resistant cells in vitro by a single anticancer drug, such as an anthracycline, a Vinca alkaloid, or a taxane often leads to cells with cross-resistance to all of those and other drugs (101–104). Cross-insensitivity to various anticancer drugs seems to correlate with overexpressed P-glycoprotein and/or MRP (77, 88, 90, 105–110). Both proteins are members of the ATP-binding cassette superfamily of transport proteins (77, 111). Overexpression of P-glycoprotein does not correlate with development of resistance to 9NC (86, 112). A correlation of MRP with 9NC resistance has not been reported yet, but preliminary studies have shown that human leukemia cells that acquired resistance to etoposide, doxorubicin, or vincristine exhibit 9NC sensitivity similar to or higher than those that retain or exceed the 9NC sensitivity inhibited by the parental wild-type cells.

In general, the findings reviewed in this section indicate that sequential combination treatments involving 9NC are effective when the cells are first treated with 9NC for a prolonged period followed by a topo II-directed drug or doxorubicin or vincristine, and conversely, when the cells are first treated with

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5 P. Pantazis et al., unpublished results.
a topo II-directed drug or doxorubicin or vincristine for an extensive period followed by 9NC treatment.

**Combined Modality Treatments.** Combined modality therapy is a treatment in which a chemotherapeutic agent(s) is used in combination with nonchemotherapeutic intervention, such as surgery and/or radiation or hyperthermia. There are various forms of combined modality treatment. For example, the tumor may be initially treated with an anticancer drug(s) to increase cytotoxicity to a subsequent treatment such as radiation or heat. In other words, in addition to having antitumor activity on its own, an anticancer drug sensitizes the malignant cells or tumors to radiation or heat. Alternatively, pretreatment with radiation or hyperthermia may increase the sensitivity of the cells to subsequent chemotherapy. The theoretical basis of combined modality therapy using radiation and hyperthermia therapy and experimental and clinical literature has been critically reviewed (113–115). Following is a review of studies of combined modality treatments *in vitro* that include combination treatment with CPT and radiation and CPT and hyperthermia.

**Combination Treatment of CPT with Ionizing Radiation.** There have been several studies to elucidate the determinants of the interacting response to the combined treatment of CPT with ionizing radiation (116–121). There is agreement that confluent-arrested cells, although highly resistant to CPT-induced toxicity, exhibit increased radiosensitivity on irradiation after CPT exposure (116, 117). However, controversial results and, consequently, views have been generated from studies of proliferating cultured cells treated with CPT and radiation. One view is that pretreatment of cells with the water-soluble CPT analogue Topotecan increases cell radiosensitization and, therefore, reduces survival of the irradiated cells (118). In this regard, it has been proposed that CPT increases radiation-induced cytotoxicity because of alterations of the cell cycle progression by either agent and the marked S-phase specificity of the lethal action of the drugs (119, 120). Radiation alone preferentially kills cells at G1-M (121). In contrast, recent studies with human HeLa cells and Chinese hamster V-79 fibroblasts have shown no evidence of mutual potentiation of CPT and radiation in terms of cell survival as well as with regard to the formation and rejoining of DNA double-strand breaks (122). Specifically, radiation and CPT applied concomitantly or in close temporal proximity were shown to interact with each other in a purely additive mode, whereas when radiation was applied 2 h or more prior to CPT treatment, CPT-induced toxicity was apparently decreased following CPT treatment (122). These controversial results probably correlate with a number of parameters contributing to the complexity of CPT- and radiation-induced mechanisms, and therefore, these results should be evaluated in this context. Experimental parameters that may vary include cell type, drug concentration used, duration of drug treatment, whether CPT treatment precedes or follows radiation, expression of genes that control check points of the cell cycle, and others. For example, it has been shown that CPT may induce G2 arrest or killing of cells by apoptosis, and this has been correlated with the ability of the cells to induce tumors following inoculation in nude mice (35–37). Also important in CPT radiation studies is the drug concentration used. Although CPT specifically targets S-phase cells, the exact stage may vary within the S-phase as a result of the drug concentration. In this regard, relatively high CPT concentrations block DNA synthesis at early S-phase, *i.e.*, near G1, whereas low drug concentrations block DNA synthesis at late S-phase, *i.e.*, near G2 (37). Therefore, radiation administered after initiation of CPT treatment will probably generate various results, depending on the stage of S-phase, *i.e.*, early, middle, or late, in which the cells have accumulated when irradiated. The presence of functionally active topo I is another parameter. CPT added concomitantly with or immediately after radiation requires a long time for any additive or synergistic cytotoxicity to be observed, because immediately following ionizing radiation, there is a dramatic decrease in topo I enzymatic activity (123), which apparently results in decreased CPT-induced toxicity. Down-regulation of topo I activity in irradiated cells results from posttranslational modification rather than decreased synthesis of mRNA and protein (123). Finally, the presence of wild-type p53 function is required for CPT- or ionizing radiation-induced cell death by apoptosis (124–128). The loss of p53 function has been correlated with intrinsic or acquired resistance of cells to ionizing radiation (127), and cells having ionizing radiation-induced G1 arrest are more radiosensitive (129).

It should be emphasized that, in addition to p53, there are other key genes, kinases, and phosphatases that form a complex reaction that evidently controls, perhaps not in identical fashion, normal and cancer cells (Ref. 68 and references therein). Therefore, a better understanding of the cell cycle events involved in CPT- and ionizing radiation-induced cytotoxicity is a prerequisite to developing protocols of combination treatments of CPT with radiation, so that cancer cells will be killed while normal cells will be only minimally affected or unaffected.

**Combination of CPT with Hyperthermia.** The first recorded observation that cultured neoplastic mouse and rat sarcoma cells have higher thermosensitivity than normal mesenchymal cells was made in 1912 (130). Since then, it has been established that elevated temperatures have a selective lethal effect on cancer cells both experimentally and clinically (Refs. 114 and 131, and references therein). Furthermore, application of hyperthermia combined with anticancer drugs, *i.e.*, thermochemotherapy, has been explored as a clinical strategy to enhance the therapeutic effect of several anticancer drugs, even in drug-resistant cells (reviewed in Ref. 115). For example, heat reportedly potentiates the cytotoxicity of cisplatin (132), and tumor necrosis factor used to treat cultured cells and human tumors xenografted in nude mice (133–137). However, etoposide-induced toxicity was not enhanced by hyperthermia (137), and in some instances, hyperthermia protected human and rodent cells from the cytotoxic action of etoposide and amssacrine (138, 139). These results led to the conclusion that when the cells are heated prior to etoposide treatment, the decrease in drug-induced cytotoxicity does not correlate with any reduction in the number of topo II-DNA cleavable complexes (139).

With regard to CPT, a recent report has shown no significant increase in the cytotoxicity of CPT *in vitro* when mouse mammary tumor cells were exposed to the drug at 42°C compared with the same drug exposure at 37°C (132). However, combination treatment of CPT and hyperthermia killed more cells than CPT treatment alone in mice carrying FsaIIC fibrosarcoma (140). Furthermore, a recent study in our laboratory has demonstrated that the sequence of treatments with hyperthermia
and 9NC is important to increase or decrease the cytotoxic action of 9NC against human leukemia cells in vitro.\(^7\)

**Concluding Remarks and Future Directions**

*In vitro* studies described in this review indicate that, depending on the type of target cell and CPT concentration, CPT treatment alone may activate mechanisms that lead to reversible cytostasis, cell killing (apoptosis), or differentiation. Any of these results can be beneficial to the cancer patient as long as the side effects are absent or minimal. Optimization of the therapeutic action of CPT may be possible to achieve, with either a reduction in or no side effects, by combining the use of CPT with a second therapeutic treatment as shown by the studies presented in this review. These combination treatments are based on a variety of rationales.

Of clinical importance is the observation that water-insoluble CPT congeners exert their cytotoxic action on malignant cells regardless of overexpression of P-glycoprotein, which confers resistance to anticancer drugs of diverse natural origin. It is also possible that CPT overcomes overexpression of the MRP, but this has not been confirmed yet. On the other hand, development of resistance to CPT by malignant cells results in increased sensitivity of these cells to topo II-directed drugs and very likely to other drugs of diverse origins. Furthermore, cells that acquire resistance to any water-insoluble CPT congener exhibit cross-resistance to other water-insoluble CPT congeners.\(^4\)

Extensive investigations must also be directed toward establishment of schedules for combined modality treatments alternating CPT with radiation or hyperthermia to understand better the importance of preclinical results and subsequent potential clinical benefits, so that: (a) each modality will kill cells resistant to the other modality and prevent the emergence of doubly resistant populations; (b) one modality will enhance the cytotoxic effect of the other modality; and (c) nonsimultaneous administration will lessen the risk of adverse side effects and will allow full doses of each modality to be applied.

The studies described in this review have indicated that water-insoluble CPT congeners can target a wide variety of human cancers. However, these congeners differ from each other in several aspects, including metabolism. Therefore, each combination treatment using a CPT congener will have to be studied, developed, and refined separately. It is encouraging that the studies have indicated that water-insoluble CPT congeners are very promising anticancer drugs, regardless of whether they are used as single drugs or in combination with other drugs and modalities. The explosive increase in published reports over the last 5 years dealing with the positive chemotherapeutic effects of CPT congeners on all types of cancer certainly warrants investigations of these drugs in both preclinical and clinical studies.

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\(^7\) P. Pantazis, D. Coil, and B. Giovanella, unpublished data.

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**References**


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