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ORIGINAL ARTICLE

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Enhancement of camptothecin-induced cytotoxicity with UCN-01 in breast cancer cells: abrogation of S/G_2 arrest

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Abstract *Purpose*: To determine the ability of UCN-01 to abrogate the cell cycle arrest induced by camptothecin (CPT) in tumor cells that lack p53 function, and therefore enhance the cytotoxicity of CPT in these cells in relation to normal cells with wild-type p53. *Methods*: The responses of MDA-MB-231 and GI 101A breast cancer cells were compared to those of normal bovine endothelial cells. Cytotoxicity was assessed by the MTT assay, and the resulting data were modeled using median-effect analysis. Inhibition of DNA synthesis was determined by loss of [^{3}H]thymidine incorporation, and cell cycle status was determined by flow cytometric analysis of propidium-iodide-stained nuclei. *Results*: UCN-01, a specific inhibitor of protein kinase C (PKC) presently in clinical trials, abrogated CPT-induced activation of S and G_2 checkpoints in human MDA-MB-231 and GI 101A breast carcinoma cells, both of which are mutants for the p53 gene. This abrogation occurred with the use of sublethal doses (100 n*M*) of UCN-01 and correlated with the enhancement of CPT-induced cytotoxicity. Median-effect analysis showed that synergistic cytotoxic interactions existed between CPT and UCN-01 against these tumor cells. In normal cells, however, abrogation of the S phase arrest caused accumulation in G_0/G_1 phase, perhaps by the presence of wild-type p53 activity, with no change in CPT-induced cytotoxicity. *Conclusion*: We have shown previously that the cytotoxicity of CPT is correlated with cell cycle response in normal and tumor cells. Low doses of CPT arrest cells in the G_2/M phase and inhibit DNA synthesis, but higher doses cause arrest of cells in S phase. Thus modulation of events at the S and G_2 checkpoints may provide an opportunity to enhance CPT-induced cytotoxicity in tumor cells. The results of this study indicate that UCN-01 enhances the progression of tumor cells through S phase thus greatly increasing CPT-induced cytotoxicity. Normal cells, however, are able to arrest in G_0/G_1 and thus avoid the increased toxicity induced by CPT. Our findings suggest potential usefulness of combining UCN-01 in topoisomerase I inhibitor-based drug therapy for the treatment of breast cancer with a dysfunctional p53 gene.

Key words Camptothecin · UCN-01 · Cell cycle · Drug synergism · Breast cancer · Endothelial cells

Abbreviations *BVEC* bovine venular endothelial cells · *cdk* cyclin-dependent kinase · *CI* combination index · *CPT* camptothecin · *DMSO* dimethyl sulfoxide · *Fa* fraction affected · IC_{50} concentration causing 50% inhibition of cell growth · *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (thiazolyl blue) · *PBS* phosphate-buffered saline · *PKC* protein kinase C · *topo I* DNA-topoisomerase I · *UCN-01* 7-hydroxystaurosporine

Introduction

Camptothecin (CPT) and its clinical analogs such as topotecan and CPT-11 are a new class of chemotherapeutic agents with a novel mechanism of action targeting the nuclear enzyme topoisomerase I (topo I), causing single- and double-strand DNA breaks and subsequent cell death (for reviews, see references 5 and 31). The

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cytotoxicity of these agents is predominantly exerted during the S phase of the cell cycle [9]. This inhibition is the result of a passive collision of the advancing DNA replication forks with the CPT-topo I-DNA cleavable complexes which are expected to cause an arrest of DNA replication and to kill cells by generating DNA strand breaks [6, 29].

The sensitivity of cells to CPT and its analogs cannot be completely explained by the collision model. Recent evidence from our laboratory and from others indicates that the sensitivity of cells to CPT is also determined by their ability to activate checkpoints in the S and G_2 phases of the cell cycle [10, 13, 14, 24, 38]. The activation in the S phase occurs with high doses of CPT and at G_2/M with low doses of CPT, presumably to avoid high and low levels of DNA damage, respectively. DNA damage extends the time of at least two stages or checkpoints in the cell cycle, the G_1-S and the G_2-M transitions [16]. A critical component of the G_1 checkpoint is the p53 gene product, which when inactivated by mutations, renders a cell incapable of G_1 arrest following DNA damage [17, 18]. Instead, cells arrest in G_2 phase [2, 32]. The G_2 arrest can permit repair of DNA and ensures that DNA replication is complete before the cell enters mitosis.

Based on such findings it has been proposed and is now largely accepted that the main function of normal p53 is to preserve genomic integrity by acting as the "guardian of the genome" [19]. As a consequence, tumor cells with no or mutated p53 function loose their sensitivity to a wide variety of DNA-damaging agents [11, 20, 21]. It is possible that this phenomenon occurs because p53 stimulates a more efficient DNA repair process. Therefore, treatment of tumor cells deficient in p53 normal function with topo I inhibitors alone is unlikely to be curative, since G_2 arrest induced by the use of low doses would allow DNA repair to occur prior to mitosis, thus preventing potentially lethal lesions from killing tumor cells, while S phase arrest induced with the use of high doses may inflict high levels of DNA damage on the normal cells. One way to increase the sensitivity of these tumor cells to DNA-damaging agents is to modulate events at checkpoints in the S and G_2 phases to which only damaged tumor cells with mutant p53 would progress. At the same time, normal cells that pass the G_1 checkpoint during this modulation would also progress to G_2 and would also be sensitive to modulation at the S and G_2 checkpoints. However, the wild-type p53 seems to protect these cells from abrogation at these checkpoints [26, 28].

A variety of agents such as caffeine and other methylxanthines can override the DNA damage-dependent G_2-checkpoint and enhance drug-induced cytotoxicity [12, 26, 28]. However, plasma drug concentrations higher than the maximum tolerated doses are required to achieve this effect in clinical settings. In search of new G_2-checkpoint inhibitors, a staurosporine analog, 7-hydroxystaurosporine (UCN-01; Fig. 1), has been found to abrogate the cisplatin-induced S and G_2 checkpoints and to enhance its cytotoxicity in CHO and HT-29 cells lacking normal p53 function [3, 37].

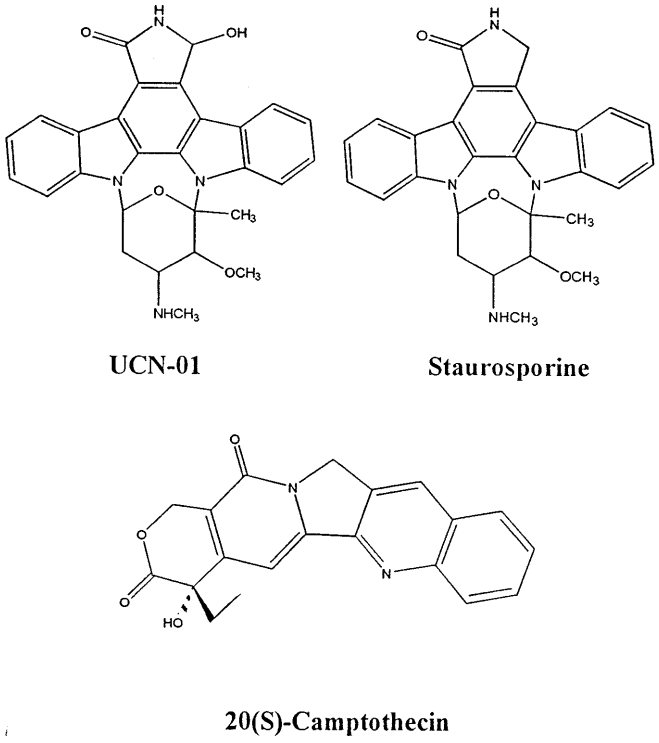

Fig. 1 Structures of UCN-01 (7-hydroxystaurosporine), staurosporine and camptothecin

In this report we present the results of studies performed to determine the ability of UCN-01 to abrogate the CPT-induced S phase arrest and to enhance CPT-induced cytotoxicity in human breast cancer cells lacking normal p53 function compared to normal endothelial cells with wild-type p53. The results of this study suggest that: (1) UCN-01 can inhibit CPT-induced S phase arrest in tumor cells defective in p53 function and can enhance CPT-induced cytotoxicity in a synergistic manner, (2) normal endothelial cells with normal p53 function are protected from CPT-induced damage by arresting in the G_0/G_1 phase, and (3) the abrogation of S phase arrest occurs with the use of sublethal doses of UCN-01. We propose that the use of UCN-01 in combination chemotherapy with topo I inhibitor-based regimens will improve the therapeutic effectiveness and clinical application of these agents against breast cancer. Parts of this study have been presented previously as an abstract [15].

Materials and methods

Cell lines and culture

The two human breast carcinoma cell lines with mutant p53, MDA-231 and GI 101A, were maintained as monolayer cultures in RPMI-1649 medium (Gibco, Grand Island, N.Y.) supplemented with 10% bovine calf serum (Hyclone, Logan, Utah) at 37 °C in a humidified atmosphere containing 5% CO_2. Normal bovine venular endothelial cells (BVEC) were maintained in DMEM medium (Gibco) supplemented with 1 m*M* sodium pyruvate

(Gibco), and 20% bovine calf serum (Hyclone). Cell viability was determined using the trypan blue exclusion test. To ensure exponential growth, cells were plated in fresh medium 24 h before each treatment.

Drugs and chemical reagents

CPT (NSC 94600) and UCN-01 (NSC 638850) were obtained from the Drug Development Branch, National Cancer Institute, NIH (Bethesda, Md.) dissolved in dimethyl sulfoxide (DMSO) at 4 m*M* and 1 m*M*, respectively, aliquoted and stored at −70 °C. Further dilutions were made in culture medium just before use. The final concentration of DMSO in culture did not exceed 0.1% (v/v) which is nontoxic to cells. Thiazolyl blue (MTT) was purchased from Sigma Chemical Co. (St. Louis, Mo.), [methyl-^{3}H]thymidine (7 Ci/mmol) was obtained from Andotek (Irvine, Calif.). All other chemicals were reagent grade.

Drug treatment and survival curves

Cytotoxicity studies were initiated by plating 2×10^4 cells obtained from exponentially growing cultures in 24-well tissue culture plates (Corning-Costar, Cambridge, Mass.) in the appropriate medium. Following a 24-h incubation at 37 °C, drugs were added to quadruplicate wells and incubated for 24 h, the wells were washed twice with prewarmed phosphate-buffered saline (PBS) and then incubated with drug-free medium for two to three doubling times.

Cell survival was determined using a semiautomated tetrazolium (MTT)-based colorimetric assay, as previously described [7, 8]. The effect of UCN-01 on CPT-induced cytotoxicity was evaluated by exposing cells to graded concentrations of the latter drug in the presence and absence of subtoxic doses of UCN-01. The concentration of drugs causing 50% inhibition of cell growth (IC_{50}) for the drug combination was calculated by logarithmic analysis.

Drug combinations and data analysis

In this study, cells were exposed to UCN-01 and CPT either alone or in combination at fixed dose ratios for 24 h. The surviving cell fraction was determined using the MTT assay (cytotoxicity effects) as described above or [^{3}H]thymidine incorporation assay (antiproliferative effects) as previously described [14]. Synergy of activity was analyzed according to the median-effect principle and plotted as a combination index (CI) versus fraction affected (Fa) as previously reported [4]. A CI of 1 at the IC_{50} value indicates an additive interaction, a CI of >1 indicates antagonism and a CI of <1 indicates synergism.

Cell cycle analysis

Flow cytometric analysis of the cell cycle was performed as previously described [14]. Briefly, cells treated with 100 n*M* CPT, 100 n*M* UCN-01 or drug combinations were scraped into ice-cold PBS. Cell suspensions were centrifuged (1000 rpm for 10 min) and then washed twice with PBS. After the final wash, cell pellets were resuspended in 1 ml PBS and fixed with 70% (v/v) ethanol at 4 °C overnight. DNA was then stained by incubating cells in 0.1% Triton-X-PBS buffer containing 50 μg/ml propidium iodide and 100 μg/ml RNaseA overnight at 4 °C. DNA content was determined on a Becton Dickinson FACScan flow cytometer. Propidium iodide-stained nuclei were excited with a 488-nm air-cooled argon laser, and fluorescence emission above 680 nm was recorded on a linear scale. Cell cycle distribution was quantitated by gating control cells and maintaining gates for treated cells using a doublet discrimination module.

Results

Enhancement of CPT-induced cytotoxicity by UCN-01 in tumor cells

The response of MDA-231 and GI 101A tumor cells to CPT and the effect of UCN-01 on the sensitivity of these cells for a 24-h drug exposure as compared to the response of normal endothelial cells (BVEC) is illustrated in Fig. 2. In the presence of CPT alone, GI 101A and MDA 231 cells (Fig. 2B and 2C, respectively) were inhibited by 50% by 300 n*M* and 200 n*M* CPT, while growth of normal endothelial cells (Fig. 2C) was inhibited by 50% at doses of >1 μ*M* CPT. Sublethal doses of UCN-01 (100 n*M*) shifted the CPT dose-response curves in tumor cells to the left, with minimal effect on normal endothelial cells. As seen in Fig. 2B, C, the presence of 100 n*M* UCN-01 for 24 h greatly enhanced CPT cytotoxicity in GI 101A and MDA 231 cells (about 30- to 40-fold, respectively), as compared to the absence of UCN-01. For BVEC (Fig. 2C), sublethal doses of

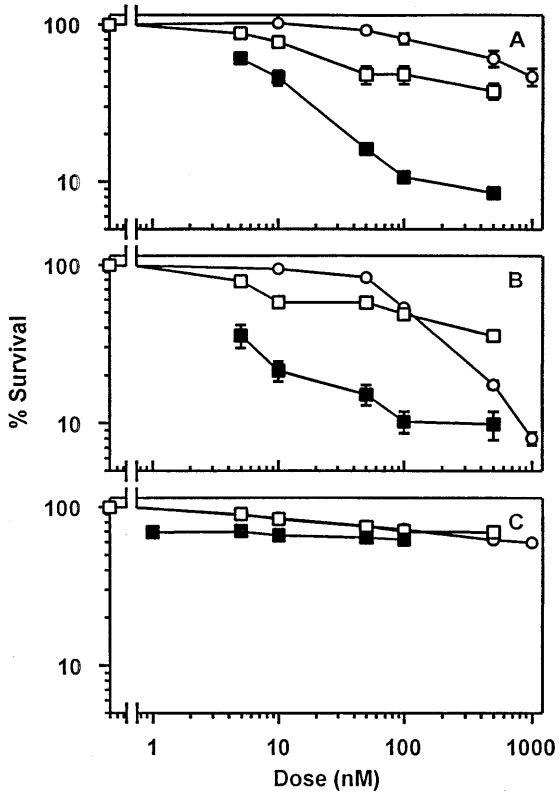

Fig. 2A–C Dose-response curves for the exposure of GI 101A (**A**), MDA-231 (**B**) and endothelial cells (**C**) to combinations of CPT and sublethal doses of UCN-01. Cells were exposed to various doses of CPT (□), UCN-01 (○) and 100 n*M* UCN-01 plus CPT (■) for 24 h. Following drug treatment, the cells were reincubated in drug-free medium, and the surviving cell fraction was determined by the MTT assay. The points represent the means of quadruplicate determinations ±SE, obtained in two or three independent experiments

UCN-01 produced a minimal leftward shift in the dose-response curves to CPT.

Synergy between UCN-01 and CPT in tumor cells

To determine whether the interaction between UCN-01 and CPT was truly synergistic, tumor cells in culture were exposed to UCN-01 and CPT either alone or in combination over a wide range of doses but at a fixed dose ratio (1:1 molar ratio) for 24 h. In these experiments, the median-effect doses, D*m*, for CPT were 104 n*M* and 28 n*M* in GI 101A and MDA 231, respectively. Computed regression coefficients for the linearized dose-effect curves were >0.9, indicating that the data fulfilled the criteria for computation of the CI according to Chou and Talalay [4]. The composite Fa-CI plot for these experiments is presented in Fig. 3A. Analysis of the data for these experiments suggested that the two drugs acted synergistically over the majority of concentrations tested. The data were analyzed under mutually nonexclusive conditions, since we assumed that the two drugs act toward different targets. However, similar results were obtained when the data were analyzed under mutually exclusive conditions (data not shown).

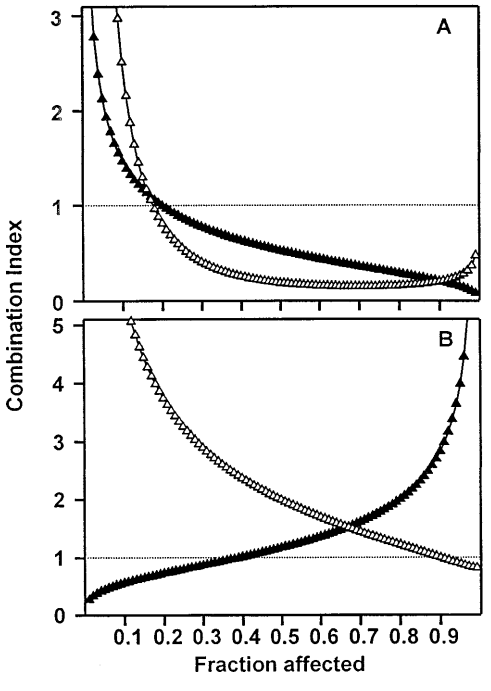

Fig. 3A,B Combined effect of a 24-h exposure of GI 101A (▲) and MDA-231 (△) cells to CPT plus UCN-01 at a fixed dose ratios of 1:1. Computer-generated curves represent the combined effects of CPT plus UCN-01. The results are plotted as a function of the fraction of treated cells affected as determined by cytotoxicity assays (**A**) or [^{3}H]thymidine incorporation assays (**B**) versus the combination index (Fa-CI plot) under a mutually nonexclusive assumption. All points lying above a CI of 1 represent antagonism, those lying below a CI of 1 represent synergism, and those lying at a CI of 1 represent additivity. Interactions of CPT and UCN-01 are strongly synergistic in both cell lines when analyzed for growth inhibition, while antagonistic when analyzed for DNA synthesis

When the antiproliferative activity of the drug combination was determined by [^{3}H]thymidine incorporation assay, an antagonistic effect was observed (Fig. 3B). In these experiments, cells were treated with each drug alone and in combination (CPT/UCN-01 molar ratio 1:10) and the DNA synthesis was determined following incubation for 4 h with [^{3}H]thymidine. Analysis of the data of these experiments (Fig. 3C) showed that the two drugs acted antagonistically over the majority of concentrations tested (25–100%). Thus the results of the above experiments indicated that UCN-01 enhanced CPT-induced cytotoxicity (growth inhibition) in tumor cells that lack p53 function with minimal effects on normal endothelial cells. The antiproliferative activity (DNA synthesis) of CPT on tumor cells was inhibited in the presence of UCN-01.

Abrogation of CPT-induced S/G_2 activation with UCN-01 in tumor cells

To determine whether the synergistic interaction between CPT and UCN-01 is the result of abrogation of S/G_2 checkpoint activation, the effect of sublethal doses of UCN-01 on CPT-induced S phase arrest in tumor and normal cells was determined by flow cytometry. In these experiments, asynchronized cells were simultaneously treated for 24 h with 100 n*M* CPT, 100 n*M* UCN-01 and drug combinations. Following incubation in drug-free medium for an additional 24 h, cells were analyzed for cell cycle distribution (Fig. 4). As indicated previously [14], the addition of 100 n*M* CPT caused an arrest of tumor cells as well as normal cells in S phase. However, when these cells were treated with 100 n*M* UCN-01, a different effect on the rate of passage of cells was observed. While 100 n*M* UCN-01 had little effect on any of the cell lines by itself, it had significant effects on CPT-induced S phase arrest. On normal cells, 100 n*M* UCN-01 eliminated the CPT-induced S phase accumulation, causing cells to accumulate in G_0/G_1 compared to CPT-treated cells (Fig. 4C,F). Although, the response of MDA 231 cells to 100 n*M* CPT was similar to that of the normal endothelial cells, the response to the drug combination was quite different. Incubation of these cells with 100 n*M* UCN-01 shifted the CPT-induced S phase arrest later in the cell cycle, with no accumulation of cells in G_0/G_1 phase (Fig. 4B,E). Further investigation is underway to determine if early mitosis is a result of this shift. Similar results were obtained when GI-101A cells were treated with the drug combination (Fig. 4A,D). Interestingly, the drug combination in this case led to a much greater number of cells staining with sub-G_0/G_1 phase amounts of DNA. This may have been due to cells undergoing apoptosis or necrosis. Thus these data indicate that normal cells treated with sublethal doses of UCN-01 were protected against CPT-induced cytotoxicity by arresting in G_0/G_1 phase. In contrast, UCN-01

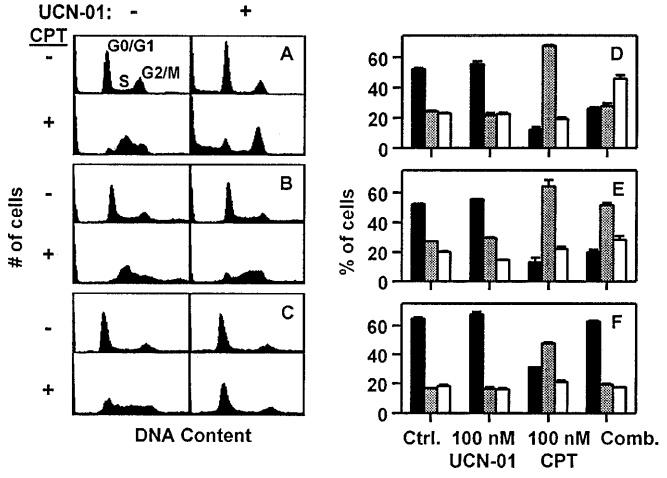

Fig. 4A–F The effect of UCN-01 on abrogation of CPT-induced S/G_2 checkpoint activation in GI 101A (**A**, **D**), MDA-231 (**B**, **E**) and normal endothelial (**C**, **F**) cells. Asynchronous cells were treated for 24 h with 100 n*M* CPT in the presence or absence of 100 n*M* UCN-01. Cells were fixed, stained with propidium iodide and analyzed by flow cytometry for DNA content. In tumor cells (**A**, **D** GI 101A cells; **B**, **E** MDA-231 cells) the addition of UCN-01 caused acceleration of passage to mitosis and loss of cells from S phase. In contrast, normal endothelial cells (**C**, **F**) arrested in G_0/G_1 phase with no acceleration of passage of cells from S phase to mitosis. The percentages of the cell populations in G_0/G_1 (■), S (□) and G_2/M (□) phases in response to drug treatment are shown and are the means ± SE of at least two independent experiments

abrogated S phase-arrested tumor cells that lack p53 function by accelerating the passage of cells to mitosis.

Discussion

We investigated the ability of UCN-01 to potentiate CPT-induced cytotoxicity in two human breast carcinoma cell lines defective for p53 function through modulation of CPT-activated S and G_2 checkpoints.

The sensitivity of cells to CPT treatment is determined by their ability to activate checkpoints in S and G_2 phases of the cell cycle [10, 14, 24, 38]. At low doses, CPT causes an accumulation of breast carcinoma cells that lack p53 function (MDA 231 and GI 101A) in the G_2 phase while at high doses cells are arrested in the S phase [14]. This dual arrest is well correlated with the low and high levels of DNA damage, respectively [13]. Therefore, pharmacological modulation of events during the S and G_2 phases can potentially enhance the therapeutic index of CPT and analogs. This can simply happen by accelerating the progression of cells that lack p53 normal function through S phase, hence inducing early mitosis which can occur prior to sufficient repair of DNA damage in G_2. Normal cells that pass the G_1 checkpoint during this modulation would also progress to G_2 and would also be sensitive to modulation in G_2. However, the wild-type p53 seems to protect these cells from abrogation at the S and G_2 checkpoints [26, 28]. We tested this approach in cultures with the use of UCN-01, a new G_2 abrogator [28].

UCN-01 (7-hydroxystaurosporine; Fig. 1) is in phase I clinical trials following evidence of preclinical activity [1, 22]. The drug was originally isolated from a strain of *Streptomyces* as a protein kinase C (PKC)-selective inhibitor [34] although many studies have suggested that PKC inhibition is unlikely to be directly responsible for UCN-01 cytotoxicity [30, 34]. UCN-01 has been shown to abrogate S and G_2 checkpoints following DNA damage preferentially in cells with defective p53 function compared to those with wild-type p53 [23]. Thus when the drug is combined with radiation or cisplatin, a synergistic interaction is only observed in cells with defective p53 function [3, 20]. In the present study, we showed that UCN-01 at sublethal doses can enhance CPT-induced cytotoxicity in tumor cells as compared to normal endothelial cells (Fig. 2). When this enhancement was assessed according to inhibition of cell growth, the IC_{50} values obtained for CPT in the presence and absence of 100 n*M* UCN-01 during a 24-h exposure were 300 n*M* and 10 n*M* for GI 101A cells and 200 n*M* and 5 n*M* for MDA-231 cells, respectively (Fig. 2A,B). There was no enhancement of CPT-induced cytotoxicity in normal endothelial cells as clearly indicated in Fig. 2C.

The potentiating effect of UCN-01 on CPT-induced cytotoxicity in tumor cells with defective p53 function raises the question as to whether this phenomenon is additive or synergistic. To address this question, we assessed the outcome of the drug combination (growth and proliferation inhibition) using median-effect analysis [4]. Previously we have shown that the antiproliferative activity (DNA synthesis) of CPT on breast cancer cells with defective p53 is more pronounced than its growth inhibitory effect [14]. Thus when growth inhibition of the drug combination was analyzed a synergistic cytotoxic effect was clearly indicated in both cell lines, as shown in Fig. 3A. However, antagonistic interaction was observed for the antiproliferative activity of the drug combination (Fig. 3B). This effect indicates that UCN-01 counteracts CPT-induced inhibition of DNA synthesis by increasing the rate of DNA synthesis in tumor cells. Thus the DNA content of treated cells was determined by flow cytometry (Fig. 4).

As expected, UCN-01 preferentially abrogates only the DNA damage-dependent activation of the S/G_2 checkpoint induced by CPT. Acceleration of the passage of the tumor cells through the S phase of the cell cycle was observed when cells were incubated with sublethal doses of UCN-01, thereby increasing the cytotoxic activity of CPT. This hypothesis is supported by the results of the [^{3}H]thymidine incorporation assays (Fig. 3B) that showed that UCN-01 was able to eliminate suppression of DNA synthesis and thus an antagonistic interaction was observed. While the normal endothelial cells showed a loss of S phase-arrested cells with UCN-01 treatment, they accumulated in G_0/G_1 (Fig. 4C) and were relatively resistant to the cytotoxicity of the drug combination (Fig. 2C). This was as expected from normal cells expressing wild-type p53. Thus the cell cycle response of the normal cells to CPT and UCN-01 was markedly

different from that of the tumor cells, and may be responsible for their lower drug sensitivity.

The molecular events responsible for the observed synergism between UCN-01 and CPT has not been fully established. One possible explanation of the results might be related to DNA damage-dependent mechanisms and the components of cell cycle regulatory proteins. For example, the activity of cyclin-dependent kinases (cdk) is known to be essential for progression through the cell cycle, but the specific substrates for those kinases, and the way they promote cell cycle progression remain a mystery. However, there is enough evidence on the role of some molecules to enable conclusions to be drawn based on their status. Arrest of the cell cycle in the G_2 phase is likely regulated by the cdk, $p34^{cdc2}$ (reviewed in reference 25). The direct regulators of $p34^{cdc2}$ are wee1 kinase, that phosphorylates and inactivates $p34^{cdc2}$, and cdc25 phosphatase, that activates $p34^{cdc2}$ [27, 33]. Previous studies by Tsao et al. [35] have shown that CPT treatment leads to a loss of $p34^{cdc2}$ activity in HeLa cells. This inactivation is associated with G_2 arrest in these cells. However, cyclin B levels are maintained at a high level despite a decrease in the rate of cyclin B synthesis. The authors also noted that subsequent to CPT treatment, there is a loss in the dephosphorylation of $p34^{cdc2}$. This suggests that CPT causes, either directly or indirectly, the inactivation of $p34^{cdc2}$ by affecting its phosphorylation state, and this leads to a G_2 arrest.

While the precise mechanisms underlying the antitumor activity of UCN-01 remain unclear, Wang et al. [36] have demonstrated that UCN-01-induced apoptosis is correlated with inappropriate activation of cdk2 and $p34^{cdc2}$ in cultured T lymphoblasts. Thus it is possible that UCN-01 abrogates S and G_2 checkpoints by this activation. Furthermore, as cdk2 is active during S phase of the cell cycle [25], its inappropriate activation by UCN-01 may be enhancing progression through S phase as well. If this occurs, and if a failure to arrest at the G_2/M phase or slow DNA synthesis during S phase does increase DNA damage induced by CPT treatment, then UCN-01 could act synergistically with CPT. Furthermore, in cells that lack other regulators of the cell cycle such as p53, there may be a greater increase in cytotoxicity with drug combination, owing to the lack of other checkpoints where the cell cycle could be arrested, and further DNA damage avoided or repaired. Alternate cdk inhibitors might be involved in these checkpoint controls, and alternative explanations are possible.

In conclusion, our work showed that UCN-01, an agent entering phase I clinical trials, can abrogate CPT-induced activation of the S/G_2 checkpoint in breast tumor cells with mutant p53 gene. This abrogation occurred with the use of sublethal doses of UCN-01 and was correlated with enhancement of CPT-induced cytotoxicity in tumor cells. Normal endothelial cells which express normal p53 function were arrested in G_0/G_1 phase with no potentiation of CPT-induced cytotoxicity. These observations suggest that UCN-01 may be able to enhance the therapeutic index of topo I inhibitors as a result of tumor-specific differences at cell cycle checkpoints.

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