Potent antitumor activity of 10-methoxy-9-nitrocamptothecin

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Abstract
The present data showed that 10-methoxy-9-nitrocamptothecin (MONCPT), a family of camptothecin analogues, possessed high antitumor activity in vitro and in vivo. Cytotoxicity assays showed that MONCPT was a potential and highly efficient antitumor compound with IC50 values of 0.1 to 500 nmol/L in nine tumor cell lines. The high cytotoxic potency of MONCPT was paralleled with its ability to increase the cellular accumulation of DNA damage. DNA relaxation assay also showed that MONCPT exerted high potency as a topoisomerase I inhibitor. Moreover, administration of MONCPT (5–20 mg/kg) for 15 to 17 days significantly inhibited tumor growth in human androgen-independent prostate tumor (PC3) and human non–small cell lung tumor (A549) xenografts; the inhibition rates ranged from 29.6% to 98%. The cytotoxic effect of 1,000 nmol/L of MONCPT in PC3 cells was associated with causing an arrest in G0-G1 phase, whereas that of 10 and 100 nmol/L MONCPT was relative to a persistent block in G2-M phase. Furthermore, down-regulation of CDK2, CDK4, and cyclin D1 was observed in PC3 cells treated with 1,000 nmol/L of MONCPT, whereas overexpression of CDK7, CDK1, and cyclin B1 was seen in PC3 cells treated with 10 and 100 nmol/L of MONCPT. These results suggested that cell cycle regulation might contribute to the anticancer properties of MONCPT and strongly support the further anticancer development of MONCPT. [Mol Cancer Ther 2006;5(4):962–8]

Introduction
Camptothecin, an alkaloid with antitumor efficacy, was isolated from the Chinese “happy tree,” Camptotheca acuminata, by Wall and coworkers (1) in 1966, and then evaluated clinically in the U.S. However, due to unpredictable and severe toxicities, including myelosuppression, vomiting, diarrhea, and severe hemorrhagic cystitis, it was suspended from clinical trials until its anticancer activities could be found (2). It was reported that camptothecin could bind to topoisomerase I (Topo I), resulting in DNA damage by collision with replication forks and subsequent cell death (3). Because tumor cells have been shown to contain more Topo I compared with normal tissue, camptothecin and its analogues make up an important class of anticancer drug. It has been shown that camptothecin is an S phase–specific DNA-damaging agent (4) and can kill both S phase and non–S phase cells at high concentrations (5). The mechanism of cytotoxicity of camptothecin in non–S phase tumor cells may be through apoptosis (6).

Since its mechanism of action, the inhibition of Topo I, was identified (7), camptothecin has again attracted the attention of scientists to modify its structure and develop new analogues for anticancer therapy. Two camptothelin derivatives, irinotecan and topotecan, are the most widely used camptothecin analogues in clinical applications and in clinical trials against colorectal and ovarian cancers (2). Other compounds, including 9-aminocamptothecin, 9-nitrocamptothecin, GG-211, and DX-8951f, have also been clinically evaluated (8). However, the response rate remains low, and the overall survival rate has not improved substantially (9). The structure-activity relationship analysis (10) of the camptothecins has shown that these camptothecins contain a terminal lactone ring that makes them unstable in aqueous solutions after undergoing a rapid, pH-dependent, nonenzymatic hydrolysis to form an open-ring hydroxyl carboxylic acid. This hydrolysis results in camptothecin’s much less potent inhibition on Topo I. Thus, substitutions at the 7-, 9-, or 10-positions of most camptothecin analogues have been shown to enhance their antitumor activity. Although the induction of hydroxyl or alkoxy at position 10, such as, 10-methoxycamptothecin, has antitumor activity, its low therapy index limited further research (11). The introduction of a nitro at position 9, such as 9-nitrocamptothecin, has shown satisfactory activity with low toxicity. However, 9-nitrocamptothecin was difficult to prepare by nitration of camptothecin because 12-nitrocamptothecin was the main product (12). In contrast, 10-alkoxy-9-nitrocamptothecin could easily be prepared from 10-hydroxycamptothecin (13, 14). To our knowledge, there are few reports about the systematic evaluation on anticancer activity of 10-alkoxy-9-nitrocamptothecin, except for antileukemic activity research of 10-methoxy-9-nitrocamptothecin (MONCPT) by Wani et al. (13). In this article,
we investigated the antitumor effects of MONCPT in vitro and in vivo, and reported for the first time that MONCPT-induced cell cycle inhibition may play a key role in its antitumor activity.

**Materials and Methods**

**Chemicals**

MONCPT (structure in Fig. 1) was prepared according to Wani’s method (13). Stock concentrations of MONCPT (10 mmol/L) and SN38, an active metabolite of irinotecan (10 mmol/L; HengRui Medicine, Inc., LianYungang, JiangSu, China), were prepared with DMSO and stored at −20°C for in vitro test. The stock solution was further diluted with the appropriate culture medium immediately before use. For in vivo tests, MONCPT (4 mg/mL) was dissolved and further diluted in injectable oleum camelliae before injection. Irinotecan (4 mg/mL) was dissolved in sodium chloride before injection.

**Cell Lines and Cell Culture**

All of the cell lines were purchased from the Institute of Cell Biology in Shanghai and grown at 37°C in a 5% CO₂ atmosphere (15). Breast cancer MCF7 was maintained in LG-DMEM (Life Technologies, 2 g/L glucose), glioma U251 was maintained in HG-DMEM (Life Technologies, 4.5 g/L glucose), androgen-independent prostate tumor PC3, non–small cell lung tumor A549, erythromyeloid tumor K562, hepatocellular carcinoma Bel-7402, ovarian cancer HO8910, acute lymphoblastic leukemia MOLT-4, and promyelocytic leukemia HL-60 cell lines were maintained in RPMI 1640. All media were supplemented with 10% FCS plus 2 mmol/L of glutamine and 50 units/mL of penicillin.

**Cytotoxicity Assay**

Cell lines were seeded in 96-well microtiter plates (4,000 cells/well). After 24 hours of incubation in the appropriate medium, cells were treated with various concentrations of MONCPT or SN38 for another 72 hours of culture. Afterwards, 10 μL of stock 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, St. Louis, MO) solution was added to each well (final, 0.5 mg/mL) for an additional 4 hours of incubation (37°C, 5% CO₂). Then, 200 μL of DMSO was added to each well and absorbance was read at 570 nm. The IC₅₀’s were calculated using the PrismPad computer program (GraphPad Software, Inc., San Diego, CA) and were defined as concentration of drug causing 50% inhibition in absorbance compared with control (vehicle) cells (16). Each treatment was tested in sextuplicate (six wells of the 96-well plate per experimental condition). All of the experiments were done in triplicate.

**Antitumor Effect on Prostate Cancer PC3 and Non–Small Cell Lung Cancer A549 Xenografted Athymic Mice**

Tumors were established by injection of PC3 cells or A549 cells (5 x 10⁶ cells per animal, s.c.) into the armpit of the athymic nude mice (National Rodent Laboratory Animal Resource, Shanghai, China). Treatments were initiated when tumors reached a mean group size of ~100 mm³ (PC3) or 320 mm³ (A549). Tumor volume (mm³) was measured with calipers and calculated as \((W^2 \times L) / 2\), where \(W\) is width; and \(L\) is length. The tumor volume at day \(n\) was expressed as RTV according to the following formula: \(RTV = TV_n / TV_0\), where \(TV_n\) is the tumor volume at day \(n\) and \(TV_0\) is the tumor volume at day 0. The T/C% was determined by calculating RTV as \(T/C\% = 100 \times (\text{mean RTV of treated group}) / \text{mean RTV of control group}\).

The tumor growth inhibition rate was calculated using the formula \(\text{IR} (%) = (1 - \text{WTW/t/Wi}) \times 100\), where \(\text{WTW}/t\) and \(\text{WTW}/i\) are the mean tumor weight of treated and control groups, respectively. MONCPT (5, 10, and 20 mg/kg) was formulated in injectable oleum camelliae and administered i.m. once every 2 days for 15 days (A549) or 17 days (PC3). The positive control group was treated i.m. with 10 mg/kg of irinotecan according to the same schedule as MONCPT’s in PC3 models. Mice weight and tumor volume were recorded every 2 days until animals were sacrificed at 15 days (A549) or 17 days (PC3). Animal care was in accordance with institutional guidelines (17).

**DNA Relaxation Assay**

The ability of camptothecins to relax supercoiled plasmid DNA was detected as described previously (18). Supercoiled plasmid PB322 DNA was incubated with Topo I in the presence of the MONCPT (100 and 200 μmol/L), DMSO was diluted in the same way as MONCPT and was used as negative controls. Reactions were carried out for 30 minutes at 37°C. Samples were run on electrophoresis in a 0.8% agarose gel in Tris-borate EDTA buffer (100 mmol/L Tris-borate, 2 mmol/L EDTA) at 50 V for 1 hour. The gels were further stained with 1 μg/mL ethidium bromide. Each experiment was run in duplicate.

**Cell Cycle Analysis**

For cell cycle analysis, PC3 cells (5 x 10⁴ cells/mL, 5 mL) were cultured in 25 cm² flasks, with or without MONCPT (10, 100, and 1,000 nmol/L) for 48 hours. Cells were then harvested, washed in PBS, centrifuged, and resuspended in 1 mL of 0.1% sodium citrate containing 0.05 mg of propidium iodide and 50 μg of RNaše for 30 minutes at room temperature in the dark. DNA content was measured with Coulter Epics Elite flow cytometer (19).

**Western Blot**

PC3 cells were treated with MONCPT (10–1,000 nmol/L) for 24 or 48 hours. Proteins were extracted with lysis buffer (50 mmol/L NaCl, 50 mmol/L Tris, 1% Triton X-100,
1% sodium deoxycholate, and 0.1% SDS) and 50 μg of total protein was loaded per lane. Proteins were fractionated on 12% Tris-glycine gels, transferred to nitrocellulose membrane (Pierce Biotechnology, Inc., Rockford, IL) and probed with primary antibodies (CDK1, CDK7, cyclin B1, CDK2, CDK4, cyclin E, cyclin D1, and h-actin) and then horseradish peroxidase–labeled secondary antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Antibody-positive band were visualized using enhanced chemiluminescence Western blot detection reagents (Pierce Biotechnology; ref. 20).

Results

Effect of MONCPT on Cell Viability

The antiproliferative activities of MONCPT and SN38 were determined on various human tumor cell lines from a diverse set of target organs, including leukemia and solid tumors (prostate, ovary, lung, liver, and breast cancer cell lines). As shown in Table 1, MONCPT was an antiproliferative agent on the cell lines tested. Among the nine tested cell lines, MOLT-4, K562, HO-8910, A549, and PC3 exhibited more sensitivity to MONCPT (IC_{50} values, 0.1–40 nmol/L) than to SN38 (IC_{50} values, 0.3–115 nmol/L). Particularly, they were ~9-fold more sensitive to MONCPT than to SN38 in A549 and PC-3 cell lines. These results suggested that MONCPT had promising antitumor activity against a broad spectrum of human tumors.

Effect of MONCPT on Tumor Growth In vivo in PC3 and A549 Xenograft Models

In the study with PC3 cells, tumor size was ~100 mm^3 at the initiation of treatment (day 1). MONCPT or irinotecan was administered i.m. once every 2 days for 17 days. The tumor volumes were significantly inhibited ($P < 0.05–0.001$) from days 5 to 17 in the groups treated with MONCPT (10 and 20 mg/kg). There was a significant inhibition of tumor growth ($P < 0.05–0.01$) from days 12 to 17 in the group treated with MONCPT (5 mg/kg). As shown in Fig. 2, from days 1 to 17, the tumor volumes in the control group achieved a 5.2-fold increase, whereas tumor volumes in MONCPT treatment groups obtained 3.7-fold (5 mg/kg) and 1.9-fold (10 mg/kg) elevations, respectively.

Similar to PC3 cells, a 69.2% decline in the tumor volume was obtained in the group treated with 20 mg/kg MONCPT. As shown in Table 2, MONCPT showed a significant effect on tumor weight, but not on athymic mice body weight at day 17, and the inhibition rates caused by MONCPT (5–20 mg/kg) ranged from 29.6% to 77.7% compared with 33.3% in the group treated with irinotecan (10 mg/kg).

In the study with A549 cells, the tumor size was ~320 mm^3 at the initiation of treatment (day 1). MONCPT was administered i.m. once every 2 days for 15 days. The tumor volumes were significantly inhibited ($P < 0.05–0.001$) in 20 mg/kg MONCPT treatment group from days 7 to 15. MONCPT (5 and 10 mg/kg) exhibited significant inhibition of tumor growth ($P < 0.05–0.001$) from days 11 to 15. As shown in Fig. 3, from days 1 to 15, the tumor volumes in the control group achieved a 5.6-fold increase, and in MONCPT treatment groups, obtained 3.9-fold (5 mg/kg) and 1.7-fold (10 mg/kg) elevations, respectively. Similar to PC3 cells, a 69.2% decrease in the tumor volume

Table 1. Cytotoxicity of MONCPT and SN38 on a panel of nine human tumor cell lines

<table>
<thead>
<tr>
<th>Original tumor types</th>
<th>Cell lines</th>
<th>IC_{50} (nmol/L) MONCPT (nmol/L)</th>
<th>SN38 (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>MOLT-4*</td>
<td>0.22</td>
<td>0.30</td>
</tr>
<tr>
<td>Erythromyeloid tumor</td>
<td>K562*</td>
<td>0.10</td>
<td>10.1</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>HO-8910*</td>
<td>40</td>
<td>85</td>
</tr>
<tr>
<td>Non–small cell lung tumor</td>
<td>A549*</td>
<td>6.2</td>
<td>50</td>
</tr>
<tr>
<td>Androgen-independent prostate tumor</td>
<td>PC-3*</td>
<td>10.7</td>
<td>115</td>
</tr>
<tr>
<td>Promyelocytic leukemia</td>
<td>HL60</td>
<td>12.5</td>
<td>5.1</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>MCF-7</td>
<td>230</td>
<td>309</td>
</tr>
<tr>
<td>Glioma</td>
<td>U251</td>
<td>120</td>
<td>51</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>Bel-7402</td>
<td>500</td>
<td>50</td>
</tr>
</tbody>
</table>

NOTE: Cells were exposed with various concentrations of MONCPT or SN38 for 72 hours.
*Compared with SN38, cell lines were significantly ($P < 0.05–0.001$) more sensitive to MONCPT according to IC_{50} values.

Figure 2. MONCPT antitumor potency in human androgen–independent prostate tumor PC3 xenograft model. Treatment was initiated when average tumors reached a mean group size of 100 mm^3. MONCPT was administered (i.m.) every 2 d for 17 d ($n = 6–11$ per group).
was observed in the group treated with a high dose of MONCPT (20 mg/kg). Table 3 showed the effect of MONCPT on A549 tumor weight. At day 15, MONCPT had a significant effect on A549 tumor weight, but not on athymic mice body weight, and the inhibition rates caused by MONCPT (5–20 mg/kg) ranged from 32% to 98%.

**Effect of MONCPT on Topo I-Mediated Relaxation of Supercoiled DNA**

The ability of MONCPT to inhibit Topo I-catalyzed relaxation of supercoiled DNA was illustrated in Fig. 4 (R, relaxed PBR322 DNA; S, supercoiled PBR322 DNA). In the absence of Topo I (lane A), MONCPT did not induce any DNA band changes in the gel. The inhibition of Topo I by MONCPT was visualized at the concentrations of 100 μmol/L (lane E) and 200 μmol/L (lane D). Supercoiled DNA entirely relaxed without MONCPT treatment (lanes B and C). It was obvious that MONCPT retained Topo I poisoning activity.

**Effect of MONCPT on the Cell Cycle**

PC3 cells were treated with MONCPT (10–1,000 ng/mL) for 48 hours, and the cell cycle patterns were analyzed by flow cytometry. MONCPT caused G2-M arrest at doses of 10 and 100 nmol/L, and induced G1-G0 arrest at a dose of 1,000 nmol/L (Fig. 5). The percentages of G1-G0 phase were 12.5% (10 nmol/L), 23.4% (100 nmol/L), and 60% (1,000 nmol/L), and the proportions (%) of G2-M phase were 74.3% (10 nmol/L), 36.4% (100 nmol/L), and 5.8% (1,000 nmol/L). These data showed that the MONCPT-induced cycle changes were dose-dependent.

**Effect of MONCPT on Cell Cycle Regulatory Proteins**

To investigate the potential mechanisms involved in MONCPT-induced cell cycle arrest, we examined several regulatory proteins associated with the G1-G0 phase and G2-M phase in the cell cycle. We first analyzed the possible alterations of CDK2, CDK4, cyclin E, and cyclin D1 because these factors have been reported to play important roles in the regulation of G1-G0 phase. Figure 6A showed that the level of CDK2 was obviously changed with the increase in the concentrations of MONCPT, although there was no effect on the protein level of cyclin E. CDK2 protein expression was elevated in PC3 cells treated with 10 and 100 nmol/L of MONCPT, but declined in cells treated with 1,000 nmol/L of MONCPT. Meanwhile, the protein level of cyclin D1 was down-regulated by MONCPT in a dose-dependent manner (Fig. 6B).

In addition, we examined three important G2-M checkpoint proteins: CDK7, CDK1, and cyclin B1. The results showed that 10 and 100 nmol/L MONCPT, but not 1,000 nmol/L MONCPT, induced overexpression of CDK7, CDK1, and cyclin B1 protein in PC3 cells (Fig. 6C).

**Discussion**

Camptothecin and its various derivatives have been reported to have potent antitumor activity against several experimental tumors through the inhibition of Topo I. However, their clinical application has been restrained due to their physiologic stability and toxicity (21). Previous investigations have shown that the addition of hydroxy or alkoxy to position 10 could strengthen antitumor activity, such as 10-methoxy camptothecin (11). Moreover, the addition of a nitro to position 9 has satisfactory activity with low toxicity, like 9-nitrocamptothecin (22). Thus, we
chose MONCPT, a compound which conjugated two groups in the 9 and 10 positions, to test its antitumor potential, to investigate its mechanism, and to evaluate its possibility for development.

The antitumor activity of MONCPT was first determined by the value of IC\textsubscript{50} from various tumor cell lines, including leukemia and solid tumors (prostate, lung, liver, breast, ovary, and glioma). Our results showed that MONCPT exhibited strong antiproliferative activity on all of the tested cell lines (IC\textsubscript{50} values, 0.1–500 nmol/L). Moreover, the IC\textsubscript{50} values of MONCPT for five of nine cancer cell lines were lower than those of SN38, indicating that MONCPT possessed higher antiproliferative activities in certain cancer species, such as A549 and PC3 cancer cell lines. It is well known that the clinical efficacy of anticancer agents, including some topoisomerase inhibitors, is limited by drug resistance mechanisms (23). This resistance can result from a decrease in the levels and/or activities of topoisomerase, a decrease in intracellular drug accumulation (24), or the presence of nonproliferating tumor cells during the treatment period (25). Therefore, we are preparing camptothecin-resistant cell lines, and will treat these cell lines with MONCPT to show whether MONCPT still has an active antiproliferative effect on the three critical situations.

With the support of the in vitro data which showed the antiproliferative activity of MONCPT in a wide spectrum of tumor cell lines, the antitumor activity of MONCPT was further evaluated in animal models. In two different tumor models, androgen-independent prostate cancer PC3 and non–small cell lung cancer A549, MONCPT was shown to have strong antitumor activity with a dose-dependent administration (i.m.) regimen. The tumor inhibition rates achieved 77.7% for PC3 at day 17 and 98% for A549 at day 15. Only limited weight loss was observed, even at the highest dose. In PC3 tumor xenograft, MONCPT exerted 2-fold more potent than irinotecan when delivered by treatment schedule.

Inhibition of Topo I has been implicated in the mechanism of cytotoxicity induced by camptothecin and its derivatives (26). It has been hypothesized that this inhibition is involved in radiation enhancement by camptothecin analogues, because the levels of Topo I correlated with the levels of radiopotentiation in various cell lines (27, 28). The relaxation of supercoiled DNA assay confirmed that MONCPT had Topo I poisoning activity as a Topo I inhibitor.

Previous studies have addressed the role of camptothecin derivatives on the cell cycle. Studies using 9-nitrocamptothecin alone showed an accumulation of tumor cells at the S-G\textsubscript{2} boundary (29), whereas irinotecan was reported to increase the proportion of G\textsubscript{2}-M phase in the tumor cells (30). To check the effect of MONCPT on the cell cycle, we did DNA analysis using PC3. The results showed that MONCPT at 10 and 100 nmol/L delayed PC3 cell transition from the G\textsubscript{2}-M phase and prolonged the duration of the G\textsubscript{2}-M phase, whereas 1,000 nmol/L of MONCPT induced a G\textsubscript{1}-G\textsubscript{0} phase arrest. The effect on the two phases of the cell cycle was similar with the previous report of topotecan, which showed an arrest of G\textsubscript{2}-G\textsubscript{0} phase at 1 \textmu mol/L or higher topotecan concentrations and an arrest of G\textsubscript{2}-M phase at <0.2 \textmu mol/L of topotecan (31). These data suggested that MONCPT exhibited anticancer activity via cell cycle regulation pathway.

Cells entering into each phase of the cell cycle is carefully regulated by receptor collectives, termed cell cycle checkpoints (32). Passage through the four phases of the cell cycle is regulated by a family of cyclins that act as

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of animals</th>
<th>Body weight (g)</th>
<th>Tumor size (mm\textsuperscript{3})</th>
<th>Tumor weight (g)</th>
<th>Inhibition rate (%)</th>
<th>T/C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>23 ± 1</td>
<td>356 ± 187</td>
<td>1999 ± 847</td>
<td>2.00 ± 0.84</td>
<td>—</td>
</tr>
<tr>
<td>MONCPT (20 mg/kg)</td>
<td>8</td>
<td>22 ± 2</td>
<td>312 ± 166</td>
<td>96 ± 138*</td>
<td>0.04 ± 0.03*</td>
<td>98</td>
</tr>
<tr>
<td>MONCPT (10 mg/kg)</td>
<td>8</td>
<td>23 ± 2</td>
<td>369 ± 207</td>
<td>639 ± 714*</td>
<td>0.43 ± 0.49*</td>
<td>78.5</td>
</tr>
<tr>
<td>MONCPT (5 mg/kg)</td>
<td>8</td>
<td>22 ± 1</td>
<td>346 ± 169</td>
<td>1357 ± 691</td>
<td>1.36 ± 0.84*</td>
<td>32</td>
</tr>
</tbody>
</table>

NOTE: Athymic mice with A549 transplant tumor were treated with (i.m.) MONCPT 20, 10, or 5 mg/kg every 2 days for 15 days. Criteria for therapeutic activity: % T/C, optimal growth inhibition <10 (optimal); optimal growth inhibition <25 (good); and optimal growth inhibition <50 (moderate).

\*P < 0.05.

\textsuperscript{†}P < 0.001.

Figure 4. Effect of MONCPT on plasmid DNA relaxation induced by Topo I. Native supercoiled PBR322-DNA was incubated with Topo I in the presence of MONCPT at 200 \textmu mol/L (lane D) and 100 \textmu mol/L (lane E). Double-distilled water (lane B) and DMSO (solvent diluted in the same way as MONCPT, lane C) were used as negative or vehicle control, respectively. DNA without Topo I (lane A). DNA samples were separated by electrophoresis on agarose gels. Gels were then stained in ethidium bromide and photographed under UV light.
regulatory subunits for cyclin-dependent kinases (CDK).
The activity of the various cyclin/CDK complexes that regulate the progression through G1-S-G2 phases of the cell cycle is controlled by the synthesis of the appropriate cyclins during a specific phase of the cell cycle. Cyclin D1 is synthesized in late G1 and seems to be required for progression into S phase. As cells enter the cycle from G0 phase, cyclin D1 is induced as part of the delayed early responses to growth factor stimulation, and its synthesis and assembly with its catalytic partner, CDK4, depend on mitogenic stimulation. Down-regulation of cyclin D1 leads to the increase of length of G1. Cyclin E/CDK2 accumulates during late G1 phase and triggers the passage into S phase. In proliferating cells, the expression of cyclin E and CDK2 are normally periodic and maximal at the G1-S transition. By Western blot, we observed that 1,000 nmol/L of MONCPT caused CDK4 and CDK2 degradation, whereas 10 and 100 nmol/L of MONCPT were found to increase these protein’s expressions. Meanwhile, down-regulation of cyclin D1, but not cyclin E, was observed with concentrations of 10 to 1,000 nmol/L. These results were consistent with the cell cycle analysis data which showed that 1,000 nmol/L MONCPT caused a G1-G0 phase arrest. It is widely believed that the CDK1/cyclin B1 complex plays a critical role during the G2 phase. During the G2 phase, CDK1 forms a heterodimeric complex with cyclin B1 and remains inactive, the CDK1/cyclin B1 complex could be activated by CDK7 (33). The up-regulation process of CDK1 and cyclin B seem to be necessary to block the cell at G2-M phase (34). The increase in CDK1 or cyclin B1 protein expression during the G2-M transition has been shown in a number of models in which cells arrested in G2-M phase (35). CDK7 is necessary for G2-M transition that can activate CDK1/cyclin B complex, and its activity decreases to undetectable levels during the M phase of mitosis (36). The present studies also revealed that up-regulation of CDK1, CDK7, and cyclin B1 was correlated with the block in G2-M progression of MONCPT at the doses of 10 and 100 nmol/L. These findings provide additional evidence that regulation of the cell cycle plays a crucial role in MONCPT-induced antitumor.

In conclusion, MONCPT, a potent Topo I poison, is a broad-spectrum antiproliferative agent, and has remarkable antitumor efficacy with low side effects in the PC3 and A549 xenograft models. The mechanism of its antitumor activity may be associated with the control of cell cycle
regulation proteins, resulting in inhibition of cell growth. Therefore, the overall profile of MONCPT, particularly the plasma stability and the mechanistic differences with respect to the related molecules, merit further study and clinical antitumor evaluation of the compound.

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