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β-Lapachone Induces Cell Cycle Arrest and Apoptosis in Human Colon Cancer Cells

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Abstract

Background: Human colon cancers have a high frequency of p53 mutations, and cancer cells expressing mutant p53 tend to be resistant to current chemo- and radiation therapy. It is thus important to find therapeutic agents that can inhibit colon cancer cells with altered p53 status. β-Lapachone, a novel topoisomerase inhibitor, has been shown to induce cell death in human promyelocytic leukemia and prostate cancer cells through a p53-independent pathway. Here we examined the effects of β-lapachone on human colon cancer cells.

Materials and Methods: Several human colon cancer cell lines, SW480, SW620, and DLD1, with mutant or defective p53, were used. The antiproliferative effects of β-lapachone were assessed by colony formation assays, cell cycle analysis, and apoptosis analysis, including annexin V staining and DNA laddering analysis. The effects on cell cycle and apoptosis regulatory proteins were examined by immunoblotting.

Results: All three cell lines, SW480, SW620, and DLD1, were sensitive to β-lapachone, with an IC_{50} of 2 to 3 μM in colony formation assays, a finding similar to that previously reported for prostate cancer cells. However, these cells were arrested in different stages of S phase. At 24 hr post-treatment, β-lapachone induced S-, late S/G2-, and early S-phase arrest in SW480, SW620, and DLD1 cells, respectively. The cell cycle alterations induced by β-lapachone were congruous with changes in cell cycle regulatory proteins such as cyclin A, cyclin B1, cdc2, and cyclin D1. Moreover, β-lapachone induced apoptosis, as demonstrated by annexin V staining, flow cytometric analysis of DNA content, and DNA laddering analysis. Furthermore, down-regulation of mutant p53 and induction of p27 in SW480 cells, and induction of pro-apoptotic protein Bax in DLD1 cells may be pertinent to the anti-proliferative and apoptotic effects of β-lapachone on these cells.

Conclusions: β-Lapachone induced cell cycle arrest and apoptosis in human colon cancer cells through a p53-independent pathway. For human colon cancers, which often contain p53 mutations, β-lapachone may prove to be a promising anticancer agent that can target cancer cells, especially those with mutant p53.

Introduction

β-Lapachone is a natural compound derived from the lapacho tree (*T. avellanedae*) native to South America. It has a broad spectrum of pharmacological effects including antibacterial, antifungal, antiparasitic, antiviral, and antitumor activities (1–12). Induction of hydrogen peroxide and superoxide anions, which can damage DNA, may account for the antibacterial and antiparasitic activities of β-lapachone (1,13). The antiviral activity of β-lapachone is demonstrated by its inhibition of reverse transcriptase of avian myeloblastosis and Rausher murine leukemia viruses (4) and by its inhibition of transcription from the long terminal repeat of the human immunodeficiency virus type 1 (HIV-1) (5).

The molecular mechanisms underlying the antineoplastic activities of β-lapachone have not been well elucidated. One candidate molecular

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target for β-lapachone is the eukaryotic topoisomerase I (Topo I). β-Lapachone directly interacts with Topo I and thus inhibits its catalytic activity (14) via a mechanism different from camptothecin, a classical Topo I inhibitor, which induces the formation of stabilized enzyme–DNA cleavable complexes (15). β-Lapachone also poisons Topo II by inducing reversible Topo II–DNA cleavable complexes and thus protein-linked DNA breaks (16). Topoisomerases are ubiquitously expressed nuclear enzymes involved in a variety of cellular functions including DNA repair, DNA replication, transcription, recombination, and chromatid segregation during mitosis (17), and they are important targets of many anticancer chemotherapeutic agents (18,19). Consistent with its inhibition of topoisomerases, β-lapachone inhibits repair of damaged DNA (20–22) and induces high occurrences of chromosomal aberrations and sister-chromatid exchanges, as does camptothecin (23).

Recent studies suggest that β-lapachone is a promising antineoplastic chemotherapeutic agent. β-Lapachone induces apoptosis in several kinds of human tumor cells, including promyelocytic leukemia and prostate cancer cells (6,7), malignant glioma cells (8), hepatoma cells (9), breast cancer cells (10), and ovarian cancer cells (11,12). More significantly, β-lapachone-induced apoptosis is independent of p53 status in cancer cells such as human promyelocytic leukemia and prostate cancer cells (6,7). Since inactivation of p53 by mutations has been shown to reduce both radio- and chemosensitivity (24–26), agents such as β-lapachone, which are capable of inducing cell death independent of p53 status, would be of particular interest and can be potentially useful in eradicating cancer cells with defective p53 function.

The *p53* tumor suppressor gene is frequently mutated in colon cancers (27). In order to better manage colon cancers, it is important to find drugs that can inhibit colon cancer cells with altered p53 status, especially those expressing mutant p53. In this study, we examined the effects of β-lapachone on several human colon cancer cell lines with mutant or defective p53. SW480 and SW620 cells express mutant p53, whereas DLD1 cells are p53-deficient (26,28,29). Our results showed that β-lapachone induced cell cycle alterations and apoptosis in human colon cancer cells. Cell cycle and apoptosis regulatory proteins were dysregulated after β-lapachone treatment in these colon cancer cells, which may contribute to β-lapachone-mediated cytotoxicity. Overall, our results suggest that β-lapachone promises to be an anticancer agent that can target human colon cancer cells with p53 mutations.

Materials and Methods

Cell Culture

Human colon cancer cell lines SW480, SW620, and DLD1 were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C in a humidified incubator with 5% CO_2.

Chemicals

β-Lapachone (3,4-dihydro-2,2-dimethyl-2H-naphthol[1,2-b]pyran-5,6-dione) was a generous gift from Dr. A. Matter (Ciba-Geigy, Basel, Switzerland). It was dissolved in DMSO at a concentration of 20 mM and stored in small aliquots at −70°C. A fresh aliquot was used for each experiment.

Colony Formation Assays

Cells were seeded into six-well plates at a density of 500 cells per well. After overnight incubation, β-lapachone was added to achieve final concentrations ranging from 0.5 to 20 μM. Control cells were treated with an equal volume of DMSO. After 4-hr treatment, cells were rinsed to remove β-lapachone or DMSO, and further incubated in drug-free medium for 14 days. Cells were then fixed and stained with Wright-Giemsa Stain (Sigma Chemical Co., St. Louis, MO). Colonies of >50 cells were counted.

Cell Cycle and Apoptosis Analysis

Cells were seeded into 60-mm dishes at a density of 7×10^5 per dish. After overnight incubation, cells were treated with β-lapachone at a final concentration of 5 μM or an equal volume of DMSO for 4 hr, and further incubated in drug-free medium for additional times as indicated. Cells were then harvested by trypsinization, and incubated for 30 min at room temperature in staining solution consisting of propidium iodide (PI; 50 μg/ml), sodium citrate (0.1%), Triton X-100 (0.1%), and DNase-free RNase (20 μg/ml). Stained cells were then analyzed for DNA content by flow cytometry.

Annexin V Staining

Following β-lapachone treatment, cells were harvested by trypsinization and then incubated for 10 min at room temperature in binding buffer containing PI and fluorescein isothiocyanate (FITC)–labeled enhanced annexin V, provided by ApoAlert Annexin V Apotosis Kit (Clontech, Palo Alto, CA). PI-negative cells containing early apoptotic cells were gated and analyzed for annexin V labeling by flow cytometry.

DNA Laddering Analysis

Cells (2×10^6 per dish) were plated into 100-mm dishes. After overnight incubation, cells were treated with β-lapachone (5 μM) or DMSO alone for 4 hr and incubated in drug-free medium for additional times as indicated. Cells were harvested and incubated for 3 hr at 50°C in lysis buffer consisting of NaCl (100 mM), EDTA (25 mM), Tris-HCl (pH 8; 10 mM), SDS (0.5%), and proteinase K (0.5 mg/ml). Samples were heated for 5 min at 80°C and further incubated for 3 hr at 50°C in the presence of DNase-free RNase (100 μg/ml). After addition of loading buffer (10 mM EDTA, 40% sucrose, and 0.25% bromophenol blue), samples were loaded into a 2% agarose gel and resolved by electrophoresis.

Immunoblotting

Total protein extracts were prepared by lysing cells in extraction buffer consisting of NaCl (150 mM), Tris-HCl (pH 8; 50 mM), NP-40 (1%), sodium deoxycholate (0.5%), SDS (0.1%), phenylmethylsulfonyl fluoride (2 mM), aprotinin (1 μg/ml), leupeptin (1 μg/ml), and sodium orthovanadate (1 mM). Nuclear extracts were prepared as described elsewhere (30). Protein concentrations were determined using the BioRad protein assay (BioRad, Hercules, CA). Total or nuclear protein extracts (20 μg per lane) were resolved by 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. The membranes were blocked in Tris-buffered saline containing 0.2% Tween 20 (TBS-T) and 5% nonfat dried milk, and then probed with various primary antibodies. After washing with TBS-T, blots were further probed with horseradish peroxidase (HRP)–conjugated secondary antibodies. Proteins were detected using the ECL chemiluminescence detection system (Amersham, Little Chalfont, England). Each blot was subsequently stripped and reprobed with other antibodies. The following antibodies were used: mouse monoclonal antibodies to cyclin B1 (GNS1), cyclin A (BF683), cyclin D1 (R-124), cdc2 (17), cdk2 (M2), and Bcl-2 (100) (Santa Cruz Biotechnology, Santa Cruz, CA); rabbit polyclonal antibodies to p21 (C-19), p27 (C-19), and Bax (P-19) (Santa Cruz); and HRP-conjugated goat anti-mouse antibody (Sigma) and goat anti-rabbit antibody (Santa Cruz). All blots were stripped and reprobed with rabbit polyclonal antibody to actin (Sigma), which served as a loading control.

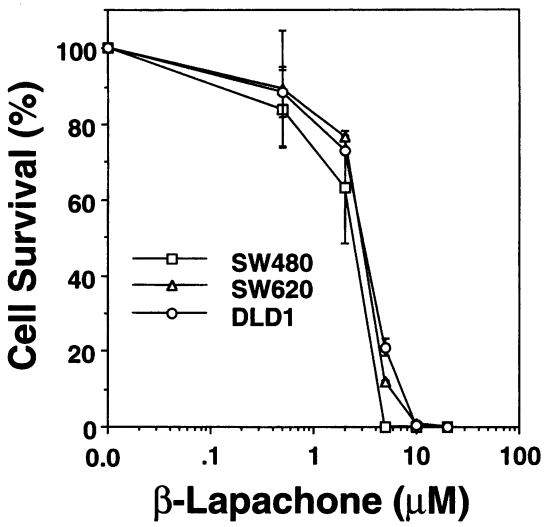

Fig. 1. Effects of β-lapachone on cell survival of human colon cancer cells. After 4 hr treatment with β-lapachone at concentrations ranging from 0.5 to 20 μM, SW480, SW620, and DLD1 cells were incubated in drug-free medium for 14 days and then analyzed for cell survival by colony formation assays. The bars represent standard deviation of three independent experiments.

Results

Effects of β-Lapachone on Cell Survival of Human Colon Cancer Cells

Human colon carcinoma cell lines SW480, SW620, and DLD1 were tested for their susceptibility to β-lapachone-mediated growth inhibition. As assessed by colony formation assays, SW480, SW620, and DLD1 cells were quite sensitive to β-lapachone, with an IC_{50} of 2 to 3 μM (Fig. 1), which is similar to that found for prostate cancer cells (7).

Effects of β-Lapachone on Cell Cycle Progression in Human Colon Cancer Cells

To examine if β-lapachone induced cell cycle alterations in colon cancer cells, SW480, SW620,

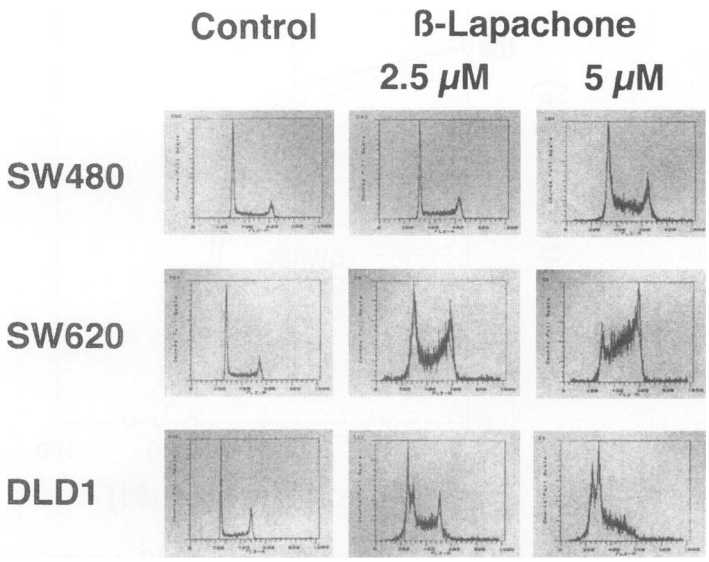

Fig. 2. Effects of β-lapachone on cell cycle progression in human colon cancer cells. SW480, SW620, and DLD1 cells were treated with DMSO alone (control) or β-lapachone (2.5 or 5 μM) for 4 hr, further incubated in drug-free medium for 20 hr, and then analyzed for cell cycle distribution by flow cytometry. Shown here are DNA histograms.

and DLD1 cells were transiently exposed to β-lapachone (2.5 or 5 μM) for 4 hr, further incubated in drug-free medium for 20 hr, and then analyzed for cell cycle distribution by flow-cytometric analysis. As shown in Figure 2, β-lapachone induced primarily S-phase arrest in SW480 cells, late S- and G2/M-phase arrest in SW620 cells, and early S-phase arrest in DLD1 cells. The cell cycle alterations were concentration-dependent. At 0.5 μM, β-lapachone did not induce any cell cycle alterations (data not shown). However, at 2.5 μM, β-lapachone induced S-phase arrest in all cell lines. When the concentration was increased to 5 μM, more SW480 cells were arrested in S phase (53.1% at 5 μM as compared to 43% at 2.5 μM and 34.5% at control), more SW620 cells were arrested in S/G2 phase (93.7% at 5 μM as compared to 64.5% at 2.5 μM and 56.6% at control), and more DLD1 cells were arrested in early S phase. Notably, in DLD1 cells, β-lapachone consistently induced two populations of cells. One population had approximately 20% more DNA content than the other. The cell number in the population with higher DNA content increased as the concentration of β-lapachone increased.

We then determined if β-lapachone exhibited any time-dependent effect on cell cycle progression. After 4-hr treatment with β-lapachone (5 μM), SW620 cells were incubated in drug-free medium for various times and analyzed for cell cycle distribution. As early as 12 hr post-treatment, β-lapachone led to 59% of cells in S phase, as compared to 38% in control cells (Fig. 3A,B). The percentage of cells in S phase further increased to 72% at 24 hr, and returned to control levels after 48 hr. The percentage of cells in G2/M phase began to increase at 24 hr, further increased to 54% at 48 hr, and remained high at 72 hr. Overall, after β-lapachone treatment, SW620 cells accumulated in S phase earlier, then slowly passed through S phase, and were blocked in G2/M phase later. In SW480 and DLD1 cells, however, β-lapachone did not exhibit any marked time-dependent effects on cell cycle progression for up to 72 hr after treatment (data not shown).

β-Lapachone Induces Apoptosis in Human Colon Cancer Cells

Following β-lapachone treatment, the growth of SW480, SW620, and DLD1 cells was suppressed, and cell death could be observed, particularly at later time points. To determine whether colon cancer cells underwent apoptotic cell death after β-lapachone treatment, we used the annexin V staining method to detect apoptotic cells. Labeling with annexin V, which binds the phospholipid phosphatidylserine translocated from the inner face of the plasma membrane to the cell surface soon after initiating apoptosis, allows detection of apoptotic cells at very early stages, long before the appearance of DNA fragmentation (31). After treatment with β-lapachone (5 μM) for 4 hr and further incubation in drug-free medium for 20 hr, SW480, SW620, and DLD1 cells were harvested and stained for annexin V and with PI. In order to distinguish early apoptotic cells from late apoptotic and necrotic cells, which

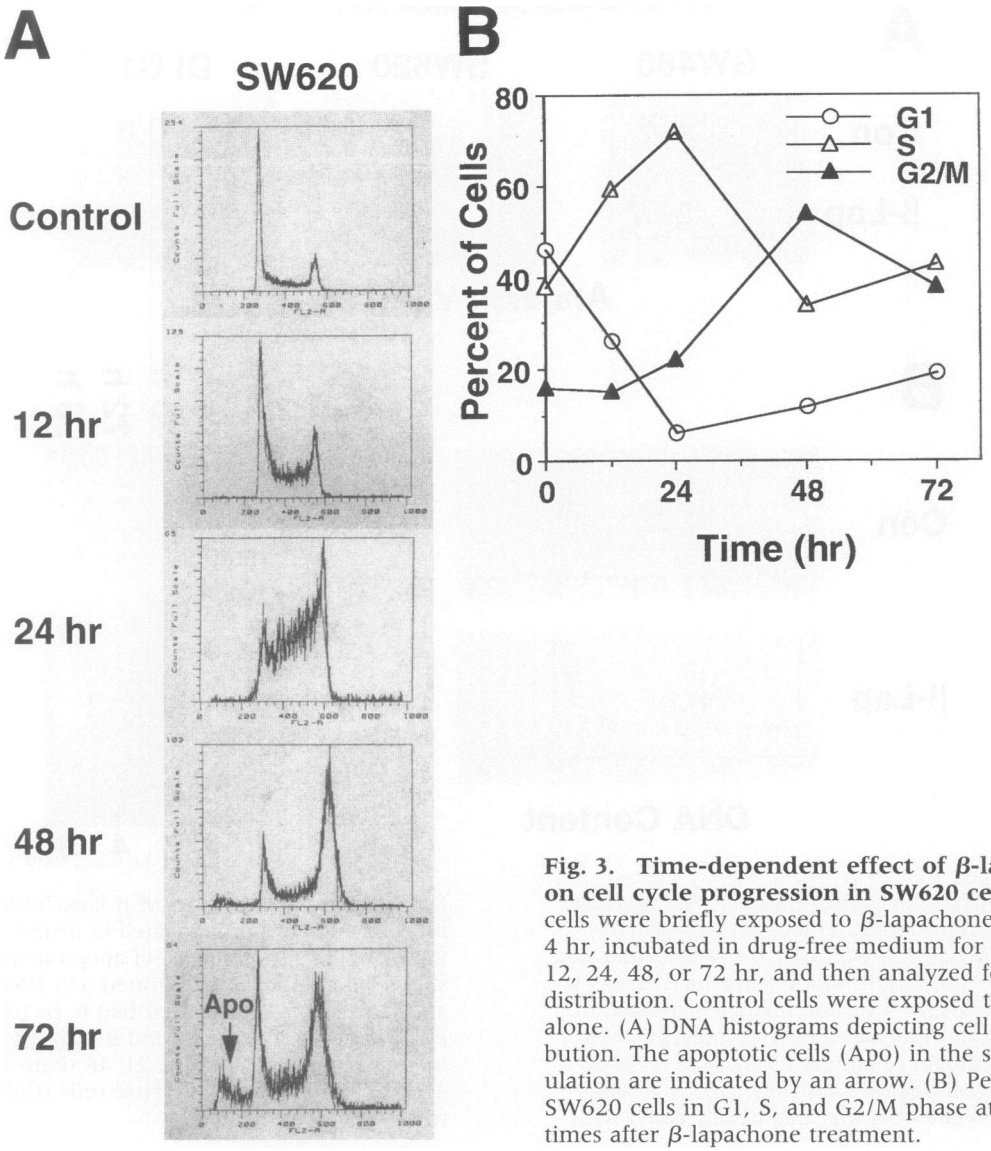

Fig. 3. Time-dependent effect of β-lapachone on cell cycle progression in SW620 cells. SW620 cells were briefly exposed to β-lapachone (5 μM) for 4 hr, incubated in drug-free medium for a total of 12, 24, 48, or 72 hr, and then analyzed for cell cycle distribution. Control cells were exposed to DMSO alone. (A) DNA histograms depicting cell cycle distribution. The apoptotic cells (Apo) in the sub-G_1 population are indicated by an arrow. (B) Percentages of SW620 cells in G1, S, and G2/M phase at various times after β-lapachone treatment.

take up PI under nonpermeable conditions, we gated PI-negative cells, which contain early apoptotic cells, and analyzed for annexin V labeling. β-Lapachone treatment caused an approximately 26-, 30-, and 26-fold increase in PI-negative, annexin V–positive cells in SW480, SW620, and DLD1 cells, respectively (Fig. 4A), indicating that β-lapachone induced apoptosis in these cells.

We further analyzed apoptosis by flow-cytometric analysis of DNA content and DNA laddering assays. Unlike annexin V staining, which detects apoptotic cells at significantly earlier stages, both these methods only detect late apoptotic cells with the appearance of DNA fragmentation. Since more cell death was observed in SW620 cells after β-lapachone treatment, and a higher percentage of early apoptotic cells was detected by annexin V staining, we focused on SW620 cells for late apoptotic analysis by using these two methods. As shown in Figure 4B, at 24 hr post-treatment, β-lapachone induced 6.9% of SW620 cells in sub-G_1 population, which represents late-stage apoptotic cells, as compared to 0.22% in untreated cells. The percentage of late apoptotic cells was quite low as compared to early apoptotic cells detected by annexin V staining (36%; Fig. 4A), suggesting that apoptosis occurred quite slowly in colon cancer cells after treatment. This is consistent with the observation by others that colon cancer cells do not undergo apoptosis rapidly (32). The slowly occurring apoptosis was further demonstrated by DNA laddering analysis, which showed that DNA ladders

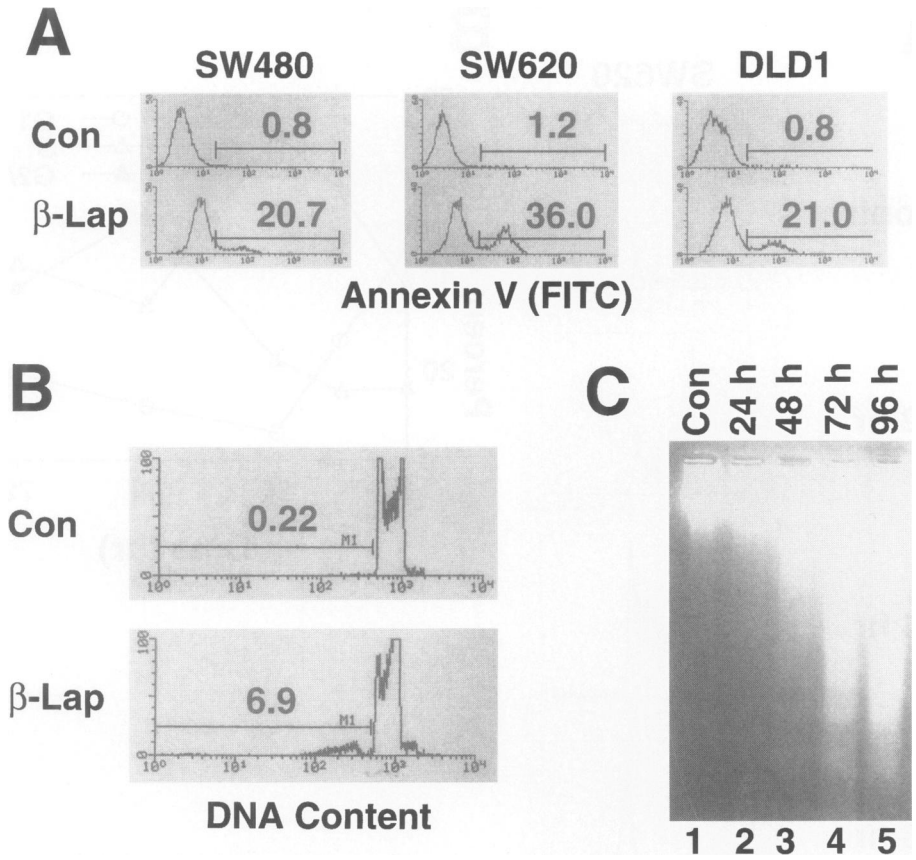

Fig. 4. Induction of apoptosis by β-lapachone in human colon cancer cells. (A) Annexin V staining of SW480, SW620, and DLD1 cells that had been treated with DMSO alone (Con) or 5 μM β-lapachone (β-Lap) for 4 hr and further incubated in drug-free medium for 20 hr. The numbers shown are the percentages of annexin V–positive cells obtained, based on negative controls. (B) Apoptotic analysis of SW620 cells that had been treated with DMSO alone (Con) or 5 μM β-lapachone (β-Lap) for 4 hr and further incubated in drug-free medium for 20 hr. The percentages of apoptotic cells in sub-G_1 population are indicated. (C) DNA laddering analysis of SW620 cells that had been treated with 5 μM β-lapachone for 4 hr and incubated in drug-free medium for up to 24 (lane 2), 48 (lane 3), 72 (lane 4), and 96 hr (lane 5). Control cells (Con) were treated with DMSO alone.

were not detected until 48 hr post-treatment (Fig. 4C), and by flow cytometric analysis of DNA content, which showed that the percentages of sub-G_1 population representing late apoptotic cells increased at later time points (Fig. 3A).

β-Lapachone Differentially Modulates Cell Cycle and Apoptosis Regulatory Proteins in Human Colon Cancer Cells

In an attempt to explore the molecular mechanisms for β-lapachone-induced cell cycle alterations and apoptosis, we examined the effects of β-lapachone on cell cycle and apoptosis regulatory proteins. Nuclear or whole-cell protein extracts were prepared at 24 hr after treatment with β-lapachone (5 μM) and analyzed for protein levels by immunoblotting.

First, we examined the effects of β-lapachone on the expression of the S and G2/M phase–related cyclin A and cyclin B1 proteins in the nucleus (Fig. 5). In SW480 cells, the expression of the S phase–specific cyclin A was moderately up-regulated, but the expression of the G2/M phase–specific cyclin B1 was prominently down-regulated, which is consistent with the S-phase arrest induced by β-lapachone at 24 hr post-treatment. In SW620 cells, both cyclin A and cyclin B1 expression was prominently up-regulated, a finding consistent with the late S- and G2/M-phase arrest induced by β-lapachone at 24 hr post-treatment. In DLD1 cells, cyclin A was not altered, but cyclin B1 was prominently down-regulated, which is consistent with the early S-phase arrest induced by β-lapachone at 24 hr post-treatment.

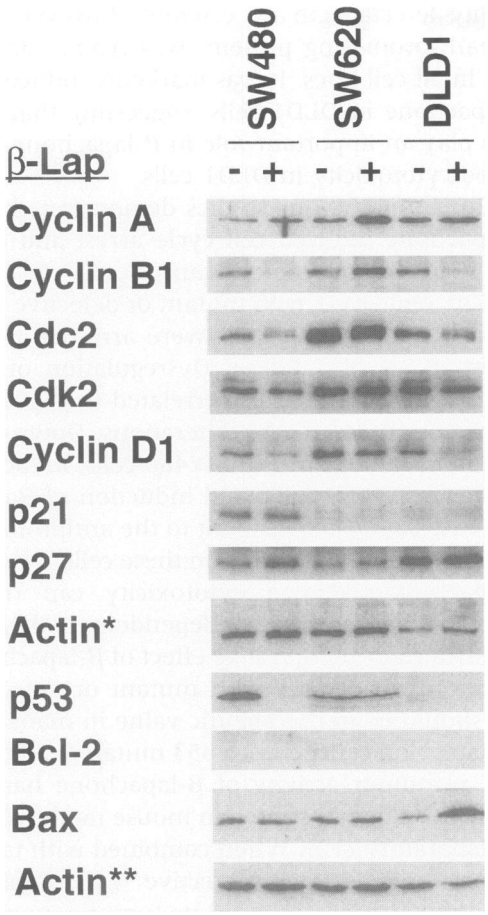

Fig. 5. Differential modulation of cell cycle and apoptosis regulatory proteins by β-lapachone in human colon cancer cells. SW480, SW620, and DLD1 cells were treated with DMSO alone (−) or 5 μM β-lapachone (+) for 4 hr, further incubated in drug-free medium for 20 hr, and then analyzed for nuclear (i.e., cyclin A, cyclin B1, cdc2, cdk2, cyclin D1, p21, p27, and actin*), and whole-cell (i.e., p53, Bcl-2, Bax, and actin**) protein levels by immunoblotting. Nuclear and whole-cell actin levels served as loading controls for nuclear and whole-cell protein, respectively.

We further examined the effects of β-lapachone on the expression of cyclin B1–and cyclin A-associated protein kinases. Cdc2, which interacts with cyclin B1 and cyclin A, was prominently down-regulated in both SW480 and DLD1 cells but not in SW620 cells (Fig. 5). Cdk2, which interacts with cyclins A, E, and D, was not significantly affected in any cell line.

Cyclin D1 plays a key role in regulating the G1 phase of the cell cycle. Its down-regulation in the nucleus is essential for the relocation of proliferating cell nuclear antigen and repair DNA synthesis (33). Since these colon cancer cell lines were arrested predominantly in S phase by β-lapachone, we would expect cyclin D1 to be down-regulated in the nucleus of these cells. As predicted, cyclin D1 was significantly reduced in the nucleus of SW480, SW620, and DLD1 cells (Fig. 5). Cyclin E, another G1-specific cyclin, was not affected by β-lapachone (data not shown).

We next examined the effects of β-lapachone on the expression of other cell cycle regulatory and apoptosis-related proteins. The p53 tumor suppressor is involved in the control of cell cycle checkpoints and apoptosis (34). As expected, p53 was quite abundant in mutant p53-expressing SW480 and SW620 cells, whereas it was not detectable in p53-deficient DLD1 cells (Fig. 5). After exposure to β-lapachone, mutant p53 in SW480 cells was drastically down-regulated to an almost undetectable level. However, in SW620 and DLD1 cells, p53 was not significantly affected by β-lapachone.

p21 and p27 are inhibitors of cyclin-dependent kinases. Induction of these inhibitors is associated with cell cycle arrest and apoptosis (35–37). As shown in Figure 5, the basal levels of p21 were readily detectable in SW480 cells but not in SW620 and DLD1 cells. In any cell line, p21 was not induced by β-lapachone at 24 hr post-treatment. p27 was readily detectable in all cell lines. It was moderately up-regulated by β-lapachone in SW480 cells.

Bcl-2 and its dominant inhibitor Bax protein are key regulators of apoptosis (38,39). Bcl-2 promotes cell survival, whereas Bax promotes cell death. As shown in Figure 5, Bcl-2 was not readily detectable in any cell line. The same Bcl-2 antibody detected Bcl-2 protein in other cell lines, such as breast cancer cell line MCF7 (data not shown). Its level was not affected by β-lapachone at 24 hr post-treatment. Unlike Bcl-2, Bax was readily detected in all cell lines. Its level was markedly up-regulated by β-lapachone in DLD1 cells. Bax was up-regulated in total protein extract (Fig. 5), but not in nuclear extract of DLD1 cells (data not shown), indicating that cytoplasmic Bax was up-regulated, which is consistent with its function in the cytoplasm.

Discussion

In this study, we demonstrated that human colon cancer cells SW480, SW620, and DLD1 were quite sensitive to β-lapachone-mediated cyto-

toxicity. These colon cancer cells were arrested in different stages of S phase, following β-lapachone treatment. The cell cycle alterations were congruous with changes in cell cycle regulatory proteins. In SW480 cells, consistent with the induced S-phase arrest, β-lapachone up-regulated the S phase–specific cyclin A, but down-regulated the G2/M phase–specific cyclin B1 and its associated kinase cdc2. In SW620 cells, consistent with the induced late S/G2-phase arrest, β-lapachone up-regulated both cyclin A and B1. In DLD1 cells, consistent with the induced early S-phase arrest, β-lapachone did not affect cyclin A, but down-regulated cyclin B1 and cdc2.

β-Lapachone-induced S-phase arrest is not peculiar to colon cancer cells, since S-phase blockage by β-lapachone is also found in human hepatoma cells (9) and Chinese hamster ovary cells (40). In prostate cancer cells, however, β-lapachone induces G1-phase arrest (6), indicating that different mechanisms for β-lapachone-mediated cytotoxicity may be involved in different cancer cell types.

β-Lapachone-induced cell death of these colon cancer cells is by apoptosis, as demonstrated by annexin V staining, flow-cytometric analysis of DNA content, and DNA laddering analysis. The apoptosis occurred quite slowly, over many hours, as in camptothecin-induced apoptosis in colon cancer cells (32).

As for other cancer cells, such as human promyelocytic leukemia cells and prostate cancer cells (6,7), the antiproliferative effects of β-lapachone on colon cancer cells can be through a p53-independent pathway, since cells with mutant or defective p53 were sensitive to β-lapachone. β-Lapachone did not affect p53 levels in SW620 and DLD1 cells. However, it drastically down-regulated the mutant p53 level to an almost undetectable level in SW480 cells. Since mutant p53 can be oncogenic (41) and enhance genetic instability (42), such strong down-regulation of mutant p53 may play an important role in β-lapachone-mediated cytotoxicity in SW480 cells.

p21, a p53 targeted gene product, was readily detectable in SW480 cells which have mutant p53, indicating that p21 can be regulated via a p53-independent mechanism. However, p21 was not detectable in SW620 and DLD1 cells. In any cell line, p21 was not induced by β-lapachone, suggesting that β-lapachone-mediated cytotoxicity in colon cancer cells was independent of the p21 pathway.

Bcl-2, a survival-promoting protein, was not readily detectable in any cell line. However, Bax, a death-promoting protein, was readily detectable in all cell lines. It was markedly induced by β-lapachone in DLD1 cells, suggesting that Bax may play an important role in β-lapachone-mediated cytotoxicity in DLD1 cells.

In summary, our studies demonstrated that β-lapachone induced cell cycle arrest and apoptosis in human colon cancer cells SW480, SW620, and DLD1 with mutant or defective p53. Various colon cancer cells were arrested at different stages of S phase. Dysregulation of cell cycle regulatory proteins correlated with β-lapachone-induced cell cycle alterations. Down-regulation of mutant p53 in SW480 cells, induction of p27 in SW480 cells, and induction of Bax in DLD1 cells may be pertinent to the antiproliferative effects of β-lapachone on these cells. Overall, β-lapachone-mediated cytotoxicity can occur through p53- and p21-independent pathways. The strong antiproliferative effect of β-lapachone on colon cancer cells with mutant or defective p53 should be of therapeutic value in managing human colon cancers with p53 mutations. The in vivo antitumor activity of β-lapachone has recently been demonstrated in mouse models from our laboratory (12). When combined with taxol, β-lapachone is very effective in inhibiting growth of ovarian and prostate tumor xenografts in mice with little host toxicity.

Acknowledgments

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