β-Lapachone-induced apoptosis is associated with activation of caspase-3 and inactivation of NF-κB in human colon cancer HCT-116 cells

Byung Tae Choi[a], JaeHun Cheong[b] and Yung Hyun Choi[c]

β-Lapachone is a naturally occurring quinone obtained from the bark of the lapacho tree (*Tabebuia avellanedae*) with cancer chemopreventive properties. The objective of the present study was to investigate the effect of β-lapachone on the cell growth and apoptosis in human colon carcinoma tumor cell line HCT-116. Exposure of HCT-116 cells to β-lapachone resulted in growth inhibition and induction of apoptosis in a dose-dependent manner as measured by hemocytometer counts, fluorescence microscopy and flow cytometric analysis. This increase in apoptosis was associated with a decrease in Bcl-2 protein expression, an increase in caspase-3 activity, a decrease in intact poly(ADP-ribose) polymerase protein levels and degradation of β-catenin. After β-lapachone treatment, the nuclear protein levels of NF-κB and the activity of NF-κB–DNA binding were markedly decreased. β-Lapachone treatment also resulted in inhibition of the transcriptional activity of NF-κB–luciferase reporter plasmid suggesting that β-lapachone-induced apoptosis may be partly regulated through the inactivation of NF-κB. *Anti-Cancer Drugs* 14:845–850 © 2003 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2003, **14**:845–850

Keywords: β-lapachone, apoptosis, Bcl-2, caspase-3, NF-κB

[a]Department of Anatomy, [c]Department of Biochemistry, Dong-Eui University College of Oriental Medicine and Research Institute of Oriental Medicine, Busan, Korea and [b]Department of Molecular Biology, Pusan National University, Busan, Korea.

Sponsorship: Supported by a grant (R05-2001-000-00480-0) from the Korea Science and Engineering Foundation.

Correspondence to Y. H. Choi, Department of Biochemistry, Dong-Eui University College of Oriental Medicine, Yangjung-dong, Busanjin-gu, Busan 614-052, Korea.
Tel: +82 51 850 7413; fax: +82 51 853 3578;
e-mail: choiyh@dongeui.ac.kr

Received 5 April 2003 **Revised form accepted** 20 August 2003

Introduction

β-Lapachone (3,4-dihydro-2,2-dimenthyl-2*H*-napthol [1,2-b]pyran-5,6-dione) is a naturally occurring quinone obtained from the bark of the lapacho tree (*Tabebuia avellanedae*) native to South America [1]. Unlike camptothecin, this compound acts as an inhibitor of DNA topoisomerase I catalytic activity without inducing topoisomerase I-cleavable complex [2]. β-Lapachone exhibits a number of pharmacological actions including anti-bacterial, anti-fungal, anti-trypanocidal and cytotoxic activities [3–5], which are linked to the formation of reactive oxygen species [6,7]. While β-lapachone has been shown to induce apoptosis in a variety of cancer cell lines [8–13], the mechanism of this observed programmed cell death has not been well described.

The purpose of this study was to investigate the pathway of β-lapachone-induced apoptosis and β-lapachone's effect on NF-κB activity in human colon cancer cells. We report here that exposure of HCT-116 cells to β-lapachone resulted in a dose-dependent growth inhibition and apoptosis. This increase in apoptosis by β-lapachone was associated with a decrease in Bcl-2 expression and an increase in caspase-3 activity. Down-regulation of NF-κB protein expression in nuclei by β-lapachone treatment was associated with decreased NF-κB–DNA binding and NF-κB transcriptional activity. These results suggest that β-lapachone-induced apoptosis may be regulated in part through inactivation of NF-κB in HCT-116 cells.

Materials and methods

Cell culture and β-lapachone

The human colon cancer HCT-116 cells were obtained from ATCC (Rockville, MD) and cultured in DMEM medium (Gibco/BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin in a 37°C incubator with 5% CO_2. β-Lapachone was purchased from Biomol (Plymouth Meeting, PA), dissolved in ice-cold absolute alcohol as a stock solution at 10 mM concentration and stored in aliquots at –20°C.

Growth inhibition study

For growth inhibition analysis, HCT-116 cells were plated at 1×10^3 cells per 60-mm plate and incubated for 24 h. Cells were cultured in the presence or absence of variable concentrations of β-lapachone in culture medium enriched with 10% FBS for 24 h. The cells were trypsinized, washed with phosphate-buffered saline (PBS) and the viable cells were scored using the Trypan blue method.

0959-4973 © 2003 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000097712.69687.bf

Assessment of DNA degradation by agarose gel electrophoresis

Cells were incubated with different concentrations of β-lapachone for 24 h and trypsinized. Cells were washed with PBS and resuspended in lysis buffer [1 mM EDTA, 10 mM Tris (pH 8.0), 1% SDS aand 1 μg/ml proteinase K]. After 1 h incubation at 37°C, RNase A was added and incubation continued for 1 h. Crude DNA preparations were extracted twice with phenol : chloroform : isoamyl alcohol (25 : 24 : 1). Cell lysate samples were subsequently run at 120 V on a 1.0% agarose gel containing ethidium bromide (EtBr; Sigma). The gel was examined on an ultraviolet light source and photographed.

Flow cytometry analysis

After treatment with β-lapachone the cells were collected by trypsinization, washed with cold PBS and resuspended in PBS. DNA contents of cells were measured using a DNA staining kit (CycleTEST Plus; Becton Dickinson, Heidelberg, Germany). Propidium iodide-stained nuclear fractions were obtained by following the kit protocol. Fluorescence intensity was determined using a FACScan flow cytometer and analyzed by CellQuest software (Becton Dickinson).

Western blot analysis and antibodies

Whole-cell lysates were lysed in extraction buffer as previously described [14]. For nuclear extracts, cells were harvested, washed with PBS and resuspended in cytoplasmic extraction buffer (10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl and 0.5 mM DTT). After 15 min, the cytoplasmic fraction was separated by centrifugation (14 000 *g* for 5 min at 4°C). The pellet was resuspended in nuclear extraction buffer (20 mM HEPES, 1.5 mM $MgCl_2$, 25% glycerol, 420 mM NaCl, 0.2 mM EDTA and 0.5 mM DTT) for 30 min on ice and centrifuged (14 000 *g* for 10 min at 4°C). Protein concentration determined with the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). For Western blot analysis, proteins were separated by SDS–PAGE and then electrotransferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH). The membrane was subjected to immunoblot analysis and proteins were visualized by the enhanced chemiluminescence (ECL) method (Amersham, Arlington Heights, IL). Monoclonal antibody to Bcl-2, and polyclonal antibodies to β-catenin, actin, Bax, NF-κB were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal anti-poly(ADP-ribose) polymerase (PARP) antibody was purchased from Calbiochem (Cambridge, MA). Peroxidase-labeled donkey anti-rabbit immunoglobulin and peroxidase-labeled sheep anti-mouse immunoglobulin were purchased from Amersham.

Caspase-3 activity assay

Caspase-3 activity was assayed by cleavage of Asp-Glu-Val-Asp (DEVD)-pNA using a commercially available kit (Clontech, Palo Alto, CA). Briefly, cells were cultured in the presence or absence of β-lapachone and then 2×10^6 cells were incubated with DEVD-pNA in the presence of DTT for 60 min at 37°C. The reaction was measured by changes in absorbance at 405 nm using the VERSAmax tunable microplate reader (Molecular Devices, Palo Alto, CA).

Electrophoretic mobility shift assay (EMSA)

EMSA was performed as follows. A reaction mixture of binding buffer (50 mM KCl, 20 mM HEPES–KOH, pH 7.5, 10 mM $MgCl_2$, 10% glycerol, 0.5 mM DTT and 1% NP-40), 0.5 ng ^{32}P-labeled oligonucleotide probe, 10 μg sonicated salmon sperm DNA, 2 μg poly(dI–dC) (Pharmacia Biotech, Piscataway, NJ) and 10 μg nuclear protein was incubated at 25°C for 10 min, and the reaction products separated on a 4% polyacrylamide gel in 0.25 × TBE (22.5 mM Tris–borate and 0.5 mM EDTA). The double-stranded DNA oligonucleotide for NF-κB is commercially available from Santa Cruz Biotechnology and contains the consensus binding site for NF-κB.

Transfections and luciferase assay

To measure NF-κB activation, cells were transiently transfected with a plasmid in which the luciferase gene was driven by four tandem NF-κB binding motifs (Clontech, Palo Alto, CA) using Lipofectamine transfection reagent (Gibco/BRL), according to the manufacturer's recommendations. After transfection for 6 h, cells were allowed to recover for 12 h and were then treated with β-lapachone for an additional 24 h. The cells were then lysed and luciferase activity in the lysates was assayed using a Dynatech ML1000 luminometer (Dynatech, Chantilly, VA). Luciferase activity was normalized according to protein content.

Results

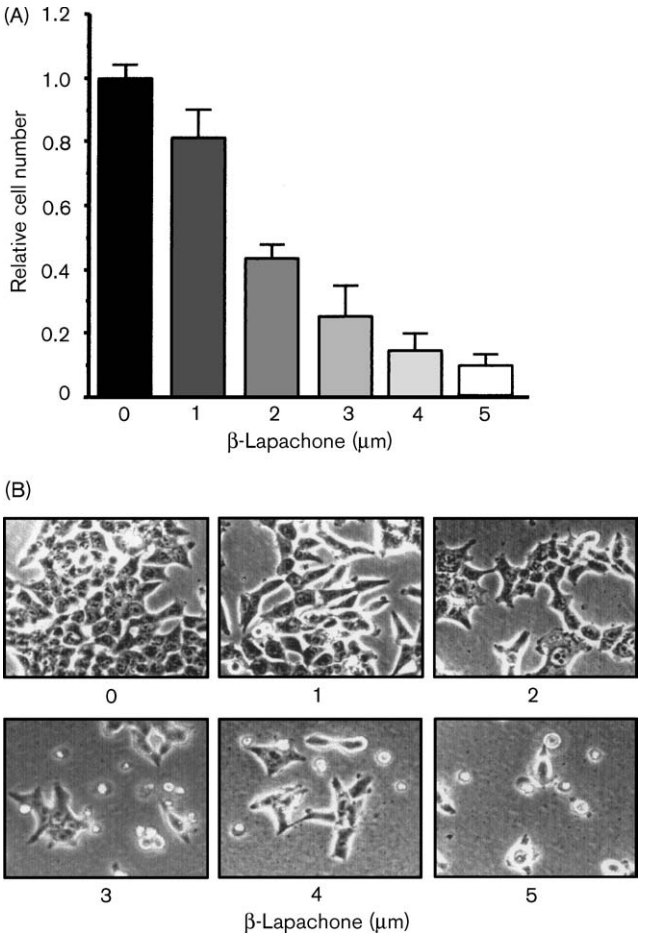
Growth Inhibition by β-lapachone

Initial experiments were designed to determine the growth-inhibitory activity of β-lapachone in a colon cancer cell model. HCT-116 cells were treated with β-lapachone for 24 h and viable cells were measured by hemocytometer counts of Trypan blue-excluding cells. Exposure of β-lapachone to HCT-116 colon cancer cells resulted in a significant decrease in viable cells in a concentration-dependent fashion (Fig. 1A). After exposure with β-lapachone, the cells became vacuolated and detached from the plate (Fig. 1B).

Apoptotic cell death by β-lapachone

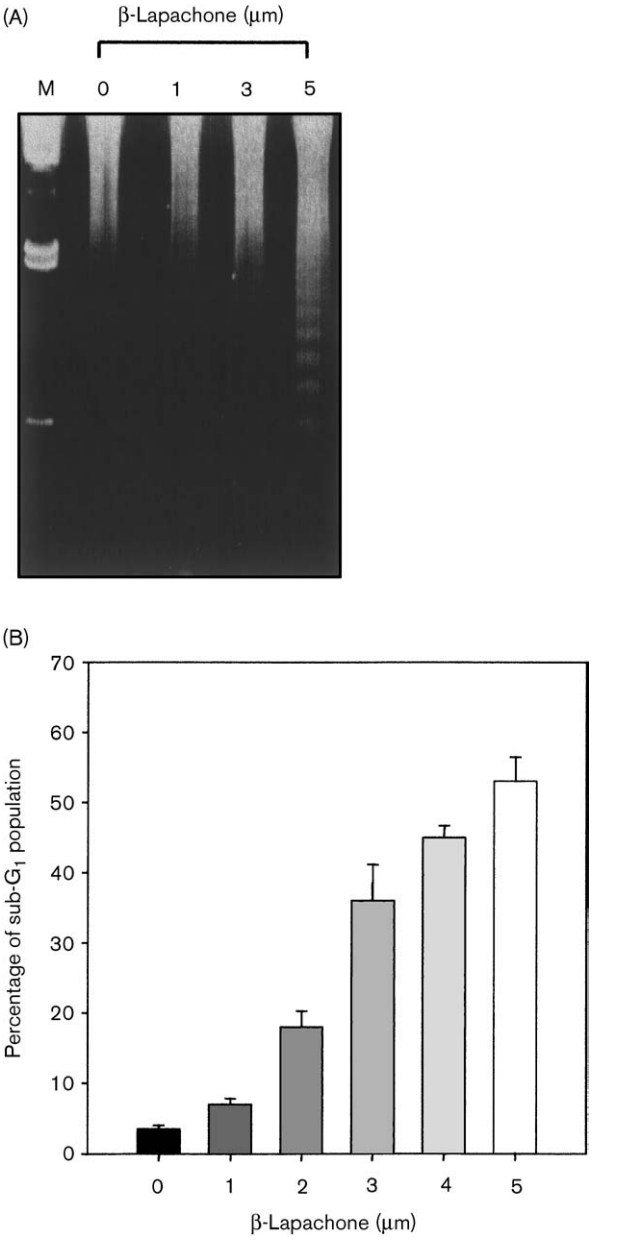
To determine whether an increase in apoptosis was associated with the observed decrease in cell number after β-lapachone treatment, HCT-116 cells were not exposed to β-lapachone (0) or treated with β-lapachone for 24 h, and DAPI staining and agarose gel electrophoresis were performed. Nuclei with chromatin condensation

Fig. 1



Growth inhibition and morphological changes of human colon cancer HCT-116 cells after treatment with β-lapachone. As described in Materials and methods, the cells were plated at 1×10^3 cells per 60-mm plate and incubated for 24 h. Cells were treated with variable concentrations of β-lapachone for 24 h. (A) The cells were trypsinized, washed with PBS and the viable cells were scored by hemocytometer counts of Trypan blue-excluding cells. Each point represents the mean ± SE of three independent experiments. (B) Cells were incubated with variable concentrations of β-lapachone. After 24 h incubation cells were sampled and examined under light microscopy. Magnification × 200.

Fig. 2



Exposure of HCT-116 cells to β-lapachone induces apoptosis. (A) After incubation of cells in the presence of various concentrations of β-lapachone for 24 h, DNA was extracted, and resolved in a 1.0% agarose gel and visualized using EtBr. (B) Cells treated for 24 h with increasing concentration of β-lapachone showed a dose-dependent increase in the number of apoptotic cells as measured by flow cytometry. The profile represents the increase of the sub-G_1 population and each point represents the mean of two independent experiments.

and formation of apoptotic bodies, a characteristic of apoptosis, were seen in cells cultured with β-lapachone in a dose-dependent manner, but very few in control cells (data not shown). In addition, agarose gel electrophoresis of DNA extracted from cells treated with β-lapachone showed DNA fragmentation (Fig. 2A), indicating that β-lapachone induced apoptosis in HCT-116 cells. Thus we quantified the extent of apoptosis by measuring the fraction of nuclei that contained subdiploid DNA content using flow cytometry. As shown by data in Figure 2(B), β-lapachone treatment for 24 h resulted in 35.8, 44.7 and 52.5% of apoptotic cells at 3, 4 and 5 μM. While the induction of apoptosis was almost negligible (4.7% compared to 3.2% of control) at the lowest concentration (1 μM), the highest concentration (5 μM) resulted in a significant increase in apoptosis as determined by flow cytometry.

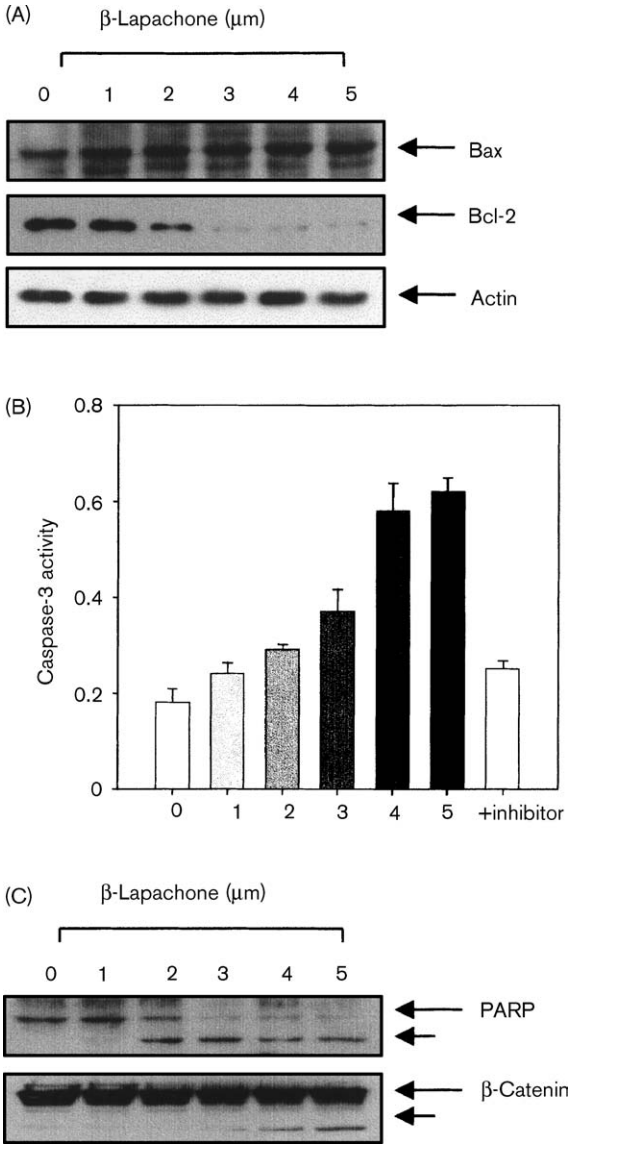
Down-regulation of Bcl-2 and activation of caspase-3 by β-lapachone

To investigate the apoptotic cascade involved by β-lapachone, HCT-116 cells were exposed to β-lapachone, and the levels of Bax and Bcl-2 expression, and *in vitro* caspase-3 activity levels were measured. Western immunoblotting indicated that treatments of cells with β-lapachone significantly resulted in a down-regulation of Bcl-2, however, Bax expression showed no change (Fig. 3A). Lysates equalized for protein from cells treated with β-lapachone were assayed for caspase-3 activity using DEVD-pNA as a substrate. β-Lapachone exposure significantly increased caspase-3 activity, which could be partially reversed by the addition of the caspase-3 inhibitor, Ac-DEVD-CHO (Fig. 3B). Associated with the increase in caspase-3 activity after β-lapachone exposure, protein analysis of PARP, a target of caspase-3 [15], demonstrated a loss of intensity of its 112-kDa band with accumulation of the 85-kDa band after β-lapachone treatment, suggesting cleavage by caspase-3 (Fig. 3C). As illustrated in Figure 3(C), treatment of HCT-116 cells with β-lapachone caused a dose-dependent proteolytic cleavage of β-catenin, which is also reported as a substrate for active caspase-3 during apoptosis [16].

Inactivation of NF-κB in β-lapachone-induced apoptosis

Since β-lapachone treatment induced apoptosis in HCT-116 cells and NF-κB is an important regulator of programmed cell death [17,18], we next investigated the effects of β-lapachone on the expression level and binding activity of NF-κB. HCT-116 cells were incubated in the presence or absence of β-lapachone for 24 h, harvested, and then nuclear extracts prepared and immunoblotting performed using an anti-NF-κB antibody. As shown by immunoblot analysis (Fig. 4A), β-lapachone treatment resulted in a dose-dependent inhibition of NF-κB protein expression. We further performed EMSA and reporter assay using an oligonucleotide containing the consensus binding site for NF-κB and NF-κB promoter construct. As compared to untreated controls, treatment with β-lapachone inhibited NF-κB DNA binding and the NF-κB promoter construct was significantly inactivated (Fig. 4B and C).

Fig. 3

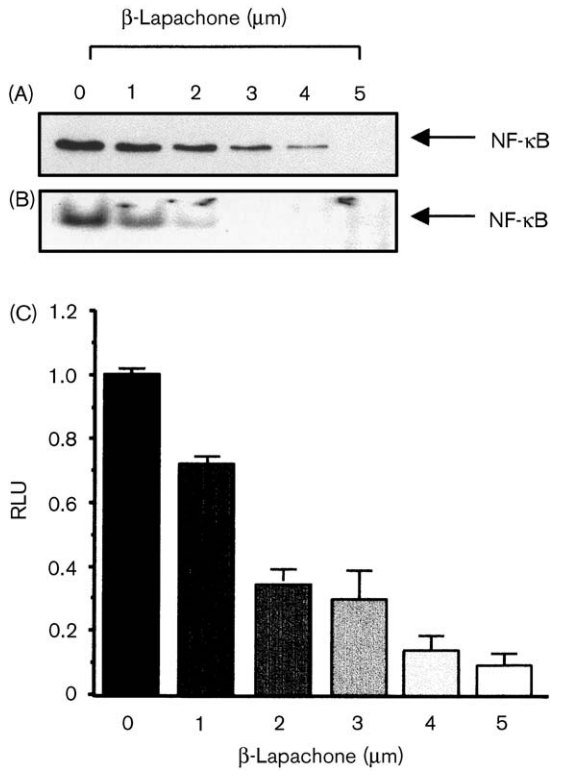


β-Lapachone exposure inhibits Bcl-2 expression and increases caspase-3 activity in HCT-116 cells. (A) Western blot analysis of Bax and Bcl-2 after β-lapachone treatment for 24 h. Whole-cell extracts were prepared, and the expression levels of Bax and Bcl-2 protein were examined by Western blotting with anti-Bax and Bcl-2 antibodies, and the ECL detection system. Actin was used as an internal control. (B) Cell lysates from cells treated with β-lapachone for 24 h were assayed for *in vitro* caspase-3 activity using DEVD-pNA as a substrate. β-Lapachone exposure significantly increased caspase-3 activity, which could be partially reversed by the addition of the caspase-3 inhibitor, Ac-DEVD-CHO. Each point represents the average of two independent experiments. (C) Cleavage of PARP and β-catenin protein during β-lapachone-mediated apoptosis in HCT-116 cells. Following treatment with different concentrations of β-lapachone for 24 h, equal intracellular proteins were resolved on 10% SDS–PAGE. Proteins were visualized by Western blotting using anti-PARP and anti-β-catanin antibodies, and ECL detection.

Discussion

Recently, increasing interest has been paid to β-lapachone as a candidate cancer chemopreventive agent. Several mechanisms of the anticarcinogenic effect of β-lapachone were investigated. β-Lapachone induces G_1 arrest of the cell cycle and induces apoptosis in various tumor cells, including human leukemic HL-60, breast carcinoma MCF-7 and prostate carcinoma PC3 cells [8–11], suggesting that β-lapachone interferes with proliferation and induces apoptosis in close association with the G_1 arrest. The present data clearly demonstrates that β-lapachone induces apoptosis in human colon cancer HCT-116 cells, which appears to account for its anti-proliferating activity. The induction of apoptotic cell death by β-lapachone was associated with characteristic

Fig. 4



Effect of β-lapachone on expression level, DNA binding and promoter activity of NF-κB in HCT-116 cells. (A) As detailed in Materials and methods, nuclear extracts from untreated control and cells treated with β-lapachone for 24 h were prepared, and 40 μg of protein was subjected to 10% SDS–PAGE and transferred onto nitrocellulose membranes. Western blot was detected with anti-NF-κB antibody and the ECL detection system. (B) EMSA was performed using an oligonucleotide containing the consensus binding site for NF-κB. Treatment with β-lapachone significantly inhibited the NF-κB–DNA binding in a concentration-dependent manner. (C) The NF-κB promoter constructs fused to the luciferase gene were transiently transfected into HCT 116 cells. As described in Materials and methods, the cells were treated with different concentrations β-lapachone for 24 h and compared the luciferase activity. The results are expressed as the mean ± SD of data from three separate experiments.

morphological changes, such as chromatin condensation and apoptotic bodies (Fig. 2A). We also observed internucleosomal DNA fragmentation and an increase of the sub-G_1 population by β-lapachone treatment in a concentration-dependent manner (Fig. 2B and C), which support the progress of apoptosis in the β-lapachone-treated cells.

Apoptosis is a tightly regulated process, which involves changes in the expression of distinct genes. One of the major genes involved in regulating apoptosis is the proto-oncogene Bcl-2 that encodes a 26-kDa mitochondria-associated protein [19]. It has been reported that Bcl-2 protects against multiple signals that lead to cell death, suggesting that Bcl-2 regulates a common cell death pathway and functions at a point where various signals converge. Bcl-2 also acts to inhibit cytochrome *c* translocation from mitochondria to cytoplasm, thereby blocking the caspase activation step of the apoptotic process [19]. Thus, it has been suggested that the ratio between the level of pro-apoptotic Bax protein and that of the anti-apoptotic factor Bcl-2 determines whether a cell responds to an apoptotic signal. In the present study, β-lapachone had no effect on the expression levels of Bax, whereas the expression of Bcl-2 protein was significantly decreased in a concentration-dependent fashion (Fig. 3A).

It becomes evident that the caspase family plays an important role in driving apoptosis and the key components of the biochemical pathways of caspase activation have been recently elucidated [20]. Among them, caspase-3, a main executioner of apoptosis, cleaves several important intracellular proteins, leading to the morphological and biochemical changes associated with apoptosis [19,20]. We observed that the induction of apoptosis after β-lapachone exposure was associated with the activation of caspase-3, and cleavages of PARP and β-catenin, its downstream targets (Fig. 3C). In addition, increased caspase-3 activity by β-lapachone was reversed by the addition of a specific inhibitor of caspase-3. Therefore, our data indicate that the pathway for apoptosis by β-lapachone in HCT-116 human colon cells is in part through a down-regulation of the anti-apoptotic protein Bcl-2 without alteration of the expression of Bax and activation of caspase-3.

Since NF-κB plays a pivotal role in the regulation of apoptosis, which binds to a set of DNA target sites and directly regulates expression of genes involved in cell cycle regulation and apoptosis [21], we explored the effects of β-lapachone on the expression levels, DNA binding and transcriptional activities of this transcription factor. β-Lapachone exposure of HCT-116 cells resulted in both the down-regulation of NF-κB expression in nuclei and reduction of NF-κB DNA binding (Fig. 4A and B). Transcriptional activity of NF-κB in β-lapachone-treated HCT-116 cells was also significantly down-regulated in a concentration-dependent manner (Fig. 4C) due to attenuated levels of NF-κB protein. Supporting the role of NF-κB as an anti-apoptotic factor includes the fact that it regulates the expression of multiple pro-survival Bcl-2 homologs [22] and cells overexpressed with NF-κB have been shown to be resistant to pro-apoptotic therapy [23]. Moreover, decreases in NF-κB binding and transcriptional activity by β-lapachone have been reported with tumor necrosis factor-treated human myeloid U937 cells [24]. Studies by Planchon *et al.* also demonstrated that Bcl-2 overexpression in human myeloid leukemia (HL-60) cells prevented all aspects of β-lapachone-mediated cytotoxicity including an enhancement of survival of β-lapachone-treated cells [25].

In summary, we demonstrated here that β-lapachone inhibits not only the expression of Bcl-2 protein, but also NF-κB DNA binding/transcriptional activities in cultured HCT-116 human colon cancer cells, suggesting that this represents an event in the apoptotic cascade by β-lapachone. Additionally, proteolytic degradation of several target proteins was associated with activation of caspase-3 in β-lapachone-treated HCT-116 cells.

References

1 Schaffner-Sabba K, Schmidt-Ruppin KH, Wehrli W, Wehrli W, Schuerch AR, Wasley JW. β-Lapachone: synthesis of derivatives and activities in tumor models. *J Med Chem* 1984; **27**:990–994.

2 Li CJ, Averboukh L, Pardee AB. β-Lapachone, a novel DNA topoisomerase I inhibitor with a mode of action different from camptothecin. *J Biol Chem* 1993; **268**:22463–22468.

3 Lopes JN, Cruz FS, Docampo R, Vasconcellos ME, Sampaio MC, Pinto AV, *et al. In vitro* and *in vivo* evaluation of the toxicity of 1,4-naphthoquinone and 1,2-naphthoquinone derivatives against *Trypanosoma cruzi*. *Ann Trop Med Parasitol* 1978; **72**:523–531.

4 Goijman SG, Stoppani AO. Effects of β-lapachone, a peroxide-generating quinone, on macromolecule synthesis and degradation in *Trypanosoma cruzi*. *Arch Biochem Biophys* 1985; **240**:273–280.

5 Guiraud P, Steiman R, Campos-Takaki GM, Seigle-Murandi F, Simeon de Buochberg M. Comparison of antibacterial and antifungal activities of lapachol and β-lapachone. *Planta Med* 1994; **60**:373–374.

6 Cruz FS, Docampo R, Boveris A. Generation of superoxide anions and hydrogen peroxide from β-lapachone in bacteria. *Antimicrob Agents Chemother* 1978; **14**:630–633.

7 Docampo R, Cruz FS, Boveris A, Muniz RP, Esquivel DM. β-Lapachone enhancement of lipid peroxidation and superoxide anion and hydrogen peroxide formation by sarcoma 180 ascites tumor cells. *Biochem Pharmacol* 1979; **28**:723–728.

8 Planchon SM, Wuerzberger S, Frydman B, Witiak DT, Hutson P, Church DR, *et al.* β-lapachone-mediated apoptosis in human promyelocytic leukemia (HL-60) and human prostate cancer cells: a p53-independent response. *Cancer Res* 1995; **55**:3706–3711.

9 Weller M, Winter S, Schmidt C, Esser P, Fontana A, Dichgans J, *et al.* Topoisomerase-I inhibitors for human malignant glioma: differential modulation of p53, p21, bax and bcl-2 expression and of CD95-mediated apoptosis by camptothecin and β-lapachone. *Int J Cancer* 1979; **73**: 707–714.

10 Wuerzberger SM, Pink JJ, Planchon SM, Byers KL, Bornmann WG, Boothman DA. Induction of apoptosis in MCF-7:WS8 breast cancer cells by β-lapachone. *Cancer Res* 1998; **58**:1876–1885.

11 Choi YH, Kang HS, Yoo MA. Suppression of human prostate cancer cell growth by β-lapachone via Inhibition of pRB phosphorylation and induction of Cdk inhibitor p21. *J Biochem Mol Biol* 2003; **36**:223–229.

12 Pink JJ, Wuerzberger-Davis S, Tagliarino C, Planchon SM, Yang X, Froelich CJ, *et al.* Activation of a cysteine protease in MCF-7 and T47D breast cancer cells during β-lapachone-mediated apoptosis. *Exp Cell Res* 2000; **255**:144–155.

13 Planchon SM, Pink JJ, Tagliarino C, Bornmann WG, Varnes ME, Boothman DA. β-Lapachone-induced apoptosis in human prostate cancer cells: involvement of NQO1/xip3. *Exp Cell Res* 2001; **267**:95–106.

14 Choi YH, Lee SJ, Nguyen P, Jang JS, Lee J, Wu ML, *et al.* Regulation of cyclin D1 by calpain protease. *J Biol Chem* 1997; **272**:28479–28484.

15 Tewari M, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, *et al.* Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* 1995; **81**:801–809.

16 Fukuda K. Apoptosis-associated cleavage of β-catenin in human colon cancer and rat hepatoma cells. *Int J Biochem Cell Biol* 1999; **31**:519–529.

17 Karin M, Lin A. NF-κB at the crossroads of life and death. *Nat Immunol* 2002; **3**:221–227.

18 Bours V, Bentires-Alj M, Hellin AC, Viatour P, Robe P, Delhalle S, *et al.* Nuclear factor-κB, cancer, and apoptosis. *Biochem Pharmacol* 2000; **60**:1085–1089.

19 Antonsson B. Bax and other pro-apoptotic Bcl-2 family 'killer-proteins' and their victim the mitochondrion. *Cell Tissue Res* 2001; **306**:347–361.

20 Shi Y. Mechanisms of caspase activation and inhibition during apoptosis. *Mol Cell* 2002; **9**:459–470.

21 Mayo MW, Baldwin AS. The transcription factor NF-κB: control of oncogenesis and cancer therapy resistance. *Biochem Biophys Acta* 2000; **1470**:55–62.

22 Pahl HL. Activators and target genes of Rel/NF-κB transcription factors. *Oncogene* 2000; **18**:6853–6866.

23 Anto RJ, Maliekal TT, Karunagaran D. L-929 cells harboring ectopically expressed RelA resist curcumin-induced apoptosis. *J Biol Chem* 2000; **275**:15601–15604.

24 Manna SK, Gad YP, Mukhopadhyay A, Aggarwal BB. Suppression of tumor necrosis factor-activated nuclear transcription factor-kappaB, activator protein-1, c-Jun N-terminal kinase, and apoptosis by β-lapachone. *Biochem Pharmacol* 1999; **57**:763–774.

25 Planchon SM, Wuerzberger-Davis SM, Pink JJ, Robertson KA, Bornmann WG, Boothman DA. Bcl-2 protects against β-lapachone-mediated caspase 3 activation and apoptosis in human myeloid leukemia (HL-60) cells. *Oncol Rep* 1999; **6**:485–492.