Synergistic antitumor effect of brusatol combined with cisplatin on colorectal cancer cells

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Abstract. Colorectal cancer (CRC) is a common and life-threatening type of malignant cancer, which is associated with a high mortality rate. Cisplatin (CDDP) is a commonly used chemotherapy drug with significant side effects. Brusatol (BR) is one of the principal chemical compounds isolated from the Chinese herb Bruceae Fructus, which has been reported to markedly inhibit the proliferation of numerous cancer cell lines. The present study aimed to investigate the possible synergistic anticancer effects of CDDP combined with BR on CT-26 cells, and to evaluate the underlying mechanisms of action. The growth inhibitory effects of BR, CDDP, and BR and CDDP cotreatment on CT-26 cells were assessed by MTT assay. Cell apoptosis were determined by flow cytometry and western blot analysis. The results indicated that compared with single-agent treatment, cotreatment of CT-26 cells with CDDP and BR synergistically inhibited cell proliferation and increased cellular apoptosis. Furthermore, treatment of CT-26 cells with CDDP and BR resulted in a marked increase in the release of cytosolic cytochrome *c*, decreased expression of procaspase-3 and procaspase-9, and upregulation of the B-cell lymphoma 2 (Bcl-2)-associated X protein/Bcl-2 ratio compared with treatment with BR or CDDP alone. These results strongly suggested that the combination of CDDP and BR was able to produce a synergistic antitumor effect in CRC cells, thus providing a solid foundation for further development of this combination regimen into an effective therapeutic method for CRC.

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Abbreviations: BR, brusatol; CDDP, cisplatin; CRC, colorectal cancer; PI, propidium iodide; PS, phosphatidylserine; TBS-T, Tris-buffered saline containing 0.1% Tween-20

Key words: brusatol, cisplatin, synergistic effect, apoptosis, CT-26

Introduction

Colorectal cancer (CRC) is one of the most common types of cancer in the Western world, and is the second most common cause of cancer-associated mortality in the world (1-3). It is estimated that >1.3 million people worldwide are affected by CRC annually (4). The worldwide threat posed by CRC is increasing, which is largely due to an aging population and the increased adoption of Westernized diets in developed and developing countries (5-7).

At present, surgery, chemotherapy and radiotherapy are applied as the main therapeutic approaches for the treatment of CRC in clinical practice (8-10). Among them, systemic chemotherapy is regarded as a promising therapeutic approach, due to its ability to elicit a good therapeutic response, improve quality of life and prolong survival (9). Cisplatin (CDDP) is one of the most frequently used chemotherapy drugs, which exerts a strong therapeutic effect; however, some tumor types, including colon, ovarian and lung cancer, have not exhibited satisfactory results in response to CDDP (11). Therefore, the enhancement of efficacy by specific compounds may provide a valuable contribution to the treatment of cancer based on CDDP chemotherapy. For this purpose, the development of

combined application of CDDP with other safe and effective agents has been the focus of research.

Currently, herbal medicines or natural compounds, either used as a monotherapy or combined with conventional chemotherapeutic agents, have been reported to exert beneficial effects on the treatment of various types of cancer (12). Bruceae Fructus refers to the fruit of *Brucea javanica* (L.) Merr. ('Ya-Dan-Zi' in Chinese), and was initially recorded in Supplementations to the Compendium of Chinese Materia Medica. Bruceae Fructus has been applied to treat various ailments, including cancer, amoebic dysentery and malaria, since the Ming Dynasty (1364-1644 AD) (13,14). The antitumor activity of Bruceae Fructus is regarded as one of the most important biological activities of this plant, and it has been commonly prescribed to treat various types of cancer in China. In previous years, emerging evidence has been provided with regards to the antitumor activity of Bruceae Fructus (13).

B. javanica is rich in quassinoids, which are considered the predominant ingredients responsible for its marked antitumor activity (15). Brusatol (BR; $C_{26}H_{32}O_{11}$), the chemical structure of which is presented in Fig. 1, is one of the major quassinoids isolated from *B. javanica*. This compound has been reported to exert marked anti-inflammatory (16), antimalarial (17) and antitumor activities (18-21). In addition, BR has been demonstrated to uniquely block the nuclear factor erythroid 2-related factor 2 pathway, thus sensitizing various cancer cells *in vitro* and A549 mouse xenografts to chemotherapeutic agents, including CDDP. These findings suggested that BR may be considered a promising candidate for combating chemoresistance and for further development into an effective adjuvant for chemotherapy drugs (22). However, whether CDDP combined with BR exerts synergistic antitumor activity on CT-26 CRC cells remains unclear. Therefore, the present study aimed to investigate the possible effects of BR alone, and in combination with CDDP, on CT-26 CRC cells, and to evaluate the potential mechanism.

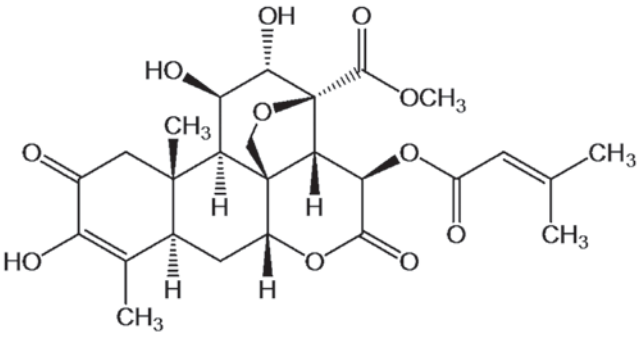

Figure 1. Chemical structure of brusatol.

Materials and methods

Reagents and chemicals. BR (CAS: 14907-98-3; PubChem CID: 73432) was isolated from Bruceae Fructus in our laboratory. Briefly, the seeds of *B. javanica* were extracted twice with 95% EtOH for 2 h, concentrated to give a crude extract and suspended in H_2O. The aqueous layer was further extracted with EtOAc and evaporated under vacuum to afford extracts and subjected to silica gel column chromatography eluted with a gradient of CH_2Cl_2-MeOH (100:0-100:20). The CH_2Cl_2-MeOH (100:1) eluate was evaporated to yield a residue, which was further purified by repeated recrystallization to obtain a white powder. The chemical structure of BR was confirmed and purity was determined to be >98% (21). CDDP and MTT were obtained from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany). Antibodies against caspase-3 (sc-113427) and caspase-9 (sc-56073), cytochrome *c* (sc-13156), B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax; sc-20067), Bcl-2 (sc-509) and β-actin (sc-47778) were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). All other chemicals and reagents were of analytical grade.

Cell culture. The murine CT-26 CRC cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). CT-26 cells were routinely grown in RPMI-1640 medium supplemented with 10% fetal bovine serum (both from Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 1% penicillin-streptomycin at 37˚C in a humidified atmosphere containing 5% CO_2.

In vitro cytotoxicity assays. *In vitro* BR and CDDP cytotoxic effects against the CRC cell line were measured using an MTT assay. Briefly, CT-26 cells in logarithmic growth were seeded onto a 96-well plate at a density of $4x10^3$ cells/well. After 24 h of incubation at 37˚C, fresh medium containing a series of concentrations of BR (0.05, 0.15, 0.45, 1.35, 4.05 and 12.15 μg/ml) and CDDP (0.05, 0.15, 0.45, 1.35, 4.05 and 12.15 μg/ml) was added at 100 μl/well; each concentration was used to treat six replicate wells. After 48 h of incubation at 37˚C, the cells were further incubated with MTT (10 mg/ml) at 37˚C for 4 h. The supernatant was then removed and the precipitate was dissolved with 100 μl dimethyl sulfoxide. Absorbance was measured using a microplate reader (EXL808; BioTek Instruments, Inc., Winooski, VT, USA) at a wavelength of 490 nm. Cytotoxicity was expressed as the concentration of BR and CDDP that inhibited cell growth by 50% [half maximal inhibitory concentration (IC_{50}) value]. The inhibitory rate was calculated according to the following formula: Inhibitory rate (%) = $(1\text{-}OD_{experiment\ group}/OD_{control\ group}) \times 100\%$; where OD refers to optical density. The possible synergistic effect of BR combined with CDDP was investigated by exposing CT-26 cells to various concentrations of each agent alone or in combination for 48 h. The synergistic effect was assessed using CalcuSyn software 2.0 (Biosoft, Cambridge, UK), which determines the combination index (CI) based on that described by Chou and Talalay (23,24). CI=1, CI<1 and CI>1 represented an additive effect, synergism and antagonism, respectively (23).

Morphological observation of nuclear alterations. CT-26 cancer cells were grown on coverslips placed in 6-well plates and were treated with a single drug (BR or CDDP) or combination for 48 h (incubation at 37˚C). After washing twice, Hoechst 33342 (Hoechst staining kit; Beyotime Institute of Biotechnology, Beijing, China) was used to stain the cells for 1 h at room temperature. Subsequently, cell morphology was observed and images were captured from random visual fields using a fluorescence microscopy (Zeiss GmbH, Jena, Germany).

Flow cytometric analysis of apoptosis. The Annexin V-fluorescein isothiocyanate (FITC) kit (Thermo Fisher Scientific, Inc.) was

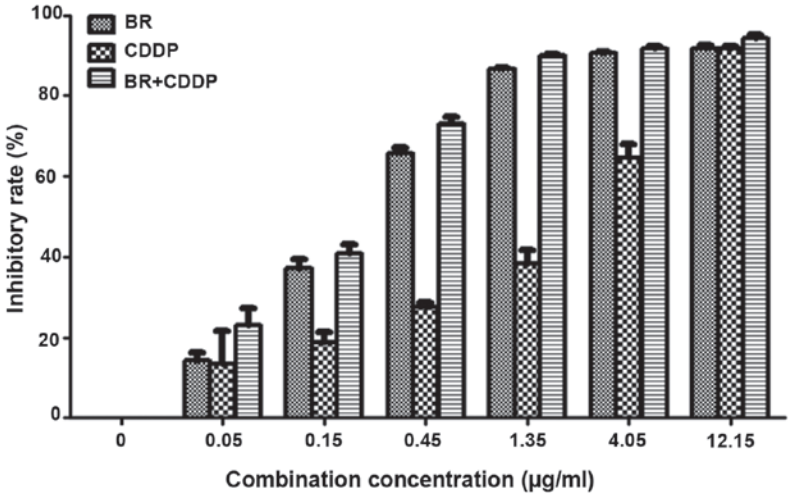

Figure 2. Inhibitory rate of CT-26 cells following treatment with various concentrations of BR (0.75-15 μg/ml) and CDDP (0.75-15 μg/ml) either alone or in combination. Data are presented as the means ± standard deviation of three independent experiments. BR, brusatol; CDDP, cisplatin.

Table I. IC_{50} values of BR and CDDP either alone or in combination on CT-26 cells.

Agent	IC_{50} value (μg/ml)
BR	0.27±0.01
CDDP	1.44±0.22
BR + CDDP	0.19±0.02

BR, brusatol; CDDP, cisplatin; IC50, half maximal inhibitory concentration.

used to determine cellular apoptosis. After exposure to BR (0.27 μg/ml), CDDP (1.44 μg/ml), or their combination for 48 h in 37˚C, CT-26 cells were collected, washed twice with PBS and subjected to centrifugation at 180 x g for 5 min at room temperature. Subsequently, the cell pellet was resuspended and treated with Annexin V-FITC and propidium iodide (PI) solutions. After incubating for 15 min at room temperature in the dark, additional Annexin V binding buffer (400 μl) was added to each tube and the cells were analyzed using a Cytomics™ FC500 flow cytometer (Beckman Coulter, Inc., Brea, CA, USA) and disposed with FlowJo 7.6.5. (FlowJo, LLC, Ashland, OR, USA).

Western blot analysis. After treatment, the cells were harvested and lysed with radioimmunoprecipitation assay buffer (Cell Signaling Technology, Inc., Boston, MA, USA) supplemented with cocktail (Roche, Penzberg, Germany). Protein concentration was determined using a BCA Protein Assay kit (cat. no. 23225, Thermo Fisher Scientific, Inc.) and about 40 μg protein were separated with 10% SDS-PAGE by electrophoresis and were transferred to polyvinylidene fluoride (PVDF) membranes (Immobilon; EMD Millipore, Billerica, MA, USA) using trans-blotting apparatus (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Non-fat milk (5%, w/v) dissolved in Tris-buffered saline containing 0.1% Tween-20 (TBS-T) was used to block the PVDF membranes. The membranes were then incubated with the following primary antibodies: Procaspase-3 (1:200), procaspase-9 (1:200), cytochrome *c* (1:200), Bcl-2 (1:200), Bax (1:200) and β-actin (Santa Cruz Biotechnology, Inc.) overnight at 4˚C. After washing with TBS-T three times, the membranes were incubated with the appropriate secondary antibodies (1:1,000; sc-2350, sc-2005 and sc-2370; Santa Cruz Biotechnology, Inc.). Finally, the protein bands were developed using enhanced chemiluminescence western blot detection reagents (GE Healthcare, Chicago, IL, USA) and were analyzed using ImageJ software 1.51s (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. SPSS 19.0 (IBM Corp., Armonk, NY, USA) was used to conduct all statistical analyses. Data are presented as the means ± standard deviation. One-way analysis of variance was used for multiple group comparisons, followed by Dunnett's test to detect intergroup differences. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Synergistic cytotoxic effects of BR in combination with CDDP on CT-26 CRC cells. To explore the possible synergistic cytotoxicity of BR in combination with CDDP, the present study investigated the effects of BR and CDDP cotreatment on CT-26 cell viability using an MTT assay. CT-26 cells were treated with various concentrations of BR (0.05, 0.15, 0.45, 1.35, 4.05 and 12.15 μg/ml) and CDDP (0.05, 0.15, 0.45, 1.35, 4.05 and 12.15 μg/ml) for 48 h, either alone or in combination.

The inhibitory effects on the proliferation of CT-26 cells and IC_{50} values are presented in Fig. 2 and Table I, respectively. Following treatment with BR and CDDP for 48 h, the viability of CT-26 cells was reduced in a dose-dependent manner, with IC_{50} values of 0.27±0.01 and 1.44±0.22 μg/ml, respectively. When BR was combined with CDDP at a constant concentration ratio of 1:1, cell growth inhibition was markedly enhanced compared with single-agent treatment; the IC_{50} value of BR and CDDP cotreatment was 0.19±0.02 μg/ml.

The effects of CDDP and BR cotreatment on cell proliferation were revealed to be synergistic, as determined by calculating the CI values (Fig. 3). Isobologram analysis indicated that the

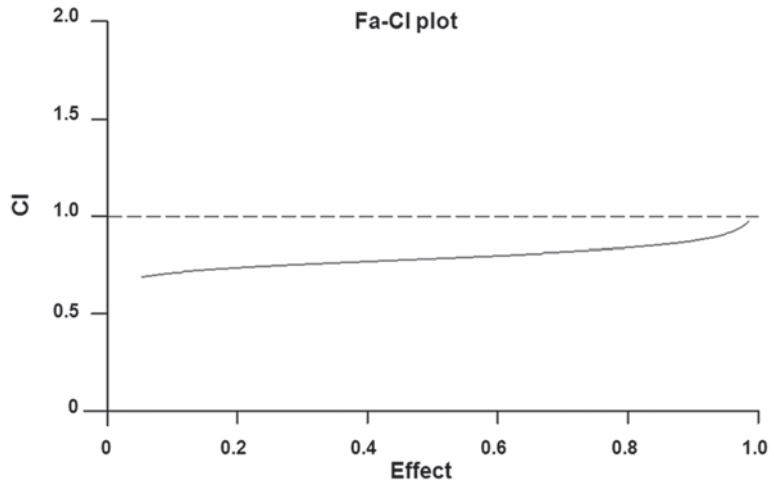

Figure 3. Fa-CI plot for brusatol and cisplatin cotreatment on CT-26 cells. CI, combination index.

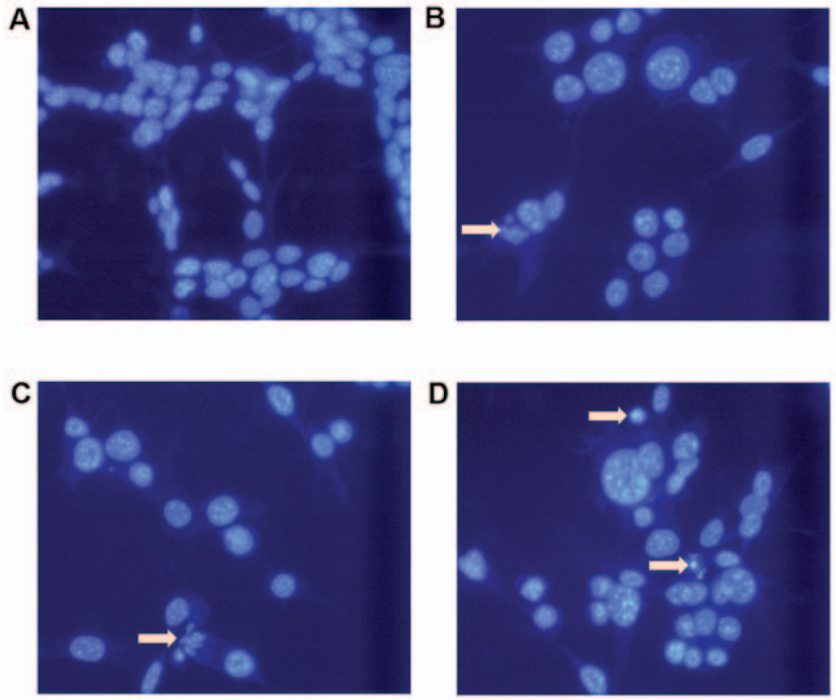

Figure 4. Cellular apoptosis observed with Hoechst 33342 staining (magnification, x200). CT-26 cells were treated with BR (0.27 μg/ml), CDDP (1.44 μg/ml), or their combination for 48 h. Chromatin condensation, nuclear fragmentation and apoptotic bodies are indicated by small arrows. (A) Control group; (B) BR (0.27 μg/ml)-treated group; (C) CDDP (1.44 μg/ml)-treated group; and (D) BR (0.27 μg/ml) + CDDP (1.44 μg/ml)-treated group. BR, brusatol; CDDP, cisplatin.

CI value was <1, thus suggesting that there was a synergistic effect of BR in combination with CDDP on CT-26 cell inhibition.

Morphological alterations in CT-26 cells. To investigate whether cellular apoptosis was involved in the cytotoxic effects of BR and CDDP cotreatment on CT-26 CRC cells, morphological alterations were observed using Hoechst 33342 nuclear staining (Fig. 4). Compared with the control cells, cells treated with BR and CDDP underwent chromatin condensation, and nuclear fragmentation and shrinkage, which are characteristics of apoptosis. Compared with in the BR and CDDP single treatment groups, cells treated with a combination of BR and CDDP exhibited a more obvious increase in the levels of apoptotic chromatin condensation and the number of dead cells.

Synergistic induction of apoptosis of CT-26 cells by BR and CDDP. To further confirm whether the antitumor effects of BR and CDDP cotreatment on CT-26 cells were associated with the induction of apoptosis, Annexin V/PI double staining was used to detect apoptosis of CT-26 cells, which were treated with BR, CDDP and their combination. The proportions of early and late apoptotic cells were quantified using flow cytometric analysis, after labeling cells with PI and Annexin V. As shown in Fig. 5, there was a marked increase in the number of apoptotic cells when CT-26 cells were treated with BR or CDDP. The results indicated that BR and CDDP, either individually or in combination, were able to generate a significant increase in the apoptotic population of CT-26 cells ($P<0.01$; Fig. 5B). Compared with the BR or CDDP groups, a

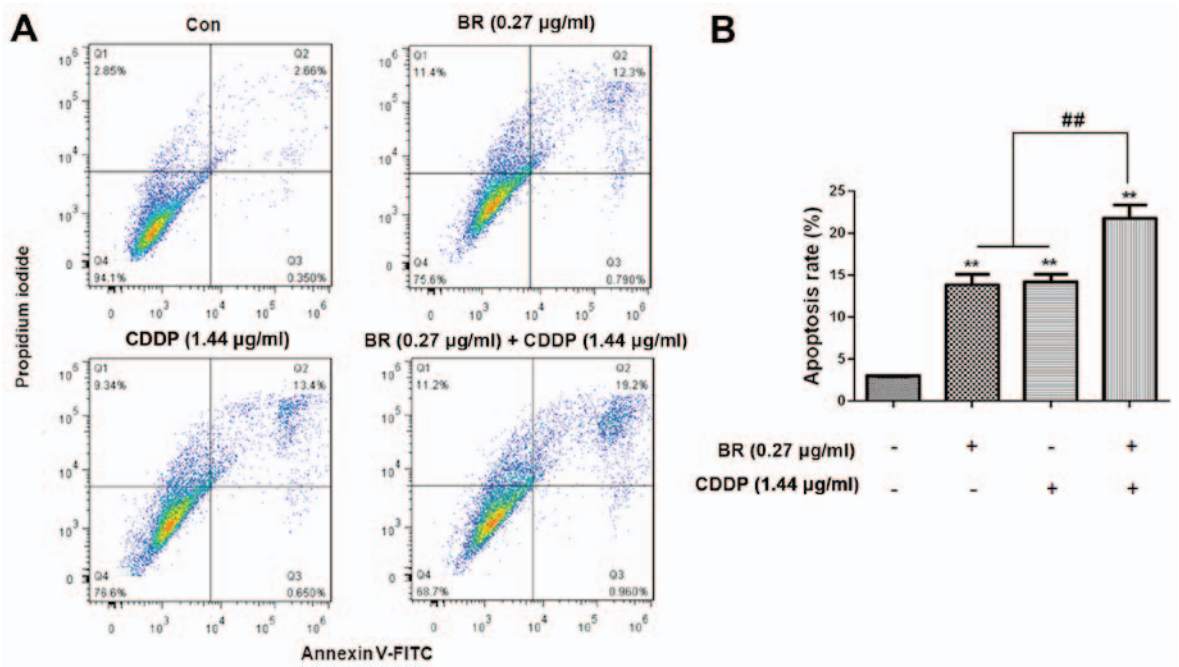

Figure 5. Apoptosis of CT-26 cells mediated by BR and CDDP, alone or in combination. (A) Apoptosis was measured by flow cytometry after PI/Annexin V-FITC staining. Q1, PI^+ (cells undergoing necrosis); Q2, Annexin V-$FITC^+$ PI^+ (cells in the late period of apoptosis and undergoing secondary necrosis); Q3, Annexin V-$FITC^+$ PI^- (cells in the early period of apoptosis); Q4, Annexin V-$FITC^-$ PI^- (living cells). Total apoptotic rate was calculated as Q2 + Q3. (B) Apotoic rates were calculated. The proportion of early and late apoptotic cells stained with Annexin V and PI is presented for each group. Data are presented as the means ± standard deviation of three independent experiments. **P<0.01 compared with the Con group; $^{\#\#}$P<0.01 compared with the BR and CDDP monotherapy groups. BR, brusatol; CDDP, cisplatin; Con, control; FITC, fluorescein isothiocyanate; PI, propidium iodide

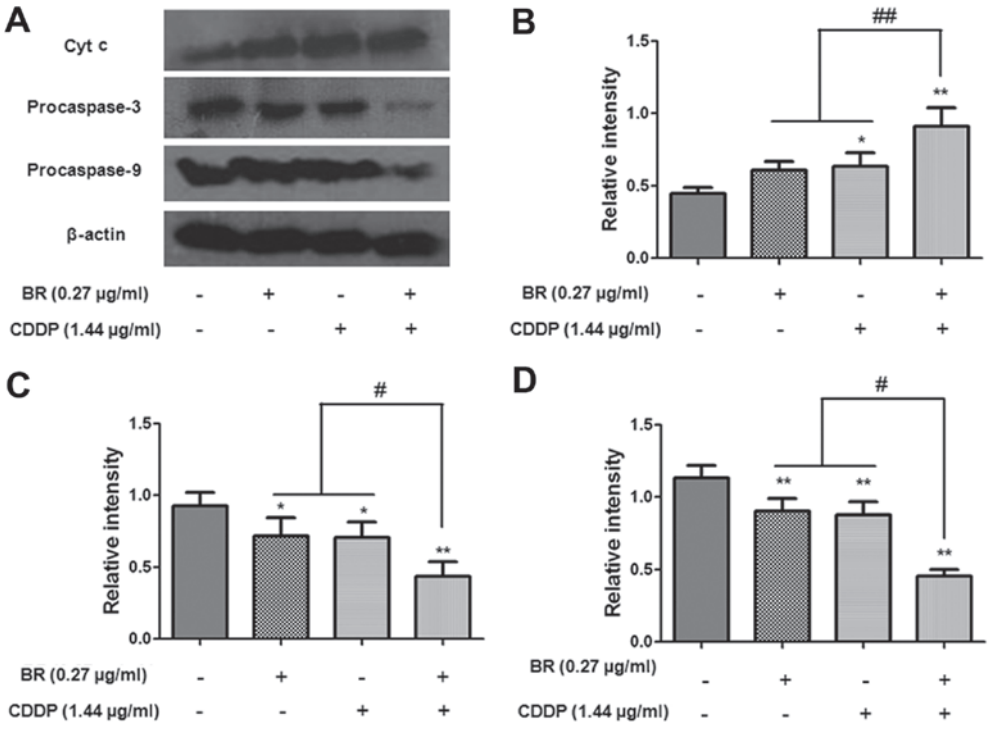

Figure 6. Protein expression levels of procaspase-3, procaspase-9 and cyt *c* in CT-26 cells treated with BR and CDDP, alone or in combination. (A) Total cell extracts were prepared and subjected to western blot analysis to monitor the protein expression levels of procaspase-3, procaspase-9 and cyt *c* in CT-26 cells. β-actin was used as the protein loading control. (B-D) Protein expression levels (relative to β-actin) of (B) cyt *c*, (C) procaspase-3 and (D) procaspase-9 were determined. All data are presented as the the means ± standard deviation of at least three independent experiments. *P<0.05 and **P<0.01 compared with the control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared with the BR or CDDP monotherapy groups. BR, brusatol; CDDP, cisplatin; cyt *c*, cytochrome *c*.

significantly greater apoptotic rate was observed in the BR and CDDP cotreatment group (P<0.01; Fig. 5B).

Effects of BR and CDDP on the expression levels of apoptosis-associated proteins in CT-26 cells. According to the

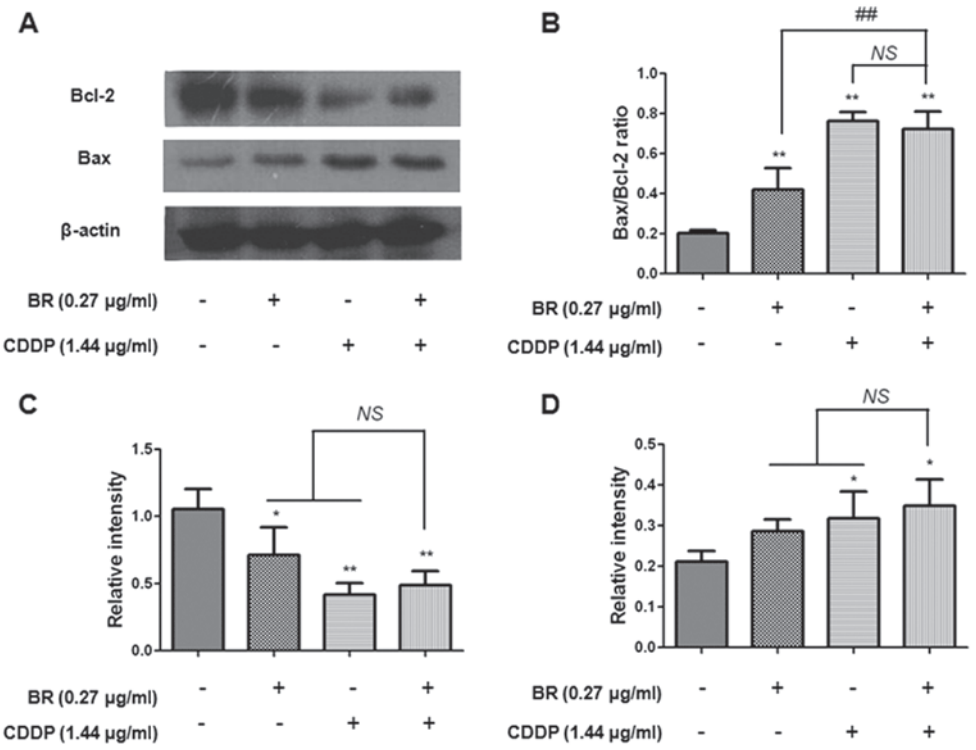

Figure 7. Protein expression levels of Bax, Bcl-2 and Bax/Bcl-2 ratio in CT-26 cells treated with BR and CDDP, alone or in combination. (A) Total cell extracts were prepared and subjected to western blot analysis to monitor the protein expression levels of Bax and Bcl-2 in CT-26 cells. β-actin was used as the protein loading control. (B) Bax/Bcl-2 ratio, and protein expression levels (relative to β-actin) of (C) Bcl-2 and (D) Bax were determined. All data are presented as the means ± standard deviation of at least three independent experiments. *P<0.05 and **P<0.01 compared with the control group; $^{\#\#}$P<0.01 compared with the BR or CDDP monotherapy groups; NS, no statistical significance, P>0.05. Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; BR, brusatol; CDDP, cisplatin

aforementioned results, the present study aimed to further determine the mechanisms underlying the synergistic antitumor effects of BR and CDDP. Since BR and CDDP cotreatment markedly induced synergistic regulation of apoptosis, the present study focused on the molecular mechanisms underlying apoptosis. In the present study, western blot analysis was used to detect the protein expression levels of procaspase-3, procaspase-9, cytochrome *c*, Bax and Bcl-2. As shown in Fig. 6, the expression levels of procaspase-3 and procaspase-9 were markedly decreased following treatment with BR or CDDP alone. Compared with in the monotherapy groups, BR and CDDP cotreatment significantly downregulated the protein expression levels of procaspase-3 and procaspase-9 (P<0.05) and upregulated the protein expression levels of cytosolic cytochrome *c* (P<0.01). The present study also measured the expression levels of Bax and Bcl-2 (Fig. 7). Treatment with CDDP was able to markedly increase the expression levels of Bax (P<0.05) and decrease Bcl-2 expression (P<0.01). In addition, BR monotherapy significantly decreased the expression levels of Bcl-2 (P<0.05). However, there was no significant difference between BR or CDDP monotherapy and cotreatment on the expression levels of Bax and Bcl-2. Furthermore, the Bax/Bcl-2 ratio was also increased (P<0.01) following monotherapy or cotreatment. These results indicated that BR and CDDP induced cellular apoptosis via a caspase-dependent signaling pathway.

Discussion

The present study aimed to evaluate the synergistic effects of BR and CDDP on CT-26 CRC cells and to evaluate the possible underlying mechanism. BR is the major active constituent of *B javanica,* and has been reported to exert potent anti-inflammatory (16), antimalarial (17) and antitumor activities (18-21). CDDP is a chemotherapy drug commonly used in cancer therapy; however, the side effects, including digestive tract reactions, renal toxicity, bone marrow suppression and auditory neurotoxicity, cannot be ignored (25). Furthermore, long-term use of CDDP can induce drug resistance (26,27). Compared with single-drug therapy, it has been reported that combination therapy offers numerous advantages, including several critical molecular targets, lower dose and toxicity, and increased sensitivity (28). Therefore, the present study investigated the synergistic effects of BR and CDDP on CT-26 cells.

The proliferative capacity of tumor cells is deemed vital for the growth and development of tumors (29). The present study demonstrated that a series of concentrations of BR and CDDP dose-dependently suppressed the proliferation and growth of CT-26 cells (Fig. 2). In addition, the results revealed that BR may exhibit a synergistic effect with CDDP on CT-26 cells, with a CI value <1.

Apoptosis serves a central role in regulating normal tissue equilibrium, and dysregulation of apoptosis presents a key factor in the growth of cancer (30). Therefore, strategies that target the apoptotic process may inhibit CRC development. Apoptotic cells exhibit characteristics, including cell shrinkage, and chromatin and nuclear condensation (31,32). To determine whether the inhibition of CT-26 cellular proliferation induced by BR and CDDP was associated with apoptosis, morphological alterations were detected by Hoechst 33342 staining following treatment with BR and CDDP for 48 h. The treated

cells displayed marked apoptotic characteristics, including cell shrinkage, formation of small vesicles, cytoplasmic condensation, pyknotic chromatin and nuclear fragmentation (Fig. 4). The nuclei of CT-26 cells in the cotreatment group appeared to be slightly smaller with brighter fluorescence compared with those of the monotherapy and control groups.

During early apoptosis, phospholipid asymmetry takes place prior to disintegration of the cellular membrane (33,34). Phosphatidylserine (PS) may translocate to the outer layer of the plasma membrane from the inner layer, where it is finally exposed to the external surface of the cell. Therefore, surface exposure of PS is regarded as a sensitive marker for assessing cellular membrane function and apoptosis. Annexin V is a type of calcium-dependent phospholipid-binding protein with a high affinity for PS; its application with PI (a supravital fluorescent dye) is commonly used to detect apoptotic and/or necrotic cells (34,35). To further quantify the apoptotic rate of CT-26 cells following various treatments, cells were stained with Annexin V and PI, and were subjected to flow cytometry. Compared with the percentage of apoptotic cells in the control group (3.00%), BR, CDDP and cotreatment significantly increased the percentage of apoptotic cells to 13.88, 14.21 and 21.81%, respectively; apoptotic rate was relatively higher in the BR and CDDP cotreatment group.

Activation of caspase cascades is vital for the initiation of apoptosis (27,36). It has been reported that the initiation of apoptosis involves the participation of at least two distinct apoptotic pathways, including the intrinsic mitochondrial apoptotic pathway, which is associated with caspase-9 activation, and the extrinsic apoptotic pathway, which is associated with caspase-3 activation (37,38). To elucidate the molecular mechanism underlying the apoptosis of CT-26 cells induced by BR and CDDP cotreatment, the present study further investigated the possible activation of intrinsic and extrinsic caspase cascades. In the present study, the protein expression levels of procaspase-3 and procaspase-9 in CT-26 cells treated with BR or CDDP monotherapy, or with a combination of BR and CDDP, were significantly decreased compared with in the untreated cells ($P<0.05$), whereas the expression of cytosolic cytochrome *c* was significantly upregulated ($P<0.05$). BR combined with CDDP led to synergistic regulation of the protein expression of initiator and effector caspases in CT-26 cells (Fig. 6). Therefore, it may be suggested that apoptosis of CT-26 cells is induced by BR and CDDP via downregulation of procaspase-3 and procaspase-9, and upregulation of cytochrome *c*, which may be associated with both intrinsic and extrinsic mitochondrial pathway.

The Bcl-2 family proteins, including Bcl-2 and Bax, also serve an important role in regulation of the mitochondrial apoptotic pathway (39). Released cytochrome *c* binds with cytosolic apoptosis protease activating factor, and induces the activation of caspase-9 (38). Bcl-2 suppresses the initiation of apoptosis and promotes cell survival by inhibiting the release of cytochrome *c*. Conversely, Bax elicits apoptosis and evoked cell death through its promotion of cytochrome *c* release from the mitochondria (40). Positive modulation of the Bax/Bcl-2 ratio can lead to decreased mitochondrial membrane potential and the release of cytochrome *c*, thereby contributing to activation of the intrinsic apoptotic pathway. Therefore, the ratio of Bax/Bcl-2 is commonly employed as an important index for the assessment of mitochondria-mediated apoptosis (39). The present study detected the protein expression levels of proapoptotic Bax, anti-apoptotic Bcl-2 and the Bax/Bcl-2 ratio in CT-26 cells by western blotting. Compared with in the control cells, the expression levels of Bax were significantly enhanced in the treated cells, whereas the protein expression levels of Bcl-2 were markedly decreased (Fig. 7). This observation may result in the release of cytochrome *c* from the mitochondria, which may further induce apoptosis.

In conclusion, the present study demonstrated that BR could synergistically enhance the antitumor effects of CDDP on CT-26 cells via the intrinsic and extrinsic apoptotic pathways, as indicated by activation of Bax and cytochrome *c*, and negative modulation of procaspase-3, procaspase-9 and Bcl-2. These findings suggested that BR and CDDP cotreatment may be a beneficial option to enhance the antitumor effects of CDDP on the treatment of CRC.

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Competing interests

The authors declare that they have no competing interests.

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