Original Article

DOI: 10.5582/bst.2016.01115

Reversal of the multidrug resistance of human ileocecal adenocarcinoma cells by acetyl-11-keto-β-boswellic acid *via* down-regulation of P-glycoprotein signals

Xia Xue[1], Fang Chen[1], Anxin Liu[2], Deqing Sun[1], Jing Wu[1], Feng Kong[3], Yun Luan[3], Xianjun Qu[4], Rongmei Wang[1,*]

[1] *Department of Pharmacy, the Second Hospital of Shandong University, Ji'nan, Shandong, China;*
[2] *Care Ward, Rizhao Hospital of TCM, Rizhao, Shandong, China;*
[3] *Central Laboratory, the Second Hospital of Shandong University, Ji'nan, Shandong, China;*
[4] *Department of Pharmacology, School of Basic Medical Sciences, Capital Medical University, Beijing, China.*

Summary **Multidrug resistance (MDR) represents a clinical obstacle to cancer chemotherapy since it causes cancer recurrence and metastasis. Acetyl-11-keto-β-boswellic acid (AKBA), an active ingredient derived from the plant *Boswellia serrata*, has been found to inhibit the growth of a wide variety of tumor cells, including glioma, colorectal cancer, leukemia, human melanoma, hepatocellular carcinoma, and prostate cancer cells. However, the actions of AKBA in multidrug-resistant cancer cells have not been fully elucidated. The current study examined the reversal of MDR by AKBA in a human ileocecal adenocarcinoma cell line with vincristine-induced resistance, HCT-8/VCR. A 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay indicated that cytotoxicity increased drastically and the IC_{50} of VCR in HCT-8/VCR cells decreased in the presence of AKBA. AKBA had a maximum "fold reversal" of MDR (FR) of 9.19-fold. In addition, HCT-8/VCR cells treated with AKBA and VCR exhibited a higher percentage of apoptotic tumor cells according to flow cytometry. The reversal of MDR by AKBA was evident in an intracellular increase in Rhodamine (Rh123), indicating that the activity of P-glycoprotein (P-gp) was blocked. Furthermore, AKBA inhibited the expression of P-gp and decreased levels of expression of multidrug resistance gene 1 in HCT-8/VCR cells. The current results indicated that AKBA might be a potential agent to reverse MDR in human ileocecal adenocarcinoma.**

Keywords: Multidrug resistance (MDR), acetyl-11-keto-β-boswellic acid (AKBA), P-glycoprotein (P-gp), reversal of multidrug resistance

1. Introduction

Acquired multidrug resistance (MDR) has emerged as a major obstacle to effective chemotherapy (*1*). P-glycoprotein (P-gp) is encoded by multidrug resistance gene 1 (MDR1) and is a member of the ATP-binding cassette (ABC) superfamily (*2*). High levels of P-gp expression have been linked to the efflux of chemotherapeutic drugs in cancer cells. Inhibition of P-gp-mediated drug efflux is an effective way to overcome cancer drug resistance (*3*). However, no agents targeting P-gp have been approved for clinical use (*4*). Therefore, agents to overcome drug resistance must be promptly identified in order to treat malignancies.

Acetyl-11-keto-β-boswellic acid (AKBA), a pentacyclic triterpene extracted from the fragrant gum resin of the *Boswellia serrata* tree, has been found to be effective against inflammatory diseases such as rheumatoid arthritis (*5*), ulcerative colitis (*6*), and trinitrobenzene sulphonic acid-induced colitis (*7*). Recently, AKBA was reported to exhibit antitumor action in several human cell lines, including malignant glioma (*8*), colon cancer (*9*), prostate cancer (*10*), and leukemia

Released online in J-STAGE as advance publication August 19, 2016.

**Address correspondence to:*
Dr. Rongmei Wang, Department of Pharmacy, the Second Hospital of Shandong University, Ji'nan, 250033, China.
E-mail: wrm_yxb001@163.com

(*11*) cell lines. In addition, AKBA has been found to suppress nuclear factor-κB and STAT3-related pathways (*12,13*). However, whether AKBA can modulate acquired MDR in tumors has yet to be determined.

The current study examined the reversal of MDR in HCT-8/VCR cells by AKBA. HCT-8/VCR is a human ileocecal adenocarcinoma cell line with vincristine-induced resistance. The ability of AKBA to reverse MDR is evident in an increase in the intracellular accumulation of VCR and inhibition of the expression of P-gp. Therefore, AKBA might be a potential agent to reverse MDR in human ileocecal adenocarcinoma.

2. Materials and Methods

2.1. *Drugs*

AKBA (Sigma-Aldrich, St. Louis, USA) was dissolved in dimethylsulfoxide (DMSO, Sigma-Aldrich) at 20 mM as a stock solution. Dilutions of all of the reagents were freshly prepared before each experiment.

2.2. *Cell lines and cell culture*

The human ileocecal adenocarcinoma cell line HCT-8 and its MDR counterpart HCT-8/VCR were purchased from Keygen Biotech (Nanjing, China). Cancer cells were maintained in RPMI-1640 (GIBCO, NY, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (GIBCO) and penicillin-streptomycin (100 IU/mL to 100 μg/mL) at 37°C in a humidified atmosphere (5% CO_2-95% air). Cells were harvested by brief incubation in trypsin 0.25% (w/v) and 0.53mM EDTA in PBS. HCT-8/VCR cells were maintained in a complete RPMI-1640 medium containing 1 mg/mL of vincristine (VCR, Wanle, Shenzhen, China) at 37°C in a humidified atmosphere of 5% CO_2. The cells were cultured for 2 weeks in drug-free medium prior to their use in experiments.

2.3. *Determination of P-gp*

P-gp expression was determined with a Western blot analysis as described elsewhere (*14*). A mouse monoclonal antibody against amino acids 1040-1280 of *MDR1* of human origin (sc-13131, Santa Cruz, CA, USA) was used to determine the expression of P-gp in HCT-8 and HCT-8/VCR cells. P-gp bands were quantified with densitometry using an electrophoresis image analysis system (FR980, Furi Science & Technology, China).

2.4. *Assay of cytotoxicity and reversal of MDR*

To test for reversal of P-gp-mediated MDR by AKBA, the cytotoxicity of AKBA to HCT-8/VCR and HCT-8 cells was first measured with an MTT assay (*15*). Briefly, HCT-8/VCR and HCT-8 cells (1×10^4/well) were seeded in 96-well plates. After incubation for 12 h, the cells were treated with various concentrations of AKBA for 24 h, 48 h, and 72 h. Cell viability was assessed by adding 20 μL of MTT reagent (5 mg/mL, Sigma-Aldrich) and incubating cells for 4 h. Light absorbance of the solution was measured at 570 nm on a plate reader (TECAN, Austria).

The reversal of MDR by AKBA was then detected using the same method. Cells seeded in 96-well plates were treated with varying concentrations of VCR in the absence or presence of various concentrations of AKBA (1.25 μM, 2.5 μM, and 5 μM) for 72 h. IC_{50} values (the concentration resulting in 50% inhibition of cell growth) were calculated for VCR. The "fold reversal"of MDR (FR) was calculated by fitting data for ¼ the IC50 of VCR alone/the IC50 of VCR in the presence of AKBA. Experiments were performed in triplicate with three samples. Control cultures included an equivalent amount of DMSO (as the solvent control), which does not modulate the growth or drug sensitivity of these cells at the concentrations used in this study. In all of the experiments, verapamil (Wanle, Shenzhen, China) served as a positive control agent.

2.5. *Annexin V/FITC and 7-AAD staining analysis*

HCT-8/VCR and HCT-8 cells were seeded in 6-well plates (1.5×10^5 per well) and treated with VCR alone or treated with a combination of AKBA and VCR for 24 h. Cells were harvested and washed with cold PBS. The cell surface phosphatidylserine in apoptotic cells was quantitatively estimated using Annexin V/FITC and 7-AAD apoptosis detection kits according to manufacturer's instructions (Becton Dickinson, CA, USA). Cell apoptosis was analyzed on a FACScan flow cytometry system (Becton Dickinson) (*16*). Experiments were performed in triplicate with three samples.

2.6. *Rh123 accumulation*

The effect of AKBA on P-gp activity was assessed by measuring the intracellular accumulation of Rh123 (*17*). HCT-8/VCR cells were seeded into 96-well plates at a density of 1×10^4/well. Cells were pretreated with AKBA (1.25 μM, 2.5 μM, or 5 μM) for 90 min and were then incubated with 200 nM of Rh123 in culture medium in the dark at 37°C in 5% CO_2 for another 90 min. The cells were washed twice with ice-cold PBS. The MFI associated with Rh123 was measured using a multilabel counter with excitation/emission wavelengths of 485/535 nm.

2.7. *Real-time RT-PCR for MDR1 mRNA detection*

Real-time RT-PCR was performed to determine the level of expression of MDR1 mRNA. HCT-8/VCR

cells were seeded in 6-well plates and treated with various concentrations of AKBA for 24 h. Total RNA was extracted using the RNAeasy kit according to the manufacturer's instructions (Sangon, Shanghai, China). RNA quality was determined based on the ratio of A260/A280 (1.8-2). The concentration of total RNA was measured by detecting absorbance at 260 nm (A260) (*18*). Then, reverse transcription (RT) was performed with 2 μg of extracted RNA using the First Strand cDNA Synthesis Kit (Toyobo, Osaka, Japan) according to the supplied protocol. A 25-μL mixture for PCR included 2× SYBR Green real-time PCR Master Mix (Toyobo, Japan), 2 μg of the cDNA template, and 0.3 μM of each primer. The thermal profile of the reaction was as follows: 3 min at 95°C, followed by 40 cycles consisting of 20 s at 95°C, 30 s at 60°C, and 30 s at 70°C. The fold-change in the level of expression of MDR1 mRNA was calculated using the $2^{-\Delta\Delta CT}$ method. Experiments were performed in triplicate with three samples.

The following primers (Genecore Biotech, Guangzhou, China) were used for the specific amplification of MDR1 (forward: 5′-AGACATGACCAGGTATGCCTAT-3′ and reverse: 5′-AGCCTAT CTCCTGTCGCATTA-3′). The expression of GAPDH (forward: 5′-GAGGGGCCATCCACAGTCTT-3′ and reverse: 5′-TTCATTGACCTCAACTACAT-3′) served as an internal control.

2.8. *Western blot analysis*

HCT-8/VCR cells were treated with 1.25 μM, 2.5 μM, or 5 μM AKBA in cell growth medium for 48 h. Cells were harvested, lysed, and centrifuged as described previously. Supernatants were collected and protein concentration was determined using the Bradford assay (*19*). Samples containing 50 μg of protein were subjected to 10% SDS-PAGE and electro-transferred to nitrocellulose membranes that were blocked with 3% non-fat milk/0.1% Tween 20/TBS, 100 mM NaCl in 10 mM Tris, pH 7.5, incubated with anti-P-gp antibody (sc-13131, Santa Cruz, CA, USA) for 1 h at room temperature followed by horseradish-peroxidase-conjugated secondary antibody for another 1 h at room temperature. Protein bands were detected with ECL.

2.9. *Statistical analysis*

Data are expressed as the mean ± S.D. Data were analyzed using the Student's *t*-test. *p*-values below 0.05 were considered to be statistically significant.

3. Results

3.1. *High levels of P-gp expression in the HCT-8/VCR cell line*

Western blot analysis was used to determine the expression of P-gp in multidrug-resistant HCT-8/VCR and parental HCT-8 cells (Figure 1A). Results indicated that drug-sensitive HCT-8 cells had markedly lower levels of P-gp when probed using an anti-P-gp monoclonal antibody, while drug-resistant HCT-8/VCR cells exhibited a stronger signal corresponding to P-gp (Figure 1B). These results indicated that HCT-8/VCR was an MDR cell line characterized by over-expression of P-gp.

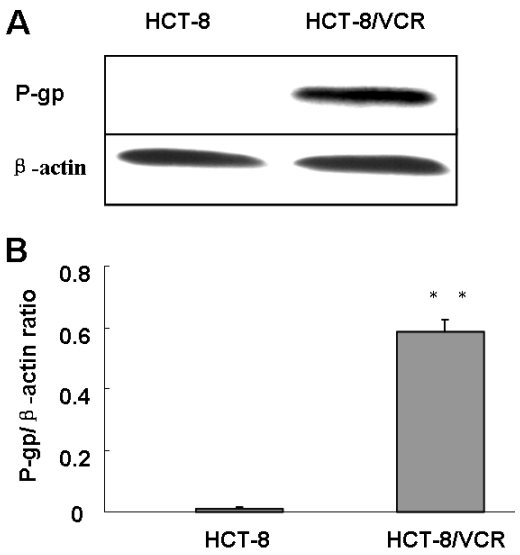

Figure 1. The expression of P-gp in HCT-8 and HCT-8/VCR cells. (A) Levels of P-gp in HCT-8 and HCT-8/VCR cells were estimated using Western blot analysis. **(B)** P-gp bands were quantified using densitometry. Bars indicate the mean ± S.D. $n = 3$). $^{**}p < 0.01$ compared to HCT-8 cells.

3.2. *Effects of AKBA on proliferation of HCT-8 and HCT-8/VCR cells*

The effects of AKBA on HCT-8/VCR and parental HCT-8 cell proliferation were examined. As shown in Figures 2A and 2B, AKBA at concentrations ranging from 2.5 μM to 5 μM weakly inhibited HCT-8/VCR and HCT-8 cell proliferation, but significant differences in inhibition of cell proliferation were noted at higher concentrations (10 μM to 60 μM). Therefore, lower concentrations of AKBA (2.5 μM to 5 μM) were selected and short periods of incubation in were used in experiments to examine reversal of MDR.

3.3. *Effects of AKBA on sensitivity to VCR*

To assess the synergistic effects of AKBA on VCR-induced cytotoxicity, an MTT assay was performed first to determine the "fold reversal"of MDR in HCT-8/VCR and HCT-8 cells. Incubation with VCR at concentrations ranging from 8 to 256 μg/mL weakly inhibited HCT-8/VCR cell proliferation, but HCT-8 cells were sensitive to VCR at all concentrations. As shown in Table 1, VCR-induced cytotoxicity drastically

increased and the IC50 of VCR in HCT-8/VCR cells decreased in the presence of AKBA. AKBA at a concentration of 2.5 μM had a "fold reversal"of MDR (FR) of 8.20-fold and AKBA at a concentration of 5 μM had an FR of 9.19-fold.

The synergistic effects of AKBA on VCR were also evident in the induction of apoptosis. As indicated by the flow cytometry results shown in Figure 2, annexin V-positive cells increased significantly with 24 h of incubation in the presence of VCR and AKBA compared to VCR alone. At AKBA concentrations from 2.5-5 μM, the percentage of apoptotic HCT-8/VCR cells increased from 7.24% to a maximum of 22.22% (Figures 3B-3D). However, 2.5-5 μM of AKBA alone had almost no effect on apoptosis of HCT-8/VCR cells. The percentage of apoptotic HCT-8/VCR cells was 0.23% and 0.31% (Figures 3E and 3F).

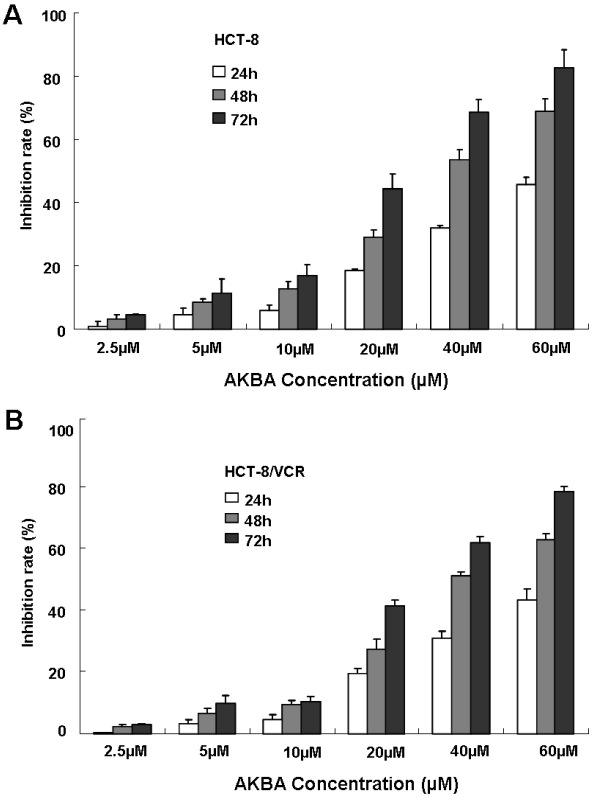

Figure 2. Effects of P-gp on the proliferation of HCT-8 and HCT-8/VCR cells. HCT-8 **(A)** and HCT-8/VCR **(B)** cells were treated with various concentrations of AKBA for 24, 48, and 72 h. Viable cell numbers were evaluated with an MTT assay and were denoted as a percentage of untreated controls at the corresponding time point. Data are expressed as the mean ± S.D. from three independent experiments.

3.4. *Increased accumulation of Rh123*

To further assess the role of AKBA in regulating P-gp expression, the transport activity of P-gp was examined by measuring the intracellular accumulation of Rh-123. As shown in Figure 4A, the fluorescence intensity of Rh-123 increased markedly in HCT-8/VCR cells treated with AKBA in comparison to cells in the control group. The average Rh123 accumulation MFI in 1.25μM AKBA group was 1,627 ± 82, compared to 536 ± 26 in control group. Incubation of HCT-8/VCR cells with 2.5 μM of AKBA resulted in Rh123 accumulation of 2,071 ± 67. AKBA at a concentration of 5 μM completely restored Rh123 accumulation in HCT-8/VCR cells, with an MFI 2,514 ± 92. The intracellular accumulation of Rh-123 increased significantly in the presence of AKBA in comparison to the control group (Figure 4B, $^{**}p < 0.01$).

3.5. *AKBA decreased P-gp expression in HCT-8/VCR cells*

Levels of P-gp and MDR1 mRNA in HCT-8/VCR cells were also examined. As shown in Figure 5A, the level of MDR1 mRNA decreased significantly after 24 h of treatment with AKBA according to RT-PCR. Further examination indicated that the level of P-gp expression also markedly decreased as a result of AKBA and was accompanied by a notable decrease in P-gp in HCT-8/VCR cells treated with 2.5 μM or 5 μM of AKBA (Figure 5B, $^{\#\#}p < 0.01$).

4. Discussion

The development of resistance to chemotherapeutic agents remains a major challenge to chemotherapy to treat ileocecal adenocarcinoma. Chemotherapeutic agents such as vincristine sulphate, 5-fluorouracil, and

Table 1. Effects of AKBA on the sensitivity of HCT-8/VCR and HCT-8 cells to VCR

Treatment	HCT-8/VCR		HCT-8	
	IC_{50} (μg/mL)	FR	IC_{50} (μg/mL)	FR
VCR alone	99.2 ± 3.58		18.66 ± 1.97	
VCR + 5 μg/mL of verapamil	15.2 ± 1.34**	6.53	15.37 ± 1.25	1.21
VCR + 1.25 μM of AKBA	21.4 ± 0.89**	4.63	15.89 ± 1.13	1.17
VCR+2.5 μM of AKBA	12.1 ± 0.75**	8.20	14.23 ± 1.07	1.31
VCR+5 μM of AKBA	10.8 ± 0.56**	9.19	16.27 ± 0.84	1.15

he effects of AKBA on the sensitivity of HCT-8/VCR cells to VCR were examined using an MTT assay. Cells were treated with varying concentrations of VCR in the presence of AKBA for 72 h. IC_{50} values for VCR were calculated and the "fold reversal" of MDR (FR) was compared. Data are the mean ± S.D. from three independent experiments. $^{**}p$ <0.01 *vs*. VCR alone, untreated group.

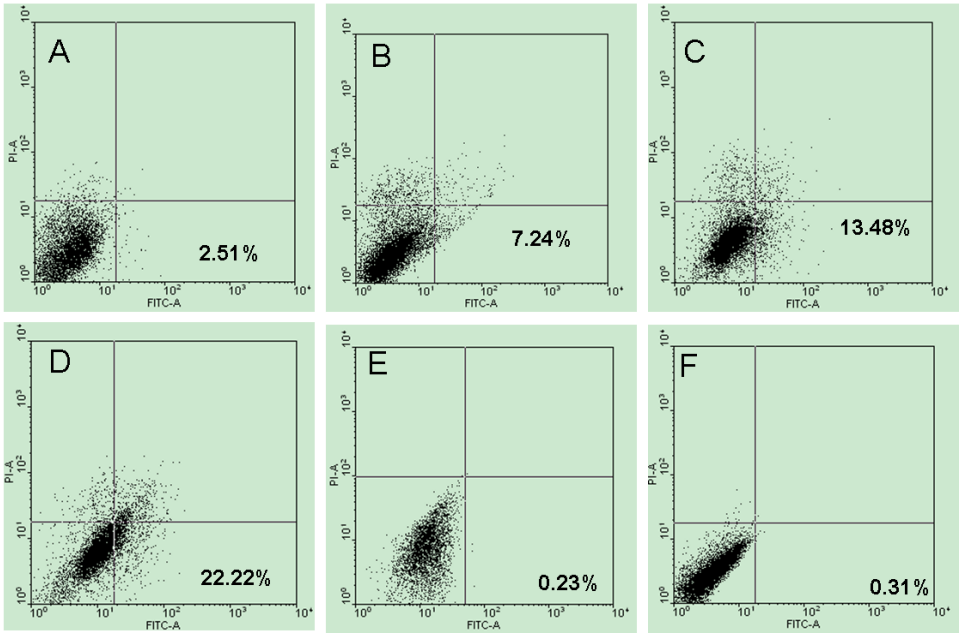

Figure 3. Detection of apoptotic cells with flow cytometric analysis after Annexin V/7-AAD staining. HCT-8/VCR cells were exposed to VCR and increasing concentrations of AKBA for 24 h. Cells were harvested and stained with AnnexinV/7-AAD. **(A)** Vehicle control; **(B)** VCR 20 μg/mL; **(C)** VCR 20 μg/mL + AKBA 2.5 μM; **(D)** VCR 20 μg/mL + AKBA 5 μM; **(E)** AKBA 2.5 μM **(F)** AKBA 5 μM.

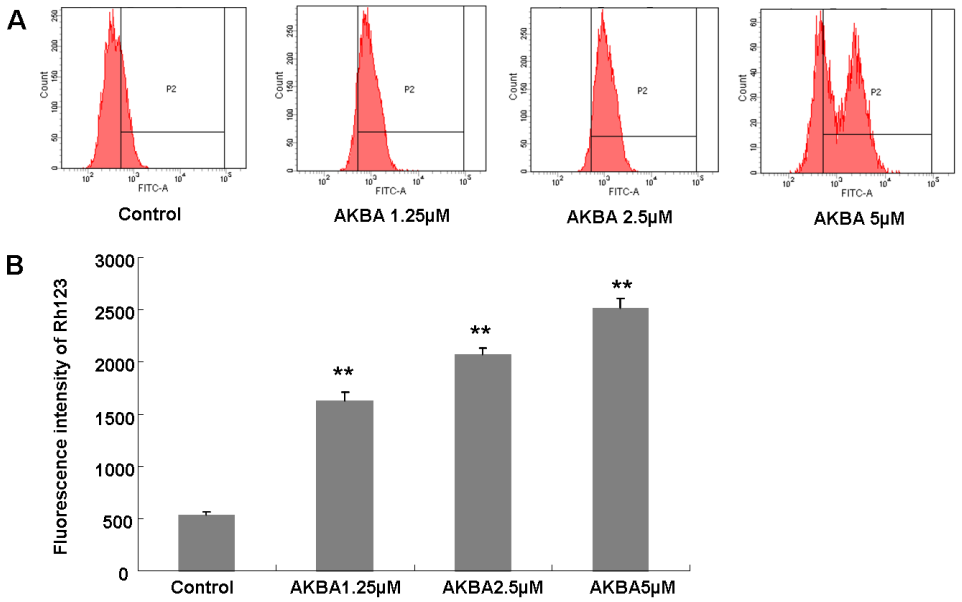

Figure 4. Effect of AKBA on accumulation of Rhodamine 123 (Rh-123) in HCT-8/VCR cells. HCT-8/VCR cells were cultured in RPMI 1640 supplemented with 200 nm of Rh-123 for 30 min at 37°C. At the end of incubation, the cells were washed twice with PBS to remove the free Rh-123 and kept in dye-free medium. **(A)** The fluorescence intensity of Rhodamine 123 in cells was measured with FACS. **(B)** Quantification of Rh-123 fluorescence intensity. Data are expressed as the mean ± S.D. from three independent experiments. $^{**}p < 0.01$ compared to the control group.

capecitabine have been used in chemotherapy to treat ileocecal adenocarcinoma (*20*). However, cancer cells become resistant when these chemotherapeutic agents are used for a prolonged period. MDR has multiple causes, including (*i*) upregulation of drug efflux pumps (*21*), (*ii*) increased signaling *via* AKT (*22*), and (*iii*) decreased apoptosis (*23,24*). A key cause of MDR is enhanced expression of drug efflux proteins mediated by members of the superfamily of ABC transporters. Four members of ABC family function as drug efflux pumps: P-glycoprotein (P-gp/MDR1/ABCB1), multidrug resistance-associated protein 1 (MRP1/ABCC1), breast cancer resistance protein (BCRP/ABCG2), and lung resistance protein (LRP) (*25*). P-gp is known to interact with over 300 compounds and why it is able to achieve this has not yet been revealed

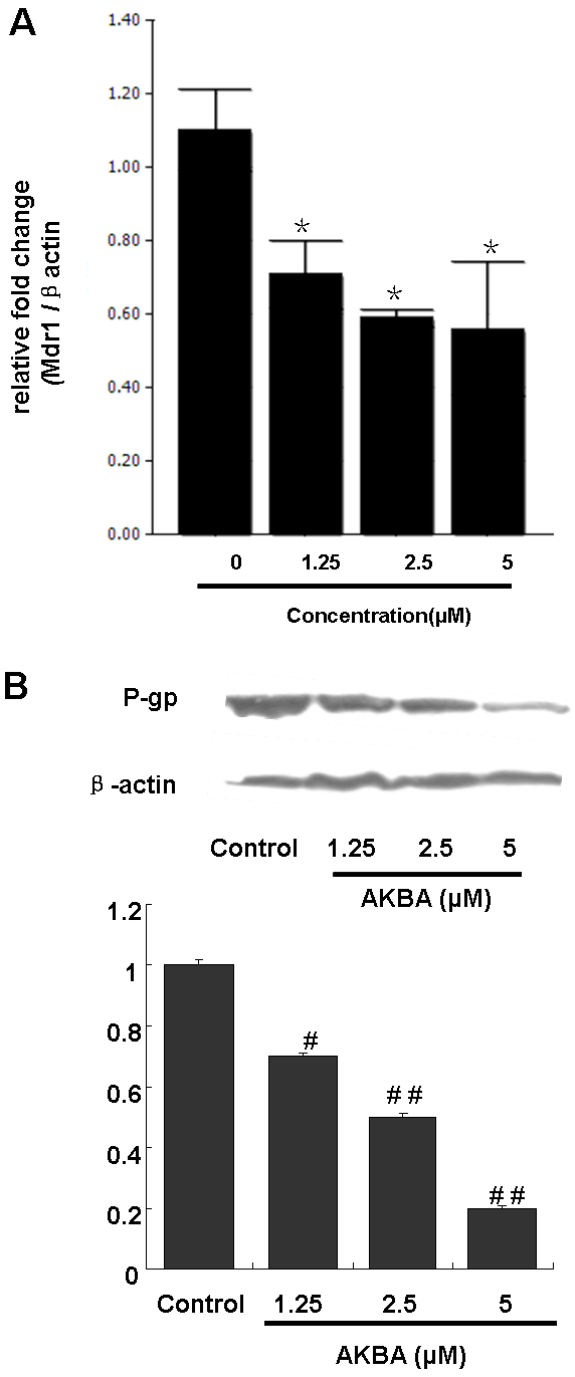

Figure 5. RT-PCR (A) and Western blot (B) analysis of P-gp in HCT-8/VCR cells after treatment with different concentrations of AKBA. Total cell proteins were separated with 10% SDS-PAGE, transferred to nitrocellulose filters, and incubated with anti-P-gp antibody. Immunoreactive proteins were visualized using ECL. Decreases in Mdr1 levels **(A)** and P-gp expression **(B)** in HCT-8/VCR cells after treatment with different concentrations of AKBA were detected. HCT-8/VCR cells were exposed to AKBA for the indicated time. Total RNA or proteins was extracted for P-gp or MDR1 analysis. **(A)** Decrease in MDR1 mRNA in HCT-8/VCR cells exposed to AKBA for 24 h. Levels of MDR1 mRNA were detected with a real-time PCR assay. Data are expressed as the mean ± S.D. from three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to untreated controls. **(B)** Decrease in P-gp expression in HCT-8/VCR cells exposed to AKBA for 48 h. Data are expressed as the mean ± S.D. from three independent experiments. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the untreated controls.

(*26*). P-gp prevents the intracellular accumulation of anticancer drugs by an efflux mechanism. Furthermore, a range of agents have been developed to reverse the MDR phenotype and restore drug sensitivity to cancer cells (*27*). However, most agents to reverse MDR have proven to be intrinsically toxic or to decrease the pharmacokinetic effects of accompanying anticancer drugs (*28*). Therefore, new effective compounds that can increase the sensitivity of resistant cells to chemotherapeutic agents must quickly be identified.

Previous studies by the current authors indicated that orally administered AKBA resulted in an inhibition of intestinal tumorigenesis in $APC^{Min/+}$ mice. AKBA treatment significantly reduced polyp number and size and the degree of cytological dysplasia in the small intestine and colon (*29*). These effects might be produced by the inhibition of the Wnt/β-catenin and NF-κB/cyclooxygenase-2 signaling pathways. Moreover, numerous studies have indicated that NF-κB plays a critical role in resistance to chemotherapeutic agents (*30*). Inhibition of the transcription activity of NF-κB causes a decrease in the anti-apoptotic proteins Bcl-2 and Bcl-(XL), and this appears to be a synergistic effect. Combining NF-κB inhibitors with conventional chemotherapeutics might overcome the drug resistance of cancer cells. In addition, previous Western blotting results have indicated a decrease in NF-κB-p65RelA and its active form p-NF-κB Ser536 in intestinal polyps from mice treated with AKBA. AKBA reduced levels of NF-κB-p65RelA by 51.8% and p-NF-κB Ser536 by 48.6% in the small intestine and colon. The inhibitory effect of AKBA on COX-2, 5-LO, and TNF-α in intestinal polyps is also evident in Western blotting. Therefore, the hypothesis was that AKBA could be a potential agent to reverse MDR.

In the current study, AKBA markedly reversed MDR in HCT-8/VCR, a human ileocecal adenocarcinoma cell line with vincristine-induced resistance, according to an MTT assay and flow cytometry. AKBA had a maximum FR of MDR of 9.19-fold. In addition, the reversal of MDR by AKBA was evident in an intracellular increase in Rh123, indicating that the activity of P-gp was blocked. Furthermore, AKBA inhibited the expression of P-gp and decreased the levels of MDR1 gene expression in HCT-8/VCR cells. These results indicated that AKBA might be a potential agent to reverse MDR in human ileocecal adenocarcinoma. The mechanism of AKBA as a P-gp inhibitor has yet to be studied. In the current study, the inhibition of P-gp was evident both at the level of biosynthesis and also at the level of activity. Accumulating evidence suggests that P-gp, a member of the ATP-binding cassette superfamily, exports structurally diverse hydrophobic compounds from cells through a process driven by ATP hydrolysis (*31*). Efflux occurs depending on the energy produced by ATP hydrolysis when substrates bind with nucleotide-binding domains (NBDs) (*32*).

In the current study, AKBA probably blocked the activity of P-gp by inhibiting ATPase. That said, the exact regulatory mechanism is unclear and warrants further study. Furthermore, AKBA has been studied for its inhibition of Wnt/β-catenin signaling pathways. Previous studies found that the Wnt/β-catenin signal pathway was constitutively activated in cancer cells with doxorubicin-induced MDR. Specific knockdown of β-catenin by RNAi-mediated depletion eliminated MDR1 transcription and expression, resulting in a complete reversal of P-gp-dependent efflux function and restoration of sensitivity to doxorubicin-induced cytotoxicity (*33*). Therefore, the suppression of Wnt/β-catenin signaling by AKBA might be related to the inhibition of P-gp activity and function. However, the precise mechanisms by which AKBA reverses MDR need to be explored.

Acknowledgements

This study was supported by a grant from the Youth Foundation of the Second Hospital of Shandong University (grant No. S2013010022), grants from the Shandong Provincial Foundation for Natural Science (grant No. ZR2013HM085, grant No. ZR2014HQ036), and a grant from the Natural Science Foundation of China (grant No.81402962). The authors wish to thank the Central Research Laboratory, the Second Hospital of Shandong University for its technical assistance and generous support.

References

1. Xi G, Hayes E, Lewis R, Ichi S, Mania-Farnell B, Shim K, Takao T, Allender E, Mayanil CS, Tomita T. CD133 and DNA-PK regulate MDR1 *via* the PI3K-or Akt-NF-κB pathway in multidrug-resistant glioblastoma cells *in vitro*. Oncogene. 2015; 35:1-10.
2. Teodori E1, Dei S, Martelli C, Scapecchi S, Gualtieri F. The functions and structure of ABC transporters: Implications for the design of new inhibitors of Pgp and MRP1 to control multidrug resistance (MDR). Curr Drug Targets. 2006; 77:893-909.
3. Ye P, Xing H, Lou F, Wang K, Pan Q, Zhou X, Gong L, Li D. Histone deacetylase 2 regulates doxorubicin (Dox) sensitivity of colorectal cancer cells by targeting $ABCB_1$ transcription. Cancer Chemother Pharmacol. 2016; 77:613-621.
4. Kadioglu O, Saeed ME, Valoti M, Frosini M, Sgaragli G, Efferth T. Interactions of human P-glycoprotein transport substrates and inhibitors at the drug binding domain: Functional and molecular docking analyses. Biochem Pharmacol. 2016; 104:42-51.
5. Abdel-Tawab M, Werz O, Schubert-Zsilavecz M. *Boswellia serrata*: An overall assessment of *in vitro*, preclinical, pharmacokinetic and clinical data. Clin Pharmacokinet. 2011; 50:349-369.
6. Takada Y, Ichikawa H, Badmaev V, Aggarwal BB. Acetyl-11-keto-β-boswellic acid potentiates apoptosis, inhibits invasion, and abolishes osteoclastogenesis by suppressing NF-κB and NF-κB-regulated gene expression. J Immunol. 2006; 176:3127-3140.
7. Syrovets T, Buchele B, Krauss C, Laumonnier Y, Simmet T. Acetyl-boswellic acids inhibit lipopolysaccharide-mediated TNF-α induction in monocytes by direct interaction with IκB kinases. J Immunol. 2005; 174:498-506.
8. Hostanska K, Daum G, Saller R. Cytostatic and apoptosis-inducing activity of boswellic acids toward malignant cell lines *in vitro*. Anticancer Res. 2002; 22:2853-2862.
9. Liu JJ, Nilsson A, Oredsson S, Badmaev V, Zhao WZ, Duan RD. Boswellic acids trigger apoptosis *via* a pathway dependent on caspase-8 activation but independent on Fas/Fas ligand interaction in colon cancer HT-29 cells. Carcinogenesis. 2002; 23:2087-2093.
10. Lu M, Xia L, Hua H, Jing Y. Acetyl-keto-β-boswellic acid induces apoptosis through a death receptor 5-mediated pathway in prostate cancer cells. Cancer Res. 2008; 68:1180-1186.
11. Hostanska K, Daum G, Saller R. Cytostatic and apoptosis-inducing activity of boswellic acids toward malignant cell lines *in vitro*. Anticancer Res. 2002; 22:2853-2862.
12. Catanzaro D, Rancan S, Orso G, Dall'Acqua S, Brun P, Giron MC, Carrara M, Castagliuolo I, Ragazzi E, Caparrotta L, Montopoli M. *Boswellia serrata* preserves intestinal epithelial barrier from oxidative and inflammatory damage. PLoS One. 2015; 105:e0125375.
13. Kunnumakkara AB, Nair AS, Sung B, Pandey MK, Aggarwal BB. Boswellic acid blocks signal transducers and activators of transcription 3 signaling, proliferation, and survival of multiple myeloma *via* the protein tyrosine phosphatase SHP-1. Mol Cancer Res. 2009; 7:118-128.
14. Park EJ, Shin JW, Seo YS, Kim DW, Hong SY, Park WI, Kang BM. Gonadotropin-releasing hormone-agonist induces apoptosis of human granulosa-luteal cells *via* caspase-8, 9 and -3, and poly-(ADP-ribose)-polymerase cleavage. Biosci Trends. 2011; 5:120-128.
15. Shi YQ, Qu XJ, Liao YX, Xie CF, Cheng YN, Li S, Lou HX. Reversal effect of a macrocyclic bisbibenzyl plagiochin E on multidrug resistance in adriamycin-resistant K562/A02 cells. Eur J Pharmacol. 2008; 584:66-71.
16. Wang G, Chen H, Huang M, Wang N, Zhang J, Zhang Y, Bai G, Fong WF, Yang M, Yao X. Methyl protodioscin induces G2/M cell cycle arrest and apoptosis in HepG2 liver cancer cells. Cancer Lett. 2006; 241:102-109.
17. Sun LR, Li X, Cheng YN, Yuan HY, Chen MH, Tang W, Ward SG, Qu XJ. Reversal effect of substituted 1,3-dimethyl-1H-quinoxalin-2-ones on multidrug resistance in adriamycin-resistant K562/A02 cells. Biomed Pharmacother. 2009; 63:202-208.
18. Xue X, Qu XJ, Gao ZH, Sun CC, Liu HP, Zhao CR, Cheng YN, Lou HX. Riccardin D, a novel macrocyclic bisbibenzyl, induces apoptosis of human leukemia cells by targeting DNA topoisomerase II. Invest New Drugs. 2012; 30:212-222.
19. Zhang XF, Pan QZ, Pan K, Weng DS, Wang QJ, Zhao JJ, He J, Liu Q, Wang DD, Jiang SS, Zheng HX, Lv L, Chen CL, Zhang HX, Xia JC. Expression and prognostic role of ubiquitination factor E4B in primary hepatocellular carcinoma. Mol Carcinog. 2016; 55:64-76.
20. Wu S, Guo Z, Hopkins CD, Wei N, Chu E, Wipf P,

Schmitz JC. Bis-cyclopropane analog of disorazole C1 is a microtubule-destabilizing agent active in ABCB1-overexpressing human colon cancer cells. Oncotarget. 2015; 6:40866-40879.
21. Seebacher NA, Richardson DR, Jansson PJ. Glucose modulation induces reactive oxygen species and increases P-glycoprotein-mediated multidrug resistance to chemotherapeutics. Br J Pharmacol. 2015; 172:2557-2572.
22. Mao Z, Zhou J, Luan J, Sheng W, Shen X, Dong X. Tamoxifen reduces P-gp-mediated multidrug resistance *via* inhibiting the PI3K/Akt signaling pathway in ER-negative human gastric cancer cells. Biomed Pharmacother. 2014; 68:179-183.
23. Sui H, Zhou LH, Zhang YL, Huang JP, Liu X, Ji Q, Fu XL, Wen HT, Chen ZS, Deng WL, Zhu HR, Li Q. Evodiamine suppresses ABCG2 mediated drug resistance by inhibiting p50/NF-κB pathway in colorectal cancer. J Cell Biochem. 2016; 117:1471-1481.
24. Lee YY, Jeon HK, Hong JE, *et al*. Proton pump inhibitors enhance the effects of cytotoxic agents in chemoresistant epithelial ovarian carcinoma. Oncotarget. 2015; 6:35040-35050.
25. Vasiliou V, Vasiliou K, Nebert DW. Human ATP-binding cassette ABC transporter family. Hum Genomics. 2009; 3:281-290.
26. Callaghan R. Providing a molecular mechanism for P-glycoprotein; why would I bother? Biochem Soc Trans. 2015; 43:995-1002.
27. Ye S, Zhang J, Shen J, Gao Y, Li Y, Choy E, Cote G, Harmon D, Mankin H, Gray NS, Hornicek FJ, Duan Z. NVP-TAE684 reverses multidrug resistance (MDR) in human osteosarcoma by inhibiting P-glycoprotein (PGP1) function. Br J Pharmacol. 2016; 173:613-626.
28. Previs RA, Armaiz-Pena GN, Lin YG, Davis AN, Pradeep S, Dalton HJ, Hansen JM, Merritt WM, Nick AM, Langley RR, Coleman RL, Sood AK. Dual metronomic chemotherapy with Nab-paclitaxel and topotecan has potent antiangiogenic activity in ovarian cancer. Mol Cancer Ther. 2015; 14:2677-2686.
29. Liu HP, Gao ZH, Cui SX, Wang Y, Li BY, Lou HX, Qu XJ. Chemoprevention of intestinal adenomatous polyposis by acetyl-11-keto-β-boswellic acid in $APC^{Min/+}$ mice. Int J Cancer. 2013; 132:2667-2681.
30. Nabekura T, Hiroi T, Kawasaki T, Uwai Y. Effects of natural nuclear factor-κ B inhibitors on anticancer drug efflux transporter human P-glycoprotein. Biomed Pharmacother. 2015; 70:140-145.
31. Jones PM, George AM. Opening of the ADP-bound active site in the ABC transporter ATPase dimer: Evidence for a constant contact, alternating sites model for the catalytic cycle. Proteins. 2009; 75:387-396.
32. Matsunaga T, Kose E, Yasuda S, Ise H, Ikeda U, Ohmori S. Determination of P-Glycoprotein ATPase activity using luciferase. Biol Pharm Bull. 2006; 29:560-564.
33. Xia Z, Guo M, Liu H, Jiang L, Li Q, Peng J, Li JD, Shan B, Feng P, Ma H. CBP-dependent Wnt/β-catenin signaling is crucial in regulation of MDR1 transcription. Curr Cancer Drug Targets. 2015; 15:519-532.

(Received June 21, 2016; Revised July 30, 2016; Accepted July 30, 2016)