Bioorganic & Medicinal Chemistry 16 (2008) 8397–8402

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Obovatol inhibits colorectal cancer growth by inhibiting tumor cell proliferation and inducing apoptosis

Su-Kyung Lee [a], Hye-Nan Kim [a], Yeong-Rim Kang [a], Chang Woo Lee [a], Hwan-Mook Kim [a], Dong Cho Han [a], Jongheon Shin [b], KiHwan Bae [c], Byoung-Mog Kwon [a,*]

[a] Korea Research Institute of Bioscience and Biotechnology, University of Science and Technology, 52 Uendong, Yuseonggu, Daejon 305-600, Republic of Korea
[b] Natural Products Research Institute, College of Pharmacy, Seoul National University, Sillim San 56-1, Gwanakgu, Seoul 151-742, Republic of Korea
[c] College of Pharmacy, Chungnam National University, Gung-Dong 220, Yuseonggu, Daejon 305-764, Republic of Korea

ARTICLE INFO

Article history:
Received 7 July 2008
Revised 13 August 2008
Accepted 14 August 2008
Available online 19 August 2008

Keywords:
Obovatol
Honokiol
Magnolia obovata Thunb
Apoptosis
Antitumor

ABSTRACT

Neolignans such as obovatol, honokiol, and magnolol have been previously reported to show various biological activities including anti-inflammation and antitumor effects. This is the first demonstration on the in vivo antitumor effect of obovatol on human colorectal carcinoma SW620 cells. Nude mice were implanted with SW620 cells and fed with vehicle or 5 mg/kg/d dose of obovatol for 20 days. Obovatol inhibited tumor growth that accounted for 50% decrease in tumor volume and 44.6% decrease in tumor weight at the end of the experiment without any adverse health effect. In nude mice bearing SW620-incubated tumor, obovatol exhibited more potent antitumor activity than honolkiol. In addition, DNA flow cytometric analysis shows that obovatol progresses to apoptosis as detected by flow cytometry after double staining with annexin V and propidium iodide. Thus, we suggest that obovatol is a potent inducer of cell apoptosis in SW620 cells, and a potent antitumor agent.

1. Introduction

Colorectal cancer (CRC) is a major public health concern around the world. Initiation and progression of CRC are a complex process that results from the loss of the normal regulatory pathways that govern a balance between cell proliferation and death. And the epigenetic alterations controlling tumor cell proliferation, survival, inflammation, and angiogenesis also play critical roles in CRC growth and progression. Therapeutic strategies for colorectal cancer have improved greatly over the past decade but are still not satisfactory.[1] Therefore, significant effort has been desired to identify novel compounds for the treatment of this disease.

Natural products are considered as rich sources for the discovery of new therapeutic agents.[2,3] Plants contain a variety of chemo-preventive compounds that have been shown to prevent the development of malignancies.[4] Natural products, isolated from Chinese herbal medicines, were reported to be antineoplatic activities both in experimental and in clinical studies.[5]

Magnolia officinalis and obovata have been used as herbal medicine, and neolignans such as magnolol, honokiol, and obovatol (Fig. 1) were isolated from the extracts of bark or leaves of the herbal medicines. It has been reported that there are a variety of pharmacological activities such as antitumor,[6] antidepressant-like,[7] and anti-inflammatory effects.[8] Especially, magnolol and honokiol showed antitumor effects through inhibition of tumor

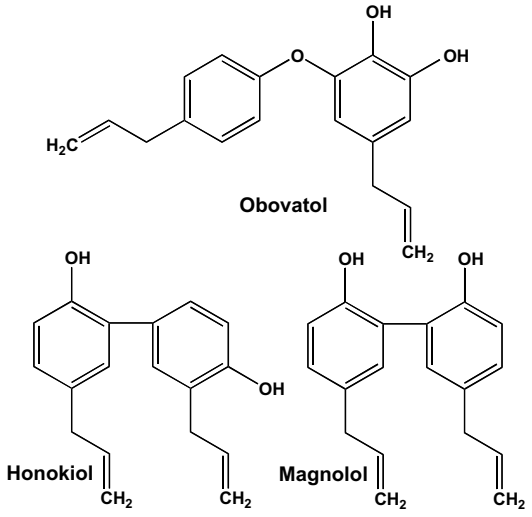

Figure 1. Stucture of obovatol, honokiol, and magnolol.

* Corresponding author. Tel.: +82 42 860 4557; fax: +82 42 861 2675.
E-mail address: kwonbm@kribb.re.kr (B.-M. Kwon).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.08.033

growth,[9] tumor cell invasion,[10] metastasis,[11] angiogenesis,[12] and induction of apoptosis.[13] Based on these reports, allylated biphenol analogs were synthesized to improve the potent activity of these two compounds, and the analogs showed antiviral and anti-proliferative activities.[14,15]

Obovatol is a compound isolated from the extracts of *Magnolia obovata* Thunb,[16] there are studies about its biological activities including anxiolytic-like,[17] antifungal,[18] and anti-inflammatory effects.[19] Although it has been reported that obovatol inhibits cell growth through induction of apoptotic cell death in prostate and colon cancer by blocking of NF-κB,[20] the in vivo efficacy of obovatol was not reported.

In the present study, we examined the effects of obovatol on cell cycle regulation, apoptosis, and in vivo using human colon tumor xenograft in nude mice. The findings show that obovatol inhibits the tumor growth through cell cycle arrest and inducing apoptosis. In vivo efficacy of obovatol and honokiol using human colon tumor xenograft in nude mice was also examined.

2. Results and discussion

2.1. In vivo antitumor activity

To study obovatol in vivo efficacy on CRC development, SW620 tumor xenograft model of nude mice was used. SW620 cells were implanted subcutaneously into the right flank of a nude mouse on day 0 and obovatol feeding was started to nude mice on day 1 after the SW620 xenograft implantation. Tumor-bearing nude mice were injected intraperitoneally with vehicle (100 μL, 0.5% Tween 80) or obovatol (5 mg/kg, diluted in 0.5% Tween 80) each day. Compared with control (vehicle), obovatol treatment showed suppression of tumor xenograft growth throughout the study (Fig 2A). On day 21, the mice were sacrificed, and tumor volume and weight were measured. At the end, tumor volume per mouse was 277.0 ± 54 mm^3 in obovatol treatment group compared with 554.4 ± 63 mm^3 in control, accounting for a 50% decrease in tumor volume (Fig. 2A). Consistent with this observation, obovatol caused 44.6% decrease in tumor weight ($P = 0.01$) compared with control (Fig 2B). Doxorubicin as positive control suppressed the tumor growth 58% in tumor volume and 53.7% in tumor weight (fed at concentration of 2 mg/kg/day). To compare the antitumor activity of obovatol and honokiol, the compounds were fed by oral gavage at 5 mg/kg/day, 5 days a week for 26 days, showed suppression of tumor xenograft growth (Table 1). Compared with honokiol, obovatol showed greater antitumor efficacy. We monitored any adverse health effect during the study by measuring body weight gain and diet consumption profiles, which are widely used primary indicators to assess gross toxicity of a test compound. Obovatol and honokiol did not affect body weight gain and diet consumption profiles, which were almost comparable with their respective control groups. However, 13.1% ($P < 0.001$) of body weight loss was found in doxorubicin-treated nude mice. Previous studies reported that honokiol significantly inhibited the growth of RKO colorectal cancer cells and PC-3 prostate cells xenografts in nude mice without causing weight loss.[21,22] Therefore, obovatol will be a potent antitumor agent for the treatment of colorectral cancer.

2.2. Obovatol inhibited growth of the tumor cells

To understand biological activity of the obovatol on cancer cells, we investigated whether the compound is able to inhibit growth of the SW620 cells (colon cancer carcinoma cells) with counting cells after treatment with different concentrations of the compound in the cell proliferation assay at a time course (24, 48, 72, and 92 h). As shown in Table 2, obovatol could inhibit growth of SW620 cells in a time and concentration dependent. Additionally, we evaluated the cytotoxicity of obovatol in various human cell lines using an MTS cell proliferation assay representing different tumor types, namely, colorectal cancer (SW620, HCT116, HT29, and DLD-1), breast adenocarcinoma (MCF7 and MDA-MB231), prostate cancer (Du145 and PC3), and erythroleukemia cell (K562) after a continuous exposure of 48 h. The results are summarized in Figure 3.

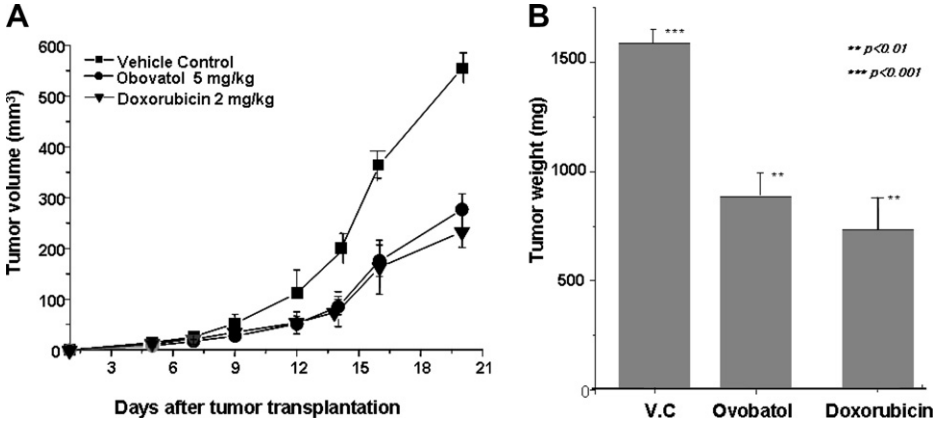

Figure 2. Antitumor effects of obovatol on SW620 colorectal tumor xenograft growth. For the evaluation of in vivo antitumor activity of obovatol, SW620 cells were implanted subcutaneously into the right flank of nude mice on day 0. Compound was dissolved 0.5% Tween 80 and was daily administered intraperitoneally for 20 days.

Table 1
Antitumor effects of obovatol and honokiol SW620 human colon cancer cells in a human tumor xenograft model in nude mice

Treatment	Dose (mg/kg)	Number of mice		Tumor volume (mm^3)				
		Start	End	Day 5	Day 10	Day 16	Day 23	Day 27
Vehicle	0	8	8	100 ± 11	160 ± 17	450 ± 42	1050 ± 110	1500 ± 145
Honokiol	5	8	8	100 ± 12	150 ± 16	270 ± 29	600 ± 66	900 ± 105
Obovatol	5	8	8	100 ± 11	130 ± 17	150 ± 18	400 ± 51	750 ± 80

Table 2
Effect of obovatol on growth of SW620 cells

Concentration (μM)		0	30	50	100
Time	24	0	7 ± 1.1	21 ± 0.3	36 ± 0.07
	48	0	26 ± 0.3	57 ± 0.5	92 ± 0.05
	72	0	33 ± 1	64 ± 0.2	97 ± 0.01
	96	0	18 ± 1	85 ± 0.1	98 ± 0.01

All experiments were independently performed three times (% of cell inhibition).

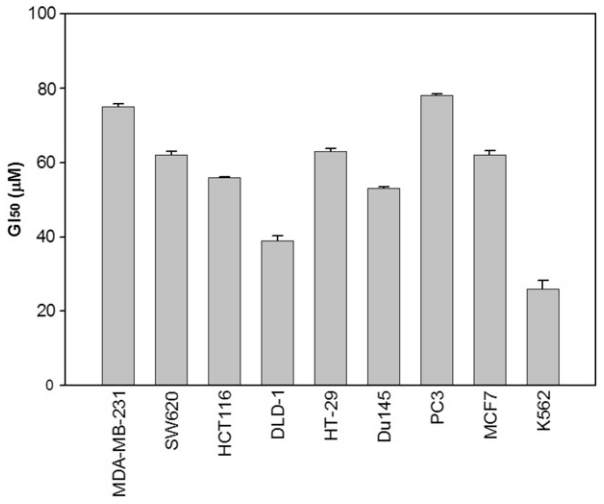

Figure 3. Obovatol inhibited various human tumor cells. .Obovatol was treated in various human cells such as colorectal cancer (SW620, HCT116, HT29, and DLD-1), breast adenocarcinoma (MCF7 and MDA-MB231), prostate cancer (Du145 and PC3), and erythroleukemia cell (K562) for 48 h.

2.3. Cell cycle profile effects of obovatol in SW620 cells

It was reported that honokiol treatment decreased the viability of PC-3 and LNCaP human prostate cancer cells in a concentration-dependent manner, which correlated with G0-G1 phase cell cycle arrest[23] and also magnolol inhibited the growth of 5637 bladder cancer cells through inducing inhibition and G1-, G2/M-phase cell cycle arrest.[9] To determine the effect of obovatol on the cell cycle, FACS analysis was performed using the SW620 cells. Cells were harvested at 72 h after treatment of obovatol and analyzed with a FACScalibur. After incubation with 50 mM concentration of obovatol, the sub-G_1 portions were increased by 22.27% versus the untreated control and S population increased from 19.4% in the control to 30.4% in cells treated with obovatol (Fig. 4). While honokiol and magnolol induced G0-G1 or G2/M-phase cell cycle arrest, the percentage of G2/M phase cells was not profoundly affected in obovatol-treated cells.

2.4. Obovatol induced apoptosis in SW620 cells

Obovatol treatment cells induced morphological changes into rounding form, which is a primary indication of apoptosis and cell death (data not shown). And because treatment of obovatol in SW620 cells could induce sub-G1 population (Fig. 4), in order to study obovatol induced cell death in detail, we tested whether obovatol could induce apoptotic signal by flow cytometry. To distinguish between necrosis and apoptosis, FACS analysis was carried out after staining cells with annexin V-FITC and propidium iodide. As shown in Fig. 5A, 50 μM obovatol induced late apoptosis from <50% SW620 cells and also caused necrosis of SW620 cells (<12%) compared with control cells. To examine further effect of the compounds on apoptosis, we tested whether obovatol could induce chromosomal DNA fragmentation. As shown in Figure 5B, obovatol caused chromosomal degradation (Fig. 5B). These results collectively suggest that obovatol inhibits the growth of SW620 cells by induction of apoptosis.

2.5. Apoptosis induced by obovatol, is it through generation of ROS?

Apoptosis and cell cycle arrest by many naturally occurring anticancer agents correlate with ROS (reactive oxygen species) generation.[24,25] And also it was reported that honokiol generated ROS.[26] We studied the possible involvement of obovatol-mediated ROS generation in the apoptotic process. To explore the meaning of reactive oxygen species formation in obovatol-treated MDA-MB-231 cells, we used the general antioxidant *N*-acetylcysteine (NAC), which serves both as a redox buffer and as a reactive oxygen intermediate scavenger.[26] ROS production was not elevated in tumor cells by treatment of obovatol, and obovatol-induced morphological changes were not abolished by treatment with NAC (Fig. 6). These results clearly indicated that reactive oxygen species production was not involved in obovatol-induced cell cycle arrest and apoptosis. These results imply that obovatol and honokiol have different mechanisms on inducing apoptosis of human tumor cells.

3. Conclusion

Collectively, present findings provide in vivo evidence for anti-proliferative effects of obovatol, which were associated with the

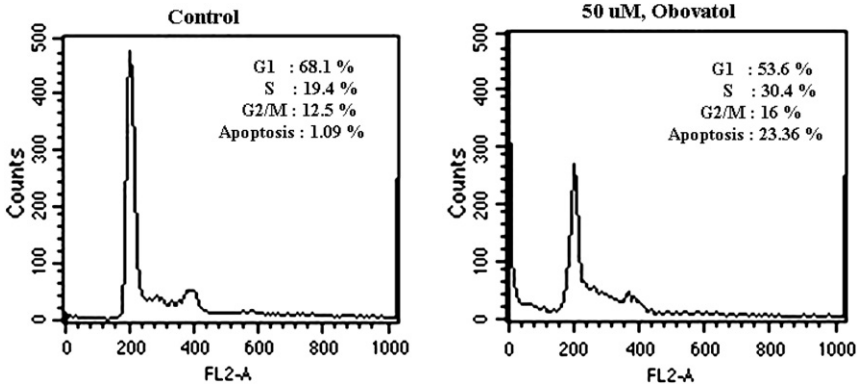

Figure 4. Representative cell cycle distribution of SW620 cells in the presence of obovatol. SW620 cells were treated with 50 μM obovatol or with 0.1% DMSO. Cells were harvested, fixed, and stained with propidium iodide. 20,000 stained cells were then subjected to FACScalibur analysis to determine the distribution of cells.

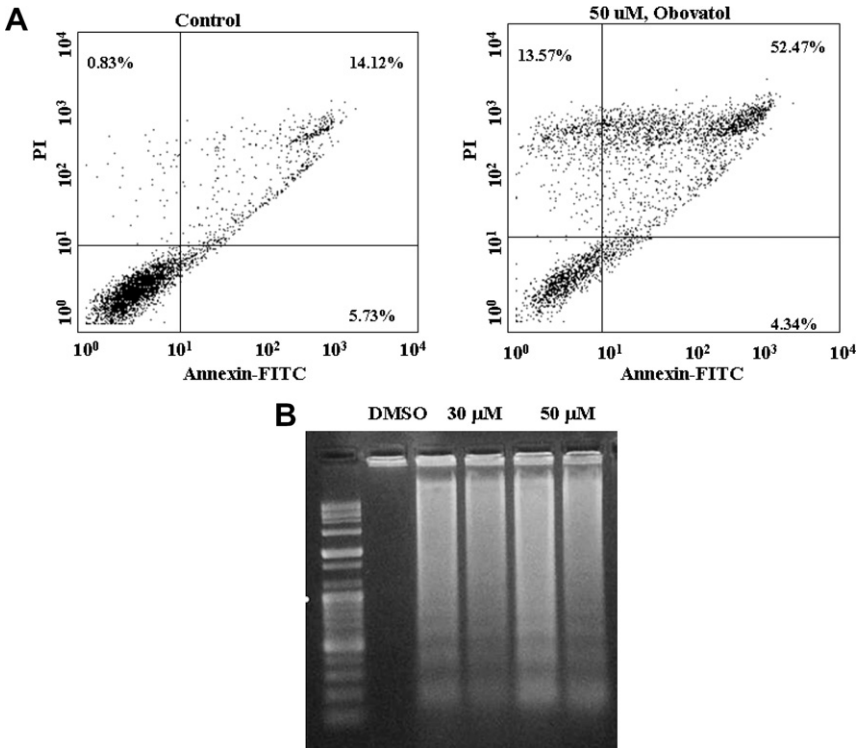

Figure 5. Obovatol induces apoptosis of SW620 cells. SW620 cells were treated with 50 μM obovatol or with vehicle solvent (0.1% DMSO) for 72 h and stained with annexin V-FITC and propidium. Stained cells then were subjected to FACScalibur analysis to determine the distribution of cells.

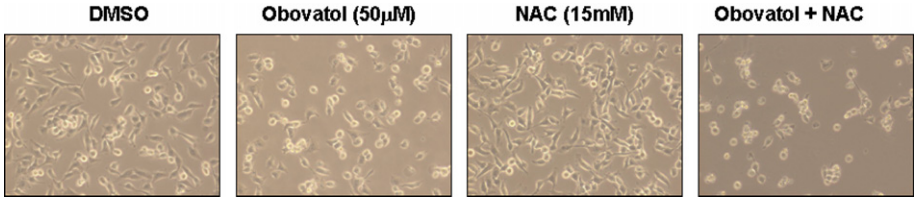

Figure 6. Obovatol-induced morphological changes were not abolished by treatment with NAC. MDA-MB-231 cells were treated with NAC (15 mM). One hour after the treatment, 50 mM obovatol was added and incubated for an additional 24 h.

inhibition of human CRC tumor xenograft growth without any toxicity in mice. Inducing apoptosis by obovatol could be potential in vivo mechanisms to inhibit CRC growth. In human SW620 tumor xenograft growth, obovatol shows more potent antitumor effects than honokiol, the finding provides preclinical evidence for antitumor efficacy of oral obovatol against human CRC, because honokiol is a well-known antitumor agent. Honokiol and magnolol induced through cell cycle arrest at Go/G1 and G2/M-phases, respectively, and honokiol generates ROS. However, obovatol does not arrest cells at Go/G1 and G2/M-phases and also produce ROS. Therefore, obovatol-induced inhibition of cell growth in vitro and in vivo will be different mechanisms. Further studies will be required to investigate the issue of what kinds of cellular mechanisms are involved in the obovatol-induced growth inhibition in SW620 cells.

4. Experimental

4.1. General

Chemicals and solvents were reagent grade and used without further purification. The column fractions were monitored by thin layer chromatography (TLC) on pre-coated Merck Silica gel $60F_{254}$ plates; the spots were visualized by exposure to UV radiation. ^1H NMR was recorded on a Varian 400 MHz spectrometer in $CDCl_3$. Column chromatography (CC) separations were carried out by using silica gel 60 (0.04–0.063 mm) and LiChroprep RP-18 (40–63 μm) supplied by E. Merck.

4.2. Isolation of obovatol and honokiol

The dried leaves (1 kg) of *M. obovata* Thunb were extracted with MeOH for 48 h at room temperature. After the combined extract was filtrated and concentrated, the residue was partitioned between H_2O and EtOAc (1:1, v/v) to give EtOAc-soluble fraction (15.2 g). EtOAc-soluble fraction was concentrated, and then the residue was chromatographed on a silica gel (1 kg) column, eluted with a gradient of *n*-Hexane–EtOAc to provide 10 fractions. Active fractions were collected and concentrated to yield 0.8 g. The active fraction was re-subjected to a C18 column and it was eluted with a gradient of MeOH–H_2O from 60% MeOH/water to 80% MeOH/water to provide obovatol (220 mg), magnolol (31 mg), and honokiol (17 mg). ^1H NMR (300 MHz, $CDCl_3$) for obovatol: δ 7.15 (2H, d, J = 9 Hz), 6.94 (2H, d, J = 9 Hz), 6.59 (1H, d, J = 1.5 Hz), 6.30 (1H, d, J = 1.5 Hz), 5.97 (2H, m), 5.06 (4H, m), 3.38 (2H, d, J = 6.6 Hz), 3.20 (2H, d, J = 6.6 Hz) ppm. ^1H NMR (300 MHz, $CDCl_3$) for magnolol: δ 6.88–7.26 (6H, m), 6.01 (2H, m), 5.08 (4H, m), 3.36 (4H, d,

J = 6.6 Hz) ppm. ^{1}H NMR (300 MHz, $CDCl_3$) for honokiol: δ 6.95–7.16 (6H, m), 5.95 (2H, m), 5.11 (4H, m), 3.46 (2H, d, J = 6.6 Hz), 3.35 (2H, d, J = 6.6 Hz) ppm.

4.3. Cells culture and compounds treatment

Human cancer cells such as colorectal cancer (SW620, HCT116, HT29, and DLD-1), breast adenocarcinoma (MCF7 and MDA-MB231), prostate cancer (Du145 and PC3), erythroleukemia cell (K562) were purchased from ATCC. Cancer cells were maintained as monolayer cultures in DMEM/F12 supplemented with 5% horse serum, hydrocortisone (0.5 μg/ml), insulin (10 μg/ml), epidermal growth factor (20 ng/ml), cholera toxin (0.1 μg/ml), penicillin–streptomycin (100 units/ml), L-glutamine (2 mM), and amphotericin B (0.5 μg/ml). SW620 cells were maintained in RPMI1640, supplemented with 10% heat-inactivated FBS (Gibco/BRL). Solutions (10 mM) of obovatol or obovatol-rd were prepared with dimethyl sulfoxide (DMSO), stored at −20 °C, and then diluted as needed in the cell culture medium.

4.4. Cell proliferation assay

Cells were seeded at a density of 5000 cells/well in a 96-well microtiter plate. After 24 h, cells were replenished with fresh complete medium containing various concentrations of obovatol, obovatol-rd, or 0.1% DMSO. Viable cells were counted after incubated at 37 °C for 24, 48, 72, and 96 h in a 5% CO_2 atmosphere.

4.5. Western blot analysis

A 40 μg protein was resolved by 10% SDS–polyacrylamide gel and transferred to the PVDF (polyvinylidene difluoride) membrane (Roche, Germany). The membrane was blocked with TBS-T (50 mM Tris–HCl, pH 7.6, 150 mM NaCl, 0.1% Tween 20) containing blocking reagents (Roche) for 1 h. MMP-2 and actin primary antibody were purchased from Cell Signaling Technology. The antibodies were used at dilutions recommended by the manufacturers. The membrane was incubated with MMP-2 antibody for overnignt at 4 °C. Incubation in horseradish peroxidase-conjugated secondary antibody (Jackson immunology) was for 1 h at room temperature and then washed three times with TBS-T, and visualized with Chemiluminence POD (peroxidase) reagents (Roche, Germany). Quantity analysis is based on the intensity of the band using the Quantity ONE software (Bio-Rad).

4.6. Cell cycle analysis

To analyze the DNA content by flow cytometry, cells were trypsinized from the culture flask. After centrifugation at 300g for 5 min at room temperature, the supernatant was removed. The cells were then washed twice with PBS solution and fixed with 3 ml of ice cold 70% EtOH overnight. Fixed cells were harvested by centrifugation at 300g for 3 min at room temperature and washed twice with PBS containing 1% FBS. Collected cells were resuspended in PBS (100 μl/1×10^5 cells) and treated with 100 μg/ml of RNase A at 37 °C for 30 min. Propidium iodide was then added to a final concentration of 50 μg/ml for DNA staining, and 20,000 fixed cells were analyzed on a FACScalibur (Becton Dickinson, San Jose, CA). Cell cycle distribution was analyzed using the Modifit's program (Becton Dickinson).

4.7. Annexin V-FITC staining

To analyze the apoptosis by flow cytometry, cells were collected 48 h after obovatol or obovatol-rd treatment, washed twice with phosphate-buffered saline, and resuspended in 1× binding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, and 2.5 mM $CaCl_2$) at a concentration of 1×10^6 cells/ml. Cells (100 μl) were transferred to a 5-ml culture tube containing 5 μl of annexin V-FITC and 10 μl of propidium iodide, and were mixed and incubated for 15 min at room temperature in the dark. Four-hundred microliters of 1× binding buffer were added into each tube, and stained cells were analyzed by flow cytometry.

4.8. Detection of DNA fragmentation

SW620 cells were treated with 30, 50 μM of obovatol and/or obovatol-rd for 48 h. Cells were harvested and resuspended with 0.5 ml of RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% Triton X100, 0.1% SDS, 5 mM EDTA, 30 mM Na_2HPO_4, 50 mM NaF, 0.5 mM $NaVO_4$, 2 mM PMSF, and 1% aprotinin). After incubation on ice for 30 min, the samples were centrifuged for 30 min at 13,000g, genomic DNA (the supernatant) was extracted by removing proteins with 1:1 mixture of phenol:chloroform (gentle agitation for 5 min followed by centrifugation) and precipitated with ethanol and one-tenth equivalence of 3 M sodium acetate. After centrifuging the sample at 13,000g, 20 min, 4 °C, supernatant was discarded and the pellet was resuspended in 30 μl of 20 mM Tris–HCl, pH 8.0, RNase solution (RNase 0.1 mg/ml). Samples were separated on 1.5% agarose gel and DNA in the gel was stained with ethidium bromide and photographed under UV light.

4.9. Nude mouse xenograft assay

For the evaluation of obovatol for antitumor activity in vivo, SW620 human colon adenocarcinoma cells (3×10^7 cells/ml) were implanted subcutaneously into the right flank of nude mice on day 0. Compound was emulsified in 0.5% Tween 80 and was administered intraperitoneally or orally for 20 days or orally for 26 days at a concentration of 5 mg/kg/day. Tumor volumes were estimated as length (mm) × width (mm) × height (mm)/2. To determine the toxicity of the compound, the body weight of tumor-bearing animals was measured. On day 21 or 27, the mice were sacrificed and the tumors were weighed.

4.10. Statistical analysis

All data are presented as means ± SD. The statistically significant differences compared with untreated group were calculated by Student's t test.

Acknowledgments

This research was financially supported by grants from Plant Diversity Research Center of 21st Century Frontier Research Program, KRIBB Research Initiative Program, and the Korea Food and Drug Administration program for the Standardization of Oriental Herbal Medicines.

References and notes

1. Wang, W. S.; Chen, P. M.; Su, Y. *Cell. Mol. Life Sci.* **2006**, *63*, 663–671.
2. Baker, D. D.; Chu, M.; Oza, U.; Rajgarhia, V. *Nat. Prod. Rep.* **2007**, *24*, 1225–1244.
3. Butler, M. S. *Nat. Prod. Rep.* **2008**, *25*, 475–516.
4. Surh, Y. J. *Nat. Rev. Cancer* **2003**, *3*, 768–780.
5. Tang, W.; Hemm, I.; Bertram, B. *Planta Med.* **2003**, *69*, 97–108.
6. Huang, S. H.; Chen, Y.; Tung, P. Y.; Wu, J. C.; Kuo-Hsin Chen, K. H.; Wu, J. M.; Wang, S. M. *J. Cell. Biocehm.* **2007**, *101*, 1011–1022.
7. Xu, Q.; Pan, Y.; Wang, X.; Li, Y. C.; Li, J. M.; Wang, C. P.; Kong, L. D. *Prog. Neurosychopharmacol. Biol. Psychiatry* **2008**, *32*, 715–725.
8. Lin, Y. R.; Chen, H. H.; Ko, C. H.; Chan, M. H. *Life Sci.* **2007**, *81*, 1071–1078.
9. Lee, S. J.; Cho, Y. H.; Park, K.; Kim, E. J.; Jung, K. H.; Park, S. S.; Kim, W. J.; Moon, S. K. *Biochem. Pharmacol.* **2008**, *75*, 2289–2300.
10. Ahn, S. S.; Shishodia, S.; Sung, B.; Arbiser, J. L.; Aggarwal, B. B. *Mol. Cancer Res.* **2006**, *4*, 621–633.

11. Shigemura, K.; Arbiser, J. L.; Sun, S. Y.; Zayzafoon, M.; Johnstone, P. A.; Fujisawa, M.; Gotoh, A.; Weksler, B.; Weksler, H. E.; Chung, L. W. *Cancer* **2007**, *109*, 1279–1289.
12. Bai, X.; Cerimele, F.; Ushio-Fukai, M.; Waqas, M.; Campbel, P. M.; Govindarajan, B.; Der, C. J.; Battle, T.; Frank, D. A.; Ye, K.; Murad, E.; Dubiel, W.; Soff, G.; Arbiser, J. L. *J. Biol. Chem.* **2003**, *278*, 35501–35507.
13. Wolf, I.; O'Kelly, J.; Wakimoto, N.; Nguyen, A.; Amblard, F.; Karlan, B. Y.; Arbiser, J. L.; Koeffler, H. P. *Int. J. Oncol.* **2007**, *30*, 1529–1537.
14. Kong, Z. L.; Tzeng, S. C.; Liu, Y. C. *Bioorg. Med. Chem.* **2005**, *15*, 163–166.
15. Amblard, F.; Govindarajan, B.; Lefkove, B.; Rapp, K. L.; Detorio, M.; Arbiser, J. L. J.; Schinazi, R. F. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4428–4431.
16. Kwon, B. M.; Kim, M. K.; Lee, S. H.; Kim, J. A.; Lee, I. R.; Kim, Y. K.; Bok, S. H. *Planta Med.* **1997**, *63*, 550–551.
17. Seo, J. J.; Lee, S. H.; Lee, Y. S.; Kwon, B. M.; Hong, J. T.; Oh, K. W. *Prog. Neuropsychopharmacol. Biol. l Psychiatry* **2007**, *31*, 1363–1369.
18. Hwang, E. I.; Kwon, B. M.; Lee, S. H.; Kim, N. R.; Kang, T. H.; Kim, Y. T.; Park, B. K.; Kim, S. W. *J. Antimicrob. Chemother.* **2002**, *49*, 95–101.
19. Choi, M. S.; Lee, S. H.; Cho, H. S.; Kim, Y.; Yun, Y. P.; Jung, H. Y.; Jung, J. K.; Lee, B. C.; Pyo, H. B.; Hong, J. T. *Eur. J. Pharmcol.* **2007**, *556*, 181–189.
20. Lee, S. Y.; Yuk, D. Y.; Yoon, D. Y.; Jung, J. K.; Moon, D. C.; Lee, B. S.; Hong, J. T. *Eur. J. Pharmcol.* **2008**, *582*, 17–25.
21. Hahm, E. R.; Arlotti, J. A.; Marynowski, S. W.; Singh, S. V. *Clin. Cancer Res.* **2008**, *14*, 1248–1257.
22. Chen, F.; Wang, T.; Wu, Y. F.; Gu, Y.; Xu, X. L.; Zheng, S.; Hu, X. *World J. Gastroenterol.* **2004**, *10*, 3459–3463.
23. Hahm, E. R.; Singh, S. V. *Mol. Cancer Ther.* **2007**, *6*, 2686–2695.
24. Singh, S. V.; Srivastava, S. K.; Choi, S., et al *J. Biol. Chem.* **2005**, *280*, 19911–19924.
25. Han, D. C.; Lee, M. Y.; Shin, K. D.; Jeon, S. B.; Kim, J. M.; Son, K. H.; Kim, H. C.; Kim, H. M.; Kwon, B. M. *J. Biol. Chem.* **2004**, *279*, 6911–6920.
26. Hahm, E. R.; Sigh, S. V. *Mol. Cancer Ther.* **2007**, *6*, 2686–2695.