Modulation of P-Glycoprotein Expression by Honokiol, Magnolol and 4-*O*-Methylhonokiol, the Bioactive Components of *Magnolia officinalis*

HYO-KYUNG HAN and LUU THI VAN ANH

College of Pharmacy, Dongguk University-Seoul, Jung-gu, Seoul, Korea

Abstract. *Aim: This study aimed to evaluate the effect of honokiol and its structural analogs on the functional activity and gene expression of P-glycoprotein (P-gp) in order to identify effective P-gp inhibitors from natural products which have additional health-promoting effects. Materials and Methods: The interaction characteristics of honokiol, magnolol and 4-O-methylhonokiol with P-gp were determined in NCI/ADR-RES cells overexpressing P-gp. Results: All three compounds down-regulated the expression of P-gp in a concentration- and time-dependent manner, leading to 2.5- to 4.1-fold reductions of P-gp expression in NCI/ADR-RES cells. Accordingly, down-regulation of P-gp resulted in the significant enhancement of the intracellular accumulation of calcein, a P-gp substrate. Furthermore, pre-treatment with honokiol, magnolol or 4-O-methylhonokiol significantly increased the susceptibility of cancer cells to daunorubicin-induced cytotoxicity in NCI/ADR-RES cells. Conclusion: The present study suggests that honokiol, magnolol and 4-O-methylhonokiol could be promising agents for reducing the multidrug resistance of cancer cells to anticancer drugs via the down-regulation of P-gp expression.*

Cancer is one of the leading causes of death worldwide (1). Despite the increase in the survival rates, the number of cancer patients will continuously increase and deaths from cancer worldwide are projected to be over 17 million in 2030 (2). Treatment for cancer adopts a series of interventions including psychosocial support, surgery, radiotherapy and chemotherapy, which aim to cure the disease or considerably prolong the patient's life-time by offering a better quality of life. Among the treatment options, chemotherapy plays a critical role in the treatment of various types of cancer. However, multidrug resistance, the principal mechanism by which many types of cancer develop resistance to chemotherapy, often limits the efficacy of anticancer drugs. Multidrug resistance affects patients with a variety of blood cancer types as well as those with solid tumors, including breast, ovarian, lung and lower-gastrointestinal cancer (3).

It is generally accepted that the principal mechanism of multidrug resistance is the active transport of drugs out of cells (3, 4). Among the efflux transporters pumping-out drugs, P-glycoprotein (P-gp, gene symbol *ABCB1*) plays a prominent role in the resistance of cancer cells to a variety of chemotherapeutic drugs (*e.g.* doxorubicin, taxol and etoposide) (4). In addition, P-gp is widely distributed in the body and interacts with structurally diverse drugs to limit their bioavailability. Therefore, the development of effective inhibitors of P-gp expression and/or functional activity should reverse drug resistance and enhance the bioavailability of P-gp substrates. To this end, first-, second- and third-generation P-gp inhibitors have been discovered but these compounds suffer from disadvantages, such as toxicity at the doses required for attenuating P-gp activity, poor specificity, or unpredictable pharmacokinetic interactions (5). Therefore, there is a need for continuous effort to discover P-gp inhibitors with more favorable safety profiles.

Honokiol, magnolol and 4-*O*-methylhonokiol are bioactive constituents extracted from *Magnolia officinalis* (Figure 1). They exhibit various pharmacological effects, such as anti-platelet aggregation, antioxidative activity, anxiolytic potency, anti-inflammatory effects, and anti-microbial and anticancer effects (6-11). In addition, Xu *et al.* (12) have suggested that honokiol is able to down-regulate the gene expression of P-gp in MCF-7/ADR cells after 1-3 days incubation. However, they did not observe any competitive inhibition of P-gp-mediated drug efflux *via* the co-incubation with honokiol, although honokiol was reported to be a P-gp substrate in Caco-2 cells by Zeng *et al.* (13). Therefore, there should be further clarification on the inhibition potential of P-gp by honokiol. In addition, magnolol and 4-*O*-

Correspondence to: Hyo-Kyung Han, Ph.D., College of Pharmacy, Dongguk University-Seoul, Pil-dong-3ga, Jung-gu, Seoul 100-715, Korea. Tel: +82 222603957, e-mail: hkhan@dongguk.edu

Key Words: Honokiol, magnolol, 4-*O*-methylhonokiol, P-glycoprotein, gene expression, NCI/ADR-RES cells.

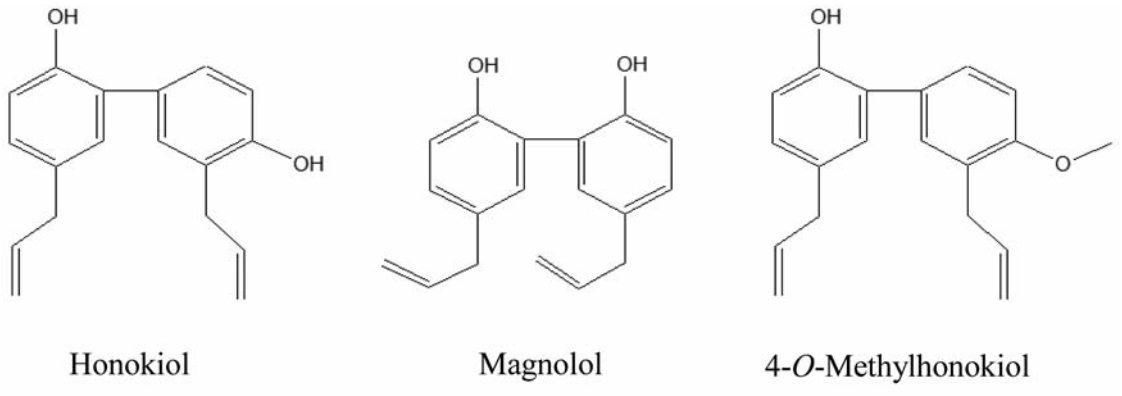

Figure 1. *Structures of honokiol, magnolol and 4-O-methylhonokiol.*

methylhonokiol are structural analogs of honokiol and their effects on the functional activity and gene expression of P-gp have not been studied yet. Therefore, the present study aimed at evaluating the effect of honokiol, magnolol and 4-*O*-methylhonokiol on the functional activity and gene expression of P-gp, as well as the effect of the structural features upon interaction with P-gp, in order to identify more effective P-gp inhibitors from natural products offering additional health- promoting effects.

Materials and Methods

Materials. Calcein AM, cyclosporin A, daunorubicin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma Co. (St. Louis, MO, USA). The BCA protein assay kit was purchased from EMD Chemicals Inc. (Gibbstown, NJ, USA). Honokiol and magnolol were purchased from Chengdu Biopurify Phytochemical Ltd. (Chengdu, Sichuan, China). 4-*O*-Methyl honokiol was a gift from Dr. YH Kim (R&D Center, Bioland Ltd., Cheonan, Korea). Fetal bovine serum (FBS) and cell culture media were purchased from Seolin Science Co. (Seoul, Korea). NCI/ADR-RES and OVCAR-8 cells (human ovarian carcinoma cells) were obtained from the National Cancer Institute (Frederick, MD, USA). The P-gp antibody was purchased from Calbiochem (Merck KGaA, Damstadt, Germany). Alkaline phosphatase donkey anti-mouse IgG and horseradish peroxidase-conjugated donkey anti-mouse IgG were purchased from Jackson ImmunoReasearch (West Grove, PA, USA). Actin antibody and most of the reagents used for the western blot assays were obtained from Sigma Co. (St. Louis, MO, USA). All other chemicals were of reagent-grade and all solvents were of high-performance liquid chromatography (HPLC) grade.

Cells. NCI/ADR-RES cells were routinely maintained in RPMI-1640 containing 10% FBS and penicillin (50 IU/ml)/streptomycin (50 μg/ml). OVCAR-8 cells were routinely maintained in RPMI-1640 culture medium containing 10% FBS. All the cells were maintained in an atmosphere of 5% CO_2 and in 90% relative humidity at 37˚C.

Assessment of the inhibitory effect on P-gp activity. NCI/ADR-RES cells were seeded into 24-well plates at a density of 2.5×10^5 cells/well and incubated for 24 h. Cells were then incubated with honokiol, magnolol or 4-*O*-methylhonokiol, respectively, at 10 μM. After 0-12 h incubation, the medium was removed, cells were washed three times with phosphate-buffered saline buffer (PBS) and then incubated with calcein AM (2.5 μM, which is hydrolyzed by intracellular esterases into the calcein, a P-gp substrate). At the end of 30-min incubation, calcein AM solution was removed and cells were washed three times with ice-cold PBS. After cell lysis (0.1% Triton X, 0.3% NaOH in distilled water), samples were transferred into a 96-well plate. The fluorescence intensity of each sample was measured at excitation and emission wavelengths of 496 nm and 516 nm, respectively. The amount of protein in each sample was determined with the BCA protein assay kit following the manufacturer's instructions (EMD Chemicals Inc.).

Determination of the P-gp expression. Cells were washed twice with PBS and were ruptured with a lysis buffer containing 50 mM Tris–HCl (pH 7.5), 120 mM NaCl, 1 mM EDTA (pH 8.0), 50 mM NaF, 1% Igapal CA-630 and a combination of protease inhibitors (0.2 μg/ml aprotinin, 1 mM benzamide–HCl, 0.5 μg/ml chymostatin, 0.5 μg/ml leupeptin, 0.002 μg/ml pepstatin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 μM Na_3VO_4). Cell lysates were centrifuged at 10,000 ×*g* for 10 min to remove the cell debris. Protein amounts in the soluble extracts were determined by the BCA protein assay kit following the manufacturer's instruction (EMD Chemicals Inc.). Proteins were fractionated using a 10% separating gel. Fractionated proteins were then electrophoretically transferred to nitrocellulose paper and were immunoblotted with specific antibodies. The secondary antibodies used were horseradish peroxidase- or alkaline phosphatase-conjugated anti-IgG antibodies (Jackson ImmunoReasearch, West Grove, PA, USA). Nitrocellulose papers were developed using an ECL chemiluminescence system.

Effect of compounds on the cytotoxicity of daunorubicin. Cells were seeded into 96-well plates at a density of 2×10^4 cells/well and were then incubated for 24 h. Cells were washed three times with PBS buffer and then incubated with honokiol, magnolol or 4-*O*-methylhonokiol at a concentration of 0-50 μM for 72 h. In addition, to evaluate the effect on the anticancer activity of daunorubicin, cells were also incubated with daunorubicin (0-100 μM) for three days after 48-h pre-treatment with or without 10 μM of one of the three compounds (honokiol, magnolol or 4-*O*-methylhonokiol). At

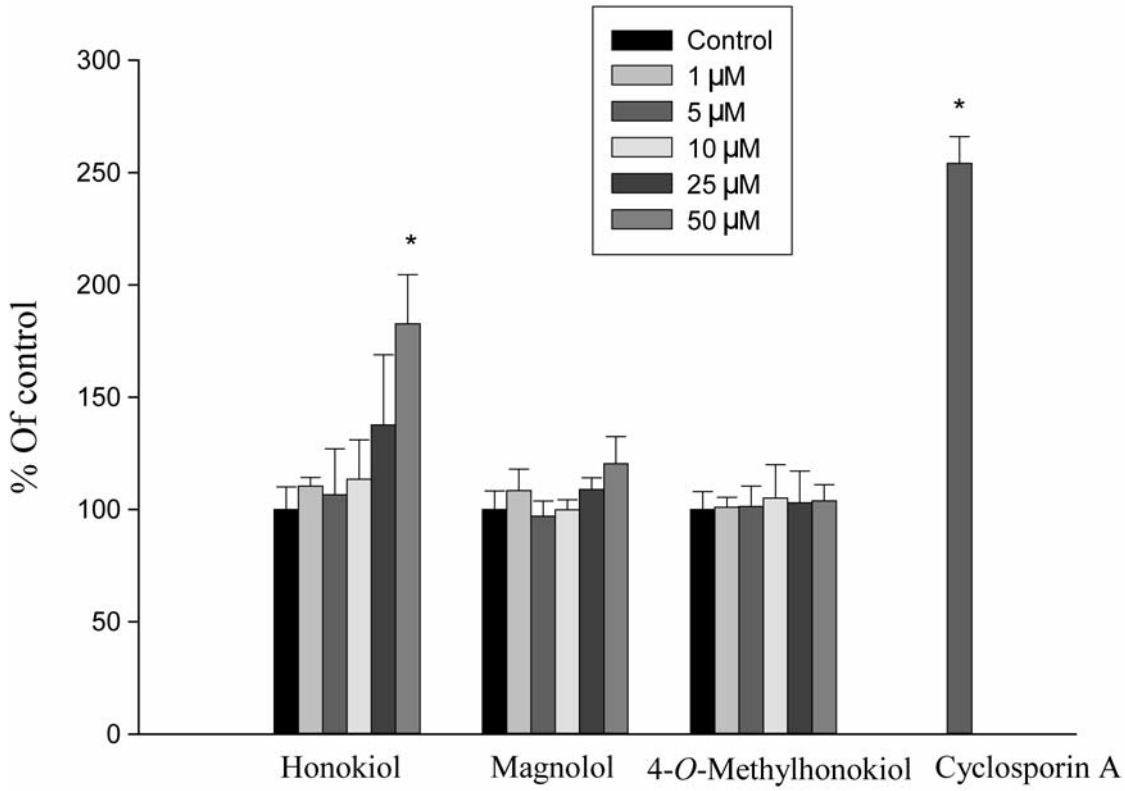

Figure 2. *Cellular accumulation of calcein in NCI/ADR-RES cells after co-incubation with honokiol, magnolol and 4-O-methylhonokiol at 10 μM for 30 min (mean±SD, n=6). *p<0.05 compared to the control group.*

the end of incubation, cell viability was determined by a modified colorimetric assay, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT). Briefly, the medium with the drug was removed and replaced by fresh medium (200 μl/well), containing 0.5 mg/ml MTT. After a 4-h incubation at 37˚C, the medium was aspirated and cells were extracted with 100 μl of DMSO. The concentration of the extracted formazan metabolite was determined by the measurement of absorbance at 560 nm in a 96-well plate reader. The 50% cytotoxic concentration (CC_{50}) was determined from the nonlinear regression of a dose-response curve using Sigma Plot 9.0 (Systat Software Inc., Point Richmond, CA, USA).

Statistical analysis. All mean values are presented with their standard deviations (mean±S.D.). Statistical analysis was conducted using a one-way ANOVA, followed by a posteriori testing with Dunnett correction. A *p*-value less than 0.05 was considered statistically significant.

Results and Discussion

Inhibitory effect on the P-gp activity. ABC transporters play a critical role in the development of multidrug resistance and include major efflux transporters such as P-gp, multidrug resistance-associated protein 1 (MRP1, gene symbol ABCC1), multidrug resistance-associated protein 2 (MRP2, gene symbol *ABCC2*), and breast cancer resistance protein (BCRP, gene symbol *ABCG2*). Taking into account the significant overlap in the substrate specificity across ABC transporters, *in vitro* models predominantly overexpressing a single efflux transporter have been often adopted to investigate the interaction with a particular transporter. Some previous studies indicated that NCI/ADR-RES cells have overexpression of P-gp but have no detectable level of other efflux transporters such as MRP1 and BCRP (14, 15). In addition, the expression of MRP2 seems to be minimal (15). Therefore, NCI/ADR-RES cells were selected as an *in vitro* model for the evaluation of the interaction with P-gp, and cyclosporine A, a well-known P-gp inhibitor was used as a positive control in this study.

The inhibitory effect of honokiol, magnolol and 4-*O*-methylhonokiol on P-gp-mediated cellular efflux was evaluated by using the calcein AM assay. Due to the intracellular conversion of calcein AM to calcein, a P-gp substrate, co-incubation of cells with P-gp inhibitors could significantly alter the cellular accumulation of calcein. As shown in Figure 2, magnolol and 4-*O*-methyl honokiol did not alter the intracellular accumulation of calcein over the concentration range of 1-50 μM. In contrast, honokiol

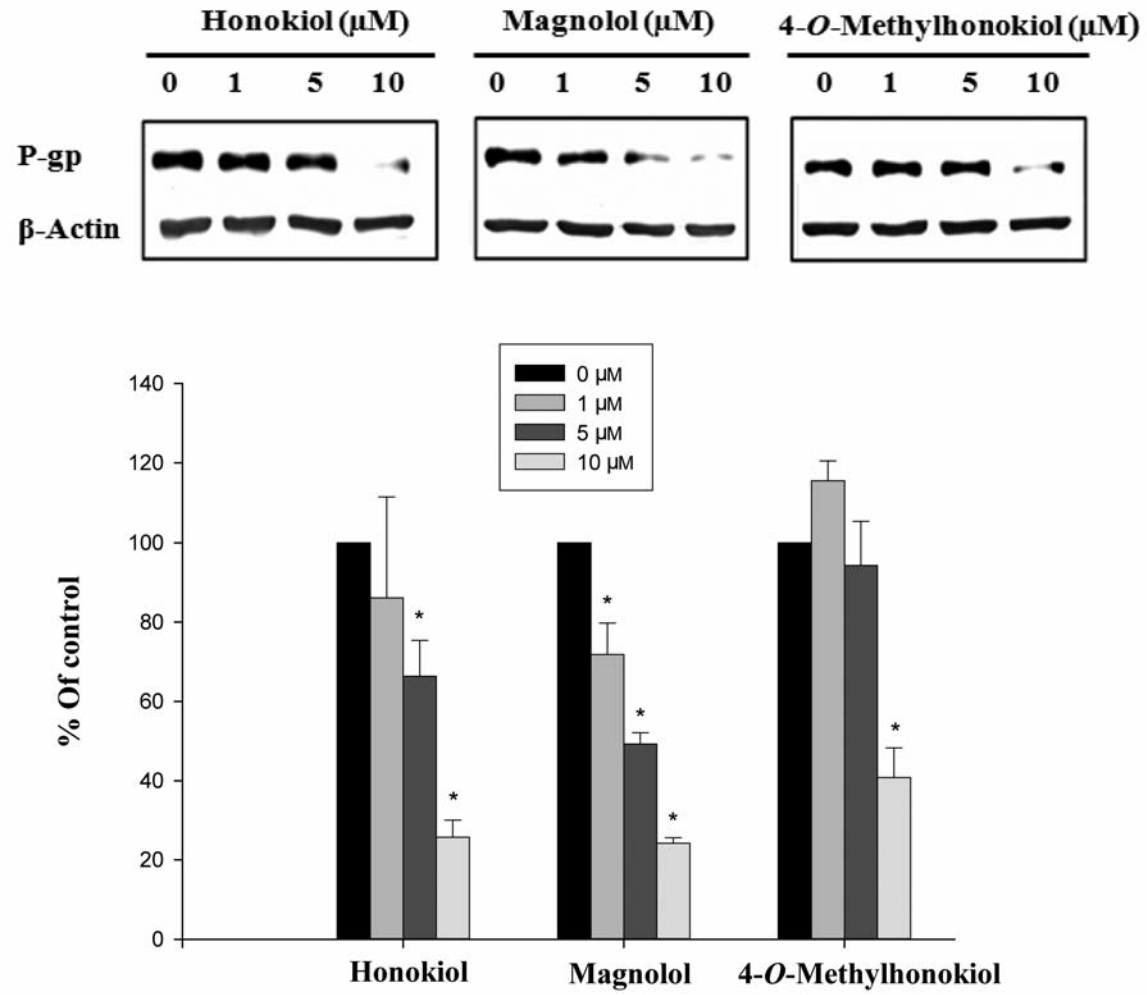

Figure 3. *Concentration dependency of the effect of honokiol, magnolol and 4-O-methylhonokiol on the expression of P-glycoprotein (P-gp) in NCI/ADR-RES cells (mean±SD, n=3). The expression levels of P-gp in NCI/ADR-RES cells exposed to the three compounds for 48 h were determined by western blot analysis. *p<0.05 compared to the control group (0 μM).*

significantly enhanced the intracellular accumulation of calcein at 50 μM, while it did not show any inhibitory effects at concentrations of 1-25 μM. Considering that honokiol was found to be a substrate of P-gp in Caco-2 cells (13), honokiol may inhibit P-gp-mediated cellular efflux of calcein by a competitive mechanism. However, our finding suggests that the affinity of honokiol for P-gp may be weak judging by the inhibitory effect at a concentration as high as 50 μM. Taken together, the concomitant use of honokiol may directly inhibit P-gp activity in a concentration-dependent manner, while magnolol and 4-*O*-methylhonokiol do not.

Effect of compounds on the P-gp expression. The effect of honokiol, magnolol and 4-*O*-methylhonokiol on the expression of P-gp was evaluated by western blot analysis. During a 72-h incubation, none of the tested compounds (honokiol, magnolol, 4-*O*-methylhonokol) exhibited any cytotoxicity over the concentration range of 0-10 μM in NCI/ADR-RES cells (data not shown). Therefore, the interaction of honokiol, magnolol and 4-*O*-methylhonokiol with P-gp was evaluated using concentrations of 0-10 μM.

Firstly, NCI/ADR-RES cells were incubated with the three compounds at different concentrations (0-10 μM) for 48 h. Among the tested compounds, magnolol was effective at inhibiting the expression of P-gp at as low as 1 μM and significantly ($p<0.05$) reduced P-gp expression by 2.0- to 4.1-fold over the concentration range of 1-10 μM. Honokiol significantly reduced P-gp expression by 1.5- to 3.9-fold at 5-10 μM, while 4-*O*-methyl honokiol appeared to be effective only at 10 μM, leading to the inhibition of P-gp expression by 2.5-fold (Figure 3). In addition, the time-dependency in the inhibitory effect on P-gp expression was

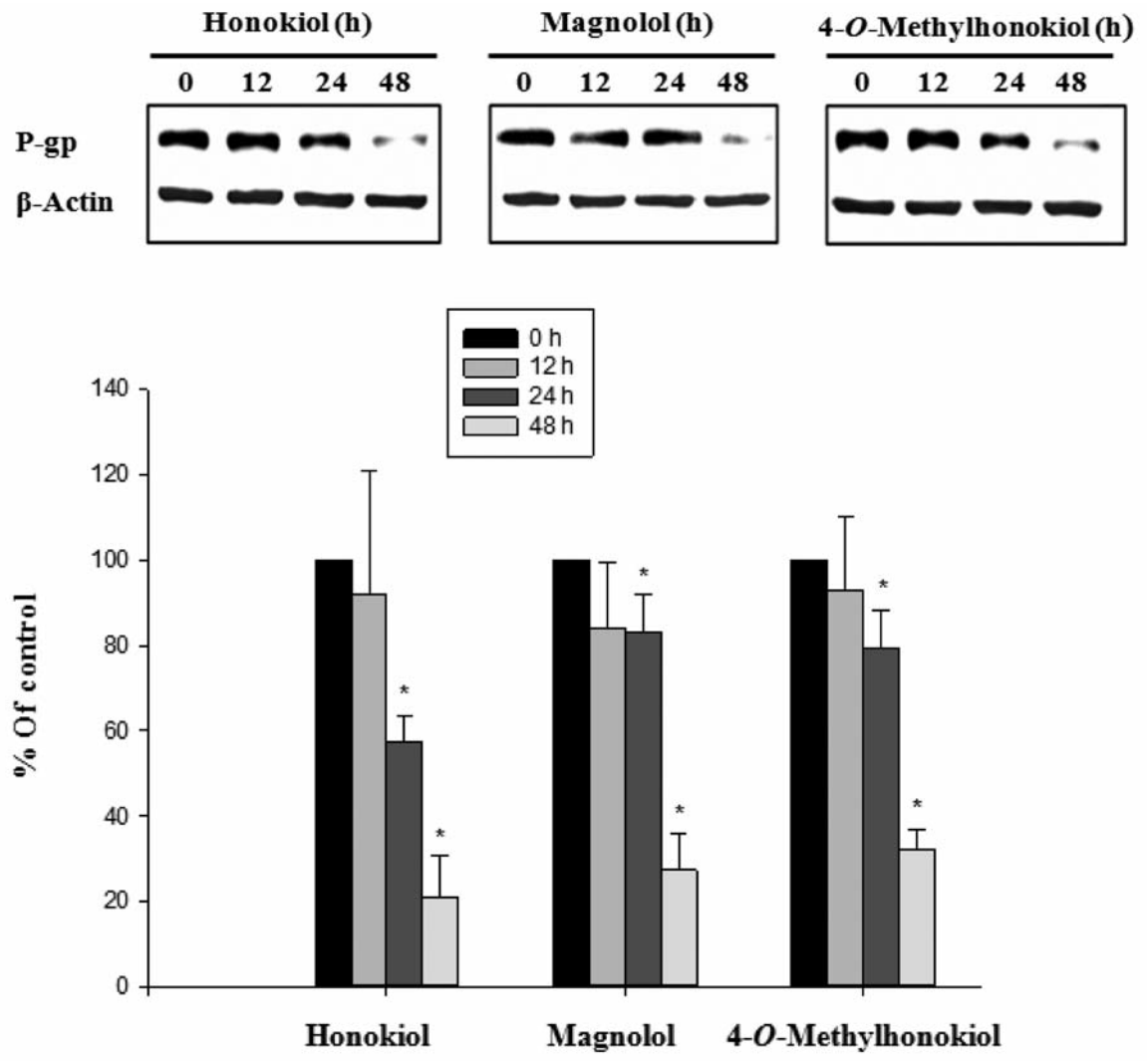

Figure 4. *Time dependency of the effect of honokiol, magnolol and 4-O-methylhonokiol on the expression of P-glycoprotein (P-gp) in NCI/ADR-RES cells (mean±SD, n=3). The expression levels of P-gp in NCI/ADR-RES cells exposed to the three compounds (10 μM) for 0-48 h were determined by western blot analysis. *p<0.05 compared to the control group (0 h).*

also evaluated in NCI/ADR-RES cells. After a 12-h incubation, none of the tested compounds had any inhibitory effect on P-gp expression, however, all of three compounds significantly ($p<0.05$) reduced P-gp expression after 24-h and 48-h incubations (Figure 4). Western blot analyses indicated that all three compounds reduced the expression of P-gp in a concentration- and time-dependent manner.

Functional assays of P-gp were conducted to determine whether the down-regulation of P-gp expression was correlated with the reduction of P-gp-mediated cellular efflux. NCI/ADR-RES cells were pre-treated with the three compounds (at 10 μM) for 0, 12, 24, 48 and 72 h, respectively and then the calcein AM assay was performed as described above. The pre-treatment with honokiol, magnolol, and 4-*O*-methylhonokiol for up to 24 h did not affect the intracellular accumulation of calcein. However, after pre-treatment for 48 h or 72 h, the intracellular accumulation of calcein significantly ($p<0.05$) increased (Figure 5). After 72-h incubation, all three compounds (10 μM) enhanced intracellular calcein accumulation by 2- to 3-fold. Therefore, the down-regulation of P-gp expression appeared to be well-correlated with the reduced P-gp-mediated cellular efflux of calcein, a P-gp substrate.

Previous studies indicated that reactive oxygen species (ROS) are a leading cause of elevated P-gp expression in cancer cell lines. ROS increase P-gp expression *via* multiple pathways including phosphatidylinositol 3-kinases (PI3K), extracellular signal-regulated kinases (ERK), c-Jun N-terminal

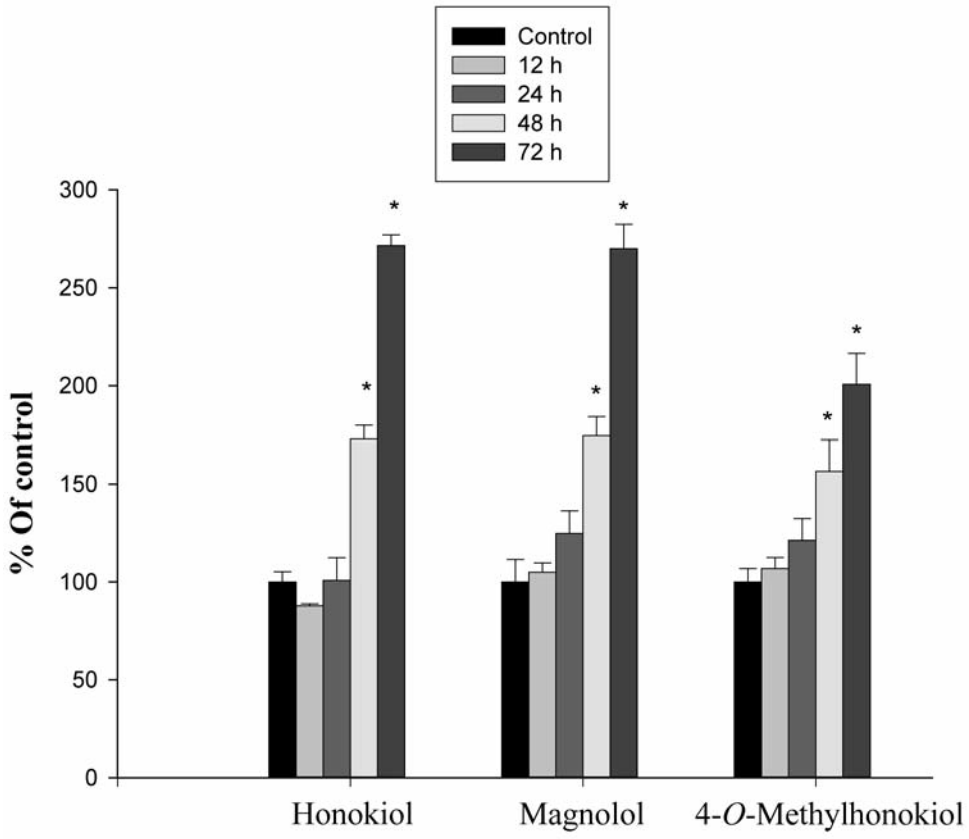

Figure 5. *Cellular accumulation of calcein in NCI/ADR-RES cells under pre-treatment with honokiol, magnolol and 4-O-methylhonokiol for 0-72 h (mean±SD, n=6). *p<0.05 compared to the control group (without pretreatment).*

kinases (JNK), protein kinase C (PKC) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways (16). On the other hand, a number of studies have indicated that honokiol and magnolol inhibit ROS production (17-19). Therefore, honokiol, magnolol and 4-*O*-methylhonokiol may down-regulate the expression of P-gp *via* the inhibition of ROS production. They might inhibit ROS production by several mechanisms (16-19): by diminishing the activity of assembled-NADPH oxidase, a major ROS producing enzyme; by inhibiting two important enzymes for ROS generation, namely myeloperoxidase and cyclooxygenase; by enhancing activity of glutathione (GSH) peroxidase activity, an enzyme that triggers the metabolism of hydrogen peroxide; and by being an effective scavenger of ROS. Among these mechanisms, the free radical-scavenging capacity is primarily attributed to the high reactivity of hydroxyl substituent (20). Therefore, the higher inhibitory effects of honokiol and magnolol on P-gp expression compared to 4-*O*-methylhonokiol might be explained, at least in part, by the difference in the number of hydroxyl groups (honokiol and magnolol have two hydroxyl groups, while 4-*O*-methyl honokiol has one) (20).

Effect of compounds on the cytotoxicity of daunorubicin. Daunorubicin is an anticancer drug and a P-gp substrate. Since the present study demonstrated that honokiol, magnolol and 4-*O*-methylhonokiol effectively down-regulated the P-gp expression, the effect of the three compounds on daunorubicin-induced cytotoxicity was also investigated in NCI/ADR-RES cells.

Honokiol, magnolol and 4-*O*-methylhonokol alone did not have any cytotoxicity at the tested concentration. However, after pre-treatment with these compounds, NCI/ADR-RES cells became more susceptible to the cytotoxicity of daunorubicin (Figure 6). The CC_{50} of daunorubicin was 29.2, 28.0 and 41.4 μM under pretreatment with honokiol, magnolol and 4-*O*-methylhonokiol, respectively, while the CC_{50} of daunorubicin-alone was >100 μM. Therefore, all three compounds appeared to be effective at enhancing daunorubicin-induced cytotoxicity in NCI/ADR-RES cells. This result might be explained by the increased net influx of daunorubicin *via* the down-regulation of P-gp expression by the three compounds. For clarification, the CC_{50} of daunorubicin was also determined with and without pre-

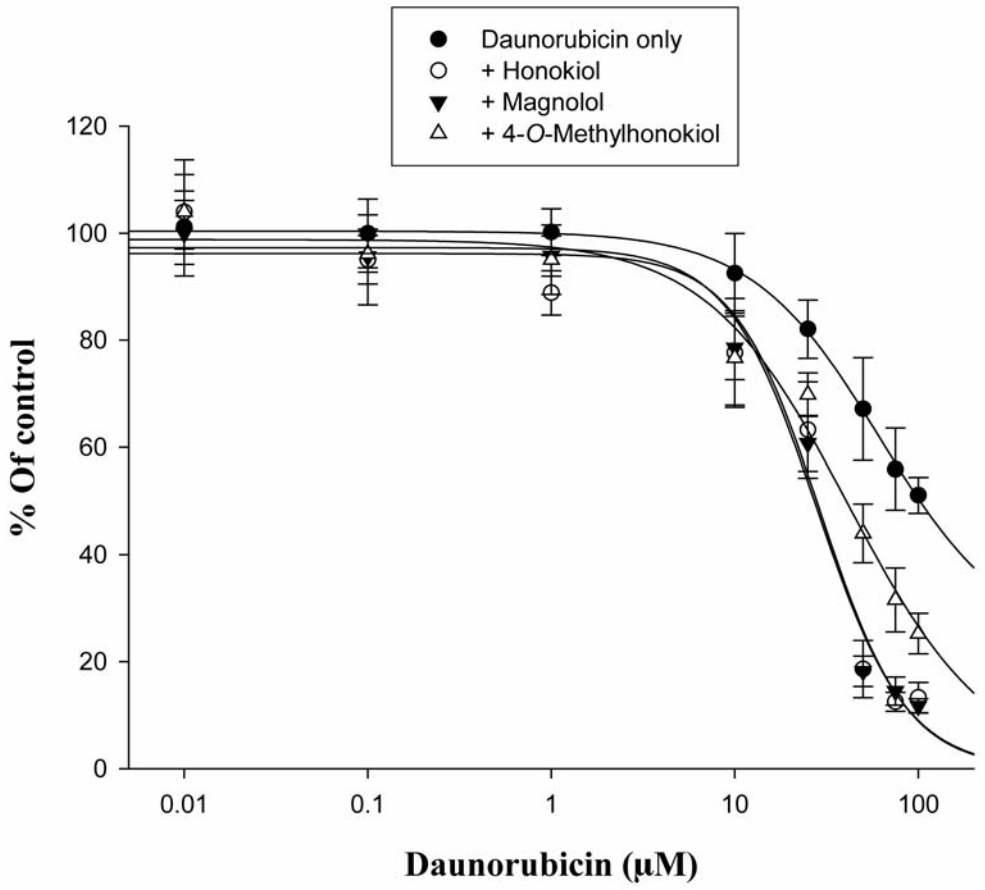

Figure 6. *Cytotoxicity of daunorubicin in NCI/ADR-RES cells with and without 48-h pretreatment with honokiol, magnolol, or 4-O-methylhonokiol (mean±SD, n=6).*

treatment with 4-*O*-methylhonokiol in OVCAR-8 cells, which lack P-gp expression (15). The CC_{50} values of daunorubicin in OVCAR-8 cells were 28.4 μM and 28.1 μM in the presence and the absence of 4-*O*-methylhonokiol, implying that the sensitivity of daunorubicin was not affected by 4-*O*-methylhonokiol in cells lacking P-gp expression.

Given the fact that many anticancer drugs are substrates for P-gp, current findings suggest that the concurrent use of honokiol, magnolol and 4-*O*-methylhonokiol should be beneficial in improving the effectiveness of cancer chemotherapy. In addition, these compounds exhibit various pharmacological activities and thus their concurrent use may provide with additional therapeutic benefits. For examples, honokiol has anticancer activity *via* various mechanisms, including the inhibition of angiogenesis and induction of apoptosis (21, 22). Therefore, the concurrent use of honokiol may improve the efficacy of cancer chemotherapy by its own anticancer effect, as well as by the inhibition of P-gp-mediated cellular efflux of anticancer drugs.

Conclusion

The present study suggests that honokiol, magnolol and 4-*O*-methylhonokiol could be promising agents for reducing multidrug resistance *via* the down-regulation of P-gp expression.

Acknowledgements

This work was supported by the Dongguk University Research Fund of 2010.

References

1 Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin *61*: 69-90, 2011.
2 Thun MJ, DeLancey JO, Center MM, Jemal A and Ward EM: The global burden of cancer: priorities for prevention. Carcinogenesis *31*: 100-110, 2010.
3 Persidis A: Cancer multidrug resistance. Nat Biotechnol *17*: 94-95, 1999.

4 Schinkel AH and Jonker JW: Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Adv Drug Deliv Rev *55*: 3-29, 2003.
5 Szakács G, Paterson JK, Ludwig JA, Booth-Genthe C and Gottesman MM: Targeting multidrug resistance in cancer. Nat Rev Drug Discov *5*: 219-234, 2006.
6 Teng CM, Chen CC, Ko FN, Lee LG, Huang TF, Chen YP and Hsu HY: Two antiplatelet agents from *Magnolia officinalis*. Thromb Res *50*: 757-765, 1988.
7 Shen JL, Man KM, Huang PH, Chen WC, Chen DC, Cheng YW, Liu PL, Chou MC and Chen YH: Honokiol and magnolol as multifunctional antioxidative molecules for dermatologic disorders. Molecules *15*: 6452-6465, 2010.
8 Maruyama Y, Kuribara H, Morita M, Yuzurihara M and Weintraub ST: Identification of magnolol and honokiol as anxiolytic agents in extracts of saiboku-to, an oriental herbal medicine. J Nat Prod *61*: 135-138, 1998.
9 Kim BH and Cho JY: Anti-inflammatory effect of honokiol is mediated by PI3K/AKT pathway suppression. Acta Pharmacol Sin *29*: 113-122, 2008.
10 Ho KY, Tsai CC, Chen CP, Huang JS and Lin CC: Antimicrobial activity of honokiol and magnolol isolated from *Magnolia officinalis*. Phytother Res *15*: 139-141, 2001.
11 Liu H, Zang C, Emde A, Planas-Silva MD, Rosche M, Kühnl A, Schulz CO, Elstner E, Possinger K and Eucker J: Antitumor effect of honokiol alone and in combination with other anticancer agents in breast cancer. Eur J Pharmacol *591*: 43-51, 2008.
12 Xu D, Lu Q and Hu X: Down-regulation of P-glycoprotein expression in MDR breast cancer cell MCF-7/ADR by honokiol. Cancer Lett *243*: 274-280, 2006.
13 Zeng B, Wu A, Chen J and Lai X: Study on transport characteristics of honokiol in Caco-2 cell model by HPLC. Zhongguo Zhong Yao Za Zhi *36*: 1286-1290, 2011.
14 Zhang S, Yang X and Morris ME: Flavonoids are inhibitors of breast cancer resistance protein (ABCG2)-mediated transport. Mol Pharmacol *65*: 1208-1216, 2004.
15 Qiang F, Lee BJ, Ha I, Kang KW, Woo ER and Han HK: Effect of maceligan on the systemic exposure of paclitaxel: *In vitro* and *in vivo* evaluation. Eur J Pharm Sci *41*: 226-231, 2010.
16 Callaghan R, Crowley E, Potter S and Kerr ID: P-Glycoprotein: So many ways to turn it on. J Clin Pharmacol *48*: 365-378, 2008.
17 Liou KT, Shen YC, Chen CF, Tsao CM and Tsai SK: The anti-inflammatory effect of honokiol on neutrophils: Mechanisms in the inhibition of reactive oxygen species production. Eur J Pharmacol *475*: 19-27, 2003.
18 Sheu ML, Chiang CK, Tsai KS, Ho FM, Weng TI, Wu HY and Liu SH: Inhibition of NADPH oxidase-related oxidative stress-triggered signaling by honokiol suppresses high glucose-induced human endothelial cell apoptosis. Free Radic Biol Med *44*: 2043-2050, 2008.
19 Dikalov S, Losik T and Arbiser JL: Honokiol is a potent scavenger of superoxide and peroxyl radicals. Biochem Pharmacol *76*: 589-596, 2008.
20 Heim KE, Tagliaferro AR and Bobilya DJ: Flavonoid antioxidants: chemistry, metabolism and structure activity relationships. J Nutr Biochem *13*: 572-584, 2002.
21 Bai X, Cerimele F, Ushio-Fukai M, Waqas M, Campbell PM, Govindarajan B, Der CJ, Battle T, Frank DA, Ye K, Murad E, Dubiel W, Soff G and Arbiser JL: Honokiol, a small molecular weight natural product, inhibits angiogenesis *in vitro* and tumor growth *in vivo*. J Biol Chem *278*: 35501-35507, 2003.
22 Battle TE, Arbiser J and Frank DA: The natural product honokiol induces caspase-dependent apoptosis in B-cell chronic lymphocytic leukemia (B-CLL) cells. Blood *106*: 690-697, 2005.

Received June 13, 2012
Revised August 8, 2012
Accepted August 9, 2012