

## 23-Hydroxyursolic Acid Causes Cell Growth-Inhibition by Inducing Caspase-Dependent Apoptosis in Human Cervical Squamous Carcinoma HeLa Cells

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**Abstract.** *Background:* There are few reports on the biological activities of 23-hydroxyursolic acid (23-HUA). The mechanism of growth-inhibition induced by 23-HUA, isolated from *Cussonia bancoensis*, in human cervical squamous carcinoma HeLa cells is hereby investigated. *Materials and Methods:* The growth-inhibitory activity was measured by MTS assay. Caspases activation and expression of apoptosis-related proteins were detected by Western blotting. Apoptotic cells were observed by morphological analysis with Hoechst 33342. *Results:* 23-HUA inhibited the growth of HeLa cells in a concentration dependent manner. Proteolytically generated fragments of caspase-3, -8 and -9 were observed in HeLa cells treated with 60  $\mu$ M 23-HUA. The expression of Bcl-X<sub>L</sub>, an anti-apoptotic protein, was markedly decreased by 60  $\mu$ M 23-HUA. Morphological analysis showed that apoptotic changes occurred after treatment with 60  $\mu$ M 23-HUA, and the changes were

inhibited by a pan-caspase inhibitor, Z-VAD-FMK. *Conclusion:* These results indicate that 23-HUA causes potent growth-inhibition by the induction of apoptosis via activation of caspases in HeLa cells.

Bioactive natural products are major sources of leads for the development of novel agents with pharmacological applications (1). Triterpenes represent a diverse class of compounds widely distributed in plants, and have a variety of biological activities, including anticancer and anticarcinogenic activities (2-6). Among them, pentacyclic triterpenes such as asiatic acid and ursolic acid have been reported to have anticancer activity *in vitro* (3-11). For example, asiatic acid has been described to induce apoptosis of various types of cancer cells, including glioblastoma, prostate, colon adenocarcinoma, breast cancer and hepatoma cells (3, 7-11).

23-Hydroxyursolic acid (23-HUA) has anti-nociceptive and anti-inflammatory activity (12, 13). It is structurally very similar to asiatic acid, the only difference being the absence of an OH-group at position 2. However, to date, the anticancer activity of 23-HUA has not been examined. In the present study, it was found that 23-HUA, isolated from the stem bark of *Cussonia bancoensis*, inhibited the growth of human cervical squamous carcinoma HeLa cells through induction of apoptosis. Further, the mechanism of apoptosis induction by 23-HUA in HeLa cells was investigated.

**Abbreviations:** DISK, death initiating signal complex; FADD, Fas-associated death domain; FBS, fetal bovine serum; 23-HUA, 23-hydroxyursolic acid; IC<sub>50</sub>, 50% growth-inhibitory concentration; MEM, minimum essential medium; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NF- $\kappa$ B, nuclear factor-kappaB; PBS, phosphate-buffered saline; Z-VAD-FMK, benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone.

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**Key Words:** 23-Hydroxyursolic acid, pentacyclic triterpenes, growth-inhibition, apoptosis, caspases.

### Materials and Methods

**Materials.** 23-Hydroxyursolic acid (23-HUA) from *Cussonia bancoensis* and asiatic acid from *Centella asiatica* (Figure 1), previously isolated at Kyung-Hee University, were used (12, 14). These compounds were checked by HPLC and were >97% pure. Minimum essential medium (MEM) and fetal bovine serum (FBS) were from Invitrogen (Carlsbad, CA, USA); penicillin/streptomycin

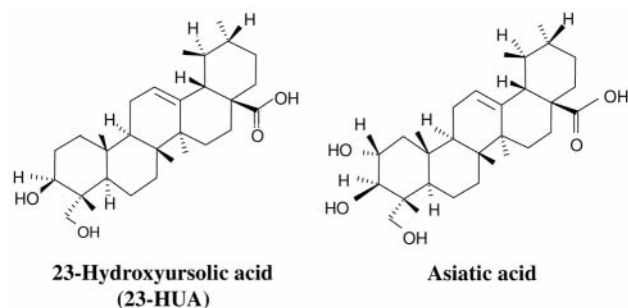


Figure 1. Chemical structures of pentacyclic triterpenes, 23-hydroxyursolic acid (23-HUA) and asiatic acid.

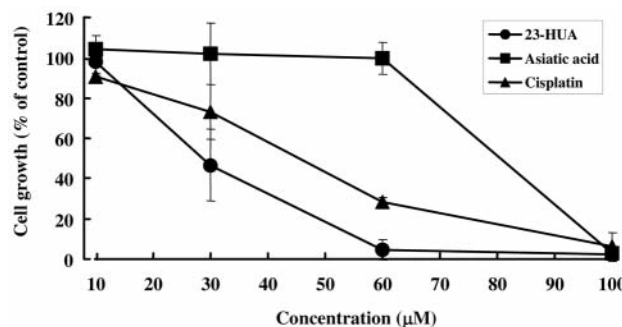
was from Cambrex Bio-Sciences (Baltimore, MD, USA); CellTiter 96 AQueous One Solution cell proliferation assay was from Promega (Madison, WI, USA); benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-FMK), cisplatin and Hoechst 33342 were from Sigma (St. Louis, MO, USA); antibodies for caspase-3, caspase-8, caspase-9, Fas-associated death domain (FADD), Bid, Bcl-X<sub>L</sub>, Bcl-2 and Bax were from Cell Signaling Technology (Beverly, MA, USA);  $\beta$ -actin antibody was purchased from Abcam (Cambridge, UK).

**Cell culture and in vitro growth-inhibitory assay.** Human cervical squamous carcinoma HeLa cells were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were cultured in MEM supplemented with 10% FBS and 1% penicillin/streptomycin in a CO<sub>2</sub> incubator at 37°C.

The cell growth-inhibitory effects of 23-HUA, asiatic acid and cisplatin were measured with the CellTiter 96 AQueous One Solution cell proliferation assay (Promega). This assay is a colorimetric method, using a tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and an electron coupling reagent (phenazine ethosulfate), to determine the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays. HeLa cells were plated at a density of  $2 \times 10^3$  cells/0.1 mL/well in 96-well plates and cultured for 24 hr in a CO<sub>2</sub> incubator at 37°C. Then, the cells were treated with or without 23-HUA, asiatic acid or cisplatin in the presence or absence of a pan-caspase inhibitor, Z-VAD-FMK, for 72 hr in a CO<sub>2</sub> incubator at 37°C. After 72 hr incubation, 20  $\mu$ L of the CellTiter 96 AQueous One Solution Reagent was added and incubation was continued for 3 hr in a CO<sub>2</sub> incubator at 37°C. The quantity of formazan product, which is directly proportional to the number of living cells in the culture, was measured in terms of absorbance at 492 nm (620 nm for background) with a 96-well plate reader, Sunrise Remote (Tecan Austria GmbH; Grodig, Austria). The cell growth (%) was calculated as follows:

$$\text{Cell growth (\%)} = \frac{[\text{OD}_{490} - \text{OD}_{620}](\text{treated})}{[\text{OD}_{490} - \text{OD}_{620}](\text{untreated})} \times 100$$

**Western blotting.** HeLa cells ( $1 \times 10^6$ ) cultured for 24 hr were treated with or without 60  $\mu$ M 23-HUA for the indicated periods in a CO<sub>2</sub> incubator at 37°C. Then, the cells were harvested using a cell scraper and washed twice with ice-cold phosphate-buffered saline (PBS). The cells were lysed with 50  $\mu$ L of cell lysis buffer [20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM ethyleneglycol-bis ( $\beta$ -aminoethylether)-N,N'-tetraacetic acid (EGTA), 1% Triton



Treatment	IC <sub>50</sub> (μM) <sup>a)</sup>
23-HUA	30.9 ± 6.7
Asiatic acid	80.4 ± 1.6
Cisplatin	57.8 ± 6.8

Figure 2. Cell growth-inhibitory effect of 23-HUA on human cervical cancer HeLa cells. HeLa cells ( $2 \times 10^3$  cells/well) were cultured in 96-well plates for 24 hr. Then, the cells were treated with 23-HUA, asiatic acid or cisplatin for 72 hr. After the 72 hr incubation, 20  $\mu$ L of the CellTiter 96 AQueous One Solution Reagent was added and incubation was continued for 3 hr. The absorbance at 492 nm was measured as a parameter of cell viability. Data are the means  $\pm$  SE of three experiments.

a) IC<sub>50</sub> is defined as the concentration that results in a 50% decrease in the number of cells compared to that of the untreated control. The values are the means  $\pm$  SE of three experiments.

X-100, 2.5 mM sodium pyrophosphate, 1 mM  $\beta$ -glycerophosphate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mg/mL leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF)], and then sonicated and centrifuged at  $14,000 \times g$  for 10 min at 4°C. The quantity of protein in the supernatant fraction was normalized against the untreated control, and this fraction was used as a Western blotting sample. The samples were loaded and separated by SDS-polyacrylamide gel electrophoresis. The proteins were transferred to a PVDF membrane and probed with antibodies for caspase-3, caspase-8, caspase-9, FADD, Bid, Bcl-X<sub>L</sub>, Bcl-2 and Bax. The antibody-bound proteins were detected by fluorescence assay with an ECF Western Blotting Kit (GE Healthcare; Little Chalfont, Buckinghamshire, UK), and bands were analyzed using a Typhoon 9410 imaging analyzer (GE Healthcare).

**Morphological analysis.** HeLa cells were treated with or without 60  $\mu$ M 23-HUA in the presence or absence of 100  $\mu$ M Z-VAD-FMK for 6 hr in a CO<sub>2</sub> incubator at 37°C. Then, the cells were trypsinized and washed twice by centrifugation at  $240 \times g$  for 5 min with PBS. After centrifugation, 20  $\mu$ L of 0.2 mg/mL Hoechst 33342 in PBS were added and the cells were examined with a confocal laserscan microscope, LSM510 META (Carl Zeiss; Oberkochen, Germany).

## Results

**Growth-inhibition of HeLa cells by 23-hydroxyursolic acid (23-HUA).** The growth of HeLa cells treated with 23-HUA for 72 hr decreased in a concentration-dependent manner and was 6.8% of the untreated control at the concentration of 60  $\mu$ M

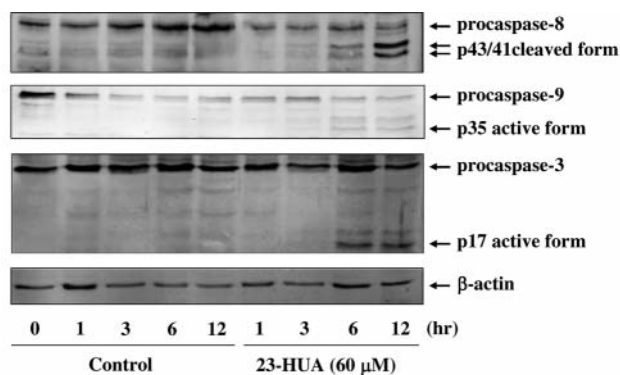


Figure 3. Activation of caspase-8, -9 and -3 in HeLa cells treated with 23-HUA. HeLa cells were treated with 60  $\mu$ M 23-HUA for the indicated periods. The cleavage of procaspases was detected by Western blotting.

(Figure 2). The concentration giving 50% inhibition ( $IC_{50}$ ) was  $30.9 \pm 6.7$   $\mu$ M. The growth-inhibitory effect was stronger than those of asiatic acid ( $IC_{50}=80.4 \pm 1.6$   $\mu$ M) and cisplatin ( $IC_{50}=57.8 \pm 6.8$   $\mu$ M).

**Activation of caspases by 23-HUA in HeLa cells.** The activation of an executioner caspase, caspase-3, and the initiator caspases, caspase-8 and -9, was assessed by detection of cleaved or active caspases by means of Western blotting. The p43/41 cleaved form of caspase-8, p35 active form of caspase-9 and p17 active form of caspase-3 were observed in HeLa cells treated with 60  $\mu$ M 23-HUA for 6-12 hr (Figure 3). Compared with caspase-3 and -9, the cleaved form of caspase-8 was increased in HeLa cells treated with 23-HUA for 12 hr.

**Effects of 23-HUA on the expression of FADD and Bcl-2 family proteins in HeLa cells.** The expression levels of FADD, an adaptor protein, which is essential for activation of caspase-8 in the extrinsic apoptotic pathway *via* death receptors, and Bid, a target protein of caspase-8, were estimated by Western blotting. A decrease of FADD protein was observed when HeLa cells were treated with 60  $\mu$ M 23-HUA for 3-12 hr (Figure 4). The expression of Bid also decreased in the cells treated with 60  $\mu$ M 23-HUA for 6-12 hr (Figure 4). The pattern of decrease of Bid protein was consistent with that of the increase of cleaved caspase-8 (Figures 3 and 4).

Bcl-2 family proteins, including Bcl-2, Bcl- $X_L$ , Bax and Bid, activate the intrinsic apoptotic pathway, with release of cytochrome *c*, activation of caspase-9 and subsequent activation of executioner caspases, such as caspase-3, through control of mitochondrial membrane permeability (14). As shown in Figure 5, the expression of Bcl- $X_L$ , an anti-apoptotic protein, decreased in HeLa cells treated with 60  $\mu$ M 23-HUA for 3-12 hr. The decrease of Bcl- $X_L$

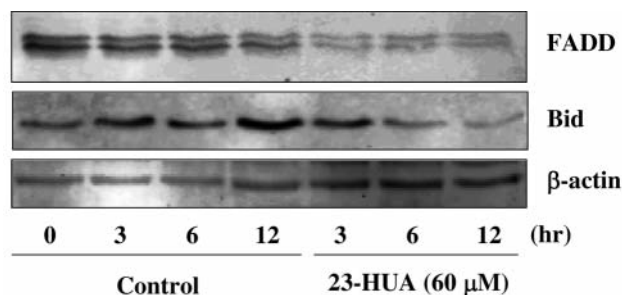


Figure 4. Expression of Fas-associated death domain (FADD) and Bid in HeLa cells treated with 23-HUA. HeLa cells were treated with 60  $\mu$ M 23-HUA for the indicated periods. The expression of proteins was detected by Western blotting.

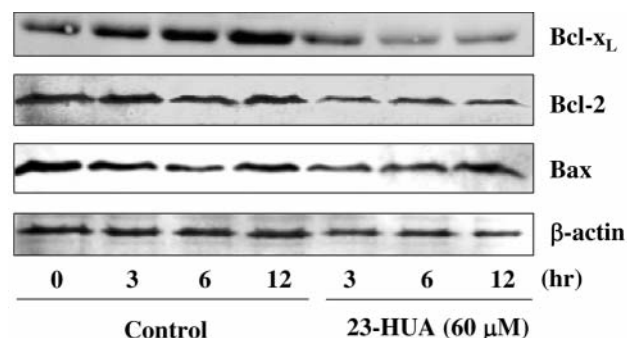


Figure 5. Expression of Bcl-2 family proteins in HeLa cells treated with 23-HUA. HeLa cells were treated with 60  $\mu$ M 23-HUA for the indicated periods. The expression of proteins was detected by Western blotting.

expression was marked at 6 hr and 12 hr. In addition, the expression of Bcl-2, another anti-apoptotic protein, also tended to be decreased by treatment with 23-HUA for 3-12 hr. On the other hand, the expression of Bax, a pro-apoptotic protein, hardly changed.

**Involvement of caspases in apoptosis induced by 23-HUA.** Morphological analysis with Hoechst 33342 also showed that apoptotic changes, including nuclear condensation and fragmentation, occur in HeLa cells treated with 60  $\mu$ M 23-HUA for 6 hr (Figure 6). The 23-HUA-induced apoptotic changes were inhibited by treatment with a pan-caspase inhibitor, Z-VAD-FMK (100  $\mu$ M) (Figure 6). Z-VAD-FMK also significantly inhibited cell growth-inhibition by 23-HUA in a concentration-dependent manner (Figure 7).

## Discussion

It was found that 23-HUA, a pentacyclic triterpene from *Cussonia bancoensis*, was a more potent growth inhibitor of HeLa cells than asiatic acid, an apoptosis-inducing

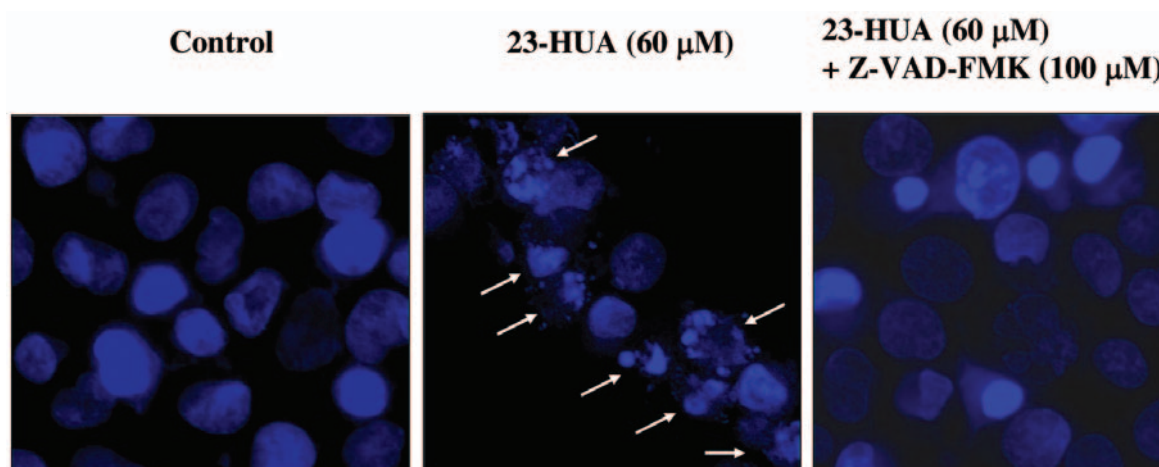


Figure 6. Effect of caspase inhibitor Z-VAD-FMK on morphological apoptotic change in HeLa cells treated with 23-HUA. HeLa cells were treated with 60  $\mu$ M 23-HUA for 6 hr in the presence or absence of 100  $\mu$ M Z-VAD-FMK. The nuclei were stained with Hoechst 33342 and visualized under a confocal laser-scanning microscope. Apoptotic cells showed nuclear condensation and fragmentation (arrow).

pentacyclic triterpene, or cisplatin, an anticancer drug well-used to treat cervical cancer (Figure 2). 23-HUA is structurally very similar to asiatic acid, the only difference being the absence of an OH-group at position 2 (Figure 1), which is presumably significant for the growth-inhibitory effect of pentacyclic triterpenes.

Several reports have shown that asiatic acid causes cell growth-inhibition through induction of apoptosis in various cancer cell lines (3, 7-11). Hsu *et al.* (10) reported that asiatic acid exhibited growth inhibition of breast cancer cells by inducing S-G<sub>2</sub>/M phase arrest and apoptosis. The cell cycle distribution in HeLa cells treated with 23-HUA was also investigated. 23-HUA (60  $\mu$ M) had no impact on the cell cycle distribution up to 24 hr, but an increase of hypodiploid content (sub-G<sub>1</sub> peak) indicative of apoptosis was observed (data not shown). Induction of apoptosis of cancer cells is an important strategy in cancer chemotherapy (15, 16), and caspases, a family of cysteine aspartic acid proteases, regulate apoptosis by cleaving target proteins (17). Therefore, the growth-inhibitory effect of 23-HUA may be related to apoptosis induction through the activation of caspases. The appearance of proteolytic fragments (active or cleaved forms) of caspase-8, -9 and -3 was observed, reflecting activation of these caspases in HeLa cells treated with 23-HUA for 6-12 hr (Figure 3). Furthermore, treatment of HeLa cells with 23-HUA for 6 hr induced morphological changes characteristic of apoptosis (Figure 6).

Caspase-8 is known to be activated in the extrinsic apoptotic pathway from death receptors such as CD95/Fas (18, 19). Activated caspase-8 promotes apoptosis by cleavage (activation) of proteins such as procaspase-3 and Bid (18, 19). The results showed that the expression of FADD protein was decreased by 23-HUA treatment for 3-12 hr (Figure 4).

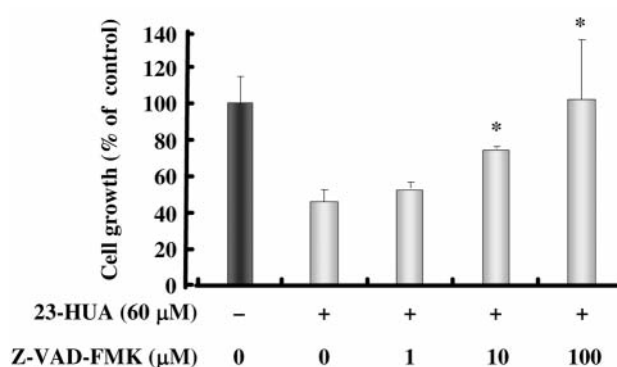


Figure 7. Effect of Z-VAD-FMK on cell growth-inhibition of HeLa cells by 23-HUA. HeLa cells ( $2 \times 10^3$  cells/well) in 96-well plates were cultured for 24 hr. Then, the cells were treated with 60  $\mu$ M 23-HUA for 6 hr in the presence or absence of Z-VAD-FMK (1-100  $\mu$ M). After 72 hr incubation, 20  $\mu$ L of the CellTiter 96 Aqueous One Solution Reagent was added and incubation was continued for 3 hr. The absorbance at 492 nm was measured as a parameter of the cell viability. Data are the means  $\pm$  SE of three experiments. \*Significantly different from 23-HUA alone at  $p < 0.05$ .

FADD is an adaptor protein that couples the signals received by death receptors to an intracellular death initiating signal complex (DISC) that causes activation of caspase-8 and/or -10 (17-19). In DISC formation, the binding of the prodomains of procaspase-8 and/or -10 to FADD leads to oligomerization of these procaspases, causing a conformational change that results in acquisition of enzymatic activity (17-19). Therefore, though the cause of the decrease of FADD protein by 23-HUA is unclear, the decrease of FADD suggested that the activation of caspase-8 is independent of the FADD-mediated extrinsic pathway. In



addition, the increase of cleaved caspase-8 and the decrease of Bid protein on treatment with 23-HUA for 12 hr (Figure 4) also suggested that the activation of caspase-8 is not the primary initiation signal in 23-HUA-induced apoptosis.

Caspase-9 is a primary initiator caspase that activates caspase-3 in response to the release of cytochrome *c* from mitochondria (17, 20). Bcl-2 family proteins regulate apoptosis through control of mitochondrial membrane permeability and release of cytochrome *c* (14, 17). Since 23-HUA activated caspase-3 and -9 (Figure 3), the effects of 23-HUA on expression of Bcl-2 family proteins, Bcl-2, Bcl-X<sub>L</sub> and Bax, were examined. Treatment with 23-HUA decreased the expression of anti-apoptotic proteins, Bcl-X<sub>L</sub> and Bcl-2, but the expression of a pro-apoptotic protein, Bax, did not change (Figure 5). Therefore, 23-HUA-induced apoptosis may be related, in part, to the activation of caspases such as caspase-9 and -3 *via* the decrease of anti-apoptotic proteins. Shin *et al.* (12) have demonstrated that 23-HUA inhibited the lipopolysaccharide-induced DNA binding activity of nuclear factor-kappaB (NF-κB) and decreased NF-κB p65 protein level in the nucleus. The expression of Bcl-2 family proteins, such as Bcl-2 and Bcl-X<sub>L</sub>, is regulated through NF-κB activation (21-23). Therefore, the decrease of Bcl-2 family proteins by 23-HUA may be due to the inhibition of NF-κB activation.

To estimate whether the activation of caspases is involved in 23-HUA-induced apoptosis, the effect of a pan-caspase inhibitor Z-VAD-FMK on the apoptotic changes and growth-inhibition induced by 23-HUA was examined. Z-VAD-FMK inhibited the induction of apoptosis by 23-HUA (Figure 6), and also almost completely blocked 23-HUA-induced HeLa cell growth-inhibition (Figure 7). These results suggested that the growth-inhibition of HeLa cells by 23-HUA results from induction of apoptosis *via* activation of caspases.

In this study, it was found that 23-HUA, a pentacyclic triterpene from *Cussonia bancoensis*, caused potent HeLa cell growth-inhibition by inducing caspase-dependent apoptosis. This is the first report that 23-HUA has growth-inhibitory effects on cancer cells. Indeed, 23-HUA was a more potent inhibitor than cisplatin, an anticancer agent used for treatment of cervical cancer. Asiatic acid is a similar apoptosis-inducing pentacyclic triterpene; the only structural difference is that 23-HUA lacks the OH-group at position 2. It is noteworthy that the loss of this group increased the growth-inhibitory potency. 23-HUA appears to be an interesting compound for the development of novel anti-cancer drugs.

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## References

- 1 Newman DJ and Cragg GM: Natural products as sources of new drugs over the last 25 years. *J Nat Prod* 70: 461-477, 2007.
- 2 Huang J, Tang XH, Ikejima T, Sun XJ, Wang XB, Xi RG and Wu LJ: A new triterpenoid from *Panax ginseng* exhibits cytotoxicity through p53 and the caspase signaling pathway in the HepG2 cell line. *Arch Pharm Res* 31: 323-329, 2008.
- 3 Cho CW, Choi DS, Cardone MH, Kim CW, Sinskey AJ and Rha C: Glioblastoma cell death induced by asiatic acid. *Cell Biol Toxicol* 22: 393-408, 2006.
- 4 Sun HX, Zheng QF and Tu J: Induction of apoptosis in HeLa cells by 3beta-hydroxy-12-oleanen-27-oic acid from the rhizomes of *Astilbe chinensis*. *Bioorg Med Chem* 14: 1189-1198, 2006.
- 5 Ovesná Z, Vachálková A, Horváthová K and Tóthová D: Pentacyclic triterpenoic acids: new chemoprotective compounds. *Minireview. Neoplasma* 51: 327-333, 2004.
- 6 Manu KA and Kuttan G: Ursolic acid induces apoptosis by activating p53 and caspase-3 gene expressions and suppressing NF-kappaB mediated activation of bcl-2 in B16F-10 melanoma cells. *Int Immunopharmacol* 8: 974-981, 2008.
- 7 Gurfinkel DM, Chow S, Hurren R, Gronda M, Henderson C, Berube C, Hedley DW and Schimmer AD: Disruption of the endoplasmic reticulum and increases in cytoplasmic calcium are early events in cell death induced by the natural triterpenoid asiatic acid. *Apoptosis* 11: 1463-1471, 2006.
- 8 Bunpo P, Kataoka K, Arimochi H, Nakayama H, Kuwahara T, Vinitketkumnuen U and Ohnishi Y: Inhibitory effects of asiatic acid and CPT-11 on growth of HT-29 cells. *J Med Invest* 52: 65-73, 2005.
- 9 Park BC, Bosire KO, Lee ES, Lee YS and Kim JA: Asiatic acid induces apoptosis in SK-MEL-2 human melanoma cells. *Cancer Lett* 218: 81-90, 2005.
- 10 Hsu YL, Kuo PL, Lin LT and Lin CC: Asiatic acid, a triterpene, induces apoptosis and cell cycle arrest through activation of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways in human breast cancer cells. *J Pharmacol Exp Ther* 313: 333-344, 2005.
- 11 Lee YS, Jin DQ, Kwon EJ, Park SH, Lee ES, Jeong TC, Nam DH, Huh K and Kim JA: Asiatic acid, a triterpene, induces apoptosis through intracellular Ca<sup>2+</sup> release and enhanced expression of p53 in HepG2 human hepatoma cells. *Cancer Lett* 186: 83-91, 2002.
- 12 Shin KM, Kim RK, Azefack TL, David L, Luc SB, Choudhary MI, Park HJ, Choi JW and Lee KT: *In vitro* anti-inflammatory activity of 23-hydroxyursolic acid isolated from *Cussonia bancoensis* in murine macrophage RAW 264.7 cells. *Planta Med* 70: 803-807, 2004.
- 13 Tapondjou LA, Lontsi D, Sondengam BL, Choi J, Lee KT, Jung HJ and Park HJ: *In vivo* anti-nociceptive and anti-inflammatory effect of the two triterpenes, ursolic acid and 23-hydroxyursolic acid, from *Cussonia bancoensis*. *Arch Pharm Res* 26: 143-146, 2003.
- 14 Cory S, Huang DC and Adams JM: The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene* 22: 8590-8607, 2003.
- 15 Kornblau SM: The role of apoptosis in the pathogenesis, prognosis, and therapy of hematologic malignancies. *Leukemia* 12: S41-46, 1998.
- 16 Melet A, Song K, Bucur O, Jagani Z, Grassian AR and Khosravi-Far R: Apoptotic pathways in tumor progression and therapy. *Adv Exp Med Biol* 615: 47-79, 2008.

- 17 Nuñez G, Benedict MA, Hu Y and Inohara N: Caspases: the proteases of the apoptotic pathway. *Oncogene* 17: 3237-3245, 1998.
- 18 Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH and Peter ME: FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *EMBO J* 16: 2794-2804, 1997.
- 19 Fischer U, Stroh C and Schulze-Osthoff K: Unique and overlapping substrate specificities of caspase-8 and caspase-10. *Oncogene* 25: 152-159, 2006.
- 20 Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD, Wang HG, Reed JC, Nicholson DW, Alnemri ES, Green DR and Martin SJ: Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J Cell Biol* 144: 281-292, 1999.
- 21 Viatour P, Bentires-Alj M, Chariot A, Deregowski V, de Leval L, Merville M-P and Bours V: NF- $\kappa$ B2/p100 induces Bcl-2 expression. *Leukemia* 17: 1349-1356, 2003.
- 22 Lee HH, Dadgostar H, Cheng Q, Shu J and Cheng G: NF- $\kappa$ B-mediated up-regulation of Bcl-x and Bfl-1/A1 is required for CD40 survival signaling in B lymphocytes. *Proc Natl Acad Sci USA* 96: 9136-9141, 1999.
- 23 Liu X, Togo S, Al-Mugotir M, Kim H, Fang Q, Kobayashi T, Wang X, Mao L, Bitterman P and Rennard S: NF- $\kappa$ B mediates the survival of human bronchial epithelial cells exposed to cigarette smoke extract. *Respir Res* 9: 66-76, 2008.

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