

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/274511669>

Antiproliferative and Pro-Apoptotic Activity of Diarylheptanoids Isolated from the Bark of *Alnus japonica* in Human Leukemia Cell Lines

Article in *The American Journal of Chinese Medicine* · June 2015

DOI: 10.1142/S0192415X15500470

CITATIONS

8

READS

192

8 authors, including:



Regina Appiah-Opong

Noguchi Memorial Institute for Medical Research

70 PUBLICATIONS 559 CITATIONS

[SEE PROFILE](#)



Abigail Aning

Noguchi Memorial Institute for Medical Research

3 PUBLICATIONS 10 CITATIONS

[SEE PROFILE](#)



Dominic Edoh

University of Ghana

16 PUBLICATIONS 52 CITATIONS

[SEE PROFILE](#)



Alexander Kwadwo Nyarko

University of Ghana

141 PUBLICATIONS 1,184 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Chemical investigation of dichapetalum species [View project](#)



Valuation of African Traditional Medicine: Pharmacological and Toxicological evaluation of medicinal plant used in the management of non-communicable diseases. [View project](#)

Antiproliferative and Pro-Apoptotic Activity of Diarylheptanoids Isolated from the Bark of *Alnus japonica* in Human Leukemia Cell Lines

Takuhiro Uto,^{*,a} Nguyen Huu Tung,^{*,§,a} Regina Appiah-Opong,[†] Abigail Aning,[†] Osamu Morinaga,^{*}
Dominic Edoh,[‡] Alexander K. Nyarko[†] and Yukihiro Shoyama^{*}

^{*}Department of Pharmacognosy, Faculty of Pharmaceutical Sciences
Nagasaki International University, Nagasaki 859-3298, Japan

[†]Department of Clinical Pathology, Noguchi Memorial Institute for Medical Research
University of Ghana, Ghana

[‡]Centre for Scientific Research into Plant Medicine
Mampong-Akuapem 73, Ghana

[§]School of Medicine and Pharmacy, Vietnam National University, Hanoi
Cau Giay, Hanoi, Vietnam

Published 26 June 2015

Abstract: *Alnus japonica* Steud is a tree that grows in damp areas of mountain valleys and has been used as a traditional medicine in Asia. We investigated the antiproliferative activity of hirsutanone (Hir) and oregonin (Ore) in human cancer cell lines and elucidated their mechanisms of action. A cytotoxicity study using a panel of 12 human cancer and 4 normal cell lines indicated that Hir exhibited potent antiproliferative activity against 4 leukemia (Jurkat, U937, THP-1, and HL-60) and 2 colon cancer cell lines (HCT-15 and Colo205). Although Ore suppressed the cell growth of Jurkat and THP-1, its inhibitory potency was weaker than that of Hir. The IC₅₀ values of Hir and Ore in Jurkat were 11.37 μ M and 22.16 μ M, respectively. Further analysis on Jurkat cells demonstrated that Hir caused a sequence of events involved in apoptosis, including nuclear morphological changes and accumulation of cells with sub-G1 DNA content. Hir led to the cleavage of poly(ADP-ribose) polymerase (PARP) and activation of caspase-3, -8, and -9. In addition, Hir-induced PARP cleavage was completely abolished by specific inhibitors to these caspases. Our data suggested that Hir is a potent antiproliferative compound against the 4 leukemia cell lines and the 2 colon cancer cell lines tested. Furthermore, Hir exerts antiproliferative actions via caspase-dependent apoptotic cell death.

Correspondence to: Prof. Yukihiro Shoyama, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan. Tel: (+81) 956-20-5653, Fax: (+81) 956-20-5653, E-mail: shoyama@niu.ac.jp

^aThese authors contributed equally to this work.

Keywords: *Alnus japonica*; Hirsutanone; Oregonin; Diarylheptanoid; Antiproliferation; Apoptosis; Leukemia Cells.

Introduction

Alnus japonica Steud (Betulaceae) is a tree found in the damp areas of mountain valleys, and has been used as a traditional medicine in Asia as a remedy for fever, hemorrhage, diarrhea, alcoholism, various skin infections, and inflammation. Phytochemical investigations of *A. japonica* led to the isolation of diarylheptanoids, triterpenoids, and flavonoids (Nomura *et al.*, 1981; Lee *et al.*, 1992; Wada *et al.*, 1998; Kuroyanagi *et al.*, 2005). Among them, diarylheptanoids, which are classified into linear and cyclic types, were reported to possess potent biological activities *in vitro* and *in vivo*. Kuroyanagi *et al.* (2005) also showed that diarylheptanoids from *A. japonica* have anti-oxidative activity. Furthermore, diarylheptanoids from *A. japonica* are reported to have significant low-density-lipoprotein anti-oxidizing and radical scavenging activities (Lee *et al.*, 1992). Kim *et al.* (2004) examined the anti-inflammatory effect of 9 diarylheptanoids and suggested that the presence of a keto-enol group in the heptane moiety or a caffeoyl group in the aromatic ring were important for the efficacy on the inhibitory activities of nitric oxide (NO) and prostaglandin E₂ (PGE₂) production. Our previous study demonstrated that several diarylheptanoids from *A. japonica* exhibit anti-oxidative and anti-influenza properties (Tung *et al.*, 2010a,b). Recently, we found that 2 diarylheptanoids, hirsutanone (Hir) and oregonin (Ore) (Fig. 1), exhibited potent *in vitro* inhibitory activity against *Trypanosoma brucei* growth in the bloodstream (Tung *et al.*, 2014a). Furthermore, we reported that Ore restrained ischemia-reperfusion-induced mesentery oxidative stress by inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation (Tung *et al.*, 2014b). Several recent papers have examined the anticancer effects of Hir or Ore in cancer cell lines. Hir exhibited cytotoxic activity against HT-29 human colon carcinoma cells, and reactive oxygen species (ROS) participated in Hir-induced cancer cell death

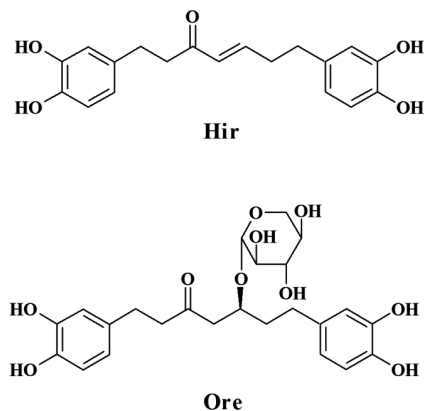


Figure 1. Chemical structures of Hir and Ore.

(León-Gonzalez *et al.*, 2014). Kang *et al.* (2014) indicated that Hir suppressed anchorage-dependent and independent cell growth of human prostate cancer cells via the direct inhibition of Akt1/2. In addition, Lee *et al.* (2012) reported that Hir enhanced the apoptotic effect of tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) on ovarian carcinoma cell lines by increasing the activation of the caspase-8- and Bid-dependent pathways and the mitochondria-mediated apoptotic pathway. However, the anti-proliferative properties of Hir and Ore in cancer and normal cell lines and their molecular mechanisms are not fully understood.

In the present study, we investigated the antiproliferative activities of Hir and Ore using a cell panel of human cancer and normal cell lines. Furthermore, we examined apoptosis induction and its molecular mechanisms in the human leukemia Jurkat cell line.

Materials and Methods

Reagents

Antibodies against caspase-3, -8, -9, and PARP were obtained from Cell Signaling Technology (Beverly, MA). Antibody against β -actin was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Fetal bovine serum (FBS) was supplied by GIBCO (Gaithersburg, MD). Caspase inhibitors were purchased from Calbiochem (San Diego, CA). All other chemicals were obtained from Wako Pure Chemical Industries.

Plant Material and Purification of Hir and Ore

The bark of *A. japonica* was collected in the Kyushu University Forest in January 2011 by Prof. Takao Setsu. The voucher specimen was deposited at the Herbarium of the Faculty of Pharmaceutical Science, Nagasaki International University. The air-dried bark of *A. japonica* (1.0 kg) was extracted with 50% aqueous EtOH (2.0 L \times 3 times) at 40°C under sonication. After the removal of the solvent, the resulting residue (115 g) was resuspended in 1.0 L of water and successively partitioned with CH₂Cl₂ and EtOAc (each 1.0 L \times 3 times) to obtain soluble fractions of CH₂Cl₂ (35.0 g) and EtOAc (53.5 g). A part of the EtOAc fraction (40.0 g) was fractionated over a silica gel column using a gradient of CHCl₃-MeOH (15:1-0:1, v/v) to give 6 fractions (fractions 1.1-1.6). Fraction 1.2 (2.6 g) was further chromatographed using a silica gel column with CHCl₃-MeOH (10:1), followed by reversed-phase chromatography on a C18 (RP) column with MeOH-H₂O (1:1) to produce Hir (60 mg). Fraction 1.4 (4.5 g) was studied with an RP column with MeOH-H₂O (6:5) to obtain 7 subfractions (fractions 2.1-2.7). Then, fraction 2.1 (1.8 g) was rechromatographed using a silica gel column with CHCl₃-MeOH (6:1, v/v) to yield Ore (1600 mg). The structures of Hir and Ore have been previously confirmed (Tung *et al.*, 2014a).

Oregonin (Ore): pale syrup; ESI-MS m/z 479 $[M + H]^+$; and ¹³C NMR (CD₃OD, 100 MHz): δ_C 29.9 (C-1), 46.3 (C-2), 212.0 (C-3), 48.6 (C-4), 76.3 (C-5), 38.4 (C-6), 31.6 (C-7), 134.0/135.1 (C-1'/1''), 116.3/116.5 (C-2'/2''), 145.8/145.9 (C-3'/3''), 144.0/144.2

(C-4'/4''), 116.3/116.5 (C-5'/5''), 120.6/120.7 (C-6'/6''), 104.1 (xylose-1), 75.0 (xylose-2), 77.7 (xylose-3), 71.1 (xylose-4), and 66.8 (xylose-5).

Hirsutanone (Hir): pale solid; ESI-MS m/z 329 $[M + H]^+$; and ^{13}C NMR (CD_3OD , 100 MHz): δ_C 30.9 (C-1), 42.6 (C-2), 203.2 (C-3), 131.5 (C-4), 149.5 (C-5), 35.6 (C-6), 34.7 (C-7), 134.0/134.1 (C-1' /1''), 116.4/116.6 (C-2' /2''), 146.0/146.1 (C-3' /3''), 144.4 (C-4'/4''), 116.4/116.5 (C-5'/5''), 120.7/120.8 (C-6'/6'').

Cell Culture and Treatment

All cancer cell lines and NB1RGB were obtained from RIKEN BioResource Center Cell Bank. PNT2, Chang Liver, and Hs 888Lu were obtained from ECACC. HepG2, MCF-7, and Hs 888Lu were maintained in Dulbecco's modified Eagle's medium (DMEM). Chang Liver and NHSF46 were grown in Eagle's minimum essential medium (EMEM) and Minimum essential medium- α (MEM- α), respectively. The other cell lines were maintained in RPMI1640 medium. All these media were supplemented with 10% FBS and 1% penicillin–streptomycin and were then incubated at 37°C under 5% CO_2 in fully humidified conditions. For the cell treatment, Hir, Ore, and caspase inhibitors were dissolved in dimethyl sulfoxide (DMSO) and were stored at $-20^\circ C$ before use. DMSO concentrations in the cell culture medium did not exceed 0.2% (v/v), and the controls were always treated with the same amount of DMSO as the actives used in the corresponding experiments.

Cell Viability Assay

Cell viability was determined by a MTT assay. In brief, the cells were seeded in 96-well plates at a density of 1×10^4 cells/well for suspension cells or 0.5×10^4 cells/well for attached cells. After incubation for 24 h, the cells were treated with Hir or Ore at various concentrations for 48 h. At the end of treatment, MTT solution was added to each well, and the cells were incubated for another 4 h. The precipitated MTT-formazan was dissolved with 0.04 N HCl-isopropanol, and the amount of formazan was measured at 595 nm using a microplate reader (Immuno Mini NJ-2300, Nihon InterMed, Tokyo, Japan) (Liu *et al.*, 2013). Cell viability was expressed as a percentage of the control culture.

Nuclear Staining by Hoechst 33258

After treatment with the isolated compounds for 48 h, the cells were harvested, washed with PBS, and fixed with 1% glutaraldehyde for 30 min. After washing with PBS, the cells were stained with Hoechst 33258 for 10 min. The cells were washed with PBS, and nuclear morphology was observed by fluorescence microscopy (Eclipse E600, Nikon, Tokyo, Japan).

Flow Cytometric Detection of Apoptotic Cells by Propidium Iodide Staining

Cell cycle analysis to detect sub-G1 phase cells was performed using a cell cycle phase determination kit (Cayman Chemical, Ann Arbor, MI), according to the manufacturer's

instructions. In brief, the cells were treated with Hir for 48 h, then washed and fixed with a fixative, and suspended with a staining solution containing propidium iodide (PI) and RNase A (Lin *et al.*, 2014). The sub-G1 peak was measured and analyzed in the FL2 channel of a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) with a 488 nm excitation laser. A total of 10,000 cells were analyzed per sample.

Western Blotting

After treatment with Hir for various periods, the harvested cells were lysed, and the supernatants were boiled for 5 min. Protein concentration was determined using a dye-binding protein assay kit according to the manufacturer's manual (Bio-Rad, Richmond, CA) (Hu *et al.*, 2013). Equal amounts of lysate protein were subjected to SDS-PAGE. Proteins were electrotransferred to PVDF membranes and were detected as described previously (Uto *et al.*, 2013).

Results

Effect of Hir and Ore on Cell Proliferation in Human Cancer and Normal Cell Lines

Hir and Ore were isolated from *A. japonica* as previously reported (Tung *et al.*, 2014a) and spectroscopically determined (See Section "Materials and Methods"). The antiproliferative activities of Hir and Ore were evaluated by a MTT assay against a panel of human cancer cell lines including leukemia, colon cancer, prostate cancer, stomach cancer, liver cancer, and breast cancer, and against normal cell lines including prostate, liver, lung, and fibroblast cells. The cells were treated with Hir or Ore at concentrations below 50 μM for 48 h. Table 1 shows the IC_{50} values of Hir and Ore. Hir strongly suppressed cell growth in all leukemia cell lines (Jurkat, $\text{IC}_{50} = 11.37 \mu\text{M}$; U937, $\text{IC}_{50} = 19.87 \mu\text{M}$; THP-1, $\text{IC}_{50} = 27.03 \mu\text{M}$; and HL-60, $\text{IC}_{50} = 33.59 \mu\text{M}$) and 2 colon cancer cell lines, including HCT-15 ($\text{IC}_{50} = 39.34 \mu\text{M}$) and Colo205 ($\text{IC}_{50} = 43.91 \mu\text{M}$). In contrast, Hir had no effect on the proliferation of other cancer and all normal cell lines. Moreover, Ore showed antiproliferative activities in 2 leukemia cell lines (Jurkat, $\text{IC}_{50} = 22.16 \mu\text{M}$; THP-1, $\text{IC}_{50} = 46.87 \mu\text{M}$), but these antiproliferative potencies were weaker than those of Hir. Ore had no influence on cell viabilities except in Jurkat and THP-1. Figure 2 indicates the effects of Hir and Ore on Jurkat cells, suggesting that both Hir and Ore suppressed cell growth in a dose- and time-dependent manner. Taken together, the results indicated that Hir is a potent antiproliferative compound against all of the leukemia cell lines and the two colon cancer cell lines tested, whereas it had low cytotoxicity in other cancer cell lines and normal cell lines. Among four leukemia cell lines, Jurkat was most sensitive to Hir; therefore, we further investigated the detailed mechanisms underlying the antiproliferative activity of Hir in the Jurkat cell line.

Table 1. Anti-Proliferative Effects (IC₅₀) of Hir and Ore Measured by MTT Assay Using Human Cancer and Normal Cell Lines

Cell Type	Cell Line	IC ₅₀ (μM) ^a	
		Hir	Ore
Cancer Cell Line			
Leukemia	Jurkat	11.37	22.16
	U937	19.87	>50
	THP-1	27.03	46.87
	HL-60	33.59	>50
Colon Cancer	HCT-15	39.34	>50
	Colo205	43.91	>50
	DLD1	>50	>50
Prostate Cancer	LNCap FGC	>50	>50
	PC-3	>50	>50
Stomach Cancer	KATO III	>50	>50
Liver Cancer	HepG2	>50	>50
Breast Cancer	MCF-7	>50	>50
Normal Cell Line			
Prostate	PNT2	>50	>50
Liver	Chang Liver	>50	>50
Lung	Hs 888Lu	>50	>50
Fibroblast	NB1RGB	>50	>50

Note: ^a IC₅₀ value of each compound was defined as concentration (μM) causing 50% inhibition of cell viability of the control culture.

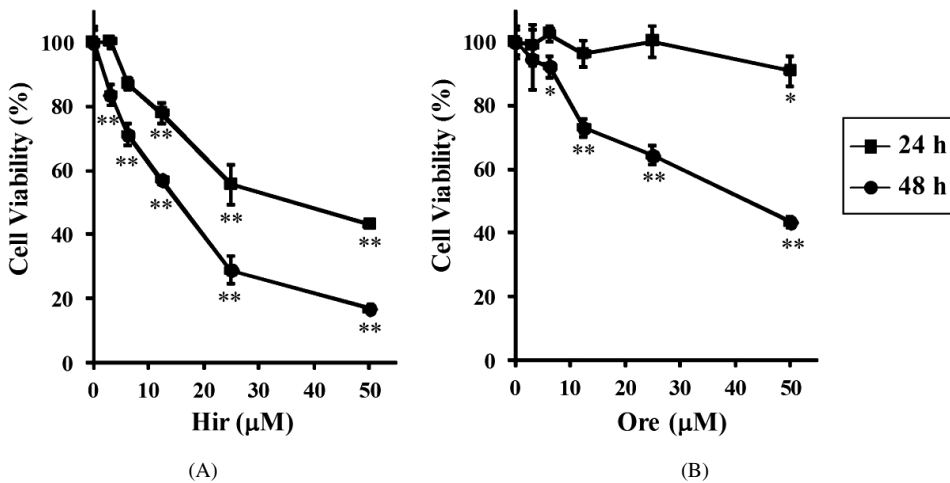


Figure 2. Effects of Hir (A) and Ore (B) on cell proliferation in Jurkat cells. Cells were treated with Hir or Ore at various concentrations for 24 h or 48 h, and the cell viability was determined using the MTT assay. Data represent the mean ± SD for three individual experiments. **p* < 0.05, ***p* < 0.01 compared with the control group.

Effect of Hir on Apoptosis Induction

To explore whether the antiproliferative activity of Hir is related to apoptosis induction, we conducted nuclear morphological observation and examined the number of cells with sub-G1 DNA content (characteristic of apoptosis) in Jurkat cells. Cells were treated with Hir at 10 or 20 μM for 48 h, and the nuclear morphology of the cells was observed using Hoechst 33258 staining. The control cells exhibited normal nuclear morphology, whereas the cells treated with Hir displayed chromatin condensation (Fig. 3A). Furthermore, we also analyzed the sub-G1 phase by flow cytometry after the cellular DNA had been stained with PI. Hir increased the accumulation of cells in the sub-G1 phase in a dose-dependent manner, and the proportion of cells in the sub-G1 phase increased from 2.2% to 14.3% and 34.2% at concentrations of 10 μM and 20 μM , respectively (Fig. 3B). Thus, these results clearly indicate that Hir exerted its antiproliferative effect via the induction of apoptotic cell death.

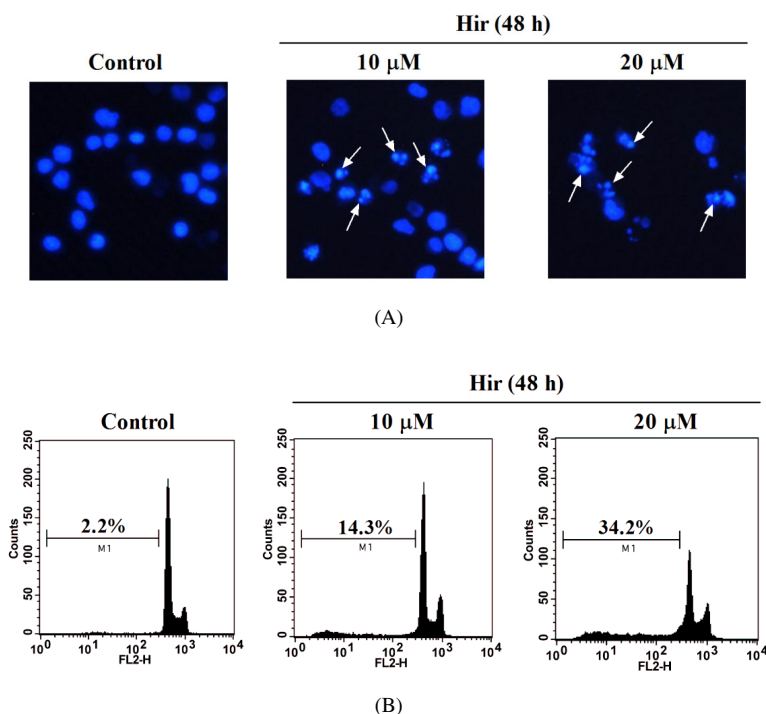
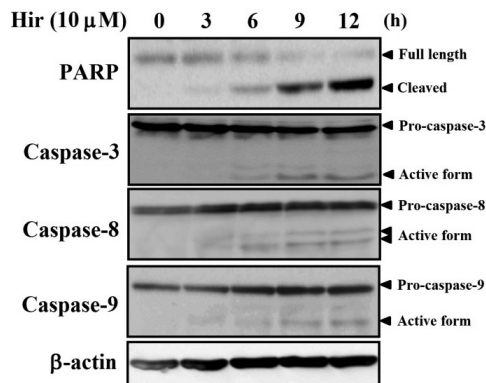


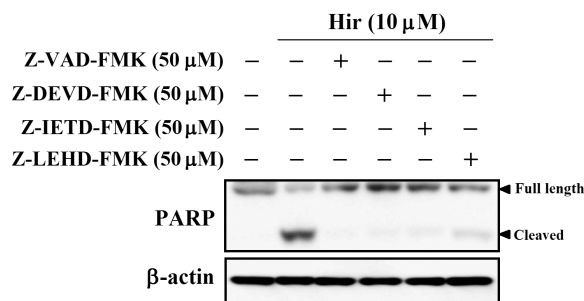
Figure 3. Effect of Hir on apoptosis induction. (A) Induction of chromatin condensation by Hir. Jurkat cells were treated with the indicated concentration of Hir for 48 h and were stained with Hoechst 33258. (B) Increase in the sub-G1 phase cells by Hir. Jurkat cells were treated with the indicated concentration of Hir for 48 h and were then analyzed by flow cytometry after staining with PI. Data shown are representative of three independent experiments that had similar results.

Involvement of the Caspase Cascade on Hir-Induced Apoptosis

In order to confirm the role of caspases in apoptosis induction by Hir, we used western blotting to examine the levels of PARP and, caspase-3, -8, and -9. PARP is the endogenous substrate protein of caspase-3, and caspase-3 activation induces specific proteolytic cleavage of PARP, a hallmark of apoptosis (D'Amours *et al.*, 1998). Hir caused the cleavage of PARP and activated caspase-3 in a time-dependent manner (Fig. 4A). Caspase-8 has been identified as the initiator caspase induced by death receptors, and caspase-9 has been proposed as another initiator caspase in the mitochondria-dependent apoptotic pathway (Degterev *et al.*, 2003). The activation of caspase-3 requires the activation of initiator caspases, such as caspase-8 and -9. Our data demonstrated that Hir activated both caspase-8 and -9. To further determine the involvement of caspases in Hir-induced apoptosis, the



(A)



(B)

Figure 4. Involvement of the caspase cascade in Hir-induced apoptosis. (A) The effect of Hir on PARP cleavage and the activation of caspase-3, -8, -9. Jurkat cells were treated with 10 μ M Hir for the times indicated. The cells were lysed, and PARP, caspase-3, -8, -9, and β -actin protein levels were determined by Western blotting. (B) The effect of caspase inhibitors on Hir-induced PARP cleavage. After pretreatment with 50 μ M caspase inhibitors for 1 h, Jurkat cells were treated with 10 μ M Hir for 12 h, and then Western blotting was performed. Data shown are representative of three independent experiments with similar results.

cells were treated with Hir in the absence or presence of caspase inhibitors, and then the PARP cleavage was analyzed. PARP cleavage induced by Hir was completely abolished by Z-VAD-FMK (broad caspase inhibitor), Z-DEVD-FMK (caspase-3 inhibitor), Z-IETD-FMK (caspase-8 inhibitor), and Z-LEHD-FMK (caspase-9 inhibitor) (Fig. 4B). These results indicate that Hir-induced apoptosis via a caspase-dependent pathway is involved in caspase-3 and initiator caspases of both caspases-8 and -9.

Discussion

Apoptosis is a highly regulated programmed cell death process in which cells undergo inducible nonnecrotic cellular suicide, and thus plays an important role in anticarcinogenesis (Chen *et al.*, 2013; Ma *et al.*, 2013). Apoptosis is characterized by distinct morphological changes such as cell shrinkage, chromatin condensation, plasma membrane blebbing, and oligonucleosomal DNA fragmentation (Elmore, 2007). Apoptosis induction involves the activation of caspases, which can be divided into 2 groups: initiator caspases and their downstream effector caspases (Degterev *et al.*, 2003). Initiator caspases, such as caspase-8 and -9, are self-activated in response to apoptotic stimulations, whereas the activation of effector caspases, such as caspase-3, requires the activation of initiator caspases (Ulukaya *et al.*, 2011). The induction of apoptosis is the most frequently observed mechanism of anticancer agents (Okun *et al.*, 2008; Hensley *et al.*, 2013). The strategy to selectively induce apoptosis in cancer cells is important for cancer chemoprevention and chemotherapy (Wang *et al.*, 2014; Zhang *et al.*, 2015).

In this study, we demonstrated that Hir exerts antiproliferative activity against 4 human leukemia cell lines (Jurkat, U937, THP-1, and HL-60) and two human colon cancer cell lines (HCT-15 and Colo205). Ore also suppressed cell proliferation of Jurkat and THP-1, but its inhibitory potency was weaker than that of Hir. Moreover, the results indicate that the antiproliferative activity of Hir in Jurkat cells is mainly due to apoptosis induction via a caspase-dependent pathway. This result could be explained based on the structures of Hir and Ore. Hir possesses an α,β -unsaturated ketone function resembling that of the chalcone skeleton, which has the possibility of forming of cyclic hydrogen bonds with high affinity to drug targets, and has been featured for the anticancer activity of chalcone analogs (Sahu *et al.*, 2012; Karthikeyan *et al.*, 2015). On the other hand, Ore cannot interact like Hir because it possesses a xylose moiety in a molecule. However, Ore will be able to be hydrolyzed to give hydrated Hir which is a precursor of Hir, and hydrated Hir can easily be transformed to Hir during metabolism, suggesting that Ore has a possibility as a prodrug of Hir. In fact, it is evident that the difference of cell viability between 24 h and 48 h in Ore is bigger than that of Hir (Fig. 2). This phenomenon might occur via the metabolism of Ore in cells during 48 h incubation. Although we have previously isolated other diarylheptanoid glycosides (Tung *et al.*, 2014a), they are supposed to have low antiproliferative activities depending on the positive relation between anti-parasite and anticancer activity (Huijsduijnen *et al.*, 2013) because their IC_{50} of anti-trypanosome activities are around 20–50 μ M. In addition, it is well-known that several natural products can exhibit their effects in a prodrug-like

manner, such as sennosides, which are metabolized by intestinal bacteria to release active anthrone which acts as a laxative (Hattori *et al.*, 1982, 1988). From these arguments, we assumed that Ore might function as a prodrug of Hir. Thus, the results of this study indicate that Hir and Ore isolated from the bark of *A. japonica* may be a potential candidate for the development of anticancer drugs.

Acknowledgments

This work was conducted partially under the Science and Technology Research Partnership for Sustainable Development (SATREPS), which was supported by the Japan Science and Technology (JST) and Japan International Cooperation Agencies (JICA). We are grateful to Prof. Takao Setsu, University Forest of Kyushu University, for his help with collection of the bark of *A. japonica*.

References

- Chen, Y.C., H.Y. Chang, J.S. Deng, J.J. Chen, S.S. Huang, I.H. Lin, W.L. Kuo, W. Chao and G.J. Huang. Hispolon from *Phellinus linteus* induces G0/G1 cell cycle arrest and apoptosis in NB4 human leukaemia cells. *Am. J. Chin. Med.* 41: 1439–1457, 2013.
- D'Amours, D., M. Germain, K. Orth, V.M. Dixit and G.G. Poirier. Proteolysis of poly(ADP-ribose) polymerase by caspase 3: Kinetics of cleavage of mono(ADP-ribosyl)ated and DNA-bound substrates. *Radiat. Res.* 150: 3–10, 1998.
- Degterev, A., M. Boyce and J. Yuan. A decade of caspases. *Oncogene* 22: 8543–8567, 2003.
- Elmore, S. Apoptosis: A review of programmed cell death. *Toxicol. Pathol.* 35: 495–516, 2007.
- Hattori, M., G. Kim, S. Motoike, K. Kobashi and T. Namba. Metabolism of sennosides by intestinal flora. *Chem. Pharm. Bull.* 30: 1338–1346, 1982.
- Hattori, M., T. Namba, T. Akao and K. Kobashi. Metabolism of sennosides by human intestinal bacteria. *Pharmacology* 36: 172–179, 1988.
- Hensley, P., M. Mishra and N. Kyprianou. Targeting caspases in cancer therapeutics. *Biol. Chem.* 394: 831–843, 2013.
- Hu, W.S., Y.M. Lin, T.J. Ho, R.J. Chen, Y.H. Li, F.J. Tsai, C.H. Tsai, C.H. Day, T.S. Chen and C.Y. Huang. Genistein suppresses the isoproterenol-treated H9c2 cardiomyoblast cell apoptosis associated with P-38, ERK1/2, JNK, and NFkappaB signaling protein activation. *Am. J. Chin. Med.* 41: 1125–1136, 2013.
- Huijsduijnen, R.H.V., R.K. Guy, K. Chibale, R.K. Haynes, I. Peitz, G. Kelter, M.A. Philips, J.L. Vennerstrom, Y. Yuthavong and T.N.C. Wells. Anticancer properties of distinct antimalarial drug classes. *PLoS One* 8: e82962, 2013.
- Karthikeyan, C., N.S. Moorthy, S. Ramasamy, U. Vanam, E. Manivannan, D. Karunagaran and P. Trivedi. Advances in chalcones with anticancer activities. *Recent Pat. Anticancer Drug Discov.* 10: 79–115, 2015.
- Kang, S., J.E. Kim, Y. Li, S.K. Jung, N.R. Song, N.R. Thimmegowda, B.Y. Kim, H.J. Lee, A.M. Bode, Z. Dong and K.W. Lee. Hirsutenone in *Alnus* extract inhibits akt activity and suppresses prostate cancer cell proliferation. *Mol. Carcinog.* “Ahead of Print” 2014.
- Kim, S.T., J.D. Kim, S.H. Ahn, G.S. Ahn, Y.I. Lee and Y.S. Jeong. Hepatoprotective and antioxidant effects of *Alnus japonica* extracts on acetaminophen-induced hepatotoxicity in rats. *Phytother. Res.* 18: 971–975, 2004.

- Kuroyanagi, M., M. Shimomae, Y. Nagashima, N. Muto, T. Okuda, N. Kawahara, T. Nakane and T. Sano. New diarylheptanoids from *Alnus japonica* and their antioxidative activity. *Chem. Pharm. Bull. (Tokyo)* 53: 1519–1523, 2005.
- Lee, C.S., E.R. Jang, Y.J. Kim, S.C. Myung, W. Kim and M.W. Lee. Diarylheptanoid hirsutenone enhances apoptotic effect of TRAIL on epithelial ovarian carcinoma cell lines via activation of death receptor and mitochondrial pathway. *Invest. New Drugs*. 30: 548–557, 2012.
- Lee, W.S., J.R. Kim, K.R. Im, K.H. Cho, D.E. Sok and T.S. Jeong. Antioxidant effects of diarylheptanoid derivatives from *Alnus japonica* on human LDL oxidation. *Planta Med.* 71: 295–299, 2005.
- León-Gonzalez, A.J., N. Acero, D. Muñoz-Mingarro, M. López-Lázaro and C. Martín-Cordero. Cytotoxic activity of hirsutanone, a diarylheptanoid isolated from *Alnus glutinosa* leaves. *Phytomedicine* 21: 866–870, 2014.
- Lin, C.C., P.Y. Chao, C.Y. Shen, J.J. Shu, S.K. Yen, C.Y. Huang and J.Y. Liu. Novel target genes responsive to apoptotic activity by *Ocimum gratissimum* in human osteosarcoma cells. *Am. J. Chin. Med.* 42: 743–767, 2014.
- Liu, M., W. Wang, X. Li, D. Shi, H. Mei, X. Jin and J. Zhu. *Wedelia chinensis* inhibits nasopharyngeal carcinoma CNE-1 cell growth by inducing G2/M arrest in a Chk1-dependent pathway. *Am. J. Chin. Med.* 41: 1153–1168, 2013.
- Ma, H., Q. Fan, J. Yu, J. Xin and C. Zhang. Novel microemulsion of tanshinone IIA, isolated from *Salvia miltiorrhiza* Bunge, exerts anticancer activity through inducing apoptosis in hepatoma cells. *Am. J. Chin. Med.* 41: 197–210, 2013.
- Nomura, T., T. Tokoroyama and T. Kubota. Biarylheptanoids and other constituents from wood of *Alnus japonica*. *Phytochemistry* 20: 1097–1104, 1981.
- Okun, I., K.V. Balakin, S.E. Tkachenko and A.V. Ivachtchenko. Caspase activity modulators as anticancer agents. *Anticancer Agents Med. Chem.* 8: 322–341, 2008.
- Sahu, N.K., S.S. Balbhadra, J. Choudhary and D.V. Kohli. Exploring pharmacological significance of chalcone scaffold: A review. *Curr. Med. Chem.* 19: 209–225, 2012.
- Tung, N.H., S.K. Kim, J.C. Ra, Y.Z. Zhao, D.H. Sohn and Y.H. Kim. Antioxidative and hepatoprotective diarylheptanoids from the bark of *Alnus japonica*. *Planta Med.* 76: 626–629, 2010a.
- Tung, N.H., H.J. Kwon, J.H. Kim, J.C. Ra, J.A. Kim and Y.H. Kim. An anti-influenza component of the bark of *Alnus japonica*. *Arch. Pharm. Res.* 33: 363–367, 2010b.
- Tung, N.H., M. Suzuki, T. Uto, O. Morinaga, K.D. Kwofie, N. Ammah, K.A. Koram, F. Aboagye, D. Edoh, T. Yamashita, Y. Yamaguchi, T. Setsu, S. Yamaoka, N. Ohta and Y. Shoyama. Anti-trypanosomal activity of diarylheptanoids isolated from the bark of *Alnus japonica*. *Am. J. Chin. Med.* 42: 1245–1260, 2014a.
- Tung, N.H., K. Sun, J.Y. Fan, Y. Shoyama and J.Y. Han. Oregonin from the bark of *Alnus japonica* restrained ischemia-reperfusion induced mesentery oxidative stress by inhibiting NADPH oxidase activation. *Microcirculation* 21: 688–695, 2014b.
- Ulukaya, E., C. Acilan and Y. Yilmaz. Apoptosis: Why and how does it occur in biology? *Cell Biochem. Funct.* 29: 468–480, 2011.
- Uto, T., A. Sakamoto, N.H. Tung, T. Fujiki, K. Kishihara, S. Oiso, H. Kariyazono, O. Morinaga and Y. Shoyama. Anti-proliferative activities and apoptosis induction by triterpenes derived from *Eriobotrya japonica* in human leukemia cell lines. *Int. J. Mol. Sci.* 14: 4106–4120, 2013.
- Wada, H., H. Tachibana, H. Fuchino and N. Tanaka. The new diarylheptanoid glycosides from *Alnus japonica*. *Chem. Pharm. Bull.* 46: 1054–1055, 1998.
- Wang, C.Z., Z. Zhang, S. Anderson and C.S. Yuan. Natural products and chemotherapeutic agents on cancer: Prevention vs. treatment. *Am. J. Chin. Med.* 42: 1555–1558, 2014.
- Zhang, Z., Z. Li, X. Wu, C.F. Zhang, T. Calway, T.C. He, W. Du, J. Chen, C.Z. Wang and C.S. Yuan. TRAIL pathway is associated with inhibition of colon cancer by protopanaxadiol. *J. Pharmacol. Sci.* 127: 83–91, 2015.