ELSEVIER

Biochemical Pharmacology 63 (2002) 1389–1396

Biochemical Pharmacology

Apoptosis of a human non-small cell lung cancer (NSCLC) cell line, PLA-801, induced by acutiaporberine, a novel bisalkaloid derived from *Thalictrum acutifolium* (Hand.-Mazz.) Boivin

Qi Chen, Wenlie Peng, Anlong Xu*

Department of Biochemistry and Center for Biopharmaceutical Research, School of Life Sciences, Zhongshan University, Guangzhou 510275, China

Received 2 January 2001; accepted 31 August 2001

Abstract

Acutiaporberine is a novel ether-linked bisalkaloid isolated from the traditional Chinese medicinal herb *Thalictrum acutifolium* (Hand.-Mazz.) Boivin (TAB). The present study demonstrates for the first time, by means of nuclear staining, DNA agarose gel electrophoresis, and flow cytometry, that acutiaporberine induces apoptosis in PLA-801 cells, a cultured human non-small cell lung cancer (NSCLC) cell line. An immunohistochemical assay and western immunoblot analysis showed down-regulation of the *bcl-2* gene and up-regulation of the *bax* and c-*myc* genes in the acutiaporberine-treated cells. The observations also indicate that acutiaporberine induces apoptosis of PLA-801 cells in a concentration- and time-dependent manner. These results suggest that acutiaporberine may be a potential, natural apoptosis-inducing agent for NSCLC. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Acutiaporberine; Apoptosis; NSCLC; Antitumor drug; *Thalictrum acutifolium*; TCM

1. Introduction

Apoptosis is a physiological process leading to cell death. It is responsible for the deletion of excess cells from normal tissues and for specific pathologic events. Evidence has shown that the possible mechanisms of various current antitumor drugs are related to their ability to induce apoptosis in target tumor cells [1]. Biochemically and morphologically distinct from cellular necrosis, apoptosis involves chromatin condensation, cell shrinkage, DNA fragmentation, plasma membrane blebbing, and the formation of membrane-enclosed apoptotic bodies containing well-preserved organelles. Apoptotic cells are phagocytosed and digested by nearby resident cells without inducing any associated inflammation [2,3]. Therefore, induction of apoptosis has become a target strategy for antitumor drug discovery in recent years, and an apoptosis-inducing agent specific for tumor cells may be an ideal antitumor drug.

TCM has been used for a long time, with well-documented efficacy, in cancer therapy in China. However, the underlying mechanisms in most cases remain unknown. Recent research has revealed that inducing apoptosis is one of the anti-neoplastic mechanisms of TCM [4–8]. Based on this knowledge, and following the new strategy of anti-cancer drug screening methods proposed by the National Cancer Institute of the USA, our laboratory has established an *in vitro* system for the fast screening of novel anti-neoplastic compounds, based on their apoptosis-inducing ability in tumor cells. Acutiaporberine (Fig. 1), a novel bisalkaloid isolated from the root of a frequently used Chinese medicinal herb, TAB, was found to induce apoptosis in the human NSCLC cell line PLA-801. A previous study of ours has shown that this alkaloid causes death in several cultured human cancer cell lines [9]. The apoptosis-inducing function of acutiaporberine and the possible associated molecular mechanism are reported here.

* Corresponding author. Tel.: +86-20-84113655; fax: +86-20-84038377.

E-mail address: ls36@zsu.edu.cn (A. Xu).

Abbreviations: TCM, traditional Chinese medicine; TAB, *Thalictrum acutifolium* (Hand.-Mazz.) Boivin; NSCLC, non-small cell lung cancer; FCM, flow cytometry; IHC, immunohistochemistry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI, propidium iodide; DAB, diaminobenzidine.

0006-2952/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0006-2952(02)00871-7

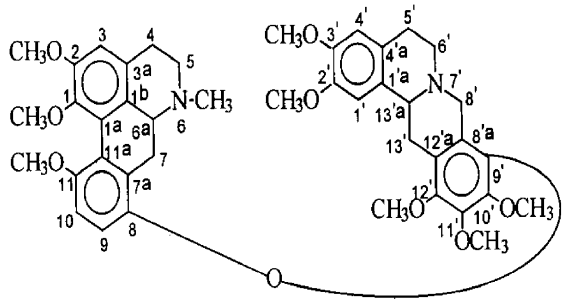

Fig. 1. Chemical structure of acutiaporberine.

2. Materials and methods

2.1. Acutiaporberine

Acutiaporberine, provided by Dr. Lin Cuiwu and coworkers of the Chemistry Department, Zhongshan University, is a light yellow crystalline flake with a relative molecular mass of 724.85, m.p. 183.5–184°, optical rotation [α] −14.2° (3% CH_3OH). It was readily solubilized in an aqueous solution of 10% DMSO and diluted with serum-free RPMI 1640 medium (Life Technology) before use.

2.2. Cell culture and exposure to acutiaporberine

The human NSCLC cell line PLA-801 was obtained from the Institute of Biophysics, Chinese Academy of Science. This cell line was derived from a male patient diagnosed with adenocarcinoma and previously treated with chemotherapy. Other cell lines used in the cytotoxicity assay, i.e. the human hepatocellullar cancer cell line HepG2, the mammary cancer cell line MCF-7, and a normal mouse fibroblast cell line (NIH3T3), were obtained from the Shanghai Cell Institute, Chinese Academy of Science. Cells were cultured in RPMI 1640 medium (Life Technology) containing 10% fetal bovine serum (HyClone Co.), 100 U/mL of penicillin, and 100 μL/mL of streptomycin at 37° in a humidified atmosphere of 5% CO_2/95% air.

Cells in the log-phase of growth were exposed to concentrations of acutiaporberine ranging from 0.003 to 0.14 μmol/mL for different times.

2.3. MTT assay

The cytotoxicity of acutiaporberine was assessed by the MTT assay. Cells at a density of 1×10^5/mL were seeded into 96-well tissue culture plates (Falcon Co.). After about 24 hr, supernatants were replaced by drug-containing medium. After 48 hr of incubation, 20 μL of 5 mg/mL MTT was added to each well, and the plates were incubated at 37° for an additional 4 hr. The supernatant in each well was removed carefully. Formazan crystals were redissolved in DMSO for 10 min with shaking. Each plate was read immediately on a microplate reader (ELX800, BIO-TEK Instruments Inc.) at a wavelength of 490 nm. The IC_{50} values were calculated using SPSS software.

2.4. Nuclear staining and morphology

After being treated with the drug, the cells were washed with PBS and fixed in 4% paraformaldehyde at 4° for 20 min. The cells were stained with 10 μg/mL of Hoechst 33258 (Molecular Probes) at room temperature for 10 min and then examined under a fluorescence microscope (Olympus).

2.5. FCM

About 1×10^6 cells were harvested, washed with PBS, and fixed in 70% ethanol at 4° overnight. The cells were then incubated with 50 μL RNase (50 μg/mL) and 450 μL PI (50 μg/mL) at 37° in the dark for half an hour. After filtration to remove cellular debris, the cell suspension was analyzed by FCM (Coulter EPICS XL) (Ex 448 nm; Em 620 nm).

2.6. Analysis of DNA fragmentation (DNA ladder)

Analysis of DNA fragmentation in PLA-801 cells was performed by the method of Han [10]. About 10^7 cells were collected and suspended in denaturing solution (10 mM Tris–HCl, pH 8.0, 100 mM NaCl, 25 mM EDTA, pH 8.0, and 100 μg/mL of Proteinase K), and incubated at 50° for 12–18 hr. Total DNA was isolated by phenol/chloroform extraction and ethanol precipitation, and then solubilized in TE buffer (10 mM Tris–HCl, 100 μM EDTA, pH 8.0). Ten micrograms of the collected DNA was electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and then visualized under UV illumination.

2.7. IHC assay

The IHC assay was carried out to detect the protein products of some apoptosis-associated genes. The cells, cultured on glass coverslips, were treated with acutiaporberine for different times, fixed in a solution of 45% acetone, 25% formaldehyde, 30% 1 mM phosphate, pH 6.1–6.2, and then transferred to PBS. A fresh solution of 0.3% H_2O_2 in methanol was used to treat the cells for 30 min at room temperature to inhibit endogenous peroxidase activity. Then, after blocking with 1.5% serum, the cells were mixed with Bcl-2 mouse monoclonal antibody (1:100), Bax mouse monoclonal antibody (1:100), c-Myc mouse monoclonal antibody (1:100), or p53 mouse monoclonal antibody (1:100) (all purchased from Santa Cruz Biotechnology Inc.), and incubated at 4° overnight. After washing with PBS, the cells were incubated with biotinylated goat anti-mouse IgG (15 μg/mL) and then with a

horseradish peroxidase streptavidin solution (5 μg/mL) (Vector Lab.). Finally, DAB/H_2O_2 (Vector Lab.) was used for development, and cells were counter-stained with Mayer's hematoxylin solution (Nanjin Jiancheng Co.).

2.8. Western immunoblot analysis

Western immunoblot analysis was carried out using a BM Chromogenic Western Blotting Kit (Roche Molecular Biochemicals). SDS-PAGE and western blotting were performed by the method of Sambrook *et al*. [11].

3. Results

3.1. IC_{50} Values

Drug concentrations ranging from 0.003 to 0.14 μmol/mL were used to determine the IC_{50} values of acutiaporberine in three epithelial cancer cell lines (PLA-801, HepG2, and MCF-7) and one non-cancerous fibroblast cell line (NIH3T3). The NIH3T3 cells were used to determine the cytotoxicity of acutiaporberine on non-cancerous cells. The results are shown in Table 1.

Table 1
IC_{50} Values of acutiaporberime to several cultured cell lines[a]

Cell line	Acutiaporberine IC_{50} (μmol/mL)
PLA-801	0.07
HepG2	>0.14
MCF-7	>0.14
NIH3T3	0.12

[a] The IC_{50} values are the means of the results of three experiments.

3.2. Morphological changes

PLA-801 cells were treated with acutiaporberine concentrations of 0.03, 0.06, 0.09, 0.11, and 0.14 μmol/mL for different times. Morphological changes compatible with apoptosis (chromatin condensation, nuclear fragmentation, appearance of apoptotic bodies) were first observed in cells treated with 0.06 μmol/mL of acutiaporberine for 22 hr. As the drug concentration and the incubation time increased, the number of cells having morphological changes increased accordingly. The cells having apoptosis-specific morphological changes reached a peak when treated for 48 hr.

When cells were incubated for 48 hr with a low concentration of acutiaporberine (0.03 μmol/mL), nuclear chromatin began to condense, but it was not very obvious (Fig. 2B) compared with the control cells (Fig. 2A). When treated with 0.06 μmol/mL of acutiaporberine, cells with morphological changes were mainly in the early phase of apoptosis [condensation of nuclear chromatin, forming uniformly dark crescentic masses that migrated toward the nuclear envelop or forming some blocks of condensed chromatin in the inner leaflet of the nuclear membrane (Fig. 2C and D)]. When treated with 0.09 μmol/mL of acutiaporberine, the apoptotic cells were mainly in the middle phase of apoptosis [presence of condensed chromatin in the cytoplasm by nuclear blebbing [12] (Fig. 2E)]; and when treated with the higher drug concentration (0.11 μmol/mL), the cells reached the later phase of apoptosis (appearance of apoptotic bodies formed by plasma membrane blebbing) (Fig. 2F). However, when the concentration of acutiapoberine was increased further to 0.14 μmol/mL, the cells entered a necrosis-like phase associated with PI staining (Fig. 2G).

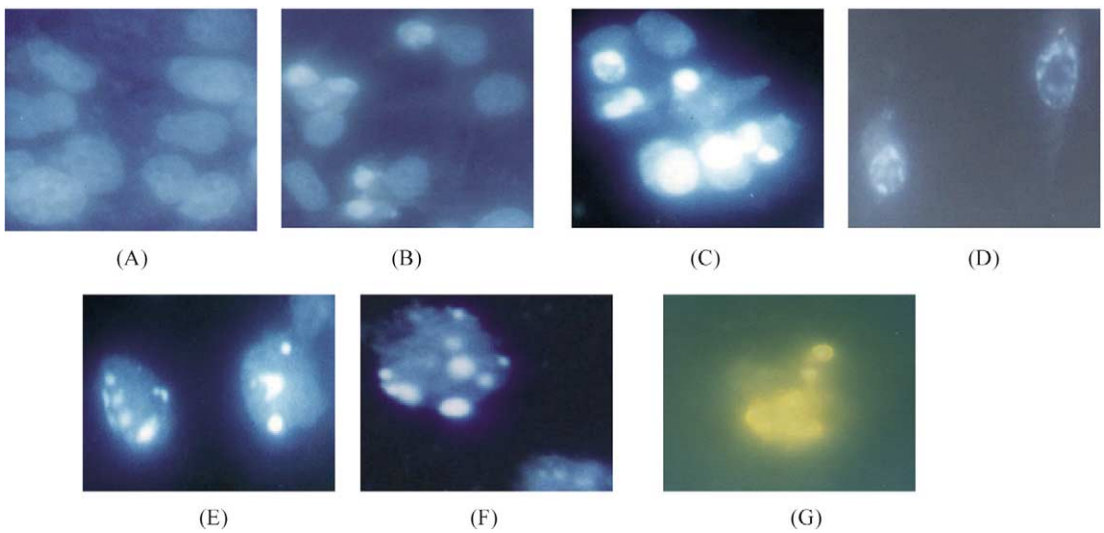

Fig. 2. Fluorescence photomicrographs of cells stained with Hoechst 33258 and PI after being treated with acutiarporberine. (A) Control; (B, C, and D) chromatin condensation in the nucleus; (E) chromatin condensation in the cytoplasm; (F) formation of an apoptotic body; (G) membrane permeability change of apoptotic cells. Cells were treated with 0.03 μmol/mL of acutiaporberine in panel B, 0.06 μmol/mL in panels C and D, 0.09 μmol/mL in panel E, 0.11 μmol/mL in panel F, and 0.14 μmol/mL in panel G.

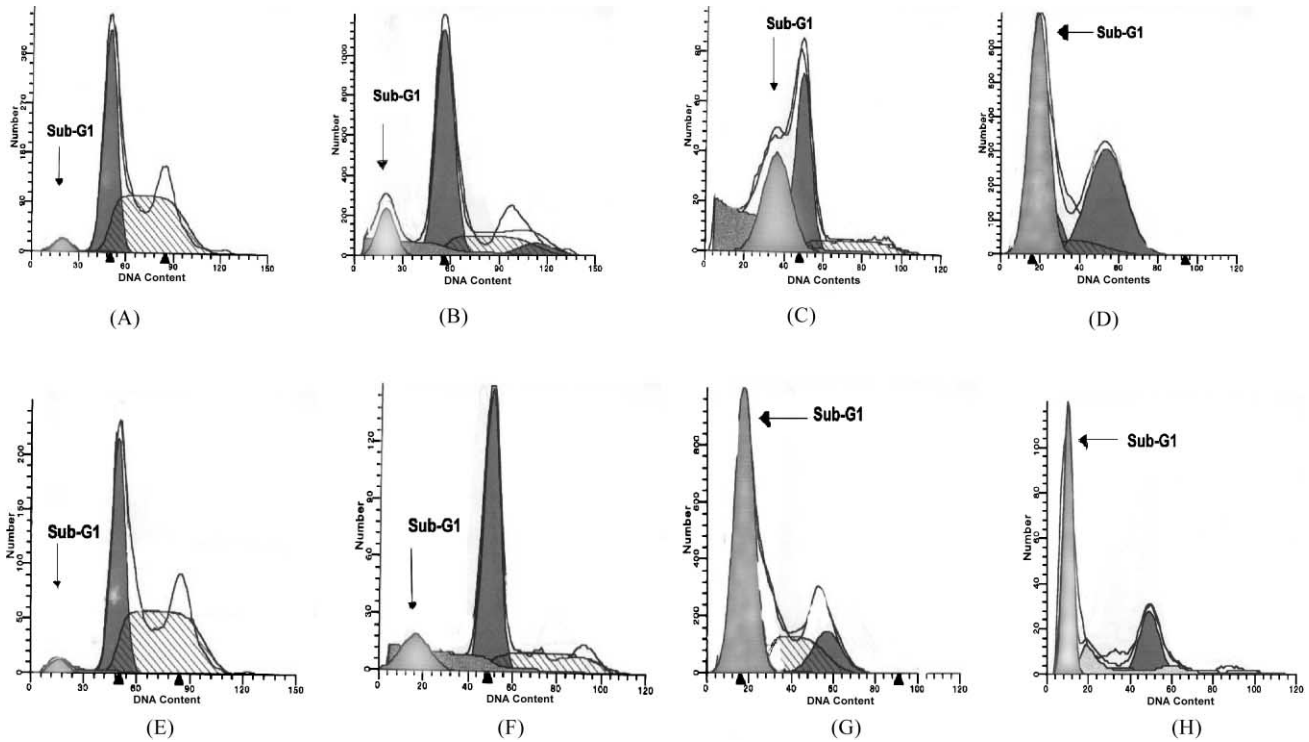

Fig. 3. FCM analysis of apoptosis induced by acutiaporberine. The gray peaks show the ''sub-G_1'' peaks. The black peaks represent cells in the G_0–G_1 phase. The hatched areas show the S-phase cells. Other areas are debris. For the control cells (A) and (E), the percentage of apoptotic cells was 3.04 and 3.05%, respectively. For cells treated with 0.09 μmol/mL of acutiaporberine for 24 hr (B), 48 hr (C) or 72 hr (D), the percentage of apoptotic cells was 12.59, 42.01, and 46.17%, respectively. For cells treated with 0.11 μmol/mL of acutiaporberine for 24 hr (F), 48 hr (G) or 72 hr (H), the percentage of apoptotic cells was 14.47, 58.43, and 50.09%, respectively. In group H, most of the cells disintegrated into debris, so the percentage of apoptotic cells was reduced. In these panels, the peaks are calibrated results, and the curves represent the results before calibration.

3.3. Percentage of cells undergoing apoptosis

FCM can be used to identify and quantify apoptotic cells based on DNA content analysis of ethanol-fixed cells following the effusion of degraded low molecular weight DNA. When a population of cells containing apoptotic cells are stained with a DNA-fluorochrome (PI) and measured by FCM, an additional DNA peak (''sub-G_1 peak'' or ''apoptotic peak'') may be observed. FCM showed that the exposure of exponentially growing PLA-801 cells to acutiaporberine led to the presence of a ''sub-G_1 peak.'' Furthermore, the percentage of cells undergoing apoptosis

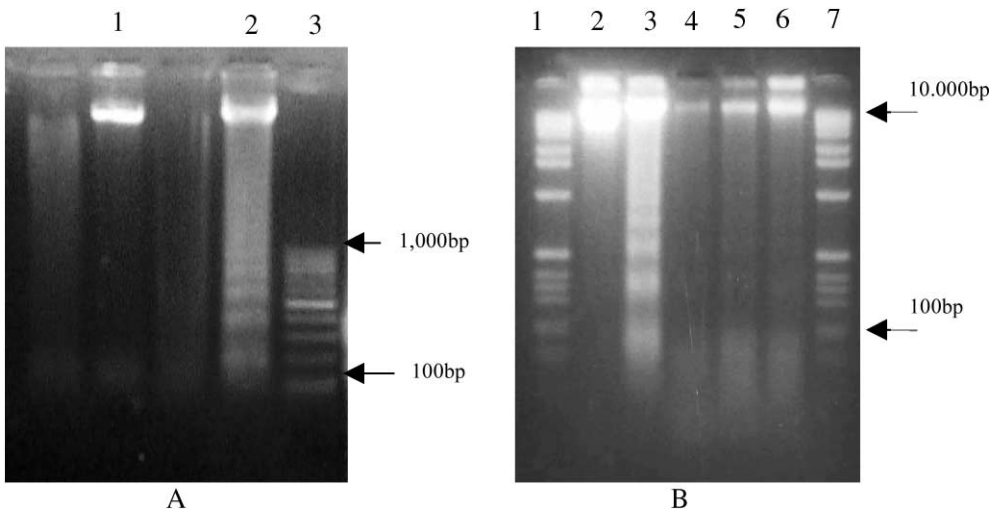

Fig. 4. DNA ladder of PLA-801 cells treated with acutiaporberine. (A) Cells were treated with 0.06 μmol/mL (lane 1) and 0.11 μmol/mL (lane 2) of acutiaporberine for 48 hr; lane 3 shows a 100 bp DNA marker. (B) Cells were treated with 0.11 μmol/mL of acutiaporberine for 48 hr (lane 3), 36 hr (lane 4), 24 hr (lane 5), and 18 hr (lane 6); lane 2 shows the control, and lanes 1 and 7 were markers.

increased with the increase in drug concentration and incubation time (Fig. 3). The percentage of cells undergoing apoptosis reached the highest point (about 58%) when treated with 0.11 μmol/mL of acutiaporberine for 48 hr.

3.4. DNA ladder

A typical biochemical change indicative of apoptosis is DNA fragmentation. In apoptotic cells, an endogenous restriction enzyme is activated to cut the genome DNA into multiples of 180–200 bp in length, forming the typical "DNA ladder" on a DNA electrophoresis gel [13]. When PLA-801 cells were treated with 0.06 or 0.11 μmol/mL of acutiaporberine for 48 hr, a DNA ladder appeared only in the cells treated with 0.11 μmol/mL (Fig. 4A). DNA fragmentation was found to increase with the lengthening of the incubation time (Fig. 4B).

3.5. Results of IHC

We examined the effect of acutiaporberine on the expression in PLA-801 cells of several apoptosis-associated genes, namely c-*myc*, *bax*, *bcl-2*, and *p53*. The

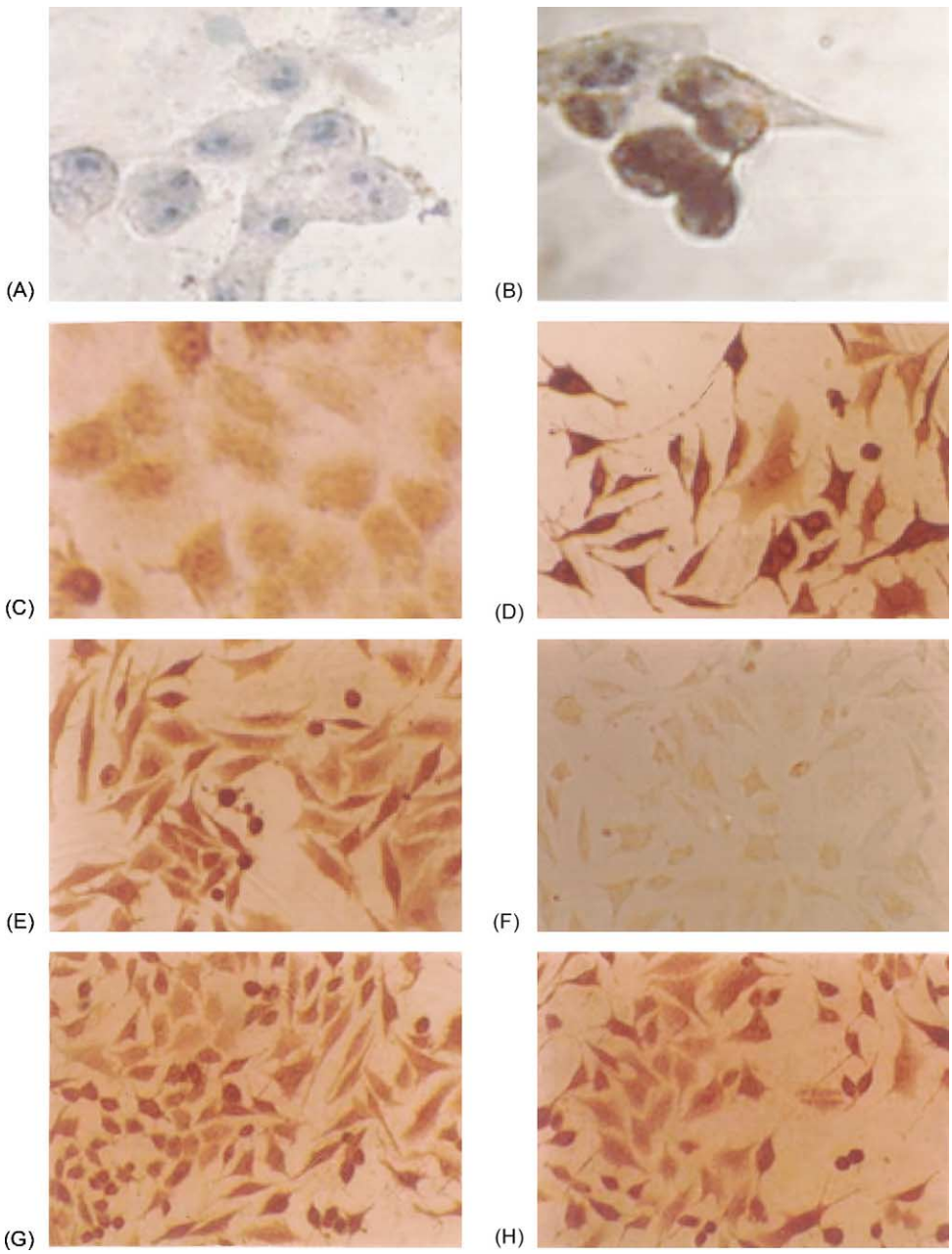

Fig. 5. IHC analysis of c-*myc, bax, bcl-2* and *p53* gene expression. Panels A, C, E, and G indicate, respectively, c-*myc*, *bax*, *bcl-2*, and *p53* gene products in the control cells. Panels B, D, F, and H indicate, respectively, c-*myc*, *bax*, *bcl-2*, and *p53* gene products in cells treated with 0.11 μmol/mL of acutiaporberine for 24 hr.

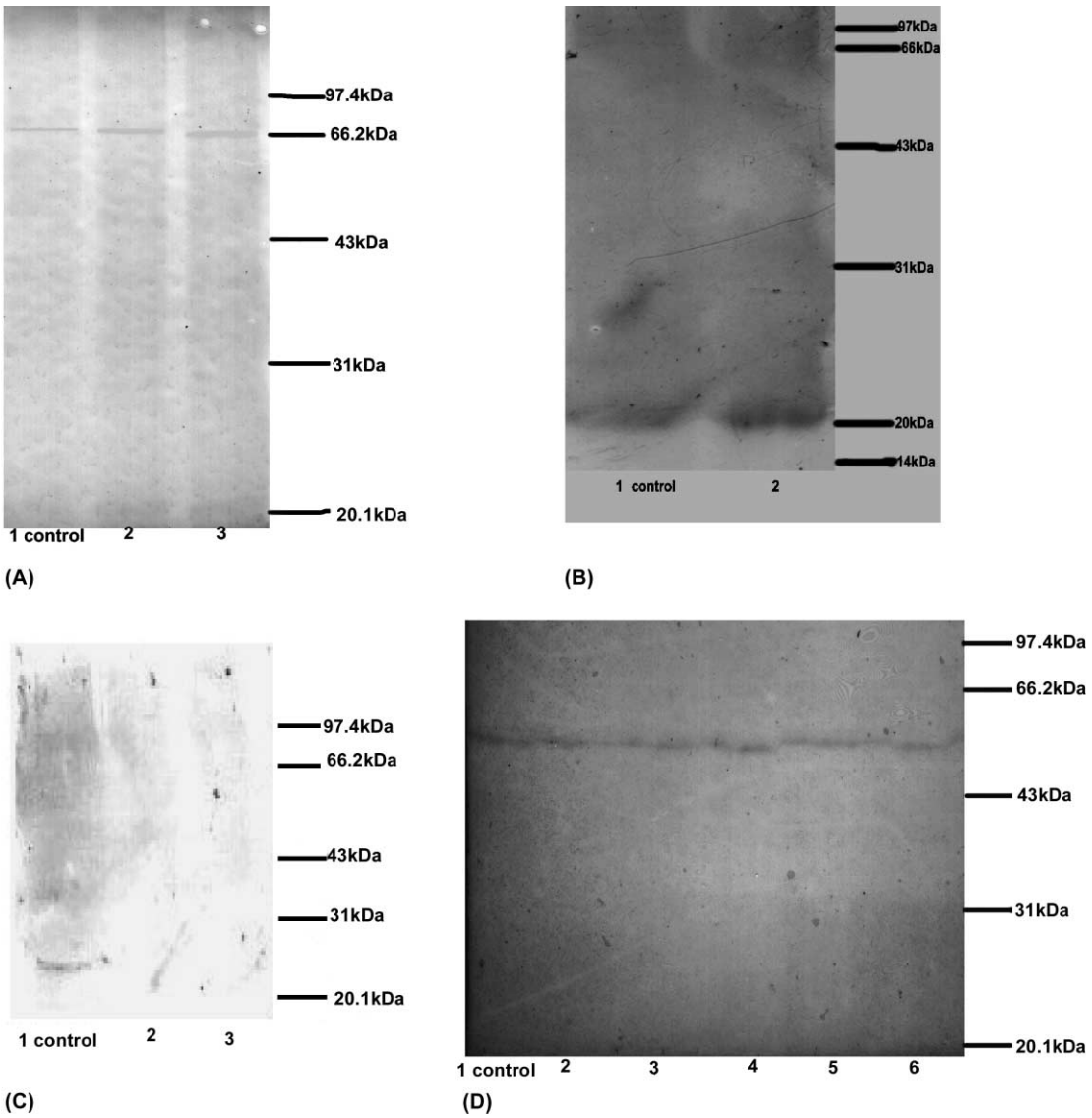

Fig. 6. Western immunoblot analysis for c-*myc* (A), *bax* (B), *bcl-2* (C), and *p53* (D) gene expression. Cells were treated with 0.11 μmol/mL of acutiaporberine. In all of the panels, lane 1 represents the control. (A) The c-*myc* gene product is about 67 kDa. Lanes 2 and 3 show cells treated with drug for 24 and 48 hr, respectively. An increment of the product can be seen. (B) The *bax* gene product is about 21 kDa. Lane 2 shows cells treated with the drug for 24 hr. An increment of the Bax protein is shown. (C) The *bcl-2* gene product is about 26 kDa. Lanes 2 and 3 show cells treated with the drug for 24 and 48 hr, respectively. A significant reduction of the Bcl-2 protein can be seen. (D) The p53 protein is about 53 kDa. Cells were treated with the drug for 6 hr (lane 2), 8 hr (lane 3), 12 hr (lane 4), 24 hr (lane 5), and 48 hr (lane 6). No reduction or obvious increments were observed.

results of the IHC in PLA-801 cells are shown in Fig. 5 after treatment with 0.11 μmol/mL of acutiaporberine for 24 hr, with the brown particles indicating protein expression. The darker the color, the higher the protein expression. As shown in Fig. 5, expression of the c-*myc* and *bax* gene products was elevated greatly after treatment with acutiaporberine (A, B, C, D), but the *bcl-2* gene product was inhibited significantly (E, F). No obvious change was seen for the *p53* gene product (G, H).

3.6. Results of western immunoblot analysis

The results of the western immunoblot analysis (Fig. 6) provided further support for the results obtained with the IHC analysis. c-*myc* and *bax* gene expression was up-regulated when cells were treated with acutiaporberine for 24 hr, whereas *bcl-2* gene expression was inhibited significantly at the same time point. *p53* gene expression showed little change during exposure of the cells to the drug from 6 to 48 hr.

4. Discussion

Natural products have been shown to be excellent and reliable sources for the development of new drugs. As an important part of natural products in the world, TCM should play a pivotal role in the discovery of new drugs,

including new anticancer drugs. Many species of the *Thalictrum* genus are often used in traditional Chinese herbal medicine. They contain various kinds of chemical components that have antiproliferative activities in a broad range of animal and human tumor cell lines *in vitro* [14–16] and have antitumor activities in several tumor models *in vivo* [17–19]. Among them, thaliblastine (chemical name thalicarpine) [20] and thalidasine [21], low toxic alkaloids, are good examples.

A new bisalkaloid, acutiaporberine, was recently isolated from the root of the herb *T. acutifolium* (Hand.-Mazz.) Boivin [22]. This novel chemical structure (Fig. 1) is comprised of an aporphine and a protoberberine ring complex. The MTT assay showed that this alkaloid can inhibit the growth and cause the death of several human cancer cell lines *in vitro* [9]. The IC_{50} values in Table 1 show that the human NSCLC cell line, PLA-801, is more sensitive to acutiaporberine than are the HepG2 and MCF-7 cancer cell lines, which may imply that acutiaporberine is more effective in some kinds of tumors than others. Interestingly, the IC_{50} value for the non-cancerous fibroblast cell line, NIH3T3, was much higher than that for the PLA-801 cancer cell line, so a relatively low toxicity might be expected for this compound in further clinical research.

The present study provided supportive evidence for the hypothesis that acutiaporberine induces apoptosis in the PLA-801 cell line, i.e. chromatin condensation, the formation of apoptotic bodies, DNA fragmentation in a ladder pattern, and the appearance of a sub-G_1 peak in FCM analysis.

The up-regulation of the c-*myc* gene product in cells treated with acutiaporberine shows that the c-*myc* gene plays an important role in the apoptosis of PLA-801 cells induced by acutiaporberine. A high expression level of c-*myc* gene product might increase the sensitivity of cells to apoptosis-inducing factors.

A high expression of the *bcl-2* gene product significantly protects cells from apoptosis induced by various factors, whereas the Bax protein reverses this process. The present study showed that acutiaporberine inhibited the expression of the *bcl-2* gene significantly and activated the expression of the *bax* gene, thus causing a change in the proportion of Bcl-2 and Bax proteins in the treated cells. This change might result in the dissociation of the Bcl-2–Bax protein dimer and the formation of the Bax protein homo-dimer, thus causing cell apoptosis. Surprisingly, we did not see any significant change in the expression level of the *p53* gene product in the apoptotic cells, suggesting that acutiaporberine-induced apoptosis may be independent of the *p53* gene.

Further investigation is being carried out in our laboratory to determine in more detail the molecular mechanism of apoptosis in PLA-801 cells induced by acutiaporberine, e.g. the pathway of this apoptotic process, the signal transduction pathway(s) involved, and the possible changes in expression of other apoptosis-related genes. However, this study does indicate that the novel alkaloid acutiaporberine may serve as a potent apoptosis-inducing drug for human NSCLC.

Acknowledgments

This work was supported by grants from the National New Drug Fund for Doctors (96–901–06–36) of the Ministry of Science & Technology, the National Natural Science Foundation (027–413046), and the Guangzhou Commission of Science and Technology (97–Z–12–01).

References

[1] Hickman JA. Apoptosis induced by anticancer drugs. Cancer Metastasis Rev 1992;11:121–39.
[2] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972;26:239–57.
[3] Kerr JF, Winterford CM, Harmon BV. Apoptosis: its significant in cancer and cancer therapy. Cancer 1994;73:2013–6.
[4] Zhang GQ, Ren RG, Kong QY, Li YJ, Guan WM. Effects of radix salivas miltiorrhizae on proliferation apoptosis and c-*myc* protein expression of fibroblast in culture of kidney with lupus nephritis. Chin J Integrated Traditional Western Med 1997;17:473–5.
[5] Dong Y, Yang MM, Kwan CY. *In vitro* inhibition of proliferation of HL-60 cells by tetrandrine and coriolus versicolor peptide derived from Chinese medicinal herbs. Life Sci 1997;60:PL135–40.
[6] Zhang X, Yang XP, Pan QC. Studies on the anticancer effect and apoptosis induction in human liver cancer cell line BEL-7402 by sodium artesunate. Chin J Herbal Drugs 1998;29:467.
[7] Tanizawa A, Fujimori A, Fujimori Y, Pommier Y. Comparison of topoisomerase I inhibition DNA damage and cytotoxicity of camptothecin derivatives presently in clinical trials. J Natl Cancer Inst 1994;86:836–42.
[8] Ma J, Peng WL, Liang D, Fu NY, Pang DB, Xu AL. The extract of glycyrrhiza uralensis fisch induces apoptosis in MGC-803 cells and its molecular mechanisms. Biomed Res 2000;21:129–37.
[9] Chen Q, Qi SJ, Peng WL, Xu AL. Cytotoxic activity of acutiaporberine, a novel bisalkaloid of plant origin on several human carcinoma cell lines. Acta Sci Natural Univ Sunyatseni 2001;40:9–12.
[10] Han R. Methods of apoptosis study. Research and development of anticancer drugs and experimental techniques. Beijing, China: The Press Union of Peking Medical University and China Xiehe Medical University, 1997. p. 389–405.
[11] Sambrook J, Fritsch EF, Maniatis T. Detection and analysis of expressed proteins of cloned genes. Molecular cloning, a laboratory manual. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989. p. 880–97.
[12] Fang M, Zhang HQ, Xue SB. Apoptosis of HL-60 cells induced by harringtonine: membrane blebs, nucleus blebs and chromatin condensation. Acta Biol Exp Sinica 1996;29:221–3.
[13] Smith CA, Williams GT, Kingston R, Jenkinson EJ, Owen JJ. Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. Nature 1989;337:181–4.
[14] Todorou DK, Damyanova MS. Investigations into acute toxicity of the antitumour alkaloid thaliblastine. C R Acad Bulg Sci 1975;28:709–11.
[15] Deliconstantinos G, Ramantanis G, Todorou DK. Interaction of ^{99m}Tc-labeled liposomes with Walker tumour cells *in vitro*. Liposome-mediated introduction of thaliblastine into resistant Walker tumour cells. Gen Pharmacol 1983;14:407–11.

[16] Todorov DK, Zeller WJ. Antiproliferative activity of the non-myelotoxic antitumour agent of plant origin thaliblastine on two human glioma cell lines. J Cancer Res Clin Oncol 1992;118:83–6.
[17] Lin LZ, Hu SF, Zaw K, Angerhofer CK, Chai H, Pezzuto JM, Cordell GA. Thalifaberidine, a cytotoxic aporphine-benzylisoquinoline alkaloid from *Thalictrum faberi*. J Nat Prod 1994;10:1430–6.
[18] Mircheva J, Stoychkov J. Effect of thaliblastine on trans-plantable tumors in mice. Biomed Exp 1976;25:280–1.
[19] Ilarionova M, Maneva K, Todorov D. Pathomorphological studies on animals bearing experimental tumors treated with thaliblastine. C R Acad Bulg Sci 1980;33:719–22.
[20] Kupchan S, Chakravarti K, Yokoyoma N. Thalicarpine a new hypotensive alkaloid from *Thalictrum dasycarpum*. J Pharm Sci 1963;52:985–8.
[21] Kupchan SM, Yang TH, Vasilikiotis GS, Barnes MH, King ML. Tumor inhibitors. XLII. Thalidasine, a novel bisbenzylisoquinoline alkaloid tumor inhibitor from *Thalictrum dasycarpum*. J Org Chem 1969;34:3884–8.
[22] Lin CW, Su JY, Zeng LM, Xu AL, Pen WL. Structure determination of a novel ether-linkaged bisalkaloid acutiaporberine. Chem J Chin Univ 2000;12:1820–3.