ELSEVIER

Biochemical Pharmacology 63 (2002) 951–957

Biochemical Pharmacology

Jolkinolide B induces neuroendocrine differentiation of human prostate LNCaP cancer cell line

W.K. Liu[a,*], J.C.K. Ho[a], Guowei Qin[b], Chun-Tao Che[c]

[a]*Department of Anatomy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong*
[b]*Shanghai Institute of Materia Medica, The Chinese Academy of Sciences, Shanghai, People's Republic of China*
[c]*School of Chinese Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong*

Received 31 July 2001; accepted 12 December 2001

Abstract

Euphorbia fischeriana is a Chinese herbal medicine which has been reported to possess chemotherapeutic effects, yet the underlying mechanism is unclear. In order to understand its possible anti-tumor property, we have isolated a number of chemical compounds from the roots of this plant [Phytochemistry 52 (1999) 117] and studied their *in vitro* effects by using human prostate LNCaP cancer cell line. Among the six compounds tested, jolkinolide B exhibited the most potent anti-proliferative activity (IC_{50} = 12.5 μg/mL = 40 μM) and it inhibited DNA synthesis by down-regulating bromodeoxyuridine (BrdU) incorporation in LNCaP cells in a dose-dependent manner. Jolkinolide B, at concentrations up to 25 μg/mL, induced G1 arrest and neuroendocrine differentiation of LNCaP cells. Immunoblotting analysis confirmed the increased expression of neuroendocrine markers, keratin 8/18 (K8/18) and neuron specific enolase (NSE), in these cells. Apoptotic bodies and DNA fragmentation were observed by fluorescence microscopy and flow cytometry when the cells were exposed to a concentration higher than 25 μg/mL jolkinolide B. Taken all data together, jolkinolide B seems to play a role in the regulation of proliferation, differentiation, and apoptosis of LNCaP cells.

Keywords: Jolkinolide B; Neuroendocrine differentiation; Prostate cancer cell lines; Growth arrest; Apoptosis

1. Introduction

E. fischeriana Steud. (Euphorbiaceae) is a traditional Chinese medicine occasionally used for the treatment of a wide range of ailments, including edema, indigestion, as well as liver and lung cancers [2]. In an early report, the ethanolic and aqueous extracts of *E. fischeriana* were observed to inhibit growth of Lewis lung carcinoma and ascitic hepatoma in mice [3]. Later antibacterial activity was reported [4]. Chemical investigations have led to the isolation of sterols, triterpenes, tannins, and a number of diterpene compounds from the plant extracts [5–7]. The diterpene constituents of *E. fischeriana* include the abietane-type lactones (jolkinolides) and tigliane-type derivatives.

Prostate cancer is one of the most prevalent male cancers in western countries, and the incidence is also on a rise in Asia along with the westernization of diet and lifestyle [8]. Growth and proliferation of prostatic cells rely on a continuous supply of androgen, and thus hormone therapy has been a standard treatment for prostate cancer. Finasteride is one of the best examples of a successful drug which reduces the available physiological potent dihydrotestosterone by more than 90%, and consequently induces prostatic epithelial atrophy [9]. However, relapse occurs in few years because of the heterogeneous nature of both androgen-dependent and -independent cells in the prostate, making hormone therapy not entirely curative [9]. Other therapeutic approaches, such as apoptosis induction, cytotoxic chemotherapy, inhibition of transduction and surgical operation, have been introduced, but no satisfactory results can be achieved. Differentiation therapy is another approach whereby terminal differentiation is induced in advanced prostate cancer cells whose proliferation is suppressed [10]. Phosphodiesterase inhibitors, papaverine [10] and butyrate, have been reported to inhibit cell proliferation and induce differentiation of prostate cancer cell lines, and butyrate is currently under clinical trial for the treatment of advanced prostate cancer [11].

As a continuation of our interest in bioactive natural products against prostate cancer, we have assessed the

* Corresponding author. Tel.: +852-2609-6896; fax: +852-2603-5031.
E-mail address: ken-liu@cuhk.edu.hk (W.K. Liu).

0006-2952/02/$ – see front matter
PII: S0006-2952(01)00938-8

anti-proliferative activity of jolkinolide B in three prostate cancer cell lines, including the androgen-dependent (LNCaP) and -independent (DU145 and PC3) cells, as well as the concomitant changes in cell cycles using flow cytometry.

2. Materials and methods

2.1. Test compound

Jolkinolide B is a diterpene isolated from the roots of *E. fischeriana* [1] (Fig. 1). It has a molecular weight of 308 and its molecular formula is $C_{20}H_{26}O_4$ as determined by mass spectrometry and elemental analysis [1]. The compound was dissolved in DMSO to make a stock solution at a concentration of 40 mg/mL which was then diluted to appropriate concentrations with culture medium before each experiment. The final concentration of DMSO did not exceed 0.5% in any experiment.

2.2. Cell cultures

Three human prostate cancer cell lines, LNCaP (CRL-1740), DU145 (HTB-81) and PC3 (CRL-1435), were obtained from the American Type Culture Collection (ATCC). They were routinely maintained in RPMI supplemented with 10% fetal bovine serum, 100 μg/mL streptomycin and 100 IU/mL penicillin at 37° in a humidified atmosphere of 5% CO_2.

2.3. Cytotoxicity of jolkinolide B

All three prostate cancer cell lines (1×10^4 cells/0.1 mL/well) were incubated in the presence of serial dilutions of jolkinolide B in 96-well culture plates (Costar) or on 8-chamber culture slides (Nunc, 177402) for 24 or 48 hr for DU145 and PC3, and LNCaP cells, respectively. They were then reacted with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at 37° for 2 hr. The reaction product, formazan, was extracted with DMSO and the absorbance was read at 540 nm. Data points represented the mean values and standard deviations of triplicate samples [12]. Since LNCaP cells were most susceptible to the anti-proliferative activity of jolkinolide B, bioassays were conducted to investigate its underlying growth inhibitory mechanism.

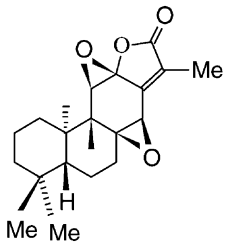

Fig. 1. Chemical structure of jolkinolide B.

2.4. Fluorescent staining for morphological observation

LNCaP cells were cultured on the chamber slides with serial concentrations of jolkinolide B for 48 hr, washed briefly with PBS before they were fixed with 70% cold ethanol. The cells were then stained with 0.01% acridine orange and differentiated with 0.1 M calcium chloride. Fluorescence micrographs were taken on a fluorescence microscope (Axioskop) with a 450–490 nm excitation block filter and a 520 nm barrier filter [13].

2.5. Flow cytometry

Only jolkinolide-treated LNCaP cells were subjected to flow cytometry analysis. Cells were cultured in the presence of jolkinolide B for 1–6 days, fixed in 70% cold ethanol, and resuspended in 1 mL PBS containing 200 μg/mL RNase A (Sigma, R4875) and 20 μg/mL propidium iodide (PI), (Sigma, P4170) at 4° for 30 min. The red fluorescence of DNA-bound PI in individual cells was measured at 575 nm by using a Coulter ALTRA™ flow cytometer. The results obtained from 10,000 cells were analysed using Expo II software (Beckman Coulter Company).

BrdU is an analog of thymidine which is incorporated into DNA during the S phase of the cell cycle. To detect the anti-proliferative activity of jolkinolide B, LNCaP cells were pulsed with 10 μM BrdU (Sigma, B5002) for 1 hr before they were trypsinized and fixed with ice-cold 70% ethanol. Cellular DNA was denatured with 2 M HCl, washed with PBS containing 0.5% BSA, and the residual acid was neutralized with 0.1 M sodium borate (pH 8.5). The cells were then incubated in the presence of FITC-conjugated anti-BrdU antibody (Pharmingen, 33284X) for flow cytometric analysis of incorporated BrdU in the S phase of LNCaP cells.

2.6. Immunoblotting analysis of differentiation marker

Jolkinolide-treated LNCaP cells were washed with PBS twice, resuspended in lysis buffer (50 mM Tris–Cl, 150 mM NaCl, 0.2% Triton X-100, 10 μg/mL aprotinin, 0.5 mM PMSF), and centrifuged at 10,600 g at 4° for 10 min. Lysates were normalized for protein content using the protein assay reagent (Bio-Rad Laboratories, 500-0006). Equal amount of protein samples were separated on a 12% SDS-PAGE gel and transferred onto a PVDF membrane using a semi-dry transfer cell (Bio-Rad Laboratories). The blot was then rinsed with Tris-buffered saline containing 0.1% Tween 20 (TBS/T) (pH 7.6) and soaked in blocking reagent to prevent non-specific binding before it was reacted overnight at room temperature with monoclonal antibodies against human NSE (Zymed Laboratories

Inc., 18-0196) and K8/18 (Neomarkers, MS743), respectively. The blot was rinsed with TBS/T, linked with HRP-conjugated antibody and detected using enhanced chemiluminescence blotting reagents (Amersham, RPN2108) for 1 min and exposed to a X-OMAT film (Kodak). The amount of translational K8/18 was analysed using a Flour-Chem Imager (Alpha Innotech Corporation).

3. Results

3.1. Cytotoxicity of jolkinolide B

Prostate cancer LNCaP is an androgen-dependent cell line whose cell cycle is about 50 hr (Fig. 2) while DU145 and PC3 are androgen-independent cell lines whose cell cycles are about 20 hr. In order to compare the drug effect on these three cell lines, they were treated with jolkinolide B for a period of one cell cycle and their ability to reduce MTT into formazan was analysed and shown in Fig. 2. All three cell lines maintained at a normal range at doses of jolkinolide B lower than 3 μg/mL, and then started to inhibit the ability of LNCaP and DU145 cells to reduce MTT, the maximal suppression was achieved at 25 and 200 μg/mL, respectively. The PC3 cells were the least susceptible cells to the cytotoxicity of jolkinolide B because it maintained its normal MTT reduction activity at doses up to 25 μg/mL when most of the LNCaP cells had already lost their activity. The IC_{50} is 12.5, 45 and 75 μg/mL (=40, 145 and 244 μM) for LNCaP, DU145 and PC3, respectively, indicating that the androgen-dependent LNCaP cells are more sensitive to the toxicity of the

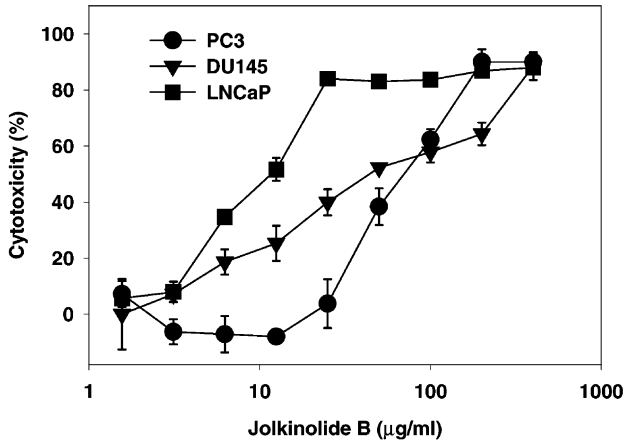

Fig. 2. Cytotoxicity of jolkinolide B on three different human prostate cancer cell lines for one cell cycle as determined by MTT assay. Data points represent the mean values and standard deviations of triplicate samples.

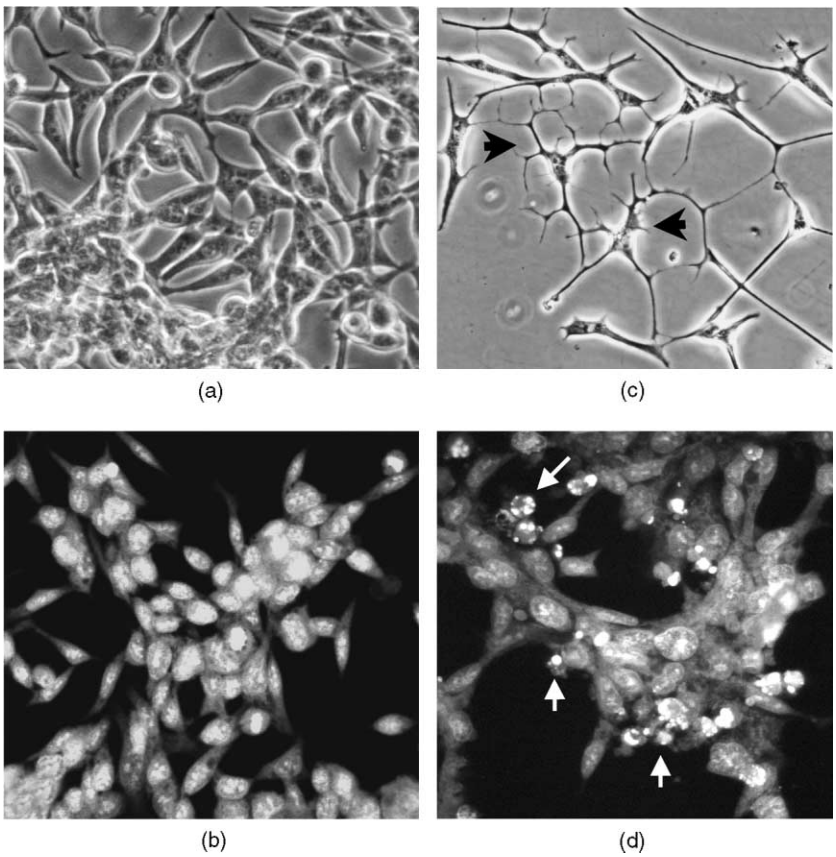

Fig. 3. Normal LNCaP cells were spindle-shaped and they proliferated to form colonies in 48 hr (a and b). Some of the cells became elongated with dendrite-like cytoplasmic processes (arrow heads) in the presence of 12.5 μg/mL jolkinolide B for 48 hr (c), and some cells showed apoptotic changes (arrows) at 25 μg/mL jolkinolide B for 48 hr (d).

compound, while the androgen-independent DU145 and PC3 cells are less sensitive. Since LNCaP is the most sensitive cell line to the cytotoxicity of jolkinolide B, thereby only LNCaP cells were subjected for further cell cycle analysis.

3.2. Fluorescent microscopy of LNCaP cells treated with jolkinolide B

Normal LNCaP cells are spindle-shaped and adhered loosely on the culture surface (Fig. 3a and b). They proliferated and aggregated into colonies after 2–3 days. However, few cell colonies were observed and a monolayer of cells in neuroendocrine phenotype appearance (Fig. 3c) was observed when the cells were treated with jolkinolide B for 2–3 days. Cells became elongated with dendrite-like cytoplasmic processes connecting to each other, indicating that a neuroendocrine differentiation occurred in these cells. Apoptotic changes were observed when the cells were exposed to 25 μg/mL of jolkinolide B for 48 hr (Fig. 3d).

3.3. Cell cycle analysis on LNCaP cells treated with jolkinolide B

A kinetic cell cycle analysis on LNCaP cells treated with/without jolkinolide B was shown in Fig. 4. The level of G1 cells in normal LNCaP was 60% at days 1, 3 and 5, while that for days 2, 4 and 6 was 80%, indicating that a cell cycle of LNCaP cells was around 2 days (Fig. 4a). A higher percentage of cells in S phase and more than 20% of BrdU uptake, and only 60% of cells at G1 phase were

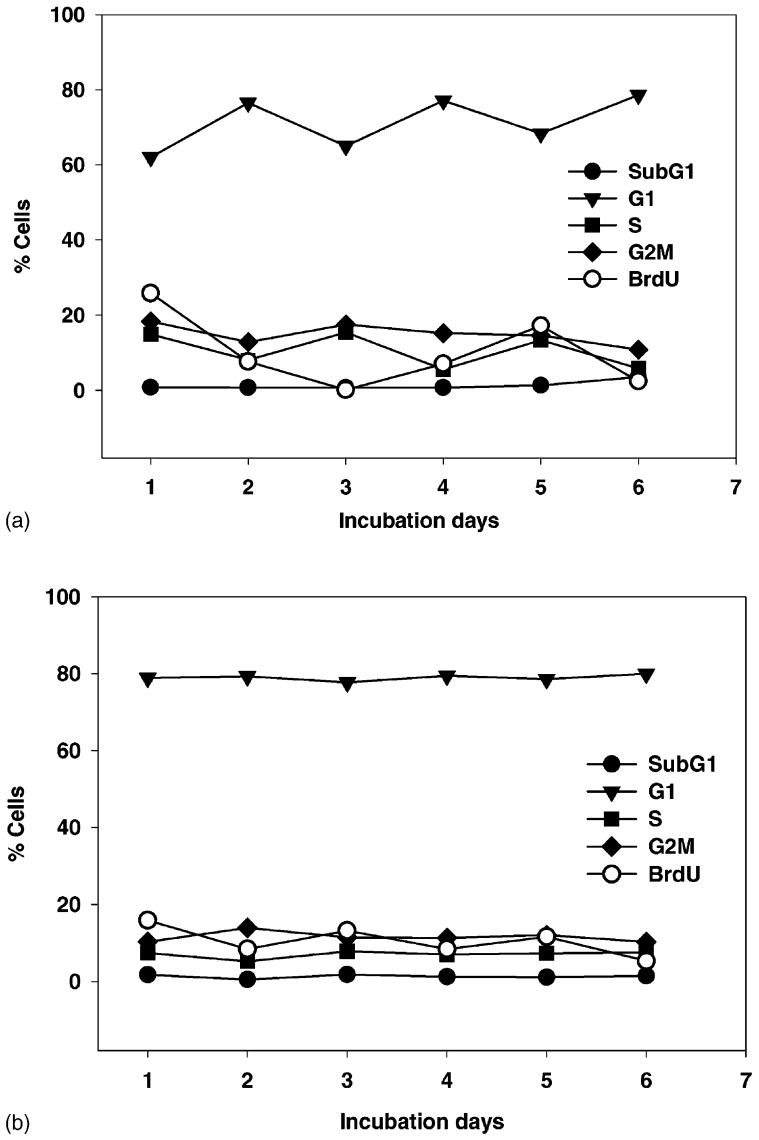

Fig. 4. A kinetic cell cycle analysis on untreated LNCaP cells (a) and those treated with 12.5 μg/mL jolkinolide B (b) for 6 days. Untreated cells showed active proliferation (G1 phase peaked at every 2 days), whereas cells treated with 12.5 μg/mL jolknolides B arrested at G1 phase, indicating the anti-proliferative activity of jolknolides B on LNCaP cells.

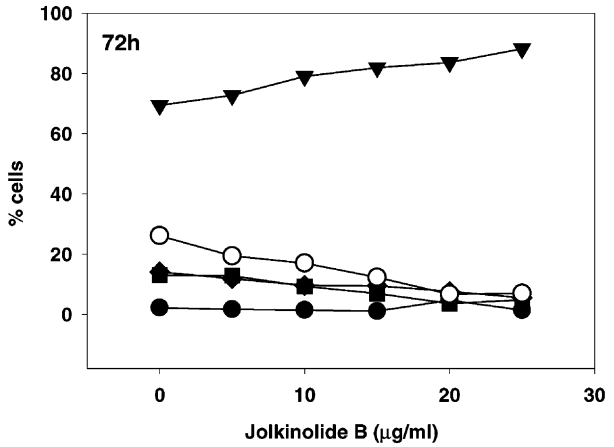

Fig. 5. A cell cycle analysis on LNCaP cells treated with a serial concentration of jolkinolide B for 72 hr. A higher percentage of cells in S phase and more than 20% of BrdU uptake were measured in LNCaP cells when only 60% of cells at G1 phase, indicating that an active proliferation occurred in control cells. By comparison with cells treated with 10–25 μg/mL jolkinolide B, 80% of cells were in G1 phase and only 5–10% of cells had proceeded to S phase, strongly indicating cells were arrested in G1 phase.

measured in untreated LNCaP cells, indicating that control LNCaP cells were proliferating. In contrast, cells treated with 12.5 μg/mL jolkinolide B, 80% of cells were in G1 phase and less than 10% of cells had proceeded to S phase, strongly indicating that the cells were arrested in G1 phase (Fig. 4b). When the cells were incubated with different concentrations of jolkinolide B for 72 hr, cells accumulated in G1 phase (about 20% increase) but the uptake of BrdU declined by 20% from 0 to 25 μg/mL, and accompanied with a slight increase of cells in sub-G1, indicating high doses of jolkinolide B induced apoptotic changes in LNCaP cells (Fig. 5).

3.4. Immunoblotting analysis of jolkinolide-treated LNCaP cells

NSE protein (MW = 46 kDa), keratin 8 (MW = 52.5 kDa) and keratin 18 (MW = 45 kDa) are well documented neuroendocrine markers, because they are constitutively expressed in neuroendocrine cells [14,15]. In order to confirm the ability of jolkinolide B to induce differentiation of LNCaP cells, the jolkinolide B-treated cells were subjected to immunoblotting analysis with these differentiation markers. A dose-dependent increase of K8/18 and NSE was observed in LNCaP cells treated with jolkinolide B, which coincided with neuroendocrine phenotypic changes, indicating that the compound induced neuroendocrine differentiation of LNCaP cells (Fig. 6).

4. Discussion

This study demonstrated the growth inhibitory activity of jolkinolide B, a diterpene isolated from a Chinese medicinal herb, on human prostate LNCaP cancer cell line. The effect is mediated by arresting the cells at G1 phase, blocking the incorporation of BrdU and thus inhibiting DNA synthesis, and inducing a neuroendocrine differentiation of the epithelial cells. The expression of the biomarkers, K8/18 and NSE, confirmed the neuroendocrine differentiation of the LNCaP cells. LNCaP, together with DU145 and PC3 are three commercially available cell lines which are commonly used in *in vitro* bioassays. LNCaP is androgen-dependent while DU145 and PC3 are androgen-independent cell lines, which reflects the heterogeneity of cell populations in the prostate gland and also underlines the varying degrees of behavior and response of prostate cancers to any single therapy. The current standard treatment for prostate hypertrophy and cancer is hormone therapy, thereby inhibiting the growth of androgen-dependent population and leaving the remainder for other treatments [16], including surgical removal, induction of apoptosis and differentiation of cancer cells. A number of chemicals, including retinoids [17], 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) [18] and ginsenoside Rg3 [13], have been reported to induce apoptosis of prostate cancer cells mediated by oxidative stress and/or activation of cell cycle inhibitors, thus inhibiting the cell proliferation. In addition, both retinoids and $1,25(OH)_2D_3$ can induce terminal differentiation of cultured cancer cells [18] and also diminish tumor growth in nude mice [19]. $1,25(OH)_2D_3$ arrests LNCaP cells in G1 phase of the cell cycle and triggers cell differentiation [20]. However,

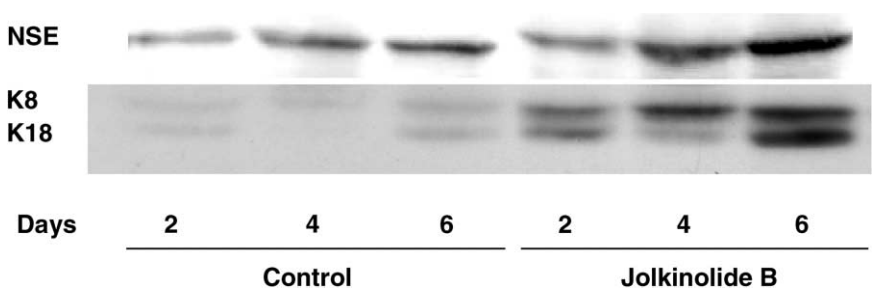

Fig. 6. Constitutive expression of differentiation markers, K8/18 and NSE, in jolkinolide B-treated LNCaP cells as determined by immunoblotting analysis.

cytotoxicity and side effects may preclude their wide applications [18].

In the present study, the response to jolkinolide B varies among three prostate cancer cell lines, with LNCaP being the most sensitive, followed by DU145 and PC3 (Fig. 2). LNCaP is an androgen-dependent cell line whose 5α-reductase can convert testosterone into the physiologically more potent dihydrotestosterone. Whether such differential cytotoxicity is attributable to the action of 5α-reduction is not clear. Experiments are being conducted to elucidate if jolkinolide B plays any role in androgen conversion. We have previously demonstrated that lemnabourside, a diterpene glycoside isolated from a soft coral, *Nephthea chabroli*, inhibited the growth of LNCaP cells by inhibiting 5α-reduction of testosterone and triggering the capsase-3 apoptotic pathway [21]. In the present study, cells were also arrested at G1 phase and apoptosis was induced at doses higher than 25 μg/mL in 48 hr (Figs. 3d and 5). Normal LNCaP cells grew in loosely adherent colonies after 48 hr, but in the presence of jolkinolide B they acquired a neuron-like appearance with a small cell body and multiple long cytoplasmic processes, a differentiation phenotype similar to those treated with other differentiation inducers [10,22]. The differentiation also manifests a concomitant expression of neuroendocrine markers, NSE [15] and K8/18 [14]. The differentiation phenotype was replaced by apoptosis when the cells were exposed to higher doses (<25 μg/mL). No existing data indicates if apoptosis-related genes, e.g. *bcl*-2 and *bax*, were only upregulated by jolkinolide B at high doses. Further study is warranted to investigate the molecular mechanism underlying the apoptotic action of the compound.

Retinoids have been demonstrated to improve the cure rates in acute promyelocytic leukemia by inducing growth arrest and terminal maturation, and thus forming the basis of differentiation therapy in leukemia [23]. Evidence of differentiation therapy has also been reported in glioma [24], colon carcinoma [25], prostate cancers [11], and mammary cancers [26]. A number of differentiation inducers, including carboxypeptidase A3 [11], papaverine [10], retinoids [27], silibinin [14], activin A [28], and butyrate [20,29] promoted neuroendocrine differentiation of prostate epithelial cancer cells which were mutually exclusive with uncontrollable proliferation. Butyrate and its analogs have recently entered clinical trails as a potential drug for differentiation therapy of prostate cancer [12]. However, the relationship between neuroendocrine differentiation and prostatic cancer has been controversial because a poor prognosis and tumor progression are associated with neuroendocrine differentiation [30]. Neuroendocrine cells are devoid of androgen receptors and their proliferation cannot be inhibited by androgen ablation, and prostate cancer thus becomes hormone-refractory. The complexities of neuroendocrine differentiation of prostate cancer cells require further studies before the differentiation therapy can be implemented.

Acknowledgments

The authors thank N. Wong for her technical assistance. This study was supported by a grant awarded to C.-T. Che from the Hong Kong Research Grant Council.

References

[1] Che CT, Ma QG, Qin GW, Williams ID, Wu HM. Diterpenes and aromatic compounds from *Euphorbia fischeriana*. Phytochemistry 1999;52:117–21.
[2] Yang CL. Materia medica of toxic herbs, Beijing (China): Chinese Medicine and Materials Publisher, 1993. p. 1021–3.
[3] Tu SJ, Wei P, Wang A. A study of the antitumor effect of *Euphorbia fischeriana* Steud on mice with implantative tumor. Chinese J Integrated Trad West Med 1984;4:46–7.
[4] Lin X, Zhu JH. Pharmacological research and clinical application of *Euphorbia fischeriana* Steud. Zhejiang J Chinese Med 1992;27:331–3.
[5] Lee SH, Tanaka T, Nonaka GI, Nishioka I, Zhang B. Allose gallates from *Euphorbia fischeriana*. Phytochemistry 1991;30:1251–3.
[6] Liu WZ, Wu XY, Yang GJ, Ma QG, Zhou TX, Tang XC, Qin QW. 12-Deoxyphorbol esters from *Euphorbia fischeriana*. Chinese Chem Lett 1996;7:917–8.
[7] Ma QG, Liu WZ, Wu HM, Zhou TX, Qin GW. Diterpenoids from *Euphorbia fischeriana*. Phytochemistry 1997;44:663–6.
[8] Hsing AW, Tsao L, Devesa SS. International trends and patterns of prostate cancer incidence. Int J Cancer 2000;85:60–7.
[9] Buttyan R, Shabsigh A, Perlman H, Colombel M. Regulation of apoptosis in the prostate gland by androgenic steroids. Trends Endocrinol Metab 1999;10:47–54.
[10] Goto T, Matsushima H, Kasuya Y, Hosaka Y, Kitamura T, Kawabe K, Hida A, Ohta Y, Simizu T, Takeda K. The effect of papaverine on morphologic differentiation, proliferation and invasive potential of human prostatic cancer LNCaP cells. Int J Urol 1999;6:314–9.
[11] Huang H, Reed CP, Zhang JS, Shridhar V, Wang L, Smith DI. Carboxypeptidase A3 (CPA3): a novel gene highly induced by histone deacetylase inhibitors during differentiation of prostate epithelial cancer cells. Cancer Res 1999;59:2981–8.
[12] Van de Loosdrecht AA, Beelen RHJ, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MMAC. A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells. J Immunol Methods 1994;174:311–20.
[13] Liu WK, Xu SX, Che CT. Anti-proliferative effect of ginseng saponins on human prostate cancer cell line. Life Sci 2000;67:1297–306.
[14] Zi X, Agarwal R. Silibinin decreases prostate-specific antigen with cell growth inhibition via G1 arrest, leading to differentiation of prostate carcinoma cells: implications for prostate cancer intervention. Proc Natl Acad Sci USA 1999;96:7490–5.
[15] Cox ME, Deeble PD, Bissonette EA, Parsons SJ. Activated 3′, 5′-cyclic AMP-dependent protein kinase is sufficient to induce neuroendocrine-like differentiation of the LNCaP prostate tumor cell line. J Biol Chem 2000;275:13812–8.
[16] Garnick MB, Fair WR. Prostate cancer. Sci Am 1998;12:45–53.
[17] Sun SY, Yue P, Lotan R. Induction of apoptosis by N-(4-hydroxyphenyl)retinamide and its association with reactive oxygen species, nuclear retinoic acid receptors, and apoptosis-related genes in human prostate carcinoma cells. Mol Pharmacol 1999;55:403–10.
[18] Brawer MK, Ellis WJ. Chemoprevention for prostate cancer. Cancer 1995;75S:1783–9.
[19] Schwartz GG, Hill CC, Oeler TA, Becich MJ, Bahnson RR. 1,25-Dihydroxy-16-ene-23-yne-vitamin D-3 and prostate cancer cell proliferation *in vivo*. Urology 1995;46:365–9.

[20] Blutt SE, Allegretto EA, Pike JW, Weigel NL. 1,25-Dihydroxyvitamin D-3 and 9-cis-retinoic acid act synergistically to inhibit the growth of LNCaP prostate cells and cause accumulation of cells in G1. Endocrinology 1997;138:1491–7.

[21] Che CT, Liu WK. Chemical constituents of *Nephthea chabroli* and anti-proliferative effects on prostate cancer cells. In: Proceedings of the UNESCO Regional Symposium on Chemistry of Medical Plants. 1999, March 17–19, Kunming, China.

[22] Bang YJ, Pirnia F, Fang WG, Kang WK, Sartor O, Whitesell L, Ha MJ, Tsokos M, Sheahan MD, Nguyen P, Niklinski WT, Myers CE, Trepel JB. Terminal neuroendocrine differentiation of human prostate carcinoma cells in response to increased intracellular cyclic AMP. Proc Natl Acad Sci USA 1994;91:5330–4.

[23] Jansen JH, De Ridder MC, Geertsma WMC, Erpelinck CAJ, Van Lom K, Smit EME, Slater R, Vd Reijden BA, De Greef GE, Sonneveld P, Lowenberg B. Complete remission of t(11;17) positive acute promyelocytic leukemia induced by all-trans retinoic acid and granulocyte colony-stimulating factor. Blood 1999;94:39–45.

[24] Driever PH, Knupfer MM, Cinatl J, Wolff JEA. Valproic acid for the treatment of pediatric malignant glioma. Klinische-Padiatrie 1999; 211:323–8.

[25] Nojiri H, Manya H, Isono H, Yamana H, Nojima S. Induction of terminal differentiation and apoptosis in human colonic carcinoma cells by brefeldin A, a drug affecting ganglioside biosynthesis. FEBS Lett 1999;453:140–4.

[26] Petersen OW, Ronnov-Jessen L, Weaver VM, Bissell MJ. Differentiation and cancer in the mammary gland: shedding light on an old dichotomy. Adv Cancer Res 1998;75:135–61.

[27] Lu XP, Fanjul A, Picard N, Shroot B, Pfahl M. A selective retinoid with high activity against an androgen-resistant prostate cancer cell type. Int J Cancer 1999;80:272–8.

[28] Zhang Z, Zhao Y, Batres Y, Lin MF, Ying SY. Regulation of growth and prostatic marker expression by activin A in an androgen-sensitive prostate cancer cell line LNCAP. Biochem Biophys Res Commun 1997;234:362–5.

[29] Maier S, Reich E, Martin R, Bachem M, Altug V, Hautmann RE, Gschwend JE. Tributyrin induces differentiation, growth arrest and apoptosis in androgen-sensitive and androgen-resistant human prostate cancer cell lines. Int J Cancer 2000;88:245–51.

[30] Abrahamsson PA. Neuroendocrine differentiation in prostatic carcinoma. Prostate 1999;39:135–48.