Acacetin inhibits cell growth and cell cycle progression, and induces apoptosis in human prostate cancer cells: structure-activity relationship with linarin and linarin acetate

Rana P. Singh, Puja Agrawal, Dongsool Yim, Chapla Agarwal and Rajesh Agarwal

1Department of Pharmaceutical Sciences, School of Pharmacy and 2Department of Pharmacy, Sahm Yook University, Seoul, Korea and 3University of Colorado Cancer Center, University of Colorado Health Sciences Center, Denver, CO 80262, USA

This study was carried out to assess the anticancer efficacy of linarin (LN), linarin acetate (LA) and acacetin (AC), the flavonoid compounds with the same flavone ring structure but different substitution, against human prostate cancer (PCA), LNCaP and DU145 cells. LN was isolated and purified from Chrysanthemum zawadskii; LA was chemically synthesized from LN, and AC obtained commercially. In each case, the cells were treated with these agents at 25−100 μM doses for 24−72 h. LN and LA showed moderate cell growth inhibition with different time kinetics as compared to AC. LN caused up to a 5-fold increase in cell death and LA enhanced cell death by up to 4-fold with the increase in treatment time in both cell lines. AC showed a time- as well as dose-dependent stronger cell growth inhibition (20−70%) accompanied by cell death as compared to LN and LA in both the cell lines. LN or LA did not show any profound effect on cell cycle arrest except for a moderate G1 arrest, whereas, AC showed a stronger G1 and/or G2–M arrest depending on the doses and treatment times. G1 arrest was associated with an increase in Cip1/p21 and a decrease in CDK2, CDK4 and CDK6 protein levels. G2-M arrest was associated with a decrease in Cdc25C, Cdc2/p34 and cyclin B1, which were more prominent in LNCaP compared to DU145 cells. LN, LA and AC induced cell death was associated with significant increase in apoptosis induction (up to 5−6-fold) accompanied by poly-(ADP-ribose) polymerase cleavage. Overall, AC showed more potent anticancer efficacy among these three flavonoids, which was diminished when its flavone ring was modified by disaccharide rhamnose substitution at C7 (LN) or acetylation of this substituted group (LA). These findings, for the first time, revealed the structural determinants in anticancer efficacy and mechanisms of these three flavonoids against human PCA cells.

Introduction

Prostate cancer (PCA) continues to be a major problem in the developed world. Owing to the high mortality and unsatisfactory treatment options available, PCA remains the most common malignancy and is the second leading cause of cancer-related deaths among males in the USA (1–3). PCA usually progresses from androgen-dependent to -independent stage, making antiandrogen therapy ineffective leading to an increase in metastatic potential and incurable malignancy (4). In such cases, chemoprevention and intervention strategies using anticancer agents are suggested as promising alternative options. Generally, natural or synthetic chemical agents are employed in cancer chemoprevention to reverse, suppress or prevent cancer progression (5). Flavonoids constitute one of the most characteristic classes of compounds in higher plants and many flavonoids are easily recognized as flower pigments in most angiosperm families (6). Flavonoids from flowers have demonstrated positive antimutagenic activity (7). For the first time, in the present study, we investigated the anticancer activity and associated the mechanisms of action of three flavonoids having the same flavone ring structure but different substitution, linarin (LN) (Figure 1A), linarin acetate (LA) (Figure 1B) and acacetin (AC) (Figure 1C), against human PCA in cell culture.

LN is the main active compound found in the herb Chrysanthemum zawadskii var. latilobum (Compositae), which has been used in traditional medicine in Korea for the treatment of pneumonia, bronchitis, cough, common cold, pharyngitis, bladder-related disorders, gastroenteric disorders and hypertension. LN is also reported to have sedative and sleep enhancing properties in mice (8), and activates macrophages and modulates cytokine production (9). It also possesses antiinflammatory, analgesic, diaphoretic, hypotensive, antistress/anxiety, antipyretic and amebicidal activities (10). LA, a synthetic derivative of LN was synthesized in the present study.

The flavonoid AC, present in Cirsiwm rhinoceros Nakai (Compositae), a herbaceous perennial native to Korea, is...
used in folklore medicine. It is shown to have antiperoxidant, antimitogenic (7), anti-inflammatory and antiplaasmoidal (10) effects. More recent studies have shown that AC inhibits the proliferation of human liver and lung cancer cells, HepG2 and A549 cells respectively, by inducing apoptosis and blocking cell cycle progression (11,12). It has also been shown to reduce cholesterol content in cultured HepG2 cells (13). AC has also been reported to exert anticancer efficacy against human colon carcinoma cells (14), and to enhance differentiation-inducing activity in HL-60 cells. In addition, other reports show inhibitory effect of AC on glutathione reductase and cytochrome P450 (15,16).

Taken together, based on the above rationales and observations, this study was undertaken to investigate the effects of LN, LA and AC on cell growth and death in human PCA cells and associated biological events such as induction of programmed cell death, impairment of cell cycle progression and associated molecular events. The data obtained provide first evidence that these flavonoids impart anticancer activity to different extents in human prostate carcinoma DU145 and LNCaP cells.

Materials and methods

Cell lines and reagents

Human prostate carcinoma LNCaP and DU145 cell lines were purchased from American Type Culture Collection (Manassas, VA), and cultured in RPMI 1640 with 10% fetal bovine serum (Hyclone, Logan, UT) under standard culture conditions (37°C, 95% humidified air and 5% CO2). LN was isolated and purified from Chrysanthemum zawadskii var. latilobum (Compositae), LA synthesized from LN and AC used in the present study was from Sigma-Aldrich Chemical Co. (St Louis, MO), abundant in Chironoceros Nakai (Compositae), RPMI 1640 and other culture materials were from Life technologies, Inc. (Gaithersburg, MD). The primary antibody for anti-Cip1/p21 was from Calbiochem (Cambridge, MA), and for anti-Ki-67/p27 from Neomarkers (Fremont, CA). Antibodies to CDK2, CKD4, CKD6, Cdc2, cyclin D1, cyclin E, cyclin B1 and Cdc25C were from Santa Cruz Biotechnology (Santa Cruz, CA). Anticleaved PARP and secondary antibody for polyclonal primary antibodies were purchased from Cell Signaling, Inc. (Beverly, MA). Antibody for β-actin was from Sigma. Secondary antibody for monoclonal primary antibodies and ECL detection system were from Amersham (Arlington Heights, IL); and bis-benzimide (Hoechst 33342) was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO).

Plant material and extraction of LN

The herb, C.zawadskii var. latilobum Kitamura (Compositae) was purchased from Kyung Dong Market, Seoul, Korea and verified by Prof. S.Y.Lee, Sahm Yook University, Korea. A voucher specimen of this plant was deposited at the Yook University, Korea. A voucher specimen of this plant was deposited at the collections of the American Type Culture Collection (Manassas, VA) and the Korean Academy of Science and Technology, and cultured in RPMI 1640 medium containing 10% fetal bovine serum under standard culture conditions. At 60% confluency, cultures were treated with desired doses of LN, LA or AC for 24 and 48 h. Following these treatments, cell lysates were prepared in non-denaturing lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 0.2 mM sulfonyl fluoride, 0.5% NP-40 and 5 U/ml aprotinin). For lystate preparation, the medium was aspirated and the cells were washed with ice-cold PBS twice followed by incubation in lysis buffer for 15 min on ice. The cells were then scraped and kept on ice for 30 min, and finally cell lysates were cleared by centrifugation at 4°C for 30 min at 14 000 r.p.m. as reported earlier (17). Protein concentrations in lysates were determined using Bio-Rad DC protein assay kit (Bio-Rad laboratories, Hercules, CA).

Western immunoblotting

For immunoblotting, equal amount of cell lysate protein from each treatment, was denatured with 2× SDS-PAGE sample buffer and subjected to SDS-PAGE on 8, 12 or 16% Tris-glycine gel and separated proteins were transferred onto nitrocellulose membrane followed by blocking with 5% non-fat milk powder (w/v) in Tris-buffered saline (10 mM Tris, 100 mM NaCl and 0.1% Tween-20) for 1 h at room temperature and as desired, probed with primary antibody for Cip1/p21, Kip1/p27, CKD2, CKD4, CKD6, Cyclin E, Cyclin D1, Cdc25C, Cdc2, Cyclin B1, cleaved PARP and β-actin over night at 4°C followed by peroxidase-conjugated appropriate secondary antibody and ECL detection as reported earlier (17).

Statistical analysis

 Autoradiograms of the immunoblots were scanned using Adobe Photoshop, Adobe System Inc. (San Jose, CA). Statistical significance of differences between control and treated samples were calculated by Student’s t-test (SigmaStat 2.03). P < 0.05 was considered significant. Unless otherwise mentioned, all the data shown in the study for cell growth inhibition, cell cycle phase distribution, immunoblotting and quantitative apoptosis are representative of 2–3 independent studies.

Results

**NMR characterization of LN and LA**

Linarin (mol. wt 592)-1H NMR (DMSO-d6, δ): 1.12 (d, J = 6 Hz, 3H, CH3 of rhamnose), 3.30–3.80 (9H, sugar protons), 3.87 (s, 3H, ÓCH3), 4.63 (s, 1H, H rhamnose), 9.50 (1H, Ar-CHO).
LN, LA and AC inhibit growth and induce death of human prostate carcinoma LNCaP and DU145 cells

First, we investigated the growth inhibitory effect of these flavonoids against human PCA cell lines LNCaP (androgen-dependent) and DU145 (androgen-independent). It was observed that treatment of LNCaP cells with 25, 50 and 100 μM doses for 24–72 h showed a moderate cell growth inhibition by LN and LA accounting for up to 58% (P < 0.01) (Figure 2A) and 55% (P < 0.01) (Figure 2B) growth inhibition, respectively. As for AC, it showed a strong and both time- and dose-dependent cell growth inhibition accounting for 30–63% (P < 0.01), 45–77% (P < 0.01–0.001) and 50–80% (P < 0.01–0.001) inhibition at 25, 50 and 100 μM doses, respectively, for 24–72 h of treatment in LNCaP cells (Figure 2C). When compared with LNCaP cells, LN and LA were not very effective in inhibiting the cell growth of DU145 cells. In similar treatments, LN and LA showed 5–33% (P < 0.05–0.001) and 8–18% (P < 0.05–0.001) growth inhibition of DU145 cells, respectively (Figure 2D and E). Whereas AC showed almost similar growth inhibitory effect in DU145 cells as compared with LNCaP cells. The quantitative data of AC for DU145 cells showed 25–62% (P < 0.01), 29–68% (P < 0.05–0.01) and 45–83% (P < 0.01–0.001) cell growth inhibition at 25, 50 and 100 μM doses, respectively (Figure 2F). These results imply that growth inhibitory effect of these flavonoids against human PCA cells are in the order of AC > LN > LA.

These compounds were also effective in causing death of human PCA cells. A dose-dependent increase in cell death was observed for all the three compounds in most cases for both the cell lines with few exceptions (Figure 3). In LNCaP cells, similar treatment with LN, LA and AC as in cell growth assay caused up to 5-fold (P < 0.01–0.001), 3.5-fold (P < 0.01–0.001) and 4-fold (P < 0.05–0.001) increase in cell death, respectively (Figure 3A–C). Similar treatment to DU145 cells showed up to 4-fold (P < 0.05–0.001), 3-fold (P < 0.01–0.001) and 4-fold (P < 0.01–0.001) increase in cell death by LN, LA and AC, respectively (Figure 3D–F). Overall, these data suggest that acetate substitution in LN (i.e. LA) reduces its efficacy for growth inhibition as well as inducing cell death effect. However, substitution of hydroxyl group in AC with rhamnose disaccharide (i.e. LN) reduces its growth inhibitory efficacy but almost did not affect its cell death-inducing effect.

LN, LA and AC inhibit cell cycle progression of LNCaP and DU145 cells

To assess whether LN, LA and AC induced cell growth inhibition is mediated via alterations in cell cycle progression, we evaluated the effect of these compounds on cell cycle phase distribution. As shown in Table I, consistent with growth inhibitory effects, LN, LA and AC (25–100 μM) caused a significant (P < 0.05–0.001) G1 arrest at the expense of S and/or G2–M phase cell population following 24 h of treatment but this effect invariably started diminishing with the increase in treatment time (48 and 72 h) in LNCaP cells. However, interestingly, only in the case of AC this decrease in G1 arrest was accompanied by an increase (P < 0.01) in G2–M phase cell population at 48 and 72 h of treatment (Table I).

Further, we did similar LN, LA and AC treatment to DU145 cells and performed cell cycle distribution analysis to compare their effect with LNCaP cells. Our data show that LN was almost completely ineffective in causing any cell cycle arrest in DU145 cells while LA could slightly induce G1 arrest (up to ~3–5%, P < 0.05–0.01, increase over controls at 24 and 72 h of treatments) (Table II). AC showed a similar trend in G1 arrest in terms of its time-dependent decreasing effect showing maximum efficacy at 24 h of treatment as observed in LNCaP cells; however, this effect was totally different when compared in terms of increase in doses (Tables I and II). The lower doses of AC (25 and 50 μM) showed 8% (P < 0.01) increase in G1 arrest together with a 1–5% (P < 0.05–0.01) increase in G2–M arrest, while 100 μM dose showed a strong increase only in G2–M arrest (21%, P < 0.001) at the expense of both G1 and S phase cell population (Table II). Our data also showed that with the increase in treatment time, the effect on G1 arrest kept on decreasing, showing almost no G1 arrest at 72 h for AC; however, it was sustained significantly for G2–M arrest after 48–72 h of treatments with similar efficacy at lower doses but with a reduced efficacy at 100 μM dose of AC (Table II). In morphological analysis, we observed that these compounds cause cell elongation and rounding of the cells following their detachment from the surface, which was more prominent in AC treatments (data not shown). Overall, these data suggest considerable effect on the extent as well as nature of the cell cycle arrest in PCA cells when flavone ring is modified by substitution at C7 position with different chemical moieties.

Effect of AC on G1 cell cycle regulators in LNCaP and DU145 cells

Based on the above results showing maximum efficacy for AC, we used 25–100 μM of AC treatment for 24 and 48 h to study its effect on cyclin dependant kinase inhibitor (CDKIs), cyclins and CDKs involved in G1 phase of cell cycle regulation by western blot-analysis. Association of cyclin with CDK forms active kinase complexes, which are regulated and inhibited by binding with CDKI. Our data show that AC did not change the level of Kip1/p27 in LNCaP cells except for showing a moderate decrease at 100 μM dose for 48 h treatment (Figure 4A). Interestingly, in DU145 cells, AC decreased the level of Kip1/p27 at all the doses (25–100 μM) for both the treatment times (Figure 4B). These results excluded the possibility of the involvement of Kip1/p27 in AC-induced G1 arrest in both the PCA cell lines. Next, we analyzed the level of Cip1/p21 that increased in a dose-dependent manner in both the cell lines except for a decrease at 100 μM dose for 48 h treatment in LNCaP cells (Figure 4A and B). We did not observe any prominent change in cyclin D1 and cyclin E following AC treatment in both the cell lines (data not shown). Next, we assessed the effect of AC on G1 CDKs that showed a strong dose-dependent decrease in CDK2 and a moderate dose-dependent decrease in CDK4 and 6 protein levels in LNCaP cells at both the treatment times (Figure 4A). In DU145 cells,
strong inhibitory effect of AC was observed for CDK4 and 6 at both the treatment times and an almost similar effect was observed for CDK2 decrease following 48 h of treatment (Figure 4B). Protein loading was checked by reprobing the membranes with β-actin antibody, which did not show any change in the protein level (Figure 4A and B). Overall, these results suggested that AC-induced G1 arrest in PCA cells involves a decrease in G1 CDK levels with a moderate increase in Cip1/p21 (except for 48 h treatment with 100 μM dose in LNCaP cells).

**AC modulates G2-M cell cycle regulators in PCA cells**

Based on the results, also showing G2-M arrest following AC treatment in LNCaP and DU145 cells, we assessed its effect on G2-M cell cycle regulators including Cdc25C, Cdc2/p34 and cyclin B1. As shown in Figure 5A, AC caused a dose-dependent decrease in the levels of these proteins after 24-48 h of treatment in LNCaP cells except for 48 h treatment at 100 μM dose for cyclin B1 (Figure 5A). In DU145 cells, AC showed a moderate dose-dependent decrease in Cdc25C protein level (Figure 5B). Cdc2 and cyclin B1 were slightly
decreased or remained unchanged by AC in DU145 cells, and overall the effects were not as pronounced as in the case of LNCaP cells (Figure 5A and B). Protein loading was checked by reprobing the membranes with β-actin antibody, which did not show any change in the protein level (Figure 5A and B).

**LN, LA and AC induce apoptosis in LNCaP and DU145 cells**

In the following experiments, we assessed whether LN-, LA-, and AC-caused cell death (as observed by trypan blue dye exclusion) was accompanied by an induction of apoptosis in LNCaP and DU145 cells. Cells were treated with 50 and 100 μM doses of LN, LA and AC for 24 and 48 h, and stained with PI and Hoechst 33342 followed by observation and quantification of apoptotic cells under fluorescence microscope as reported earlier (18). In LNCaP cells, we observed an increase in apoptotic cell population by 3.2 to 4.2-fold ($P$ $<$ 0.001) and 2.8 to 3.5-fold ($P$ $<$ 0.001) by LN (Figure 6A); 4.1 to 4.5-fold ($P$ $<$ 0.001) and 4 to 4.1-fold ($P$ $<$ 0.001) by LA (Figure 6B); and 4.3 to 5.5-fold ($P$ $<$ 0.001) and 4.8 to 5.6-fold ($P$ $<$ 0.001) by AC (Figure 6C) after 24 and 48 h of treatments, respectively. Similarly, an increase in apoptotic cell population in DU145 cells was 2 to 5-fold ($P$ $<$ 0.001) and 2.4 to 2.6-fold ($P$ $>$ 0.05 $<$ 0.001) by LN (Figure 6D); 3 to 5-fold ($P$ $<$ 0.001) and 3.6 to 5.5-fold ($P$ $<$ 0.001) by LA (Figure 6E);
and 4.5 to 5-fold \((P < 0.001)\) and 3 to 3.2-fold \((P < 0.001)\) by AC (Figure 6F) after 24 and 48 h of treatments, respectively. A slight increase in apoptotic effect with the increase in doses was evident for LN and AC in LNCaP cells; and LN and LA in DU145 cells at both the treatment times. Overall, on comparison among the three compounds, AC was more effective in LNCaP cells, and all the three compounds were almost equally effective (24 h) in DU145 cells where only LA showed a sustained effect (48 h).

**LN, LA and AC induce cleavage of PARP in LNCaP and DU145 cells**

The 116-kDa-PARP, which is normally involved in DNA repair, stability and other cellular events, is cleaved (89 kDa fragment) by the members of the caspase family thereby marking the onset of early apoptosis as well as caspases activation (19). To further confirm the apoptotic effects of LN, LA and AC, cells were treated with 50–100 \(\mu\)M doses of these flavonoids for 24 and 48 h, and western blot-analysis was performed in cell lysates to analyze PARP cleavage. In terms of doses, we did not observe any prominent change in the level of cleaved PARP by these compounds, except for a decreased level in 100 \(\mu\)M dose of AC treated for 24 h (Figure 7). In LNCaP cells, 24 h LA treatment showed more levels of cleaved PARP as compared with LN and AC; however, after 48 h the levels of cleaved PARP by these compounds were in the order of AC \(\gg\) LA \(\gg\) LN (Figure 7A). In DU145 cells, AC-induced PARP cleavage was greater as compared with LN and LA at both the time points, and was in the similar order (AC \(\gg\) LA \(\gg\) LN) after 48 h as in LNCaP cells (Figure 7A and B). Overall, these results indicate the potency of apoptosis induction by these compounds in PCA cells.

**Discussion**

Flavonoids are widely recognized as naturally occurring antioxidants. Recently, several flavonoid antioxidants have been reported to have chemopreventive effects on cancer (20). In the present study, we evaluated three flavonoids, LN, LA and AC, for their activities in inhibiting the growth of human prostate carcinoma LNCaP and DU145 cells. We observed that LN, LA and AC inhibited PCA cell growth in both androgen-responsive LNCaP cells as well as hormone-refractory DU145 cells. Whereas LN and LA exhibited moderate growth inhibitory effect, AC was found to be the most potent among the three and resulted in a dose- and time-dependent decrease in cell number of both LNCaP and DU145 cells. LN and LA showed greater growth inhibitory efficacy in LNCaP cells compared with DU145 cells. Overall, data in the present study suggest that growth inhibitory effect of these agents in human PCA cells is in the order of AC \(\gg\) LN \(\gg\) LA. Therefore, it is
evident that modification of C-7 in flavone ring of AC by adding disaccharide-rhamnose decreases its efficacy, and subsequent modification by acetylation of the substituted group at C-7 in LN further reduces or does not have any considerable effect on its growth inhibitory efficacy. We also observed that growth inhibitory effect of these flavonoids was accompanied by an induction of cell death. Overall, the order of cell death inducing effect was LN > AC > LA. These data suggest that conversion of AC into LN either does not affect or slightly increases its cell death inducing effect, while acetylation of rhamnose moiety always decreases this efficacy in both the PCA cell lines.

To further explore the underlying mechanisms of cell growth inhibition we conducted cell cycle analysis which revealed that although AC caused both G1 and G2–M arrests in cell cycle progression of LNCaP and DU145 cells, LN and LA lack such a profound effect. Based on these results, it could be suggested that modification of 7-OH of flavone ring in AC leading to formation LN or LA results in decrease in its biological effect of inducing G1 and G2–M arrests in PCA cells. Based on AC efficacy in causing cell cycle arrest, we next analyzed whether it alters cell cycle regulatory proteins involved in G1 and G2–M checkpoints in both LNCaP and DU145 PCA cell lines. Our data show that AC increased the level of CDKI Cip1/p21. It is known that CDKI Cip1/p21 can induce cell cycle arrest in G1 and/or G2–M by inhibiting kinase activity of CDKs as it is regarded as a universal inhibitor of CDKs (21). Further, AC also decreased the protein levels of CDK2, CDK4 and CDK6 in PCA cells.

Previous studies have shown an association between deregulated cell cycle progression and cancer, and suggest that inhibition of cell cycle could be an important target for the management of cancer (21–23). Multiple proteins are responsible for coordinating advancement through each phase of the cycle, particularly G1 phase and its restriction point (23–25). The formation of two major protein complexes and their associated kinase activities are required for G1–S progression; cyclin D-CDK 4/6 is active in early G1 phase, whereas cyclin E-CDK2 is required for entry into S phase (23,24,26). CDKs bound to their regulatory subunit cyclins, drive the events of the eukaryotic cell cycle progression (24). In complex cell cycles, they are also the information processors that integrate extracellular and intracellular signals to ensure the smooth coordination of cell cycle events in the face of environmental change or mechanical failure (24). Catalytic activity of CDKs is regulated by phosphorylation and
abundance of their cyclin partners and by association with CDK inhibitors of the Cip/Kip or INK family proteins (21,22). In light of these reports, a straightforward interpretation for the increased expression of Cip1/p21 together with decreased levels of CDK2, 4 and 6 by AC could be that the decreased levels of G1 cyclin-CDK complexes and subsequently reduced CDKs activities resulting in an inhibition or delay of the cell cycle progression in the G1 phase, as we observed in the present study.

In this study, we also observed that AC causes G2-M arrest along with G1 arrest in both LNCaP and DU145 cells, particularly at higher doses. Similar to G1-S transition, cell cycle

Fig. 6. LN, LA and AC induce apoptosis in LNCaP (A-C) and DU145 cells (D-F). Cells were treated with vehicle control (DMSO) or 50 and 100 μM doses of LN, LA and AC for 24 and 48 h. At the end of the treatments, cells were collected and subjected to Hoechst staining as described in Materials and methods. Quantitative data shown are the mean ± SE of three independent plates. #, P < 0.05; $, P < 0.01; *, P < 0.001. LN, linarin; LA, linarin acetate; AC, acacetin.

R.P. Singh et al.
progression through G2–M is regulated by activation of CDK, whose activity is dependent upon their association with regulatory cyclins (27,28). The major regulator of G2–M transition is a complex consisting of catalytic subunit Cdc2 and regulatory subunit cyclin B1 that controls the entry into mitosis (27–29). Cdc2 is inactive in phosphorylated form, and is dephosphorylated by Cdc25C phosphatase to form active complex with cyclin B1 (29,30). Consistent with these reports, AC decreased Cdc25C, Cdc2 and cyclin B1 protein levels in PCA cells, being more effective in LNCaP cells. Overall, the decreasing effect on these proteins could be the underlying molecular events contributing to G2–M arrest caused by AC in PCA cells.

Increasing evidence indicates that impaired ability to undergo apoptosis plays an important role in the evolution from androgen-dependent to androgen-independent PCA as well as drug resistance (31). Many reports also suggest that cell cycle arrests are often followed by or associated with apoptotic death of cancer cells by many cancer therapeutic agents (32). Apoptosis or cell suicide is a form of cell death that is morphologically and biochemically distinct from necrosis, and is regarded as an efficient way to eliminate cells (33). Therefore, agents that can induce apoptosis may be useful in management and therapy of cancer (31–33). In this regard, our data show that LN, LA and AC induced apoptosis in PCA cells, which was also accompanied by PARP cleavage. The cleavage of PARP by activated caspase 3 or 7 is considered to be one of the hallmarks of apoptosis (34). However, in terms of PARP cleavage we observed an overall efficiency in the order of AC > LA > LN. This could imply that AC might be more effective in activating caspase pathway which was decreased by it chemical modification into LN or LA. However, more studies are needed to confirm this anticipation.

The findings in the present study have also significance to the different stages of the PCA as LNCaP and DU145 cell lines have different status of tumor suppressor p53 and retinoblastoma (Rb) genes, and represent from less to more advanced progressive stages of PCA. These flavonoids showed more sensitivity to cell growth inhibition, cell cycle arrest in G1 phase and apoptosis in LNCaP cells which have functional p53 and Rb genes, as compared with DU145 cells lacking both these functional genes. These observations suggest that there could be a, most likely, role of p53 and/or pRb in increased efficacy of these compounds in LNCaP cells; however, more studies are needed to confirm such anticipation.

In conclusion, our results indicated that LN, LA and AC inhibit human PCA cell growth and induce apoptotic cell death; however, growth inhibitory effect by AC through G1 and G2–M cell cycle arrest was more prominent as compared with LN or LA. These results also suggest that in vivo efficacy studies with AC in preclinical PCA models are warranted where a positive outcome would be useful in moving this natural agent forward as a chemopreventive agent against PCA.

Acknowledgement

This work was supported in part by USPHS grants CA91883 and CA102514 from the National Cancer Institute, NIH.

References


Received November 16, 2004; revised December 20, 2004; accepted December 21, 2004