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Induction of human promyelocytic leukemia HL-60 cell differentiation into monocytes by silibinin: involvement of protein kinase C

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Abstract

The effect of silibinin, an active component of *Silybum marianum*, on cellular differentiation was investigated in the human promyelocytic leukemia HL-60 cell culture system. Treatment of HL-60 cells with silibinin inhibited cellular proliferation and induced cellular differentiation in a dose-dependent manner. Cytofluorometric analysis and morphologic studies indicated that silibinin induced differentiation of HL-60 cells predominantly into monocytes. Importantly, strongly synergistic induction of differentiation into monocytes was observed when silibinin was combined with 5 nM 1α,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], a well-known differentiation inducer of HL-60 cells into the monocytic lineage. Silibinin enhanced protein kinase C (PKC) activity and increased protein levels of both PKCα and PKCβ in 1,25-$(OH)_2D_3$-treated HL-60 cells. PKC and extracellular signal-regulated kinase (ERK) inhibitors significantly inhibited HL-60 cell differentiation induced by silibinin alone or in combination with 1,25-$(OH)_2D_3$, indicating that PKC and ERK may be involved in silibinin-induced HL-60 cell differentiation.

Keywords: Silibinin; HL-60 cell differentiation; 1α,25-Dihydroxyvitamin D_3; Protein kinase C; Extracellular signal-regulated kinase

1. Introduction

Silibinin is a polyphenolic flavonoid derived from the fruits and seeds of the milk thistle also called *Silybum marianum* [1]. It has received the special attention of many chemists and clinicians, since it has been shown to have cancer-preventive and anticarcinogenic effects [2,3] as well as a hepatoprotective effect [4,5]. Silibinin also possesses a variety of biological activities such as an antioxidative activity scavenging free radicals and inhibiting lipid peroxidation [6,7], an anti-inflammatory effect due to the inhibition of nuclear factor-κB activation [8], and an inhibitory action on tumor necrosis factor-α expression and ornithine decarboxylase activity [9,10].

Human promyelocytic leukemia HL-60 cells are differentiated into the monocytic lineage when treated with 1,25-$(OH)_2D_3$ [11]. HL-60 cell culture has been employed as an excellent model system for studying cellular differentiation *in vitro*. Several studies provide evidence that activation of PKC is necessary for differentiation of HL-60 cells, especially along the monocytic pathway. Continuous treatment of HL-60 cells with 1,25-$(OH)_2D_3$ increased PKC levels and cell differentiation [12], which were significantly inhibited by PKC inhibitors [13] or a PKC anti-sense construct [14].

In this report, we investigated the effect of silibinin on cellular differentiation in the human promyelocytic leukemia HL-60 cell culture system. We also investigated the effects of combinations of silibinin and 1,25-$(OH)_2D_3$ on HL-60 cell differentiation. 1,25-$(OH)_2D_3$ was chosen for this study because it has been widely used as an endogenous stimulator of differentiation in HL-60 cells. In addition, analogues of 1,25-$(OH)_2D_3$ have been used clinically for the treatment of psoriasis [15].

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Abbreviations: 1,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; ERK, extracellular signal-regulated kinase; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; NBT, nitroblue tetrazolium; and PKC, protein kinase C.

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2. Materials and methods

2.1. Materials

The HL-60 cell line was obtained from the American Type Culture Collection and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum. A stock solution of 1 mM 1,25-$(OH)_2D_3$ was dissolved in absolute ethanol and stored at −70°. Silibinin was also dissolved in absolute ethanol to make a stock solution of 40 mg/mL. The solutions were diluted in the growth medium such that the final concentration of ethanol had no effect on the expression of the differentiation. All manipulations were performed in subdued light.

2.2. Determination of cell number and viability

To quantify the number of cells after each treatment, cells were counted at different times of incubation using a hemocytometer under a light microscope. Cell viability was determined by the trypan blue exclusion assay. Viability was calculated as the percentage of live cells in the total cell population.

2.3. Immunofluorescent staining and cytofluorometric measurements

Quantitative immunofluorescence measurements were performed in an Epic V flow cytofluorograph equipped with a multiparameter data acquisition and display system (MDADS) as previously described [16]. Briefly, single-cell suspensions were collected from the various cultures and washed twice with ice-cold PBS (pH 7.4). Thereafter, fluorescein isothiocyanate-conjugated anti-human CD14 or phycoerythrin-conjugated anti-human 11b mAbs were added and incubated at 4° for 1 hr. After incubation, the cells were washed and fixed in PBS containing 1% paraformaldehyde, after which cytofluorometric analysis was performed. Background staining was determined by staining cells with fluorescein isothiocyanate- or phycoerythrin-conjugated isotype control mAbs. One-parameter fluorescence histograms were generated by analyzing at least 1×10^4 cells.

2.4. NBT reduction assay

HL-60 cell differentiation was assessed by the NBT reduction assay as previously described [17]. The assay is based on the ability of phagocytic cells to produce superoxide upon stimulation with tissue plasminogen activator. For this assay, 2×10^5 cells were harvested by centrifugation and incubated with an equal volume of 0.2% NBT dissolved in PBS containing 1 ng/mL of freshly diluted tissue plasminogen activator at 37° for 30 min in the dark. Cytospin slides were prepared and examined for blue–black nitroblue diformazan deposits, indicative of a tissue plasminogen activator-stimulated respiratory burst. At least 200 cells were assessed for each experiment.

2.5. Morphologic studies

Single-cell suspensions were prepared and 2×10^5 cells were loaded into cytofunnel and spun at 27 *g* in a cytospin centrifuge. The slides were fixed with methanol and dried. The slides were stained with Giemsa staining solution for 20 min and rinsed in deionized water, and assessed under microscope with a camera.

2.6. PKC activity assay

PKC activity was determined using a commercially available kit as previously described [18]. Briefly, the HL-60 cells were homogenized in extraction buffer containing 20 mM Tris (pH 7.5), 0.5 mM ethylenediaminetetraacetic acid, 0.5 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 0.5% Triton X-100, 25 μg/mL of leupeptin, and 25 μg/mL of aprotinin. These crude extracts were partially purified by eluting with the extraction buffer and eluted with buffer containing 20 mM Tris (pH 7.5), 0.5 mM ethylenediaminetetraacetic acid, 0.5 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 10 mM β-mercaptoethanol, and 0.2 M Na. Subsequently, each sample was incubated with lipid, peptide, and PKC substrates containing [γ-^{32}P]ATP for 5 min at 30°. The terminated reaction mixture was pipetted onto phosphocellulose disc, and the radioactivity was quantified by scintillation counting.

2.7. Western blot analysis

The protein levels of PKC isoforms in HL-60 cells were analyzed as previously described [19]. In brief, the HL-60 cells were washed twice with PBS and then resuspended in lysis buffer (0.2 M Tris, 0.5 mM leupeptin, 0.4 mM phenylmethylsulfonyl fluoride, pH 7.5). The protein content of the total cell homogenates was determined by the Bradford protein assay. Equal amounts of protein from each experimental group were boiled for 10 min and run on a 10% polyacrylamide gel. The proteins were subsequently electroblotted into nitrocellulose paper. The blot was blocked with PBS containing 0.1% Tween 20 and 5% milk for 1 hr at 22°. The blots were incubated in fresh blocking solution with an appropriate dilution of each rabbit anti-human PKC isoform mAb or anti-TBP (TATA box-binding protein) mAb for 1 hr at 22°. Subsequently, the blots were washed three times with blocking buffer and incubated for 1 hr with a biotinylated anti-rabbit immunoglobulin G for 1 hr at 22°. After washing, horseradish peroxidase–streptavidin was added. The blots were again washed three times with blocking solution and then developed by enhanced chemiluminescence.

2.8. Statistical analysis

The Student's *t*-test and one-way analysis of variance (ANOVA) were used to determine the statistical differences between various experimental and control groups. *P* values <0.05 were considered significant.

3. Results

3.1. Effects of silibinin on HL-60 cell proliferation and differentiation

The HL-60 cells were seeded at a density of 2×10^5 cells/mL and treated with medium alone or with 10–60 μg/mL of silibinin for 72 hr. The cell number for each group was determined. The cell density of the untreated group after 72 hr was 12.5×10^5 cells/mL. Treatment of the cells with more than 10 μg/mL of silibinin significantly inhibited cell proliferation in a dose-dependent manner (Fig. 1A).

Next, the effect of silibinin on HL-60 cell differentiation was assessed by the NBT reduction assay. As shown in Fig. 1B, incubation with silibinin significantly induced HL-60 cell differentiation in a dose-dependent manner. Independent experiments were performed to determine silibinin-induced HL-60 cell differentiation by analyzing CD11b (Mac-1) expression on the cell surface. CD11b is a cell surface marker for differentiation into either monocytes or granulocytes [20]. As shown in Fig. 2B, treatment with silibinin markedly increased the number of cells showing high fluorescence intensity. This is shown by a shift in the fluorescence peak to the right of the negative control peak (Fig. 2B vs 2A). Silibinin-induced cell differentiation was also confirmed by morphologic studies. As shown in Fig. 2C, Giemsa-stained undifferentiated control HL-60 cells treated with medium alone were predominantly promyelocytes with round and regular cell margins, large nuclei, and very little cytoplasm, suggesting that the cells were highly active in DNA synthesis and rapidly proliferating. Silibinin-treated cells exhibited relatively small changes in cell morphology such as irregular cell margins, decreased cell size, and denser chromatin (Fig. 2D). Some cells showed a horseshoe-shaped nucleus, which is a sign of monocytic differentiation. These results indicate that silibinin was an inducer of HL-60 cell differentiation.

3.2. Effects of silibinin on the differentiation pathway of HL-60 leukemia cells

To determine the differentiation pathway taken by HL-60 cells after treatment with silibinin, HL-60 cells were first treated with the flavonoid, and cytofluorometric analysis using mAb for the monocytic surface antigen CD14 was determined. CD14 is expressed exclusively when cells are differentiated into monocytes [21]. As shown in Fig. 3, HL-60 cells treated with silibinin reacted very strongly with anti-CD14 mAb in a dose-dependent manner. These results indicate that silibinin stimulated HL-60 cell differentiation along the monocytic pathway.

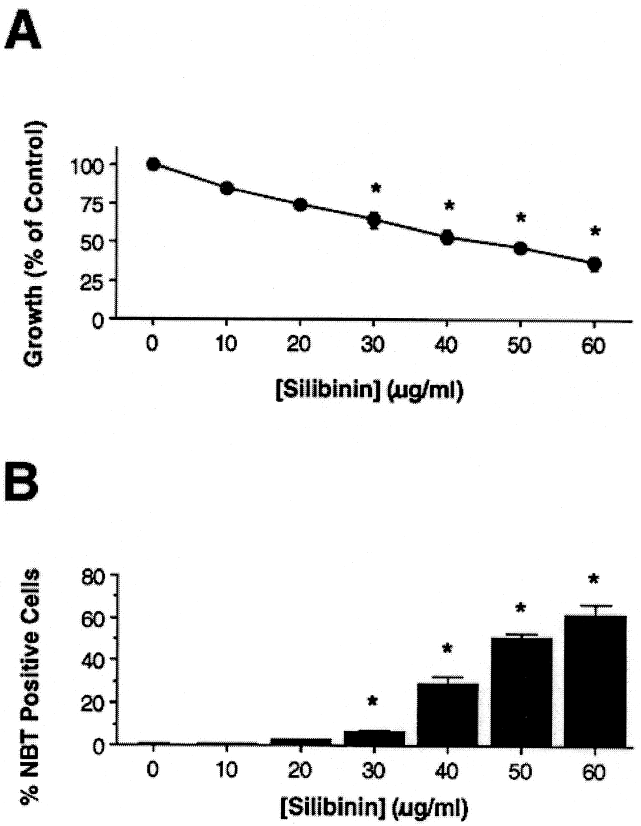

Fig. 1. Effects of silibinin on HL-60 cell proliferation and differentiation. HL-60 cells were treated for 72 hr with medium alone or 10–60 μg/mL of silibinin. The cell number was measured using a hemocytometer under a light microscope and represented as a percentage of the cell number of each treated group relative to the untreated control group (A). Aliquots were removed and the NBT reduction assay was performed to determine cell differentiation (B). Each value represents the mean ± standard deviations of triplicate determinations from one representative experiment. The experiments were repeated at least twice with similar results. $*P < 0.01$, relative to an untreated group.

3.3. Synergistic effects of silibinin on 1,25-$(OH)_2D_3$-induced HL-60 cell differentiation

1,25-$(OH)_2D_3$ is well known to induce HL-60 cell differentiation into the monocytic lineage [11]. To determine the effect of silibinin on 1,25-$(OH)_2D_3$-induced cell differentiation, HL-60 cells were treated with combinations of silibinin and 1,25-$(OH)_2D_3$, and cellular differentiation was assessed by the NBT reduction assay. As controls, the cells were treated with either silibinin or 1,25-$(OH)_2D_3$ alone. As shown in Fig. 4, silibinin synergistically potentiated 1,25-$(OH)_2D_3$-induced HL-60 cell differentiation. For example, the observed effect of 5 nM 1,25-$(OH)_2D_3$ in combination with 30 μg/mL of silibinin (72.1% NBT-positive cells) was significantly higher than the sum of the effects of the individual treatments (32.3% NBT-positive cells).

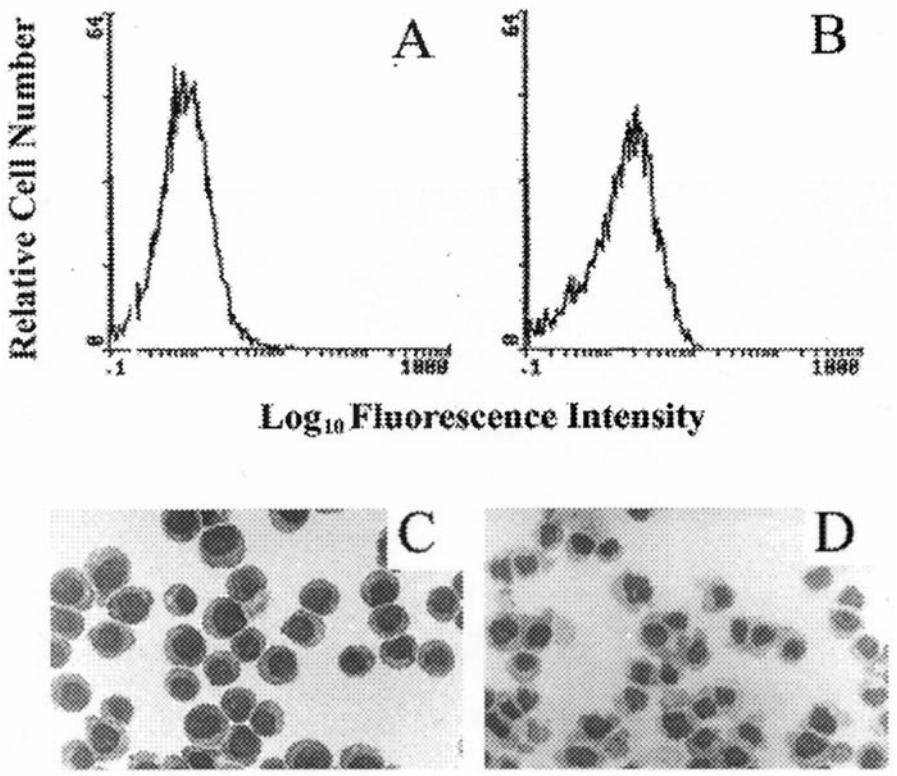

Fig. 2. Silibinin-induced HL-60 cell differentiation as determined by cytofluorometic and morphologic analysis. HL-60 cells were treated with either medium alone (A and C) or 40 μg/mL of silibinin (B and D) for 72 hr. The cells were assessed by cytofluorometric analysis using anti-CD11b mAb (A and B) or by morphologic analysis using Giemsa stain (C and D). The data are representative of three separate experiments.

To determine the differentiation pathway taken by HL-60 cells after treatment with combinations of silibinin and 1,25-$(OH)_2D_3$, the HL-60 cells were treated with silibinin alone or with a mixture of silibinin and 1,25-$(OH)_2D_3$, and cytofluorometric analysis using mAb for the monocytic surface antigen CD14 was determined. As shown in Fig. 5, HL-60 cells treated with silibinin and 1,25-$(OH)_2D_3$ reacted very strongly with anti-CD14 mAb. Cells treated with either silibinin or 1,25-$(OH)_2D_3$ alone also reacted with anti-CD14 mAb, but to a lesser extent than those treated with silibinin in combination with 1,25-$(OH)_2D_3$. These results indicate that silibinin synergistically stimulated 1,25-$(OH)_2D_3$-induced HL-60 cell differentiation along the monocytic pathway.

3.4. Effects of PKC or ERK/MAPK inihbitors on HL-60 cell differentiation induced by silibinin alone or in combination with 1,25-$(OH)_2D_3$

Previous studies provide evidence that activation of PKC is necessary for the differentiation of HL-60 cells [12,22].

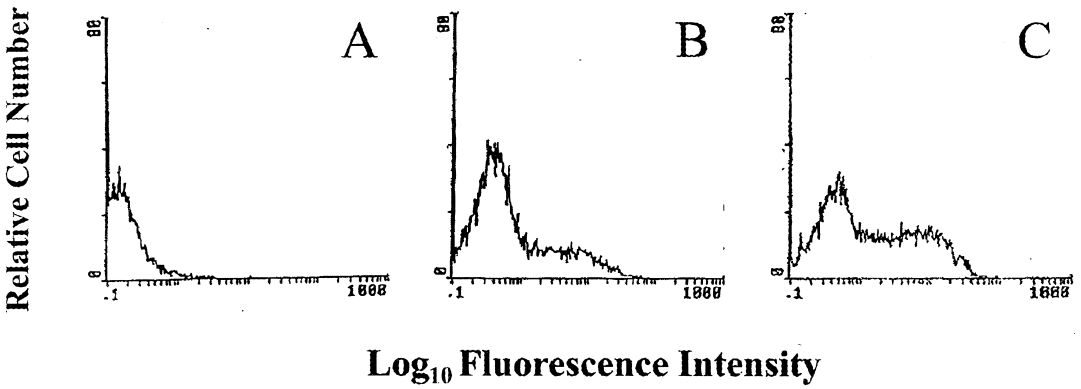

Fig. 3. Cytofluorometric analysis of silibinin-induced HL-60 cell differentiation using monoclonal antibody for monocytic cell surface marker CD14. HL-60 cells were treated for 72 hr with medium alone (A), 40 μg/mL of silibinin (B), or 50 μg/mL of silibinin (C), and the cells were assessed by cytofluorometric analysis using anti-CD14 mAb. The data are representative of four separate experiments.

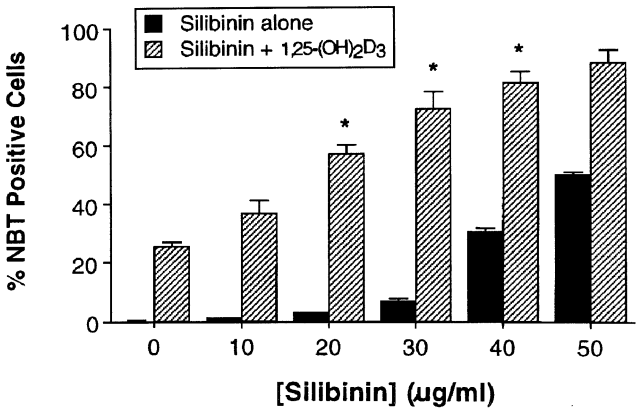

Fig. 4. Synergistic effects of silibinin on 1,25-$(OH)_2D_3$-induced HL-60 cell differentiation. HL-60 cells were treated for 72 hr with 10–50 μg/mL of silibinin alone or in combination with 5 nM 1,25-$(OH)_2D_3$. Then, the cellular differentiation was assessed by the NBT reduction assay. Each value represents the mean ± standard deviations of triplicate determinations from one representative experiment. The experiment was repeated more than three times with similar results. *$P < 0.001$, relative to groups treated with either silibinin or 5 nM 1,25-$(OH)_2D_3$.

To determine any relationship between the effect of silibinin on cellular differentiation and PKC activation, the HL-60 cells were treated with the PKC inhibitors 3-[1-[3-(dimethylamino)propyl]-1*H*-indol-3-yl]-4-(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione monohydrochloride (GF109203X) [23], chelerythrine, or H-7 [24] in the presence of silibinin alone or in combination with 1,25-$(OH)_2D_3$, and cell differentiation was assessed by the NBT reduction assay. As shown in Fig. 6, all three PKC inhibitors significantly inhibited HL-60 cell differentiation induced by silibinin alone or in combination with 1,25-$(OH)_2D_3$. GF-109203X in particular completely inhibited HL-60 cell differentiation induced by silibinin or 1,25-$(OH)_2D_3$ alone or in combination at a relatively low dose (2.5 μM). Previous reports have shown that GF-109203X acts as a competitive inhibitor of the ATP-binding site of PKC, and the IC_{50} value of GF-109203X against PKC activity is significantly lower than that of other PKC inhibitors [25]. In addition, the PKC inhibitors were not toxic to the HL-60 cells at concentrations used in the experiments, as demonstrated by the trypan blue exclusion assay (data not shown).

To further investigate the involvement of PKC in HL-60 cell differentiation enhanced by silibinin, HL-60 cells were treated with silibinin alone or in combination with 1,25-$(OH)_2D_3$, and PKC activity in the treated cells was determined. As shown in Fig. 7A, silibinin induced PKC activity in HL-60 cells while enhancing it in 1,25-$(OH)_2D_3$-treated HL-60 cells, whereas this activity was significantly inhibited by GF-109203X, a PKC inhibitor. In addition, to de-

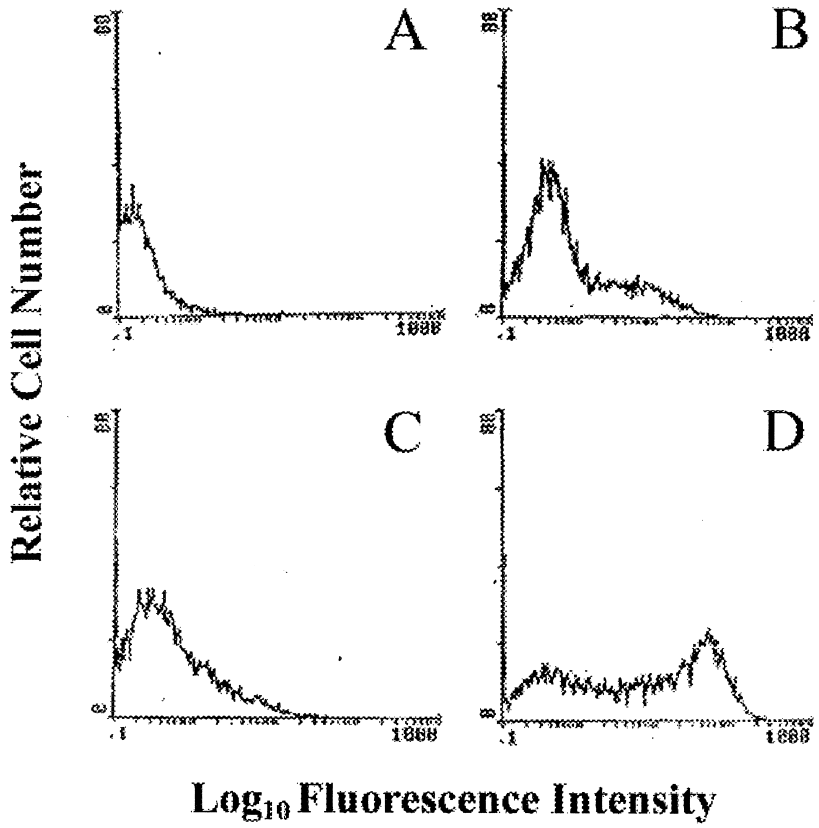

Fig. 5. Cytofluorometric analysis of HL-60 cell differentiation using anti-CD14 mAb after treatment with silibinin and 1,25-$(OH)_2D_3$. HL-60 cells were treated for 72 hr with medium alone (A), 40 μg/mL of silibinin (B), 5 nM 1,25-$(OH)_2D_3$ (C), or 40 μg/mL of silibinin in combination with 5 nM 1,25-$(OH)_2D_3$ (D). The cells were assessed by cytofluorometric analysis using anti-CD14 mAb. The data are representative of three separate experiments.

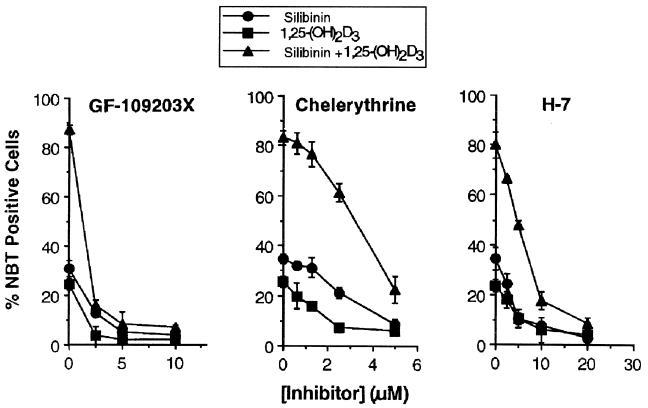

Fig. 6. Effect of PKC inhibitors on HL-60 cell differentiation induced by silibinin alone or in combination with 1,25-$(OH)_2D_3$. HL-60 cells were treated for 40 min with varying concentrations of PKC inhibitors (GF-109203X, chelerythrine, or H-7), followed by incubation with 40 μg/mL of silibinin, 5 nM 1,25-$(OH)_2D_3$, or 40 μg/mL of silibinin in combination with 5 nM 1,25-$(OH)_2D_3$ for 72 hr. Cellular differentiation was assessed by the NBT reduction assay. Data are presented as a percentage of differentiated cells with the mean ± standard deviations of triplicate determinations. The experiment was repeated twice with similar results.

termine PKC isoforms induced by silibinin, HL-60 cells were treated with silibinin alone or in combination with 1,25-$(OH)_2D_3$, and the protein levels of PKC isoforms were determined by Western blot analysis using mAbs for each PKC isoform. As shown in Fig. 7B, silibinin increased the protein levels of both PKCα and PKCβ, while enhancing the levels in 1,25-$(OH)_2D_3$-treated HL-60 cells.

MAPK is a downstream element in the PKC signaling pathway of HL-60 cells [26]. To determine the involvement of MAPK in 1,25-$(OH)_2D_3$-induced cell differentiation enhanced by silibinin, HL-60 cells were treated with a selective ERK MAPK inhibitor PD98059 [2-(2′-amino-3′-methoxyphenyl)-oxanaphthalen-4-one], [27] or with a selective p38 MAPK inhibitor SB203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl) 1*H*-imidazole] [28], in the presence of silibinin alone or in combination with 1,25-$(OH)_2D_3$. As shown in Fig. 8, PD98059 significantly inhibited HL-60 cell differentiation induced by silibinin alone or in combination with 1,25-$(OH)_2D_3$ in a dose-dependent manner. In contrast, SB203580 increased HL-60 cell differentiation induced with silibinin. Therefore, silibinin may, at least in part, potentiate 1,25-$(OH)_2D_3$-induced HL-60 cell differentiation via the PKC–ERK signaling pathway.

4. Discussion

In the present study, we have demonstrated that silibinin significantly induced differentiation in HL-60 promyelocytic leukemia cells that are widely used as a model system for differentiation studies. HL-60 cells were synergistically differentiated into monocytes when treated with silibinin in combination with 1,25-$(OH)_2D_3$. Many previous studies have reported some chemical combinations which exerted an additive or synergistic effect on HL-60 cell differentiation. These combinations include butyrate and retinoic acid or hexafluoro-1,25-$(OH)_2D_3$ [29,30], 1,25-$(OH)_2D_3$ and interferon-γ [31], 1,25-$(OH)_2D_3$ and tumor necrosis factor-α [32], retinoic acid and α-tocopherol [33], and 1,25-$(OH)_2D_3$ and vitamin E succinate [34].

The mechanism by which silibinin potentiates 1,25-$(OH)_2D_3$-induced HL-60 cell differentiation is not clear. Previous studies have shown that 1,25-$(OH)_2D_3$ may mediate biological responses including cell differentiation as a consequence of its interaction with nuclear receptor to regulate gene transcription [35] and with a putative cell membrane receptor to generate rapid non-genomic effects [36], including the opening of voltage-gated calcium and chloride channels [37]. The importance of PKC in 1,25-$(OH)_2D_3$ promotion of HL-60 cell differentiation is now generally accepted. 1,25-$(OH)_2D_3$ has been demonstrated to induce an increase in PKC levels [38]. The classical PKC inhibitors H-7 and sphinganine blocked 1,25-$(OH)_2D_3$ promotion of cell differentiation [13]. In addition, involvement of MAPK pathways in the differentiation of HL-60 cells has been studied in several laboratories [26,39,40]. A recent study has shown that monocytic differentiation of HL-60 cells induced by moderate concentrations of 1,25-$(OH)_2D_3$ was accompanied by prolonged activation of the ERK pathway [28]. In our study, inhibitors selective for PKC or ERK MAPK significantly decreased HL-60 cell differentiation induced with silibinin alone or with combinations of silibinin and 1,25-$(OH)_2D_3$ (Figs. 6 and 8). In addition, silibinin enhanced PKC activity and protein levels of PKCα and PKCβ in 1,25-$(OH)_2D_3$-treated HL-60 cells (Fig. 7), suggesting that potentiation of 1,25-$(OH)_2D_3$-induced cell differentiation by silibinin may be, at least in part, via activation of the PKC-ERK signaling pathway. Silymarin, a structural isomer of silibinin, is known to activate MAPK, a downstream element in the PKC signaling pathway [41]. Interestingly, inhibition of p38 MAPK activity by SB203580 increased HL-60 cell differentiation induced with silibinin alone or in combination with 1,25-$(OH)_2D_3$ (Fig. 8). Recent studies have shown that inhibition of p38 MAPK activity up-regulated multiple MAPK pathways and potentiated 1,25-$(OH)_2D_3$-induced differentiation of HL-60 cells [28]. It is likely that p38 MAPK activity is not required for 1,25-$(OH)_2D_3$-induced monocytic differentiation, but that a sustained activation of the c-Jun N-terminal kinase (JNK) and ERK MAPK pathways markedly enhances this form of differentiation. Some studies have reported the involvement of ERK MAPK in 1,25-$(OH)_2D_3$-induced differentiation of HL60 and other cell types [42,43].

Furthermore, HL-60 cells treated with silibinin reacted very strongly with antibody against CD14 monocytic surface marker in a dose-dependent manner (Fig. 3), indicating that silibinin stimulated HL-60 cell differentiation along the monocytic pathway. CD14-positive cells were significantly increased when HL-60 cells were treated with silibinin in combination with 1,25-$(OH)_2D_3$, a well-known differentia-

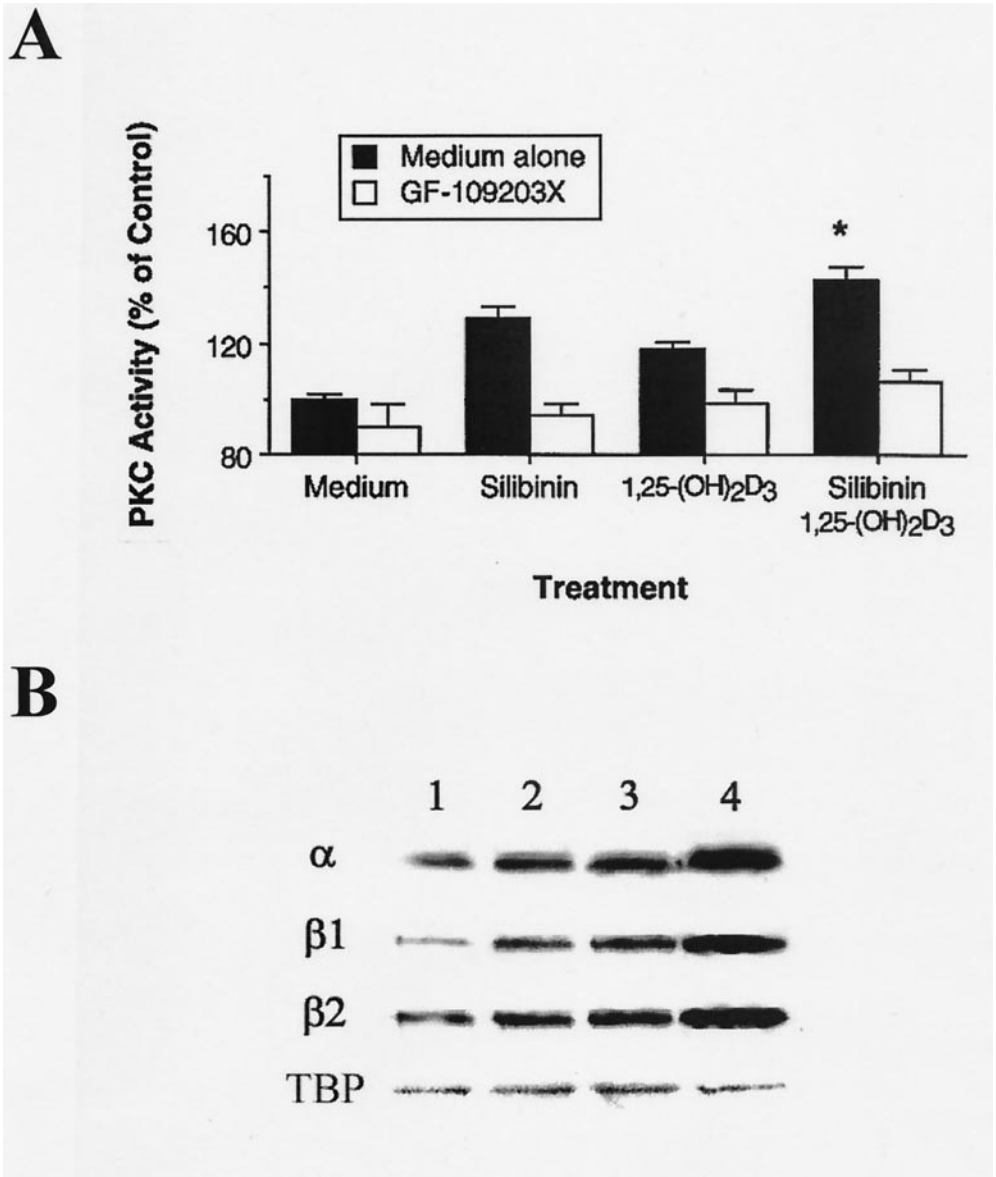

Fig. 7. Effect of silibinin on PKC activity and protein levels of PKC isoforms in 1,25-$(OH)_2D_3$-treated HL-60 cells. (A) HL-60 cells were treated with medium alone, 40 μg/mL of silibinin, or 40 μg/mL of silibinin in combination with 5 nM 1,25-$(OH)_2D_3$ for 2 hr in the absence or presence of GF-109203X, and total PKC activity in the treated cells was determined. PKC activity represents the percentage of PKC activity of each treated group relative to the untreated control group. The values represent the means ± standard deviations of triplicate determinations. The experiment was repeated twice with similar results. *$P < 0.01$, relative to any other groups. (B) HL-60 cells were treated with medium alone (lane 1), 5 nM 1,25-$(OH)_2D_3$ (lane 2), 40 μg/mL of silibinin (lane 3), or 40 μg/mL of silibinin in combination with 5 nM 1,25-$(OH)_2D_3$ (lane 4) for 48 hr, and PKC isoforms and TBP (TATA box-binding protein) as a control were visualized by Western blot analysis.

tion inducer of HL-60 cells into the monocytic lineage (Fig. 5). Morphologic analysis also exhibited a horseshoe-shaped nucleus in silibinin-treated HL-60 cells, which is a sign of monocytic differentiation (Fig. 2D).

1,25-$(OH)_2D_3$ and some of its analogues are also used for the treatment of psoriasis [44]. The results presented here suggest that treatment of patients with combinations of silibinin and 1,25-$(OH)_2D_3$ may provide a greater therapeutic response than 1,25-$(OH)_2D_3$ alone, possibly with less toxicity. It is possible that many dietary chemicals such as curcuminoids, tocopherols, carotenoids, and other substances in fruits, vegetables, and some edible plants can prevent human cancer in part by synergizing with endogenously produced stimulators of differentiation such as 1,25-$(OH)_2D_3$. Epidemiological studies suggest that people who eat large amounts of fruits and green-yellow vegetables containing these compounds have a lower risk of many kinds of cancer [45]. Although many compounds that synergize with 1,25-$(OH)_2D_3$ are antioxidants, antioxidant properties may not be associated with the differentiation-enhancing properties. Curcumin synergized 1,25-$(OH)_2D_3$-induced differentiation in HL-60 cells. In contrast, ferulic acid, which is a commonly ingested dietary substance structurally related to curcumin with similar levels of antioxidant

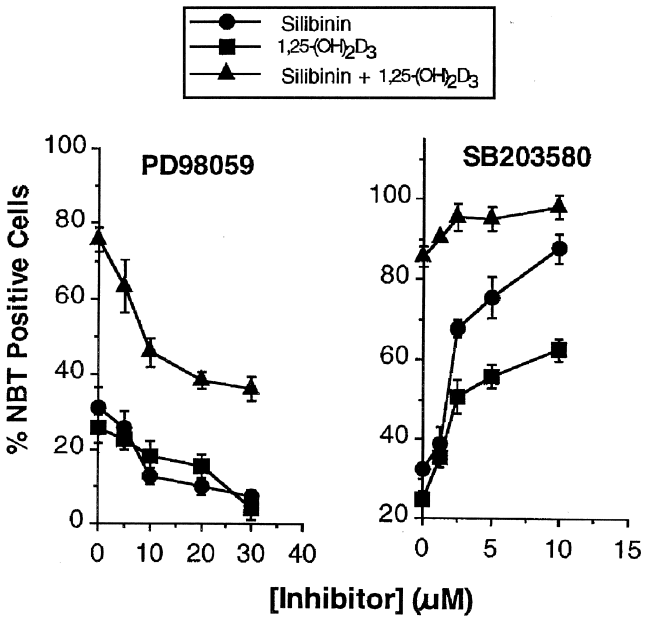

Fig. 8. Effect of ERK and p38 MAPK inhibitors on HL-60 cell differentiation induced by silibinin alone or in combination with 1,25-$(OH)_2D_3$. HL-60 cells were treated for 40 min with varying concentrations of the ERK MAPK inhibitor PD98059 or the p38 MAPK inhibitor SB203580, followed by incubation with 40 μg/mL of silibinin, 5 nM 1,25-$(OH)_2D_3$, or 40 μg/mL of silibinin in combination with 5 nM 1,25-$(OH)_2D_3$ for 72 hr. Data are presented as a percentage of differentiated cells with the mean ± standard deviations of triplicate determinations. The experiment was repeated twice with similar results.

properties, did not enhance 1,25-$(OH)_2D_3$-induced cell differentiation [34].

In conclusion, silibinin induces differentiation of HL-60 leukemia cells predominantly into monocytes and, furthermore, potentiates 1,25-$(OH)_2D_3$-induced cellular differentiation via the PKC signaling pathway. These results may explain some known activities of silibinin, including its anticarcinogenic effects, and strongly suggest a possible use of silibinin in the treatment of neoplastic diseases.

Acknowledgments

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