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Geraniol and β-ionone inhibit proliferation, cell cycle progression, and cyclin-dependent kinase 2 activity in MCF-7 breast cancer cells independent of effects on HMG-CoA reductase activity

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Abstract

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase catalyzes the formation of mevalonate, a precursor of cholesterol that is also required for cell proliferation. Mevalonate depletion results in a G1 phase cell cycle arrest that is mediated in part by impaired activity of cyclin-dependent kinase (CDK) 2, and decreased expression of positive regulators of G1 to S phase progression. Inhibition of mevalonate synthesis may, therefore, be a useful strategy to impair the growth of malignant cells. Plant isoprenoids, including β-ionone and geraniol, have previously been shown to inhibit rodent mammary tumor development, and rodent and avian hepatic HMG-CoA reductase activity. We hypothesized that the putative anti-proliferative and cell cycle inhibitory effects of β-ionone and geraniol on MCF-7 human breast cancer cells in culture are mediated by mevalonate depletion resulting from inhibition of HMG-CoA reductase activity. Flow cytometric analysis showed a G1 arrest in isoprenoid-treated MCF-7 cells, and also a G2/M arrest at higher concentrations of isoprenoids. These compounds minimally affected the growth of MCF-10F normal breast epithelial cells. Both β-ionone and geraniol inhibited CDK 2 activity and dose-dependently decreased the expression of cyclins D1, E, and A, and CDK 2 and 4, without changing the expression of $p21^{cip1}$ or $p27^{kip1}$. Although both β-ionone and geraniol also inhibited MCF-7 proliferation, only geraniol inhibited HMG-CoA reductase activity. While these effects were significantly correlated ($r^2 = 0.89$, $P < 0.01$), they were not causally related, since exogenous mevalonate did not restore growth in geraniol-inhibited cells. These findings indicate that mechanisms other than impaired mevalonate synthesis mediate the anti-proliferative and cell cycle regulatory effects of β-ionone and geraniol in human breast cancer cells.

Keywords: MCF-7 human breast cancer cells; Plant isoprenoids; 3-Hydroxy-3-methylglutaryl coenzyme A reductase; Mevalonate; Cell cycle; Cyclin-dependent kinase 2

1. Introduction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [EC 1.1.1.34], the rate-limiting enzyme in the biosynthesis of cholesterol and other sterols, catalyzes the formation of mevalonate [1]. Mevalonate is also required for other cellular processes including DNA synthesis and proliferation [1], and is the precursor of non-sterol isoprenoids that have a variety of functions such as the isoprenylation of growth-regulatory proteins and oncoproteins [2]. Mevalonate depletion by statins that are competitive inhibitors of HMG-CoA reductase, results in a characteristic G1 phase cell cycle arrest [3]. Conversely, increased levels of mevalonate promote cell growth [4,5]. Indeed, increased HMG-CoA reductase activity is characteristic of a number of tumor types [6–10] and inhibition

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; FBS, fetal bovine serum; CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate buffered saline; SDS, sodium dodecyl sulphate

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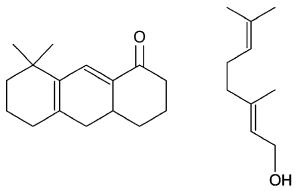

β-ionone geraniol

Fig. 1. Chemical structure of β-ionone and geraniol.

of mevalonate synthesis is an important focus for the development of new agents for cancer therapy as well as chemoprevention [11].

The plant isoprenoids are a group of compounds that have been suggested to inhibit cancer growth and development through inhibition of HMG-CoA reductase activity [12–16]. This group includes over 20,000 different compounds derived from plant mevalonate metabolism [14]. A number of these compounds are known to inhibit hepatic HMG-CoA reductase activity in rodent and avian models [14]. Although their effects on mammary HMG-CoA reductase activity have not been characterized, several isoprenoids have been shown to inhibit experimental mammary carcinogenesis [14,17–19]. Elson and colleagues have reported that the potency with which a plant isoprenoid suppresses hepatic reductase is predictive of its ability to inhibit the development of mammary tumors in rats [14]. These compounds also inhibit the growth of breast and other cancer cells in culture [16,20–24]. γ-Tocotrienol, a molecule with an isoprenoid side-chain, has been shown to inhibit HMG-CoA reductase activity in cultured human hepatoma cells [25]. However, the effect of plant isoprenoids on HMG-CoA reductase activity in normal or malignant extra-hepatic cells, including cells of the breast, is unknown. We hypothesized that these compounds inhibit the growth of breast cancer cells by down-regulating HMG-CoA reductase activity, resulting in mevalonate depletion that subsequently impairs G1 phase progression. In support of this notion, cancer cells treated with isoprenoids in culture have been shown to undergo a G1 arrest with associated changes in cell cycle regulatory proteins [20,21,26] that is similar to effects observed in statin-treated cells [27–32]. Additionally, mevalonate has been shown to reverse the inhibitory effects of some plant isoprenoids on cell proliferation [24,33], although not all studies have found this effect [34,35].

Here, we describe the effects of the acyclic isoprenoid geraniol, and the cyclic isoprenoid β-ionone (Fig. 1), on HMG-CoA reductase activity in MCF-7 human breast cancer cells. Geraniol and β-ionone have both been shown to inhibit hepatic HMG-CoA reductase activity [14] and rodent mammary tumor development [14,22]. We also determined the effects of β-ionone and geraniol on proliferation, cell cycle distribution, and levels and activity of protein regulators of the G1 to S phase transition. To test whether decreased HMG-CoA reductase activity mediated the growth-inhibitory effects of the isoprenoids we assessed the ability of exogenous mevalonate to restore proliferation.

2. Materials and methods

2.1. Materials

Plant isoprenoids, protein agarose A, antibiotics for cell culture, and all chemicals used in staining, flow cytometry, buffers, and thin-layer chromatography were purchased from Sigma. Fetal bovine serum (FBS) was from Gibco BRL. Culture medium was prepared by the University of Toronto tissue culture facility. Histone H1, and antibodies against cyclins D1/D2, E, and A, cyclin-dependent kinase (CDK) 2 and 4, and the CDK inhibitor (CDKI) $p27^{kip1}$ were from Upstate Biotechnology Inc., and horseradish peroxidase-conjugated secondary antibodies and the antibody to β-actin were from Santa Cruz Biotechnology Inc. The enhanced chemiluminescence kit was from Amersham Life Sciences. [^{14}C]HMG-CoA reductase and [γ-^{32}P]ATP were from Perkin-Elmer Life Sciences Inc. Silica gel G plates for chromatography were obtained from Analtech Inc. and were autoradiographed in an Instant Imager® (Packard, Canberra, Canada). Roche Molecular Biochemicals supplied the cell proliferation enzyme-linked immunosorbent assay (ELISA) kit. Flow cytometric analysis was performed using the FACStation Software package from Beckton Dickinson FACS Systems.

2.2. Cell culture

Cells were purchased from the American Type Cell Culture collection and routinely cultured in 150 dL flasks at 37 °C and 5% CO_2 in 1:1 DME/F12 with 1% penicillin/streptomycin. MCF-7 cells were supplemented with 10% FBS. MCF-10F cells were supplemented with 5% horse serum, 0.1 μg/mL cholera toxin, 10 μg/mL insulin, 0.5 μg/mL hydrocortisone, and 20 ng/mL epidermal growth factor. Geraniol and β-ionone were dissolved in absolute ethanol and stored at −20 °C in the dark before use. Final ethanol concentration in the tissue culture media was 0.1%.

2.3. Crystal violet staining

In order to assess relative cell numbers, cells grown in 12-well plates in triplicate and treated with increasing concentrations of test isoprenoid for 2, 4, 7, and 10 days were washed with phosphate buffered saline (PBS), fixed with methanol for 20 min, stained with crystal violet [36] for an additional 20 min, washed with water, and air dried. Dye was eluted from cells at room temperature by the addition of 1 mL of 1% sodium

dodecyl sulphate (SDS), and absorbances were measured at 595 nm. In experiments with mevalonate, cells were grown in six-well plates and treated with geraniol or mevastatin and mevalonate concurrently for 1 week prior to staining.

2.4. Flow cytometry

Cells grown in 150 dL flasks were harvested by trypsinization, washed repeatedly with ice cold PBS (minus Ca/Mg) and then treated with 70% ethanol for 30 min to permeabilize membranes. Nuclei were stained with propidium iodide in a 0.38 mM sodium citrate buffer containing 100 μL RNAse. DNA content was determined using a FACStar Plus flow cytometer and the proportion of cells in each cell cycle phase was calculated from DNA histograms using the FACStation Software package.

2.5. Immunoblotting

Cells harvested by trypsinization were washed repeatedly with ice cold PBS (minus Ca/Mg), and lysed using a glass mini-homogenizer in buffer A (5 mM Tris–HCl (pH 7.5) containing 5 mM EDTA, 10 mM EGTA, 50 mM NaF, 150 mM NaCl, 0.1% Nonidet P-40, 50 μg/mL PMSF, 1 μg/mL aprotinin, 2 μg/mL leupeptin). Homogenates were then centrifuged for 10 min at 12,000 × *g*, and aliquots of supernatant containing 2–40 μg protein were mixed with 2× Laemmli buffer, heated for 5 min at 95 °C, electrophoresed in 10 or 12% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes. After blocking overnight, membranes were probed first with antibodies to cyclins D1/D2, E and A, CDK 2 and 4, and $p27^{kip1}$ or $p21^{cip1}$ then with horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies, and signals were detected by enhanced chemiluminescence. Band densities were quantified in arbitrary units, then expressed as relative density compared with the untreated controls that were taken as 100%. β-Actin was used as a loading control.

2.6. CDK 2 activity assay

Cells were trypsinized, washed repeatedly with PBS and then lysed using a glass mini-homogenizer in buffer A. After centrifugation of homogenates for 10 min at 12,000 × *g*, 200 μL of the supernantants were diluted in buffer A to 1 mg/mL. Samples were pre-cleared by gentle rocking for 1 h at 4 °C with 30 μL of protein agarose A beads followed by immunoprecipitation with antibody to CDK 2 (2 μg) overnight. Immunocomplexes were captured by rocking with 100 μL of protein agarose A beads at 4 °C for 2 h, washed three times with buffer A, then four times with kinase reaction buffer (50 mM Tris–HCl (pH 7.5) containing 10 mM $MgCl_2$, 2 mM EGTA, 1 mM DTT, 1 mM NaF, 0.1 mM $NaVO_4$, 10 mM β-glycerophosphate). Immunoprecipitates were incubated for 30 min at 37 °C with 30 μL of kinase reaction buffer containing 25 μM cold ATP, 5 μg histone H1, and 10 μCi of [γ-^{32}P]ATP. Reactions were stopped by the addition of 30 μl of Laemmli buffer with 2-mercaptoethanol followed immediately by boiling for 5 min. The products of the reaction were then electrophoresed on a 13% SDS-polyacrylamide gel. Radioactive bands corresponding to histone H1 were visualized and quantified using a Packard Instant Imager®. Coomassie blue staining of protein bands was used to ensure equal loading in gels.

2.7. Cell proliferation

Cells seeded in 96-well plates at an average density of 2×10^3 cells per well were treated with geraniol or β-ionone for 7 days. Proliferation rates on day 7 were measured according to manufacturer's instructions by quantifying bromodeoxyuridine incorporation into DNA of actively proliferating cells using a cell proliferation ELISA kit.

2.8. HMG-CoA reductase assay

Cells were harvested by trypsinization, washed repeatedly with PBS, and then homogenized in 20 mM Tris–HCl (pH 7.2) containing 0.25 M sucrose, 70 mM KCl, 5 mM EDTA, 5 mM EGTA, 50 μM leupeptin, and 1 mM DTT. HMG-CoA reductase activity in samples of supernatant prepared by centrifugation of homogenates at 5000 × *g* for 1 min was determined as described previously [37]. Briefly, 100 μg samples of total protein were pre-incubated at 37 °C for 5 min in 100 mM phosphate (pH 7.4) containing 70 mM KCl, 10 mM dithiothreitol, 5 mM EDTA, 5 mM EGTA, and 50 μM leupeptin. After a further 5 min incubation with an NADPH regenerating system (1 U glucose-6-phosphate dehydrogenase, 20 mM glucose-6-phosphate, 2 mM NADP), HMG-CoA containing 8.12 nCi/nmol [^{14}C]HMG-CoA was added to yield a final concentration of 55.4 μM and assay mixtures (75 μL final volume) were incubated at 37 °C for 30 min. The assay was terminated by addition of 5 μL of 10N HCL containing 16 mM mevalonolactone as a carrier for thin-layer chromatography. Samples were incubated for 1 h to allow complete lactonization of product, and then centrifuged for 1 min at 3000 × *g* to remove denatured protein. Forty microliters of supernatant was applied to a silica gel G plate and chromatographed in toluene/acetone (1:1). Plates were then autoradiographed in a Packard Instant Imager® for 24–48 h, after which bands corresponding to mevalonolactone (R_f, 0.7) were visualized and quantified in counts per minute. Enzyme activity in samples treated with plant isoprenoids was normalized to the mean activity of the control samples in each individual experimental set. Relative enzyme activity was then expressed as a percentage of control values.

2.9. Statistical analysis

All values shown are means ± S.E.M. Differences between treated groups and controls were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Differences between cells grown with or without mevalonate were analyzed by Student's *t*-test. Linear regression analysis was used to determine correlations between HMG-CoA reductase activity and cell proliferation rates. Significance was accepted at $P < 0.05$.

3. Results

3.1. Time- and concentration-dependent effects of β-ionone and geraniol on cell growth

β-Ionone and geraniol inhibited the growth of MCF-7 human breast cancer cells in a concentration- and time-dependent manner (Fig. 2A and B) resulting in significant effects by day 7. For all other experiments, therefore, cells were treated for 7 days prior to harvesting or assay. MCF-10F normal human breast epithelial cells were not significantly inhibited by treatment with geraniol for 10 days at any concentration tested (data not shown). MCF-10F cell growth, however, was significantly inhibited after 10 days by ~24% by 300 μM β-ionone, ~33% by 400 μM β-ionone, and by a maximum of 38% after treatment with 500 μM β-ionone (data not shown). These effects were considerably less than the inhibition seen in MCF-7 cells treated under the same conditions, in which 500 μM β-ionone inhibited cell growth by ~80%.

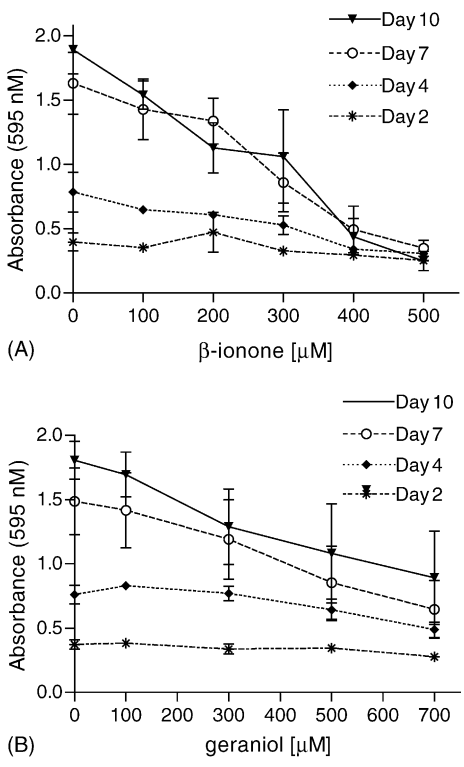

Fig. 2. Plant isoprenoids inhibit the growth of human breast adenocarcinoma cells in a concentration- and time-dependent manner, and with a greater potency than normal breast epithelial cells. MCF-7 cells were grown in 12-well plates in the presence of increasing concentrations of β-ionone (A) or geraniol (B) for the times indicated. Medium was replaced every 48 h, and growth was assessed by crystal violet staining of fixed cells. Data points are means ± S.E.M.

Table 1
Cell cycle distribution of MCF-7 cells following treatment with β-ionone or geraniol[a]

Treatment (μM)	Percentage of cells in			
	G0/G1	S	G2/M	Apoptosis
Control	53.7 ± 1.8	30.4 ± 2.5	16.0 ± 2.7	3.5 ± 1.3
β-Ionone				
100	64.5 ±1.7**	19.1 ± 2.0*	16.4 ± 0.9	8.2 ± 4.6
300	58.5 ± 0.3	14.9 ± 3.8**	26.6 ± 3.5*	0.4 ± 0.1
500	58.7 ± 2.6	17.2 ± 0.8*	24.1 ± 1.9	4.9 ± 0.6
Geraniol				
100	63.0 ± 0.7*	23.2 ± 4.2	13.8 ± 3.5	3.5 ± 1.5
300	64.2 ± 2.2*	21.9 ± 2.6	13.9 ± 0.4	1.5 ± 0.7
500	62.6 ± 3.2*	18.3 ± 2.5*	19.1 ± 1.1	2.1 ± 1.5
700	56.4 ± 0.7	17.8 ± 0.2*	25.8 ± 0.9*	0.5 ± 0.2

[a] MCF-7 cells were grown in the absence (control) or presence of test compounds at the concentrations indicated. After 1 week, cells were harvested by trypsinization, stained with propidium iodide, and analyzed for DNA content by flow cytometry. Values are means ± S.E.M. ($N = 3$).
* $P < 0.05$ vs. control value for same cell cycle phase.
** $P < 0.01$ vs. control value for same cell cycle phase.

3.2. Effects of β-ionone and geraniol on cell cycle regulation

Flow cytometric analysis showed that growth inhibition of MCF-7 cells treated with 100 μM β-ionone, or 100, 300, or 500 μM geraniol, was mediated primarily by a G0/G1 arrest as evidenced by an increased proportion of cells in G0/G1 (Table 1). At higher concentrations, however, inhibition of cell growth was also mediated by a slowing of G2/M progression. The net result was a significant decrease in the percentage of cells actively synthesizing DNA at all concentrations of β-ionone tested, and at 500 and 700 μM geraniol. There were no significant differences in the percentage of apoptotic cells detected by flow cytometry in any of the groups compared to the control cells.

3.3. Effects of β-ionone and geraniol on expression of G1 and S phase protein regulators

The changes in cell cycle control were associated with alterations in the activity and expression of protein regulators of G1 and S phase progression (Fig. 3). A significant, concentration-dependent decrease in expression of the early G1 phase cyclin D1 was observed in MCF-7 cells treated with either β-ionone or geraniol. Cyclin D2 expression was less sensitive to treatment, decreasing only in cells treated with 700 μM geraniol. Expression of CDK

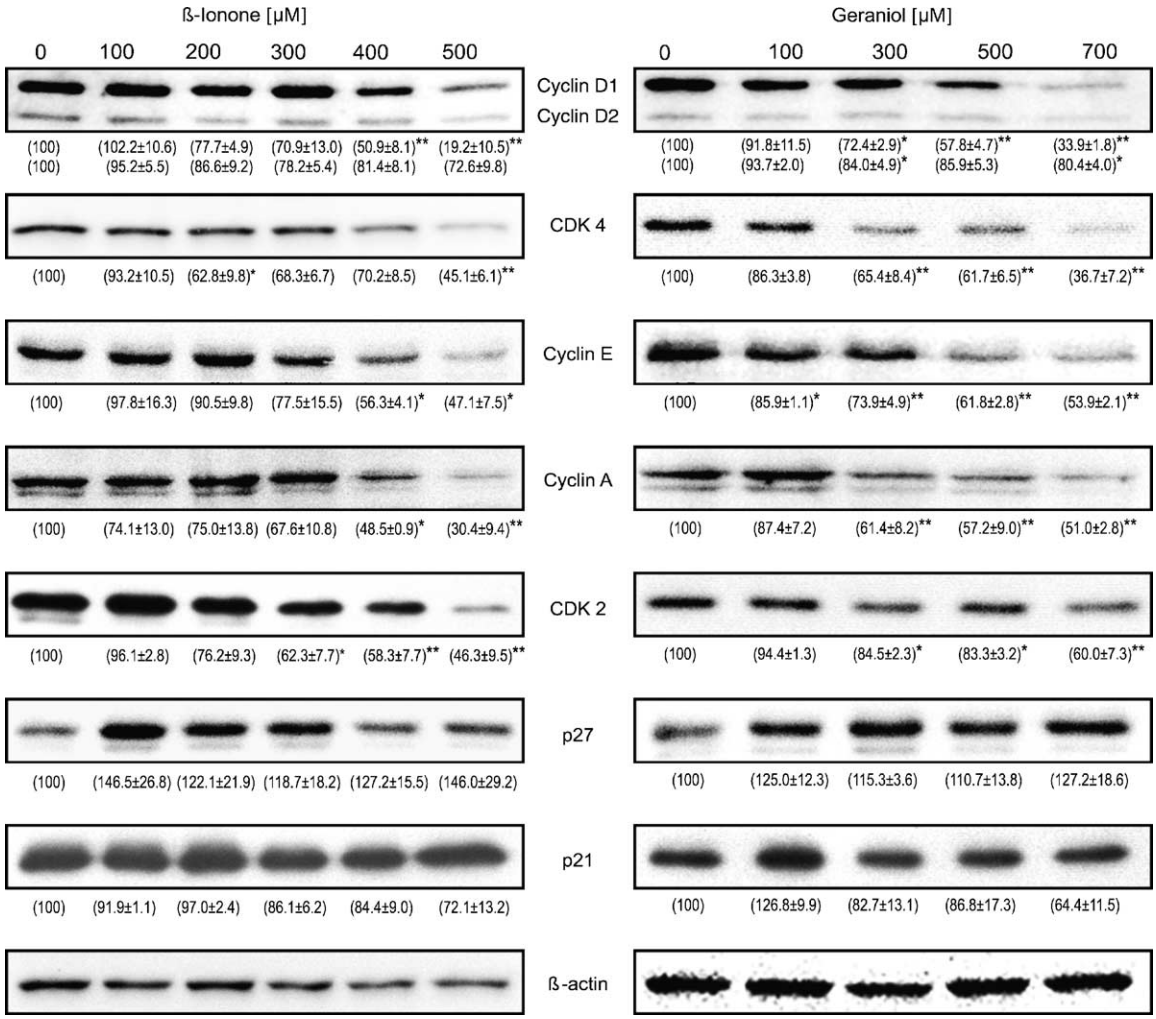

Fig. 3. Plant isoprenoids inhibit the expression of G1 and S phase regulatory proteins. Extracts from MCF-7 cells grown in the presence of either β-ionone or geraniol for 7 days were subjected to Western immunoblot analysis. Results from a representative experiment are shown. Protein expression levels in treated cells were measured in arbitrary densitometric units and then expressed as a percentage of the untreated control group. Numbers in parentheses are means ± S.E.M. calculated from relative protein expression levels determined in three separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. protein expression level in untreated control cells.

4, the catalytic binding partner of the D-type cyclins, also decreased significantly in cells treated with either isoprenoid. Expression levels of late G1 regulators were similarly reduced. Protein levels of cyclin E, the G1 phase activator of CDK 2, decreased significantly in cells treated with geraniol at all concentrations, and in cells treated with 400 or 500 μM β-ionone. Expression of cyclin A, the S phase CDK 2 activator, decreased significantly following treatment of cells with either geraniol or with β-ionone. CDK 2 also decreased in cells treated with either compound. Expression of the CDKI $p27^{kip1}$ appeared to increase in cells treated either with geraniol or β-ionone. However, this change was not statistically significant when analyzed by ANOVA or for linear trend. Expression of $p21^{cip1}$ also did not change significantly with increasing concentrations of either geraniol or β-ionone. There was, however, a significant linear trend towards decreased expression levels of $p21^{cip1}$ ($P < 0.05$) in cells treated with either isoprenoid.

3.4. Effect of β-ionone and geraniol on CDK 2 activity

Since inhibition of CDK 2 activity is an important mediator of the cellular growth arrest that follows mevalonate depletion in cells treated with competitive inhibitors of HMG-CoA reductase [27,31,32], we determined the effect of β-ionone and geraniol on its activity. Treatment of cells with either of the isoprenoids caused a concentration-dependent reduction in the catalytic activity of CDK 2, as measured by its ability to phosphorylate histone H1 (Fig. 4).

3.5. Effects of β-ionone and geraniol on proliferation and HMG-CoA reductase activity

Treatment of MCF-7 cells with 100–500 μM β-ionone for 7 days significantly inhibited cell proliferation but, at this time point, had no significant effect on HMG-CoA reductase activity at any of the concentrations (Fig. 5A).

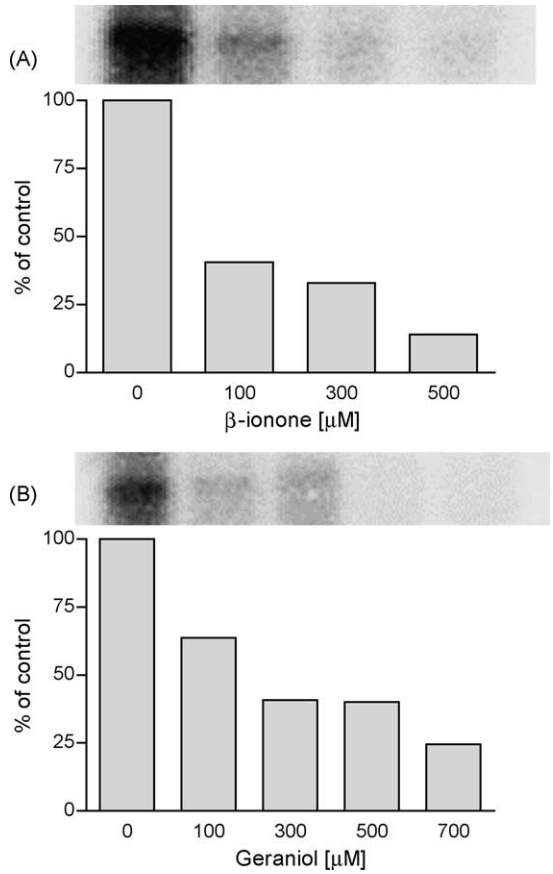

Fig. 4. CDK 2 activity is inhibited by both β-ionone and geraniol. Immunoprecipitates of CDK 2 from MCF-7 cells treated for 7 days with increasing concentrations of either β-ionone (A) or geraniol (B) were assayed for ability to phosphorylate histone H1. Graphs show the mean activity of CDK 2 in isoprenoid-treated cells grown and assayed in two separate experiments. Results from a representative autoradiograph are shown.

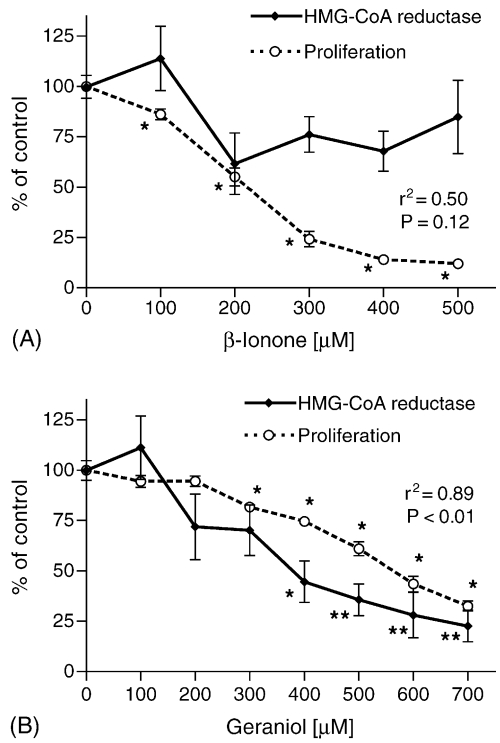

Fig. 5. HMG-CoA reductase activity and cell proliferation are coordinately inhibited in MCF-7 cells treated with geraniol but not β-ionone. Cells were grown for 7 days in β-ionone (A) or geraniol (B) at the concentrations indicated. HMG-CoA reductase activity was determined by radiochemical assay of whole cell extracts. Data points are means ± S.E.M. calculated from values obtained in at least three independent experiments. Cell proliferation was assessed by ELISA of bromodeoxyuridine incorporation into DNA of replicating cells. Data points are means ± S.E.M. ($N = 8$). $^{*}P < 0.01$, $^{**}P < 0.001$ vs. control values. Linear regression analysis was used to generate correlation coefficients for the relationship between HMG-CoA reductase activity and cell proliferation.

Geraniol, however, inhibited both HMG-CoA reductase activity and cell proliferation in a similar, concentration-dependent manner, and these effects were significantly correlated (Fig. 5B). To determine whether effects of the isoprenoids on cell proliferation and HMG-CoA reductase were causally related, we assessed the ability of exogenous mevalonate to restore cell growth. The addition of mevalonate to the culture medium did not affect cell growth at any concentration of geraniol tested up to 600 μM (Fig. 6). As a positive control, we observed that exogenous mevalonate significantly restored cell growth in mevastatin-treated cells.

4. Discussion

β-Ionone and geraniol caused a cell cycle arrest at G0/G1 and at higher concentrations, a slowing of passage through G2/M that resulted in significant growth inhibition of MCF-7 human breast cancer cells. Concentrations utilized were comparable to those reported in blood for similar isoprenoids in human pharmacokinetic studies [38,39]. Geraniol had no effect on the growth of MCF-10F normal breast epithelial cells, and β-ionone was only weakly inhibitory at high concentrations. No significant effect of isoprenoid treatment on apoptosis in MCF-7 cells was detected. Analysis of immunoblots indicated that

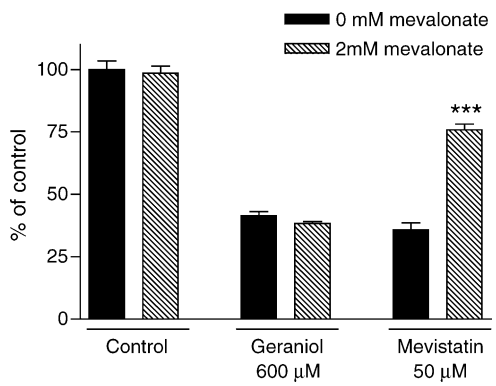

Fig. 6. Mevalonate does not restore cell proliferation in human mammary adenocarcinoma cells inhibited by geraniol. MCF-7 cells were seeded in six-well plates and grown for 1 week in medium containing the treatments indicated, either with or without the addition of 2 mM mevalonate. Cell growth was assessed by crystal violet staining. Mevastatin-treated cells were included as positive controls. Values are means ± S.E.M. $^{***}P < 0.0001$ vs. cells grown without mevalonate.

inhibition of early G1 phase progression by the isoprenoids may have been mediated by decreased expression of CDK 4 and its activating subunits cyclins D1 and D2. Decreased passage of cells from late G1 into and through S phase was likely mediated by reduced activity of CDK 2. This resulted, in part, from reduced expression of the kinase, as well as reduced expression of its activating subunits cyclins E and A, at least in cells treated with higher concentrations of isoprenoids. At concentrations as low as 100 μM, however, impaired G1 phase progression and decreased CDK 2 activity cannot be explained by decreased kinase or cyclin expression. It is possible that at lower concentrations, these effects are mediated primarily by changes in binding of the kinase with CDKIs. Indeed, Rao et al. have shown that mevalonate depletion in MCF-7 cells by lovastatin significantly inhibited CDK 2 activity without affecting its expression, or the expression of $p21^{cip1}$ or $p27^{kip1}$ [10]. Instead, mevalonate depletion caused an increase in the binding of these CDKIs to CDK 2. A similar mechanism may explain our observation of a significant decrease in CDK 2 activity at concentrations of isoprenoids that produced no change in CDK 2, $p21^{cip1}$ or $p27^{kip1}$ expression. CDK 2 activity is also regulated by an activating phosphorylation on threonine 160/161, and by a deactivating phosphorylation on tyrosine 15 [40]. Changes in the activity of kinase and phosphatase regulators of these phosphorylations may, therefore, also have contributed to the decrease in CDK 2 activity evident at all levels of isoprenoid treatment, without altering expression of CDK 2.

Similar to results from the present study, mevalonate depletion by statins has been shown previously to inhibit proliferation by imposing a cell cycle block at G0/G1 [3] that is mediated by reduced activity of CDK 2 [27,31,32], decreased expression of CDK 2 and 4, and cyclins D, E, and A [28–30], and by an impaired ability of cells to down-regulate expression of $p27^{kip1}$ [28]. Therefore, we investigated whether depletion of mevalonate by inhibition of HMG-CoA reductase activity mediated the anti-proliferative effects of β-ionone and geraniol in MCF-7 breast cancer cells.

In malignant cells, the level and catalytic efficiency of HMG-CoA reductase is increased compared to normal cells [6–10], and is resistant to transcriptional feedback regulation by sterols [41]. Post-transcriptional regulation of reductase synthesis is, however, intact [6,15]. Plant isoprenoids have been shown to inhibit HMG-CoA reductase activity through post-transcriptional events including increased proteolytic degradation and decreased efficiency of translation of reductase mRNA [25,42]. HMG-CoA reductase in malignant cells, therefore, appears to retain sensitivity to regulation by these compounds [25]. It has been suggested that plant isoprenoids may inhibit tumor cell proliferation by inhibiting HMG-CoA reductase activity, thereby depleting cellular mevalonate required for growth [12–16]. Our initial results showed a close correlation between effects of geraniol on MCF-7 cell proliferation and HMG-CoA reductase activity, suggesting that this relationship may, indeed, be causal. Exogenous mevalonate, however, was unable to reverse the growth inhibition. This result indicates that depletion of mevalonate by inhibition of HMG-CoA reductase activity is unlikely to play any role in the inhibition of MCF-7 cell proliferation by geraniol. β-Ionone had no significant effect on HMG-CoA reductase activity in MCF-7 cells, and clearly does not inhibit growth via this pathway. Based on these findings we can also conclude that a mechanism other than impaired HMG-CoA reductase activity is responsible for the cell cycle regulatory effects of β-ionone and geraniol.

Inhibition of protein prenylation may be an alternative mechanism to explain the growth and cell cycle inhibitory effects of β-ionone and geraniol on MCF-7 cells. A G0/G1 arrest with impaired CDK 2 activity, as was observed in the present study, is known to occur when geranylgeranyl protein transferase (GGPTase) I activity is blocked [28,43]. Likewise, inhibition of farnesyl protein transferase (FPTase) causes a G2/M arrest [43] such as we observed when MCF-7 cells were treated with higher concentrations of β-ionone or geraniol. The plant isoprenoids perillic acid and perillyl alcohol have previously been shown to competitively inhibit GGPTase I and II, and FPTase in several different cell lines [34,44–46]. Whether inhibition of protein prenylation by β-ionone and/or geraniol mediated the growth- and cell cycle-inhibitory effects that we observed in the present study merits further investigation. It is also possible that growth inhibition by β-ionone and geraniol is independent of any effects on the mevalonate pathway. The plant isoprenoids limonene and perillyl alcohol have been shown to inhibit expression of the growth-regulatory protein Ras without affecting its activation by farnesylation [47]. Plant isoprenoids have also been shown to inhibit cancer cell growth by inducing apoptosis [23] and differentiation [48].

In summary, we have shown that the plant isoprenoid geraniol inhibits HMG-CoA reductase activity in MCF-7 cells, and that this effect is closely correlated with inhibition of cell proliferation. Exogenous mevalonate, however, does not restore proliferation in geraniol-inhibited cells, indicating that this relationship is not causal. Unexpectedly, β-ionone does not inhibit HMG-CoA reductase activity, although it does inhibit cell proliferation in a dose-dependent manner. Although mevalonate depletion is not responsible for cellular growth inhibition by β-ionone and geraniol, both compounds produce cell cycle regulatory effects that are, nonetheless, similar to those previously reported in mevalonate-depleted cells. These findings indicate that understanding the growth and cell cycle inhibitory effects of plant isoprenoids will require investigation of mevalonate-independent mechanisms.

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