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Inhibitory effect of 1-O (2 methoxy) hexadecyl glycerol and phenylbutyrate on the malignant properties of human prostate cancer cells

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Abstract

The ability of the naturally occurring ether lipid, 1-O (2 methoxy) hexadecyl glycerol (MHG), and phenylbutyrate (BP) to inhibit cellular proliferation, anchorage-independent growth and cellular invasion in the human prostate cancer LnCap and DU145 cells was determined. Both MHG and PB inhibited the malignant properties of these prostate cancer cells. The concentrations required to achieve similar inhibitory effect, however, were significantly different for these two agents. MHG inhibited cell growth with equal potency in these cell lines with an IC-50 value of 93 μM for LnCap, and 97 μM for DU145. The IC-50 values for PB were 1.3 mM and 7.3 mM, respectively, for LnCap and DU145 cells. Both MHG and PB (IC-50 concentrations) inhibited the anchorage-independent growth and cellular invasion in these cells. Over 50% inhibition of anchorage-independent growth was achieved for both LnCap and DU145 cells by PB, while a lesser degree of inhibition was achieved with MHG. Both MHG- and PB-treated cells showed a reduced propensity to invade matrigels. Invasion of PB-treated LnCap and DU145 cells was reduced, respectively, by approximate 41 and 30% when compared to untreated control cells, while invasion of MHG-treated LnCap and DU145 cells was reduced to a lesser extent. Because differentiation-inducing agents may possess chemopreventive properties, the use of naturally occurring MHG and nontoxic PB in the chemoprevention of malignant diseases warrants further investigation.

Introduction

Lipids are integral cellular components of living organisms and play important roles in cellular physiology [1]. The function of a lipid is determined by its chemical structure [1]. The inositol phospholipids participate in signal transduction [2–5], while ether phospholipids are multifunctional and play important physiologic roles in platelet aggregation [6], inflammatory and immune responses [7–11], cellular proliferation [12–14] and cell migration [15]. Other ether lipids of different molecular structures have been shown to be cytotoxic towards cancer cells [16–18].

Alkylglycerols are naturally occurring ether lipids [7, 19–21]. Alkylglycerols have been reported to possess antitumor activities in human and the administration of alkylglycerols has been shown to decrease the toxic side-effects of radiation therapy [22–24]. About 20 different types of alkylglycerols exist, and the three most common species are the chimyl-, batyl- and selachyl- alcohols [21, 25]. Methoxy-substituted alkylglycerols constitute about 2% of alkylglycerols [25]. We have previously reported that a methoxy substituted alkylglycerol – 1-O (2 methoxy) hexadecyl glycerol (MHG) can promote a benign and differentiated phenotype in a variety of human colon cancer cancer cells, including some highly malignant cell lines [26]. The biologic effect of MHG on prostate cancer cells, however, is not known.

Phenylbutyrate (PB), an aromatic short-chain fatty acid, is a differentiation-inducing agent. PB modulates hemoglobulin synthesis [27] and induces differentiation in leukemia [28] and solid tumor cell lines [29–30]. In human, serum levels of PB up to 2 mM can be achieved without limiting clinical toxicity [31–32] and PB has been used to treat thalassemia [33], Cooley's anemia [34] and leukemia [35].

Because differentiation-inducing agents can promote or restore a more normal or benign phenotype to malignant cells [36–43], they may possess chemopreventive properties, i.e., preventing or delaying the development of cancer [44]. Because chemoprevention of cancer is likely to be a long-term process, the use of naturally occuring and nontoxic differentiation-inducing agents in chemoprevention is particularly attractive.

In this study, we evaluated the effect of MHG and PB on the malignant properties of the human prostate cancer cell lines LnCap and DU145. Both agents inhibited cellular proliferation and the propensity of these cells to grow in soft agarose (anchorage-independent growth) and to invade a matrigel matrix. The inhibitory effect of MHG was acheived with μM concentrations while similar inhibitory effect of

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PB was achevied with mM concentrations. It is concluded that both MHG and PB inhibited the malignant behavior of prostate cancer cells and that the concentrations required to achieve similar inhibitory effect were significantly different for these two agents.

Materials and methods

Materials and cell culture

MHG and PB were obtained from Triple Crown America Inc. (Perkasie, Pennsylvania). MHG was dissolved in a minimal volume of dimethyl sulfoxide and then diluted to a stock solution of 1 mg/ml in serum-free RPMI 1640 culture medium. PB was directly dissolved in the same medium. 3-(4,5-dimethyl-thiazol-2-1)-2,5-diphenyltetrazolium bromide (MTT) and trypan blue dye were purchased from Sigma (St. Louis, Missouri). Low melting agarose was purchased from Gibco/BRL (Gaithersburg, Maryland). Matrigel invasion chambers were purchased from Becton Dickinson (Franklin Lakes, New Jersey). Human prostate cancer cell lines LnCap and DU 145 were purchased from the American Type Culture Collection (ATCC, Rockville, Maryland) and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum.

Growth inhibition in monolayer culture

Cells were seeded into 96-well culture plates (20,000 cells/well) and allowed to attach overnight. The medium was then replenished with fresh medium containing various concentrations of MHG or PB. Six replicate determinations for each concentration were performed in each experiment. Cells cultured in medium without MHG or PB served as controls. The cells were then incubated for three days in a humidified CO_2 incubator and the number of viable cells in each well was estimated by the MTT assay as previously described [45]. Briefly, 40 μl of MTT (2 mg/ml) solution was added to each well of the culture plates and the plates were then incubated for 3 h at 37 °C to allow the formation of tetrazolium crystals. The solution in each well was then carefully removed with a 25 G needle attached to a vacuum source and the crystals dissolved in 200 μl of DMSO. The optical density of the solution in the culture wells was then determined by a microplate reader at 570 nm. The percent inhibition of growth exerted by MHG or PB was determined by the following formula:

$$\frac{\text{OD untreated cells} - \text{OD treated cells}}{\text{OD untreated cells}} \times 100$$

Anchorage-independent growth and invasion of matrigel

Cells maintained in 25 cm^2 flasks (in the log-phase of growth) were used in these experiments. The culture medium of these cells was replenished with fresh medium (control cells) or with medium containing MHG or PB. The cells were then incubated for three days at 37 °C and were then used in the anchorage-independent growth and invasion assays as previously described [26].

Anchorage-Independent Growth: healthy, viable untreated control or treated cells (5,000 cells/dish) were seeded into 0.35% soft agarose cloning medium (RPMI 1640 medium supplemented with 10% fetal calf serum) in 60 × 15 mm Petri tissue culture dishes. After 10 days of incubation at 37° in a humidified CO_2 incubator, the colonies were stained with p-iodonitrotetrazolium violet and counted manually with the aid of a magnifying glass.

Invasion of Matrigel-coated porous membranes: healthy viable control and treated cells were collected, washed with serum-free medium and then diluted to a concentration of 250,000 cells/ml in the same. A 200 μl volume (50,000 cells) was seeded into each rehydrated invasion chamber immersed in chemoattractant (NIH3T3 conditioned medium). After 4 days of incubation at 37 °C, the bottoms of the chambers were scraped to collect cells that had invaded through the membranes and pooled with cells that had invaded and fallen through into the bottom chambers. The total number of invaded cells was then determined by counting with the aid of a hemocytometer. The effect of each treatment with its corresponding control was performed in triplicate chambers. The results presented are an average and standard error of three independent experiments.

Table 1. Inhibitory effect of MHG and PB on cellular proliferation.

Cell lines	MHG	PB
	IC-50[a]	
LnCap	93 μM	1.3 mM
DU145	97 μM	7.3 mM

[a]Concentrations required to achieve 50% inhibition of growth.
The IC-50 values were estimated from growth curves generated from the MTT assays as described in 'Materials and methods'.

Results

Effect of MHG and PB on cellular proliferation

Both cell lines responded to the antiproliferative effect of MHG in a similar manner. MHG inhibited cell growth with equal potency in these cell lines with an IC-50 value of 93 μM for LnCap, and 97 μM for DU145 (Table 1). The concentrations of PB needed to achieve equivalent level of inhibition were found to be much higher. The IC-50 values for PB were 1.3 mM and 7.3 mM, respectively, for LnCap and DU145 cells. Thus, compared to the DU145 cells, the LnCap cells were at least five times more sensitive to the antiproliferative effect of PB. This difference in sensitivity to the antiproliferative effect of PB may reflect on the inherent differences in the biological phenotype of these cells lines.

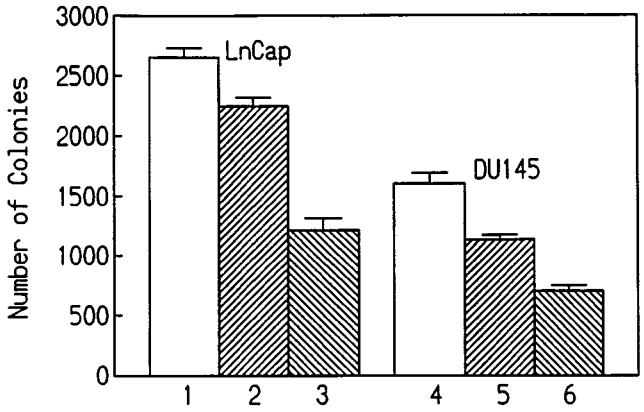

Figure 1. Effect of MHG and PB on anchorage-independent growth. Healthy, viable control and treated cells (treated with IC-50 concentrations of MHG or PB) were seeded into 0.35% soft agarose cloning medium and cultured for 10 days as described in 'Materials and methods'. The colonies were stained with p-iodonitrotetrazolium violet and counted manually with the aid of a magnifying glass. Each value represents the mean and standard error of three separate experiments. Lanes 1 to 3, untreated, MHG- and PB-treated LnCap cells, respectively. Lanes 4 to 6, untreated, MHG- and PB-treated DU145 cells, respectively.

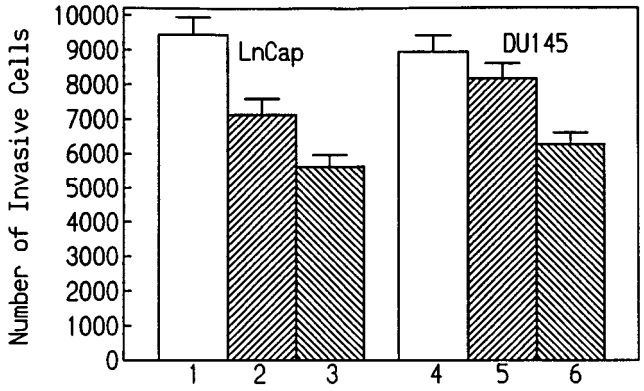

Figure 2. Effect of MHG and PB on cellular invasion. Invasion of matrigel-coated porous membranes was determined as described in 'Materials and methods'. Healthy viable control and treated cells (treated with IC-50 concentrations of MHG or PB) were used in these experiments. The number of invasive cells after 4 days of incubation at 37 °C was determined by counting with the aid of a hemocytometer. The effect of each treatment with its corresponding control was performed in triplicate invasion chambers. The results presented are the mean and standard error of three independent experiments. Lanes 1 to 3, untreated, MHG- and PB-treated LnCap cells, respectively. Lanes 4 to 6, untreated, MHG- and PB-treated DU145 cells, respectively.

Effect of MHG and PB on anchorage-independent growth and cellular invasion

The ability of MHG and PB to suppress malignant cell behavior in terms of anchorage-independent growth and invasion of matrigel was next determined. The LnCap cells had a greater propensity to grow in soft agarose than the DU145 cells (2,500 vs. 1,500 colonies, Figure 1). Both MHG and PB (IC-50 concentrations) inhibited the growth of these cells in soft agarose and inasmuch as over 50% inhibition was achieved for both LnCap and DU145 cells by PB, a lesser degree of inhibition was observed with MHG (Figure 1).

MHG- and PB-treated cells showed a reduced ability to invade a matrigel matrix (Figure 2). Both the LnCap and DU145 cells invaded the matrigel to similar extent. Invasion by PB-treated LnCap and DU145 cells was reduced, respectively, by approximate 41 and 30% when compared to untreated control cells (Figure 2). Invasion of MHG-treated LnCap and DU145 cells was reduced, respectively, by about 25 and 9% (Figure 2).

Discussion

Carcinogenesis may be viewed as aberrant differentiation and many differentiation-inducing chemicals possess chemopreventive properties [44]. Unlike many other differentiation-inducing chemicals the alkylglycerols constitute a class of ether lipids that are naturally occurring in living organisms [19–21]. We have previously shown that MHG, a species of alkyglycerols, is biologically active and promotes a more benign or differentiated phenotype in human colon cancer cells [26]. In human colon cancer cells, MHG inhibits cellular proliferation, anchorage-independent growth, cellular invasion and induces the expression of differentiation-related markers [26]. To our knowledge, the ability of MHG to promote differentiation or inhibit the malignant behavior of human prostate cancer cells has not been investigated. In this study, we found that MHG, in the μM concentrations, also inhibited cellular proliferation, anchorage-independent growth and cellular invasion in the human prostate LnCap and DU145 cells. Colon cancer cells, however, are relatively more sensitive to MHG by comparison. For examples, the IC-50 values for colon cancer cells are about 12 μM, and MHG in the 50 μM concentration exerts a good inhibitory effect (35–50% reduction) in anchorage-independent growth and cellular invasion [26].

The ability of PB to promote differentiation in colon cancer cells has not been thoroughly investigated. PB, however, has been reported to induce growth arrest and promote differentiation in fluorodeoxyuridine-treated human colon cancer HT29 cells [46]. Because serum levels of PB up to 2 mM can be achieved without limiting clinical toxicity, the idea of using PB to enhance a clinical response following cytoxic therapy in patients with refractory colon carcinoma is quite attractive [46].

We found a significant difference in the IC-50 values of MHG (93–97 μM) and PB (1.3–7.3 mM) for these prostate cancer cell lines. PB in mM concentrations has also been reported to inhibit cell growth and induce apoptosis in human prostate cancer cell lines [30]. At equivalent IC-50 concentrations, we found that PB was more potent than MHG in inhibiting anchorage-independent growth and cellular invasion. The IC-50 values of PB for the LnCap and DU145 cells were 1.3 and 7.3 mM, respectively; while the IC-50 values of MHG for these cells were 93 and 97 μM, respectively (Table 1). We could not compare the effect of MHG to that of PB using equivalent concentrations because concentrations higher than the IC-50 values for MHG (but not PB) were toxic to the cells. The inhibitory effect of these compounds on anchorage-independent growth and cellular invasion (following treatment with IC-50 concentrations and

the use of viable live cells in the assays), therefore, could not be attributable to cell death.

The use of this group of natural substances in differentiation induction of malignant cells or in chemoprevention of malignant diseases has not been thoroughly investigated. Thus, the differentiation-promoting and/or chemoprevention properties of MHG and PB in prostate cancer or other malignant diseases warrants further investigation.

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