Antiproliferative evaluation of tall-oil docosanol and tetracosanol over CHO-K1 and human melanoma cells

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A R T I C L E I N F O
Article history:
Received 21 January 2015
Accepted 2 May 2015
Available online 28 May 2015

Keywords:
CHO-K1 cells
Growth-arrested
Human melanoma cells
Polycosanols

A B S T R A C T
Background: Polycosanols derived from plant species have traditionally been used in medicine as antiproliferative agents for treating various viruses (primarily the herpes simplex virus). However, few studies have studied their effects on hyperproliferative cell lines. In this work, the antiproliferative capacity of polycosanols from tall-oil pitch, obtained from black liquor soaps in the kraft pulping process of cellulose (specifically from Pinus radiata, Pinus taeda, and Eucalyptus globulus), was evaluated on CHO-K1 and CRL-1974 human melanoma cell lines.

Results: The proliferative capacities and cell viabilities were measured for 72 and 140 h, respectively. Treatment with docosanol produced differential effects on the CHO-K1 and human melanoma cells and significantly affected their proliferation rates, but not their cell viabilities. Tetracosanol produced a significant negative effect on the proliferation of human melanoma cells, and this effect was less than that caused by docosanol. However, it had no effect on the proliferation of CHO-K1 cells and did not induce any significant effect on the viability of the studied cell lines.

Conclusion: Docosanol and tetracosanol induced antiproliferative effects on the studied cell lines and exhibited significantly greater effects on the oncogenic cell lines. Prior to this study, the capacity of these polycosanols has never been investigated. Future studies will be necessary to determine their mechanisms of action on these cell systems.

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1. Introduction

Long-chain aliphatic alcohols (polycosanols) and fatty acids with more than 20 carbon atoms are of great interest as medical, personal care, and pharmaceutical products. Polycosanols have primarily been used to treat the herpes simplex virus (HSV-1) [1,2,4,5,6] and the respiratory syncytial virus [3]. Hyperproliferative skin lesions [7], which can be benign or malignant (keloids, skin cancer), can be treated and cured with these alcohols. Additionally, polycosanols can be used as anti-inflammatory agents [8] in epithelial prostate cells [9, 10] and in the treatment of certain conditions caused by enveloped viruses, such as Kaposi sarcomas [11]. However, this type of alcohol is extremely rare in nature and is only found in small quantities in sugarcane, spinach, evening primrose oil [12], beeswax [13,14], the native Chilean plant Myoschilos oblongum (Orocoipo, Codocoipo) [10], the Brazilian shrub Gallesia gorazema (Phytolaccaceae) [15], and some Oriental medicinal plants [16,17,18].

The discovery of new utilities for polycosanols has created new opportunities to exploit the sources that contain these alcohols. One of these sources is the black liquor soap, from the kraft pulping process of cellulose, which is a byproduct resulting from the processing of these soaps in the recovery of other compounds, such as wood sterols [19].

Tall-oil is a fraction obtained from black liquor soap in the kraft pulping process. After further fractionation to isolate a heavy component called pitch [20,21], a light fraction rich in polycosanols can be recovered by distillation. Subsequently, a short-path distillation yields three additional fractions rich in docosanol (C22), tetracosanol (C24) and higher fatty alcohols (C > 24), respectively [19] (Fig. 1). Because of these compounds, black liquor soap has been considered one of the most suitable raw materials for the commercial production of docosanol (D-nol) and, in particular, tetracosanol (T-nol).

There have been several studies conducted on the therapeutic uses of polycosanols. Extensive investigations on the herpes simplex virus in vivo and in vitro have shown that docosanol exerts potent inhibitory effects on the ability of the virus to infect target cells. Current evidence suggests that docosanol inhibits viral replication by interfering with early intracellular events surrounding viral entry into target cells [3]. However, for a few cell lines, the effects of docosanol are scarce. In mammalian cells, there is no information about the cytotoxic effects of these alcohols. Recent studies have reported the antiproliferative properties of natural extracts that are known to contain molecules
with high numbers of carbon atoms. However, few of these molecules are polycosanols as most are flavonoids, terpenoids or quinones [22, 23,16,24,25,26].

The objective of this work was to determine the antiproliferative effects of polycosanols on the growth of CHO-K1 mammalian cells and melanoma human cells. These polycosanols were obtained from the tall-oil pitch of black liquor soaps byproducts of the kraft pulping process of cellulose, derived from plants of Pinus radiata, Pinus taeda, and Eucalyptus globulus in Chile.

2. Materials and methods

2.1. Raw materials

Polycosanols in the light fraction of tall oil (Härting S.A. Santiago, Chile) were concentrated by short path distillation (KDL5, UIC GmbH, Alzenau-Goerstein, Germany) from 7 to 21.8%. Then, a mixture with over 90% of polycosanols was obtained by crystallization. This mixture was subjected to fractional distillation in a packed glass column (stainless steel), and three high purity fractions were obtained (i.e., docosanol > 98%; tetracosanol > 99% and hexacosanol > 95%) [27].

2.2. Formulation of long-chain alcohols in Pluronic® F-68

Long-chain alcohols (docosanol > 98% and tetracosanol > 99%) were obtained by a previously described protocol and were suspended in Pluronic® F-68 (Poloxamer 188; Mr 4000; BASF, Parsippany, NJ) at 37°C in 0.15 % C02 atmosphere with 95% relative humidity. All cell lines were seeded at 1.5 × 10⁵ cell/mL and cultured in 12-well plates (Orange Scientific, 4430400) for 12 h to allow complete adherence. To assess the antiproliferative effect of the long-chain aliphatic alcohols.

2.3. Cell culture assays

Chinese hamster ovary cells K1 (CHO-K1) obtained from Sigma-Aldrich (Sigma, 98070106), and human melanoma cells (CRL-1974™) obtained from ATCC (Manassas, USA), were grown to 8.1 × 10⁵ cell/mL, which was not significantly affected by the presence of Plu surfactant (1.25 mg/mL). However, at higher concentrations of Plu, a negative effect was observed on cellular growth (data not shown). A dose of 15 mM of docosanol was previously tested for the virus; the tests results can be found in a previous publication [3]. Higher concentrations of docosanol could not be tested due to the instability of the Plu suspension.

The effects of long-chain aliphatic alcohols (docosanol and tetracosanol) on the growth of CHO-K1 cells were investigated (Fig. 2). The maximum cell density achieved by the control culture was 8.1 × 10⁵ cell/mL, which was not significantly affected by the presence of Plu surfactant (1.25 mg/mL). However, at higher concentrations of Plu, a negative effect was observed on cellular growth (data not shown). A dose of 15 mM of docosanol was previously tested for the virus; the tests results can be found in a previous publication [3]. Higher concentrations of docosanol could not be tested due to the instability of the Plu suspension.

The presence of docosanol (15 mM) had a negative effect on cell growth population and reduced it by 15% relative to that of the control culture. Values were expressed as the means ± the standard error. An analysis of variance was used to compare the results using the Design-Expert 7 software.

2.4. Statistical analysis

Each measured experimental condition was performed in triplicate, and two independent samples were taken at each time point for every culture. Values were expressed as the means ± the standard error. An analysis of variance was used to compare the results using the Design-Expert 7 software.

3. Results and discussion

3.1. Effect of long-chain aliphatic alcohols on CHO-K1 cell growth

The effects of long-chain aliphatic alcohols (docosanol and tetracosanol) on the growth of CHO-K1 cells were investigated (Fig. 2). The maximum cell density achieved by the control culture was 8.1 × 10⁵ cell/mL, which was not significantly affected by the presence of Plu surfactant (1.25 mg/mL). However, at higher concentrations of Plu, a negative effect was observed on cellular growth (data not shown). A dose of 15 mM of docosanol was previously tested for the virus; the tests results can be found in a previous publication [3]. Higher concentrations of docosanol could not be tested due to the instability of the Plu suspension.

The presence of docosanol (15 mM) had a negative effect on cell growth population and reduced it by 15% relative to that of the control culture after 72 h of culture. However, the presence of tetracosanol did not trigger a cytotoxic effect on the growth of CHO-K1 cells.
In conclusion, long-chain aliphatic alcohols (docosanol and tetracosanol) exhibited inhibitory effects on the growth of CHO-K1 and human melanoma oncogenic cell lines. The antiproliferative capacity of these molecules is promising. A deeper understanding of their mechanisms would require additional research on the cell lines investigated and other oncogenic cell lines.

**Financial support**

This work was supported by Härting S.A., Pontificia Universidad Católica de Valparaíso, CONICYT (National Commission for Science and Technology Research) program financial support: FONDEF, Project number: DO4I1007; and CONICYT, Scientific Information Program/Fund for Scientific Journals Publishing, Year 2014, ID FP140010.

**Authors’ contribution**

Proposed the theoretical frame: CA, AO, MV. Conceived and designed the experiments: CA, MV. Contributed reagents, materials, analysis tools: CA, AO. Wrote the paper: MV, AO, CA. Performed the experiments: MV, AO. Analyzed the data: CA, AO, MV.

**Conflict of interest**

There is no conflict of interest.

**Acknowledgments**

The authors are grateful to Alejandro Markovits (Härting S.A.) for his valuable cooperation.

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**Table 1**

| Viability (%) | CHO-K1 cells | Melanoma cells |
|---------------|--------------|----------------|
| Control       | 98 ± 4       | 96 ± 3         |
| Tetracosanol  | 96 ± 6       | 94 ± 4         |
| Docosanol     | 95 ± 4       | 97 ± 5         |
| Mixture (50:50)| 97 ± 5       | 96 ± 4         |

Values shown are the mean ± SEM of triplicates.
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