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Lipid nanocarriers based on natural oils with high activity against oxygen free radicals and tumor cell proliferation

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ABSTRACT

The development of nano-dosage forms of phytochemicals represents a significant progress of the scientific approach in the biomedical research. The aim of this study was to assess the effectiveness of lipid nanocarriers based on natural oils (grape seed oil, fish oil and laurel leaf oil) in counteracting free radicals and combating certain tumor cells. No drug was encapsulated in the nanocarriers. The cytotoxic effect exerted by bioactive nanocarriers against two tumor cells, *MDA-MB 231* and *HeLa* cell lines, and two normal cells, *L929* and *B16* cell lines, was measured using the MTT assay, while oxidative damage was assessed by measuring *the* total antioxidant activity using chemiluminescence analysis. The best performance was obtained for nanocarriers based on an association of grape seed and laurel leaf oils, with a capacity to scavenge about 98% oxygen free radicals. A dose of nanocarriers of 5 $mg \cdot mL^{-1}$ has led to a drastic decrease in tumor cell proliferation even in the absence of an antitumor drug (e.g. about 50% viability for *MDA-MB 231* cell line and 60% viability for *HeLa* cell line). A comparative survival profile of normal and tumor cells, which were exposed to an effective dose of 2.5 $mg \cdot mL^{-1}$ lipid nanocarriers, has revealed a death rate of 20% for normal *B16* cells and of 40% death rate for *MDA-MB 231* and *HeLa* tumor cells. The results in this study imply that lipid nanocarriers based on grape seed oil in association with laurel leaf oil could be a candidate to reduce the delivery system toxicity and may significantly improve the therapeutic efficacy of antitumor drugs in clinical applications.

1. Introduction

Combining natural and synthetic remedies is very useful in biomedical research in order to develop a safety therapeutic strategy for a maximum treatment efficiency and low side effects [1,2]. Currently, aromatic plant species are primarily recommended and used to treat several diseases due to the therapeutic properties of their essential oils such as anti-inflammatory, antibacterial, anti-rheumatic, free radical-scavenging activities and antitumor activities [3–5]. Laurel essential oil isolated from leaves of *Laurus nobilis* L. (LLO) contains many active compounds including 1,8-cineole (eucalyptol — the major monoterpenoid from LLO), terpinyl-acetate, pinene, limonene and so on whose properties have been assessed for their efficacy and tolerability in various anticancer therapies. It was reported that nonpolar *Laurus nobilis* leaf extract had the ability to induce cytotoxicity and apoptosis towards SK-N-BE(2), SH-SY5Y and C6 brain tumor cell lines [6]. The antiproliferative activity of laurel volatile oil against human breast adenocarcinoma was proved by Jelnar et al. [7]. Other in vitro study demonstrated inhibition of cancer cell growth by laurel leaf oil in HT-29, HCT-116, Caco-2, and SW-480 human cancer cell lines, which were accompanied by variable levels of elevated apoptosis [8]. Both seed and leaf essential oils from laurel plant exhibited a scavenging effect on DPPH radical and inhibited proliferation of the K562 tumor cell line [9]. A comparative study claiming the effect of *Laurus* essential oil and its main components on α-glucosidase and reactive oxygen scavenging activity has revealed that inhibition of lipid peroxidation manifested by the essential oil was more pronounced than those of 1,8-cineole and limonene alone [10]. The synergistic effect of *Laurus nobilis* L. leaf extracts was also demonstrated by Kauroinovic et al. in an in vitro and in vivo antioxidant activity study [11].

In conjunction, the natural oils enriched in ω-3, −6, and −9 fatty acids completed the health-promoting effects of essential oils. Broad pharmacological activities of *grape seed oil* associated to its composition have already been demonstrated [12–14]; e.g. enhancing the cardiovascular health, combating the free radicals, reducing the symptoms in gastric ulcers, inhibiting the growth of certain types of cancers in cell cultures. The anticancer effects of grape antioxidants have been demonstrated in both in vitro and in vivo models [15,16]. The antioxidant compounds from grapes, especially proanthocyanidins have been shown to induce cell cycle arrest and apoptosis in cancer cells by activating DNA damage checkpoint cascade [17], and to inhibit angiogenesis in human breast cancer xenografts in vivo [18] as well as cancer progression in

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rodent models [19]. An irreparable DNA damage leading to G2/M arrest and apoptosis selectively in head and neck squamous cell carcinoma was also reported by Shrotriya et al. [20]. In addition, grape seed antioxidants were also found to selectively inhibit the human melanoma A375 and Hs294t cells, inhibiting over-expression of COX-2 and prostaglandin E2 receptors, or modifying estrogen receptor pathways, resulting in cell cycle arrest and apoptosis [21].

Many studies and significant efforts have been made to develop targeted delivery systems that can provide higher effectiveness in prevention and treatment of cancer with minimal effect on normal cells [22]. Lipid based nanoformulations, with particular emphasis on nanostructured lipid carriers (NLC) are among the most attractive strategies for encapsulation and delivery of antitumor drugs [23,24]. NLC are spherical particles with mean diameters in the 50–500 nm range, made by biocompatible lipids and surfactants [25,26], with proven safety and improved delivery features [27]. In their pure form, NLC are composed of a solid lipid core consisting of solid lipids blended with a liquid lipid [28], but new and efficient vegetable lipid based nanocarriers were recently reported by the authors for co-delivery of antioxidant, UV-A and UV-B filters [29,30]. The benefit of these nanocarriers with high content of vegetable active compounds is highly advantageous as the bioactive principles of phytochemicals can complement or even enhance the biological activity of the synthetic drug [31,32].

Conventional chemotherapy usually employs high doses of toxic antitumor drugs which often induce severe hazardous effects on healthy organs [33,34]. In addition, the delivery systems can contribute to the overall toxicity of a pharmaceutical product [35]. Alternatively, oral delivery of anti-cancer drugs is usually compromised by the low penetration and limited distribution [36] of the drug in solid tumors and its degradation during the first-pass metabolism [37]. Consequently, an ideal therapy for certain tumor cells could be based on the development of new approaches in delivery systems that can successfully meet both requirements, to improve drug and carrier safety and to provide complementary biological effect by using a combination of natural bioactive compounds and synthetic drugs.

Over the past decade solid lipid nanoparticles and nanostructured lipid carriers have been extensively investigated in the delivery of antitumor drugs such as oridonin [38], doxorubicin [39], methotrexate [40], and tetracyclines (CMT) [41]. However, to the best of our knowledge the positive/negative effect of delivery systems was not a topic of great interest. Although the pharmacological and biological effects of LLO have been thoroughly investigated in the literature, its presence inside lipid nanocarriers that could be directly associated with therapeutic efficacy has not been addressed yet. As a result, in the present work we focused on the evaluation of the effectiveness of several lipid nanocarriers prepared by association of plant and animal oils, e.g. *grape seed oil* or *fish oil* with *laurel leaf oil*, to counteract the oxygen free radicals and to combat certain tumor cells without encapsulating any antitumor drug. The chemiluminescence method and cellular proliferation tests were employed in order to demonstrate the efficiency of developed bioactive lipid nanocarriers. The study intends to underline the ability of vegetable oils to confer valuable bioactive properties to conventional lipid nanocarriers. The association of classical lipid nanocarriers with vegetable oils will significantly contribute to the removal of carrier cytotoxicity simultaneously with adding supplementary biological effects.

2. Experimental

2.1. Materials

Sodium cholate, polyoxyethylene sorbitan monolaurate (Tween 20), L-α-phosphatidylcholine, dimethylsulfoxide, and hydrogen peroxide were purchased from Merck (Frankfurter, Germany). Poloxamer 407 (block copolymer of polyethylene and polypropylene glycol) was supplied by BASF Chem Trade GmbH (Burgbernheim, Germany) and Tris[Hydroxymethyl] aminomethane (Luminol) was purchased from Sigma Aldrich Chemie GmbH (Seelze, Germany). n-Hexadecyl palmitate (*CP*) was obtained from Acros Organics (USA), glycerol monostearate (*GM*) from Cognis GmbH (Monheim, Germany) and Myritol 318 (*My*) was from Merck (Germany). Grape seed oil (GSO) with a fatty acid content of 59% linoleic, oleic, palmitic and stearic acids was from Manicos SRL (Romania). The essential oil fraction of laurel (LLO) concentrated in eucalyptol, α-terpinyl acetate and α,β-pinene [42] was obtained from dried leaves (collected from Amanos Mountain) and was provided by the Department of Field Crops, Faculty of Agriculture (Turkey). The fish oil (*FO*) with the composition of 33% eicosapentaenoic acid (*EPA*), 22.3% docosahexaenoic acid (*DHA*) and 5.6% docosapentaenoic acid (*DHA*) was supplied by Henry Lamotte Oils Gmbh (Bremen, Germany).

2.1.1. Cell cultures and treatments

HeLa human cervical cancer cell line, *MDA-MB 231* human breast cancer cell line, *B16* mouse melanoma cell line and *L929* mouse fibroblast cell line were purchased from the American Type Culture Collection (ATCC) and routinely maintained in culture in RPMI-1640 medium added with 2 mM L-glutamine and 10% fetal calf serum (Sigma Aldrich, St. Louis, MO, USA) and incubated at 37 °C/5% CO_2 humidified atmosphere.

2.2. Preparation of bioactive nanocarriers by association of natural oils

The nanocarriers were prepared by a combined HSH (High-Shear Homogenizer PRO250 type, 0–28.000 rpm; power of 300 W, Germany) and HPH (APV 2000 Lab Homogenizer, Germany). In brief, an aqueous phase which consisted of distilled water and 2.5% surfactant mixture (sodium cholate: Tween 20: block copolymer = 80: 12: 8, *w*/*w*) and various lipid phases (e.g. mixture of solid lipids, including GM/CP, and oils – GSO, FO, My and LLO) were separately prepared. After mixing the aqueous and lipid phases, the obtained pre-emulsions were kept under stirring at 80 °C for 5 min. The resulting emulsions were subjected to a high shear homogenization stage by applying 12,000 rpm for 1 min and then processed through high pressure homogenization, with eight homogenization cycles at 600 barr. The nanodispersions were cooled at room temperature to obtain bioactive lipid nanocarriers. In order to remove the excess of water, all dispersions were lyophilized, using an Alpha 1–2 LD Freeze Drying System, Germany.

The developed nanocarriers have been denoted as *NLC 1 ÷ 3* (prepared with 2% LLO and 15, 20 and 25% grape seed oil), *NLC 4 ÷ 6* (prepared with 2% LLO and 15, 20 and 25% fish oil) and *NLC 7 ÷ 9* (prepared with 2% LLO and 15, 20 and 25% Myritol).

2.3. Characterization of bioactive nanostructured carriers

2.3.1. Particle size analysis

Particle size measurements were performed by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., United Kingdom). The mean particle size (Z_{ave}) and the polydispersity index (PdI) of the NLC dispersions were measured at a scattering angle of 90° and a temperature of 25 °C. Before measurements, the dispersions were diluted with deionized water to an adequate scattering intensity. The particle size data was evaluated using intensity distribution. The average diameters (based on Stokes–Einstein equation) and the polydispersity index were given as average of three individual measurements.

The size and morphology of bioactive lipid nanocarriers were examined by transmission electron microscopy (Philips 208 S, The Netherlands). The lipid nanocarriers were diluted with water (1:100, v/v) before examination. Analyses were performed without staining.

2.3.2. Zeta potential measurements

The Zeta potential (ZP) was determined by measuring the electrophoretic mobility of the lipid nanocarriers in an electric field, by using