

SYNTHESIS OF ANTICANCER VEGETABLE-BASED LIPID NANOCARRIERS

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The lack of specificity of chemotherapeutic agents towards cancerous cells leads to systemic toxicity and adverse effects, causing a limitation of the maximum dose of drug administrated. The present study aims to use vegetable oils in combination with anticancer drugs in formulating lipid-based drug delivery systems in order to overcome the drawbacks of anticancer agents. The vegetable oils used, sesame seed oil, black caraway seed oil and olive oil, which are rich in bioactive compounds with antioxidant and antitumor properties, were coupled with an anticancer drug from the class of antimetabolites.

Keywords: Lipid nanocarriers, vegetable oils, anticancer drugs

1. Introduction

Pharmaceutical drugs available for cancer therapy today have numerous drawbacks: only a small fraction of drugs reaches the therapeutic site of action, they cause severe side effects [1, 2], and, additionally, the tumor cells are getting drug resistant [3]. This is why in the last decade researchers have started to explore the area of nanotechnology to develop new nanocarriers for anticancer drug delivery [4].

The use of nanoparticles as delivery systems of cytotoxic drugs is advantageous due to the presence at nanoscale of the drug, which improves its site-specific delivery and therapeutic effect, and reduces the toxicity of the pharmaceutical formulation by lowering the drug concentration [5, 6]. The definition of nanoparticles is particulate matter that has a size of 100 nm or less. However, in medical applications of nanotechnology the nanoparticles are defined as particles with sizes ranging from 1 to 1000 nm [7]. Among the studied nanoparticles, nanostructured lipid carriers (NLCs) are promising lipid-based colloidal systems that have a small particle size and a narrow size distribution, assure stability of drug molecules [8, 9] and possess low systemic toxicity and biodegradability [10]. Several studies performed on NLCs loaded with drugs used

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in cancer therapy (e.g. docetaxel, topotecan) demonstrated an efficient entrapment and a sustained and continuous release of the drug [11, 12].

Another approach used in cancer treatment is finding novel compounds of natural origin from plants that can be less toxic and more efficient than the chemotherapeutic drugs in use nowadays [13]. For example, vegetable extracts and oils, which have been utilized from a long time in traditional medicine for their antioxidant, anti-inflammatory and antibacterial activity, could have a great impact on the development of effective pharmaceuticals against cancer. Additionally, coupling bioactive vegetable oils with anticancer drugs in the same lipid-based nanocarrier system may offer synergistic therapeutic effects [14-16]. For example, the combined exposure of curcumin and 5-fluorouracil (5Fu) separately loaded into N,O-carboxymethyl chitosan nanoparticles proved enhanced anticancer effects in colon cancer cells (HT 29) and increased the drug bioavailability [17]. The encapsulation at nanosize also maintains the oil's stability, and improves its cellular absorption [18].

In the present study the potential of three cold-pressed oils with antioxidant and antitumor activity, black caraway seed oil (*Nigella Sativa*), sesame seed oil (*Sesamum indicum*) and olive oil (*Olea europaea*), was investigated in formulating nanostructured lipid carriers as delivery system of hydrophilic anticancer drugs.

The antioxidant activity and the protective effects of sesame seed oil are mostly attributed to the total polyphenols, lignans (sesamin and sesamol) and lignan glycosides (sesaminol and sesamolinal). Sesamol is reported to be a potent antioxidant scavenging the entire range of reactive oxygen species (ROS), and inhibiting lipid peroxidation [19]. The presence of lignans offers the anticancer activity, the sesame oil inhibiting human colon cancer growth *in vitro* [20].

Black caraway oil has a high content of antioxidants including phenols and phytosterols. The cytotoxicity assessment of the main compounds present in the oil showed that thymoquinone was the most active, inhibiting tumor cell lines, as well as longifolene that also inhibits the growth of tumor cells [21].

Olive oil contains phenolic compounds (e.g. tyrosol, hydroxytyrosol), squalene, phytosterols that are strong antioxidants and radical scavengers [22]. Phenols may also contribute to the chemopreventive effects of olive oil against cancer. In a study of Fini et al. (2008), an olive oil extract containing 62% pinosresinol showed antiproliferative and proapoptotic effect against human colon carcinoma cells [23].

The vegetable-based lipid nanocarriers formulations were characterized by mean particle size, physical stability and drug entrapment efficiency of the anticancer drugs, 5-fluorouracil or pemetrexed. The antioxidant activity of the oils and of their nanocarriers was evaluated by the chemiluminescence technique.

2. Experimental

2.1. Materials

The surfactant system was composed of polyoxyethylene sorbitan monolaurate (Tween 20) purchased from Merck, Synperonic PE/F68 (block copolymer of polyethylene and polypropylene glycol) and L- α -Phosphatidylcholine obtained from Sigma Aldrich. The solid lipids used were glyceryl monostearate (GMS) and emulgade SE/PF (Em) purchased from Cognis GmbH. Pemetrexed disodium (Pm) was purchased from Scinopharm Taiwan LTD and 5-fluorouracil was supplied by Sigma Aldrich. The vegetable oils purchased from Elemental (Romania) were: sesame seed oil – SO (44.01% linoleic acid, 39.81% oleic acid, 8.51% palmitic acid, 5.88% stearic acid, 0.68% arachidic acid, 0.41% linolenic acid, 0.17% eicosenoic acid), black caraway seed oil – BCO (55.8% linoleic acid, 24.7% oleic acid, 12.1% palmitic acid, 3.3% stearic acid, 2.5% eicosadienoic acid, 0.4% eicosenoic acid, 0.3% linolenic acid, 0.2% arachidic acid, 0.2% palmitoleic acid, 0.2% myristic acid, 0.1% erucic acid) and olive oil – OO (77.5% oleic acid, 11.0% palmitic acid, 5.5% linoleic acid, 3.4% stearic acid, 0.8% palmitoleic acid, 0.7% linolenic acid, 0.4% arachidic acid, 0.3% eicosenoic acid, 0.1% behenic acid, 0.1% lignoceric acid).

2.2. Synthesis of vegetable - lipid nanocarriers

The lipid nanocarriers were prepared by a modified high pressure homogenization (HPH) method. The lipid phase consists in a mixture of Em, GMS and one of the three vegetable oils in a ratio of 1.16:1.16:1. The anticancer drugs in a concentration of 0.3 wt% were added into the aqueous phase composed of a mixture of 2.5 wt% surfactant (Tween 20: Synperonic PE/F68: Phosphatidylcholine in a ratio of 4.66:1:1). The emulsion was heated under stirring at 85 °C for 30 min, and then homogenized by applying 15000 rpm for 1 min (High-shear homogenizer PRO250, Germany), and subjected to six homogenization cycles at 600 bar (HPH, APV 2000 Lab Homogenizer, Germany). To remove the water, the dispersions were lyophilized using a Christ Alpha 1-2 LD Freeze Dryer (Germany).

2.3. Methods

The mean particle size was analyzed by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, United Kingdom). Zeta potential (ZP) was determined by measuring the electrophoretic mobility of the nanoparticles in an electric field by using the same apparatus, Zetasizer Nano ZS. Prior to measurements, the dispersions were diluted with deionized water to an adequate scattering intensity.

The entrapment efficiency (EE %) of the drug into lipid nanocarriers was determined by measuring the amount of free drug found in the dispersion medium. The method is described in a previous study of the authors [24]. The amount of free drugs was analyzed spectrophotometric at $\lambda_{\max} = 245$ nm and $\lambda_{\max} = 265$ nm for Pm and 5Fu, respectively, by using calibration curves with a correlation coefficient of $R^2 = 0.9995$ (UV-Vis-NIR Spectrophotometer type V570 Jasco, Japan).

The antioxidant activity of the lipid nanocarriers was evaluated *in vitro* by the chemiluminescence method using Chemiluminometer Turner Design TD 20/20, USA. Luminol (10^{-5} M) and H_2O_2 (10^{-5} M) in tris-hydroxymethyl aminomethane – HCl buffer solution (pH 8.6) have been used as a generator system for free radicals [25]. Solutions with a concentration of 4.7 mg/L anticancer drug and 47 mg/L oil were prepared by using lyophilized lipid nanoparticles in dimethyl sulfoxide. The percentage of scavenged free radicals was calculated according to the equation:

$$\%AA = \frac{I_0 - I_s}{I_0} \times 100 \quad (1)$$

where I_0 and I_s are the chemiluminescence maximum of standard and of sample, respectively, at time $t = 5$ s.

All measurements were performed in triplicate at 25°C and the average was reported.

3. Results and discussion

Vegetable-based lipid nanocarriers with mean particle sizes between 103 - 140 nm were obtained. The nanocarriers presented a narrow size distribution with PDI values smaller than 0.25 (Fig. 1). Among the oils used, sesame oil resulted in obtaining lipid nanocarriers with the smallest size. By encapsulating the anticancer drug 5Fu, the size of the vegetable-NLCs has increased for those prepared with BCO (from 125 to 140 nm) and OO (from 114 to 122 nm), and has slowly decreased for NLCs based on SO (from 119 to 117 nm). In the case of encapsulating Pm, the mean particle sizes of nanocarriers has slightly decreased by 2 nm for NLCs based on BCO and OO, and by 16 nm for NLCs based on SO. In general, the PDI values have increased by encapsulating the drugs into NLCs, but they still remained below 0.25.

The vegetable oils have led to the development of lipid nanocarriers with a moderate physical stability, having zeta potential values less than -28 mV (Fig. 2). No significant difference was observed between the zeta potentials of the various vegetable-NLCs depending on the oil used or the type of the encapsulated anticancer drug.

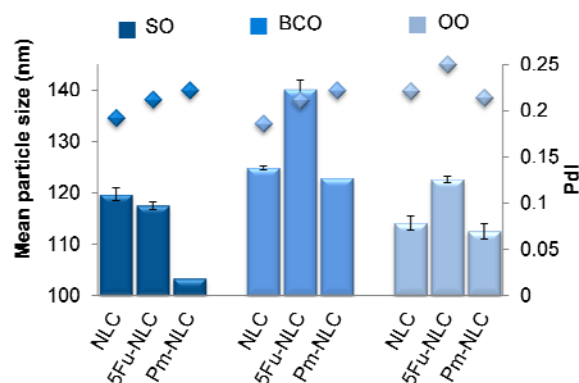


Fig. 1. Particle size and polydispersity index (PDI) of unloaded and drug-loaded vegetable NLCs

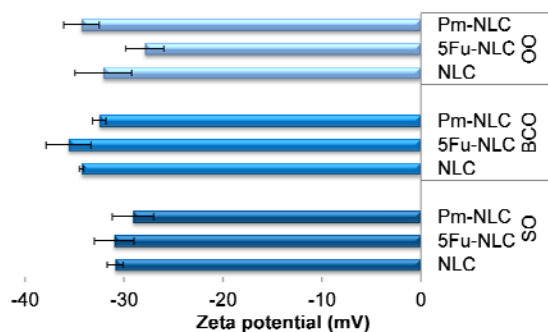


Fig. 2. Zeta potential of unloaded and drug-loaded vegetable NLCs

The entrapment capacity was evaluated to determine the efficacy of the vegetable lipid systems used as nanocarriers for hydrophilic anticancer drugs. The obtained results were very good with entrapment efficiency values of 82 - 84% for 5Fu and of 86.5 - 88% for Pm (Fig. 3). Pemetrexed has a better incorporation due to its greater affinity for the lipid matrix of the NLCs, reflected by a higher octanol-water partition coefficient compared to 5Fu. This is in accordance with the size analysis that was carried out, where lipid nanocarriers loaded with Pm presented smaller sizes than 5Fu-NLC. Among the oils used, sesame oil assures the best entrapment efficiency for 5Fu inside the lipid nanocarriers and olive oil assures the best entrapment efficiency for Pm.

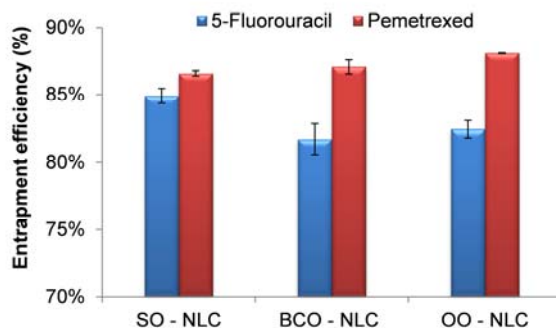


Fig. 3. Drug entrapment efficiency of NLCs based on different vegetable oils

The antioxidant activity of the vegetable oils and of the vegetable-based lipid nanostructures is shown in Fig. 4. Free (unencapsulated) olive oil presented the best antioxidant activity (78 %), followed by sesame oil with a capacity to scavenge 74% of the reactive oxygen species generated *in situ* by the chemiluminescence system. Their corresponding vegetable nanocarriers presented improved antioxidant capacity with values between 91% and 96%. The encapsulation of the anticancer drugs into the lipid nanocarriers did not influence their antioxidant capacity.

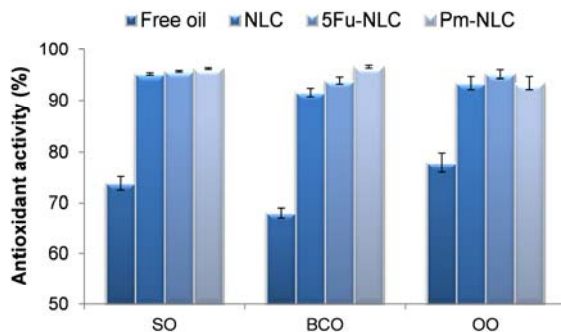


Fig. 4. *In vitro* evaluation of the antioxidant activity of anticancer NLCs based on different vegetable oils

6. Conclusion

The vegetable oils used, sesame seed oil, black caraway seed oil and olive oil, have successfully led to the development of lipid nanocarriers as delivery systems for hydrophilic anticancer drugs. Physically stable lipid nanoparticles with mean particle sizes less than 140 nm were obtained. Among the oils used,

sesame seed oil resulted in obtaining lipid nanocarriers with the smallest size (103 nm). The nanocarriers showed good entrapment efficiencies of the anticancer drugs with values over 82%. Out of the two encapsulated drugs, pemetrexed showed better encapsulation efficiency into nanocarriers (e.g. 88%) and resulted in smaller particle sizes. The studied systems have demonstrated a very good antioxidant capacity to scavenge up to 96% of reactive oxygen species.

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