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Physico-chemical characterization of asolectin–genistein liposomal system: An approach to analyze its *in vitro* antioxidant potential and effect in glioma cells viability

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ABSTRACT

In this study, the interaction between soy isoflavone genistein and asolectin liposomes was investigated by monitoring the effects of isoflavone on lipidic hydration, mobility, location and order. These properties were analyzed by the following techniques: horizontal attenuated total reflection Fourier transform infrared spectroscopy (HATR-FTIR), low-field ^{1}H nuclear magnetic resonance (NMR), high-field ^{31}P NMR, zeta potential, differential scanning calorimetry (DSC) and UV–vis spectroscopy. The antioxidant and antitumoral activities of the genistein liposomal system were also studied. The genistein saturation concentration in ASO liposomes corresponded to 484 μM. HATR–FTIR results indicated that genistein influences the dynamics of the lipidic phosphate, choline, carbonyl and acyl chain methylenes groups. At the lipid polar head, HATR–FTIR and ^{31}P NMR results showed that the isoflavone reduces the hydration degree of the phosphate group, as well as its mobility. Genistein ordered the lipid interfacial carbonyl group, as evidenced by the HATR–FTIR bandwidth analysis. This ordering effect was also observed in the lipidic hydrophobic region, by HATR–FTIR, NMR, DSC and turbidity responses. At the saturation concentration, liposome-loaded genistein inhibits the lipid peroxidation induced by hydroxyl radical in 90.9%. ASO liposome-loaded genistein at 100 μM decreased C6 glioma cell viability by 57% after 72 h of treatment. Results showed an increase of the genistein *in vitro* activities after its incorporation in liposomes. The data described in this work will contribute to a better understanding of the interaction between genistein and a natural-source membrane and of its influence on isoflavone biological activities. Furthermore, the antitumoral results

Abbreviations: ASO, soybean asolectin; DLS, dynamic light scattering; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; DMEM, Dulbecco's modified Eagle's medium; ΔH, enthalpy variation; FBS, fetal bovine serum; FID, free induction decay; FTIR, Fourier transform infrared spectroscopy; HATR–FTIR, horizontal attenuated total reflection–Fourier transform infrared spectroscopy; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMR, nuclear magnetic resonance; •OH, hydroxyl radical; S.D., standard deviation; TBARS, thiobarbituric acid reactive substances; TSP, sodium 3-(trimethylsilyl)-[2,2,3,3-2H4]-1-propionate; τ, time delay; ν, stretching vibration.

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showed that genistein-based liposomes, which contain natural-sourced lipids, may be promising as a drug delivery system to be used in the glioma therapy.

1. Introduction

Several studies have demonstrated that genistein (4,5,7-trihydroxyisoflavone, Fig. 1), one of the main soy isoflavones, has important antioxidant and antitumoral activities (Atlante et al., 2010; Choi and Lee, 2004; Jin et al., 2012; Mitchell et al., 2000; Zhou et al., 1999). Indeed, genistein inhibits the *in vitro* lipid peroxidation induced by total reactive oxygen species, as well as by other oxidant agents, such as Fe^{2+}–ADP complex, NADPH, superoxide dismutase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), peroxinitrite, nitric oxide and superoxide anion in microsomes, chick skeletal muscle cells and murine macrophages (Jha et al., 1985; Jiang et al., 2008; Jin et al., 2012). Its antitumoral properties are related to multiple action targets in cellular processes (Li et al., 1999; Matsukawa et al., 1993). For example, genistein is a specific inhibitor or protein tyrosine kinase (Akiyama et al., 1987). Also, the isoflavone may inhibit the kappa light polypeptide gene enhancer activation in B-cells (NF-κB) signaling pathway, as seen in studies related to prostate cancer (Adjakly et al., 2013). In retinal pigment epithelial cells, genistein can induce apoptosis through the opening of its mitochondrial permeability transition pore (Yoon et al., 2000). Genistein may also provoke DNA damage since inducing cytokinesis failure, through chromosome bridge formation and Ras homolog gene family (member A, RhoA) delocalization (Nakayama et al., 2014). It is important to note that Ras stimulates the DNA topoisomerase II, which has an important role in DNA replication (Chen et al., 1999; Khoshyomn et al., 2002; Okura et al., 1988). The genistein-induced DNA damage is also related to the activation of the ataxia-telangiectasia-mutated kinase (Chang et al., 2004; Tominaga et al., 2007).

Genistein affects the physico-chemical properties of biological membranes (Arora et al., 2000). Although isoflavones can protect lipid membranes from oxidants, genistein–membrane interactions have not been completely elucidated; in fact, few correlations have been established between the genistein effect on membranes and its biological activities (Pawlikowska-Pawlęga et al., 2014; Yu et al., 1999).

Some researches have reported the effect of genistein on synthetic phosphatidylcholine-based model membranes, such as dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC). It has been studied by instrumental techniques, such as fluorescence polarization anisotropy, calorimetry, nuclear magnetic resonance (NMR) and spin label electron paramagnetic resonance (EPR) (Arora et al., 2000; Maniewska et al., 2009; Pawlikowska-Pawlęga et al., 2012). These studies have evidenced that the membrane composition influenced the isoflavone–lipid interaction, as well as the magnitude of the genistein effect on the membrane order and fluidity. To correlate drug-induced changes on membrane physico-chemical properties and its biological activities, it is interesting to perform studies which use lipid membrane models that resemble natural membranes with associations of different kinds of lipids. Among the simplest membrane models, liposomes composed of unsaturated lipids have been currently used to investigate membrane behavior (Roleira et al., 2010; Simplício de Sousa et al., 2013). Pawlikowska-Pawlęga et al. (2014) characterized the effects of isoflavone addition on the biophysical properties of egg yolk phosphatidylcholine liposomes by NMR and EPR techniques and correlated them with the genistein activity against human cervix carcinoma cell line. In this context, the aim of the present study was to better understand the influence of genistein on a natural-source membrane model and to elucidate the impact of the genistein-membrane interaction on its antioxidant and antitumoral properties. It was also compared the *in vitro* antitumoral efficacy of free genistein to liposome-loaded one. Thus, this paper describes results of the genistein location and effects on hydration, mobility and order of soybean asolectin (ASO) liposomes. The ASO is a mixture of unsaturated phospholipids whose main component is soybean phosphatidylcholine (SPC), besides phosphatidylethanolamine and phosphatidylinositol. All these lipids contain oleic (C 18:1), linoleic (C 18:2) and linolenic (C 18:3) acids (De Lima et al., 2004). Thus, the characterization of genistein (liposome-loaded) location and its effects on ASO liposome properties were monitored by UV–vis, horizontal attenuated total reflection Fourier transform infrared spectroscopy (HATR–FTIR), ^{1}H NMR spin–lattice relaxation time (T_1) and ^{31}P NMR, differential scanning calorimetry (DSC), and zeta potential techniques. All these techniques are non-invasive and can be used to monitor membrane physico-chemical processes. HATR–FTIR provides important information concerning the hydration degree and mobility of different membrane groups (Severcan et al., 2005). NMR T_1 measurements may provide insights about the influence of an exogenous molecule in membrane lipid fast motions, such as rotation (Dufourc, 2006). ^{31}P NMR is useful to monitor the effect of a substance on the membrane polar region order and lipid phase, based on the ^{31}P chemical shift anisotropy (Berden et al., 1974; Seelig et al., 1981; Villasmil-Sánchez et al., 2013). Important information related to membrane acyl chain assembly transitions and order can be obtained by DSC technique (Biltonen and Lichtenberg, 1993). Zeta potential measurements may provide information concerning the membrane surface potential, stability and molecular orientation of phosphatidylcholine membranes (Disalvo and Bouchet, 2014).

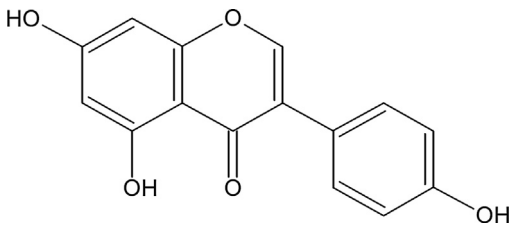

Fig. 1. Structure of genistein (4,5,7-trihydroxyisoflavone).

The results obtained by these instrumental techniques were correlated to *in vitro* antioxidant and antitumoral responses obtained for the ASO liposomal system, both pure (unloaded) or loaded with genistein. Glioma cells were chosen as tumor models since the malignant ones are the most common subtype of primary brain tumors; they are the most invasive and destructive tumors among the deadliest human cancers (Maher et al., 2001). One of the difficulties of the glioma therapy is related to the restriction caused by the blood–brain barrier (BBB) regarding the drug accessibility to the central nervous system (Bernardi et al., 2009; Mason, 2008). The possibility of a genistein-based glioma therapy is restricted due to the isoflavone size and hydrophobicity. They are additional factors which enable genistein to penetrate the BBB, but limit its delivery to the central nervous system (Kloska et al., 2012). Liposome-mediated transport mechanisms can be used for drug delivery into brain (Garg et al., 2015). In this context, the efficiency