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Enhanced anticancer effect of conjugated linoleic acid by conjugation with Pluronic F127 on MCF-7 breast cancer cells

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Abstract

This study is designed to evaluate whether conjugated linoleic acid-coupled Pluronic F127 (Plu-CLA) enhances anticancer efficacy in MCF-7 breast cancer cells when compared to conjugated linoleic acid (CLA) itself. CLA was simply coupled to Pluronic F127 through ester linkage between carboxyl group of CLA and hydroxyl one of Pluronic at melting state without solvent or catalyst. Plu-CLA significantly enhanced apoptosis with increasing concentration compared with CLA itself. Moreover, it was found that p53, p21, and Bax were up-regulated, whereas Bcl-2 and procaspase 9 were down-regulated with increasing concentration of Plu-CLA. These results were attributed to the sensitization activity of Pluronic F127.

Keywords: Conjugated linoleic acid (CLA); Pluronic F127; Apoptosis; Polymer conjugation

1. Introduction

Conjugated linoleic acid (CLA) refers to a group of polyunsaturated fatty acid (PUFA) that exists as positional and stereoisomers of octadecadienoic acid. It is found mainly in food derived from ruminants such as beef and lamb, as well as the dairy products from these sources. Recently, CLA has received considerable attention as an anticancer agent [1]. Study in animal models has shown that dietary CLA inhibits the initiation and promotion stages of carcinogenesis [2]. Feeding a synthetic mixture of CLA isomers either during or after chemical carcinogen treatment inhibited tumorigenesis in the mammary gland, colon and skin [3–8]. Both c9, t11- and t10,c12-CLA isomers and mixtures of these isomers were demonstrated to reduce the proliferation of many cancer cell lines in vitro by influencing cell cycle or increasing cell death, either by way of necrosis or apoptosis [9–14]. However, CLA was rapidly decomposed to form furan fatty acids when it was oxidized in air [15]. The susceptive oxidation and low solubility limited the clinical application of CLA.

Polymer conjugation has been developed with the aim of improving the therapeutic efficacy of low-molecular weight drugs, which can provide the opportunity to solubilize poorly water soluble

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drug, and improve stability, pharmacokinetic properties and extended circulation time in the body [16,17]. The coupling of low-molecular weight agent to polymers through a cleavable linker has been an effective method for improving the therapeutic index of clinically established agents [17]. Recently, several polymer–drug conjugates have been reported, such as *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-doxorubicin (PK1, FCE28068) [18] and HPMA copolymer-camptothecin [19], have entered Phase I/II clinical trials.

Pluronic triblock copolymer (Pluronics) is biocompatible polymer which consists of hydrophilic ethylene oxide (EO) and hydrophobic propylene oxide (PO) blocks arranged in a basic structure of: EO_a–PO_b–EO_a [20]. It is widely used in a variety of pharmaceutical applications in various dosage forms such as gels, solid polymer blends, emulsions, and nanoparticles [21–23]. Due to their lipid-like amphiphilic nature, Pluronics can be effectively incorporated into cellular membranes and inhibit drug efflux transport proteins, such as P-glycoprotein (P-gp), which interferes the entry of antineoplastic agents in MDR cells [24]. Also, Pluronics significantly inhibit GSH/GST detoxification system [25] that plays an important role in drug resistance, especially in the human breast cancer cells [26]. In fact, it was shown that Pluronics can sensitize MDR and non-MDR cancer cells to anticancer agents and can alter specific cellular responses to these agents [27–29].

In this study, CLA was simply coupled to Pluronic F127 through ester linkage between carboxyl group of CLA and hydroxyl one of Pluronic F127 without catalyst or solvent. We hypothesized that conjugation of CLA with Pluronic will enhance the solubility and stability, thereby enhancing anticancer activity of CLA. Moreover, we checked mechanism on the anticancer efficacy of the Plu-CLA in MCF-7 breast cancer cells using Western blot analysis.

2. Materials and methods

2.1. Materials

The conjugated linoleic acid mixture was purchased from HK Biotech (Seoul, Korea) containing 45.98% *cis*-9, *trans*-11 and 49.88% *trans*-10, *cis*-12 CLA. Pluronic F127 (Mw: 12,600) was provided by BASF Korea Inc. (Seoul, Korea) and was used without further purification.

2.2. Synthesis of Plu-CLA

CLA was simply coupled to Pluronic F127 at the melting phase without catalyst or solvent through ester linkages between the carboxyl group of CLA and the hydroxyl one of Pluronic F127. The synthesis procedure is as follows: CLA 5 g (17.8 mmol) and Pluronic F127 5 g (0.4 mmol) were added into a 25 ml round bottom flask and the mixture was heated with continuous stirring to produce a well-mixed molten phase and reacted at 150 °C for 5 h. Plu-CLA was obtained by dropping the resulting solution into ethyl ether to remove the unreacted CLA. Finally, the precipitated Plu-CLA was dried at room temperature under reduced pressure for 1 day. The reaction scheme of Plu-CLA is shown in Fig. 1. The composition of Plu-CLA conjugate was estimated by ^{1}H NMR spectroscopy (AvanceTM 500, Bruker, Germany).

2.3. Measurement of particle sizes

The particle sizes of Plu-CLA nanoparticles were assessed using an electrophoretic light scattering spectrophotometer (ELS 8000, Otsuka Electronics, Osaka, Japan) with 90° scattering angles at 25 °C.

2.4. Transmission electron microscopy (TEM)

The morphology of Plu-CLA nanoparticles was observed using TEM (JEM 1010, JEOL, Japan). One drop of the suspension of Plu-CLA nanoparticles was

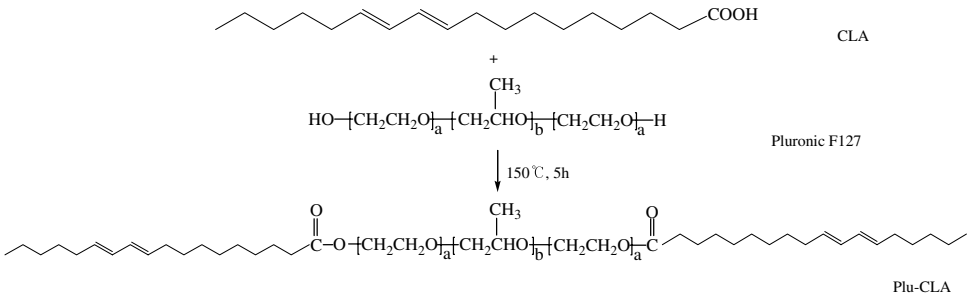

Fig. 1. Synthesis scheme of Plu-CLA.