Effects of Caffeic Acid Incorporated into Solid Lipid Nanoparticles in Cancer Cells

H.M. Kutlu*, G. Güney**, A. İşcan***, L. Genç****

*Anadolu University, Faculty of Science, Department of Biology, 26470, Eskişehir, TURKEY, hmkutlu@anadolu.edu.tr
**Anadolu University, Institute of Science, Department of Biotechnology, 26470, Eskişehir, TURKEY, gamzeguney@anadolu.edu.tr
***Anadolu University, Plant, Drug and Research Center, 26470, Eskişehir, TURKEY, aiscan@anadolu.edu.tr
****Anadolu University, Faculty of Pharmacology, Department of Pharmaceutical Technology, 26470, Eskişehir, TURKEY, lgenc@anadolu.edu.tr

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ABSTRACT

Nanotechnology has great potential to make an important role in prevention of cancer, detection, diagnosis, imaging and treatment. Solid lipid nanoparticles (SLNs) have been introduced as an alternative drug carrier system. Some of the features of SLN include good tolerability, site-specific targeting, stability, high drug payload, controlled drug release.

Caffeic acid (CA) is a widespread phenolic acid that occurs naturally in many agricultural products. CA has been reported to has antitumor activity and anti-inflammatory properties, inhibit certain enzyme activities.

We evaluated that CA-SLN interaction with H-ras transformed cell line, 5RP7. In this study, we aim to examine morphology, structural and ultrastructural changes of 5RP7 cells which exposed to CA-SLNs find by using TEM and Confocal Microscopy. We found that CA -loaded nanoparticle formulations is significantly more effective and enhanced its efficacy against experimental tumors in the treatment of cancer as compared with free CA.

Keywords: Solid lipid nanoparticles, caffeic acid, TEM, Confocal Microscopy.

1.INTRODUCTION

Cancer is a major human health problem worldwide. Over the past 30 years, significant progress has been achieved in understanding the molecular basis of cancer. Many patients being treated for cancer use dietary supplements, particularly antioxidants, in the hope of reducing the toxicity of chemotherapy and radiotherapy. The newer approaches to cancer treatment not only supplement the conventional chemotherapy and radiotherapy but also prevent damage to normal tissues and prevent drug resistance [1-2].

Caffeic acid (CA) and derived compounds have been shown their potential as agents against cancer. Caffeic acid (3,4-dihydroxycinnamic acid) (CA), is one of the most common phenolic acids and has been shown to have anti-inflammatory, immunomodulatory and antioxidant effects and also to suppress lipid peroxidation [3-4]. Caffeic acid has been reported to possess a wide spectrum of biological effects such as antioxidant activity, inhibition of enzyme activities (lipoxygenase, glutathione *S*-transferase, xanthine oxidase), antitumor activity, anti-inflammatory effect, and inhibition of HIV replication [5-6].

CA as the scavenger of reactive oxygen species (ROS) also acts in the protection of some proteins from degradation and fragmentation caused by the increase of oxygen radicals[7]. ROS are known to cause oxidative modification of DNA, proteins, lipids and small cellular molecules. ROS are associated with tissue damage and are the contributing factors for inflammation, aging, cancer, arteriosclerosis, hypertension and diabetes [8].

The use of nanoparticles as drug delivery vehicles for anticancer therapeutics has great potential to revolutionise the future of cancer therapy [9-10]. Nanoparticles based durg delivery systems widely investigated because of many advantages such as smaller size, controlled drug release potential, targeting ability, enhancement of therapeutic efficacy and reduction of toxicity. So, solid lipid nanoparticles(SLN) have recently received considerable attention as alternative drug delivery carrier [11]. SLNs combine the advantages and avoid the disadvantages of the other colloidal carriers (liposomes, emulsions, and polymeric micro- and nanoparticles). For instance; good tolerabilitysmall size, high drug loading, no biotoxicity of the carrier, controlled drug release [12-14]. However, SLN are known for insufficient drug loading, drug expulsion after polymorphic transition on storage and relative high water content of the dispersions [15].

The structure and ultrastracture effects of CA loaded SLN on cancer cells have not been known well. In this study, we

determined CA-SLNs interaction with 5RP7 cells by using transmission electron and confocal microscopy. We examined the type of cell death in 5RP7 (H-ras transform cell line) cells.

2. MATERIAL&METHODS

2.1. Characterization of CA-SLNs

Particle size measurement: The average diameter and Polydispersity Index (PI) of CA-SLNs were determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer (Malvern, UK) at a fixed angle of 90°C and at 25°C. The aqueous SLN dispersions were diluted with distilled water before analysis. Each value is the average of three measurements.
Zeta potential measurement: The particle charge was quantified as Zeta Potential (ZP) using a Zetasizer at 25°C. Before measuring, each sample had to be diluted with distilled water to an adequate intensity. Each measurement was performed at least in triplicate.

2.2. Cell lines and culture conditions

5RP7(H-ras transformed cell line) American Type Culture Collection (Rockville, MD).were chosen for the experiments. Cells were cultured in DMEM medium, supplemented with 10% fetal bovine serum (FBS), in a humidified atmosphere of 5% CO_2 in air, at 37°C. Culture media and serum were from Gibco BRL. Previously, we evaluated the effect of CA-SLNs in 5RP7 cells by using MTT assay. We examined concentration of CA-SLNs (2,5-5-7,5-10-15 and 20 μM/ml). According to MTT assay, we were given the most effective concentration of CA-SLNs (20μM/ml) to determine structure and ultrastructure effects in 5RP7 cells.

2.3. Transmission Electron Microscopy

The uptake of CA loaded solid lipid nanoparticles and CA by 5RP7 cancer cells and ultrastructure changes were determined using a Transmission Electron Microscope TEM FEI Tecnai BioTWIN. 5RP7 cells which were given the most effective concentration of CA- SLNs (20 μM/ml) according to result of MTT assay growing in DMEM medium were fixed in 2.0% glutaraldehyde in phosphate buffer at pH 7.2 at 4°Cfor at least 2 4h. After a washing in phosphate buffer at 4°C,the cells were postfixed in osmium tetroxide(%2) for2 h and dehydrated in graded ethanols then with infiltration and embedded in Araldite-Epon and sectioned on a LEICA EM UC6 ultramicrotome. Sections were stained with 2% uranyl acetate and lead citrate.

2.4. Confocal Microscopy

5RP7 the most effective concentration of the CA-SLN (20μM/ml) were incubated for 24 h at 37 C. After incubation, growth medium was removed and cells were washed with PBS, fixed with 2% gluteraldehyde for 15 min at room temparature. Then cells were washed with PBS and stained acridine orange.

3. RESULTS

3.1. Characterization of SLN

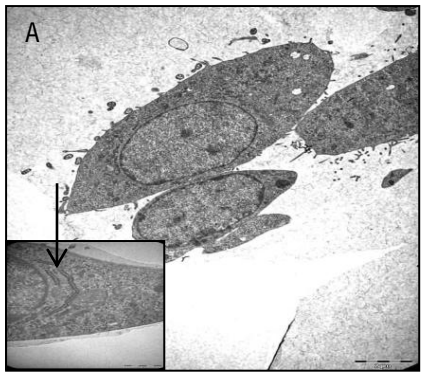
Particle size and Zeta potential with polydispersity index (PI) of caffeic acid loaded SLN. The average diameter of caffeic acid was about 350 nm and the polydispersity index was 0.5. The average diameter of caffeic acid -loaded SLN was about 100 nm, the polydispersity index was 0.3 and the zeta potential 0,6 mV in Table 1.

SLN	Particle Size(nm)	PI	Zeta Potential (mV)
SLN	102,1	0,317	0,620

Table 1. Dimensional analysis by photon correlation spectroscopy CA-SLNs prepared by hot homogenization by precipitation method.

3.2.Images of TEM

Effects of the CA- SLNs on the structure and ultrastructure changes of 5RP7 cells were investigated as seen in Figure 1. Control cells showed their normal shape and surface morphology. We show that normal endoplasmic reticulum and mitocondria. Exposure of 5RP7 cells to CA- SLNs has been shown to indicate mitochondrial changes can be characterized by the interruption or absence of the cristae and the loss of matrix density Numerous autophagic vacuoles (arrows) were observed. Shrinkage of the whole cell volume was observed.



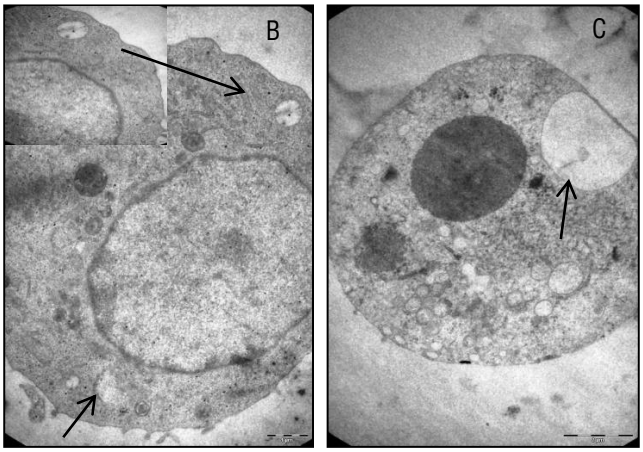


Figure 1. Morphological appearance of 5RP7 cells on electron microscopy. (A) There is normal morphology in the untreated group. (x6000). (B).After CA-SLNs treatment 72 h.(x9900). ⟶Mitochondria (B) and Vacuoles (C)

3.3. Images of Confocal Microscopy

5RP7 cells were tretated CA loaded SLN were stained more densely than control group. CA- SLNs were damaged connection between cells. CA-SLNs entered the cells especially nucleus. CA-SLN killed 5RP7 cells in vitro assays in Figure 2.

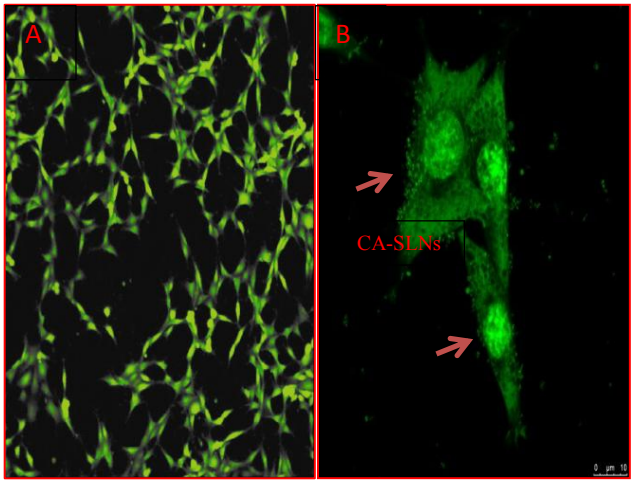


Figure 2. Confocal images of (A) Nontreated cells in their normal fibroblast shape (5RP7) (B) Changes after 20 µM/ml CA-SLNs treatment in 5RP7.

4. DISCUSSION

Various studies have shown that CA and derivative compounds have anticancer properties. For instance; studies by Huang and his colleagues evaluated the possible effects on CAPE (caffeic acid phenethyl ester) gastrointestinal carcinogenesis [16]. Chung and colleagues found that antimetastatic and anti-tumorigenic effects of CA and its derivate CAPE may be mediated through suppression of MMP-9 gene expression by the activation of NF-B and MMP-9 catalytic activity and plays an important role in cancer invasion and metastasis [17]. Studies by Bole and his colleagues evaluated that the esterification reaction of caffeic acid with methyl vanillate to achieve new molecule and may lead to new therapeutic discovery against cancer [18]. In previous studies, anti-carcer drug loaded SLNs were studied in different cancer cells. As a result of these studies, solid lipid nanoparticles display significant cytotoxicity.[19-20]. We determined that the cytotoxiic effects of CA loaded SLNs have more effective in MTT assay in 5RP7 cells. Changes of these cells have shown that CA-SLNs damage in electron and confocal microscopy.

We used CA-SLNs to target cancer cells. The various methods were used in solid lipid nanoparticles. We used hot homogenization method. Ca-SLNs could be prepared successfully.. We obtained more increase percent of death and damage cancer cells by using CA-SLNs. This study may be developed to different cancer cells, nanoparticle systems, preparation techniques and in vivo studies.

5. CONCLUSION

As further research continues in nanotechnology, more treatments will be discovered by using nanoparticle based drug delivery systems. The advantages of nanocarriers over current treatment regimes for cancer therapy include lower toxicity due entrapment of the cytotoxic drug, selectively to diseased tissue and improved bioavailability of the anti-cancer drug. Solid lipid nanoparticle has a great potential to cure the cancer, with least side effects.. In the future, it is expected that better understanding and application of solid lipid nanoparticles for drug delivery systems would ultimately improve efficacy of cancer treatment.

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