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Original paper

The Cytotoxic Effects Biocompatible Nanobubbles Carrying Quercetin on Non-small-cell Lung Carcinoma Cells

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Abstract

Background and aim: Non-small-cell lung carcinoma (NSCLC), as major lung cancer is currently considered as one of the leading causes of mortality and has become a progressively serious global public health burden. However, the conventional drug delivery approaches were unable to efficiently inhibit the proliferation and metastasis of the lung cancer cells. Exploiting nanobubbles (NBs) as a novel drug delivery system have recently a research hotspot mainly due to their outstanding characteristics such as small size, biosafety and competent drug-transporting ability. Present study aimed to establish a novel biocompatible approach for nanobubble constructions by the water-in-oil method and loading quercetin inside the obtained micelles.

Materials and methods: Dextran coating was used for more stability of NBs and the effectiveness of drug delivery to A549 NSCLC cells was evaluated. Ultrasound waves were used to stimulate the nanobubble to release the drug. Excellent drug-loading capacity and ultrasound-mediated release of quercetin were confirmed by UV spectrophotometer with the absorption about 1.6.

Results: NBs efficiently inhibited the proliferation of NSCLC cells in a concentration-dependent manner as well as the capability to achieve ultrasound enhancement. This experiment showed obtained NBs effectively delivered quercetin into lung cancer cells promoted by ultrasound irradiation.

Conclusion: In conclusion, proposed biocompatible quercetin loaded NBs are suitable for ultrasound-targeted drug delivery and are thus a promising strategy for their noninvasive clinical application.

Keywords: *Nano-bubble, Quercetin, Non-small cell lung cancer, Drug delivery*

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Introduction

Although there have been significant advances in preventing, screening and treating of lung cancer, it is still the leading cause of cancer death in many populations worldwide [1]. Indeed, lung cancer is a global health problem, among which non-small cell lung cancer (NSCLC) accounts for 80%-85%. It is estimated that more than 2 million individuals to be newly diagnosed with lung cancer annually [2]. Although there are a number of therapeutic options for NSCLC, including surgery, radiotherapy, chemotherapy, traditional medicine, targeted therapy, immunotherapy, antibody-drug conjugates, and bispecific antibodies, the lung cancer therapy still faces many challenges [3]. The tumor-specific targeting of chemotherapeutic agents for specific necrosis of cancer cells without affecting the normal cells has been the core topic of research in drug delivery systems. Carrier-based drug delivery systems for the treatment of cancerous cells has been drawn the attention of many researchers [4]. Drug delivery systems have the ability to maintain a relatively stable drug concentration for a certain time and the ability to regulate the rate of drug release, depending on the drug delivery location, the possibility of drug delivery to a specific organ or tissue, and the ability to carry multiple drug substances simultaneously [5].

Nanobubbles are nanoscopic bubbles have applications in various fields including drug delivery sytems. They exhibit several characteristic physical properties: excellent stability, high internal pressure, and high surface-to-volume ratio [6], [7]. Nanobubbles can carry the drugs and other agents and deliver them to cancer cells [8], [9].

Quercetin (2-(3,4-dihydroxy phenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one), a nontoxic flavonoids found in fruits and vegetables, is a lipophilic compound that could be absorbed by simple diffusion across the intestinal membrane [10]. Quercetin has been reported to have antioxidant, anti-apoptotic and anti-inflammatory properties, hence, may play an important role in the cancer treatment including the treatment of lung cancer [11]. Studies have shown that quercetin can induce apoptosis in cancer cells through mitochondrial depolarization and by creating an imbalance in Bcl2/Bax expression level. It has been reported that quercetin has a significant contribution to the induction of apoptosis in NSCLC and therefore, it may have a therapeutic application as a potent apoptosis inducer in lung cancer cells [12]. Present study was carried out to investigate the cytotoxic effects of biocompatible nanobubbles carrying quercetin on non-small-cell lung carcinoma cells.

Material and Methods

Making Nano-bubbles Shell with Quercetin

An ethanol solution containing Epikuron® 200 (1%, w/v) and palmitic acid (1%, w/v) was added to perfluoropentan and ultra-pure water under stirring. The solution was saturated with oxygen up to a gas concentration of 35 mg/l. A 2.7% (w/v). Dextran solution (Mw dextran = 100,000) was added drop-wise while the mixture was homogenized using a high-shear homogenizer (Ultraturrax, Germany) for 2 min at 13,000 rpm and continuing the oxygen purge. Quercetin was dissolved in 70% ethanol and stirred for 3 minutes by vertex and add to made nano-bubble. UV - assisted absorption and release was used to obtain the quercetin loading percentage.

Cell Culture

NSCLC and normal lung cells were prepared from Pasteur laboratory (Iran - Tehran) and cultured with DMEM culture medium with 10 % serum zinc for multiplication and passage and flasks were incubated in an incubator at 37 $^{\circ}$ ^{c}. After reaching 70 % confluence, the passage was carried out. For passage, the culture medium was removed and after washing the cells with PBS, trypsin was added to isolate the cells. Cells were incubated for 2 - 3 min in trypsin solution and then DMEM

medium with 10 % FBS was applied to inactivate the trypsin.

MTT Assay Test

Normal and lung cancer cells (A549 cell line) were exposed to different concentrations of quercetin, nano-bubble and loaded nano-bubble with quercetin. Cell viability was evaluated 24 and 48 hours after treatment by MTT assay method.

Results

Figures 1 and 2 show ultraviolet-visible spectra of different concentrations of quercetin. The red graph is the unknown sample and the black colors are the known samples. This analysis shows that the amount of quercetin is loaded on the carrier nanoparticle in different concentrations. At concentrations of 20 to 40 mM/L, no significant absorption peak of the drug has been observed. However, at concentrations of 40 to 500 mM/L, the peak of drug absorption was seen with greater intensity indicating that no additional drug was loaded in these areas.

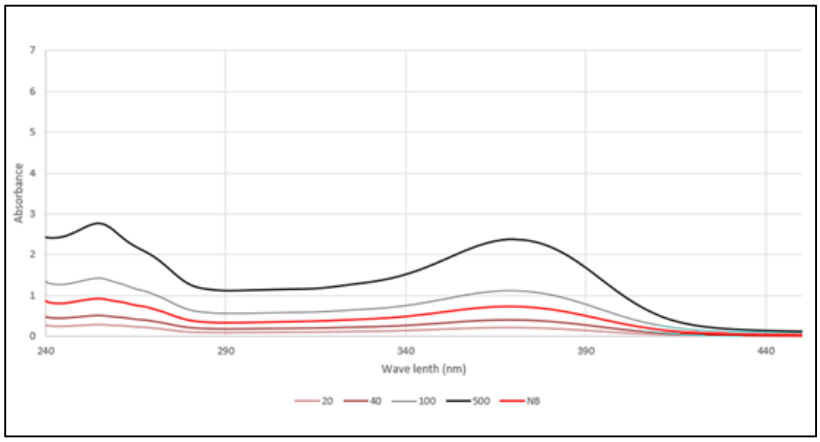

Figure 1. Ultraviolet radiation (UV) spectra of the loaded quercetin.

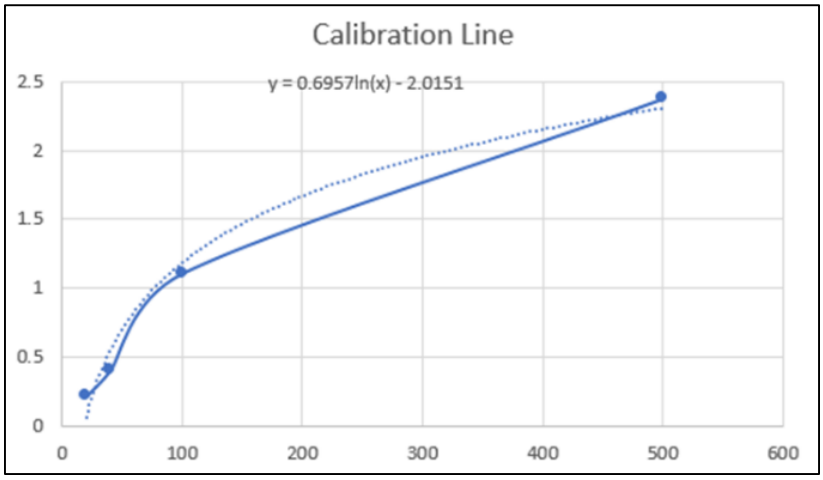

Figure2. Ultraviolet radiation (UV) spectra of quercetin into the carrier at concentrations of 20 - 500 mM/L.

MTT assay results showed that the cell viability was 90% at the highest concentration in normal cells treated with nano-bubble or nano-bubble with quercetin, indicating that nano-bubble or nano-

bubble with quercetin had no considerable cytotoxic effects of normal cells 48 hours after treatment (Fig. 3). However, in lung cancer cells the cell viability significantly decreased when treated with nano-bubble and nano-bubble with quercetin. The cytotoxic effects of nano-bubble with quercetin was higher than nano-bubble 24 and 48 hours after treatment (Fig.4). The cytotoxic effect also followed a dose dependent manner.

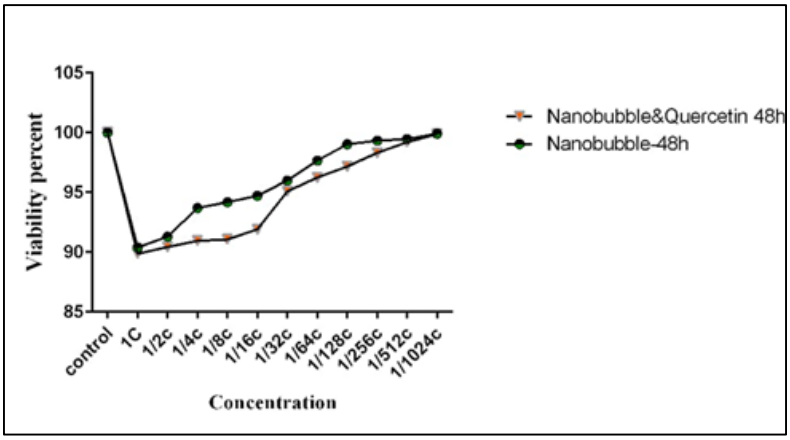

Figure 3. Cell viability of normal cells 48 hours after treatment with different concentrations (mM/L) of nano-bubble and nano-bubble with quercetin.

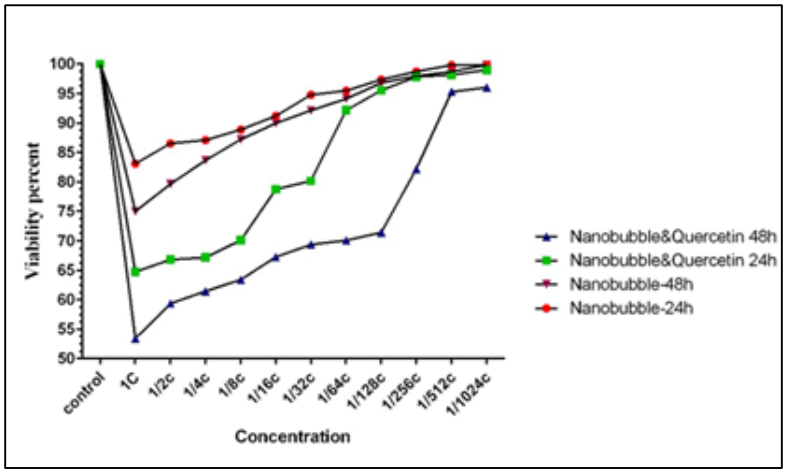

Figure 4. Cell viability of lung cancer cells (A549 cell line) 24 and 48 hours after treatment with different concentrations (mM/L) of nano-bubble and nano-bubble with quercetin.

Discussion

Lung cancer is the leading cause of cancer-related death in the world. Non-small cell lung cancer (NSCLC) comprises 85% of cases [13]. Targeted drug delivery using different nanoparticles has been carried out along with therapeutic drugs to treat lung cancer [14]. In the present study, targeted drug delivery using nano-bubble containing herbal medicine (quercetin) has been shown to have significant cytotoxic effects on cancer cells while did not show considerable cytotoxic effects on normal cells. Although many drugs have been used to treat lung cancer patients in recent years, most of these drugs have had undesirable adverse effects on body normal cells. To address this problem, drug delivery systems are imparted with unique characteristics and specifically deliver loaded drugs at lung cancer tissues [15].

Huang et al also proved the antitumor effects of nano-bubbles containing oxygen and SPION [16]. In another study, Yücel Başpınar et al have shown that bubble nanoparticles containing paclitaxel were able to decrease the cancer cell proliferation [17]. Nano-bubbles containing doxorubicin also have been reported to have cytotoxic effects on breast and cervical cancer cells [18]. Recent investigations reported successful encapsulation of quercetin in chitosan nanoparticles to target the tumour microenvironment and exhibited enhanced efficacy in cancer therapy [19]. In line with our findings it was shown that the administration of quercetin by using nanoparticles can provide a prospective strategy for the treatment of paclitaxel-resistant lung cancer [20]. The micellar formulations also may be considered as an effective drug delivery system for insoluble drugs like quercetin and possess great potential for killing lung cancer cells [21]. Furthermore, various nanoformulations have been highlighted for quercetin delivery for cancer treatment. These results suggest that quercetin nanoparticles may be a promising antitumor therapeutic agent [22].

Conclusion

Our findings indicated that synthesized nano-bubbles effectively delivered quercetin into lung cancer cells promoted by ultrasound irradiation. Indeed, quercetin loaded nano-bubbles are suitable for ultrasound-targeted drug delivery and has a potential in lung cancer treatment.

Acknowledgment

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Conflict of interests

The authors state that there are no conflicts of interest regarding the publication of this article.

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