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Original article

Comparative evaluation of curcumin and curcumin loaded- dendrosome nanoparticle effects on the viability of SW480 colon carcinoma and Huh7 hepatoma cells

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Abstract

Background and objectives: Colorectal cancer is the third most common cancer and a major cause of morbidity globally. Hepatocellular carcinoma is a leading cause of death in the world. About 80% of all anticancer drugs are somehow related to natural products. One of the most important of these natural compounds is curcumin, the main component of turmeric that has a wide range of pharmacological activities. Curcumin has been found to suppress cell proliferation and decrease cell viability in various types of cancer cells; however, owing to lack of aqueous solubility, curcumin has shown reduced bioavailability in studies. Recent studies have shown that new 400th generation of dendrosome nanoparticle can increase bioavailability of curcumin and thus enhance the cytotoxic properties. The aim of this study was to determine effectiveness of curcumin alone and in combination with 400th generation dendrosome nanoparticles (DNC) on cell viability rate in SW480 and Huh7 cells. **Methods:** SW480 and Huh7 cells were incubated with different concentrations of curcumin and DNC (0-50μM) for 24, 48 and 72 h. Then cytotoxicity was assessed by MTT assay and IC_{50} was determined. **Results:** The results suggested that the concentration-dependent inhibitory effect of DNC was stronger than curcumin on SW480 and Huh7 cells. **Conclusion:** The results suggest DNC as a more effective herbal anticancer agent for colorectal and hepatocellular tumors.

Keywords: colorectal cancer, curcumin, dendrosomal curcumin, hepatocellular carcinoma, MTT assay

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Introduction

Colorectal cancer is the third most common cancer and a major cause of morbidity and mortality globally [1,2]. Although most Asian countries show very little incidence of colon cancer, it is the second leading cause of cancer deaths in Western countries [3]. Also, hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide and its incidence is on the rise in developing countries [4]. Nowadays cancer takes place in result of different causes such as mutagenesis and carcinogen chemicals in the environment [5]. Most of mutagenic and carcinogen agents display their destructive effects through free radicals including reactive oxygen species (ROS). Antioxidants are able to reduce ROS; so, daily consumption of antioxidants enhances immunity of the body against free radicals production and serves as anticancer prevention [6]. Some fruits and vegetables are considered as the main anticancer foods, because of their abundant antioxidants such as phenols, vitamin C [7]. One of the most important of these natural compounds is curcumin (diferuloylmethane), the main component of turmeric that has a wide range of biological and pharmacological activities [8]. It has been used as a spice and a traditional medicine for many centuries in India and other Asian countries [9,10]. Chemo-preventive and therapeutic features of curcumin including anti-cancer, anti-inflammatory, antioxidant and anti-proliferative activities were previously confirmed in *in vitro* and *in vivo* models [11]. The most important property of curcumin was observed to be its selective role in targeting cancer cells against normal cells [12,13]. In addition, curcumin was found to suppress cell proliferation and decrease cell viability rate in various types of cancer cells, including colon and liver [14-20]. However, owing to lack of aqueous solubility, curcumin has shown a reduced bioavailability in both *in vitro* and *in vivo* studies. The use of nanotechnology in medicine and more specifically drug delivery is set to spread rapidly. A multitude of substances are currently under investigation for preparation of nanoparticles for drug delivery, varying from biological substances like albumin, gelatine and phospholipids for liposomes, to substances with chemical nature like various polymers and solid metal containing nanoparticles. It is obvious that the potential interaction with tissues and cells, and toxicity, greatly depends on the actual composition of the nanoparticle formulation. To overcome the reduced bioavailability, various combinations of adjuvants, liposomes, dendrosomes and other substances have been used [21]. The anticancer properties of dendrosomal curcumin (DNC) as a coadministration of curcumin and dendrosome nanoparticle have been explored in mice models of fibrosarcoma and different cancers, and toxicological analysis have indicated severe side effects [22-25]. Particularly, findings of a recent study indicated curcumin loaded dedrosome nanoparticles to be efficient in terms of solubility, bioavailability, sustainability and thus enhanced anti-cancer properties [25]. Accordingly in this study, to assess the effects of these drugs alone and in combination with carrier, we compared the efficacy of curcumin-loaded 400^{th} generation dendrosome nanoparticles (DNC) versus free curcumin on cell viability rate in SW480 (colorectal cancer) and Huh7 (hepatocellular carcinoma) cells.

Experimental

Cell lines and chemicals

The human colon cancer SW480 and hepatocellular carcinoma Huh7 cell lines were obtained from Pasteur Institute of Iran (Tehran, Iran) and cultured in RPMI 1640 medium (Invitrogen) supplemented with 10 % fetal bovine serum (Invitrogen) and 1% penicillin–streptomycin (Gibco, Scotland) in 5 % CO_2 at 37 °C. Curcumin was purchased from Sigma-Aldrich Company, USA. Dendrosome nanoparticle specified as Den O400, a nonionic biodegradable denderic glycol ester (MW: 590 Da, HLB: 12.5 Mh/M, hydroxyl value: 95 mg KOH/g and acid value: mg KOH/g) was a gift from Institute of Biochemistry and Biophysics, University of Tehran, Iran [26,27].

Dendrosomal curcumin (DNC) preparation

For DNC preparation, we used the optimized protocol in our lab as previously described [28]**;** briefly**,** different weight/weight ratios of dendrosome/curcumin ranging from 50:1 to 10:1

were examined before settling a suitable ratio of 25:1. Curcumin was dissolved in various amounts of dendrosome and checked for absorbance spectra by UV spectrophotometery (TECAN, Switzerland). Then, the appropriate mixture of dendrosome and curcumin was evaluated for excitation/emission value in comparison with curcumin dissolved in PBS and 1% methanol as control samples. The loading of dendrosome nanocarriers with curcumin molecules was performed using MaLing Gou *et al* protocol [29]. Briefly, curcumin and dendrosome were co-dissolved in 5 mL of acetone; this solution was added to 5 mL of PBS while stirring constantly. Then, acetone was evaporated in rotary evaporator. The curcumin/dendrosome micelle solution was sterilized using a 0.22 µm syringe filter (Millex-LG, Millipore Co., USA). Finally, the prepared DNC was stored in 4 °C in a light protected condition until used. For *in vitro* experiments, DNC was diluted in complete culture medium as mentioned for each assay. Just like our previous studies, the curcumin loading efficiency was very high (87%) [28].

Microculture tetrazolium (MTT) assay

Microculture tetrazolium (MTT) assay was used to assess the inhibitory effect of DNC, free curcumin and free carrier (dendrosome) on viability of SW480 and Huh7 cells. For this purpose, seeded cells were plated in 96-well plates at density of 5000 cells/100 µl PRMI in each well and then, exposed to either control or a serial dilution of DNC, free curcumin and dendrosome ranging from 0 to 50 µM for 24, 48 and 72 h (in previous studies it was found that curcumin decreased the proliferation of SW480 and Huh7 cells with an inhibitory rate of nearly 100 % after 30-40 µM curcumin treatment, as a result the above mentioned concentrations were used). Thereafter, the control medium and the media were replaced by MTT solution (0.5 mg/ mL) and after 3 h, with DMSO. The absorbance was recorded at 570 nm with an ELISA reader (BioTek, USA). The percentage of viable cells was calculated as: (%) = (OD exp/ OD con) × 100, where OD exp and OD con are the optical densities of exposed and control cells, respectively [30]. The 50 % inhibitory concentration (IC_{50}) values of DNC on SW480 and Huh7 cells at different time intervals were determined by GraphPad Prism 5 statistical package. The parameter C or IC_{50} was calculated according to the following equation: $Y= [a-d/1+(X+c^{b})] +d$ [31].

Statistical Analysis

The results were expressed as mean ± SD. All experiments were performed in triplicate. Statistical significance of difference throughout this study were calculated using a two way ANOVA analysis. p <0.05 were considered significant.

Results and Discussion

The inhibitory evaluation, which was carried out through MTT assay using various concentrations (0-50 µM) of DNC, free curcumin and dendrosome at different time intervals (24, 48 and 72 h), demonstrated that DNC and curcumin inhibited cell viability of SW480 and Huh7 cells in a concentration and time dependent manner (p<0.001).

As shown in figures 1 (a, b, c) and 2 (a, b, c), treatment of the cell lines with DNC (0–50 µM) after 24, 48 and 72 h significantly reduced cell viability of SW480 and Huh7 cells more than free curcumin. However, after DNC and free curcumin treatment at 24 and 72 h the cell viability of Huh7 cells were very close to each other after 30µM. Also, no inhibitory effect was observed for dendrosome alone in both cell lines. These findings demonstrated that dendrosome increased water solubility and entry of curcumin to cells without toxic effects correlated to dendrosomes as a carrier.

In order to evaluate the performance of curcumin versus DNC, we compared the IC_{50} of curcumin loaded on dendrosome (DNC) to curcumin in SW480 and Huh7 cells (figures 3 and 4).

Cell sensitivity to curcumin was greatly increased when they were treated with DNC in comparison to curcumin as characterized by a decrease of IC_{50} for SW480 (from 38.72 to 16.09 µM) and Huh7 (from 40.22 to 22.3 µM) after 24 h. Also, 48 and 72 h treatment confirmed the increased cell chemo-sensitivity of SW480 and Huh7 cells to DNC ccompared to curcumin (figures 3 and 4). As controls, drug-unloaded dendrosome nanoparticles did not show any significant cell toxicity when used at concentrations close to the IC_{50} of DNC. Furthermore, coadministration of

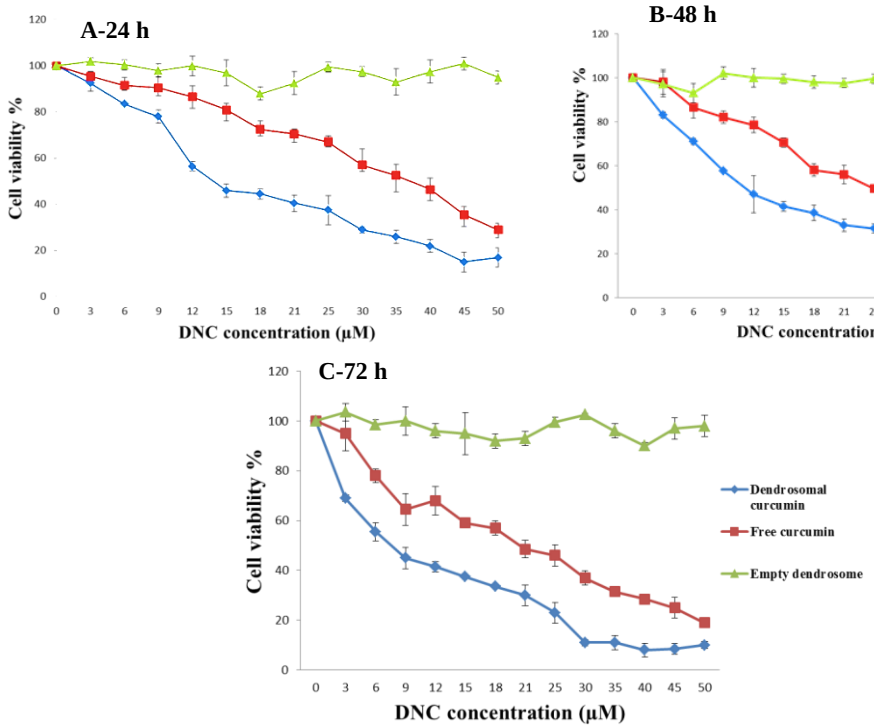

Figure 1. Effects of DNC, free curcumin and dendrosome on SW480 cell viability. Results are expressed as a percentage of viability compared to control and are presented as mean±SD from three independent experiments.

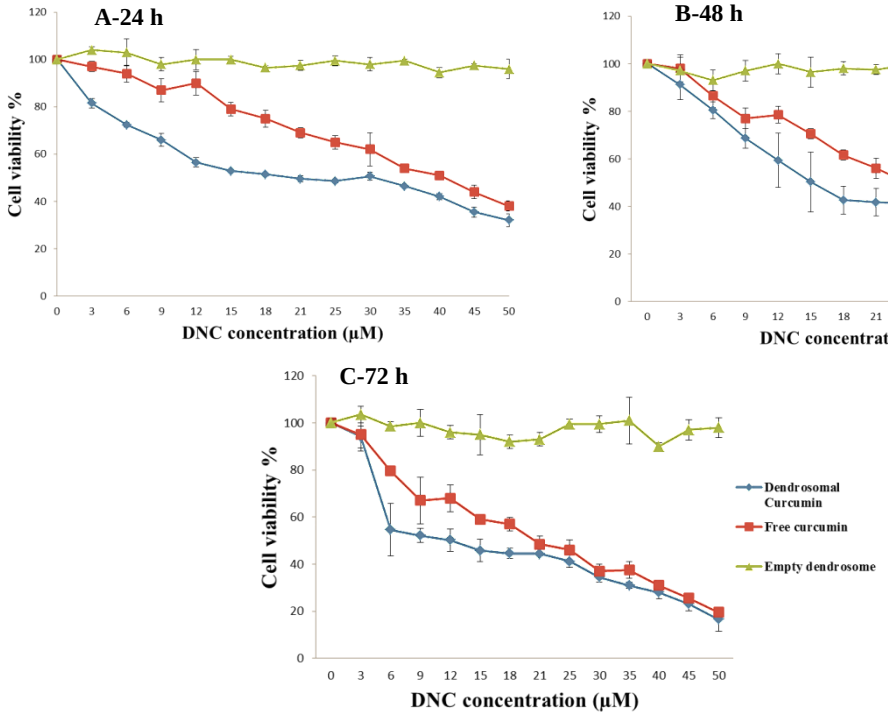

Figure 2. Effects of DNC, free curcumin and dendrosome on Huh7 cell viability. Results are expressed as a percentage of viability compared to control and are presented as mean±SD from three independent experiments.

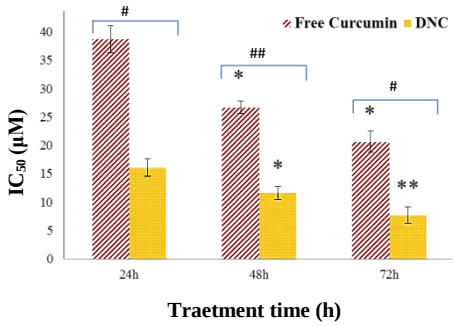

Figure. 3 IC_{50} values of DNC and free curcumin on SW480 cells at different time intervals. Data are presented as mean±SD from three independent experiments. Asterisks show significant differences between the different treatment times compared to 24 h (* P<0.05, ** p<0.01, and # indicates significant differences between DNC and free curcumin at each time point ($^{\#}$ p<0.05, $^{\#\#}$ p<0.01).

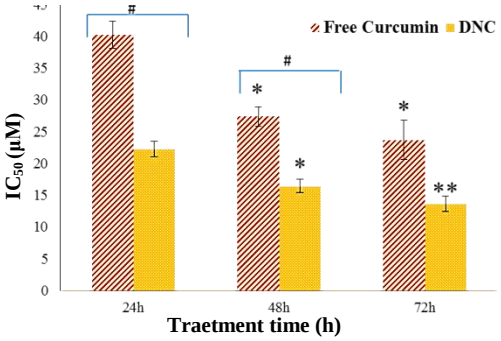

Figure 4. IC_{50} values of DNC and free curcumin on Huh7 cells at different time intervals.
Asterisks show significant differences between the different treatment times compared to 24 h (* p<0.05, ** p<0.01).

drug-unloaded dendrosome and curcumin did not increase the anti-tumor property of curcumin alone, underlining that the cytotoxic activity was linked to the encapsulation of curcumin into dendrosome nanoparticles.

Despite the tremendous progress that has been achieved in the field of cancer biology, the rate of mortality has not changed remarkably due to high toxicity to normal tissues and low efficacy of anti-cancer drugs [13,25,32-34]. Thus, interest is increasing in natural products for prevention of carcinogenesis. Curcumin has a wide range of pharmacological effects, such as inhibition of cell proliferation, cell cycle progression and apoptosis in various cancer cell lines, including colon and liver cancer. It has been suggested that curcumin does not induce side effects of other anti-cancer drugs in *in vivo* and *in vitro* studies [12,13].

The inhibitory role of curcumin on progression and invasiveness of cancer cells has been observed through different *in vitro* and *in vivo* studies [33,35-46]. Gavin P. Collett *et al.* suggested that curcumin impedes colon cancer growth by inducing of apoptosis [47]. Notarbartolo *et al*. found that curcumin exerted cell growth inhibitory and apoptotic effects, at least partly, due to free radical generation which mainly depends on the activation of apoptosis in liver cancer [48]. But its optimum potential is limited by its lack of solubility in aqueous solvents and poor oral bioavailability. Some studies have previously suggested and designed many nanocarrier formulations for increasing curcumin efficiency. Maling Gou *et al.* suggested curcumin encapsulated into monomethoxy poly (ethyleneglycol)-poly(ε-caprolactone) (MPEG-PCL) micelles that induced a stronger anticancer effect than free curcumin in colon cancer [29]. Anand *et al*. employed a polymer-based nanoparticle approach to improve the bioavailability and curcumin-loaded PLGA nanoparticles formulation had enhanced cellular uptake, and increased bioactivity *In vitro* and superior bioavailability *in vivo* over curcumin [19].

In this research we used a new formulation of 400th generation dendrosome nanoparticles as curcumin carrier that had not shown toxicity against different normal cells in our previous studies. Our results, in agreement with previous reports, have clearly demonstrated that sensitivity of SW480 and Huh7 cells to curcumin was substantially increased with curcumin loaded in dendrosome (DNC) compared to free curcumin. In other word, DNC showed a higher cytotoxic activity than free curcumin. As a result, dendrosome might increase solubility, bioavailability and sustainability of curcumin and thus enhance its cytotoxic properties.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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