RESEARCH ARTICLE

PAMAM Dendrimers Augment Inhibitory Effects of Curcumin on Cancer Cell Proliferation: Possible Inhibition of Telomerase

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Abstract

Background: Despite numerous useful anticancer properties of curcumin, its utility is limited due to its hydrophobic structure. In this study, we investigated the comparative antiproliferative effect of PAMAM encapsulating curcumin with naked curcumin on the T47D breast cancer cell line. Materials and Methods: Cytotoxic effects of PAMAM dendrimers encapsulating curcumin and curcumin alone were investigated by MTT assay. After treating cells with different concentrations of both curcumin alone and curcumin encapsulated for 24h, telomerase activity was determined by TRAP assay. Results: While PAMAM dendrimers encapsulating curcumin had no cytotoxicity on cancer cells, they decreased the IC_{50} for proliferation and also increased the inhibitory effect on telomerase activity. Conclusions: Considering the non-toxicity in addition to effectiveness for enhancing curcumin anticancer properties, dendrimers could be considered good therapeutic vehicles for this hydrophobic agent.

Keywords: Dendrimer - curcumin - telomerase - proliferation - breast cancer

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Introduction

Breast cancer is the most common cancer among female which accounts for 23% of the total cancer cases and 14% of the cancer deaths (Jemal et al., 2011). The different molecular factors are involved in breast cancer; it means that it occurs when abnormalities in the genome accumulate due to exposure to the damaging agents, which consequently results in enabling the cells to escape normal regulatory controls. Telomerase is the one of the factors which has a critical role in cell cycle and cell division, and it could promote immortalization and cell transfection (Korkola and Gray, 2010; Mukai, 2010; Adam, 2013). Telomerase is a cellular reverse transcriptase which synthesizes telomeric sequences (the ends of chromosomes) and is considerably activated in the most types of breast carcinomas (over 90%) while it has little to no activity in normal cells (Gasparini et al., 2005). Studies show that in the presence of herbal substances expression and activity of human telomerase is downregulated and decreased (Herbert et al., 2001; Cui et al., 2006, Hsina et al., 2010; Nasiri et al., 2013).

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione] is an orange-yellow component of turmeric (*Curcuma longa*). Curcumin has a long history of use in medicine because of its therapeutic properties including antitumor activity, antioxidant, antiarthritic, antiamyloid, anti-ischemic, and anti-inflammatory effect (Ringman et al., 2005; Bengmark et al., 2009; Jurenka, 2009; Araújo and Leon, 2011; Nejati-Koshki et al., 2013). Despite these valuable properties, curcumin's utility is limited due to its lack of solubility in water and its relatively low bioavailability. Nanoparticle-based drug delivery systems such as dendrimers could be helpful in bypassing these problems (Morgan et al., 2003; Bisht et al., 2007; Shi et al., 2007; Anand er al., 2008).

Dendrimers are repeatedly branched polymeric molecules with three different parts: an initiator core, the branches and the terminal functional groups (Bharali et al., 2009). Dendrimer's structure and their tunable surface make them ideal to either encapsulate or conjugate the desired drug based on the drug properties. These features introduce dendrimers as properly well-suited delivery vehicles for various anticancer agents (Alemdaroglu et al., 2008; Patil et al., 2009). Therefore, we attempted to study the efficiency of nanoparticles in delivering curcumin and enhancing its anti-proliferative effect on breast cancer cells and to compare the inhibitory effect of PAMAM dendrimers encapsulating curcumin with free curcumin on telomerase activity of cancer cells.

Materials and Methods

Cell culture

T47D breast cancer cell line (Pasteur Institute of Iran, Tehran, Iran) was cultured in RPMI1640 (Gibco,

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Invitrogen, UK) supplemented with 10% Heat-inactivated Fetal Bovin Serum (FBS) (Gibco, Invitrogen, UK), 0.05 mg/ml penicillin G (Serva Co, Germany), 0.08 mg/ml streptomycin (Merck Co, Germany). Culture flasks incubated in at 37°C in 5% CO_2.

MTT assay and cytotoxisity

Aliquots of 200μl cell suspension were plated in 96-well tissue culture plates (SPL life sciences co, Korea) (1000 cells/well) and cells were treated with different concentrations of generation 3 PAMAM dendrimer encapsulating curcumin (0.5-60μM) (kindly donated by Ardebil polymer research center). Also some wells were considered as appropriate controls (culture medium, cell control, PAMAM dissolved in PBS). The same concentrations were used for free curcumin dissolved in DMSO, in addition to appropriate controls (culture medium, cell control, DMSO). For analysis of cell viability Trypan Blue (Sigma Aldrich) was used. After 24, 48 and 72h exposure times, medium was replaced with fresh medium and let cells proliferate for two to three population-doubling times (PDTs) and then 50μl 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution (1 mg/ml) was added to each well. The plates were incubated for 4h, allowing viable cells to reduce the yellow MTT to dark-blue Formazan crystals, and then were dissolved in 200μl DMSO and 25μl Glycine Buffer (Sigma Aldrich). Finally absorbance of each individual wells was determined at 595nm using a micro plate reader STAT FAX 2100.

Cells treatment

After IC_{50} determination and cell counting, two 6-well treating plates were seeded by 1×10^6 cells per well. In the first plate, our samples include cell control, control of PAMAM in PBS, and 4 different concentrations of PAMAM encapsulating curcumin. In second plate, we used 4 different concentrations of curcumin (10, 5, 2 and 1μM) equal with the PAMAM concentrations, cell control and the control of DMSO for 24h.

Telomerase activity

Telomerase activity in cancer cells was measured according instructions of TeloTAGGG Telomerase PCR ELISA^{PLUS} kit (Cat. No. 12 013 789 001, Roche Applied Science, Germany). In brief, drug-treated or untreated cells (10^6 cells/well) were lysed on ice for 30 min and then were centrifuged at 12,000 rpm for 20 min at 4°C. Total protein concentration was measured using Quick Start™ Bradford Protein Assay kit (Cat. No. 500-0201, Bio-Rad, USA). For each sample, 1-25µg of the total extracted protein was added to 25µl of the reaction mixture include in the kit (negative and positive control). PCR conditions were cycles at 94˚C for 30 sec, 50˚C for 30 sec and 72 ˚C for 90 sec. Then the PCR products were analyzed by ELISA-based hybridization, according to the package instruction and using a microplate (ELISA) reader, the absorbance of the samples was measured at a mean wavelength of 450 nm. The intensity obtained from the lane with untreated control cells was considered as a reference for telomerase activity and accordingly, those obtained from the lanes of treated ones were normalized.

Statistical analysis

SPSS 16 was used for statistical analysis. The difference in mRNA levels of hTERT between control and treated cells was assessed by ANOVA and Tukey's test. A p value <0.05 was considered as significant difference.

Results

Effect PAMAM encapsulating curcumin and free curcumin on T47D cell growth

PAMAM encapsulating curcumin had a cytotoxic effect on T47D cells with an inhibitory concentration at 50% (IC_{50}) of concentrations of 10.5μM for 24h, 9.5μM for 48h and 9μM for 72h (Figure 1). It seems not to have a significant impact on exposure time. In other words it shows a time independence effect. Regarding free curcumin dissolved in DMSO, IC_{50} on T47D cells for 24h, 48h, 72h were 22.5μM, 19μM, 17 μM respectively

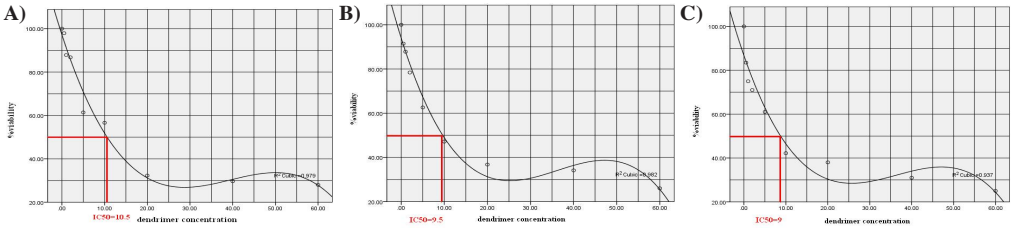

Figure 1. Cytotoxic Effect of PAMAM Encapsulating Curcumin on T47D Breast Cancer Cell Line after A) 24h; B) 48h; and; C) 72h, Exposure

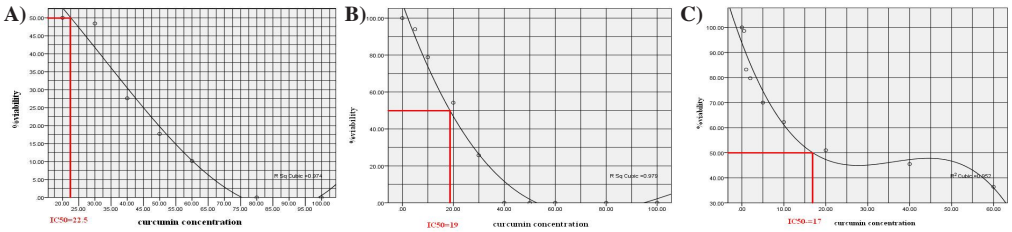

Figure 2. Cytotoxic Effect of Curcumin on T47D Breast Cancer Cell Line after A) 24h; B) 48h; and; C) 72h, Exposure

Table 1. Total Protein Extracted from Samples (Curcumin/PAMAM Encapsulating Curcumin Treated Cells and Controls) by Bradford Assay Kit

Sample	Controls			Curcumin (μM)				PAMAM (μM)			
	Cell control	DMSO	Dendrimer in PBS	1	2	5	10	1	2	5	10
Protein quantity(μg/ml)	30.01	29.84	30.02	27.8	23.2	19.9	12.3	22.7	19.3	13.4	9.8

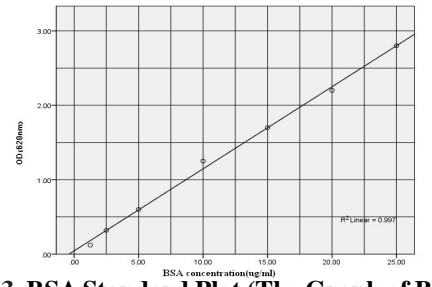

Figure 3. BSA Standard Plot (The Graph of Bradford Assay Standard Curve for BSA)

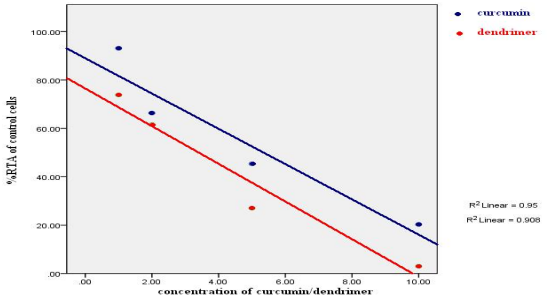

Figure 4. Correlation between Different Concentrations of Curcumin/PAMAM Encapsulating Curcumin and Telomerase Activity in T47D Breast Cancer Cell Line after 24h Treatment

and curcumin showed a time- and dose-dependent effect on T47D breast cancer cells (Figure 2).

Protein measurement

Concentrations of extracted total protein of each samples (curcumin treated cells, PAMAM encapsulating curcumin treated cells and controls) were obtained according to the standard curve which was drawn by 7 standard concentrations of BSA. Figure 3 and Table 1 show measurement total protein extracted from all samples.

Effect PAMAM encapsulating curcumin and free curcumin on telomerase activity

After exposure to PAMAM encapsulating curcumin and free curcumin for different concentrations and 24h incubation time, telomerase activity in treated T47D breast cancer cell line was assessed by TRAP assay. Quantification of %RTA of each samples showed that telomerase activity was decreased by the incubation with PAMAM encapsulating curcumin in a concentration-dependent manner. This pattern was the same for samples which were treated with free Curcumin. At 24h incubation with PAMAM encapsulating curcumin, the percent mean of the inhibition of telomerase activity in T47D cell line for concentrations of 10, 5, 2 and 1μM was 73.81, 61.65, 27 and 3%, and for free curcumin was 93.1, 66.3, 45.3 and 20.3% respectively (Figure 4). The most interesting feature is that in the same concentrations of free curcumin and encapsulated curcumin, the effect of PAMAM encapsulating curcumin is considerably more than free curcumin. This trend was kept the same manner in all 4 examined concentrations. These findings show that telomerase expression and activity are affected by curcumin and dendrimer encapsulating curcumin, and dendrimers have profound impact on enhancing potential of curcumin in inhibition of telomerase.

Discussion

In the present study PAMAM encapsulating curcumin could efficiently increase antiproliferative effect of curcumin on T47D cancer cell line. It seems that curcumin affects cancer cells through the inhibition of telomerase activity and it consequently suppresses cancer cell's proliferation.

Not surprisingly, increasing the concentration of PAMAM encapsulating curcumin, in cell treating process, decreased the %RTA (Relative Telomerase Activity) of treated cells, but the intensity of this change was noticeably higher than the %RTA of curcumin treated cells (Figure 4). Regarding the inactiveness of pure PAMAM dendrimers in cancer cells, it seems that this dramatic decrease in %RTA indicates the ability of this kind of nanoparticles in probable enhancing the effect of curcumin in inhibition of telomerase. Inhibition of telomerase was closely correlated with the antiproliferative property of these therapeutic agents on T47D cell line.

Recent work by Nomani et al. has demonstrated the cytotoxic effect of different generations of PAMAM dendrimers on T47D cell line. They have explored that the cytotoxicity of PAMAM dendrimers is closely related to their generation number. In fact generation number of dendrimers affects their cellular uptake and, consequently, the cytotoxicity of the encapsulated drugs (Nomani et al., 2010). It is noteworthy that the cytotoxic effect of the PAMAM dendrimers encapsulating drugs is not related to PAMAM structure, but it is directly related to the encapsulated drug. As such using PAMAM dendrimers dissolved in PBS as carrier control, we did not observe any cytotoxicity on treated cells. The polyamidoaminoid structure of PAMAM is biocompatible and it just enhances curcumin's uptake through the encapsulation which therefore increases cytotoxicity of curcumin. Dan Liu et al. showed that LA-PEG-b- PSD/PAMAM/DOX complexes display higher antitumor effect compared with DOX solution, due to the improved uptake of DOX by HepG2 cells (Liu et al., 2011).

Wang et al. (2010) used from a generation 4 PAMAM as a gene vector to deliver AODNs into breast cancer MDA-MB-231 cells. While dendrimers had not any significant cell toxicity, they enhanced the cellular uptake

of ODNs. The experiments went forward to human breast tumor xenograft mice model. *In vivo*, G4 PAMAM dendrimers showed more efficacies in gathering VEGF-ASODN and demonstrated more inhibitory effect on the tumor vascularization of breast tumor tissue than bare AODN. More importantly, the effect of G4 PAMAM was not only limited to the delivering of drugs across the cell wall, but also protected the DNA molecule from digestion by the cytoplasmic restriction enzymes (Wang et al., 2010). Furthermore, PAMAM dendrimers have a useful result on delivering siRNA against Bcl2 and decreasing of gene expression without any cytotoxicity on A2780 human ovarian cancer cell line (Patil et al., 2009).

Other study by Winnicka et al. (2010) showed similar effects of two modified Glycosides (digoxin and proscillaridin A) conjugated to a generation 3 polyamidoamine dendrimer (G3 PAMAM-NH2) on human breast cancer cells. They stated that conjugation with the G3 PAMAM-NH2 dendrimer enhances the cytotoxicity of both modified digoxin and proscillaridin A in MCF-7 and in MDA-MB-231 breast cancer cells. Moreover, dendrimer conjugated form of modified digoxin and proscillaridin A stimulated apoptosis significantly greater than free forms of them (Winnicka et al., 2010).

Finally, we can conclude that PAMAM dendrimers could be considered as well-suited carriers for hydrophobic therapeutic agents such as curcumin. PAMAM dendrimers encapsulating curcumin have a considerably inhibitory effect on cancer cell proliferation. Protection of the encapsulated drug, low cytotoxicity and using potential proton sponge effect, through their surface located amine groups, make PAMAM dendrimers as effective tools in drug delivery and cancer research (Patil et al., 2009). Although not having any time-dependent effect might be due to the limited release rate of the loaded drug, overall PAMAM dendrimers increase the drug uptake.

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