Case Report

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Design and Construction of Curcumin – Loaded Targeted Iron Oxide Nanoparticles for Cancer Treatment

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ABSTRACT

BACKGROUND AND OBJECTIVE: Considering the increasing number of patients with cancer and the relative ineffectiveness of existing treatments, finding a modern technique for cancer treatment has been one of the major topics of research in recent decades. The present study aims to load curcumin anticancer drug on targeted iron oxide nanoparticles.

METHODS: In this laboratory research, iron oxide nanoparticles were synthesized using polyol method. Then, they were coated with polyglycerol as a polymeric coating through ring – opening polymerization method. Folic acid (with three weight ratios of 2, 25 and 50%) was used to target the system constructed for specific penetration into the cancer cells. The experiments of loading the drug were performed with three weight ratios of 0.5, 1 and 2 µg nanoparticles on coated and targeted nanoparticles. Then, drug release rate was measured under in vitro conditions. Finally, MTT Assay was used to analyze cell toxicity of the loaded drug.

FINDINGS: Results indicated successful construction of 20 nm nanoparticles. The maximum rate of drug loading into the system was about 88 and 82% for non-targeted nanoparticles and targeted nanoparticles, respectively, while increased targeting had adverse effects on drug loading. Moreover, the loaded drug had a more successful therapeutic effect compared with the free drug.

CONCLUSION: Results of the study demonstrated that the constructed nanoparticles have the necessary efficiency to act as a system for transferring anticancer drug.

KEY WORDS: *Iron Oxide Nanoparticles, Targeting, Curcumin, Cancer*.

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Introduction

Curcumin is a low–molecular–weight hydrophobic polyphenol compound that is extracted from the rhizome of the curcuma longa plant. Most of the extracted powder, which is called Zard chobe (turmeric) in Persian language, consists of curcumin (2 and 1). Although this drug has been used in Asia for a wide range of diseases, including respiratory infections, centuries ago, it has been widely considered as an anti-inflammatory, anti-oxidant, and anti-cancer drug in western countries in recent years (3).

Despite the mentioned properties, low solubility, poor stability and rapid metabolism in internal conditions have greatly limited its use in clinical conditions (4–6). In the field of cancer treatment, drug delivery systems play a very important role in increasing the efficacy of treatment. In general, the drug delivery system is referred to carriers that have the ability to bind to the drug, hold the drug and carry it in the body (7).

Nanostructures can specifically penetrate cancerous tissues through inactive transfer systems, such as enhanced permeability and retention effect or active transfer systems, which are due to the binding of ligands such as small molecules, peptides or antibodies (8). The development of nanoparticle-based materials to release therapeutic agents provides a promising approach to improve the solubility, sustainability and biodistribution of therapeutic agents. In fact, this targeted release system leads to increased therapeutic efficacy, reduced drug dosage and reduced toxicity due to factors in healthy tissues (2).

Different structures have been developed for drug release systems, including dendrimers, micelles, liposomes, and polymer-based nanoparticles (9). Among the substances widely used in drug release systems, iron oxide magnetic nanoparticles are highly considered due to their special properties, such as their small size, low toxicity and high specific surface area, and magnetic properties (10).

So far, various types of nanosystems based on iron oxide nanoparticles have been designed and used to transfer the drug and one of the most abundant types of these systems is polymer nanosystems. Various types of natural and synthetic polymers are used to coat iron nanoparticles, including a study by Liang et al. in 2016 that used polypropylene glycol – coated nanoparticles to transfer doxorubicin (11). In another study in 2017, Patitsa et al. used nanoparticles coated with polyarabic acid polymers to transfer doxorubicin and introduced the system as a theranostic agent (12). The choice of polymer type directly depends on the biocompatibility, hydrophilicity and lack of protein absorption. Hyperbranched polyglycerol (HPG) is a group of hydrophilic polymers with properties such as high biocompatibility, low toxicity, and easy synthesis. In addition, these structures have many hydroxyl end–groups that can attach targeted ligands to them (13). This is the first reported system consisting of polyglycerol – coated and targeted iron oxide with folic acid and the present study aims to produce polyglycerol – coated iron oxide nanoparticles and target them with different percentages of folic acid. The curcumin drug is then loaded into the system and its ability to load and release the drug is evaluated. Finally, the system loaded on the cancer cell line is tested and the results are compared with the free drug sample.

Methods

Materials: Iron (III) acetylacetonate, ethyl acetate, dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP), manufactured by Merck. Glycidyl, dimethyl sulfoxide (DMSO), folic acid (FA), curcumin (CUR) and MTT, manufactured by Sigma. In addition, the required chemicals were used with laboratory grade.

Synthesis of Coated Iron Oxide Nanoparticles with Polyglycerol and Folic Acid Binding: The iron oxide nanoparticles were made using a polyol method and were coated with polyglycerol as a polymeric coating through ring – opening polymerization method (14). Three targeting ratios (5, 25 and 50% folic acid) were used to target the nanoparticles. For 5% targeting, 5 mg folic acid with 100 mg of Fe3O4 @ HPG were dissolved in 5 ml of DMSO. 1.53 mg DMAP and 2.955 mg DCC were added as catalysts and connectors to a solution containing Fe3O4–HPG, respectively. Then, the heating was carried out at 50 ° C for 36 hours. After the process completed, the solution was dialyzed using a 12 kDa dialysis bag to remove DCC, DMAP and unbound FA. After dialysis, the targeted Fe3O4 @ HPG with FA were dries using freeze dryer. Nanoparticles were targeted using 5, 25 and 50% folic acid (15).

Loading curcumin on Fe3O4 @ HPG and Fe3O4 @ HPG @ FA: In order to evaluate the loading of curcumin, three drug-nanoparticle ratios of 2:1, 1:1 and 1:2 were prepared and the samples were placed inside a polymer coating for 24 hours at 4 ° C in the shaker for penetration of curcumin. After 12 hours, samples were

centrifuged for deposition of unloaded curcumin content at 4000 rpm for 15 minutes. The drug loading efficiency in each of the ratios was calculated using the following equation:

$$\text{Curcumin loading efficiency} = \frac{\text{Initial curcumin level} - \text{unloaded curcumin level}}{\text{Initial curcumin level}} \times 100$$

Curcumin release from Fe3O4 @ HPG and Fe3O4 @ HPG @ FA: Curcumin release from Fe3O4 @ HPG and Fe3O4 @ HPG @ FA was done in phosphate buffered saline (PBS) (PBS) at 37°C and for this purpose, the drug was measured at certain intervals of up to 8 days. For this purpose, the sample of the drug – containing nanoparticles was transferred to the dialysis bag and placed in PBS solution. After the desired period, the PBS solution was centrifuged to release the curcumin deposition and the absorbance of the drug was measured at 450 nm. The concentration of released curcumin was calculated and the drug release rate was obtained using the initial curcumin content according to the following equation:

$$\text{Curcumin release percentage} = \frac{\text{Total released curcumin from the first hour until the time of test (mg)}}{\text{Initial curcumin level (mg)}}$$

MTT Assay: MTT assay was used on HeLa cell line to evaluate the toxic effect of curcumin carrier nanosystem. For this purpose, 5,000 cells were cultured in each well of 96-well plate. After 24 hours, the culture medium was removed and the medium containing drug carrier nanoparticles and equivalent concentration of free drug were added to the plate. After three intervals of 24, 48 and 72 hours, MTT assay (at 0.5 mg/ml in PBS) was performed and the optical absorption of wells was read at 492 nm by ELISA reader.

Statistical analysis: The data obtained from MTT test were analyzed using two-way analysis of variance (ANOVA) and Tukey test while $p<0.05$ was considered significant.

Results

The result of the FT-IR test of polyglycerol – coated and targeted nanoparticles with folic acid increased significantly in the vibration peaks in the range of 3300, 2900, and 1100 cm^{-1}, respectively related to O–H, C–H and C–O–C bonds, indicating glycidol polymerization on iron magnetic nanoparticles (Fig 1). In addition to confirming the polymerization, increased O–H peak rate in figure 1a indicates increased hydrophilicity of coated nanoparticles (16). As shown in Figure 1, the peak in 1100 area has decreased after nanoparticle targeting. At 3400-3500 and 1560-1640 areas, acid folic – related primary amine bonds are present and they cannot be observed in 3400-3500 area due to bonding to the hydroxyl group (figure 1b ,c, d) (17). In the 1650 area, the peak is related to the C = O amide group and in the 1700 area, the peak is related to C = O steric and carboxylic group present in the molecular structure of the folic acid, while ester peak itself is a reason for the correct attachment of folic acid to a surface polymer. In Figures 1b, c, d related to folic acid – targeted samples, the peaks of the petridine loop and aminobenzoic acid motif are observed in the 1400 and 1620 areas, respectively. In area 1640, there is also the peak of the C = C bond of the 6-carbon loops in the folic acid structure, which is well observed in the targeted samples in the form of significantly increased peaks compared with the coated sample (18).

Curcumin loading and release: Drug loading diagram with three different drug ratios: Since the changes for 1:2 ratio is lower, this ratio was selected to perform drug release analysis for coated and targeted nanoparticles. The amount of drug loading here is about 87% and 78% for the coated and targeted nanoparticles, respectively. As shown in Figure 2, drug loading decreases with increase in percentage of targeting, since carboxylic curcumin groups interact with folic acid – related carboxylic groups and prevent the entry of curcumin into the polymeric network.

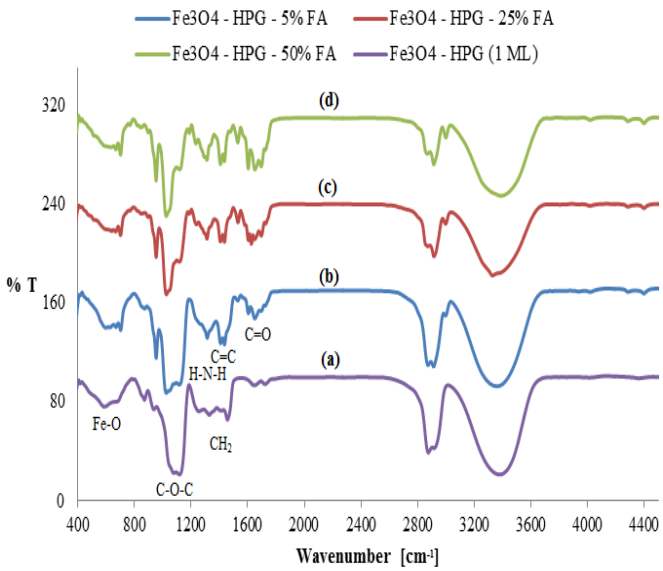

Figure 1. FT-IR test for iron oxide nanoparticles coated with (a) polyglycerol polymer and nanoparticles targeted with (b) 5 (c) 25 (d) 50% folic acid

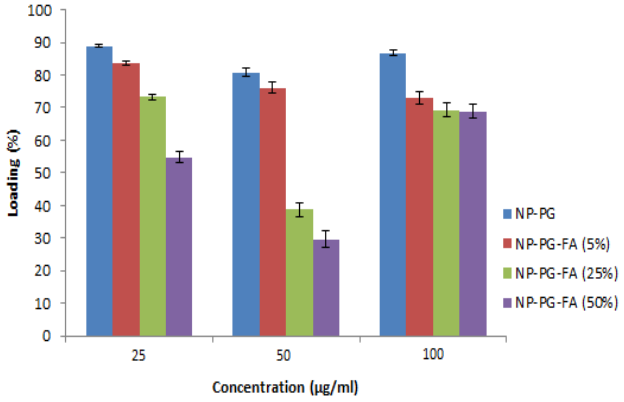

Figure 2. Curcumin loading diagram of nanoparticles coated and targeted with 3 targeting percentages (5, 25 and 50% folic acid)

The results of curcumin release from coated and targeted nanoparticles with 3 targeting percentages (5, 25 and 50%) showed that the highest drug release rate was about 75% and 60% for coated and targeted nanoparticles. The release rate of curcumin in non-targeted nanoparticles is far more than targeted nanoparticles. In addition, increase in targeting percentage decreases release rate of curcumin (Fig 3), probably due to the increase in folic acid carboxylic groups, which prevents drug withdrawal from the polymer branches by interacting with curcumin groups.

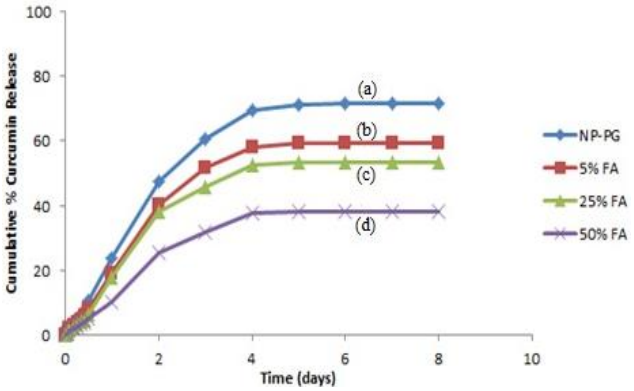

Figure 3. Curcumin release diagram for nanoparticles (a) coated with high-density polyethylene glycol and nanoparticles targeted with (b) 5 (c) 25 (d) 50% folic acid

MTT test: The result of the MTT assay on the HeLa cell line at three time intervals of 24, 48 and 72 hours showed as the period of exposure of cells to drug – carrier nanoparticles increases, cell survival decreases. In fact, by increasing the interval, curcumin release from the polymeric network increases, and among the various targeting percentages, the most inhibitory effect of growth is observed in targeted nanoparticles with 5% folic acid, in which the survival rate of the cells was reduced by about 40%, since the amount of curcumin loaded was far more than the other targeting percentages (Fig 4). The comparison between free curcumin with curcumin loaded on the nanocarrier shows that the penetration of targeted nanoparticles into the cell is much higher and shows higher toxicity. The results of variance analysis indicate that there is a significant difference between the type of nanosystem and the interactions of nanosystem/time interval and the survival of cells, while the time interval alone does not show a significant difference, and it does not affect cell survival ($p<0.05$). The results showed that the difference between mean sample with control for nanosystem samples and free curcumin samples was significant in 48 and 72 hours, whereas there is no significant difference for free curcumin in 24 hours, which indicates that the nanosystem is effective against free curcumin ($p<0.05$). In addition, the difference in mean nanosystems with free curcumin for targeted samples showed a significant difference, but for a non-targeted sample, there was no significant difference in 24 hours, which generally indicates the effect of the targeting factor in improving drug toxicity on the cancerous cell ($p<0.05$).

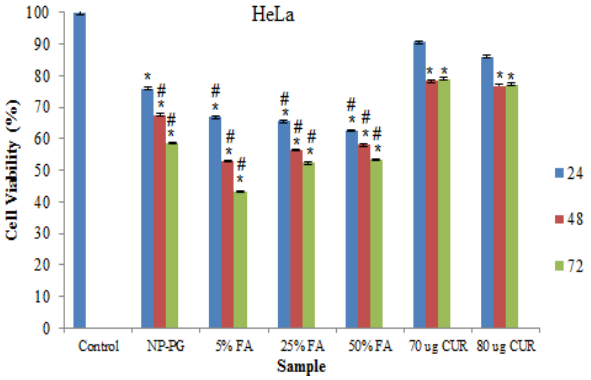

Figure 4. The results of MTT testing of the drug carrier nanosystem on the HeLa cell line in 24, 48 and 72 hours intervals. * p<0.05: significant different with control group. # p<0.05: significant difference with mean 24, 48 and 72 hours. 70 µg CUR

Discussion

The results of this study indicate the construction of a new successful nanosystem aimed at loading and transferring anticancer drugs and controlled drug release, which managed to have significant therapeutic effects compared to free drugs. So far, several studies have been dedicated to designing and manufacturing

nanosystems to be used as carriers of anticancer drugs (19). In a study by Salem et al., they used purified β – cyclodextrin – coated magnetic nanoparticles to transfer curcumin. The results of curcumin loading revealed that after targeting nanoparticles, curcumin loading efficiency decreased from 73.4% for coated nanoparticles to 41.4% for targeted nanoparticles (20). As we can see, the amount of drug loading in both cases, with and without targeting, is lower than that of the present study, and this value is particularly significant for targeted nanoparticles, indicating the superiority of the designed system in this study to the mentioned group, which can be attributed to the ability of polyglycerol to expand the loading of a higher volume of hydrophobic drug.

Nam et al. used chitosan targeted with folate to transfer curcumin. This study also proved that loading the targeted agent onto the polymer surface causes a significant reduction in the ability to load the drug by the nanosystem (21). Qi et al. used iron oxide nanoparticles coated with targeted dimercaptosuccinic acid polymer to transfer curcumin. The targeted nanoparticles in this study with primary amine end groups, have a loading percentage of 76.29% which is less than what was reported in the present study (22). In another study by Lian et al., Hydroxypropyl beta-cyclodextrin – coated iron-gold nanoparticles were used to load curcumin. Here, four-fold nanocomposite concentrations relative to the drug were used and an 88% loading rate for the drug was obtained, which is similar to that introduced by the nanocomposite introduced in this study. However, in the present study, the concentration of nanocarrier is half the drug and it can be concluded that its capacity for drug acceptance is higher (23).

Despite all the studies on the use of iron oxide nanoparticles and polyglycerol coatings as drug delivery systems, the present study was the first to investigate the use of iron oxide nanoparticles coated with polyglycerol and targeted with folic acid, which are used for controlled release of curcumin. The results of the study demonstrated that the nanoparticles made in this study have the necessary efficacy to act as a system for the transfer of anticancer drugs.

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