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Original article

Idarubicin-loaded folic acid conjugated magnetic nanoparticles as a targetable drug delivery system for breast cancer

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ABSTRACT

Conventional cancer chemotherapies cannot differentiate between healthy and cancer cells, and lead to severe side effects and systemic toxicity. Another major problem is the drug resistance development before or during the treatment. In the last decades, different kinds of controlled drug delivery systems have been developed to overcome these shortcomings. The studies aim targeted drug delivery to tumor site. Magnetic nanoparticles (MNP) are potentially important in cancer treatment since they can be targeted to tumor site by an externally applied magnetic field. In this study, MNPs were synthesized, covered with biocompatible polyethylene glycol (PEG) and conjugated with folic acid. Then, anti-cancer drug idarubicin was loaded onto the nanoparticles. Shape, size, crystal and chemical structures, and magnetic properties of synthesized nanoparticles were characterized. The characterization of synthesized nanoparticles was performed by dynamic light scattering (DLS), Fourier transform–infrared spectroscopy (FT–IR), transmission electron microscopy (TEM), scanning electron microscopy (SEM) analyses. Internalization and accumulation of MNPs in MCF-7 cells were illustrated by light and confocal microscopy. Empty MNPs did not have any toxicity in the concentration ranges of 0–500 μg/mL on MCF-7 cells, while drug-loaded nanoparticles led to significant toxicity in a concentration-dependent manner. Besides, idarubicin-loaded MNPs exhibited higher toxicity compared to free idarubicin. The results are promising for improvement in cancer chemotherapy.

1. Introduction

Chemotherapy is a systemic therapy using free drugs with poor biodistribution and targeting. Since conventional chemotherapeutics target only dividing cells without the ability of differentiation between cancer and healthy cells, their success is still questionable [1]. Targeted therapy agents have been developed to overcome the severe side effects of traditional chemotherapeutics on healthy cells. However, acquisition of drug resistance has also become a significant obstacle in targeted therapy [2]. Idarubicin, which is a synthetic analogue of daunorubicin, acts by intercalating between DNA base pairs and inhibiting topoisomerase II [3,4]. Additionally, it induces free oxygen radicals leading to destruction of DNA and cell membrane [4]. Idarubicin differs from daunorubicin due to its lack of methoxy group at position 4 of the D ring of the aglycone [5,6]. This synthetic modification leads to high lipophilicity, better DNA binding, and greater cytotoxicity. However, despite its efficiency idarubicin possesses various side effects, like myelosuppression, neutropenia, induction of secondary tumours and disease relapse due to multiple drug resistance (MDR). This necessitates its formulation into novel nanosized drug delivery vehicles to exploit its benefits in minimizing its systemic exposure and thus reducing non-specific toxicity, but retaining its efficacy at the tumour site [7]. The polymer coated magnetic nanoparticles have been widely studied as drug vectors, based on their lack of toxicity, biodegradability, good biocompatibility, and absorption [8–10]. General structure of magnetic nanoparticles (MNPs) is

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composed of an inner magnetic core and an outer polymeric shell. Magnetic core is usually a magnetite (Fe_3O_4) or maghemite (Fe_2O_3). It is covered with a polymeric shell, which renders MNPs biocompatibility, prevents their agglomeration and functions as drug reservoir [11]. Different types of polymers and molecules are used for covering surfaces of naked magnetic nanoparticles to stabilize them and for further biological applications. Starch, dextran, polyethylene glycol, fatty acids, polyvinyl alcohol, polyacrylic acid, poly lactides, gelatin and chitosan are some of the examples of widely used coating materials for different purposes [12]. PEG is one of the mostly used synthetic polymers to cover MNP. It gives MNP a hydrophilic surface and minimizes agglomeration. Thus, PEG coating enhances the circulation time of MNPs by reducing their phagocytosis by macrophages [13]. One of the most important limitations of cancer therapy is the lack of specificity of anti-cancer drug delivery. There is an increasing demand for the improvement of the effectual delivery of drugs to the targeted tumor site. There are distinctive features between cancer and healthy cells, which can be, used as preferred targets in the treatment. Cancer cells often express several proteins on the cell surface in greater amounts then the normal cells. One of the most preferable drug carrier models is multifunctional nanocarriers that comprise a high payload of drugs and targeting moieties, which bind to specific proteins that are overexpressed in cancer cells [14–16].

Folic acid receptors (FR) are found to be overexpressed on different types of cancer cells including breast cancer [17]. Normal cells do not express FR or it locates on apical surface of polarized epithelia where drugs cannot reach [18]. Besides, while only reduced folate can be transported in healthy cells, cancer cells are able to transport folate conjugates by FR via receptor mediated endocytosis. By this mechanism, folic acid covered and drug-loaded nanoparticles can overcome drug resistance caused by P-glycoprotein efflux pumps [2]. The natural characteristics of folic acid and folic acid receptors on cancer cells make them very efficient agents for drug targeting being ameliorate the severe side effects of free drugs and overcome drug resistance [18].

The aim of this study is synthesis and characterization of PEG coated, folic acid conjugated, idarubicin-loaded magnetic nanoparticles; investigating their drug release properties, internalization, and antiproliferative effects on MCF-7 cells for potential utilization as a targetable drug delivery system in breast cancer treatment.

2. Material and methods

2.1. Materials

Iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), oleic acid, polyethylene monooleate, folic acid, dicyclohexyl carbodiimide (DCC) and dimethylsulfoxide (DMSO) were purchased from Sigma–Aldrich (USA). Ammonium hydroxide solution (NH_4OH) was obtained from Merck (Germany). Dimethylsulfoxide (cell culture grade) was obtained from Applichem (Germany). MCF-7 monolayer type human epithelial breast adenocarcinoma cell line was provided from Food and Mouth Diseases Institute (Ankara), RPMI 1640 medium [(1×), 2.0 g/L $NaHCO_3$ stable glutamine], fetal bovine serum were obtained from Biochrom Ag. (Germany). Trypsin–EDTA solution (0.25% Trypsin–EDTA), gentamycin sulphate (50 mg/mL as base), tryphan blue solution (0.5%), cell proliferation kit (XTT assay) were obtained from Biological Industries, Kibbutz Beit Haemek (Israel).

2.2. Preparation of MNPs

Magnetic iron oxide (Fe_3O_4) nanoparticles were synthesized by the co-precipitation of Fe(II) and Fe(III) salts at 1:2 ratio in 150 mL deionized water within a five-necked glass balloon [19,20]. It is vigorously stirred in the presence of nitrogen (N_2) gas at 90 °C. Ammonium hydroxide (NH_4OH) is added to the system dropwise. The process ends by washing with deionized H_2O until the solution pH is 9.0.

2.3. Preparation of oleic acid coated MNPs

Oleic acid was directly added into synthesis system with iron oxide nanoparticles and stirred for 1 h by mechanical stirrer. Particles were washed 3 times with acetone and ethanol to get rid of excess amount of oleic acid and labeled as OA–MNP.

2.4. Functionalization of MNPs with PEG

Oleic acid conjugated PEG monooleate was used as polymeric surfactant. It was hypothesized that the oleate part of PEG monooleate would adsorb onto oleic acid coating on the iron oxide core. Hence, PEG could form an exterior surface layer that renders MNP hydrophilicity. After the synthesis of oleic acid coated MNPs, the temperature of the system was decreased to room temperature, and the aqueous solution of PEG monooleate was added to system and the stirring was continued for an additional 24 h at room temperature. PEG coated MNP obtained by this method was labeled as PEG–MNP.

2.5. Modification of MNPs with folic acid

Folic acid needs the activation of its carboxyl group with dicyclohexyl carbodiimide (DCC) to conjugate to surface polymer [21]. Folic acid and DCC with 1:1 ratio were added in dimethyl sulfoxide (DMSO) and stirred for 2 h. PEG–MNP sample was added into system and continuously stirred for 2 h under a nitrogen atmosphere. After washing of the nanoparticles with dH_2O, they were freeze-dried for one night. The sample stirred for 2 h labelled as FA–PEG–MNP.

2.6. Characterization of magnetic nanoparticles

The characterization of synthesized nanoparticles was performed by dynamic light scattering (DLS), transmission electron microscopy (TEM) and Fourier transform–infrared spectroscopy (FT–IR).

2.7. Light microscopy observation of FA–PEG–MNP treated cells and Prussian blue staining

MCF-7/S cells were seeded in 6-well plate (25,000 cells/well) and incubated with FA–PEG–MNPs (500 μg/mL). After 48 h of incubation, cells were washed with PBS and observed under light microscopy (40×) (Olympus, USA). Prussian blue staining kit (Sigma–Aldrich) was also used to show the cell uptake of FA–PEG–MNPs. After 8 h incubation period of MCF-7 cells with FA–PEG–MNPs (200 μg/mL), Prussian blue staining was performed according to the manufacturer's instructions. Accumulation of nanoparticles was visualized under light microscopy (40×) (Olympus, USA).

2.8. Idarubicin loading on FA–PEG–MNPs

Idarubicin (IDA) was prepared as stock solution, which dissolved in DMSO. FA–PEG–MNPs (1 mg) and idarubicin in the range of 30–400 μM (in 2 mL PBS) were rotated at 95 rpm for 24 h while being protected from light. After the incubation period, IDA-loaded FA–PEG–MNPs were separated by magnetic separation and the idarubicin loading efficiency was quantified by measuring the