DEVELOPMENT OF AMINATED STARCH COATED IRON OXIDE MAGNETIC NANOPARTICLES FOR CURCUMIN DELIVERY

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Abstract:

Aminated starch-coated iron oxide nanoparticles containing curcumin loaded with halloysite nanoclay were developed to investigate their cytotoxicity on lymphocytes and cancer cell lines. These nanoparticles were prepared using different concentrations of halloysite to study the effect of nanoclay on cytotoxicity. FTIR study confirmed the synthesis of drug loaded nanoparticles. The cytotoxicity assay revealed excellent compatibility of the system with lymphocyte cells while considerable amount of cytotoxicity on cancer cell lines. The nanoparticles with 3% halloysite showed satisfactory results.

Key words: aminated starch, curcumin, cytotoxicity, halloysite nanoclay, lymphocytes, cancer cell lines

Introduction:

As mortality rate due to cancer rises over the years, researches on this area regarding diagnosis, prevention and treatment of the disease continuously are in progresses. Different drugs of anticancer potential have been explored till date. Curcumin is a polyphenol derived from the rhizome of the herb *Curcuma longa* which is commonly known as turmeric. *Curcuma longa* is a native perennial plant of Southeast Asia, which is a member of the ginger family 'Zingiberacae' [1]. It has been extensively used as Ayurvedic medicine since it has a variety of therapeutic properties likes anti- oxidant, anti- cancer, analgesic, anti- inflammatory etc. and it is non-toxic. Despite these promising properties, curcumin has a major drawback of extremely low aqueous solubility which limits its bioavailability and clinical efficiency [2].

Extensive research have been done over the years for the development of drug delivery agents that have better therapeutic, low toxicity and can target cancer cells. Magnetite iron oxide nanoparticles have drawn much interest in this area due to their biocompatibility, low toxicity and superparamagnetic character [3]. However, the main disadvantage related to magnetic nanoparticles is their tendency for agglomeration, due to strong magnetic dipole–dipole attractions between particles, during synthesis process. Therefore surface coating of these magnetic nanoparticles become the research of interest. Various biocompatible polymers have been explored by the researchers [4]. The surface modification by these polymeric materials fulfil different tasks like stabilize the nanoparticles, control the particle size, provide biocompatibility, biodegradability, and binding sites to various functional biological molecules like peptides, folates, enzymes, etc., which will practice to formulate target specific drug delivery system. One of the engineered materials in the drug delivery applications is Halloysite nanotubes. Their specific structure draws the attention. They contain ultra-tiny hollow tubes (diameter < 100nm) composed with double layers of aluminium, silicon, hydrogen and oxygen [5]. Moreover it has no *in vivo* toxic effect. Wei et al. Reported enhanced encapsulation and sustained release of antimicrobial agents from halloysite nanotubes [6].

In this study halloysite nanotube has been incorporated into aminated starch matrix and applied as the coating material for the iron oxide nanoparticles. The curcumin loaded nanoparticles were crosslinked with genipin and studied their swelling and drug release characteristics along with the cell viability studies on human lymphocytes and HepG2 cell lines.

Experimental

Materials

Starch, ethylene diamine, genipin and halloysite were obtained from Merck, India. Curcumin, N-hydroxysuccinimide (NHS), dicyclohexyl¬ carbodiimide (DCC), [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] (MTT) (M- 5655) were purchased from Sigma Aldrich, Germany. Epichlorohydrin was purchased from SRL, India. RPMI-1640, FBS (fatal bovine serum), DMEM and Penicillin-streptomycin antibiotics were purchased from HiMedia Laboratories, India. HepG2 cell lines were obtained from NCCS, Pune, India. Rest of the chemicals were of analytical grade and used as received.

Synthesis of curcumin loaded Halloysite nanoclay incorporated aminated starch coated iron oxide nanoparticles

Starch was aminated by using the procedure given by Wang et al. [7]. For the synthesis of iron oxide core, a 2:1 solution of $FeCl_3.6H_2O$: $FeCl_2.4H_2O$ was prepared in 25 mL water under N_2 atmosphere followed by addition of the above aminated starch solution. To this solution a swelled and well sonicated solution of halloysite (1, 3 and 5% w.r.t. polymer) was added. Now a solution of NaOH was added drop-wise to this solution until black precipitation occurred.

To load curcumin in the nanoparticles, 0.01 g of curcumin in 10 mL mixture of water and minimum amount of ethanol was added dropwise to an aqueous dispersion of nanoparticles. The mixture was then stirred overnight at 500 rpm on a magnetic stirrer in order to facilitate penetration of the drug molecules into the polymer layers. Those nanoparticles were then crosslinked by 3% genipin solution.

Cytotoxicity analysis of curcumin loaded nanoparticles

The cytotoxicity studies were done two types of cells i.e. human lymphocytes and HepG2 (liver cancer cell) cells. Human blood was anti-coagulated using Histopaque (1.077 g/mL) and lymphocytes were isolated from it. Aliquotes of 200 mL of the isolated cells were cultured in RPMI supplemented with 10% heat inactivated FBS. At first cells were maintained in RPMI for 4h without FBS at 37 ºC in 5% CO_2 in an incubator. Then they were treated with the nanoparticles and maintained n presence of FBS for 12 h [8]

HepG2 cell lines were grown in DMEM supplemented with 10% FBS 10 µg/mL penicillin-streptomycin antibiotics. Cells were maintained at 37ºC and 5% CO_2 in a humidified incubator and were sub cultured when they reached 80-90% confluence.

Cell viability study was done using conventional MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay [9]. The cells were seeded in a 96 well plate at a density of 1×10^3 cells per well. Then the cells were incubated and allowed to adhere at 37 ºC for 48 h. After incubation, the medium was replaced with fresh medium and then treated with varying concentrations (50–150 µg/mL) of the samples. Control cells were treated with equivalent volume of media. After 48 h, the media was discarded and replaced with 10% MTT in PBS followed by dissolving the formazan crystals in DMSO (100 µL) and measuring the absorbance at 570 nm. The absorbance of control cells was separately set as 100% viability and calculated as percentage control.

Cellular uptake studies

Cellular uptake studies were done in HepG2 cell lines. The cells ($1x10^5$) were seeded in a six well plate in 2 mL medium containing 50 µm of the drug loaded nanoparticles. After 12 h incubation time the cells were washed with PBS and stained with Prussian blue to detect the presence of nanoparticles in the cancer cells.

Characterization

Fourier Transmission Infra-red Spectroscopy (FTIR) study:

FTIR was done by using Nicolet (model impact-410) spectrophotometer scanned in the range of 4000-400 cm^{-1}.

The FTIR study (Fig 1) confirmed the presence of different characteristics functional groups of the constituents of nanoparticles. In the spectrum of curcumin (Fig 1a) peaks appeared at 1600 cm^{-1}, 1446 cm^{-1}, 1265 $cm^{-1}$and at 1000 cm^{-1} were due to C=C stretching of benzene ring, aromatic C-O stretching vibration and C-O-C stretching vibration [10]. In the spectrum of halloysite (Fig 1b), peaks appeared at 3690 cm^{-1}, 3630cm^{-1}, 1640 cm^{-1}, 1060cm^{-1}, 893 cm^{-1}were due to OH- stretching of inner surface hydroxyl group, OH- stretching of inner hydroxyl group, deformation of water, corresponded to Si-O stretching and OH bending [11]. In the spectrum of iron oxide (Fig 1c) the peak appeared at 575 cm^{-1} indicated the stretching vibration of Fe-O and peak at 1635 cm^{-1} represented OH stretching and HOH bending vibrational bands due to absorbed water in the sample [12].The peak at 3269 cm^{-1} represents O-H stretching mode.

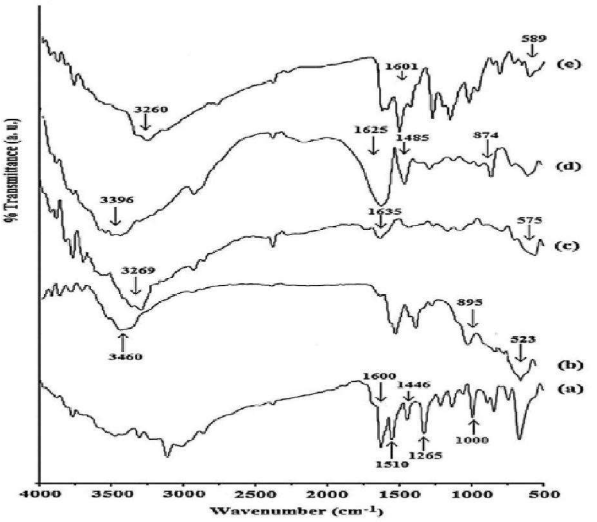

Fig 1: FTIR study

In the spectrum of aminated starch (Fig 1d), the peaks appeared at 3396 cm^{-1}, 874 cm^{-1}, 1485 cm^{-1}, 1625 cm^{-1} were due to OH- stretching which overlaps with N-H stretching in the same region, N-H bending, CO- stretching and N-H bending vibration. In the spectrum of drug loaded nanoparticles (Fig 1e), the presence of all characteristics peaks of curcumin, aminated starch, iron oxide and halloysite as well as shifting of peaks to lower wave numbers confirmed the successful interaction of all components in the nanoparticles.

Results and Discussion

Cytotoxicity studies

In human lymphocytes

Cell viability studies (MTT assay) on human lymphocytes are shown in Fig 2a. In lymphocytes, it was seen that free curcumin, genipin and halloysite did not show much toxicity. Only bare iron oxide showed 80% cell viability. However, when coated with aminated starch, the iron oxide nanoparticle system showed good cell viability. The results demonstrated that the systems had no cytotoxicity. They are biocompatible and have potentiality in biomedical applications.

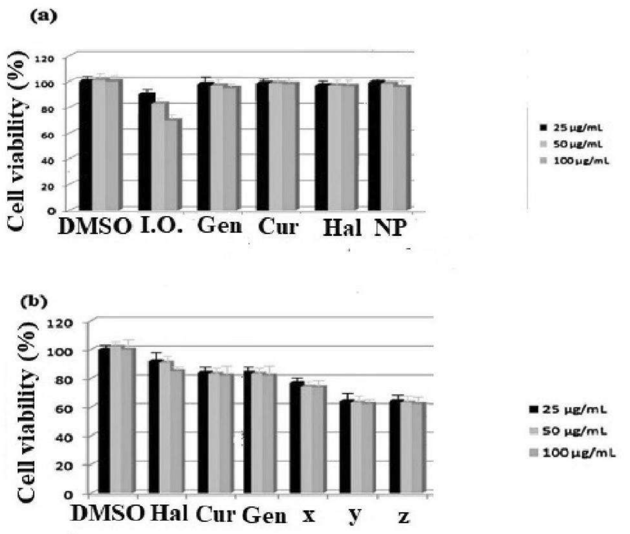

Fig 2: MTT assay in (a) human lymphocytes and (b) HepG2 cell

(I.O.: iron oxide, Gen: genipin, Cur: curcumin, Hal: halloysite, x: NP/Hal1, y: NP/Hal3, z: NP/Hal5)

In HepG2 cell line

In HepG2 cells, the cell viability studies (Fig 2b) demonstrated that free halloysite did not have any influence on cell viability while curcumin loaded nanoparticles showed dose dependent toxicity. Free genipin lowered the viability upto 85% indicating its effect on growth of cancer cells. The results showed that free curcumin shoed ~82% cell viability while curcumin loaded nanoparticles showed upto 69% cell viability. The acidic pH of cancer cells might facilitate higher amount of drug release which leads to decrease in cell viability. The genipin crosslinking might also help to reduce the cell viability which could be due to cytotoxic nature of genipin [1]. The nanoparticle toxicity was more than that of the free curcumin, suggesting better internalization of curcumin into the cells. The cell viability % was almost similar with the nanoparticles having halloysite concentration in the range of 1 to 3%. It increased marginally when halloysite concentration was increased to 5%. This could be due to slower release of drug caused by the formation of bigger particle sized nanoparticles. Smaller sized nanoparticles can easily penetrate through the cell membrane and thereby higher toxicity.

Cellular uptake studies in HepG2 cell line

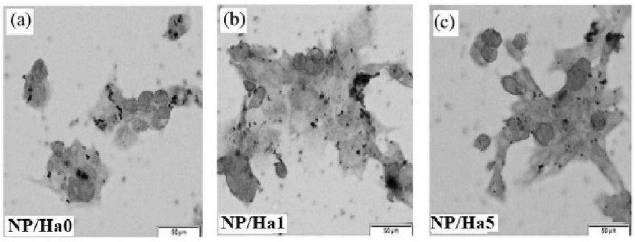

Fig 3: Cell viability studies (MTT assay)

The Prussian blue staining images of HepG2 cells internalized with four synthesized nanoparticles are sown in Fig 3. Visual assessment indicated that among the four formulations, the nanoparticle with 5% halloysite (Fig 3c) had highest cellular uptake proficiency. The findings of cellular uptake studies established linear relationship with the MTT assay results. Surface charge plays an important role in intercellular delivery [1]. At cancer cell pH (pH<7), the aminated starch coating of the magnetic nanoparticles become protonated and as a result acquired positive charge. Since plasma membrane contains negative charges, therefore the synthesized nanoparticles might have easily internalized into the cancer cells.

Conclusion

The magnetic nanoparticles coated with aminated starch were synthesized through simple co-precitation technique for controlled release of curcumin. MTT assay studies showed their compatibility with human lymphocyte cells and anticancer activity on HepG2 cancer cell lines. Curcumin loaded nanoparticles with 3% halloysite were found to reduce the cell viability in cancer cells up to 69%. Cellular uptake studies further confirmed these findings. The results suggested for the future development of targeted curcumin delivery systems the probable use in biomedical fields.

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