Nanotheranostic approach for cancer therapy and imaging

HER 2 conjugated multifunctional cubic phase nanoparticles (CPNPs): A nanotheranostic approach for cancer therapy and imaging

Abstract

Recently, cubic phase nanoparticulate systems are used as attractive vehicle in drug delivery research due to sustained release of both hydrophilic and hydrophobic drugs for cancer therapy. Currently, theranostic approach also emerged as frontier proposition for cancer therapeutics and diagnostics for better cancer management. Moreover, targeted drug delivery systems may be applied to increase the therapeutic index of the drugs and imaging agents at the targeted site. With an aim to develop a theranostic nanoformulation capable of drug delivery and imaging in a single shot, in the present study, HER 2 antibody conjugated cubic phase nanoparticles (CPNPs) were prepared by entrapping rapamycin (as a model anticancer drug) and QD 605 (as an imaging agent). The physicochemical characterisations of CPNPs were performed by different techniques. The antibody conjugated to CPNPs was confirmed by FT-IR and ^{1}H NMR spectral analysis. *In vitro* studies were performed and demonstrated that, HER 2 conjugated rapamycin loaded CPNPs showed improved therapeutic efficacy than that of native rapamycin and rapamycin loaded CPNPs in HER 2 positive SKBr 3 cell line following enhanced cellular uptake through receptor mediated endocytosis. The molecular mechanisms of the particulate system on different signalling pathways were evaluated by western blot analysis. Further, the use of above system as an imaging modality was elucidated through optical imaging under *in vitro* system. Thus, these multifunctional CPNPs can be used as an

effective targeted drug delivery system for cancer therapy and diagnosis in coming future.

Introduction

The life threatening disease cancer now becomes a challenge for survival worldwide due to its high mortality rate. So to achieve a better treatment modality, researchers are trying to develop new methodology for better management of cancer. Though chemotherapeutic agents are effective to some extent, however, clinical applications of these anticancer drugs are remained as a major problem due to their hydrophobic nature, low accessibility at the targeted site and dose limiting toxicity. With cancer tissues these drugs also affect the normal tissues with detrimental side effects [1, 2]. To overcome the lack of tumor specificity of cancer chemotherapy and diagnosis, till date nanomedicinal approaches have emerged as a hope for better therapeutics in cancer by using different nanocarriers like micelles, liposomes, solid lipid nanoparticles and nanoparticles consisting of biodegradable polymers etc. These approaches ultimately enhance the therapeutic potentiality of the drug in a sustained, controlled and targeted manner for longer period of time reducing the lethal side effects arose due to the drugs [3-5].

Recently, due to the capability to solubilise the hydrophilic, hydrophobic and amphiphilic molecules, cubic phase nanoparticulate systems are used as attractive vehicle in controlled drug delivery research [6]. In this regard, glyceryl monooleate (GMO) a synthetic lipid amphiphilic molecule approved by food and drug administration is known to form different lyotropic liquid crystalline phases depending upon water content. The cubic phase formed

from GMO is a three dimensional network of curved lipid bilayers separated by intricate network of congruent water channels, now has been used for controlled drug delivery of different water soluble and insoluble drugs in the form of CPNPs [7, 8]. The use of surfactants may modulate the phase behaviour by controlling the shape and size of the nanoparticles which is an important parameter to cross different physiological barriers. In this current scenario, usually the surfactants like poly vinyl alcohol (PVA), tween 80, Pluronic F-127, Pluronic F-68, polysorbate 80 and vitamin-E TPGS etc are used as stabilisers in clinical studies to increase the therapeutic efficacy of the drug at the tumor site. In this regard, Barauskas *et al* have demonstrated that using Pluronic F-127 as a stabilising agent, stable aqueous nanoparticle dispersions may be obtained from the cubic phase of GMO [9]. Also it is shown that Pluronic F-127 through passive targeting can enhance the transportation of the drug towards cancerous tissues by inhibiting the drug efflux transporters [10]. Similarly, a well known natural polymer vitamin-E TPGS due to its biocompatible nature and excellent emulsifier in nanotechnology, is used for lipid-based drug delivery formulations. Moreover, also it can be used as an absorption enhancer and bioavailability promoter and facilitates the nanoparticles for carrying the drugs through gastrointestinal barrier. Apart from the above properties, recently vitamin-E TPGS is reported to inhibit p-gp pump effectively [11-13].

Recently, rapamycin and it analogs has been shown to inhibit the cancer cell growth obtained from breast cancer, pancreatic cancer, prostate cancer, lung cancer, rehabdomyosarcoma, glioblastoma, neuroblastoma, osteosarcoma, leukemia and β-cell lymphoma. Rapamycin is a cytostatic

agent and it shows its function by arresting the cells in G1 phase of cell cycle. The antitumor activity of rapamycin is found by blocking mTOR pathway, proposed to be an important target for treatment of cancer due to its involvement in tumorigenesis and angiogenesis on aberrant activation [14-16]. In spite of having potent anticancer effects of rapamycin, the clinical application is restricted due to its low bioavailability and hydrophobic nature [17]. In this milieu, nowadays different nanoparticulate systems are being used by different group of researchers to improve the therapeutic efficacy of rapamycin with a smaller dose for longer period of time in a controlled manner [2, 18]. Targeted drug delivery vehicles are now emerged for improvement of treatment modality of the therapeutic agents and/or imaging agents strictly localising its pharmacological activity at the site of action through active targeting [1]. The surfaces of nanoparticles are decorated with peripheral ligands which bind specifically to the receptors those are over expressed in cancer cells arbitrating ligand-receptor interaction. Human epidermal growth factor receptor-2 (HER 2), tyrosine kinase transmembrane receptor are highly expressed in different types of cancers like breast cancer, pancreatic cancer, gastric cancer, glioblastoma, ovarian cancer etc. Humanized anti-HER2 monoclonal antibody (mAb) trastuzumab (Herceptin) was approved by U. S. Food and Drug Administration for clinical trials and it exclusively binds to HER 2 receptors preventing the cell growth in breast cancers. Targeted drug delivery with the help of mAb has drawn attention as an alternative approach for antibody mediated drug delivery of the chemotherapeutic agents for effective clinical translation [19, 20].

Nowadays optical imaging has been explored in biomedical research due to its high sensitivity, cost effectiveness, portability and lack of radiation and it shows marvellous implication by providing information for both *in vitro* and *in vivo* studies [21, 22]. The development of light emitting semiconductor nanocrystals known as quantum dots (QDs) have emerged as one of the most exciting interface of nanotechnology due to its unique optical and chemical properties over organic dyes. The unique properties of these QDs lead powerfully to study in molecular, cellular and in vivo imaging with advantages including narrow, symmetrical and tunable emission spectra according to their size and material composition, broad absorption spectra, high quantum yield, extremely photostable, more brightness and resistance to chemical degradation [23, 24].

In this study, we have formulated a theranostic nanocarrier for targeted drug delivery and imaging in treatment of cancer. Here, preparation and characterisation of HER 2 conjugated rapamycin and QD 605 loaded CPNPs based on GMO was done by blending pluronic F-127, vitamin-E TPGS, TPGS-HER 2 as a targeting moiety. Therapeutic evaluation of our formulated CPNPs was performed by taking both HER 2 positive and negative cell line through different in vitro experiments. Results confirmed that HER 2 conjugated rapamycin loaded CPNPs due to their higher uptake through receptor mediated endocytosis were more effective than that of unconjugated CPNPs and native drug treated case. Furthermore, in vitro cellular imaging study of formulated multifunctional CPNPs was validated by confocal microscopy and the results suggested that this multifunctional particulate system can act as

a single bullet capable of obtaining a new concept for better therapeutics and diagnostics in cancer.