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Integration of *in silico* modeling, prediction by binding energy and experimental approach to study the amorphous chitin nanocarriers for cancer drug delivery

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ABSTRACT

In silico modeling of the polymer–drug nanocarriers have now days became a powerful virtual screening tool for the optimization of new drug delivery systems. The interactions between amorphous chitin nanoparticles (AC-NPs) with three different types of anti-cancer drugs such as curcumin, docetaxel and 5-flurouracil were studied by integration of computational and experimental techniques. The drug entrapment and drug loading efficiency of these three drugs with AC-NPs were (98 ± 1%), (77 ± 2%), and (47 ± 12%), respectively. Further, cytotoxicity and cellular uptake studies of drug loaded AC-NPs on Gastric adenocarcinoma (AGS) cells showed enhanced drug uptake and cancer cell death. *In silico* binding energy (BE) between AC-NPs with these anti-cancer drugs were studied by molecular docking technique. Computational drug's BEs are in excellent agreement with experimental AC-NPs drug loading (R^2 = 0.9323) and drug entrapment (R^2 = 0.9741) efficiencies. Thus, present integrated study revealed significant insight on chemical nature, strength, and putative interacting sites of anti-cancer drugs with AC-NPs.

1. Introduction

Development of the polymeric nanoparticles (NPs) derived biocompatible nanocarrier systems is an empirical procedure, where the bonding of the drug to its carrier nanoparticle is generally based on its chemical nature. Other factors contributing to the stable integration of the drug and the nanoparticle are rigidity, conformation and configuration of the drug and the polymer. Experimentally, optimizing such nanoparticle drug delivery systems is not feasible and very time consuming. Therefore, instead of solely relying on the experimental techniques for identifying the suitable carrier and its drug delivery, a computational approach will certainly reduce the time and effort in studying the same (Costache, Sheihet, Zaveri, Knight, & Kohn, 2009; Allen & Geldenhuys, 2006). *In silico* modeling of the biocompatible nanocarriers with different drugs has recently contributed valuable insight in the field of nanomedicine. Drug delivery using polymeric NPs showed improved therapeutic efficiency and minimal undesirable side effects in different diseased cells (Rejinold, Chennazhi, Tamura, Nair, & Rangasamy, 2011; Duncan, 2006; Davis & Shin, 2008; Wang & Thanou, 2010; Anitha, Deepa, Chennazhi, Lakshmanan, & Rangasamy, 2014a; Anitha et al., 2014b).

The drug discovery process involves development of carefully engineered design of the novel targeted drug delivery systems at the diseased sites, for maximum therapeutic effect. Broadly, drugs which are poorly soluble have limited bioavailability and low absorption profile, which will result in poor pharmacokinetics, limiting its clinical application. It has been estimated that 50% of the newly synthesized drugs are hydrophobic in nature. Several strategies and formulations have been widely studied to overcome these limitations resulting in enhancing the bioavailability of poorly absorbed drugs. Apart from the target specificity, the NPs have the ability to improve the bioavailability of the cargos to lessen the adverse side effects (Chung, Si, & Yanli, 2014; Namgung et al., 2014). A robust computational model has not yet been designed for studying the drug–polymer interactions in order to optimize different physico-chemical properties of the nanocarrier systems. Few researchers have exploited the need of *in silico* strategies for developing the robust drug delivery systems (Allen & Geldenhuys, 2006; Haddish-Berhane et al., 2006; Patel, Lavasanifar, & Choi, 2008). Poly (lysine) based dendrimer nanocarrier system to deliver anticancer

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drug doxorubicin (DOX) at the specific site of diseased cell was being developed (Al-Jamal et al., 2013). Further, the study revealed that DOX molecule made different hydrogen bonding interactions with the dendrimer for its delivery at the target specific tumor sites for maximum therapeutic effect. Another classic example is the IT-101 polymer based nanocarrier system consisting of alternate segments of cyclodextrin and PEG together with the functional carboxylate groups, in which the camptothecin drug was conjugated to make the polymer–drug complexes (Davis, 2009; Gabizon et al., 1983).

In silico studies are developing at a high pace in the field of drug designing (structure-based drug design and ligand-based drug design) and discovery process by targeting bio-macromolecules, such as proteins and DNA (Durdagi et al., 2009; Gupta et al., 2011). But, such developments are in its initial phase in the field of nano systems using polymeric NPs (Costache et al., 2009). This analysis will definitely guide in developing the robust and biocompatible drug delivery systems. The main concern is that polymers as compared to proteins do not have specified secondary or tertiary structures, and also they do not have a precise binding site where the drug molecule interacts. But, one can exploit polymeric assemblies showing different electrophilic/hydrophobic regions of interest for drug interactions, and which in turn depend on its three dimensional (3D) folding. Thus, the importance of the present study was to develop an integrated (*in silico*/*in vitro*) model system that could have the capability of robust prediction, quantification and comparison of the chitin-drug interactions and binding affinity. Such computational models could enhance the understanding of the drug's stability and its loading/release capabilities of the systems under study (Costache et al., 2009; Patel et al., 2008).

Thus, the main objectives of the present study are (i) synthesize and characterization of AC-NPs, curcumin loaded AC-NPs (CUR-AC-NPs), docetaxel loaded AC-NPs (DOC-AC-NPs) and 5-flurouracil loaded AC-NPs (5-FU-AC-NPs). (ii) to determine the drug entrapment and drug loading efficiencies of the CUR-AC-NPs, DOC-AC-NPs and 5-FU-AC-NPs (iii) To determine the BE of AC-NPs with lipophilic, hydrophobic and hydrophilic anti-cancer drugs using molecular docking techniques, (iv) To determine the correlation coefficient between experimentally determined drug loading and drug entrapment efficiencies with the BE of AC-NPs obtained by *in silico* methodology.

2. Materials and methods

2.1. Materials

Amorphous chitin (Degree of acetylation: 65%, molecular weight 150 kDa) was purchased from Koyo Chemical Co. Ltd., Japan, Penta sodium tripolyphosphate (TPP), 5-fluorouracil (5-FU) and dialysis tubing (MWCO 12 kDa), methanol, acetic acid, DAPI, thiazolyl blue tetrazolium bromide (MTT), nutrient mixture F-12 Ham were purchased from Sigma Aldrich. curcumin (CUR) and docetaxel (DOC) drugs were purchased from MERK (Germany) and A K Scientific (USA). AGS gastric cancer cell lines were purchased from NCCS, Pune.

2.2. Preparation of nanoparticles

2.2.1. Preparation of amorphous chitin nanoparticles (AC-NPs)

AC-NPs were prepared according to the reported literature with modification in precursor concentrations (Chitin,TPP) (Smitha et al., 2013). 0.1 wt% amorphous chitin solution was prepared in 1% acetic acid. 1 ml of 2 wt% of TPP solution were added drop wise to 5 ml of this solution, kept for 15 min under constant stirring. As a result, the clear solution was changed into an opalescent nanoparticle suspension. The NPs formed were centrifuged at 10,000 rpm for 30 min. The pellets formed were washed with distilled water and lyophilized and stored at 4 °C. The pellets thus obtained were redispersed in phosphate buffered saline of pH 7.4 (PBS) prior to the experiments and used immediately.

2.2.2. Preparation of drug loaded amorphous chitin nanoparticles

CUR-AC-NPs were prepared as follows: 1 mg/ml of CUR in methanol was added to 0.1 wt% AC solution, and it was kept for stirring for 2 h. Into this solution, 2 wt% TPP was added drop-wise and stirred for 15 min until the solution became turbid. The formed CUR-AC-NPs were centrifuged at 10,000 rpm for 30 min. The pellets formed were washed with distilled water and lyophilized and stored at 4 °C. The pellets thus obtained were redispersed in PBS of pH 7.4 prior to the experiments and used immediately. The same protocol was followed for preparing *DOC-AC-NPs and 5-FU-AC NPs.*

2.3. Characterization of nanoparticles

The size distribution of the prepared bare and drug loaded chitin nanoparticles were determined by Dynamic Light Scattering (DLS) measurements (Zetasizer, Malvern Instruments, USA). The size and the surface morphology of the bare and drug loaded AC-NPs were reconfirmed by SEM (JEOL-JSM-6490LA). The surface charge and thereby the stability of the NP system were obtained by zeta potential measurements (ZP-Zetasizer, Malvern Instruments, USA). To analyze the potential chemical interaction between the constituents within the AC-NPs system, FT-IR spectra were recorded on Affinity-1S, Shimadzu, Fourier transform infrared spectrophotometer (FT-IR). Lyophilized samples were given for FT-IR analysis.

2.4. Determination of entrapment efficiency (EE) and loading efficiency (LE) of nanoparticles

To determine the EE, drug loaded nanoparticles were centrifuged (10,000 rpm; 30 min), and the supernatant was analyzed ($n = 3$) using UV–vis spectrophotometer (UV–vis 2600, Shimadzu). LE of the chitin nanoparticle–drug system was calculated with respect to the weight of the NPs obtained after centrifugation at 10,000 rpm for 30 min. The pellets obtained were redispersed in distilled water, freezed overnight and then lyophilized. The percentage EE, LE and Yield is computed using the following equations.

$$\mathrm{EE}(\%)\frac{\text{Total amount of drugs} - \text{Amount of free drug}}{\text{Total amount of drug}} \times 100$$

$$\mathrm{LE}(\%)\frac{\text{Total amount of encapsulated drug}}{\text{Total amount of nanoparticles}} \times 100$$

$$\mathrm{Yield}(\%)\frac{\text{Total amount of nanoparticles obtained}}{\text{Total amount of precursors added}} \times 100$$

2.5. Cytotoxicity studies

The anticancer activity of CUR, DOC and 5-FU released from the AC-NPs against AGS cells was evaluated by MTT assay and compared with that of 5-FU, CUR, DOC drugs alone, respectively. For this, the cells were seeded in a 96 well plate at a density of 10^4 cells/well and kept for 24 h incubation to attach the cells. After the incubation period, media was removed and replenished with 100 μl of media containing different concentrations of CUR-AC-NPs (0.1, 0.5, 1, 5, 10, 50, and 100 μM) and kept for 24 h of incubation along with triton-X (1%) treated cells as negative control and F-12 media alone containing wells as positive control. After the desired time of incubation, media containing particles were removed and