DEVELOPMENT, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF POLYSACCHARIDE BASED NANOMATERIALS FOR ENHANCED DELIVERY OF CURCUMIN TO CANCER CELLS

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by

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ABSTRACT

Chemotherapeutic drugs which are now used for the treatment of cancer have many limitations. Most of them are highly toxic, expensive and cause damage to the healthy cells. In this context, research on natural chemotherapeutic agents for cancer treatment gains great attention, since these agents can avoid many of the drawbacks of conventional drugs. Curcumin is one such natural chemotherapeutic agent derived from turmeric. Since long, curcumin is being used in Ayurvedic medicines as healing agent for a variety of diseases. Research on the therapeutic effects of curcumin on various tumors has escalated tremendously over the last few decades. Anticancer activity of curcumin is mediated through its regulation of various transcription factors, inflammatory cytokines, protein kinase and growth factors. It is effective towards many types of cancers including skin, lung, brain, pancreas etc.

Draw backs of curcumin such as low solubility, stability and fast degradation reduces the practical usage of curcumin in cancer therapy. Increasing the bioavailability of curcumin by protecting it from degradation can enhance the efficacy in cancer treatment. Developments in nanobiomaterial research pave a new way in the enhancement of therapeutic effect of curcumin. Several nanomaterials such as polymeric nanoparticles, micelles, lipid bilayers and polymer-curcumin conjugates were developed for the encapsulation and safe delivery of curcumin. This thesis deals with development of three types of nanomaterials for the delivery of curcumin to carcinoma cells. These nanomaterials are polymer-curcumin conjugates, nanogels and polyelectrolyte complex. All the three nanomaterials are prepared from polysaccharides namely pullulan, alginate and gum arabic.

Owing to their high solubility, biocompatibility and biodegradability, polysaccharides are one of the best choices for developing nanoformualtions for curcumin encapsulation followed by delivery. Biomedical applications of gum arabic, pullulan and alginate are reported, but the potential of these highly soluble polymers for curcumin delivery to carcinoma cells is not yet reported. Hence, in the present work, nanosized carriers from these polysaccharides for the safe and effective delivery of curcumin to cancer cells are developed.

Conjugation of curcumin to hydrophilic polysaccharides enhances the solubility and bioavailability of curcumin. Polymer-drug conjugates are one of the best choices to increase the solubility of curcumin. Drug molecules are covalently linked to the polymer chain in a polymer-drug conjugate. Conjugates with targeting agents show enhanced cytotoxicity than that of nontargeted conjugates. Therefore, in the present study, curcumin conjugates, based on pullulan and alginate with and without targeting groups are developed and their cytotoxicity towards the hepatocarcinoma cells is evaluated. Both galactosylated and nongalactosylated pullulan-curcumin conjugates self assemble to micelle and increase the solubility and stability of curcumin. Conjugate with targeting ligand shows enhanced uptake and toxicity towards HepG2 cells.

Alginate-curcumin conjugates are prepared to avoid the shortcomings of pullulan-curcumin conjugates. Curcumin conjugates from alginate with and without targeting group are also prepared by the same synthetic strategy used for the preparation of conjugates with pullulan. These conjugates also increase the solubility and stability of curcumin and show high negative zeta potential.

Polysaccharides containing targeting ligands in the structure itself are better choice for drug conjugation; since better toxicity may be achieved with minimum steps for the preparation. Based on this hypothesis, gum arabic-curcumin conjugate also is prepared since it contains galactose group which is identified as targeting ligand towards hepatocarcinoma cells. Gum arabic-curcumin conjugate is the best among the three systems studied. It shows the highest solubility and cytotoxicity.

Encapsulation of drugs in nanoparticles will enhance the solubility and pharmacokinetics of the drug. Nanogels and polyelectrolyte complexes based on suitable polysaccharides may act as effective carrier for curcumin delivery. Nanogels are prepared by the cross-linking of polysaccharides, namely, gum arabic and alginate with protein gelatin by inverse miniemulsion technique. In order to improve the bioavailability and therapeutic efficacy, curcumin is encapsulated in alginate aldehyde-gelatin and gum arabic aldehyde-gelatin nanogels. Physicochemical properties of both bare and curcumin loaded nanogels are analyzed by dynamic light scattering, nuclear magnetic resonance spectroscopy, thermogravimetric analysis and scanning electron microscopy. Both curcumin loaded alginate aldehyde-gelatin and gum arabic aldehyde-gelatin nanogels induce toxicity to human breast carcinoma cells. Intracellular uptake of the drug encapsulated nanogels is investigated by fluorescent imaging.

Self assembled hybrid polyelectrolyte complex nanoparticles are prepared from cationically modified gelatin and alginate by electrostatic complexation between the polymers. Cationised gelatin is prepared by the reaction of gelatin with ethylenediamine. Structural changes occurred in gelatin after modification with ethylenediamine is investigated by X-ray diffraction, nuclear magnetic resonance spectroscopy and matrix-assisted laser desorption/ionization – time of flight analysis. These polyelectrolyte complex nanoparticles can be used for the encapsulation and delivery of natural antioxidant curcumin to carcinoma cells. The cationised gelatin-alginate nanoparticles show curcumin entrapment efficiency of 69 % and also exhibit sustained release of curcumin *in vitro*. Anticancer activity of curcumin loaded cationised gelatin-alginate nanoparticles towards human breast carcinoma cells are disclosed by MTT assay. Intracellular uptake of curcumin loaded polyelectrolyte complex nanoparticle is also confirmed by fluorescent imaging.

In conclusion, solubility and stability of curcumin can be increased by conjugation to pullulan, alginate and gum arabic or encapsulation in nanogels and polyelectrolyte complex nanoparticles. Curcumin conjugates and curcumin encapsulated nanogels and polyelectrolyte complex show cytotoxicity towards hepatocarcinoma cells/human breast carcinoma cells. Hence the prepared nanomaterials could be promising candidates in cancer therapy.