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Bioconjugated solid lipid nanoparticles (SLNs) for targeted prostate cancer therapy

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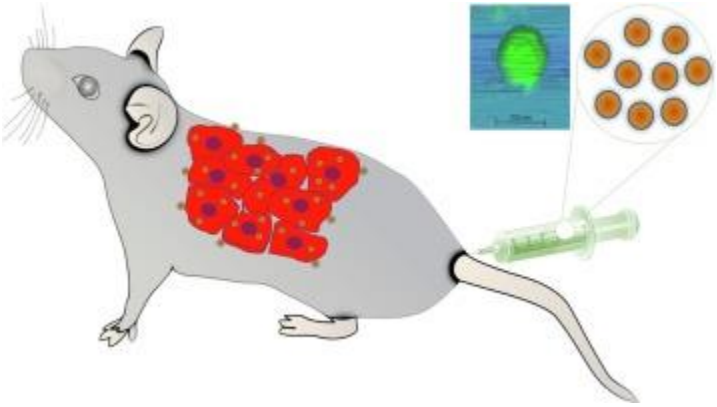
Abstract

Prostate cancer is one of the prominent causes of cancer mortality in men all over the world and a challenge to treat. In this study, transferrin (Tf) bioconjugated solid lipid nanoparticles (SLNs) were developed and loaded with curcumin (CRC) for active targeting of prostate cancer cells. Curcumin is an anticancer agent, but its clinical applications are impeded due to the poor water solubility

and bioavailability. Prepared blank Tf-SLNs showed minimal cytotoxicity while Tf-CRC-SLNs demonstrated significant *in-vitro* anti-proliferative activity compared to CRC-SLNs alone. Cellular uptake of Tf-CRC-SLNs were found to be significantly higher ($p < 0.05/=0.01$) compared to unconjugated SLNs or pure drug alone. Bioconjugated Tf-CRC-SLNs also showed improved early apoptotic and late apoptotic or early necrotic populations (6.4% and 88.9% respectively) to CRC-SLNs and CRC solution. Most importantly, *in-vivo* studies with Tf-CRC-SLNs in mice bearing prostate cancer revealed significant tumour regression (392.64 mm^3 after 4 weeks, $p < 0.001$) compared to the control group. The findings of this work encourage future investigations and further *in-vivo* clinical studies on the potential of bioconjugated SLNs for cancer cure.

Graphical abstract



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Introduction

Among many type of malignancies, prostate cancer is one of the leading cause of cancer mortality in middle age male population around the world (Melmed et al., 2002, Siegel et al., 2012, Siegel et al., 2012). The prostate cancer development inside the human body is known to be a multistage process which starts with a small latent carcinoma and progress towards high grade metastatic cancer overtime. Advanced techniques have been introduced to diagnose and categorize stage or grade of the tumour growth using digital

rectal imaging, serum prostate specific antigen measurement and transrectal ultrasound (Caplan and Kratz, 2002). Accordingly targeted therapeutic approaches have been introduced to treat prostate cancer such as folate-conjugates liposomes and polymer-peptide-enveloped drug-loaded copper sulphide nanoparticles (Patil et al., 2018, Poudel et al., 2019). Patil *et al.*, prepared folate – targeted liposomes loaded with a mitomycin C prodrug and doxurobicinfor PSMA positive cancer cells (Patil et al., 2018). Those nanoparticles presented increased cell drug levels compared to non–targeted liposomes when incubated with LNCaP cells. Another novel approach introduced by Poudel *et al.*, involved encapsulated docetaxel in copper sulphide nanostructures functionalized by a polymer peptide (Shitole et al., 2020). The conjugated nanoparticles were effectively endocytosed by somatostatin receptors while presented better loading efficiency and NIR responsive release for the anticancer agent. Furthermore, the developed nanoplatform demonstrated improved accumulation, tumour ablative behaviour, low cytotoxicity and biocompatibility.

In order to achieve prostate-specific drug delivery of a folate-targeted amphiphilic cyclodextrin was developed using fusogenic DSPE-PEG_{5000}-folate (Stephenson et al., 2014). Endosomal release of siRNA was achieved through 200 nm nanoparticles with neutral surface charge. These complexes protected siRNA from serum nucleases but when excess amount was incubated with free folate the uptake in LNCaP was decreased. Kaushik *et al*. developed solid lipid nanoparticles using a range of lipids such as palmitic acid, stearic acid, cetyl palmitate and glyceryl monostearate for the delivery of docetaxel in MCF-7 breast cancer cells (Chu et al., 2019). Among these, the stearic acid SLNs showed high apoptotic index compared to free drug, enhanced solubility but also presented lower plasma protein binding and far better pharmacokinetic and pharmacodynamic profiles in animal studies. Other examples of similar nanoparticulate drug delivery systems include proteins and polypeptides, cyclodextrins, gold nanoparticles, Zein nanoparticles, folate targeted cyclodextrin (CD) nanoparticle, and self-assembling poly(ethylene glycol)-block-polylactide conjugate nanoparticles (Tan et al., 2020, Perera et al., 2020, Yin et al., 2020, Thapa et al., 2017, Evans et al., 2016, Bharali et al., 2017).

Solid lipid nanoparticles (SLNs) are considered as a novel colloidal drug delivery system (DDS) that successfully combines the qualities of liposome and polymeric nanoparticles. SLNs have the ability to provide both stability of the solid core and also biocompatibility of lipid nanocarriers avoiding the limitations related to liposomes and polymeric nanoparticles such as long-term stability, toxicity, sterilization and scale-up (Sun et al., 2013). SLNs have been found to increase water solubility, bioavailability and therapeutic efficacy of water insoluble drugs (Bharali et al., 2017, Sun et al., 2013, Pozzi et al., 2014). However, similarly to other DDS they are prone to non-specific uptake which limits their efficiency for the prostate cancer treatment. For this purpose, surface modification of SLN has been exploited in order to avoid absorption *via* RES and enhanced tumour selectivity (Paliwal et al., 2009, Fang et al., 2012). Kuang *et al*., engineered cRGD-conjugated SLNs and encapsulated a hydrophobic IR-780 dye that could be used as imaging-guided photothermal therapeutic agent with laser irradiation (Kuang et al., 2017). The SLNs were targeted to cell lines over expressing $a_v\beta_3$ integrin and the results demonstrated tumour eradication. More recently, Karim et al. (Karim et al., 2018), conjugated a fluorescent-labelled NFL-TBS on lipid nanocapsules to enhance internalisation into human glioblastoma cells (Karim et al., 2018). The results showed that increase amounts of bioconjugated peptides enhanced internalization under *in-vitro* conditions. On the other hand, transferrin has been extensively investigated through Tf-mediated drug and gene delivery systems. Overexpression of these Tf receptors in malignant tissues compared to normal tissues is well known due to the higher iron need of malignant cells for rapid growth and division (Widera et al., 2003). Due to their high specificity, various Tf conjugated nanoparticles such as PLGA, PEGylated liposomes, gold nanoparticle/ or albumin nanoparticle have been developed for diagnostic and therapeutic purposes (Widera et al., 2003, Sahoo and Labhasetwar, 2005, Maruyama et al., 2004, Li et al., 2009).

In this study, transferrin conjugated SLNs were developed for active targeting of prostate cancer cells. The main objective of this study was to demonstrate the clinical efficacy of the bioconjugated Tf – SLNs using CRC as a model drug. CRC loaded SLNs were optimized using high pressure homogenizer and subsequently conjugated with

Tf. The stable nano–dispersions were characterized for particle size distribution, zeta potential and drug loading capacity. Further investigations include cytotoxicity studies, cellular uptake, apoptosis and *in vivo* animal trials. As drug loaded Tf -SLNs have not fully investigated particularly for the treatment of prostate cancer, the present study explores efficiency of various SLNs as drug delivery system to the tumour site using both *in vitro* and *in vivo* model.

Section snippets

Materials

Curcumin (CRC), transferrin (Tf), stearic acid (SA), was purchased from Sigma-Aldrich. Poloxamer 188 (P188) was kindly donated by BASF (Ludwigshafen, Germany). 1-ethyl-3-[3-dimethylaminopropy] carbodiimide hydrochloride (EDC) was purchased from Sigma-Aldrich. Bradford assay reagent was purchased from Bio-Rad. All other chemicals and solvents were of analytical and high-performance liquid chromatography (HPLC) grade. LNCaP cell line was purchased from American Type Culture Collection (ATTC:

Results and discussion

- 3.1 Morphology analysis of SLNs

Nanoparticles of both loaded and unloaded SLNs were prepared by using lipid and surfactant crude dispersions under high pressure homogenization processing. HPH is an established technology for the engineering of SLNs whereby controlling the temperature and the applied pressure nanodispersions with uniform particle size can be produced. It was found that SLNs with reproducible sizes could be formed with homogenization temperatures exceeding the melting point of

Conclusions

In conclusion, we have demonstrated that bioconjugated Tf- SLNs loaded with CRC can induce significant tumour suppression in mice bearing prostate cancer. The CRC encapsulation in bioconjugated

SLNs improved the drug uptake when compared to drug-ethanol solution and unconjugated SLNs. In addition, Tf-SLNs were proved as an excellent CRC carrier by preventing drug degradation while the nano-dispersions were also stable during long-term storage. Therefore, results presented in this study suggest

CRediT authorship contribution statement

Mushfiq Akanda: Writing - original draft. **Giullia Getti:** Conceptualization, Supervision, Writing - review & editing. **Uttom Nandi:** Formal analysis, Writing - review & editing. **Md Sadeque Mithu:** Formal analysis, Writing - review & editing. **Dennis Douroumis:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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  From this perspective, nanostructured drug carriers are considered a powerful tool in developing drug delivery systems. Many researchers have investigated the use and effectiveness of nanocarriers in cancer treatment due to their unique physical and chemical properties [3]. At this point, the most important hypothesis to be tested is their role in sustained or controlled release of the encapsulated drug, reducing its side effects and protecting it from degradation.

  Hide abstract

  In the present research, piroxicam entrapped core-shell lipid-polymer hybrid nanocarriers were developed and also evaluated in terms of nanoparticle features and cell-based in vitro efficacy on prostate cancer cells. Box-Behnken

optimization approach was implemented to evaluate the impact of the input variables, namely phospholipid/PLGA ratio, total lipids/lecithin molar ratio, and piroxicam concentration, on two output variables: particle size and entrapment efficiency. Surface charge, size distribution, morphological structure of particles, drug release profiles, presence of outer lipid shell, thermal profile and possible interactions and storage stability of core-shell nanocarriers of piroxicam were studied as particle features. Cell viability, apoptosis and cell cycle arrest studies were utilized for in vitro cell-based evaluation of the core-shell nanosystems. The hybrid nanocarrier formulation with a particle size of 119.2 nm and an entrapment efficiency of 91.7% at the center point of the design was selected as the optimized formulation according to the desired function (d) method applied within the scope of the Box-Behnken design approach and RSM strategy. The cell viability and apoptosis experiments were performed on the optimized nanocarrier. In conclusion, this study demonstrates that the optimized core-shell nanoformulation of piroxicam is a more promising strategy in the treatment of prostate cancer compared to the pure molecule.