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Prodrug encapsulated albumin nanoparticles as an alternative approach to manifest anti-proliferative effects of suicide gene therapy

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ABSTRACT

Conventional anticancer agents are associated with limited therapeutic efficacy and substantial nonspecific cytotoxicity. Thus, there is an imminent need for an alternative approach that can specifically annihilate the cancer cells with minimal side effects. Among such alternative approaches, CD::UPRT (cytosine deaminase uracil phosphoribosyl transferase) suicide gene therapy has tremendous potential due to its high efficacy. Prodrug 5-Fluorocytosine (5-FC) used in combination with CD::UPRT suicide gene suffers from limited solubility which subsequently leads to decline in therapeutic efficacy. In order to overcome this, 5-FC encapsulated bovine serum albumin nanoparticles (BSA-5-FC NPs) were prepared in this work by desolvation method. Physico-chemical characterizations studies revealed amorphous nature of BSA-5-FC NPs with uniform spherical morphology. Apart from increase in solubility, encapsulated 5-FC followed slow and sustained release profile. Suicide gene expressing stable clone of L-132 cells were adapted for investigating therapeutic potential of BSA-5-FC NPs. These nanoparticles were readily taken up by the cells in a concentration dependent manner and subsequently manifested apoptosis, which was further confirmed by morphological examination and gene expression analysis. These findings clearly illustrate that CD::UPRT suicide gene therapy can be efficiently utilized in combination with this nanosystem for improved suicide gene therapy and tumor eradication.

1. Introduction

In the last few years, cancer has emerged as one of the main reasons of mortality worldwide. At present, chemotherapy is among the most widely used strategies for cancer treatment. However, the various drawbacks associated with chemotherapy such as multidrug resistance and lack of specificity provokes a need to develop an alternate strategy for cancer therapy [1]. Moreover, these treatments are not specific to tumor cells and pose similar threats to normal cells. In this regard, suicide gene therapy emerged as promising tool for cancer treatment overcoming various drawbacks associated with chemotherapy. Suicide gene therapy involves delivering a gene product in proximity to the targeted cancer tissue through various targeted delivery methods followed by tissue/tumor-specific expression of the gene which then converts a prodrug into an active drug within the tumor region [2]. This makes the treatment specific to only those cells which are transfected by the suicide gene.

Recently, protein based nanoparticles have emerged as potent delivery system for cancer treatment due to its intrinsic property of preferential uptake in tumor tissue, biodegradability, lack of toxicity and immunogenicity [3,4]. With the success of food and drug administration (FDA) approved paclitaxel loaded albumin nanoparticles "Abraxane" for the treatment of advanced metastatic breast cancer and non-small-cell lung cancer [5–7], albumin nanoparticles emerged as a potent nanocarrier for anticancer agents. Albumin is the most abundant plasma protein having high solubility at physiological condition. The albumin nanocarriers are biodegradable, easy to prepare, and have well-defined sizes and reactive functional groups (thiol, amino, and carboxyl) [3,8]. The amphiphilic nature of albumin nanoparticles provide them the ability to delivery both hydrophobic as well as hydrophilic drugs.

5-FC is an established FDA approved antifungal drug. The structure of 5-FC is illustrated in Fig. 1(b). With the development of suicide gene therapy, 5-FC has emerged as potential anticancer prodrug in combination with genes encoding cytosine deaminase (CD) and uracil phosphoribosyl transferase (UPRT) as suicide genes. 5-FC itself has no significant damaging effects on normal mammalian cells as these cells are devoid of CD enzyme which converts 5-FC prodrug into a toxic 5-Fluorouracil (5-FU). Once it enters in cells expressing CD and UPRT, it gets rapidly deaminated to 5-FU by the action of CD. UPRT enzyme directly converts 5-FU to 5-fluorouridine monophosphate (5-FUMP),

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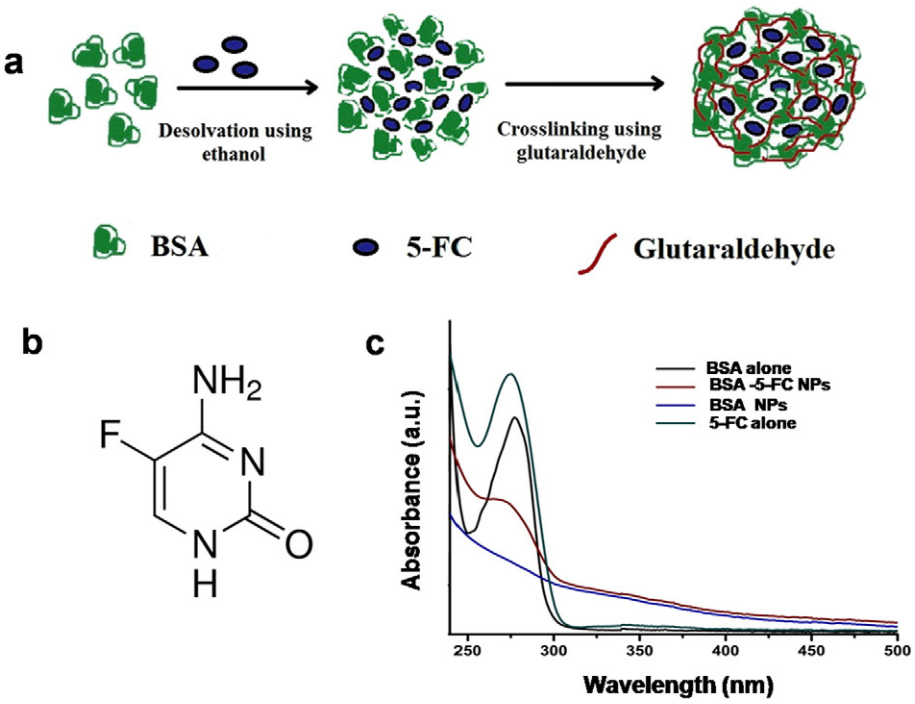

Fig. 1. (a) Schematic outline of prodrug encapsulated BSA NPs fabrication by desolvation technique. (b) Structure of 5-Fluorocytosine. (c) UV–visible absorption spectra of BSA alone, BSA NPs, 5-FC alone and BSA-5-FC NPs.

which is further converted to 5-fluoro-deoxyuridine monophosphate (5-FdUMP) that irreversibly inhibit thymidylate synthase enzyme, thus depriving DNA synthesis [2,9–10]. In mammalian cells, without UPRT enzyme, 5-FU gets converted to nontoxic β-alanine [11]. Therefore, presence of UPRT enzyme further enhances the effects of 5-FC.

The current work presents a feasible and highly reproducible method to prepare 5-FC encapsulated albumin nanoparticles with enhanced therapeutic efficacy and provide sustained release of drug over a period of time. Such nanoparticles were further investigated *in vitro* for their role in inducing apoptosis in transfected human lung epithelial (L-132) cells.

2. Experimental section

2.1. Materials

BSA was purchased from HiMedia. Sodium hydroxide and sodium chloride were purchased from SRL (Sisco Research Laboratories Pvt. Ltd.). Crosslinking agent glutaraldehyde (50% in H_2O) solution and prodrug, 5-FC was obtained from Sigma-Aldrich. For cell culture studies, L-132 cells were received from National Centre for Cell Science (NCCS), Pune, India and were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% calf serum and 1% Penicillin-streptomycin in the 37 °C incubator with 5% CO_2 and 95% air.

2.2. Synthesis of BSA nanoparticles (BSA NPs) and BSA-5-FC NPs

BSA-5-FC NPs were synthesized by desolvation method as discussed previously [12–14]. In brief, BSA solution with a concentration of 25 mg/mL was prepared in 10 mM NaCl (pH 9). 5-FC (stock concentration - 6 mg/mL) was added drop wise at a constant rate of 1 mL/min into the above solution. Solution was stirred for 15 min at 650 rpm, followed by the drop-wise addition of absolute ethanol till the turbidity just appeared. The above mixture was allowed to stir for 3 h, followed by drop-wise addition of 100 μL glutaraldehyde (Stock concentration- 8% in H_2O) solution into it. The resulted solution was left for overnight stirring. All the reactions were performed at room temperature. Overnight stirred solution was centrifuged at 14,000 × *g* at 4 °C. In order to remove the adsorbed glutaraldehyde and drug molecules from the surface of nanoparticles, the obtained pellet was washed thrice with distilled water. Washed pellet was redispersed in ultrapure water. For BSA NPs, above mentioned procedure is followed except the addition of drug. For characterization studies, dispersed nanoparticles were freeze dried in lyophilizer.

2.3. Isolation of plasmid DNA (pDNA)

The eukaryotic expression vector pVITRO2-GFP/CD::UPRT encoding CD and UPRT along with green fluorescent protein (GFP) as a reporter was used for stable expression of CD and UPRT enzyme in L-132 cell line. Plasmid DNA (pDNA) was amplified in DH5 α strain of *E. coli* and isolated using alkaline lysis method. The isolated pDNA was assessed by gel electrophoresis. Purity and quantity was checked by micro volume spectrophotometer (DeNovix, Inc. USA).

2.4. Generation of GFP/CD::UPRT expressing L-132 stable cell lines

In the present work, permanent transformed L132 cell line with stable expression of GFP/CD::UPRT suicide gene was generated by neon electroporation system. In brief, 2×10^5 L132 cells were harvested by trypsinization and electroporated with 3 μg of pVITRO2-GFP/CD::UPRT in neon electroporation system with 1050 V pulse and 30 ms pulse duration. The transformed GFP expressing L132 cells were subsequently screened with hygromycin (300 μg mL^{-1}) in order to eliminate non-transformed L132 cells. Further, the transformed L132 were serially diluted to single cells in 96 well and the single cell derived clone thereby generated, was adapted for further studies. Stable genomic integration of GFP-CD::UPRT gene was confirmed by semi-quantitative RT-PCR with primer specific amplification of GFP-CD::UPRT.

2.5. Characterization of nanoparticles

UV–Vis spectra were recorded using a Lasany double-beam L1 2800 UV–visible spectrophotometer. For measurements, samples were