Colloids and Surfaces B: Biointerfaces 155 (2017) 200–208

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Stealth recombinant human serum albumin nanoparticles conjugating 5-fluorouracil augmented drug delivery and cytotoxicity in human colon cancer, HT-29 cells

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ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form 6 April 2017
Accepted 10 April 2017
Available online 12 April 2017

Keywords:
5-Fluorouracil
Recombinant human serum albumin
Poly (ethylene glycol)
Conjugation
Cytotoxicity
Pharmacokinetic

ABSTRACT

Background and objective: 5-Fluorouracil (5-FU) is a first-line chemotherapeutic drug in colorectal cancer. However, intravenous administration of 5-FU at the dose of 7–12 mg/kg exhibits curbs like short half-life (20 min) and toxic side-effects on bone marrow cells. Therefore, in present investigation, 5-FU was conjugated to poly (ethylene glycol) anchored recombinant human serum albumin nanoparticles (5-FU-rHSA-PEG-NPs) to improve the pharmacokinetic and therapeutic profiles.

Methods and results: The mean particle size of 5-FU-rHSA-NPs was measured to be 44.3 ± 5.8-nm, significantly ($P < 0.05$) lesser than 65.7 ± 7.2-nm of 5-FU-rHSA-PEG-NPs. In addition, zeta-potential of 5-FU-rHSA-NPs was estimated to be −10.2 ± 2.6-mV significantly ($P < 0.05$) lower than −25.8 ± 3.5-mV of 5-FU-rHSA-PEG-NPs. Moreover, both 5-FU-rHSA-NPs and 5-FU-rHSA-PEG-NPs were smooth, spherical and regular in shape. *In-vitro* drug release analysis indicated that 5-FU-rHSA-NPs and 5-FU-rHSA-PEG-NPs separately released 10.9% and 9.23% of 5-FU in PBS (pH ~ 7.4) with no significant difference ($P > 0.05$) up to 48 h. However, addition of 20% v/v serum to PBS (pH ~ 7.4) boosted the drug release. 5-FU-rHSA-NPs and 5-FU-rHSA-PEG-NPs released 78.26% and 48.9% of the 5-FU up to 48 h in presence of PBS (pH ~ 7.4 and 20% serum) with significant difference ($P < 0.05$). Furthermore, 5-FU-rHSA-PEG-NPs displayed the IC_{50} of 3.7-μM significantly ($P < 0.05$) lower than 6.8-μM and 11.2-μM of 5-FU-rHSA-NPs and 5-FU solution, respectively. One compartmental pharmacokinetic elements indicated that 5-FU-rHSA-PEG-NPs demonstrated the half-life ($t_{1/2}$) of 5.33 ± 0.15-h significantly ($P < 0.001$) higher than 1.50 ± 0.08-h and 0.30 ± 0.09-h of 5-FU-rHSA-NPs and 5-FU solution, respectively.

Conclusion: 5-FU-rHSA-PEG-NPs tendered improved cytotoxicity and pharmacokinetic profile. Hence, 5-FU-rHSA-PEG-NPs must be further tested under stringent *milieu* for translating in to a clinical product.

1. Introduction

Despite noteworthy advances in cancer chemotherapy, currently used chemotherapeutic agents are unable to recover the prognosis of advanced or recurrent colorectal cancer. The transport of existing chemotherapeutic drugs by particulate or colloidal nanocomposite has displayed lucrative advantages in terms of improved penetration in tumour tissues, high bioavailability at the site of action, augmented therapeutic index and reduced toxicity to normal tissues [1]. United-States Food and Drug Administration (US-FDA) has approved various anticancer drugs including 5-fluorouracil (5-FU), bevacizumab, avastin, capecitabine, oxaliplatin and irinotecan for the treatment of colon cancer [2,3]. However, 5-FU is used as a first-line chemotherapeutic drug in colorectal cancer, and instantly after surgery, is used as an adjuvant therapy. Chemically, 5-FU (5-Fluoro-2,4-pyrimidinedione) is an antimetabolite pyrimidine analogue that exhibits potent anticancer activity against broad spectrum of solid tumours like pancreas, liver, and colon [4–6]. Mechanistically, 5-FU hinders the action of enzyme thymidylate synthase and consequently prohibits the synthesis of pyrimidine thymidine, a nucleoside requisite for DNA replication [7]. It has been reported that 5-FU metabolite, fluorouridine triphosphate (FUTP) disrupts the RNA processing and functions in both human colon as well as breast cancer cells [8].

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http://dx.doi.org/10.1016/j.colsurfb.2017.04.020
0927-7765/

Owing to erratic absorption and hampered bioavailability, 5-FU is administered as intravenous (*i.v*) infusion at the dose of 7–12 mg/kg for 4 days for successive chemotherapy [9]. However, 5-FU has certain curtails like short biological half-life (20 min) due to rapid metabolism [10] and toxic side-effects on bone marrow cells [11]. Therefore, several attempts were undertaken to improve the delivery of 5-FU from both oral and parenteral route of administration. Liposomes, microspheres, and polymeric nanoparticles have been reported to perk up the therapeutic efficacy of 5-FU [12–15]. Nevertheless, therapeutic efficacy of encapsulated 5-FU in nano/micro carriers was impinged owing to superfluous release that consequently delivered subtherapeutic pay load in vicinity of the tumour cells. In addition, only few attempts have been embarked previously including 5-FU-lipid drug conjugate, 5-FU conjugated gold nanoparticles, 5-FU-dendrimer conjugate, and dual drug conjugate (imatinib and 5-FU) for improving the anticancer potential of 5-FU in cancer cells [16–19].

Human serum albumin (HSA) with an average half-life of 19 days, is a biocompatible, biodegradable and non-immunogenic biopolymer [20,21]. Recombinant human serum albumin (rHSA) offers lucrative merits like avoidance of potential risk of contamination by blood-derived pathogens and profuse supply [22]. Moreover, rHSA is amphiphilic in nature owing to surfactant traits that prevents protein aggregation, stabilizes the conformational structure of therapeutic moiety and maintains its bioactivity throughout the shelf-life of product [23]. Besides, rHSA is also used as a cryoprotectant owing to high glass transition temperature [24]. Recently, US-FDA has approved paclitaxel-rHSA conjugate (Abraxane®) for site-specific delivery. This product offered improved tumour targeting due to enhanced permeation and retention (EPR) effect as compared to free drug [25].

Several chemotherapeutic drugs have been encapsulated in HSA nano/micro carriers for targeting tumour tissues through parenteral routes of administration [26–28]. Furthermore, albumin based nanocarriers accumulate more frequently in tumour tissues owing to EPR attribute [29,30]. The augmented EPR effect of albumin based nanocarriers may be ascribed to the fact that solid tumours commonly enjoy an immature, highly porous vasculature that is acted upon by vascular permeability enhancing factors like nitric oxide. However, tumour vasculature lacks adequate lymphatic drainage. As a consequence, macromolecules (>40 kDa) accumulate within the tumour interstitium due to EPR effect. Tumour is also a site for albumin catabolism. Tumours consume albumin as a source of energy, split albumin into amino acids in lysosomes that are subsequently exercised by cancer cells for accelerating their growth and progression [31]. This was also supported by hypoalbuminiemia in cancer patients as a result of albumin catabolism in tumour tissues [32].

PEGylation of nanoscaled drug delivery systems offered superior circulation half-life [33] and abridged toxicity of protein. Polyethylene glycol (PEG) is advantageous owing to its low toxicity, squat immunogenicity and high biocompatibility [34–36]. PEG has capability to protect the protein against enzymatic degradation and engulfment by reticuloendothelial system (RES) [37,38]. The density of PEG overlays a stearic barrier on to the surface of nanoparticles by means of its hydrophilic chains and thereby reduces the opportunity of opsonization and phagocytosis of nanoparticles. Therefore, in present investigation, initially 5-FU was conjugated to rHSA and later, 5-FU-rHSA was chemically coupled to poly (ethylene glycol) monoamine (5-FU-rHSA-PEG) for tailoring hydrophilic layer. Next, 5-FU-rHSA-PEG nanoparticles (5-FU-rHSA-PEG-NPs) and unmodified nanoparticles (5-FU-rHSA-NPs) were formulated by desolvation technique [39] and characterized *in vitro* using various analytical, spectral and biological techniques. The pharmacokinetic profile of 5-FU-rHSA-PEG-NPs, 5-FU-rHSA-NPs and 5-FU solution was analyzed in Swiss albino male mice following *i.v* route of administration *as per* the recommended dose-dosage regimen.

2. Materials and methods

2.1. Chemicals

5-FU ($M_w \sim 130.077$) was obtained as a gift sample from Arbro Pharmaceuticals, New Delhi, India. Recombinant human serum albumin (rHSA; α-lysine amino acids ~62, $M_w \sim 66.5$ kDa, Purity ~ 96–99%) was purchased from Himedia, Mumbai, India. Poly (ethylene glycol) monoamine ($M_w \sim 5000$) was obtained from Sigma-Aldrich, USA. *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDAC.HCl) and *N*-hydroxy succinimide (NHS) were purchased from Molychem, Mumbai, India. All other chemicals used were of highest analytical grade.

2.2. Cell culture and mediums

Human colon cancer cell line (HT-29) was maintained in 95% air and 5% CO_2 at 37 °C using Dulbecco's Modified Eagle's Medium (DMEM) (Biologicals, Israel) supplemented with 10% fetal bovine serum. All experiments were performed with asynchronous population in exponential growth phase (24 h after plating) [40].

2.3. Synthesis of 5-fluorouracil coupled poly (ethylene) glycol conjugated recombinant human serum albumin

2.3.1. Synthesis of 5-fluorouracil acetate

The synthesis of 5-fluorouracil acetate was instigated with alkylation that leads to the formation of an intermediate product, 5-flurouracil-methyl ester (Scheme 1) [41]. In brief, 5-FU (0.005 M, 0.65 gm) was dissolved in α-chloroacetic acid methyl ester (0.01 M, 1.8 gm) in addition to 100 mg of freshly prepared potassium carbonate. Subsequently, 40 ml of dimethyl formamide was added to the reaction mixture followed by stirring at room temperature for 14–18 h. The completion of the reaction was verified by thin layer chromatography (TLC) using 20% ethyl acetate: hexane mixture as mobile phase. Finally, the mixture was poured into the crushed ice and precipitates of 5-FU-CH_2COOCH_3 were filtered off, and dried. The yield of 5-FU-CH_2COOCH_3 was estimated to be 78.4%. The synthesis of 5-FU-CH_2-$COOCH_3$ was confirmed by ^{1}H NMR spectroscopy using BRUKER DPX 300 MHz spectrophotometer. The ^{1}H NMR of 5-FU was also captured for comparison.

Next, hydrolysis of 5-FU-CH_2COOCH_3 was carried out to yield 5-FU-CH_2COOH (Scheme 1) [42]. In an experiment, 1.0 g of 5-FU-CH_2COOCH_3 was mixed with 10 ml of 10% NaOH solution and the reaction mixture was stirred for 2–12 h at room temperature. The completion of the reaction was verified by TLC using 20% ethyl acetate: hexane as mobile phase. Finally, the solution was neutralized with 4 N HCl and extracted with 50 ml of ethyl acetate and 50 ml of water. The end product was dried over sodium sulphate followed by distillation that eventually supplied 5-FU-CH_2COOH (Scheme 1). The yield of 5-FU-CH_2COOH was measured to be 67.8%. The synthesis of 5-FU-CH_2COOH was confirmed by ^{1}H NMR spectroscopy, as specified earlier.

2.3.2. Synthesis of 5-fluorourcil coupled recombinant human serum albumin

5-Fluorourcil coupled recombinant human serum albumin (5-FU-rHSA) was synthesised by covalent-coupling method (Scheme 1) [43]. Briefly, 5-FU-CH_2COOH (0.188 g, 0.1 M), EDAC.HCl (0.384 g, 0.2 M) and NHS (0.23 g, 0.2 M) were added to 100 ml of phosphate buffer of pH ~ 4.7. Following this, 0.665 g (100 μM) of rHSA was added to the buffer and stirred overnight. Next day, 5-FU-rHSA was purified by dialysis against distilled water for 8 h.