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Gold nanoparticles enhance 5-fluorouracil anticancer efficacy against colorectal cancer cells

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ABSTRACT

5-Fluorouracil (5-FU), an antimetabolite drug, is extensively used in the treatment solid tumors. However, its severe side effects limit its clinical benefits. To enhance 5-FU anticancer efficacy and reduce its side effects it was loaded onto gold nanoparticles (GNPs) using two thiol containing ligands, thioglycolic acid (TGA) and glutathione (GSH). The GNPs were prepared at different 5-FU/ligand molar ratios and evaluated using different techniques. Anticancer efficacy of 5-FU/GSH-GNPs was studied using flow cytometry in cancerous tissue obtained from patients having colorectal cancer. The GNPs were spherical in shape and had a size of ~9–17 nm. Stability of the GNPs and drug release were studied as a function of salt concentration and solution pH. Maximum 5-FU loading was achieved at 5-FU/ligand molar ratio of 1:1 and 2:1 for TGA-GNPs and GSH-GNPs, respectively. GNPs coating with pluronic F127 improved their stability against salinity. 5-FU release from GNPs was slow and pH-dependent. 5-FU/GSH-GNPs induced apoptosis and stopped the cell cycle progression in colorectal cancer cells. They also had a 2-fold higher anticancer effect compared with free 5-FU. These results confirm the potential of GNPs to enhance 5-FU anticancer efficacy.

1. Introduction

Cancer is a leading cause of death worldwide. The number of cancer new cases worldwide is projected to be 15.4 million in 2020 (Ahmedin Jemal et al., 2004). 5-Fluorouracil (5-FU) is a pyrimidine analog that works through irreversible inhibition of thymidylate synthase (Parker and Cheng, 1990). It is used in the treatment of various cancers, such as colon, rectal, liver, gastrointestinal, ovarian, breast and skin cancers (Kennedy and Theologides, 1961). 5-FU causes several side effects including severe gastrointestinal toxicity, hematologic disturbance, severe bone marrow deficiency, skin reactions, hair thinning, hand-foot syndrome and heart toxicosis which limit its use (Macdonald, 1999). In addition, it is rapidly metabolized and has a short plasma half-life, low bioavailability and should be administrated by intravenous injection (Longley et al., 2003). To overcome these problems, several approaches have been used including preparation of 5-FU chemical derivatives and drug encapsulation into different delivery systems, such as polymeric particles, hydrogels, vesicular systems and ion exchange resins (Akalin et al., 2007; Arias, 2008; Jones et al., 1989; Paolino et al., 2008; Siepmann et al., 2004; Wang et al., 2010).

Nanoparticles (NPs) have emerged as an attractive tool to overcome drug unfavorable physicochemical and pharmacokinetic properties (Bamrungsap et al., 2012; Chidambaram et al., 2011). For instance, NPs were shown to enhance anticancer drug aqueous solubility, protect it against degradation, increase its circulation time in the blood and enhance its efficacy through passive and active targeting and avoidance of multi-drug resistance (Bertrand et al., 2014; Huynh et al., 2010; Muddineti et al., 2015). By virtue of their small size, leaky tumor vasculature and absence of tumor lymphatic drainage, NPs preferentially accumulate in the tumors

Abbreviations: 5-FU, 5-Fluorouracil; GNPs, gold nanoparticles; TGA, thioglycolic acid; GSH, glutathione.

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through the so called enhanced permeability and retention effect and increase drug concentration in the tumor by up to 2–3 fold compared with the free drug (Maeda et al., 2013). The potential of NPs in cancer chemotherapy is evidenced by the availability of some commercial nanoparticle-based products while many others are in different stages of clinical trials (Prabhu et al., 2015; Zhang et al., 2008).

Gold nanoparticles (GNPs) have shown tremendous potential for drug delivery applications due to their several advantages, such as low cytotoxicity, tunable surface features and stability under most *in vivo* conditions (Ghosh et al., 2008; Paciotti et al., 2004). They are easily synthesized, functionalized and are biocompatible (Duncan et al., 2010; Khallaf et al., 2016). GNPs can carry small drug molecules, proteins and DNA and modulate their circulation time in the blood (Kumar et al., 2012). Their surface can be modified with a wide range of functionalities allowing drug targeting and controlled release (Han et al., 2007). 5-FU/GNPs showed enhanced efficacy against several cancer cell lines (Ngernyuang et al., 2016; Nivethaa et al., 2015). However, very few 5-FU/GNPs formulations were tested against colorectal cancer cells (Mohamed et al., 2012; Wu et al., 2009). Thus, 5-FU loaded onto oligonucleotide conjugated GNPs showed about 60% cell death in SW480 colon cancer cells at a drug concentration of 1 mg/mL (Wu et al., 2009).

This work aimed to enhance 5-FU cytotoxic efficacy against colorectal cancer and overcome its shortcomings through loading onto GNPs. GNPs surface was modified with two thiol containing ligands, thioglycolic acid (TGA) and glutathione (GSH) to facilitate 5-FU loading. 5-FU/GNPs were characterized using different techniques and their *ex vivo* cytotoxic effect was evaluated in colorectal cancer cells.

2. Materials and methods

2.1. Materials

5-Flourouracil was purchased from Alfa Aesar (Ward Hill, MA, USA). Gold chloride ($HAuCl_4$) was purchased from Electron Microscopy Sciences Co. (Hatfield, PA, USA). TGA, GSH, Pluronic F127 and 5,5′-dithiobis-(2-nitrobenzoic acid) (Elleman's reagent) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Trisodium citrate was obtained from El-Nasr Chemical Co., Cairo, Egypt. Dialysis membranes (MWCO 3.5 kDa) were purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA). All other chemicals were of reagent grade and used as received.

2.2. Preparation of blank GNPs

Trisodium citrate aqueous solution (2 mL, 1.3×10^{-2} M) was added to boiling $HAuCl_4$ aqueous solution (8 mL, 3.7×10^{-4} M). The mixture was stirred vigorously on a magnetic stirrer with hot plate for 15 min. The color of the solution changed from yellow to wine-red (Liu and Lu, 2006).

2.3. Functionalization of GNPs with TGA and GSH

GNPs solution (5 mL, 5.6×10^{-4} M) was mixed with different volumes of TGA (1×10^{-1} M) or GSH aqueous solutions (4.8×10^{-2} M) to have ligand/gold molar ratio of 0.7:1–35:1 (Liu and Lu, 2006). The mixtures were stirred for 24 h at 25 °C. Ligand-functionalized GNPs were separated by centrifugation at 14,000 RPM using ultracentrifuge (Microlitre centrifuge, Micro 200R, Germany) for 1 h at 25 °C. Degree of GNPs functionalization was determined by measuring the concentration of free thiol groups in the solution using Ellman's reagent and previously reported procedures (Sedlak and Lindsay, 1968). The functionalized GNPs were separated as described above and the supernatant (0.5 mL) was treated with 1 mL Ellman's reagent (0.4 mg/mL) in phosphate buffer (100 mM, pH 8) and left in the dark for 15 min. A series of TGA and GSH solutions having known concentrations were similarly treated and used to construct a calibration curve. The absorbance of the solutions was measured spectrophotometrically at 412 nm on UV-Spectrophotometer (Shimadzu-50-02, Kyoto, Japan). The degree of gold functionalization was calculated from Eq. (1):

$$\text{Amount of loaded ligand} = (\text{amount of ligand initially added} - \text{amount of ligand in supernatant}) \quad (1)$$

2.4. Preparation of 5-FU-loaded GNPs

Thiol-functionalized GNPs solution (5 mL, 1×10^{-2} M) was mixed with different volumes of 5-FU aqueous solution (0.1–3.3 mL, 7.7×10^{-2} M) to have 5-FU/ligand molar ratios of 0.25:1–3:1. The pH of the mixtures was adjusted to 6 and they were stirred for 24 h at 25 °C. The 5-FU loaded GNPs were separated by centrifugation at 14,000 RPM for 1 h at 25 °C. 5-FU concentration in the supernatant was determined from its UV absorbance at 266 nm. 5-FU entrapment efficiency and loading capacity were calculated using equation (2) and (3). Optical properties of the GNPs were studied by measuring their UV–vis spectra in the range of 500–700 nm using a Shimadzu-50-02 spectrophotometer (Kyoto, Japan).

$$5-\text{FU entrapment efficiency 1 (weight \%)} = \frac{\text{Total } 5-\text{FU} - 5-\text{FU in the supernatant}}{\text{Total } 5-\text{FU}} \times 100 \quad (2)$$

$$5-\text{FU loading capacity 1 (weight \%)} = \frac{\text{Weight of } 5-\text{FU loaded into the nanoparticles}}{\text{Total weight of nanoparticles tested}} \times 100 \quad (3)$$

2.5. TEM measurements

Images of GNPs were captured using high resolution transmission electron microscope (HR-TEM, Tecnai G20, FEI, Netherland) equipped with eagle CCD camera with (4k*4k) image resolution at an acceleration voltage of 200 kV. TEM samples were prepared by adding 15 μL of aqueous GNPs solution onto a Formvar-coated 400 mesh grid stabilized with evaporated carbon film. The samples were allowed to dry overnight at room temperature.

2.6. Particle size and zeta-potential measurements

Dynamic light scattering measurements were performed using a Malvern ZetaSizer (Nano-ZS, Malvern Instruments, Worcestershire, UK). The instrument was equipped with a 4 mW helium/neon laser (λ = 633 nm). The particle size and ζ-potential were measured at 25 °C.

2.7. pH studies

GNPs were treated with either 1 N NaOH or 1 N HCl to obtain solutions having pH of 1–9. Samples were stirred for 5 min prior to measurement of drug loading capacity and entrapment efficiency. UV–vis spectra in the range of 500–700 nm were also measured.

2.8. Ionic strength studies

Aliquots of a NaCl stock solution (2.5 M) were added to 5-FU-loaded GNPs to have NaCl concentration of 50–300 mM. Samples were stirred for 5 min and drug loading capacity and entrapment