Saudi Pharmaceutical Journal (2017) **25**, 206–213

King Saud University

Saudi Pharmaceutical Journal

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ORIGINAL ARTICLE

Prolonged exposure of colon cancer cells to 5-fluorouracil nanoparticles improves its anticancer activity

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Received 4 May 2016; accepted 31 May 2016
Available online 6 June 2016

KEYWORDS

5-Fluorouracil;
Nanoparticles;
Colon cancer;
PLGA;
Drug delivery

Abstract In this study, we aimed to improve the anticancer effect of 5-FU on human colon cancer cell lines by incorporating in poly(D,L lactic-*co*-glycolic acid) (PLGA) nanoparticles (NPs). The 5-FU-PLGA NPs were prepared by nanoprecipitation technique. Prepared NPs were moderately dispersed with an average diameter of 133 ± 25.19 nm. Scanning Electron Microscope (SEM) images revealed spherical structures with subtle surface irregularity. Free 5-FU dose–response curves were constructed (12.5–2000 μM) using MTT assay on HCT 116 and HT-29 cell lines for 1, 3, and 5 days. The calculated IC_{50} on HCT 116 were 185 μM after 1 day, 11.3 μM after 3 days, and 1.48 μM after 5 days. On HT-29, IC_{50} was only reached after 5 days of 5-FU treatment (11.25 μM). The HCT 116 viability following treatment with 100 μM 5-FU in free or NPs forms for 3 days was 38.8% and 18.6%, respectively. Similarly, when 250 μM was applied, HCT 116 viability was 17.03% and 14.6% after treatment with free and NPs forms of 5-FU, respectively. Moreover, HT-29 cell viability after 250 μM 5-FU treatment in free or NPs forms was 55.45% and 34.01%, respectively. We also noticed that HCT 116 cells were more sensitive to 5-FU-PLGA NPs as compared to HT-29 cells. Overall, our data indicate that 5-FU activity is time dependent and the prolonged effects created by PLGA NPs may contribute, at least in part, to the noticed enhancement of the anticancer activity of 5-FU drug.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2016.05.010

1. Introduction

Colorectal cancer (CRC) has been considered a major health burden and a leading factor causing mortality and morbidity worldwide (World Health Organization, 2002). Conventional CRC chemotherapy provides marginal improvement to patients (American Cancer Society, 2006). In some cases, chemotherapy has failed to make significant impact on the prognosis of disease due to poor bioavailability, poor tissue selectivity/specificity, and *in vivo* degradation, which can lead to serious side effects (Arias, 2008; Nair et al., 2011). Moreover, chemotherapeutics are usually administered in high doses, which increase the risk of side effects and drug resistance (Duran et al., 2008; Wong et al., 2007; Zamboni et al., 2012). Thus far, many potent chemotherapeutics have been developed and extensively studied over the last few decades. However, insufficient and nonspecific drug delivery remains a major problem that adversely affects chemotherapeutic anticancer drug efficacy (Gottesman, 2002).

5-Fluorouracil (5-FU) is one of the earliest and still most commonly used anticancer drugs. Nonetheless, despite its potency in treating cancers, its clinical applications are limited due to its short half-life, disease resistance, and severe side effects such as myelosuppression, dermatitis, cardiotoxicity, neurotoxicity, nausea, vomiting and gastrointestinal, which is associated with its high non-specific *in vivo* distribution (Blanke et al., 1999; Cai et al., 2006; Cao and Rustum, 2000; Di Paolo et al., 2001; Fata et al., 1999; Schmoll et al., 1999; van Kuilenburg et al., 2000). Therefore, it is imperative to develop a strategy that overcomes the limitations as well as further improvements in the anticancer response of 5-FU. In this study, we have utilized polylactic-co-glycolic acid (PLGA) nanoparticles (NPs) to improve the anticancer activity of 5-FU in human colon cancer cells.

PLGA is a synthetic copolymer approved by the US FDA for human use (Jain, 2000). It has been extensively studied as a carrier for a wide range of drugs including chemotherapeutics (Lee et al., 2004). In addition to its biodegradability, PLGA is biocompatible, mechanically strong, soluble in a wide range of organic solvents, and can be easily processed and fabricated in various forms and sizes (Avgoustakis, 2004). In aqueous media, PLGA is hydrolyzed into lactic and glycolic acids that get consumed in the citric acid cycle and subsequently eliminated as carbon dioxide and water (Jalil and Nixon, 1990). As a drug carrier, the most widely used PLGA copolymer is PLGA 50:50 owing to its fastest degradation rate (Park, 1995).

The aim of the current study was to improve the anticancer effect of 5-FU on human colon cancer cells with the help of PLGA NPs. For this purpose, 5-FU was loaded on PLGA (50:50) NPs. In most of the previously published studies, PLGA NPs were prepared by double-emulsion solvent evaporation method (Li et al., 2008; Parikh et al., 2003; Hu et al., 2013; Lin et al., 2012; Nair et al., 2011). In this study, we have explored nanoprecipitation technique for loading of 5-FU on PLGA NPs. The 5-FU-PLGA NPs complex was further characterized for size, shape, surface charge and size distribution. Thereafter, we have studied the anticancer effects of 5-FU loaded PLGA NPs on two types of human colon cancer cell lines HCT 116 and HT-29. We observed that 5-FU-PLGA NPs showed higher cytotoxicity to both types of colon cancer cell line as compared to free 5-FU.

2. Materials and methods

2.1. Chemicals and reagents

5-FU, PLGA (50:50 MW 40,000–75,000), poloxamer 188 NF (Pluronic® F-68 NF, MW 8350), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, MW 414) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Human colon cancer cell lines HCT 116 and HT-29 were bought from the European Collection of Cell Cultures (ECACC) (Salisbury, UK). Trypsin-EDTA (0.25%), McCoy's 5a, Fetal Bovine Serum (FBS), Penicillin–Streptomycin–Glutamine (100×), and Gentamicin (10 mg/mL) were obtained from Life Technologies through Salehiya EstPO (Riyadh, Saudi Arabia).

2.2. Preparation of 5-FU-PLGA nanoparticles

5-FU-PLGA NPs were prepared by nanoprecipitation technique (Barichello et al., 1999). Briefly, 100 mg of PLGA was dissolved in 10 mL of acetone. This solution was added dropwise within 45 s to a stirring solution of 20 mL distilled water containing 5 mg 5-FU and 200 mg poloxamer 188 and kept on stirring at 1000 rpm overnight. Then, the formed suspension was washed three times with double-deionized water at 4 °C at 35,000*g* for 15 min (Optima MAX-XP Benchtop Ultracentrifuge, Brea, CA, USA) and then freeze-dried (LABCONCO FreeZone 4.5 Liter Benchtop Freeze Dry System, Kansas City, MO, USA) for 3 days to remove water.

2.3. Characterization of 5-FU-PLGA nanoparticles

2.3.1. Particle size and zeta-potential

The effective diameter and polydispersity index along with zeta potential were determined by dynamic light scattering using Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). In brief, lyophilized 5-FU and 5-FU-PLGA NPs were re-suspended in 1.5 mL distilled water and poured in a quartz cuvette. Analysis was performed at ambient temperature with an angle of detection of 90°. Zeta potential was measured by laser micro-electrophoresis. Similarly, NPs were re-suspended in 0.5 mL distilled water and added to a folded capillary cell. The Analysis was performed at ambient temperature with an applied voltage of 50 V and an applied voltage offset of −0.92 V. Each value represents the average of at least five measurements ± standard deviation (SD).

2.3.2. Surface morphology

Size and surface morphology of dry powder of 5-FU-PLGA NPs were examined under the scanning electron microscope SEM EVO LS10 (Carl-Zeiss, Cambridge, UK). Particles were mounted on double-sided adhesive carbon tape (SPI Supplies, West Chester, USA) and coated under high-vacuum evaporator with gold in a Q 150R sputter coater unit (Quorum Technologies Ltd., East Sussex, UK) in an argon atmosphere at 20 mA for 120 s. The coated samples were scanned, and photomicrographs were taken at an acceleration voltage of 1–10 kV.

2.3.3. Estimation of 5-FU content on PLGA nanoparticles

5-FU content in 5-FU-PLGA NPs complex was analyzed by high performance liquid chromatography (HPLC). The HPLC