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Preparation of new 5-fluorouracil-loaded zein nanoparticles for liver targeting

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ABSTRACT

This study proposes a new zein nanoparticle (ZP) encapsulated 5-fluorouracil (5-FU) that target liver through intravenous delivery. The ZPs were prepared by phase separation process and optimized using uniform experimental design. The physical properties, in vitro drug release and stability of optimal drug-loaded ZPs were studied. The biodistribution and the target efficiency of the particles were investigated in a mouse model. The highest drug loading was obtained using zein: 5-FU, 3:1 (v/v); zein concentration, 12.5 mg/ml, pH 9.18, mixing time, 3 h and ethanol concentration, 40%. The encapsulation efficiency and the drug loading were 60.7 ± 1.74 and 9.17 ± 0.11 respectively. The size of ZPs and zeta potential were 114.9 ± 59.4 nm and -45 ± 0.3 mV respectively. Differential scanning calorimetry (DSC) demonstrated that the drug was encapsulated within the ZPs. A sustained release profile of 5-FU was observed from ZPs. The more stable storage condition of ZPs was at a temperature of 4 °C. In vivo, ZPs was mostly accumulated in liver following intravenous injection, and the targeting efficiency increased 31.33%. The relative uptake rate of liver was 2.79. Also, nano-sized ZPs were beneficial for prolonged blood residence (7.2-fold increase). These demonstrated that the drug-loaded ZPs could be efficiently targeted at the liver by intravenous delivery.

1. Introduction

Biodegradable nanoparticles (NPs) are receiving considerable attention for the delivery of therapeutic drugs. The literature emphasizes the advantages of nanoparticles over microparticles (McClean et al., 1998) and liposomes (Soppimath et al., 2001). The submicron size of nanoparticles offers a number of distinct advantages over microparticles, including relatively higher intracellular uptake compared to microparticles. To target tumor cells more selectively, active targeting based on antibodies or receptor mediated targeting with cancer specific ligands are developed. However, recent clinical results of molecular target based drugs have shown somewhat disappointing results due to inherent heterogeneity and epitopic diversification of tumor cells even amongst the same cancer patient (Tobias et al., 2006; Greenman et al., 2007). Furthermore, the efficacy of molecular target drugs exhibited only a 4–5% response rate despite of very high expectation and very high cost of manufacturing (Maeda et al., 2009).

NPs may be delivered to specific sites by size-dependant passive targeting (Matsumura and Maeda, 1986). Passive delivery refers to NP transport through leaky tumor capillary fenestrations into the tumor interstitium and cells by passive diffusion or convection (Yuan, 1998). Nanoparticles ranging from 10 to 100 nm in size then begin to accumulate within tumors because of their ineffective lymphatic drainage. This is a phenomenon known as the enhanced permeability and retention (EPR) effect (Teicher, 2000; Sledge and Miller, 2003). Passive targeting can result in increases in drug concentrations in solid tumors of several-folds relative to those obtained with free drugs (Moghimi et al., 2001). Recently, Maeda et al. (2009) highlighted that polymeric drugs for efficient tumor targeted drug delivery were based on EPR-effect. The EPR effect can be observed in almost all human cancers with the exception of hypovascular tumors like prostate cancer or pancreatic cancer. For such a passive targeting mechanism to work, the size of the nanoparticles must be controlled to avoid uptake by the reticuloendothelial system (RES) (Gref et al., 1994). The nanoparticles injected intravenously are mainly delivered to the mononuclear phagocytes system (MPS) (liver, spleen, lungs and bone marrow). Both the polymeric compositions (type, hydrophobicity and biodegradation profile) of the nanoparticles and the associated drug (molecular weight, charge, localization in the nanospheres: adsorbed or incorporated) have a great influence on the drug distribution pattern in the reticuloendothelial organs (Couvreur et al., 1980). This effect was rapid (within 0.5 or 3 h) and compatible with endocytosis. Such propensity of MPS macrophages for endocytosis/phagocytosis provides an opportunity to efficiently deliver therapeutic agents to these cells, using un-modified nanoparticles. This biodistribution can be of benefit for the chemotherapeutic treatment of MPS localized tumors (for example, hepatocarcinoma or hepatic metastasis arising from digestive tract or gynaecological cancers).

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Biodegradable polymers such as PLGA have been extensively studied in controlled release technology. However, it had some limitations for the encapsulation sensitive therapeutic agents during bulk erosion (Park et al., 1995). Depending on the physicochemical characteristics of a drug, it is possible to choose the best polymer to achieve an efficient entrapment of the drug. Zein is an alcohol soluble protein of corn origin that exhibits hydrophobic properties. Zein showed a good biocompatibility for the development of tissue engineering (Dong et al., 2004). It has been studied as microparticle drug delivery system (Liu et al., 2005). In aqueous ethanol solution zein exists as small globules with diameters between 150 and 550 nm (Guo et al., 2005). The zein molecular has a very special bricklike shape and thus has a potential to carry other molecular inside them. In order to exhibit features of the EPR effect, a drug must have a higher molecular weight than the renal excretion threshold (typically >40 kDa) (Matsumura and Maeda, 1986; Maeda et al., 2001; Noguchi et al., 1998; Seymour et al., 1994). Zein has a molecular weight of about 40 kDa. It is a natural protein and has a good biodegradability in vivo (Wang et al., 2007). Zein and its degraded product showed good cell compatibility (Dong et al., 2004; Liu et al., 2005; Wang et al., 2005). It has the advantages vs. synthetic nanomaterials for its absorbability and for the low toxicity of the degradation end products (Liu et al., 2005). It can also overcome the drawback of hydrophilic polymeric system in order to achieve sustained drug release. These are the basis for further study. Until now, there is no study related to drug-loaded ZPs for liver targeting.

5-Fluorouracil (5-FU) has a long history of use as a chemotherapeutic agent. However, less than 20% of an injected dose undergoes enzymatic activation (Tanaka et al., 2000). The oral bioavailability of 5-FU is unpredictable due to high variability in enzymatic degradation (Grem, 1990). With the typical dose of 600 mg/m^2/day, tumor cells are only exposed to the rate-limited active metabolites for a brief time. This is due to the short half-life of 5-FU locally within tissues as well as systemically ($t_{1/2}$ = 10–20 min) (Tanaka et al., 2000). Furthermore, a problem with 5-FU therapy is its toxicity to the bone marrow and the gastrointestinal tract. It has been recognized that, optimally, this drug should be dosed once or twice a week, preferably as a long-acting injection and targeted to the desired site. Biodegradable microparticle delivery system is one promising way for achieving this (Zan et al., 2006; Lamprecht et al., 2003).

In the present study, 5-FU-loaded ZPs were prepared and optimized using uniform experimental design for liver targeting. The physical properties, stability and the in vitro release of 5-FU-loaded ZPs were investigated. Additionally, biodistribution and target efficiency of the prepared ZPs were studied in vivo in normal mice.

2. Materials and methods

2.1. Materials

The following materials and chemicals were obtained from commercial suppliers: zein (Bache Pharmaceutical Co., Wujiang, China), 5-FU (Qilu Pharmaceutical Co., Shandong, China), ethanol (Yongda Chemical Co., Tianjin, China), methanol (Guangcheng chemical Co., Tianjin, China) and rhodamine B (Sigma).

2.2. Preparation of zein nanoparticles

5-FU-loaded zein nanoparticles were prepared using a phase separation procedure. Typically certain proportional 5-FU and zein were dissolved in 5 ml 70% ethanol (w/w) ultrasonically. The resulting solution was immediately added in 9 ml distilled water. The formed dispersion was allowed to evaporate at room temperature to harden the particles. The dispersion was centrifuged, washed three times with ethyl acetate and freeze-dried.

Table 1
The levels and factors of uniform experimental design.

Levels	Factors				
	X_1 (v/v)	X_2 (mg/ml)	X_3	X_4 (h)	X_5 (%)
1	16:1	2.5	3.0	1	25
2	12:1	5	4.0	2	30
3	8:1	7.5	5.8	3	35
4	4:1	10	7.8	4	40
5	2:1	12.5	9.18	5	45

2.3. Drug encapsulation efficiency

The prepared zein nanoparticle dispersions were centrifuged at 15,000 rpm for 50 min to remove the free drug. Then the free drug was diluted by 0.1 N hydrochloride solution and determined using ultraviolet spectrophotometer at λ_{max} 266 nm. The total drugs in nanoparticles were determined by the following method. One milliliter ZPs dispersion was dissolved by 0.1 N NaOH solution, then 0.1 N HCl solution was added to aggregate zein. The solution was filtered through 0.22 μm filters and assayed spectrophotometrically. Drug encapsulation efficiency and loading were determined by following equations respectively.

$$\text{Drug loading efficiency (\%, w/w)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles}} \times 100$$

$$\text{Drug encapsulation efficiency (\%, w/w)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of feed drug}} \times 100$$

2.4. Optimization of the formulation

The optimization was applied to determine the encapsulated efficiency of the drug. The uniform experimental design U_{10} (10^5) was used (Table 1). The five factors were the ratio of the polymer and 5-FU (v/v) (X_1), zein concentration (mg/ml) (X_2), pH (X_3), stirring time (h) (X_4) and ethanol concentration (%) (X_5).

2.5. Physical properties of the ZPs

The morphology of the nanoparticles was observed by field transmission electron microscopy (TEM) (JEM-1200EX, Japan). A drop of diluted nanoparticles suspension was placed on a 400 mesh carbon-coated copper grid. After drying, the samples were dyed using 2% sodium phosphotungstate.

The particle size and size distribution of drug-loaded ZPs were elucidated by ZETASIZER (3000, Malvern, U.K.). For a better measurement the ZPs suspension was diluted with deionized water to a favorable concentration. The average of hydrodynamic particle size was expressed as the value of *z*-average size ± S.D. from three replicate samples.

Drug-loaded ZPs suspension was diluted with deionized water to ensure that the signal intensity is suitable for the instrument. The zeta potential was measured with laser Doppler velocimetry (DXD-II, Jiangsu, China) at 26 V. Values are presented as mean ± S.D. from three replicate samples.

2.6. Differential scanning calorimetry (DSC)

The physical state of 5-FU inside the ZPs was investigated by differential scanning calorimetry (Shimadzu DSC-41, Japan). The samples were purged with dry nitrogen at a flow rate of 20 ml/min. The temperature was raised at 10 °C/min.