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Pharmacokinetics of a ternary conjugate based pH-responsive 10-HCPT prodrug nano-micelle delivery system

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ABSTRACT

A pH-responsive conjugate based 10-hydroxycamptothecin-thiosemicarbazide-polyethene glycol 2000 (10-HCPT-hydro-PEG) nano-micelles were prepared in our previous study. In the present study, ultra-performance liquid chromatography (UPLC-MS) method is developed to investigate its pharmacokinetics and biodistribution in tumor bearing mice. The results demonstrated that the conjugate circulated for a much longer time in the blood circulation system than commercial 10-HCPT injection, and bioavailability was significantly improved compared with 10-HCPT. *In vivo* biodistribution study showed that the conjugate could enhance the targeting and residence time in tumor site.

1. Introduction

Multi-drug resistance (MDR) is often the main cause of chemotherapy failure. One reason is that P-glycoprotein (P-gp) on the surface of cancer cells membrane is to discharge small drug molecules [1]. Nano micelles can enter the cell through endocytosis, and due to lack of exocytosis, the concentration of drug in cells can achieve a higher level to improve efficacy [2]. Therefore, link drug with polymers by chemical bonds to form noneffective conjugate is an ideal design. Which is to release drug by specific mechanism (hydrolysis or enzymatic hydrolysis) to play efficacy after endocytosis, while to normal cells, it remains invalid form and nontoxic [3–7], . Compared with normal cells (pH7.4), tumor cells has acidic microenvironment (pH 5.8–7.2), and lysosome even lower pH value (5.0–5.5). Acid sensitive chemical bond (such as ester bond, oxime key) or chemical structure (e.g. hydrazone, acetal) can

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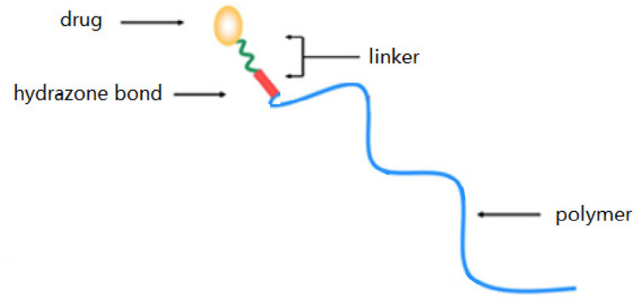

Fig. 1 – Schematic illustration of pH-responsive polymer-drug conjugate.

fracture or change structure through hydrolysis in the acidic environment of the cell, so as to control the release of drugs [8]. In the past decade, pH responsive intelligent drug research become focus in the field of drug delivery [9,10]. Hydrazone bond has been reported in design of pH responsive drug delivery system due to its good stability under the condition of physiological pH and rapid hydrolysis character under acid pH condition [11–14]. (Fig. 1).

10-Hydroxycamptothecin (HCPT) has a broad spectrum of anti-cancer activity *in vitro* and *in vivo* [15]. Its mechanism of therapeutic action is based on targeting nuclear enzyme topoisomerase I (TopoI) by stabilizing a cleavable complex to inhibit DNA S-phase replication and RNA transcription [16]. However, the clinical use of 10-HCPT has been hampered by its poor water solubility and severe side effects, such as myelosuppression and hematuria etc. Combination with a polymer as a prodrug to achieve better targeting and controlled release property is one of the most promising strategies. In our previous study, a water-soluble 10-HCPT-hydro-PEG prodrug was synthesized, as shown in Fig. 2, and it increases the solubility of 10-HCPT by more than 3000 times [17]. The linker contains a pH sensitive hydrazone bond, which breaks in the acidic environment of tumor cell and release drug. The conjugate is amphiphilic and can self-assemble into nano micelle in water. In the current study, we report a validated ultra-performance liquid chromatography (UPLC-MS) assay for the determination of its pharmacokinetics in rats.

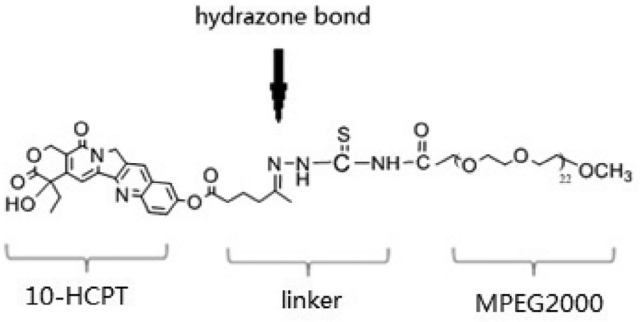

Fig. 2 – Schematic illustration of 10-HCPT-hydro-PEG.

2. Materials and methods

2.1. Materials and animals

2.1.1. Materials

10-HCPT, and EDCI (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) were obtained from Sigma Chemical Co. (St Louis, MO, USA). 5-Carbonyl caproic acid and dichloromethane were obtained from Shanghai Topbiochem Ltd. (Shanghai, People's Republic of China). Anhydrous N,N-dimethylformamide (DMF), thiosemicarbazide, and sulfoxide chloride were obtained from Aladdin Industrial Corporation (Shanghai, People's Republic of China). MeO-PEG2000-COOH (molecular weight 2,000 Da) was obtained from Xi'an Ruixi Biological Technology Co. Ltd. (Xi'an, People's Republic of China). Sephadex LH-20 was obtained from Shanghai Yuanye Bio-Technology Co. Ltd. (Shanghai, People's Republic of China). All other chemicals were of analytical grade and were used without further purification.

2.1.2. Animals

All animal research were performed following the protocol approved by the ethics committee of Henan Laboratory Animal Center and followed the guidelines of the Regulations for the administration of affairs concerning experimental animals.

Wistar rats (150~200 g), provided by Henan Academy of Medical Sciences Laboratory Animal Center (qualified number: SCXK-(army), 2007-004).

2.1.3. Statistical analysis

All statistical tests were performed using Statistical Package for Social Science, version 13.0 (SPSS Inc., Chicago, IL, USA). A minimal $P = 0.05$ was used as the significance level for all tests. One-way analysis of variance and Tukey's test were performed on the uptake data. All data are reported as mean ± standard deviation unless otherwise noted.

2.2. Characterization of conjugate and nano micelles

The conjugate was confirmed by ^{1}H NMR, ^{13}C NMR, and MALDI-TOF MS. The amphiphilic conjugate could self-assemble into nanosized micelles and the particle size, size distribution, and zeta potential of micelles were determined by dynamic light scattering. The morphology was observed by transmission electron microscopy.

2.3. Stability of nanomicelles in vitro and in plasma

Hydrazone bond is instable under certain pH conditions, so we test the amount of degradation product (10-carbonyl caproate ester of 10-HCPT) to investigate the stability of nano micelles under different pH conditions. The conjugates were dissolved in a series of PBS solutions (0.01 mol/l) at pH 5.0, 6.0, and 7.4 to give a final concentration of 0.5 mg/ml and were incubated at 37 °C for 48 h. 0.1 ml portions of each solution were withdrawn and centrifuged at 12,000 rpm for 5 min at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h and the supernatants were analyzed by UPLC/MS (Agilent Technologies 6460 Triple Quad, Palo Alto, CA, USA; ZORBAX Eclipse XDB-C18 Rapid Resolution HD 2.1 mm × 100 mm, 1.8 μm, Agilent). Solutions of conjugate

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