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Controlled release of cisplatin from pH-thermal dual responsive nanogels

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ABSTRACT

In this study, a pH-thermal dual responsive nanogel was applied for cisplatin (CDDP) delivery. CDDP was loaded into the nanogels via conjugation with the carboxyl groups in the nanogels. The conjugation was confirmed by FTIR and XPS. The bonding between CDDP and COOH can be broken by the H^+ or Cl^-. We found that the CDDP released much faster at more acidic environment. The Cl^- concentration in the human body is about 95–105 mM. The conjugated bond could be easily attacked by Cl^- while the nanosystem is injected into the body. In order to diminish the Cl^- triggering release of CDDP from the nanogels, we introduced a thermal-responsive units-NIPAm into the nanogel structure. After NIPAm introduced, the CDDP released much slower from the nanogels at 37 °C in pH = 7.38 buffer in the present of Cl^- (150 mM) than that without NIPAm. And the CDDP also released slower from the nanogels at 37 °C than at 25 °C. By in vitro release behavior studying, we found that CDDP release from the NIPAm containing nanogels can be accelerated by H^+ attacking and reduced by temperature arising. By cellular uptake observation, we found that the nanogels were mainly localized in the cytoplasm of the cancer cells. The CDDP-loaded nanogels exhibited longer circulation time in vivo while compared to free CDDP. And it has better anti-cancer performance than free CDDP in vivo therapy of breast cancer in mice model. Furthermore, some side effects of CDDP, such as renal toxicity, phlebitis, bone marrow suppression etc. have also been reduced by nanogels loading. The in vitro and in vivo results demonstrated that the dual responsible nanogel is a suitable CDDP delivery candidate.

1. Introduction

CDDP is an efficient and low-cost anticancer drug. However, the fatal side effect to the patient body [1], particular the kidney tissue [2], which restricted its clinical application. By modified with some capping agents make the CDDP derivatives (such as carboplatin, substituted (R) malonatodiammineplatinum (II), cis-dichlorobis-(cyclohexylamine) platinum (II), sulfato-1, 2-diaminocyclo-hexaneplatinum (II) [3]) more suitable for cancer therapy, but the side effects and low tumor site localization are still the main challenges in CDDP clinical application.

To date, the low tumor site localization of many anticancer drugs, such as the paclitaxel [4], docetaxel [5], honokiol [6,7], and etc. can be improved by encapsulating into the nanoparticles prepared from amphiphilic polymer (which are also called nanomedicine). For CDDP is a water soluble salt, and it is sensitive to H^+ or Cl^-. It is a challenge to load the CDDP into a hard nanoparticles formed by amphiphilic copolymer by using the same loading method applied in the drugs mentioned above. But CDDP can react with carboxyl group under the catalysis of NaOH [8–10], and the bond formed between CDDP and –COOH can be broken to form free CDDP by H^+ or Cl^- attacking. These properties give a pathway to load CDDP into the –COOH-containing nanomaterials, and some reports about the CDDP loaded nanomaterials have been published [11,12]. The delivery systems include self-assembly micelles [13–15] and nanoparticle systems [16,17]. However, most of these systems cannot control the release of CDDP efficiently.

As a soft nanomaterial, nanogels have attracted great attention in the past decade [18]. They exhibit some unique properties, such as the perfoliate network structure, high water adsorption, smart responses to some environment stimuli (including pH [19–21], temperature [22,23], some bio-catalysis [24–26], and etc). With the development of nanotechnology, nanogels with multifunctional and multiple responses to stimuli have been highlighted [27–32].

Some works have been done in the application of nanogels as drug delivery system [27–29,32]. Due to the penetration-network

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structure, drug loaded in the nanogels mainly depended on the interactions between the drug and the functional groups existing in the nanogels. The interactions include hydrogen bonding, self-assembly, hydrophobic–hydrophilic repulsion, conjugation, and etc. [33] Hydrogen bonding is the basic drug loading mechanism in many hydrogel or nanogel drug delivery systems. However, the water permeability of the nanogels makes this interaction becomes unstable, and the drug release cannot be well controlled by hydrogen bonding alone. Therefore, intelligent conjugation between the cargo and nanogel has gotten significant consideration.

In our previous report, we have successfully synthesized a kind of nanogels with pH and thermal dual responsibility, and the preparation and their responsive properties have been studied in detail [34]. The size of nanogel particles increases with the increase of the pH value, and the particles take place shrinkage while the temperature arises. By model drugs loading behavior studying, we found that the drug loading and releasing behavior of the nanogels have some connection to the inherence of the drugs. For the existence of –COOH groups in the nanogel structure, we expected to load the CDDP into the nanogels by conjugation formed between CDDP and –COOH. However, from the drug release results of some CDDP delivery systems (loaded by conjugation), the CDDP still has a very high releasing rate in pH = 7.4 PBS (containing 150 mM NaCl) [15,35–39]. It indicated by the only conjugation between CDDP and –COOH is still not strong enough to prevent the high release of CDDP in the blood stream for the high concentration of Cl^- existing in the blood (95–105 mM, normal saline is 150 mM). Therefore, another smart controlling manner is needed to reduce this drug release rate. The introduction of NIPAm units into the structure of nanogels is a preferent choice. NIPAm is one of the most well known thermal responsible monomers. While the environment temperature is higher than 32 °C, the NIPAm units change from hydrophilic to hydrophobic and aggregated with each other. A mass of research about NIPAm is published [40–43], however, in drug delivery system field, NIPAm is mainly used to form a switch to control the release of drug by magnetic heating [44] or NIR irradiated heating [45].

After the introduction of NIPAm units, the existence of thermal responsible units make the nanogels take place shrinkage in 37 °C, which can prevent the conjugation bonds formed between –COOH and Pt from Cl^- attacking, as shown in Scheme 1. By this way, we hope the release of CDDP can be reduced in blood plasma (pH = 7.38). Furthermore, the bonding between –COOH and Pt can be broken in acidic environment, and the intra- and extra-cellular environment of the tumor is a little acidic, the release of CDDP from the nanogels can be triggered by the H^+ concentration increasing. So, we expect the CDDP release from the nanogels could be reduced by the temperature arising and triggered release by H^+ concentration increasing. Therefore, the anticancer performance of the CDDP-loaded nanogels will be investigated in the study in detail.

2. Methods and experimental

2.1. Materials

N-Isopropylacrylamide (NIPAM, Aldrich) was purified by recrystallization from hexane (Chengdu KeLong Chemicals, China) prior to use. Poly(ethylene glycol) (PEG, Mw = 1000, Sigma–Aldrich), poly(ethylene glycol) methylether methacrylate (mPEGMA, Mw = 300, Aldrich), 2,2′-azoisibutyronitrile (AIBN, Sigma), N,N-methylenebis(acrylamide) (BIS, Sigma), Methacrylic acid (MAA, Aldrich), cetyltrimethylammonium bromide (CTAB, Fluka, USA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, USA), 1-ethyl-3-(3-dimethylaminopropyl) (EDAC) (Sigma–Aldrich, USA) N-Hydroxysuccinimide (NHS) (Sigma–Aldrich, USA) and 2-(4-Morpholino)ethanesulfonic acid (MES) (Sigma–Aldrich, USA) were all used as received. *cis*-diamminodichloroplatinum II dichloridle (Cisplatin, CDDP) was purchased from Sinopharm Co. Ltd. Doxorubicin chloride (doxorubicin, DOX) was supplied by Zhejiang Hisun Pharmaceutical Company, Zhejiang, China.

2.2. Preparation of nanogels

The nanogels were prepared by emulsion polymerization which has been described in our previous report [34]. Briefly, 1 g of NIPAm, MAA, and PEGMA mixture, 5 mol% total monomers of BIS were dissolved in 95 mL of degassed ultrapure water which contained 40 mg of CTAB as a surfactant. The aqueous solution was bubbled by N_2 for 15 min to remove the oxygen of the solution. Then the system was heated to 70 °C under an N_2 atmosphere with stirring (200 rpm). After being stabilized at 70 °C for 15 min, the reaction was initiated by PEGylated AIBN aqueous solution (4.8 mol% of total monomers of PEGylated AIBN dissolved in 6 mL degassed water), and the reaction maintained for 4 h after it was cooled down to room temperature. The nanogel suspension was dialyzed in water for 3 days by displacing the water every 12 h to remove the surfactant and unreacted monomers. Then the dialyzed suspension was lyophilized for further application. Another nanogel which did not contain NIPAm units was synthesized by the same methods for the drug thermal controlled release properties of the nanogel. The mole ratio of NIPAm, MAA, mPEGMA of the nanogels used in this study was 1:0.5:0.5. LCST (Lower Critical Solution Temperature) of the nanogels is 34 °C.

2.3. CDDP loading and in vitro releasing

2.3.1. Drug loading

The CDDP was loaded into the nanogels via the conjugation reaction between Pt supplied by CDDP and –COOH contained in nanogels. Briefly, in a typical loading process, 80 mg of nanogels was first dispersed in 10 mL of NaOH aqueous solution (25 mM), then 20 mg of CDDP was dissolved by the nanogels dispersion, and the reaction took place at room temperature and maintained for 72 h. The CDDP-loaded nanogels were obtained by dialyzed in 10 mM NaOH aqueous solution firstly for 24 h then in DD water for 2 days by displacing the water every 12 h to remove the free drug and NaOH. The dialyzed nanogel suspension was concentrated by ultracentrifugation with the molecular cut off of 3500, the obtained CDDP-loaded nanogels were re-dispersed in 5% glucose aqueous solution to store and further application.

2.3.2. CDDP release

To measure the effect of thermal responsibility onto the controlled release of CDDP, the CDDP-loaded nanogels were separately placed in the filter bags (molecular cut off was 3500) and immersed in 25 mL of pH = 5.0, 6.0, 7.38 buffers which contained 150 mM NaCl. And at predetermined intervals, the release mediums were all piped out and replaced by fresh buffer. At another hand, free CDDP and CDDP-loaded nanogels (which not containing NIPAm units in the nanogels structure) in the filter bags were placed in the pH = 7.38 buffer, respectively. The alternate temperature or pH release experiments were followed the same procedure.

2.4. In vivo pharmacokinetic study

SD rats (male, supplied by Beijing HFK Bioscience Co., Ltd, China) were used. After injection of NS, free CDDP, and CDDP-loaded nanogels, separately, 200 uL of blood was drawn at different predetermined times from each rat into the EP tube which contained 3 uL of EDTA-Na_2 to prevent the blood clotting. After digested by digestant (HNO_3:HCl:HPO_4 = 3:1:2), the Pt content in the blood sample was measured by ICP-AES.

2.5. Cytotoxicity of CDDP-loaded nanogels

The cell viability of free CDDP and CDDP-loaded nanogels on the MCF-7 and Hela cell lines were evaluated by MTT assay. The cells were cultured in a 5/95% CO_2–O_2 atmosphere at 37 °C. Cells were seeded on 96-well plates at a density of 10^4 cells/well in their usual culture media. After 24 h, the media were used to replace the usual ones and the cells were exposed to different compositions of nanogels for 24 h, and then 100 uL of MTT (5 mg mL^{-1}) solution was added to each well and incubated at 37 °C for 4 h. After the removal of the supernatants, 150 uL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the blue formazan crystal, and then the solution was transferred to the 96-well plates. The absorbance of the contents of each well was measured at 570 nm using an ELISA microplate reader (Bio-Rad). A mean value was obtained from the measurement of five test runs.

2.6. Cellular uptake of nanogels

2.6.1. DOX grafting

DOX was used to label the nanogels to evaluate the cellular uptake of nanogels by tumor cells. DOX were grafted to the nanogels under the catalysis of EDC/NHS. In brief, blank nanogels was dispersed in 10 mM MES buffer, then a determined amount of DOX was added to the nanogels dispersion, the mixture was stirred for 2 h at room temperature. After that, EDC was added to the above mixture, and reacted for another 45 min before the addition of NHS. The reaction maintained for 72 h at room temperature. The DOX grafted nanogels were purified by dialyzing in pH = 4.0 HCl