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ScienceDirect
Journal of Controlled Release 127 (2008) 41–49
journal of controlled release
www.elsevier.com/locate/jconrel

Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing mice

Jong-Ho Kim [a,1], Yoo-Shin Kim [b,1], Kyeongsoon Park [a], Seulki Lee [a], Hae Yun Nam [a,c], Kyung Hyun Min [a,d], Hyung Gon Jo [a,d], Jae Hyung Park [c], Kuiwon Choi [a], Seo Young Jeong [d], Rang-Woon Park [b], In-San Kim [b], Kwangmeyung Kim [a,*], Ick Chan Kwon [a]

[a] *Biomedical Research Center, Korea Institute of Science and Technology, 39-1 Hawolgok-dong, Seongbuk-gu, Seoul 136-791, South Korea*
[b] *Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, 101 Dongin-dong, Jung-gu, Daegu 700-422, South Korea*
[c] *Departments of Advanced Polymer and Nanopharmaceutical Sciences, Kyung Hee University, Gyeonggi-do 449-701, South Korea*
[d] *Department of Life and Nanopharmaceutical Science, Kyung Hee University, 1 Hoegi-dong, Dongdaemun-gu, Seoul 130-701, South Korea*

Received 28 September 2007; accepted 13 December 2007
Available online 26 December 2007

Abstract

To make a tumor targeting nano-sized drug delivery system, biocompatible and biodegradable glycol chitosan (M_w=250 kDa) was modified with hydrophobic cholanic acid. The resulting hydrophobically modified glycol chitosans (HGCs) that formed nano-sized self-aggregates in an aqueous medium were investigated as an anticancer drug carrier in cancer treatment. Insoluble anticancer drug, cisplatin (CDDP), was easily encapsulated into the hydrophobic cores of HGC nanoparticles by a dialysis method, wherein the drug loading efficiency was about 80%. The CCDP-encapsulated HGC (CDDP-HGC) nanoparticles were well-dispersed in aqueous media and they formed a nanoparticles structure with a mean diameter about 300–500 nm. As a nano-sized drug carrier, the CDDP-HGC nanoparticles released the drug in a sustained manner for a week and they were also less cytotoxic than was free CDDP, probably because of sustained release of CDDP from the HGC nanoparticles. The tumor targeting ability of CDDP-HGC nanoparticles was confirmed by *in vivo* live animal imaging with near-infrared fluorescence Cy5.5-labeled CDDP-HGC nanoparticles. It was observed that CDDP-HGC nanoparticles were successfully accumulated by tumor tissues in tumor-bearing mice, because of the prolonged circulation and enhanced permeability and retention (EPR) effect of CDDP-HGC nanoparticles in tumor-bearing mice. As expected, the CDDP-HGC nanoparticles showed higher antitumor efficacy and lower toxicity compared to free CDDP, as shown by changes in tumor volumes, body weights, and survival rates, as well as by immunohistological TUNEL assay data. Collectively, the present results indicate that HGC nanoparticles are a promising carrier for the anticancer drug CDDP.

Keywords: Hydrophobically modified glycol chitosan; Self-assembled nanoparticles; Cisplatin; Drug delivery system; Passive tumor targeting; *In vivo* antitumor efficacy

1. Introduction

Cisplatin (*cis*-diaminedichloroplatinum(II), CDDP) is an important anticancer agent that is usually administered intravenously for treatment of malignancies, including cancers of the testes, ovary, bladder, head and neck, and lung (both small cell and non-small cell lung cancers) [1]. Intravenous administration of CDDP is often unsuccessful, however, because of drug toxicity to normal tissues, which induces severe side effects such as acute nephrotoxicity and chronic neurotoxicity [2–4]. Local chemotherapies including intraperitoneal [5], transarterial [6], and intratumoral [7] administration have therefore been clinically performed. Local chemotherapy, especially intratumoral administration, has been shown to permit high antitumor CDDP efficacy and to maintain a high concentration of the CDDP at the tumor site [8]. Intratumoral administration of small molecular weight anticancer drugs (less than 1000 Da in molecular size) like CDDP may, however, cause side effects because of short

* Corresponding author. Tel.: +82 2 958 5916; fax: +82 2 958 5909.
E-mail address: kim@kist.re.kr (K. Kim).
[1] These authors contributed equally to this paper.

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doi:10.1016/j.jconrel.2007.12.014

drug retention times in tumors and rapid drug distribution into the whole body by the bloodstream.

In recent years, numerous nano-sized drug carriers, such as micelles, nanoparticles, polymer–drug conjugates, and stealth liposomes, have been investigated in order to minimize side effects of anticancer drugs and enhance the antitumoral drug efficacy in cancer therapy [9–13]. Particularly, polymeric nano-sized carriers have shown a high tumor targeting ability at tumor tissue and the nano-sized drug carriers were minimally found at normal tissue sites, leading to high antitumoral therapeutic efficacies [14–16]. The preferential accumulation of polymeric nano-sized drug carriers at tumor sites is explained by the so-called 'enhanced permeability and retention (EPR)' effect, which is caused by the disorganized vascularization and defective vascular architecture of tumors [17]. There is a wealth literature display significantly improved therapeutic efficacy of nano-sized drug carriers against different tumor model, due to the tumor targeting ability of nano-sized drug careers, compared to the free drugs [18–20].

However, only limited information is available on the interaction between nano-sized drug carriers and tumor tissue, *in vivo*. Particularly, the optimum physicochemical characteristics (particle size, surface chemistry, and stability, etc.) of nano-sized drug carriers that could effectively enhance the EPR effect have not been fully understood, because of inability to acquire direct and non-invasive information of nano-sized drug carriers, *in vivo*. Recently, non-invasive live animal imaging technology is providing new research opportunities in the preclinical and clinical development of nano-sized drug carriers in cancer therapy [21]. This new non-invasive live animal imaging technology offers the unprecedented and paradigm changing to study the essential key factors of nano-sized drug carriers that play an important role in tumor targeting ability of nano-sized drug carriers in cancer therapy. In a previous report, we already confirmed that non-invasive live animal imaging technology could be very useful tool to evaluate the tumor targeting ability of nano-sized drug carriers with different physicochemical properties [22]. The real time information of prolonged circulation profile, *in vivo* biodistribution, and tumor targeting ability of nano-sized drug carriers were easily elucidated from the live tumor-bearing mice.

In this study, we prepared CDDP-loaded hydrophobically modified glycol chitosan (CDDP-HGC) nanoparticles for cancer therapy, because water-soluble chitosan derivatives, in particular glycol chitosan, are emerging as the drug carriers of choice because of their biocompatibility and biodegradability *in vivo* [23–26]. Our group previously developed HGC nanoparticles as nano-sized drug carriers and they showed good potential as nano-sized drug carriers for anti-angiogenic peptides and anti-cancer drugs such as doxorubicin and paclitaxel [27–31]. The feasibility of CDDP-HGC nanoparticles as a nano-sized drug carrier was evaluated in the present study by measuring their drug loading efficiency, sustained drug release profile, and cytotoxicity *in vitro*. Also, the real time information of CDDP-loaded HGC nanoparticles that display prolonged circulation profile and *in vivo* biodistribution was evaluated by using non-invasive live animal imaging technology. The tumor targeting ability of CDDP-loaded HGC nanoparticles was demonstrated by the real time quantification of tumor-localized HGC nanoparticles using near-infrared fluorescence (NIRF) imaging technology in tumor-bearing mice. Finally, the antitumor efficacy of CDDP-HGC nanoparticles was evaluated by measuring changes in tumor volumes, and by employing the terminal deoxynucleotidyl-transferase-mediated nick end labeling (TUNEL) assay. The safety of CDDP-HGC nanoparticles was assessed by observing changes in the body weights and survival rates of tumor-bearing mice treated with CDDP-HGC nanoparticles.

2. Materials and methods

2.1. Materials

Glycol chitosan (M_w=250 kDa; degree of deacetylation=82.7%), cisplatin (*cis*-diaminedichloroplatinum (II), CDDP), 5β-cholanic acid, *N*-hydroxysuccinimide (HOSu), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC) were purchased from Sigma (St. Louis, MO) and they were used without further purification. Anhydrous dimethyl sulfoxide (DMSO), and methanol, and ethanol as analytical grade were obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical grade and were used without further purification.

2.2. Preparation of CDDP-HGC nanoparticles

First, the hydrophobically modified glycol chitosan–cholanic acid conjugate (HGC) was prepared by chemical grafting 5β-cholanic acids to glycol chitosan in the presence of EDAC and HOSu as previously reported [27]. Briefly, glycol chitosan (0.5 g, 2 μmol) and 5β-cholanic acid (0.02 g, 56 μmol) were dissolved in distilled water/methanol co-solvent (1:1 v/v), followed by adding 84 μmol of HOSu and EDAC at room temperature. After 1 day, the resulting mixture was dialyzed (molecular cutoff=10 kDa) for 3 days against excess water/methanol co-solvent, followed by freeze-drying to obtain glycol chitosan–cholanic acid conjugates. The synthesized conjugates were analyzed using ^{1}H NMR (3:1, v/v) D_2O/CD_3OD spectroscopy at 600 MHz on an Inova 600 ^{1}H NMR instrument. The degree of substitution of, defined as the number of 5β-cholanic acids per glycol chitosan was determined to 160 molecules by colloidal titration method [27].

Second, the CDDP-HGC nanoparticles were prepared by the dialysis method. The synthesized HGC conjugates (10 mg) were dissolved in 2 ml of water/methanol (1:1, v/v) and a predetermined amount of CDDP (0.1–2 mg) in 1 ml of methanol was added to the HGC solution. The solution was vigorously stirred for 12 h at room temperature and then dialyzed against water using a membrane with a molecular weight cutoff of 12,000–14,000 (Spectrum®, CA). After dialysis for 2 days, the solution was centrifuged at 10,000 ×g for 30 min. The supernatant was filtered through a 0.8 μm membrane filter and lyophilized.

The drug loading efficiency of CDDP-HGC nanoparticles was measured by reverse phase HPLC [32]. Twenty microliter aliquots were withdrawn at various time points, and samples were assayed for the CDDP concentration by reversed-phase