Controlled Release of All-Trans-Retinoic Acid from PEGylated Gelatin Nanoparticles by Enzymatic Degradation

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A new targeting drug carrier for anticancer drug, all-trans-retinoic acid (atRA), was proposed by using angiogenesis which is one of the specific physiological properties of cancer cells. The proposed drug carrier was prepared as PEGylated gelatin nanoparticle (176 nm size). The gelatin molecules were aggregated by coupled deoxycholic acid and the surface of the nanoparticles was covered by polyethylene glycol to reduce reticuloendothelial system (RES) uptake. To prove the feasibility of the nanoparticles as a targeting drug carrier, the degradation of the nanoparticles by collagenase IV and the release pattern of atRA from the nanoparticles by enzymatic degradation were evaluated. The PEGylated gelatin nanoparticles were significantly degraded by collagenase IV within 10 seconds, with most of them degraded within 1 min. When atRA loaded in the PEGylated gelatin nanoparticles was released in phosphate buffered saline (PBS), only twelve percent of atRA were released for one hour. However, when the nanoparticles were put into PBS with collagenase IV of 0.1 μM, a burst effect of atRA was about 40% for the initial 10 min, followed by a continuous release of atRA up to 75% for 5 hr. Therefore, the PEGylated gelatin nanoparticles released anticancer drug very sensitively by collagenase IV, which is one of major matrix metalloproteases involved in angiogenesis. These results showed a feasibility that PEGylated gelatin nanoparticles could be used as a new targeting anticancer drug carrier using angiogenesis as a specific physiological property of cancer cells.

Key words: all-trans-retinoic acid, gelatin, nanoparticle, collagenase, enzymatic degradation

INTRODUCTION

Targeting drug delivery system is to exactly deliver a drug to the target site in the body, thereby maximizing therapeutic effect and reducing side effect and toxicity. Especially for cancer treatment, targeting drug delivery has been in need because of highly severe side effects of anticancer drugs. Conjugation of several moieties such as antibodies, sugar, and low density lipoprotein (LDL) to anticancer drugs has been proposed to enhance the targeting efficiency [1-3]. It is also necessary to utilize the specific physiological properties of cancer cells to enhance the targeting efficiency of anticancer drug to the cancer cells.

All-trans-retinoic acid (atRA), a hydrophobic anticancer drug, plays important roles in the regulation of proliferation and differentiation of epithelial tissues such as skin, bladder, lung, oral cavity, and mammary gland [4-6]. However, atRA is rapidly metabolized to inactive polar metabolites such as all-trans-hydroxy retinoic acid and all-trans-4-oxo-retinoic acid [7]. This rapid metabolism of atRA is due to the induction of the cytochrome P450 by atRA [8]. To overcome such problem and to increase the therapeutic efficiency of atRA, various dosage forms have been reported such as liposomes and nanoparticles [9,10].

In this study, a new targeting drug carrier for atRA was proposed by using angiogenesis which is one of the specific physiological properties of cancer cells. Cancer cells release angiogenesis stimulating factors to endothelial cells, and endothelial cells release several matrix metalloproteases (MMP) such as collagenase and plasminogen activator [11]. The basal membrane and extracellular matrix (ECM), composed of collagen IV, V, and elastin, are degraded by the released MMP around cancer cells [12,13]. Therefore, the gelatin drug carrier was proposed in this study as a targeting drug delivery system with the hypothesis that the gelatin carriers could be degraded by MMP and release atRA at the cancer site, thereby killing the cancer cells efficiently. The gelatin drug carrier was prepared as self-assembled nanoparticle whose size was controlled for long circulation in the body. The surface of particles was also modified by polyethylene glycol to reduce reticuloendothelial system (RES) uptake.

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MATERIALS AND METHODS

Materials

Gelatin type A from porcine skin (300 Bloom), deoxycholic acid (DOCA), N,N′-dicyclohexylcarbodiimide (DCC), collagenase type IV, trichloroacetic acid, all-trans-retinoic acid, sodium sulfate, DEAE (diethylaminoethyl) Sephadex A25 were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and used without further purification. Potassium tert-butoxide, ethyl bromoacetate were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). N-hydroxysuccinimide was purchased from Acros Organics (NJ, U.S.A.). Polyethylene glycol 2000 monomethyl ether (MPEG 2000) was obtained from Fluka Chemie AG (Neu-Ulm, Switzerland) and recrystallized in CH2Cl2-diethyl ether. N,N′-Dimethylformamide was obtained by Merck Co. (Damstadt, Germany). Chloroform, dichloromethane, and diethyl ether were purchased from Junsei Chemical Co. (Tokyo, Japan) and used with purification.

Synthesis of PEGylated Gelatin Nanoparticles

The PEGylated gelatin nanoparticles were prepared as our previous study (not published). Gelatin was coupled with deoxycholic acid (DOCA) and monomethoxy polyethylene glycol 2000 (MPEG) by dichloroethylcarbodiimide (DCC) method, thereby preparing gelatin/DOCA/MPEG conjugate (GDM) [14]. The coupled DOCA played a role as a hydrophobic core, thereby aggregating gelatin molecules. The coupled MPEG chains were located at the nanoparticle surfaces and covered the nanoparticles.

Particle size, stability at room temperature, and shape of particle were determined by dynamic light scattering (DLS, Malvern Instruments Ltd., Series 4700, CA, U.S.A.) with argon ion laser system at 488 nm. The size and the shape of nanoparticles were also evaluated by atomic force micrograph (AFM, Park Scientific, Inc., Sunnyvale, CA, U.S.A.). A silicon nitride tip on a cantilever with a spring constant of 0.12 N/m was used. This instrument was recorded by a contact mode under ambient conditions.

Enzymatic Degradation of PEGylated Gelatin Nanoparticles

The concentration of collagenase IV was varied from 0.001 to 1 μM and the reaction time was also varied to 10 sec, 30 sec, 1 min, and 5 min. The reaction temperature was set at 25°C. The degradation of the gelatin nanoparticles by collagenase IV was stopped by adding trichloroacetic acid (20% v/v).

Both gel permeation chromatography (GPC) and differential scanning calorimetry (DSC) were utilized to analyze the degradation of the nanoparticles by collagenase IV. In GPC experiments, the degraded gelatin nanoparticles were dissolved in 0.1 N of sodium sulfate solution, and prepared as 0.2 wt% solution. The sodium sulfate solution was degassed under reduced pressure and eluted at 0.6 mL/min through three ultrahydrogel columns. The internal and column temperature was set at 50°C. The molecular weights of degraded fragments were calculated by PEG standard samples (Polysciences Inc., IL, U.S.A.). We checked nine standard samples and obtained a standard curve with confidence above 99%. In DSC experiments, the temperature range was from 40 to 250°C and heating rate was fixed at 10°C/min under nitrogen atmosphere. Before DSC analysis was conducted, all samples were checked for the thermal degradation with thermogravimetric analysis (TGA).

Drug Loading and Release Test

The gelatin/DOCA/MPEG conjugate (GDM, 3 mg) was dispersed in 10 mL PBS solution (pH 7.4, ionic strength 0.15) and mixed with atRA (1 wt%) in dichloromethane, followed by sonication at 28 W for 3 min. The dichloromethane was evaporated with stirring at 300 rpm for 2 hr at room temperature. The gelatin nanoparticles containing atRA were filtered with a 0.2 μm filter to remove large size particles and unloaded drug. The amount of atRA loaded into nanoparticles was measured by dissolving the nanoparticles in acetic acid and measuring UV absorbance at 365 nm.

All-trans-retinoic acid is a very hydrophobic drug and its solubility in PBS is 0.2 μM at room temperature [15]. In the release test, atRA loaded nanoparticles and collagenase IV were put in dialysis membrane (MWCO 5 000) and these membrane pouches were soaked in water bath (36 L) at 25°C to maintain a sink condition. The concentrations of collagenase IV were 0.1 and 0.01 μM. After 3, 10, 30, 60, 180, 300 min, the enzyme reaction was stopped by adding acetic acid. The remaining nanoparticles were dissolved in acetic acid, and the amount of remaining atRA in the nanoparticles was calculated by UV absorbance at 365 nm.

RESULTS AND DISCUSSION

In our previous study (not published), the PEGylated gelatin nanoparticle size was in the range of 155 to 200 nm and the average size was 176 nm. AFM images, obtained by the contact mode, showed that the nanoparticles had almost spherical shape (Fig. 1). The PEGylated gelatin nanoparticles were very stable and did not change their size at all for 7 days in PBS at room temperature.

The enzymatic degradation of PEGylated gelatin nanoparticles was evaluated by using collagenase IV since collagenase IV is one of major MMPs released from endothelial cells during angiogenesis. The concentration of collagenase IV around the cancer site has not been found. However, the concentration of collagenase IV was set as at 0.1 and 0.01 μM, based on the enzyme concentration of blood such as antithrombin III (ATIII, 1 μM), because the purpose of this study was to determine the feasibility of the degradation of the PEGylated gelatin nanoparticles by MMP. The enzyme reaction time was varied 10 sec, 30 sec, 1 min, and 5 min. It is difficult to estimate the degradation time which can show the targeting effect clearly. The time for completely degrading nanoparticles in the cancer site might be related to the resistance time of the nanoparticles.