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Flt1 peptide–hyaluronate conjugate micelle-like nanoparticles encapsulating genistein for the treatment of ocular neovascularization

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ABSTRACT

Flt1 peptide of GNQWFI is an antagonistic peptide for vascular endothelial growth factor receptor 1 (VEGFR1 or Flt1). In this work, Flt1 peptide–hyaluronate (HA) conjugates were successfully synthesized and the resulting micelle-like nanoparticles were exploited to encapsulate genistein, an inhibitor of tyrosine-specific protein kinases, for the treatment of ocular neovascularization. The mean diameter of genistein-loaded Flt1 peptide–HA conjugate micelles was measured to be 172.0 ± 18.7 nm, with a drug-loading efficiency of 40–50%. In vitro release tests of genistein from the genistein-loaded Flt1 peptide–HA conjugate micelles exhibited the controlled release for longer than 24 h. In vitro biological activity of genistein/Flt1 peptide–HA micelles was corroborated from the synergistic anti-proliferation of human umbilical vein endothelial cells (HUVECs). Furthermore, we could confirm the anti-angiogenic effect of genistein/Flt1 peptide–HA micelles from the statistically significant suppression of corneal neovascularization in silver nitrate cauterized corneas of SD rats. The retinal vascular hyperpermeability was also drastically reduced by the treatment in diabetic retinopathy model rats.

1. Introduction

A variety of micelle-like nanoparticles has been developed as novel drug delivery carriers because of the high-drug-loading capacity within the inner core and the unique deposition characteristics in the body [1–4]. Micelle-like nanostructure plays important roles in various drug delivery systems, such as increasing the bioavailability of hydrophobic drugs, reducing the cytotoxicity of drugs, long-term delivery of small molecular therapeutics, and target-specific delivery [5–7]. Hydrophobic drugs were encapsulated within amphiphilic block copolymers or conjugated to hydrophilic polymers to form a micelle-like structure. For example, poly(ethylene glycol)-*b*-poly(N-isopropylacrylamide) micelles encapsulating doxorubicin and PEGylated liposomal doxorubicin tagged with monoclonal antibody were developed for the treatment of cancers [8,9]. In addition, doxorubicin was conjugated to N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer for the treatment of breast, lung, and colorectal cancer [10]. Paclitaxel–polyglutamate conjugate was also developed to treat lung cancer [11].

We previously developed Flt1 peptide–hyaluronate (HA) conjugate in the form of micelle-like nanoparticles [12,13]. Flt1 peptide (Gly-Asn-Gln-Trp-Phe-Ile, GNQWFI) is an antagonistic hexa-peptide for VEGFR1 and inhibits binding of VEGF, placental growth factor (PlGF), and VEGF/PlGF heterodimer to VEGFR1 [14]. HA is a high-molecular-weight linear polysaccharide found in the extracellular matrix [15]. It is biocompatible, biodegradable, non-immunogenic, and non-toxic, with a unique viscoelastic property, and has been widely used for target-specific and controlled delivery of bio/pharmaceuticals [16–20]. Specifically, ophthalmic drug delivery systems using HA derivatives have been investigated to increase the ocular residence time, and enhance the bioavailability and efficacy of ophthalmic drugs [21,22]. We have previously confirmed the anti-neovascularization effect of Flt1 peptide–HA conjugate on corneal neovascularization, choroidal neovascularization, and diabetic retinopathy in animal models [12,13].

In this work, we tried to develop a combination therapy with the Flt1 peptide–HA conjugate micelles encapsulating genistein, an inhibitor of tyrosine-specific protein kinases [23,24], for the treatment of ocular neovascularization. Genistein is one of the isoflavonoids, and suppresses cell proliferation and angiogenesis by inhibiting VEGF-induced endothelial cell activation and matrix-degrading proteases, like matrix metalloproteinases [25–27]. Furthermore, genistein has shown an anti-neovascularization effect on the diabetic retinopathy and oxygen-induced retinopathy

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