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Simvastatin prodrug micelles target fracture and improve healing

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ABSTRACT

Simvastatin (SIM), a widely used anti-lipidemic drug, has been identified as a bone anabolic agent. Its poor water solubility and the lack of distribution to the skeleton, however, have limited its application in the treatment of bone metabolic diseases. In this study, an amphiphilic macromolecular prodrug of SIM was designed and synthesized to overcome these limitations. The polyethylene glycol (PEG)-based prodrug can spontaneously self-assemble to form micelles. The use of SIM trimer as the prodrug's hydrophobic segment allows easy encapsulation of additional free SIM. The in vitro studies showed that SIM/SIM-mPEG micelles were internalized by MC3T3 cells via lysosomal trafficking and consistently induced expression of both BMP2 and DKK1 mRNA, suggesting that the prodrug micelle retains the biological functions of SIM. After systemic administration, optical imaging suggests that the micelles would passively target to bone fracture sites associated with hematoma and inflammation. Furthermore, flow cytometry study revealed that SIM/SIM-mPEG micelles had preferred cellular uptake by inflammatory and resident cells within the fracture callus tissue. The treatment study using a mouse osteotomy model validated the micelles' therapeutic efficacy in promoting bone fracture healing as demonstrated by micro-CT and histological analyses. Collectively, these data suggest that the macromolecular prodrug-based micelle formulation of SIM may have great potential for clinical management of impaired fracture healing.

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1. Introduction

With a worldwide aging population, osteoporosis and osteoporosis-related bone fractures have become major public health challenges with a heavy economic burden [1]. Aging and the incipient risk of osteoporosis result in the loss of bone mass and deterioration of bone quality predisposing to fracture. In addition, the fracture repair process is delayed and less effective with aging, making the elderly population particularly susceptible to increased morbidity and mortality. Despite significant advances in the development of new drugs for osteoporosis and the intense effort to identify drugs that improve fracture healing, to date there has been no drug that has been approved by the US FDA for improved fracture healing.

Statins, which were developed as 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors, have been widely used to treat cardiovascular diseases for decades [2]. In 1999, Mundy et al. reported that two statins, simvastatin (SIM) and lovastatin, have strong bone anabolic effects that were attributed to induction of the bone inducing factor bone morphogenic protein-2 (BMP-2) [3,4]. Major efforts have been invested since then, attempting to validate this finding [5–10]. The results remain controversial [11–14], however, in part due to the hepatotropic nature of the statins. Since 95% of orally administered SIM is retained by the liver [8,11], only 5% of the drug is available for extra-hepatic activity, including effects on the skeleton system. Higher oral administration doses may increase the distribution of SIM to the bone [6,15–17] but are impractical due to the associated systemic toxicities [8,11].

Though transdermal application [18] and local delivery [3,19–32] of statins to fracture sites have been explored, most fracture sites are not within sufficient proximity to produce a clinically relevant effect and direct delivery of statins have yielded variable results. Recognizing these issues associated with the delivery of statins for fracture repair, we hypothesized that a systemically administered statin formulation that selectively localizes and retains at the injury site and gradually releases the statins locally would overcome their high hepatotropicity and low water solubility, and best facilitate their bone anabolic effects to promote fracture healing. The recently reported Extravasation through Leaky Vasculature and Inflammatory cell-mediated Sequestration (ELVIS) mechanism [33–36] provides a unique opportunity to systemically target therapeutic agents to inflammatory pathologies, including fractures. As hematoma and acute inflammation are key initial pathological features of bone fractures, we hypothesized that the leaky vasculature associated with the fracture would allow the extravasation of

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systemically administered colloids, where they could be sequestered by the inflammatory infiltrates and activated resident cells at the fracture site. The retained drug within the formulation could then be gradually released from cells at the fracture site to promote the healing process.

We here report the design and synthesis of a dendritic amphiphilic macromolecular prodrug of simvastatin (SIM-mPEG) based on ω-methoxypolyethylene glycol (mPEG) to address the poor water solubility and the lack of osteotropicity of the statins. When mixed with free SIM, the prodrug self-assembles to form stable micelles (SIM/SIM-mPEG), which can gradually release the encapsulated SIM. Our results demonstrate that the systemically delivered micelle formulation passively targets the bone fracture site and promotes fracture healing.

2. Materials and methods

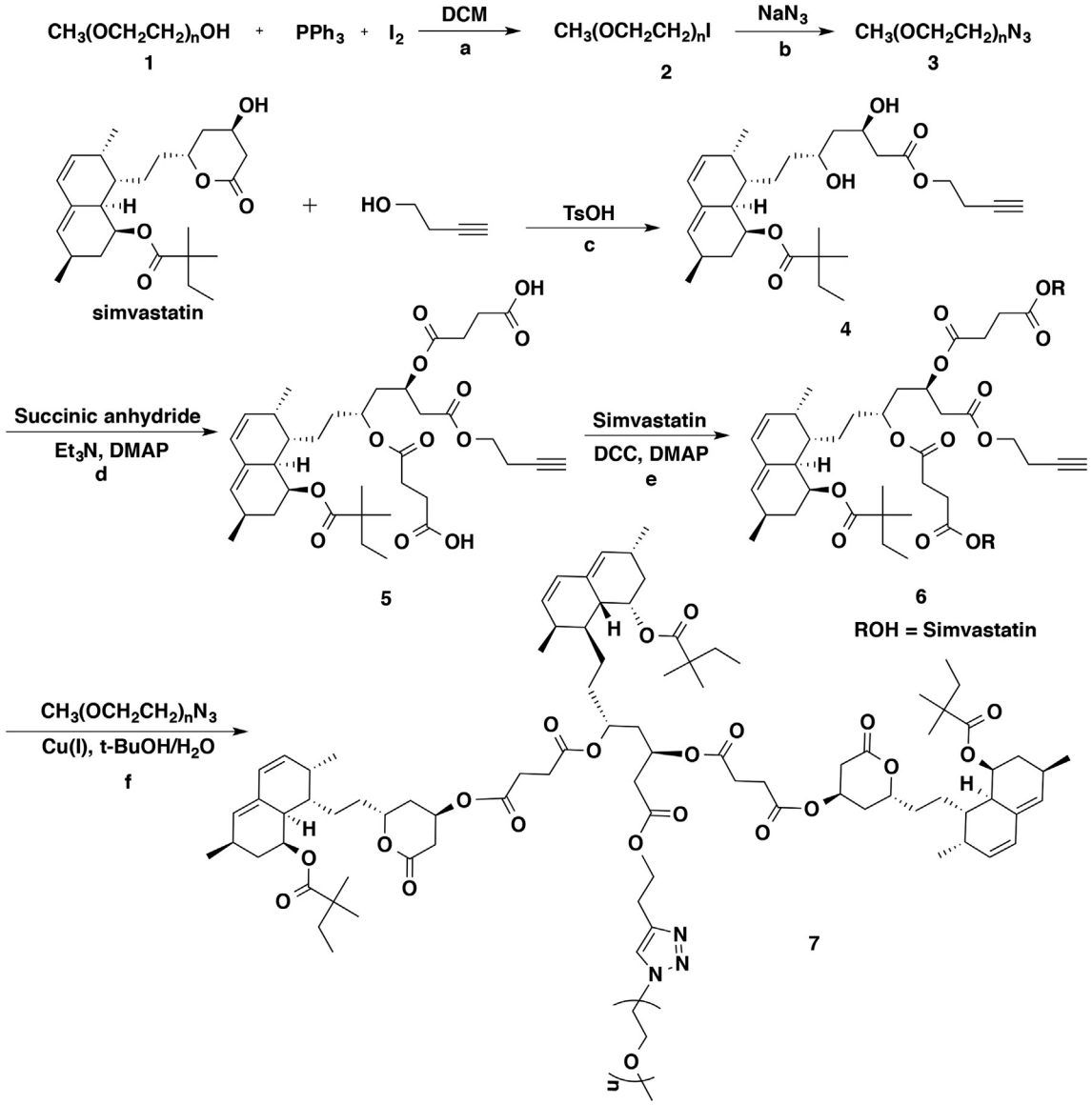
2.1. Materials

Simvastatin was purchased from Zhejiang Ruibang Laboratories (Wenzhou, Zhejiang, China). 3-Butyn-1-ol was purchased from Matrix Scientific (Columbia, SC, USA). Polyethylene glycol monomethylether (mPEG, 1.9 kDa) was purchased from Alfa Aesar (Ward Hill, MA, USA). Heterofunctional PEG (NH_2-PEG-N_3, 3 kDa) was purchased from Rapp Polymere (Tübingen, Germany). Alexa Fluor® 488 NHS ester was purchased from Invitrogen (Camarillo, CA, USA). IRDye 800CW NHS ester was purchased from LI-COR Biosciences (Lincoln, NE, USA). All other reagents and solvents were purchased from Acros Organics (Morris Plains, NJ, USA) and used as received.

2.2. The synthesis of the amphiphilic macromolecular prodrug SIM-mPEG (Scheme 1)

2.2.1. Synthesis of α-methoxy-ω-iodo-PEG (compound 2)

mPEG (1.9 kDa, 3.8 g, 2 mmol) was dissolved in anhydrous dichloromethane (DCM, 40 mL) and cooled in an ice-water bath. Under the protection of argon (Ar), triphenyl phosphine (2.62 g, 10 mmol) was added at 0 °C, and the solution was stirred for 10 min. Iodine (2 g, 8 mmol) was added in several portions at 0 °C, then the temperature of the solution was raised to room temperature and stirred for 24 h. A total of 20 mL of saturated Na_2SO_3 solution was added and stirred for 10 min. The organic phase was then separated and dried over anhydrous Na_2SO_4. The



Scheme 1. Synthesis of the amphiphilic macromolecular prodrug SIM-mPEG. a) PPh_3 (5 eq), I_2 (4 eq), DCM, RT, 24 h, 79.1%; b) NaN_3 (20 eq), DMF, 100 °C, 24 h, 93.4%; c) 3-butyn-1-ol (6 eq), TsOH·H_2O (0.1 eq), RT, 3 h, 30.3%; d) succinic anhydride (6 eq), Et_3N (4 eq), DMAP (0.4 eq), 45 °C, 20 h, 94.6%; e) DCC (2.5 eq), SIM (2.5 eq), DMAP (0.06 eq), 0 °C, 1.5 h, 58.6%; f) 6 (2.5 eq), 3 (1 eq), $CuSO_4 \cdot 5H_2O$ (1 eq), L-ascorbic acid sodium salt (2 eq), t-BuOH/H_2O, 60 h, 77.3%.

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