Biomaterials 35 (2014) 2051–2065

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Polymersomes conjugated with des-octanoyl ghrelin for the delivery of therapeutic and imaging agents into brain tissues

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ARTICLE INFO

Article history:
Received 31 August 2013
Accepted 17 November 2013
Available online 7 December 2013

Keywords:
Polymersomes
Des-octanoyl ghrelin
Blood-brain barrier (BBB)
Brain delivery
Targeting
Nanomedicine

ABSTRACT

The effective protection of the blood–brain barrier (BBB) from tight junctions and efflux transport systems ultimately results in the limited entry of 95% of drug/gene candidates, which are potentially beneficial for central nervous system (CNS) diseases. In order to enhance the brain-specific delivery, in this study we developed a targeting carrier system, which consists of poly(carboxyl ethylene glycol-g-glutamate)-co-poly(distearin-g-glutamate) (CPEGGM-PDSGM) polymersomes with the conjugation of des-octanoyl ghrelin. Des-octanoyl ghrelin across the BBB was reported to be unidirectional (blood-to-brain direction). However, there is no report about the conjugation of des-octanoyl ghrelin to a drug carrier system to confer the BBB targeting property through des-octanoyl ghrelin binding sites mediated endocytosis. To qualitatively and quantitatively investigate this carrier's properties, coumarin 6, Cy5.5 and met-enkephalin were individually encapsulated in these polymersomes. The experimental results showed that the cellular uptake was significantly higher for des-octanoyl ghrelin-conjugated polymersomes (GPs) than unconjugated polymersomes when co-incubated with the BBB cells. In addition, an enhanced accumulation in brain together with a reduced accumulation in liver and spleen was observed in animal study, indicating better brain selectivity for the GPs. In a hot-plate test, a significant inhibition of nociceptive response could be achieved for an intravenous injection of GPs encapsulated with met-enkephalin. The overall results demonstrated that GPs own a great potential for targeting delivery of drug across the BBB to treat CNS diseases.

1. Introduction

The blood–brain barrier (BBB) is formed by brain capillary endothelial cells which are closely connected together with tight junctions and the tight junctions effectively seal the capillary wall by eliminating inter-endothelial spaces [1,2]. It protects the central nervous system (CNS) from many toxic substances that may adversely affect brain functions. In the meanwhile, this effective protection also limits the entry of 95% drug candidates which are potentially beneficial for the treatment of CNS diseases [3]. Only a small class of drugs, usually lipophilic or hydrophobic molecules with molecular weight less than 400 (Da), can cross the BBB by diffusing through the capillary endothelial membrane [4,5]. Crossing the BBB is a major challenge for new drug development. The most promising approach developed so far is the receptor-mediated transport that owns a high potential to be exploited as a means to deliver drugs targeting to the brain [1,6,7]. The receptor families discovered on the BBB to date include the receptors of transferrin, lactoferrin, low-density lipoprotein, insulin, epidermal growth factor, and so on [8,9]. It is an on-going research area to identify new receptors and their respective ligands for crossing the BBB [10,11].

Delivering therapeutic agents into brain via specific receptors can obtain higher treating efficiency. In this study, des-octanoyl

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http://dx.doi.org/10.1016/j.biomaterials.2013.11.051

ghrelin was chosen as the targeting ligand for brain. Ghrelin is a multifunctional hormone that regulates food intake, gastric motility, energy balance, and many other functions displaying in several animal models [12–16]. Ghrelin consists of 28 amino acids and the *n*-octanoylation at serine-3 position is necessary for its bioactivity [17]. The acylated form of ghrelin is extremely unstable in blood and hence, the predominant form in the blood is des-octanoyl ghrelin [18,19]. The concentration in human plasma for endogenous des-octanoyl ghrelin (0.15 nM) is much lower than endogenous lactoferrin (5 nM) and endogenous transferrin (2250 nM) [20–22]. This low concentration in plasma can effectively avoid the competitive inhibition to des-octanoyl ghrelin-conjugated drug carrier system. Furthermore, des-octanoyl ghrelin was observed to transport only in the blood-to-brain direction [23] and this can result in a higher accumulation of the conjugated carriers in the brain. There is no report about the conjugation of des-octanoyl ghrelin to a drug carrier system to confer the BBB targeting property through des-octanoyl ghrelin binding sites mediated endocytosis. Another candidate for increasing BBB transport is a mouse monoclonal OX26 antibody targeting transferrin receptor and recent studies showed that it has a great potential to enhance the delivery of therapeutic agents into the brain [24–26]. However, Ji et al. argued that there was only a slight amount of OX26 antibody in the brain after intravenous administration, and the uptake of OX26 antibody and transferrin in brain was not significantly different [27]. In addition, there were reports that the biodegradable poly(glutamic acid) (PGA) improved the uptake by the BBB via PGA-specific receptor-mediated pathway [28,29]. The uptake of PGA-based nanoparticles by BBB cells was regulated by the PGA-specific receptor-mediated process with energy-dependent characteristics [30,31]. As a result, PGA played a critical role in improving the transport of drugs across the BBB via the enhanced permeability and retention effect.

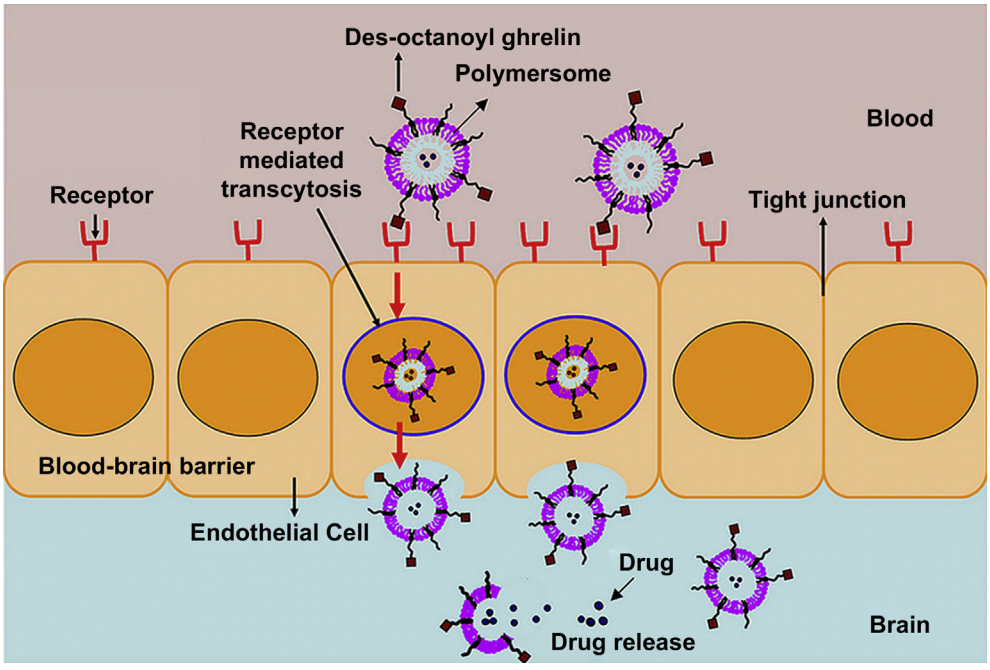
As a new class of synthetic thin-shelled capsules based on block polymer chemistry, polymersomes are self-assembly vesicles from amphiphilic block copolymers with thicker and tougher membranes than lipids [32,33]. Compared with liposomes, polymersomes broaden the range of vesicle properties through a wide choice of amphiphiles with various molecular weights and the ratios of hydrophilic to hydrophobic moiety. For example, thicker and far more stable membranes can be obtained by increasing the hydrophobic fraction of the amphiphile together with the hydrophilic fraction [34]. Moreover, physical and chemical properties of polymersomes including particle size, drug loading, surface modification, and even in-vivo behavior are broadly tunable through rich diversity of the block copolymer chemistry [35–37]. Polymersomes are great candidates as a drug carrier, which is currently being developed by many groups [26,35,37–41]. Pang et al. and Gao et al. confirmed that polymersomes could be employed as a drug carrier for brain-targeting delivery system [26,40,41].

The main objective of this study is to develop a polymersomal drug delivery system that can target the endothelial cells of the BBB and cross over it more effectively. This polymersome can achieve the objective to increase the drug transport as shown in Scheme 1. This study consists of three parts: part (1) synthesis and characterization of the targeting polymersome; part (2) met-enkephalin used as a model drug for the therapeutic study of the polymersome; part (3) coumarin 6 and Cy5.5 encapsulated into the polymersome as probes to investigate the delivery property of this carrier system.

2. Material and methods

2.1. Materials

Poly glutamic acid (PGA MW ~ 6.4 kDa) was purchased from Alamanda Polymers (Huntsville, USA). Des-octanoyl ghrelin and octanoyl ghrelin were from Bachem AG (Bubendorf, Switzerland). N-hydroxy-succinimide (NHS), ethyl(-dimethylaminopropyl) carbodiimide (EDC), dicyclohexylcarbodiimide (DCC), pyridine, coumarin 6, and distearin were purchased from Sigma (Steinheim, Germany). Met-enkephalin was from Boehringer Mannheim Biochem (Indianapolis, USA). Dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), triethylamine (TEA), N,N-dimethyl formamide (DMF), and diethyl ether, acetonitrile (ACN) were purchased from Fluka Chemical Co. (Buchs, Switzerland). 4-Dimethylaminopyridine (DMAP) and toluene were from TCI (Tokyo, Japan). Amino functionalized methoxyl polyethylene glycol (mPEG) and alpha-Amino-omega-carboxyl poly(ethylene glycol) hydrochloride (CPEG) were purchased from Nanocs Inc. (New York, USA). Cy5.5 was purchased from Biological Detection Systems (Pittsburg, USA).



Scheme 1. Mechanism of active targeting for des-octanoyl ghrelin–conjugated polymersomes via receptors to cross the blood–brain barrier.