Biomaterials 113 (2017) 18–30

Contents lists available at ScienceDirect

Biomaterials

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

Combined polymer-curcumin conjugate and ependymal progenitor/stem cell treatment enhances spinal cord injury functional recovery

Raquel Requejo-Aguilar [a, 1], Ana Alastrue-Agudo [a, 1], Marta Cases-Villar [a], Eric Lopez-Mocholi [a], Richard England [b], María J. Vicent [b, **], Victoria Moreno-Manzano [a, c, *]

[a] Neuronal and Tissue Regeneration Laboratory, Príncipe Felipe Research Center, Valencia, Spain
[b] Polymer Therapeutics Laboratory, Príncipe Felipe Research Center, Valencia, Spain
[c] Universidad Católica de Valencia, Valencia, Spain

ARTICLE INFO

Article history:
Received 22 June 2016
Received in revised form
27 September 2016
Accepted 20 October 2016
Available online 21 October 2016

Keywords:
Curcumin
Polymer Therapeutics
Spinal cord injury
Ependymal progenitor stem cells
Rho kinase

ABSTRACT

Spinal cord injury (SCI) suffers from a lack of effective therapeutic strategies. Animal models of acute SCI have provided evidence that transplantation of ependymal stem/progenitor cells of the spinal cord (epSPCs) induces functional recovery, while systemic administration of the anti-inflammatory curcumin provides neuroprotection. However, functional recovery from chronic stage SCI requires additional enhancements in available therapeutic strategies. Herein, we report on a combination treatment for SCI using epSPCs and a pH-responsive polymer-curcumin conjugate. The incorporation of curcumin in a pH-responsive polymeric carrier mainchain, a polyacetal (PA), enhances blood bioavailability, stability, and provides a means for highly localized delivery. We find that PA-curcumin enhances neuroprotection, increases axonal growth, and can improve functional recovery in acute SCI. However, when combined with epSPCs, PA-curcumin also enhances functional recovery in a rodent model of chronic SCI. This suggests that combination therapy may be an exciting new therapeutic option for the treatment of chronic SCI in humans.

1. Introduction

Traumatic spinal cord injury (SCI) leads to a devastating loss of neurological function in the affected area, and currently, there are no effective treatments for the subsequent paralysis. The failure to recover from SCI in adult mammals is attributed to both extrinsic and intrinsic factors, including low or inappropriate trophic factors in the environment, inflammation, the presence of inhibitory signals, the formation of the astrocytic scar, demyelination, and the lack of substantial axonal regeneration and plasticity [1].

The primary injury phase immediately following SCI includes massive necrotic cell death, local ischemia, vascular network loss with edema, and disruption of the blood-spinal cord barrier (BSCB) [2]. The secondary injury phase involves the activation of microglia [3], and the influx of macrophages, neutrophils, and lymphocytes alongside the continued release of inflammatory mediators [4]. This exacerbates secondary injury by promoting axonal dieback but also promotes chronic stage repair by clearing debris and promoting remyelination depending on the microenvironment and the activation state of immune cells [4]. In the following months to years, a reactive scar is formed through the activation of pericytes and astrocytes which isolates the injured area and forms an impenetrable wall with extracellular matrix proteins and chondroitin sulfate [5]. The cavities formed by the glial scar result in syringomyelia, blockage of axonal growth, and the collapse of growth cones, so inhibiting the regenerative potential of those axons to reconnect with the distal segments [6].

Anti-inflammatory, neuroprotective, and neuroregenerative combinatorial approaches constitute the key strategies for the creation of SCI treatments. However, it is important to take into account the disruption of the BSCB and the microvascular system changes that occur after trauma which lead to a reduction of blood

* Corresponding author. Neuronal and Tissue Regeneration Laboratory, Príncipe Felipe Research Center, Av. Eduardo Primo Yúfera 3, 46012 Valencia, Spain.
** Corresponding author. Polymer Therapeutics Laboratory, Príncipe Felipe Research Center, Av. Edurdo Primo Yúfera 3, 46012 Valencia, Spain.
E-mail addresses: mjvicent@cipf.es (M.J. Vicent), vmorenom@cipf.es (V. Moreno-Manzano).
[1] These authors contribute equally to the work.

http://dx.doi.org/10.1016/j.biomaterials.2016.10.032
0142-9612/

supply at the injured area [7,8]. The vascular network slowly recovers [9,10], but for weeks following the initial trauma there is a requirement for multiple local treatment administrations. Although the cord is encased within vertebral segments, the subarachnoid space where the cerebrospinal fluid flows, serves as a means for the direct local administration of bioactive treatments, molecules, or cells [10].

Curcumin is a naturally occurring anti-inflammatory component of turmeric (*Curcuma longa*) linked to NF-kβ inhibition, apoptosis regulation, and antioxidant effects in addition to neuroprotective activity in the treatment of spinal cord injuries [11–13]. However, curcumin bioavailability is low and stability is weak [14] and so efforts have been made to improve these functions by using different nanocarriers for diverse therapeutic applications [15–17]. Polymer Therapeutics are amongst the most successful polymeric nanomedicines [18,19] with 17 products in the market [20–25] and two compounds in the US Top 10 selling drugs list (Neulasta® and Copaxone®) [26]. The term Polymer Therapeutics (PT) describes complex multicomponent polymeric drugs, polymer-based conjugates, and delivery systems. They are considered "new chemical entities" rather than conventional drug-delivery systems or formulations that simply entrap, solubilize, or control drug release without resorting to chemical conjugation [17]. Due to their intrinsic characteristics at the nanoscale, this nanopharmaceutical class can be engineered to exhibit unique advantages: (i) enhanced reach compared to larger nanocarriers, (ii) ability to cross biological barriers displaying architecture specific intracellular trafficking, (iii) ability to control drug pharmacokinetics (PK) due to bioresponsive chemical conjugation, (iv) provision for carrier multivalency allowing tunable drug(s) loading capacity and combination approaches, including theranostics or active targeting, and (v) the controlled and sustained drug release of conjugated drugs through bioresponsive linkers in specific environments [27].

Biopersistent polymeric carriers can however present disadvantages if chronic parenteral administration and/or high doses are required due to the risk of "lysosomal storage disease" syndrome. Preclinical evidence of intracellular vacuolation and clinically reported hypersensitivity reactions with certain polyethylene glycol (PEG)-protein conjugates have raised awareness of the potential advantages of other biodegradable polymers with regards to safety benefit. Furthermore, the use of higher molecular weight (Mw) carriers allows PK optimization [28]. pH-responsive polyacetalic systems [29–34] have several characteristics which make them ideal drug carriers for the treatment of SCI. Firstly, they can be easily prepared by a mild polymerization method that involves the reaction of diols with commercially available divinyl ethers [29]. This also allows the incorporation of drugs with adequate diol functionalities in the polymer mainchain [30] making it possible to control the amount of drug to be incorporated into the construct. This yields a more regular pattern of drug incorporation compared to that found in the statistical pendent chain conjugates. Secondly, while being relatively stable at pH 7.4, they display pH-dependent degradation in the acidic environment encountered in endosomes, lysosomes, and the inflammatory environment often found at injury sites [29,31,32]. Finally, polyacetals have already been shown to have a suitable biodistribution patterns and toxicological profiles *in vivo* [33,34].

Ependymal progenitor/stem cells (epSPCs) are multipotent stem cells found in the adult tissue surrounding the ependymal canal of the spinal cord [35]. After SCI, epSPCs proliferate and migrate to the injured zone, giving rise to, amongst other cells, new oligodendrocyte progenitors [36]. We have previously shown that ectopic transplantation of epSPCs activated by injury (epSPCi) efficiently reversed the paralysis associated with acute SCI in rats [37,38]. The transplanted cells migrated long distances from the rostral and caudal regions to reach the neurofilament-labeled axons in and around the lesion zone, while epSPC transplanted animals always shown fewer cavities and smaller scar area [38]. However, in the chronic scenario, individual strategies have shown only partial success [39]. Chronic injuries, the more abundant and the least studied, demand additional anatomic reorganization and prolonged regenerative activity to induce the growth of large numbers of axons over long distances [40,41].

Here, we look to improve motor function in chronic severe SCI employing a polyacetal curcumin conjugate (PA-Curcumin) in combination with epSPC transplantation to generate an enhanced effect.

2. Material and methods

2.1. Materials

Polyethylene glycol (Bioultra, 4000 g/mol), Diethylene glycol divinyl ether (99%), *N*,*N*-Dimethylformamide (99.8%, anhydrous), triethylamine (≥99%), p-toluenesulfonic acid monohydrate (≥99.5%) were supplied from Sigma-Aldrich and used as supplied. Curcumin (synthetic, >97%) was purchased from TCI Europe and used as supplied. Cyanine5.5 NHS ester was purchased from GE Healthcare Life Sciences. 1,4-Dioxane (anhydrous, ≤100 ppm H_2O) was supplied by Carlo Erba Reagents and used directly. Tetrahydrofuran (THF) and hexane were supplied by Scharlab S.L. and were synthesis grade. Fmoc-Serinol was synthesized as previously reported [32].

2.2. Synthesis of control polyacetals

Please see Refs. [29–34].

2.3. Synthesis of curcumin polyacetals

An oven-dried, 50 mL Schlenk tube was equipped with a magnetic stirrer bar and glass stopper. Polyethylene Glycol (4000 g/mol)(4000 mg, 1.00 mmol) was added and the tube was evacuated to 2×10^{-3} bar for approximately 15–20 min before being refilled with nitrogen. Anhydrous dioxane (30 mL) was added and polyethylene glycol (PEG) was dissolved with gentle heating to 60 °C. After cooling, a solution of curcumin (405 mg, 1.1 mmol) in dioxane (5 mL) was added followed by a solution of p-Toluenesulfonic acid (p-TSA) solution (4 mg dissolved in 1 mL of dioxane). Finally, whilst purging rapidly with nitrogen, diethylene glycol divinyl ether (DEGVE) (572 μL, 3.5 mmol) was introduced using a Gilson pipette and the tube was sealed with a septum. The reaction was then left stirring at room temperature for 1 h in the dark. 9 mL of the solution was transferred by syringe to a separate nitrogen-purged Schlenk tube for the synthesis of PA-curcumin-serinol. The remaining reaction mixture was quenched with a large excess of triethylamine (~1 mL). Next, the sample was purified by repeat precipitations into hexane (3×, each time redissolving in THF). Finally, the sample was dissolved in deionized water (30 mL) and lyophilized for 24 h. Yield = 3.6 g/90% MW 26599–45388.

NMR (δ_H, 300 MHz, CD_3COCD_3): 1.1–1.3 (d, PEG-acetal $-CH_3$), 1.3–1.4 (d, curcumin–acetal–CH_3), 3.3–3.8 (m, PEG–O–CH_2–), 3.8–3.9 (s, curcumin $-O-CH_3$), 4.6–4.7 (q, PEG-acetal $-CH-$), 5.4–5.5 (q, curcumin-acetal $-CH-$), 5.9–6.0 (m, curcumin $-CH-$), 6.6–7.6 (m, curcumin Ar-H, m, curcumin $=CH-$). NMR (δ_C, 300 MHz, CD_3COCD_3): 19.1, 55.5, 64.3, 70.6, 82.7, 99.8, 110.7, 121.4, 142.9, 147.9, 151.2, 190.5.