Journal of Controlled Release 171 (2013) 24–32

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Human erythropoietin gene delivery for cardiac remodeling of myocardial infarction in rats

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ARTICLE INFO

Article history:
Received 11 March 2013
Accepted 13 June 2013
Available online 25 June 2013

Keywords:
Gene delivery
Myocardial infarction
Remodeling
Angiotensin

ABSTRACT

Considerable efforts have been made to exploit cardioprotective drugs and gene delivery systems for myocardial infarction (MI). The promising cardioprotective effects of recombinant human erythropoietin (rHuEPO) protein in animal experiments have not been consistently reproduced in clinical human trials of acute MI; however, the mechanisms underlying the inconsistent discrepancies are not yet fully understood. We hypothesized that the plasmid human erythropoietin gene (phEPO) delivered by our bioreducible polymer might produce cardioprotective effects on post-infarct cardiac remodeling. We demonstrated that intramyocardial delivery of phEPO by an arginine-grafted poly(disulfide amine) (ABP) polymer in infarcted rats preserves cardiac geometry and systolic function. The reduced infarct size of phEPO/ABP delivery was followed by decrease in fibrosis, protection from cardiomyocyte loss, and down-regulation of apoptotic activity. In addition, the increased angiogenesis and decreased myofibroblast density in the border zone of the infarct support the beneficial effects of phEPO/ABP administration. Furthermore, phEPO/ABP delivery induced prominent suppression on Ang II and TGF-β activity in all subdivisions of cardiac tissues except for the central zone of infarct. These results of phEPO gene therapy delivered by a bioreducible ABP polymer provide insight into the lack of phEPO gene therapy translation in the treatment of acute MI to human trials.

1. Introduction

Despite remarkable advances in guideline-based pharmacologic and interventional treatment over the last two decades, MI is the leading cause of morbidity and mortality worldwide [1,2]. The post-infarcted heart undergoes a series of structural changes, termed left ventricular (LV) remodeling, at the organ, cellular, and molecular levels, with three overlapping phases: the inflammatory phase, the proliferative phase, and the healing phase [3–5]. Although cardiac remodeling is initially an adaptive response to maintain normal cardiac function, it gradually becomes maladaptive and can lead to adverse clinical outcomes, including heart failure (HF), arrhythmia, and mortality [3–6].

Diverse efforts in experimental and clinical trials have been made to investigate cardioprotective strategies aimed at attenuating reperfusion injury, reversing adverse myocardial remodeling, and ultimately improving cardiac systolic function and clinical outcomes [7–9]. During the last two decades, the clinical indications of rHuEPO have been expanded to anemia in diverse clinical categories, including anemic patients with chronic kidney disease [10]. Beyond the conventional effect of secreted erythropoietin from the kidney in response to hypoxic stimuli, EPO was recently identified as a pleiotropic and organ-protective cytokine, mediating repair and regeneration via anti-apoptosis, anti-inflammation, anti-oxidation, pro-angiogenesis and re-endothelialization, vascular-protectant, mobilization of endothelial progenitor cells, and recruitment of stem cells into the zone of damage [10–13]. Apart from traditional erythropoietic effects, the pleiotropic organ-protective effects of erythropoietin (EPO) make it a frontline cardioprotective candidate [11]. Higher levels of endogenous EPO have been shown to have protective effects against ischemia–reperfusion (I/R) injury in acute MI in humans [14]. Along with numerous *ex vivo* and *in vivo* studies, some clinical studies with a single rHuEPO administration after the percutaneous coronary intervention showed favorable effects on infarct size, cardiac function, and patient prognosis [11,15]. However, even though the *in vitro* and *in vivo*

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http://dx.doi.org/10.1016/j.jconrel.2013.06.022

data supporting a rHuEPO cardioprotective approach are numerous, recent randomized clinical trials in acute MI patients have reported conflicting data [13,15–17].

The development of drug delivery systems (DDS) has provided new perspectives for the modification of pharmacokinetics and biodistribution of associated genes and proteins by controlling the release rates of therapeutics [18–20]. Recently, we developed a bioreducible ABP polymer, retaining the unique properties of reductive disulfide linkers coupled with the advantage of arginine residues to enhance cell penetration [21]. In addition, we reported greatly enhanced *in vitro* transfection efficiency and very low cytotoxicity, as well as increased *in vivo* erythropoietic effects over a 60-day period after a single systemic injection of phEPO/ABP polyplexes [22,23].

To date, little is known about how polymer-mediated phEPO therapy, when compared with naked phEPO gene or rHuEPO protein-alone, distinctly alters cardiac remodeling in the rat MI model. Here, we hypothesized that the sustained release of intramyocardial phEPO gene therapy delivered by ABP polymer might restore heart function and limit pathological cardiac remodeling after MI. Additionally, the present study assessed the effect of phEPO/ABP gene therapy on cardiac remodeling by evaluating the pro-fibrotic angiotensin II (Ang II) and TGF-β expression in rat hearts.

2. Materials and methods

Detailed protocols are provided in the Supplementary material online.

2.1. Preparation of phEPO/polymer polyplexes

The pCMV-hEPO DNA (phEPO) (4578 bp) was constructed and purified as previously described [22,23]. Briefly, phEPO and GFP pDNA (gWiz-GFP, Aldevron) were purified with an endotoxin-free plasmid DNA purification NucleoBond® Xtra Maxi plus EF kit (Macherey-Nagel Inc.). The arginine-grafted bioreducible poly(cystaminebisacrylamide-diaminohexane) ABP polymer was synthesized as previously described [21]. Branched poly(ethyleneimine) (bPEI, 25 kDa, Sigma-Aldrich) and rHuEPO protein (Aropotin®, TS Corporation) were used as positive controls. The 50 μg phEPO/ABP polyplexes at a weight ratio of 1:5 were prepared in a 20 mM HEPES/5% glucose buffer with a final volume of 100 μl.

2.2. Myocardial infarction model

MI was induced in 7–8-week-old male Sprague–Dawley (SD) rats (220–250 g) by surgical occlusion of the left anterior descending (LAD) coronary artery as previously described [9]. Briefly, under the mechanical ventilation, the LAD coronary artery was ligated for 60-min occlusion. Successful ischemia was verified by the blanching of the myocardium and dyskinesia of the ischemic zone, indicating interruption in coronary flow. After 60 min of occlusion, the hemostat was removed, and the temporary suture snare was released for reperfusion. Restoration of normal rubor indicated successful reperfusion of myocardium. Following successful ischemia–reperfusion (I/R), the animals were assigned to one of seven groups: sham thoracotomy, I/R only, injection of rHuEPO, injection of phEPO-alone, injection of phEPO/ABP polyplex, injection of phEPO/PEI polyplex, and injection of GFP plasmid/ABP polyplex. Right after reperfusion, the rats received a total injection volume of 100 μl delivered to four separate intramyocardial sites with three injections to the border zone of the infarct in left ventricle (LVfb) and one injection to the fibrotic central zone in left ventricle (LVf) (Fig. 1C).

2.3. Echocardiography

On post-infarct days 5 and 10 after the intramyocardial injection, echocardiography was performed using a 13 MHz linear probe (GE Vivid 7 pro, GE Medical Systems) in rats lightly anesthetized with isoflurane at 1–2 L/min and spontaneous respiration.

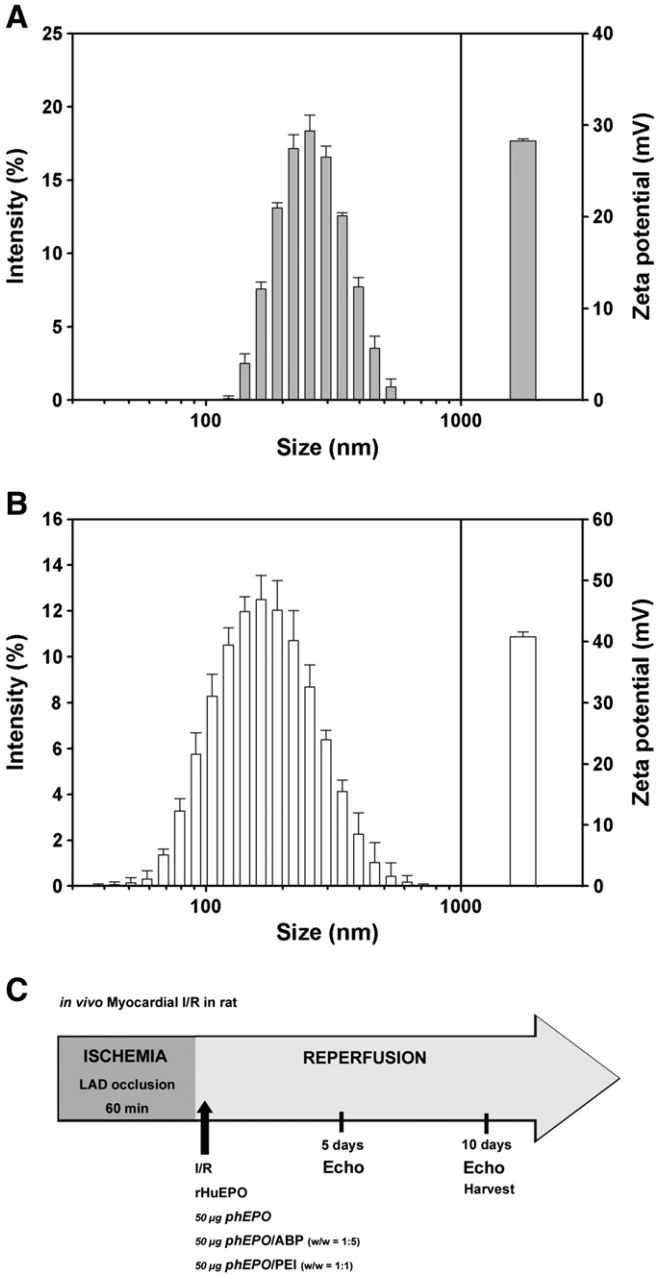

Fig. 1. Characterization of phEPO/polymer polyplexes. Average particle size and Zeta potential of 50 μg phEPO/ABP polyplex (w/w = 1:5) (A) and 50 μg phEPO/PEI polyplex (w/w = 1:1) (B). Experimental time-dependent protocol (C).

2.4. Pathological analysis

Serial 4 μm thick sections of rat myocardium were fixed, embedded, and stained with H&E stain. Fibrosis, determined by collagen contents and infarct size was evaluated by Masson's trichrome stain. Next, to evaluate the arteriolar density and the loss of cardiomyocytes after MI, heart sections were immunohistochemically (IHC) stained using α-smooth muscle actin (α-SMA) and cardiomyocyte-specific cardiac troponin T (cTnT) antibody. Finally, apoptosis in the LVfb was expressed as the number of TUNEL-positive nuclei per unit area. Analysis of all images was carried out with NIH ImageJ software (NIH) and Aperio ImageScope (Vista) and randomly chosen within the LVfb by an investigator blinded to the treatment groups.