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Encapsulation of beraprost sodium in nanoparticles: Analysis of sustained release properties, targeting abilities and pharmacological activities in animal models of pulmonary arterial hypertension

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ABSTRACT

Prostaglandin I_2 (PGI_2) and its analogues (such as beraprost sodium, BPS) are beneficial for the treatment of pulmonary arterial hypertension (PAH). The encapsulation of BPS in nanoparticles to provide sustained release and targeting abilities would improve both the therapeutic effect of BPS on PAH and the quality of life of patients treated with this drug.

BPS was encapsulated into nanoparticles prepared from a poly(lactic acid) homopolymer and monomethoxy poly(ethyleneglycol)–poly(lactide) block copolymer. The accumulation of nanoparticles in damaged pulmonary arteries was examined using fluorescence-emitting rhodamine S-encapsulated nanoparticles. The monocrotaline-induced PAH rat model and the hypoxia-induced mouse model were used to examine the pharmacological activity of BPS-encapsulated nanoparticles.

A nanoparticle, named BPS-NP, was selected among various types of BPS-encapsulated nanoparticles tested; this was based on the sustained release profile *in vitro* and blood clearance profile *in vivo*. Fluorescence-emitting rhodamine S-encapsulated nanoparticles were prepared in a similar manner to that of BPS-NP, and showed accumulation and prolonged residence in monocrotaline-damaged pulmonary peripheral arteries. Intravenous administration of BPS-NP (once per week, 20 μg/kg) protected against monocrotaline-induced pulmonary arterial remodeling and right ventricular hypertrophy. The extent of this protection was similar to that observed with oral administration (once per day, 100 μg/kg) of BPS alone. The once per week intravenous administration of BPS-NP (20 μg/kg) also exhibited an ameliorative effect on hypoxia-induced pulmonary arterial remodeling and right ventricular hypertrophy.

The beneficial effects of BPS-NP on PAH animal models seem to be mediated by its sustained release and tissue targeting profiles. BPS-NP may be useful for the treatment of PAH patients due to reduced dosages and frequency of BPS administration.

1. Introduction

Pulmonary arterial hypertension (PAH) is a rare but life-threatening disease characterized by progressive increases in pulmonary vascular resistance, vasoconstriction and arterial pressure, which lead to right ventricular (RV) hypertrophy and failure, and subsequently to death [1–3]. Although several classes of drugs have been approved for the treatment of PAH (such as endothelin receptor antagonists and phosphodiesterase-5 inhibitors), the prognosis for this disease remains poor (median survival, 2.8 years) [4,5]. While the primary cause of PAH is unclear, it is believed that pulmonary vasoconstriction, endothelial cell proliferation, inflammation, smooth muscle cell proliferation, and thrombosis play key roles in the pathogenesis of PAH [1,2,6].

The progression of PAH is associated with a reduced production of prostaglandin I_2 (PGI_2) [7,8], a potent vasodilator produced in the vascular endothelium, which also has antithrombotic effects and inhibitory effects on smooth muscle cell proliferation [5,9]. Thus, PGI_2 and its analogues

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could be therapeutically beneficial for the treatment of PAH in affected patients [5,10–16]. In fact, the intravenous administration of epoprostenol (PGI_2) is currently the most effective approach for the treatment of PAH, not only because it can ameliorate symptoms of severe PAH, but because it also prolongs life expectancy [16,17]. One major drawback of PGI_2 is that it is very unstable in blood [18,19], meaning that epoprostenol must be intravenously infused on a continuous basis, thereby drastically decreasing the quality of life (QOL) of patients due to problems associated with cosmetic appearance, transportation of an infusion pump and risk of infection [14,20]. For these reasons, stable PGI_2 analogues such as beraprost sodium (BPS) formulated for various routes of administration (oral, subcutaneous and inhalation) have been developed [5,18]. However, the half-life of these PGI_2 analogues in the blood remains short and their therapeutic efficacy in PAH patients is inferior to that of continuously infused epoprostenol [18,21]. For example, the half-life of BPS in the blood is less than 40 min following its oral administration, and it is not effective in the treatment of patients with severe PAH, even with frequent administration [5,22,23]. Therefore, a drug delivery system (DDS) that enables the sustained release of intravenously administered PGI_2 or its analogues would be clinically beneficial. On the other hand, various physiological activities of PGI_2 are related to adverse effects (such as hypotension) due to its whole-body distribution when administered systemically [24]. Furthermore, adverse events, such as headache, jaw pain, vasodilation, nausea, diarrhea, leg pain, and foot pain were observed in patients treated with BPS [23,25]. Therefore, a DDS that enables the specific targeting of PGI_2 or its analogues to the damaged pulmonary arteries is also important.

We recently established a new DDS that enables both the sustained release and specific targeting of encapsulated drugs [26–30]. By employing a nanotechnology approach, drugs are encapsulated in solid nanoparticles prepared from biodegradable and biocompatible poly(lactide) homopolymer (PLA) [31,32]. This type of nanoparticle can be used to achieve the sustained release of encapsulated drug, which takes place concomitantly with the degradation of PLA [28,31,32]. To avoid the uptake of these nanoparticles by the mononuclear phagocyte system [32,33], a monomethoxy poly(ethyleneglycol)–poly(lactide) block copolymer (PEG–PLA) was used in combination with PLA. Furthermore, the encapsulation of drug compounds in small-diameter nanoparticles (50–150 nm) would enhance the selective delivery of the drug to damaged blood vessels due to the enhanced permeability and retention (EPR) effect [34].

In the present study, we succeeded in encapsulating BPS into this type of nanoparticle (BPS-NP). BPS-NP showed a sustained release profile *in vitro* and a prolonged residence in the blood and accumulation in damaged pulmonary peripheral arteries *in vivo*. Compared to the oral administration of BPS alone, the intravenous administration of BPS-NP in animal models of PAH showed ameliorative effects even at lower doses and frequencies of administration. These results suggest that BPS-NP could serve as a useful option for the treatment of PAH patients.

2. Materials and methods

2.1. Materials and animals

BPS, monocrotaline, pentobarbital, fluorescein isothiocyanate (FITC)-labeled albumin and fetal bovine serum (FBS) were obtained from Sigma (St. Louis, MO). D,L-PLA (MW = 5000 or 20,000), 1,4-dioxane, iron chloride and rhodamine S (RS) were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). L-PLA (MW = 5000 or 20,000) was from Taki Chemical Co., Ltd. (Kakogawa, Japan). PEG–D,L-PLA (average molecular weight of PEG and PLA are 5600 and 9200, respectively) or PEG–L-PLA (average molecular weight of PEG and PLA are 5600 and 9400, respectively) was synthesized and evaluated as described previously [28,35]. Diethanolamine (DEA) was from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Wistar rats (5 weeks old, male) and C57/BL6 mice (8 weeks old, male) were from Kyudo Co., Ltd. (Kumamoto, Japan) or Charles River Laboratories Inc. (Yokohama, Japan). The experiments and procedures described here were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health, and were approved by the Animal Care Committee of Kumamoto University and Keio University.

2.2. Determination of BPS and RS levels in samples

BPS and RS levels in samples were determined by analytical high-performance liquid chromatography (HPLC) with a reverse-phase column (L-column ODS (150 × 4.6 mm, 5 μm, Chemicals evaluation research institute, Tokyo, Japan) or Eclipse XDB-C18 column (150 × 2.1 mm, 5 μm, Agilent Technologies, Santa Clara, CA)). A Waters 2695 Alliance separation module, a Waters 2996 photodiode array detector, and a Waters Alliance system running Empower software (Waters, Milford, MA) were used for HPLC analysis.

For detection of BPS, solvent A (0.1% acetic acid in Methanol) and solvent B (0.1% acetic acid in Milli-Q water) were used at a flow rate of 0.5 ml/min. After injection of the sample (0 min), the mobile phase was changed as follows; 70% solvent A (20 min), a linear gradient of 70–100% solvent A (2 min), 100% solvent A (11 min) and a linear gradient of 100–70% solvent A (2 min). Detection was performed at an optical density of 285 nm.

For detection of RS, solvent A (0.1% acetic acid in acetonitrile) and solvent B (0.1% acetic acid in Milli-Q water) were used at a flow rate of 0.05 ml/min. After injection of the sample, 80% solvent A was used as a mobile phase in isocratic elution mode for 30 min. Detection was performed at an optical density of 530 nm.

2.3. Analysis of solubility of BPS and RS

BPS or RS (0.56 mM) was mixed with DEA (various concentrations) and iron chloride (10 mM) in ethanol. After incubation for 30 min at 25 °C, the pH value of the solutions/suspensions was measured and they were then centrifuged at 13,000 rpm for 10 min to precipitate insoluble BPS or RS. The amount of BPS or RS in the supernatant was determined by analytical HPLC as mentioned above.

2.4. Preparation and characterization of nanoparticles

Nanoparticles were prepared by using the oil-in-water solvent diffusion method as described previously [27,28]. We named nanoparticle types based on the chirality (L or DL) of PLA and PEG–PLA and the molecular weight of PLA (Table 1).

For preparation of L-L20, L-DL20, DL-L20 or DL-DL20, 26 mg of L-PLA or D,L-PLA in 300 μl of 1,4-dioxane, 24 mg of PEG–L-PLA in 300 μl of

Table 1
Characterization of BPS- or RS-encapsulated nanoparticles.
Nanoparticles encapsulated with BPS (A) or RS (B) were prepared by an oil-in-water solvent diffusion method. Particle size and BPS (A) or RS (B) content in the particles were determined by the dynamic light scattering method and HPLC, respectively. Values are mean ± S.E.M. ($n = 3$).

A

NP code	PLA–PEG	PLA $_{(Mw, kDa)}$	Diameter (nm)	BPS content (%)
L-L20	L-PLA–PEG	L-PLA $_{(20)}$	121 ± 2	1.23 ± 0.02
L-DL20 $_{(BPS-NP)}$	L-PLA–PEG	DL-PLA $_{(20)}$	128 ± 3	1.08 ± 0.04
L-L5	L-PLA–PEG	L-PLA $_{(5)}$	109 ± 2	0.86 ± 0.03
L-DL5	L-PLA–PEG	DL-PLA $_{(5)}$	112 ± 1	0.88 ± 0.02
DL-L20	DL-PLA–PEG	L-PLA $_{(20)}$	112 ± 3	1.15 ± 0.08
DL-DL20	DL-PLA–PEG	DL-PLA $_{(20)}$	125 ± 3	0.86 ± 0.06
DL-L5	DL-PLA–PEG	L-PLA $_{(5)}$	108 ± 2	2.39 ± 0.09
DL-DL5	DL-PLA–PEG	DL-PLA $_{(5)}$	107 ± 1	2.16 ± 0.05

B

NP code	PLA–PEG	PLA $_{(Mw, kDa)}$	Diameter (nm)	RS content (%)
RS-NP	L-PLA–PEG	DL-PLA $_{(20)}$	107 ± 1	1.65 ± 0.04