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Intratracheal Administration of Prostacyclin Analogue–incorporated Nanoparticles Ameliorates the Development of Monocrotaline and Sugen-Hypoxia-induced Pulmonary Arterial Hypertension

Satoshi Akagi, MD, PhD, Kazufumi Nakamura, MD, PhD,* Hiromi Matsubara, MD, PhD,† Megumi Kondo, MSc,* Daiji Miura, PhD,* Tetsuya Matoba, MD, PhD,‡ Kensuke Egashira, MD, PhD,‡ and Hiroshi Ito, MD, PhD**

Abstract: Nanoparticles (NPs) have been used as novel drug delivery systems. Drug-incorporated NPs for local delivery might optimize the efficacy and minimize the side effects of drugs. Intravenous prostacyclin improves long-term survival in patients with pulmonary arterial hypertension (PAH), but it causes serious side effects such as catheter-related infections. We investigated the efficacy and safety of intratracheal administration of a prostacyclin analogue, beraprost (BPS), incorporated NPs in Sugen-hypoxia-normoxia and monocrotaline rat models of PAH and in human PAH pulmonary arterial smooth muscle cells (PASMCs). After a single administration, BPS NPs significantly decreased right ventricular pressure, right ventricular hypertrophy, and pulmonary artery muscularization in the 2 rat models. BPS NPs significantly improved the survival rate in the monocrotaline rat model. No infiltration of inflammatory cells, hemorrhage, or fibrosis was found in the liver, kidney, spleen, and heart after the administration of BPS NPs. No liver or kidney dysfunction was found in the blood examinations. BPS and BPS NPs significantly inhibited the proliferation of human PAH PASMCs after 24 hours of treatment. BPS NPs significantly continued to inhibit the proliferation of human PAH PASMCs at 24 hours after the removal of BPS NPs. BPS NPs significantly induced apoptosis in PAH PASMCs compared to that in non-PAH PASMCs. Intratracheal administration of BPS NPs ameliorates pulmonary hypertension in PAH rat models by a sustained antiproliferative effect and a proapoptotic effect on PAH PASMCs.

Key Words: pulmonary hypertension, nanomedicine, drug delivery system, prostacyclin, intratracheal administration, pulmonary arterial smooth muscle cells

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INTRODUCTION

Pulmonary arterial hypertension (PAH) is characterized by vasoconstriction, vascular remodeling caused by intimal and medial hypertrophy, and thrombosis. These changes in pulmonary arteries cause the elevation of pulmonary artery pressure and pulmonary vascular resistance, leading to right-heart failure and death. Three pathways (such as the prostacyclin pathway, endothelin pathway, and nitric oxide pathway) are involved in the development of PAH.[1] A decrease of prostacyclin plays a particularly important role in the pathogenesis of PAH. Prostacyclin is a physiological vasodilator in humans that was discovered in 1976.[2] It lowered the pulmonary vascular resistance to baseline levels, but did not decrease the systemic arterial pressure beyond baseline levels.[3] Prostacyclin is a candidate for treatment of PAH, and its replacement therapy has shown to improve hemodynamics and exercise capacity.[4–7] This therapy is one of the best treatments available for PAH, but several problems remain.

Treatment with prostacyclin involved continuous delivery intravenously through a central venous catheter with an infusion pump because of the short half-life of prostacyclin. The delivery system using a central venous catheter causes serious complications, such as catheter-related infections,[8] thrombosis, and temporary interruption of infusion due to malfunction of the pump. These complications sometimes cause deterioration of pulmonary hypertension and are life threatening. Patients with PAH must dissolve prostacyclin in a diluent themselves, which is very cumbersome. Systemic administration of prostacyclin can induce headaches, flushing, and sometimes severe hypotension at the start of prostacyclin therapy.[9] These problems could be solved if an alternative system to target the delivery of prostacyclin to the pulmonary vasculature without using a central venous catheter is developed.

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From the *Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; †Department of Clinical Science, National Hospital Organization Okayama Medical Center, Okayama, Japan; and ‡Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan.

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Reprints: Satoshi Akagi, MD, PhD and Kazufumi Nakamura, MD, PhD, Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan (e-mails: akagi-s@cc.okayama-u.ac.jp and ichibun@cc.okayama-u.ac.jp).

Nanoparticles (NPs) have been used as a novel delivery system for transport of drugs to target organs.[10] NPs are taken up by the target organ because of their small size, permeability, and their retention effect. Drug release from NPs is controlled according to the NP composition. Thus, drug-incorporated NPs for local delivery might optimize the efficacy and minimize the side effects of drugs. Therefore, local administration of prostacyclin incorporated in NPs might solve the problems caused by intravenous continuous prostacyclin therapy. The aim of this study was to investigate the efficacy and safety of the delivery of a prostacyclin analogue, beraprost (BPS), incorporated in NPs (BPS NPs) into lungs of rats with pulmonary arterial hypertension.

METHODS

Preparation of NPs

Polylactide-glycolide (PLGA) NPs encapsulated with fluorescein isothiocyanate (FITC) or BPS (Toray) were prepared using an emulsion solvent diffusion method, as previously reported.[11] In brief, PLGA with an average molecular weight of 20,000 and a copolymer ratio of lactide to glycolide of 72:25 (Wako Pure Chemical Industries, Osaka, Japan) was used as the wall material for the NPs. PLGA was dissolved in a mixture of acetone and methanol. Then, FITC or BPS was added to the solution. The PLGA–FITC or PLGA–BPS solution was dropped into 50 mL of purified water at 40°C with a speed of 400 rpm using a propeller-type agitator with 4 blades. After evaporating the organic solvent for approximately 2 hours under reduced pressure at 40°C, the prepared suspension of FITC NPs or BPS NPs was filtered using a membrane filter of 32 μm in average pore size for removing agglomerates of the NPs. The FITC NPs and BPS NPs contained 2% (wt/vol) FITC and 9% (wt/vol) BPS, respectively.

Animal Study Protocol

The animal protocol was approved by the Animal Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences (Permit number: OKU-2013195). Sugen-hypoxia-normoxia (SuHx) and monocrotaline (MCT) PAH rat models were used in this study. For the SuHx model, adult male Sprague–Dawley rats (Charles River, Yokohama, Japan; 200–250 g in body weight) received a single subcutaneous injection of SU5416 (20 mg/kg; Cayman Chemical) and were exposed to normobaric hypoxia for 3 weeks (10% O_2). The animals were assigned to either an untreated control group or a group that received a single intratracheal administration of phosphate-buffered saline (PBS), FITC NPs (1 mg of PLGA), or BPS NPs (150 μg/kg BPS per millgram of PLGA) 3 weeks after an SU5416 injection. We previously reported that high-dose prostacyclin therapy remarkably improved hemodynamics in patients with idiopathic PAH.[12] The average dose of prostacyclin in the study was 107 $ng \cdot kg^{-1} \cdot min^{-1}$, and it was nearly 100 $ng \cdot kg^{-1} \cdot min^{-1}$. We referred to the clinical results and determined the dose in this study as mentioned in the following sections. Three hundred and sixty micrograms of BPS in a human being of 60 kg in body weight are equivalent to 4 $ng \cdot kg^{-1} \cdot min^{-1}$ of prostacyclin. Thus, 6 μg/kg of BPS are equivalent to 4 $ng \cdot kg^{-1} \cdot min^{-1}$ of prostacyclin. We set the dose of BPS to 150 μg/kg (6 μg/kg × 25) in this study. We administered the FITC NPs and BPS NPs into lungs in the form of a fine mist using MicroSprayer Aerosolizers (Penn-Century, Inc) (Fig. 5A). We insert MicroSprayer into trachea of rats under inhalation of isoflurane. The tip of MicroSprayer is located at the bifurcation of the trachea. For intratracheal administration, a 0.1-mL suspension of FITC NPs or BPS NPs was injected gently into the trachea of each animal, accompanied by an equal volume of air. All surgery was performed under inhalation of isoflurane (1.8% vol/vol) anesthesia, and all efforts were made to minimize the pain. They were returned to normoxia for a period of 2 weeks, and hemodynamics and lung tissues were examined. For the MCT model, adult male Sprague–Dawley rats (Charles River; 250–300 g in body weight) received a single subcutaneous injection of 60 mg/kg MCT (Sigma-Aldrich). Two weeks after the MCT injection, the animals were assigned to either an untreated control group or a group that received a single intratracheal administration of PBS, FITC NPs, or BPS NPs. Two weeks after the treatment, hemodynamics and lung tissues were examined.

Assessment of Right Ventricular Pressure, Right Ventricular Hypertrophy and Survival

The rats were anesthetized with inhalation of isoflurane, and a high-fidelity 1.4F Millar catheter (Millar Instruments Inc) was inserted into the right ventricle (RV) through the right jugular vein. RV systolic pressure (RVSP) was measured with the PowerLab System using Chart 5.0 software. After hemodynamics had been recorded, the rats were killed and their lungs and heart were isolated. The RV wall was dissected from the left ventricle (LV) and ventricular septum (VS). The wet weight of the RV and LV + VS was determined, and RV hypertrophy was expressed as RV weight/(LV + VS) weight. Survival rate was studied in the MCT model rat.

Histological Analysis

The lung was fixed for histology in 10% neural buffered formalin. For paraffin embedding, the lungs were dissected in tissue blocks from all lobes. Sectioning at 3 μm was performed from all paraffin-embedded blocks. To assess the type of remodeling in the muscular pulmonary arteries, Elastica van Gieson staining was performed according to common histopathological procedures. In each rat, 30–40 intra-acinar arteries were categorized as muscular (those with a complete medial coat of muscle), partially muscular (those with only a crescent of muscle), or nonmuscluar (those with no apparent muscle), counted, and averaged with a range of diameters from 25 to 50 μm.

We confirmed damage of nontargeted organs using BPS NPs in the SuHx model. One week after the rats had received a single intratracheal administration of PBS, FITC NPs or BPS NPs, the liver, kidney, spleen, and heart were isolated. The liver, kidney, spleen, and heart were fixed for histology in 10% neutral-buffered formalin. Hematoxylin and

eosin staining was performed according to common histopathological procedures.

We examined the incorporation of FITC NPs in lungs of the SuHx model rats and normal rats by immunostaining. For identification of smooth muscle cells, an α-smooth muscle actin mouse monoclonal antibody was used. The primary antibody was detected with rabbit anti-mouse immunoglobulin TRITC (DakoCytomation). For identification of endothelial cells, PECAM-1 goat polyclonal antibody (M-20; Santa Cruz) was used. The primary antibody was detected with mouse anti-goat IgG-R (sc-53802; Santa Cruz). Nuclear morphology was examined by labeling with 4′,6-diamidino-2-phenylindole. Tissues were analyzed using a fluorescence microscope (Olympus IX71; Olympus Optical Co Ltd, Tokyo, Japan).

Blood Examinations

One week after the rats had received a single intratracheal administration of PBS, FITC-NPs, or BPS-NPs, blood samples were collected. Blood sample analyses were undertaken by the clinical testing laboratory SRL Co Ltd (Tokyo, Japan).

Isolation of Human Pulmonary Artery Smooth Muscle Cells

Pulmonary artery samples were obtained from 6 patients with idiopathic PAH (4 men and 2 women; mean age, 17 ± 8 years) during lung transplantation and obtained from 3 patients with lung cancer during lung lobectomy (Table 1). All human subject protocols were approved by the Human Ethics Committee of the Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences (permit number: 1233), and written informed consent was obtained from all patients before the procedure. In brief, peripheral pulmonary arteries smaller than 1 mm in outer diameter were disaggregated with collagenase in a water bath for 15 minutes at 37°C. The adventitia and intima were removed, and isolated arteries were cut into 2-mm-long sections. The cut arteries were placed in a 6-well plate with Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Sigma) and 0.1 mg/mL kanamycin (Sigma). They were incubated in a humidified 5% CO_2 atmosphere at 37°C. Isolated cells were identified by positive staining with antibodies against α-smooth muscle actin, myosin, and smoothelin. Pulmonary artery smooth muscle cells (PASMCs) were used for the scheduled experiments at passage 6.[13]

Human PASMC Proliferation Assay

We measured ^{3}H-thymidine incorporation for assessment of proliferation by methods previously described.[11] Human PASMCs were seeded in 24-well plates at a density of 1×10^5 cells/well in 10% FBS and DMEM on day 0. After 16 hours of incubation (on day 1), the culture medium was replaced with 0.1% FBS/DMEM. We measured ^{3}H-thymidine incorporation by using 2 protocols. Protocol 1: On day 2, platelet-derived growth factor (PDGF)-BB (10 ng/mL) (Sigma), BPS (1 μM) or BPS NPs containing 1 μM of BPS were added to the culture medium. After 24 hours of treatment (on day 3), these drugs were removed, and 0.1% FBS/DMEM was added and cultured. After 21 hours (on day 4), the cells were labeled with ^{3}H-thymidine at 1 μCi/mL for 3 hours. After completion of labeling, the cells were washed with ice-cold PBS, fixed with 5% trichloroacetic acid and 95% ethanol, and lysed with 200 μL/well of 0.33 mol/L NaOH. Aliquots of the cell lysates were neutralized with 1 mol/L of HCl, and the radioactivity was measured in a liquid scintillation analyzer (Tri Carb 2200CA; Packard, Downers Grove, IL). Protocol 2: After 48 hours of culture with 0.1% FBS/DMEM (on day 3), PDGF-BB (10 ng/mL), BPS (1 μM) or BPS NPs containing 1 μM BPS was added to the culture medium. After 21 hours (on day 4), the cells were labeled with ^{3}H-thymidine at 1 μCi/mL for 3 hours. Protocol 2 was the same as protocol 1 after that.

Human PASMC Apoptosis Assay

TdT-mediated dUTP nick end labeling (TUNEL) assays were performed using an ApopTag Plus Fluorescein in situ Apoptosis Detection Kit (Chemicon International Inc) according to the manufacturer's instructions. Nuclear morphology was examined by labeling with 4′,6-diamidino-2-phenylindole.

BPS Level in Serum of Rats and Culture Medium

We measured the serum BPS level in rats and BPS level in human cultured PAH PASMCs by liquid chromatography–tandem mass spectrometry (LC/MS/MS, LCMS-8040; Shimadzu Corporation). Sera were obtained from rats at 1, 3, and 7 days after the intratracheal administration of BPS

TABLE 1. Clinical Data of Patients With Idiopathic Pulmonary Arterial Hypertension Used for Experiments

Patients	Age/Sex	PAP (S/d/m)	PVR	CI	BNP	Prostacyclin Dose	Duration (yr)
1	11/M	130/51/80	2629	1.9	420	26	5
2	16/F	83/51/65	784	2.5	216	60	0.5
3	10/M	116/57/80	1864	3.5	49.6	54	2.2
4	25/M	78/30/49	429	3.0	163	156	9.7
5	12/F	125/69/97	3012	2.0	38.5	—	—
6	28/M	76/33/53	770	3.6	23.1	—	—

BNP, plasma concentration of brain natriuretic peptide (picogram per deciliter); CI, cardiac index (Liter per minute per meter); F, female; M, male; PAP, pulmonary artery pressure (millimeter of mercury); Prostacyclin dose, maximum dose of Prostacyclin given (nanogram per killogram per·minute), and duration, period between start of prostacyclin therapy and termination of PGI_2 therapy (years) PVR, pulmonary vascular resistance (dyne·sec·cm^{-5}); s/d/m, systolic/diastolic/mean.

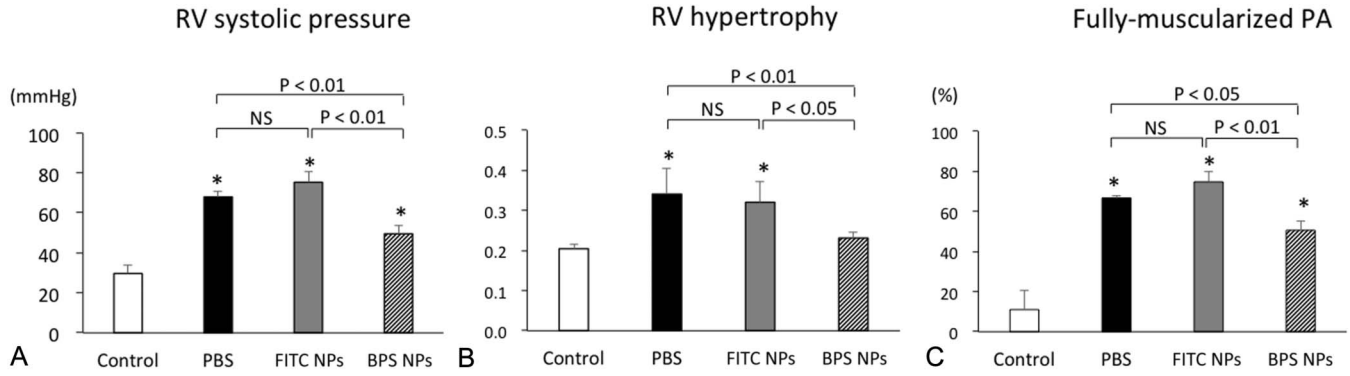

FIGURE 1. Effects of a single administration of BPS NPs in SuHx model rats. A, RVSP in the 3 experimental groups (n = 6). B, RV hypertrophy [ratio of RV/(LV + VS)] in the 3 experimental groups (n = 6). C, Percentage of fully muscularized small pulmonary arteries (PAs) in the 3 experimental groups (n = 6). *$P < 0.05$ versus control.

NPs (150 μg/kg BPS per milligram of PLGA). Human PAH PASMCs were seeded in 6-well plates at a density of 3×10^5 cells/well in 10% FBS/DMEM. After 24 hours of incubation, BPS NPs containing 1 μM BPS were added to the culture medium. After 24 hours of incubation, the culture medium was replaced with 0.1% FBS/DMEM. The plasmas were obtained at 1, 3, and 7 days after the replacement of 0.1% FBS/DMEM.

Statistical Analysis

Data are presented as means ± SD. Differences between groups were assessed by analysis of variance and post-hoc Student's *t* test for multiple comparisons, and a *P* value < 0.05 was considered significant. Survival rate was analyzed using the Kaplan–Meier method in the PBS and BPS-NP groups.

RESULTS

Effects of BPS NPs on RVSP and RV Hypertrophy

In SuHx model rats, a single intratracheal administration of PBS or FITC NPs resulted in an increase of RVSP (PBS = 68.0 ± 2.9 mm Hg, FITC-NPs = 75.3 ± 5.3 mm Hg vs. control = 29.7 ± 4.0 mm Hg; $P < 0.05$; Fig. 1A). A single intratarcheal administration of BPS NPs significantly ameliorated RVSP (49.5 ± 4.3 mm Hg vs. PBS and FITC NPs; $P < 0.01$). The RV/(LV + VS) ratio was significantly increased after a single administration of PBS and FITC NPs, compared with that in the control group (PBS = 0.34 ± 0.06, FITC NPs = 0.32 ± 0.05 vs. control = 0.20 ± 0.01; $P < 0.05$; Fig. 1B). A single intratracheal administration of BPS NPs significantly ameliorated the RV/(LV + VS) ratio (BPS NPs = 0.23 ± 0.01 vs. PBS and FITC NPs; $P < 0.05$).

In MCT model rats, a single intratracheal administration of PBS or FITC NPs resulted in an increase in RVSP (PBS = 83.9 ± 11.0 mm Hg, FITC NPs = 86.6 ± 13.3 mm Hg vs. control = 21.8 ± 3.2 mm Hg; $P < 0.05$; Fig. 2A). A single intratracheal administration of BPS NPs significantly ameliorated RVSP (62.7 ± 15.3 mm Hg vs. PBS and FITC NPs; $P < 0.05$). Also, the RV/(LV + VS) ratio was significantly increased by a single administration of PBS and FITC NPs, compared with that in the control group (PBS = 0.54 ± 0.07, FITC-NPs = 0.59 ± 0.09 vs. control = 0.23 ± 0.03; $P < 0.05$; Fig. 2B). A single intratracheal administration of BPS NPs significantly ameliorated the RV/(LV + VS) ratio (0.39 ± 0.09 vs. PBS and FITC NPs; $P < 0.05$).

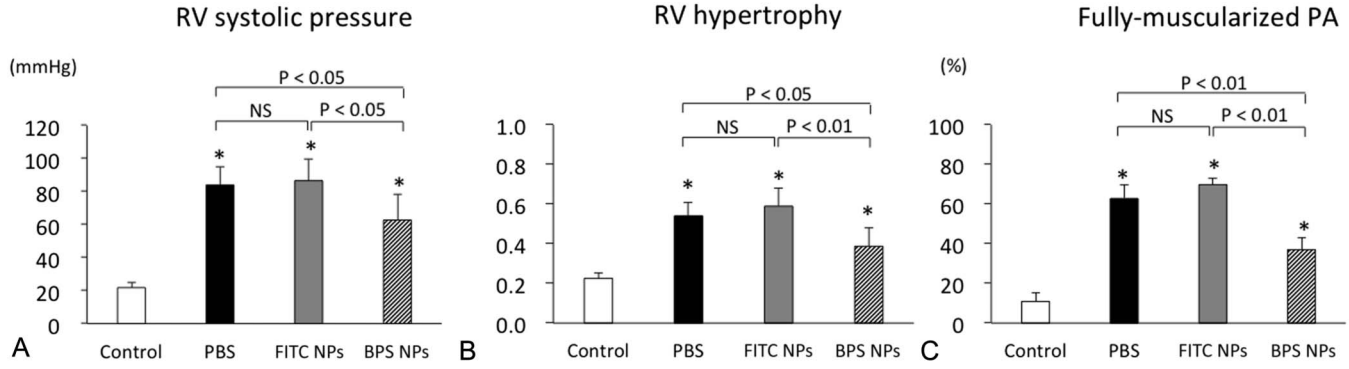

FIGURE 2. Effects of a single administration of BPS NPs in MCT model rats. A, RVSP in the 3 experimental groups (n = 6). B, RV hypertrophy [ratio of RV/(LV + S)] in the 3 experimental groups (n = 6). C, Percentage of fully muscularized small PAs in the 3 experimental groups (n = 6). *$P < 0.05$ versus control.

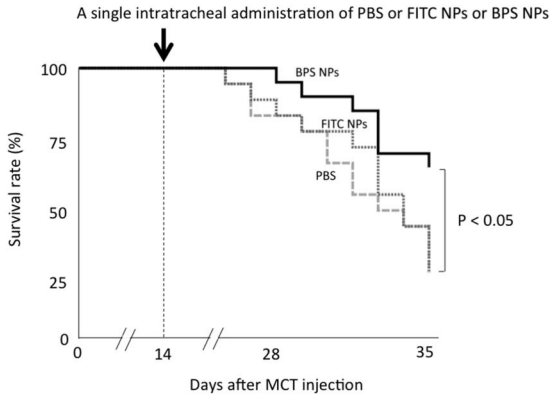

FIGURE 3. Effects of a single administration of BPS-NPs on survival rates. Survival rates analyzed by the Kaplan–Meier method in the PBS, FITC NP, and the BPS NP group are shown.

Effects of BPS NPs on Pulmonary Vascular Morphology

In SuHx model rats, the proportion of small vessels with full muscularization was greater in the PBS group (67 ± 0.8%) and FITC-NP group (75 ± 5.1%) than that in the control group (11 ± 10%) (Fig. 1C). A single administration of BPS NPs significantly reduced the percentage of small vessels with full muscularization (51 ± 4.5%, vs. PBS and FITC NPs; $P < 0.05$).

In MCT model rats, the proportion of small vessels with full muscularization was greater in the PBS group (63 ± 7%) and FITC NPs group (70 ± 3%) than that in the control group (11 ± 4%) (Fig. 2C). A single administration of BPS NPs significantly reduced the percentage of small vessels with full muscularization (37 ± 6%, $P < 0.01$).

Effects of BPS NPs on Survival

BPS NPs significantly improved survival rate to 27.8% in the PBS group (n = 18), 27.8% in the FITC NPs group (n = 18) and 65.0% in the BPS NPs group (n = 20) at 35 days after MCT injection (Fig. 3).

Distribution of FITC-NPs in the SuHx Model and Normal Rats

We examined the incorporation of FITC-NPs in the lungs of SuHx model rats and normal rats after a single intratracheal instillation of FITC-NPs. Many FITC NPs were incorporated in the media of pulmonary arteries and some FITC NPs were incorporated in interstitium of SuHX model rats, 1 day after a single intratracheal instillation of FITC NPs (Fig. 4A). FITC-NPs continued to be incorporated in the media of pulmonary arteries, 3 days after a single intratracheal instillation of FITC NPs. FITC NPs were not incorporated in the lungs of normal rats or lungs of SuHx model rats 1 week after a single intratracheal instillation of FITC NPs. FITC NPs were not incorporated in the intima of pulmonary arteries of SuHx model rats and normal rats at day 1, 3, and 7 (Fig. 4B). We examined the deposition of NPs in the airways using FITC NPs. FITC NPs were not detected in trachea, right bronchi,

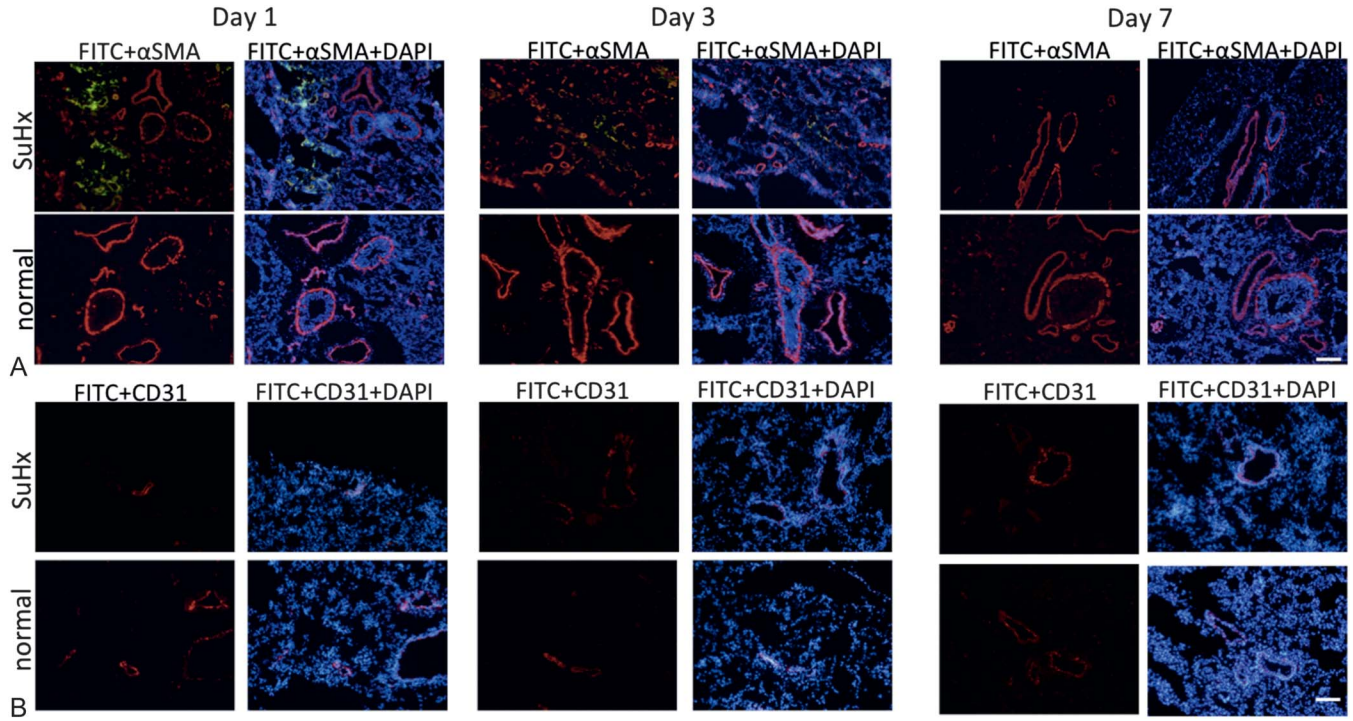

FIGURE 4. Incorporation of FITC NPs in pulmonary arteries. Representative images of the lung by immunostaining. A, Staining of α-smooth muscle actin (αSMA) in SuHx model rats and normal rats at day 1, 3, and 7. Green is FITC. Red is αSMA. Blue is 4′,6-diamidino-2-phenylindole (DAPI). Bar = 500 μm. B, Staining of CD31 in SuHx model rats and normal rats on day 1, 3, and 7. Green is FITC. Red is CD31. Blue is DAPI. Bar = 500 μm.

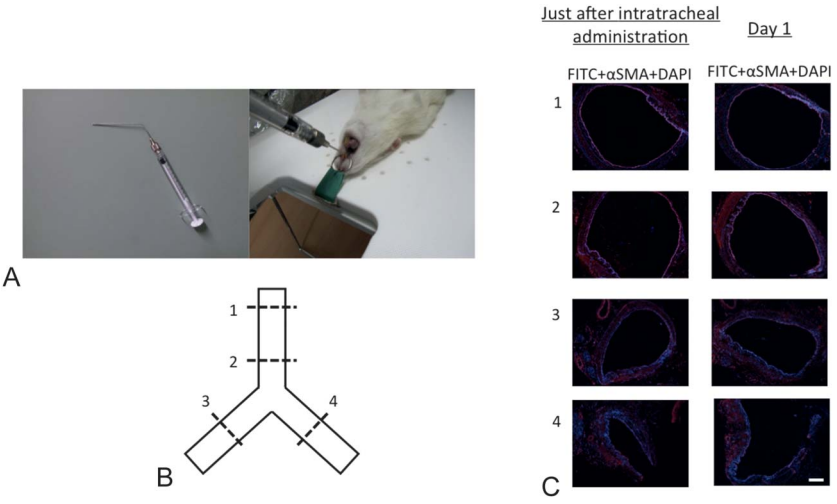

FIGURE 5. Deposition of FITC NPs in airways. A, Methods of intratracheal administration of NPs using a MicroSprayer. B, Schema of trachea and bronchi. We cut the trachea and bronchi at dotted line. Cross-sectional image of each number was shown in C. C, Cross-sectional image of trachea and bronchi. Red indicates αSMA. Blue indicates DAPI. Bar = 500 μm.

and left bronchi just after the administration of FITC NPs and 1 day after the administration of FITC NPs (Figs. 5B, C).

Effects of BPS NPs on Organ Damage

We confirmed damage of nontargeted organs by BPS NPs in the SuHx model. Hematoxylin and eosin staining images of the liver, kidney, spleen, and heart are shown in Figure 6. No infiltration of inflammatory cells, cytoclasis, hemorrhage, or fibrosis was found in the liver, kidney, spleen, and heart at 1 week after a single administration of BPS NPs. Blood examination showed that the parameters of liver and renal functions were within normal ranges in all group. The parameters in the BPS-NP group were not significantly different from those in the control group (Table 2).

Effects of BPS NPs on the Proliferation of Human PASMCs

PDGF stimulation caused a significantly higher growth rate of PAH PASMCs but not of non-PAH PASMCs (Figs. 7A, B). BPS and BPS NPs significantly inhibited PDGF-induced proliferation after 24 hours of treatment in PAH PASMCs. Twenty-four hours of this drug treatment and 24 hours after removal of these drugs, BPS and BPS NPs did not significantly inhibit PDGF-induced proliferation in non-PAH PASMCs (Fig. 7A). Twenty-four hours after the removal of these drugs, BPS NPs continued to significantly inhibit PDGF-induced proliferation, but BPS did not continue to significantly inhibit PDGF-induced proliferation in PAH PASMCs (Fig. 7B).

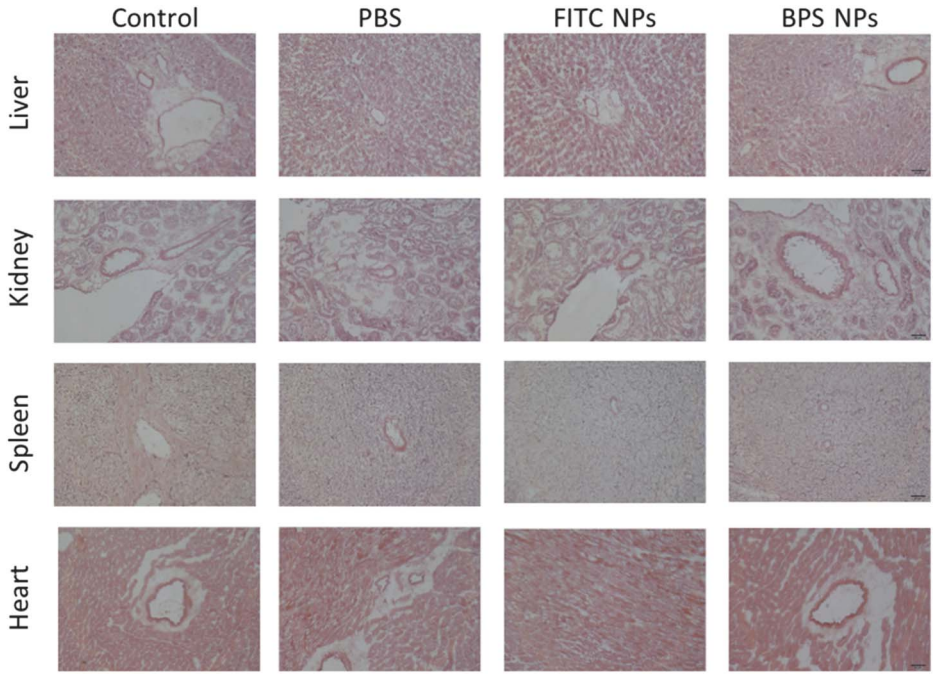

FIGURE 6. Micrograph of cross sections of the liver, kidney, spleen, and heart stained with hematoxylin and eosin.

TABLE 2. Blood Examinations

	Control	PBS	FITC NPs	BPS NPs	*P*
DBil (mg/dL)	0.02	0.02	0.03	0.01	0.471
AST (IU/L)	85	66	76	72	0.076
ALT (IU/L)	30	29	29	29	0.974
LDH (IU/L)	169	118	135	114	0.447
ALP (IU/L)	1201	1143	1329	1025	0.346
γGTP (IU/L)	<3	<3	<3	<3	NS
ChE (IU/L)	<5	<5	<5	<5	NS
BUN (mg/dL)	24.0	21.9	22.9	22.3	0.617
Cre (mg/dL)	0.30	0.26	0.27	0.26	0.113
Na (mEq/L)	145	142	143	143	0.271
K (mEq/L)	6.5	6.3	6.4	5.6	0.256
Cl (mEq/L)	102	100	101	100	0.433
Ca (mg/dL)	10.9	10.6	10.8	10.6	0.379
TP (g/dL)	5.5	5.5	5.6	5.4	0.847
Alb (g/dL)	3.9	3.9	3.9	3.5	0.097
BG (mg/dL)	206	188	188	186	0.238

Alb, albmin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; BG, blood glucose; BUN, blood urea nitrogen; Ca, calcium; ChE, cholinesterase; Cl, chloride; Cre, creatinine; DBil, direct bilirubin; K, potassium; LDH: lactate dehydrogenase; Na, sodium; TP, total protein; γGTP, γ-glutamyl transpeptidase.

Effects of BPS NPs on Apoptosis of Human PASMCs

The percentage of TUNEL-positive cells induced by BPS NPs was significantly higher in PAH PASMCs than that in non-PAH PASMCs (4.5 ± 3.3% vs. 0.3 ± 0.6%; $P < 0.05$, Fig. 8).

BPS Level in Serum of Rats and Culture Medium

BPS level in serum of rats was below the determination limit (50 ng/mL) at 1, 3, and 7 days after the intratracheal administration of BPS NPs. BPS level in culture medium was below determination limits (50 ng/mL) at 1, 3, and 7 days after the administration of BPS NPs.

DISCUSSION

A single intratracheal administration of BPS NPs ameliorates pulmonary hypertension, RV hypertrophy and pulmonary artery remodeling in SuHx and MCT model rats and improved survival in MCT model rats. This drug delivery system of BPS NPs into the lungs did not injure the liver, kidney, spleen, or heart. BPS NPs had sustained antiproliferative and proapoptotic effects on human PAH PASMCs.

Intravenous continuous prostacyclin therapy improves exercise capacity and hemodynamics, and is the only treatment method that has been shown to reduce mortality in patients with idiopathic PAH in a randomized clinical trial.[5] However, catheter-related trouble (infection and breakage) and complications caused by systemic administration (headache, flushing, and jaw pain) consistently distress the patients and make the management in treatment of PAH difficult. These shortcomings of intravenous prostacyclin therapy might be overcome by the delivery without using a central venous catheter.

Aerosolized iloprost, a stable prostacyclin analogue, is one solution for the problems caused by intravenous prostacyclin therapy. Aerosolized iloprost was shown to be more potent than inhaled NO as a pulmonary vasodilator in patients with idiopathic PAH.[14] Long-term treatment with aerosolized iloprost is safe and has beneficial effects on exercise capacity and hemodynamics in patients with idiopathic PAH.[15,16] Inhaled administration of aerosolized iloprost increased 6-minute walk distance and decreased mean pulmonary artery pressure and pulmonary vascular resistance 12 weeks after treatment in patients with PAH in a randomized clinical trial.[17] This therapy is efficient but has some problems. A high-frequency inhalation of iloprost per day is required.

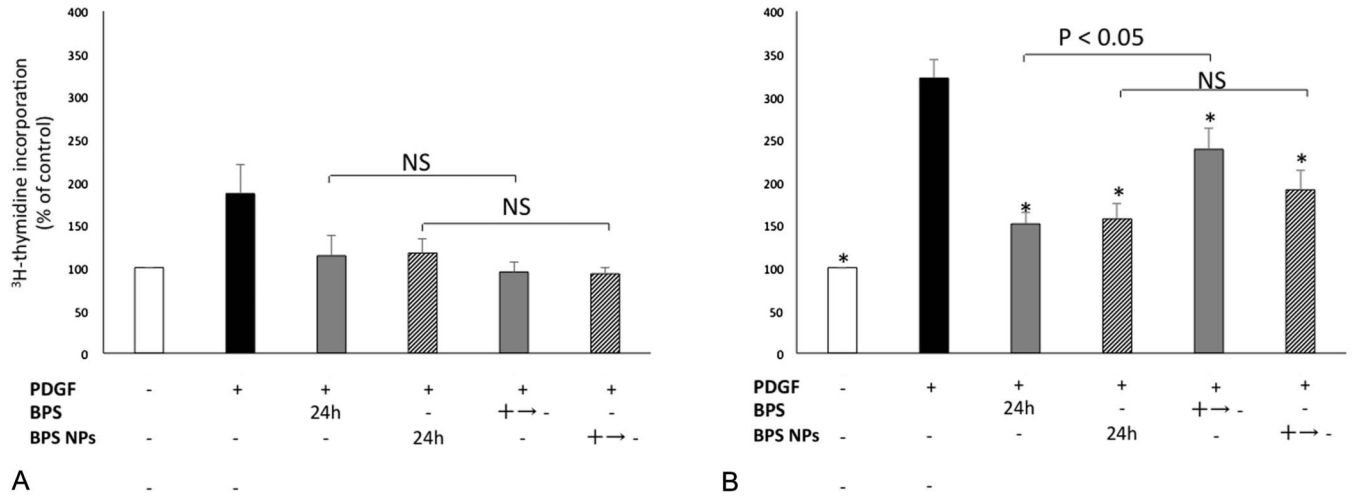

FIGURE 7. Inhibitory effect of BPS NPs on the proliferation of human PASMCs. A, Non-PAH PASMCs. B, PAH PASMCs. Counts per minute (cpm) are expressed as a percentage of cpm of PASMCs treated with a diluent (control). *$P < 0.05$ versus PDGF. + → − = Twenty-four hours after removal of BPS or BPS-NPs.

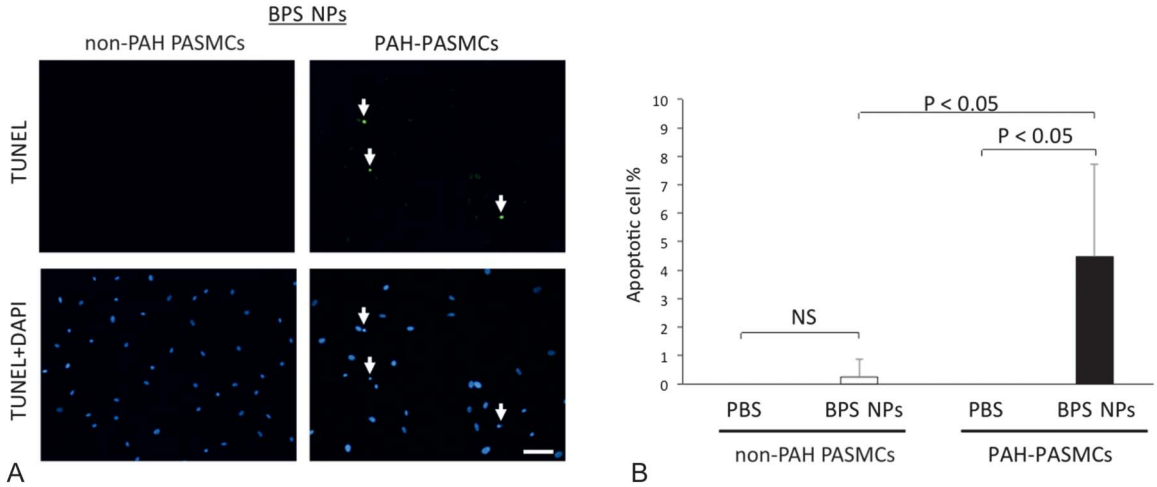

FIGURE 8. Effect of BPS NPs on the apoptosis of human PASMCs in the TUNEL assay. A, Representative images reflecting findings of induction of apoptosis in PAH PASMCs. Bar = 50 μm. B, Mean data for TUNEL-positive nuclei 24 hours after treatment with BPS NPs in non-PAH PASMCs and PAH PASMCs.

Patients inhale iloprost 6–9 times per day and inhalation takes between 4 and 10 minutes per treatment. Inhalation might therefore cumbersome as dissolution of prostacyclin everyday. It is not known whether iloprost, and intravenous prostacyclin, improves the long-term survival. We focused on using NPs as a method to solve these problems.

NPs can be used in medicine for a novel drug delivery system to aid the transport of diagnostic or therapeutic agents.[10,18] In the field of cancer, NPs can be used either as drug carriers for therapeutic applications or as contrast agents for diagnostic imaging. NPs infused into the bloodstream can accumulate in cancer because of their enhanced permeability and retention effect. Furthermore, NPs can cross the cell membrane by means of endocytosis and deliver the encapsulated agents into the cytoplasm. The PLGA NPs that we used are bioabsorbable and are decomposed in water and carbon dioxide in vivo. A drug delivery system using PLGA NPs would optimize the efficacy and minimize the side effects of drugs. There have been some reports on the efficacy of drug-incorporated PLGA NPs in a pulmonary hypertensive rat model. NP-mediated delivery of nuclear factor kB decoy,[19] pitavastatin[20] and imatinib[11] into lungs ameliorated MCT-induced PAH. In this study, a single intratracheal administration of BPS NPs ameliorated pulmonary hypertension in SuHx and MCT model rats. FITC NPs selectively accumulated in peripheral pulmonary arteries in SuHx model rats. BPS NPs did not injure nontarget organs. Selective delivery to damage blood vessels due to the enhanced permeability and retention effect of NPs would have caused these results.

Long-term antiproliferative effects and proapoptotic effects of BPS NPs on PAH PASMCs are in part attributed to the improvement of PAH. A prostacyclin analogue has an antiproliferative effect[21] and prostacyclin has proapoptotic effects.[13] The release time of drugs from NPs can be controlled by the molecular weight of PLGA, and incorporated BPS would therefore be slowly released from NPs with hydrolysis of PLGA. We previously reported that FITC NPs were incorporated into PAH PASMCs and remained in the PAH PASMCs for a long time.[11] Furthermore, the BPS level in serum of rats and in culture medium after administration of BPS NPs was very low in this study. Therefore, most BPS NPs were incorporated into PAH PASMCs and BPS was sustained to be released from NPs within PAH PASMCs, which inhibited the proliferation of PAH PASMCs for a long time and induced apoptosis of PAH PASMCs. These mechanisms provide the effects for suppression of the development of PAH by a single administration of BPS NPs.

We used BPS instead of prostacyclin as an incorporated drug in NPs because the activity of prostacyclin, which has a short half-life, might decrease when NPs are suspended in PBS. The effect of BPS is inferior to that of intravenous continuous prostacyclin.[12,22] To achieve a therapeutic effect equivalent to that of intravenous continuous prostacyclin, the maintenance of a high concentration of prostacyclin would be required. Because BPS NPs were targeted to damaged pulmonary arteries and remained in the arteries, the concentration of prostacyclin was thought to be high and thus have an effect equivalent to that of intravenous continuous prostacyclin.

CONCLUSIONS

Intratracheal administration of BPS NPs ameliorates pulmonary hypertension in rats by a sustained antiproliferative effect and proapoptotic effect on PAH PASMCs. The use of inhaled BPS NPs might be a novel approach for the treatment of PAH.

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