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ORIGINAL ARTICLE

Treatment with new organic nitrites in pulmonary hypertension of acute experimental pulmonary embolism

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Abstract

Acute pulmonary embolism may cause right heart failure due to increased pulmonary vascular resistance and arterial hypoxemia. Effective vasodilator therapy of the pulmonary hypertension is highly needed. Therefore, we investigated the effects of a newly developed effective pulmonary vasodilator, the organic mononitrites of 1,2-propanediol (PDNO), in a rabbit model of acute pulmonary embolism. In anesthetized and ventilated rabbits, systemic and pulmonary hemodynamics, exhaled nitric oxide (NO), plasma nitrite concentration, and blood gases were monitored. First, dose–response experiments with intravenous and left heart ventricle infusions of PDNO and inorganic nitrite were done in naive animals and in pulmonary hypertension induced by a thromboxane A_2 analogue. Second, acute pulmonary embolism was induced and either PDNO or placebo were administered intravenously within 20 minutes and evaluated within 1 hour after pulmonary embolization. PDNO intravenously, in contrast to inorganic nitrite intravenously, increased exhaled NO and counteracted pulmonary hypertension and vasodilated the systemic circulation, dose-dependently, thereby showing efficient NO donation. Pulmonary embolization induced pulmonary hypertension and gas exchange disturbances. PDNO significantly decreased and normalized pulmonary vascular resistance and the right ventricle rate-pressure product, without causing tolerance, with no significant side effects on the systemic circulation, nor on blood-gas values or on methemoglobin formation. In conclusion, PDNO is a NO donor and an efficient vasodilator in the pulmonary circulation. Treatment with this or similar organic nitrites intravenously may be a future option to avoid right heart failure in life-threatening acute pulmonary embolism.

KEYWORDS
alkyl nitrites, chromatography, high pressure liquid, hypertension, inorganic nitrite, lung, nitric oxide, nitric oxide donors, nitrites, organic nitrites, pulmonary, pulmonary circulation, pulmonary embolism, U46619

Abbreviations: CVP, central venous pressure; $ETCO_2$, end-tidal carbon dioxide; FENO, the fraction of nitric oxide in exhaled gas; HR, heart rate; LAP, mean left atrial pressure; MAP, mean systemic arterial blood pressure; mPAP, mean pulmonary arterial pressure; MPE, homogenized skeletal muscle tissue used for induction of pulmonary embolism; NO, nitric oxide; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; PD + nitrite, 25% v/v of 1,2-propanediol in saline plus 20 mmol L^{-1} inorganic nitrite; PD, 25% v/v of 1,2-propanediol in saline; PDNO, the composition of 2-hydroxy propyl nitrite and 2-hydroxy-1-methylethyl nitrite; PE, pulmonary embolism; PVR, pulmonary vascular resistance; RPP, cardiac rate-pressure product; Saline + NO, saline saturated with NO gas; SVR, systemic vascular resistance.

[†]Lars E Gustafsson passed away on 4 October 2017.

1 | INTRODUCTION

Acute pulmonary embolism (PE) results in acute pulmonary hypertension, induced by pulmonary vascular macro- or micro-obstruction together with active vasoconstriction, due to released vasoconstrictive mediators and scavenging of nitric oxide (NO) by cell-free hemoglobin.[1,2] In addition, acute PE severely disturbs ventilation-perfusion matching leading to hypoxemia.[3] Counteraction of the pulmonary hypertension by vasodilators or mediator antagonists could be a treatment strategy in acute PE. Since multiple vasoconstrictive mediators are involved, treatment with mediator antagonists is likely to be only partially successful.

NO is a vasodilator in the pulmonary circulation[4] and we found that the fraction of NO in exhaled gas (FENO) increased in acute PE and that *endogenous* NO production was protective in two models of experimental PE.[5,6] The NO donors nitroglycerin and sodium nitroprusside have shown some beneficial effects in animal models of PE, but the vasodilation was more pronounced in the systemic circulation and nitroglycerin may also impair the gas exchange, thereby limiting the applicability of these drugs in acute PE.[7,8] Furthermore, sildenafil, inorganic nitrite and a nitric oxide-releasing aspirin have shown beneficial effects in animal models of acute PE.[9-13] In acute PE, inorganic nitrite attenuated the increase in matrix metalloproteinase-9 and had anti-oxidant effects.[10,11] L-arginine and the NO donor diethylenetriamine/nonoate were inactive in acute PE.[14] The effects of *inhaled* NO in PE are not conclusive, with modest hemodynamic improvement but only minor or no improvement in blood gases.[15] Still, a recent single-centre phase I trial considered inhaled NO safe in submassive PE and suggested a clinical treatment protocol.[16]

NO donors of organic nitrite type are converted to NO in vivo[17,18] and in contrast to organic nitrates[19] they exhibited little or no tolerance.[20] Ethyl nitrite has been suggested as an inhaled therapeutic but might evoke significant methemoglobin formation.[21] Recently, we synthesized novel organic nitrites and found that the polarity of the individual organic nitrite molecule determined its relative selectivity for vasodilation in the pulmonary and systemic circulations.[18]

The two novel organic mononitrites, 2-hydroxy propyl nitrite and 2-hydroxy-1-methylethyl nitrite (henceforth referred to as PDNO, Supplementary Figure S1 for molecular structure) are of particular interest to investigate in experimental acute PE, because this composition exhibited increased vasodilator selectivity toward the pulmonary circulation compared to for example nitroglycerin.[18,22] In addition, the vasodilatory action of PDNO was devoid of cross tolerance with nitroglycerin in the systemic and pulmonary circulations, indicating bioactivation via another pathway.[22]

We hypothesized that PDNO intravenously can counteract the pulmonary hypertension in acute experimental PE, without inducing systemic side effects or gas exchange disturbances, nor methemoglobinemia. To distinguish the effects of the PDNO solution from inorganic nitrite and NO gas, we first performed in vivo dose–response experiments with measurement of FENO, pulmonary and systemic hemodynamics, methemoglobin and plasma nitrite both in naive animals and in animals with pharmacologically induced pulmonary hypertension. To investigate whether fast disappearance of PDNO from the circulation is one mechanism for the relatively larger effects in the pulmonary compared with the systemic circulation, we compared the effects of intravenous and left heart ventricle PDNO infusions.

We show that the NO donor PDNO, in contrast to inorganic nitrite, is a short-lived efficient vasodilator in vivo and that PDNO is effective in counteracting the pulmonary hypertension of acute PE without overt side effects.

2 | METHODS

2.1 | Test systems used

2.1.1 | Animals

Male New Zealand white rabbits (body weight 2.5-3.5 kg, n = 37) were used, since this animal species has been used previously for investigations of FENO and the pulmonary circulation.[5,6,18] The procedures were as humane as possible, and the study was conducted in accordance with the Directive 2010/63/EU on the protection of animals used for scientific purposes. The rabbits were single-housed in plastic-cages in room temperature and had free access to water and standard laboratory rabbit food. They were kept in a 12 hours day and 12 hours night cycle. The experiments were approved by the regional animal ethics committee (Stockholms Norra djurförsöksetiska nämnd, Stockholm, Sweden: Registration numbers N400/03, N148/08 and N178/11). All studies involving animals are reported according to the ARRIVE guidelines for reporting experiments involving animals.[23]

2.1.2 | Anesthesia and surgical procedures

The rabbits were anesthetized with pentobarbital, ventilated (charcoal-filtered air; fraction of inspired oxygen (FiO_2) = 0.21), and instrumented according to Nilsson et al.[6,18] A catheter was introduced via the right jugular vein to the level of the right atrium for intravenous drug infusions and measurement of central venous pressure (CVP) by a pressure transducer (PX600P, Edwards Lifesciences LLC, Irvine, CA, USA). An anesthesia monitor (AS/3, Datex, Helsinki, Finland) measured respiratory gases, airway pressure, and tidal volume by means of a Pedi-lite+ flow sensor and gas sampler (Datex) immediately after the tracheal cannula. FENO in mixed exhaled gas was measured by chemiluminescence.[6] Mean systemic arterial blood pressure (MAP) and heart rate (HR) were monitored by the AS/3 by a pressure transducer (PX600P) connected to a catheter in the left common carotid artery. In some animals the left carotid catheter was introduced into the left ventricle (position confirmed by pressure monitoring) for intracardiac drug infusions. In these animals, MAP and HR were measured and arterial blood was collected via a catheter in the right femoral artery. In the first and second sets of

dose–response experiments the preparation was complete at this point and the animals were allowed a 30-60 minutes intervention-free period.

In experiments with pharmacologically induced pulmonary hypertension (third set of dose–response experiments) and in acute PE, the animals were additionally instrumented and mean pulmonary arterial pressure (mPAP), mean left atrial pressure (LAP), and cardiac output were measured as in Nilsson et al.[18] Material for muscle tissue pulmonary embolization (MPE) was prepared from the right anterior tibial skeletal muscle.[6] After the surgery, the animals were allowed a 30-60 minutes intervention-free period.

A continuous infusion containing glucose (25.9 g L^{-1}), dextran 70 (Macrodex®, 28.2 g L^{-1}), and $NaHCO_3$ (6.6 g L^{-1}) was administered to all animals via an ear vein (STC-521 syringe pump, Terumo Corp., Tokyo, Japan). In dose–response experiments, sodium pentobarbital was added to this infusate (4.2 g L^{-1}) and the infusion was administered to the animals at a rate of 5 mL kg^{-1} h^{-1}. In pulmonary hypertension and embolism experiments sodium pentobarbital (2.1 g L^{-1}) was added to the infusate and the infusion rate was 10 mL kg^{-1} h^{-1} to compensate for the higher fluid loss.

Hemodynamic and respiratory variables including F_ENO were collected by a computerized data acquisition system (MP150; BIOPAC Inc., Goleta, CA, USA). Carotid and mixed venous blood samples were intermittently drawn and analyzed for blood gases, acid–base status, methemoglobin and total hemoglobin (ABL 520, Radiometer A/S, Copenhagen, Denmark) as well as plasma nitrite (see supplementary information).

After the experiments, the animals were killed by pulmonary air embolization in general anesthesia.

2.2 | Experimental protocols

The operator (author KFN) was not blinded to the interventions due to feasibility reasons.

2.2.1 | Protocol for the exploratory dose–response experiments

In a first set of experiments, rabbits received the following solutions intravenously: (i) the PDNO solution (0.6 mmol L^{-1} of dissolved NO gas, 9 mmol L^{-1} of the organic mononitrites, total NO/nitrites concentration 20 mmol L^{-1}, Supplementary Table S1), (ii) PD + nitrite placebo solution (1,2-propanediol dissolved in saline, 25% v/v, supplemented with 20 mmol L^{-1} inorganic nitrite), and (iii) Saline + NO which was isotonic saline treated with NO gas in the same way as the PDNO solution (1.7 mmol L^{-1} of dissolved NO gas and a total of 22 mmol L^{-1} NO/nitrite, Supplementary Table S1). The concentration of inorganic nitrite in solution 2 was thus chosen to have similar total NO/nitrite content in the control solution as in the PDNO solution. The PDNO solution was given so that the administration of the organic mononitrites was 25, 50, 100, 200, 400, 800, 1200, and 1600 nmol kg^{-1} min^{-1}. The infusion rates of the PD + nitrite and Saline + NO were the same as with the PDNO solution and are reported and compared with the corresponding PDNO dose. All animals received several of the solutions and were allowed an intervention-free period to reach stable baseline values in between the infusions. Each dose was given to at least four independent animals. Randomization was not employed in this set of experiments, since they were exploratory.

In a second set of experiments, rabbits (n = 4) received the PDNO solution at 100, 200, 400, 800, and 1600 nmol kg^{-1} min^{-1} intravenously and in a catheter in the left heart ventricle. The order of the infusions was random (intravenous or left heart ventricle) and the animals were allowed an intervention-free period between the infusions to reach stable baseline values. A bolus infusion of 2.5 mL saturated sodium bicarbonate solution at the respective infusion site was used to estimate the transit time to the pulmonary circulation.

In a third set of experiments, pulmonary hypertension was induced by a continuous intravenous infusion of U46619 (200-1200 ng kg^{-1} min^{-1}) to increase the pulmonary vascular resistance (PVR) by 100%-200%. Thereafter one group of animals (n = 4) received PDNO solution intravenously in increasing doses (200, 400, 800, and 1200 nmol kg^{-1} min^{-1}) and another group of animals (n = 3) received PD + nitrite placebo solution in corresponding doses and one higher dose.

All infusions were made by a syringe pump (CMA/100, Carnegie Medicin AB, Stockholm, Sweden) connected to a continuous saline carrier flow (864 Syringe Pump, Univentor LTD, Zejtun, Malta). The length of all infusion was 10 minutes for each dose and the effect was evaluated and an arterial blood sample was collected for blood gas analysis and plasma nitrite determination at the end of each dose. In rabbits where plasma nitrite half-life was measured, arterial blood samples were collected also at 10, 20, 40, 80 minutes post-infusion of PDNO or PD + nitrite.

2.2.2 | Protocol for pulmonary embolism experiments

Three groups of animals received an infusion of MPE (30 mg homogenized muscle kg^{-1}) administered manually via a three-way stopcock into a saline carrier flow (864 Syringe Pump) of 150 μL kg^{-1} min^{-1} entering the central venous catheter. The dose of MPE was chosen to give a significant but sub-lethal embolic challenge.[6] Twenty minutes after MPE the different drug infusions were administered intravenously as above for 40 minutes in a random fashion: one group (PDNO, n = 6) received 200 nmol kg^{-1} min^{-1} of PDNO, one group (PD, n = 6) received the same amount of 1,2-propanediol and the third group (control, n = 3) received saline. The dose of PDNO was the lowest dose that decreased the pulmonary arterial pressure maximally after MPE as determined in pilot experiments. The control group subjected to MPE but saline as drug infusion behaved like the placebo group and data in this control group is not shown. In parallel with blood gases, mixed exhaled gas samples were collected at the outlet of the exhalate mixing chamber and analyzed in the ABL520.

2.3 | Data and statistical analysis

Physiological dead space as a fraction of the tidal volume was calculated with the Bohr equation[24] and was corrected for the dead space of the ventilator system.

Pulmonary ventilation-perfusion characteristics were monitored by measurements of venous admixture, which was calculated by using the classical shunt equation, that is the true shunt as a fraction of total cardiac output.[24] The obtained venous admixture is then the sum of true shunt and any ventilation-perfusion mismatch. For calculations, see supplementary information.

Rate-pressure product (RPP) of the right ventricle was calculated as HR times mPAP. PVR was calculated as the difference between mPAP and LAP divided by cardiac output. Systemic vascular resistance (SVR) was calculated as the difference between MAP and CVP divided by cardiac output.

Half-life of inorganic plasma nitrite was determined by applying the data to a one-compartment model using the least squares method (Sigma-Stat, Jandel, San Rafael, CA, USA; supplementary information).

Data are expressed as means ± SEM. For some variables, normalization was employed to control for unwanted sources of variation. Two-way ANOVA on repeated measures was used with time or dose as one factor and drug solution or infusion site as the other factor. Tukey test was used as in post-hoc testing. $P < 0.05$ was considered statistically significant. All statistical tests were performed by using commercial software (SigmaStat v. 3.10.0, Systat Software Inc., San Jose, CA, USA). The data analysis was not blinded due to feasibility reasons.

2.3.1 | Materials

PDNO consists of the compounds 2-hydroxy propyl nitrite (compound 1 in Supplementary Figure S1, 63%) and 2-hydroxy-1-methylethyl nitrite (compound 2 in Supplementary Figure S1, 37%).[18] For preparation of PDNO, NO-saturated saline (Saline + NO) and 1,2-propanediol with inorganic nitrite (PD + nitrite) and without organic nitrite (PD) were prepared as described in supplementary information and Nilsson et al.[18] The solutions were administered to animals (see below) for the determination of biological effects and analyzed either directly in a nitrite reduction system or in HPLC coupled to nitrite reduction to quantify NO gas and inorganic and organic nitrite contents previously described[18,25] with a few modifications which are described in detail in the supplementary information (Supplementary Figure S2 shows an original recording of the chromatogram and the synchronized nitrite analysis). The contents of inorganic nitrite, organic nitrites, and NO gas are shown in supplementary information and in particular supplementary Table S1. With the same methods (see supplementary information) plasma nitrite concentrations were determined in the dose–response experiments (see below).

Heparin (Kabi Vitrum, Stockholm, Sweden), dextran 70 (Macrodex, Pharmalink, Spånga, Sweden), and sodium pentobarbital (Apoteksbolaget) were purchased from Apoteksbolaget, Stockholm, Sweden. U46619 was from Cayman Chemical Company (Ann Arbor, Michigan, USA). Other chemicals and 1,2-propanediol were from Sigma-Aldrich, St Louis, Missouri, USA. Pure NO (99.9%), certified NO for calibration, nitrogen, oxygen, and helium were from AGA gas AB, Lidingö, Sweden. Drugs and receptor nomenclature follows Alexander et al.[26]

3 | RESULTS

3.1 | Dose–response experiments

3.1.1 | Effects on FENO, systemic blood pressure, and heart rate

The animals displayed stable and similar baseline values before each set of cumulative dose infusions. MAP was 88 ± 5 mm Hg, 89 ± 4 mm Hg, and 90 ± 6 mm Hg and HR was 265 ± 14 beats min^{-1}, 279 ± 8 beats min^{-1}, and 277 ± 13 beats min^{-1} before PDNO, PD + nitrite, and Saline + NO, respectively (n = 5). PDNO infusion increased FENO and HR, and decreased MAP, dose-dependently (Figure 1A–C), whereas Saline + NO and PD + nitrite at the highest dose used had but a small effect on MAP without affecting FENO and HR (Figure 1A–C). Saline and 1,2-propanediol (25% in saline, v/v) without NO gas or inorganic nitrite had no effects (data not shown).

3.1.2 | Effects on methemoglobin concentration and blood gases

Methemoglobin increased at larger doses of PDNO, PD + nitrite, and PDNO but remained below 2.0% in all animals (Figure 1D). When comparing total nitrites doses of PDNO, PD + nitrite, and Saline + NO equi-potent for effects on MAP (200, 1600-3200, and 1600-3200 nmol kg^{-1} min^{-1} in total nitrites dose, respectively; Figure 1B), PDNO did not affect methemoglobin whereas PD + nitrite and Saline + NO increased methemoglobin (Figure 1D). With the two highest doses of PDNO there was a small but significant decrease in arterial partial pressure of oxygen (PaO_2) concomitant with a profound drop in MAP (Table 1). Other blood-gas values were not affected in infusions with PDNO, PD + nitrite, and Saline + NO (Table 1).

3.1.3 | Effects on pulmonary and systemic hemodynamics in pharmacologically induced pulmonary hypertension

At baseline both groups showed similar and normal values (Figure 2 and data not shown). Pulmonary hypertension was induced by intravenous administration of U46619 which increased mPAP, PVR (by ~150%), SVR and slightly increased CVP, and decreased cardiac output (Figure 2 and data not shown) to a similar degree in both groups, whereas MAP, HR, and LAP were not changed (data not shown). PDNO (n = 4) significantly decreased mPAP, PVR, and SVR and

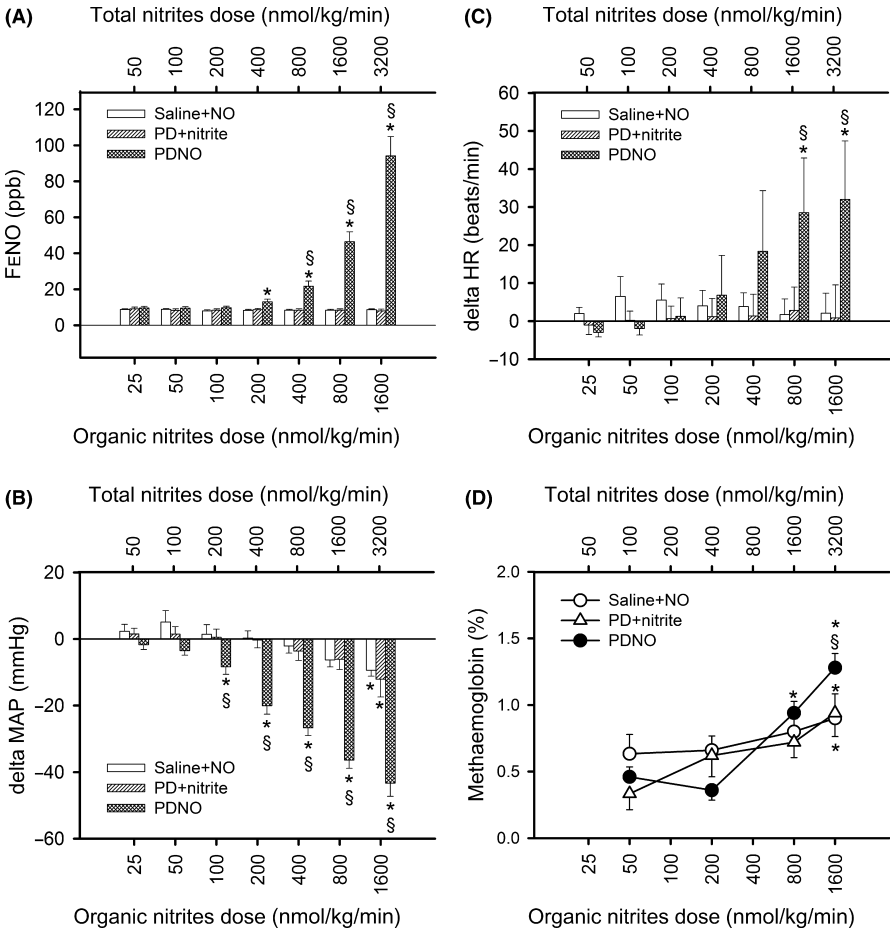

FIGURE 1 Exploratory dose–response studies of nitrites in anesthetized rabbits. Exhaled NO (FeNO, panel A), change in systemic blood pressure (MAP, panel B), change in heart rate (HR, panel C), and methemoglobin (panel D) in ventilated rabbits subjected to infusion of 1,2-propanediol (PDNO) (organic nitrite), NO gas treated saline (Saline + NO) and inorganic nitrite (20 mmol L^{-1}) in 1,2-propanediol (25% in saline, v/v) at increasing dose rates. The lower and upper *x*-axis in each panel show the dose of organic nitrite (for the PDNO) and the total dose of nitrites (organic nitrites + inorganic nitrite) of all the infusions, respectively. *denotes a significant difference compared to baseline within that group. §denotes a significant difference between the PDNO group compared to both PD + nitrite and Saline + NO groups for that particular dose. N = 4-5 for each infusion

increased cardiac output compared to PD + nitrite (n = 3), which had little or no effects at corresponding doses (Figure 2 and data not shown).

3.1.4 | Effects on plasma nitrite concentrations and relation between plasma nitrite concentrations and biological effects

PDNO (n = 3) and PD + nitrite (n = 3) dose-dependently increased plasma nitrite concentrations (Figure 3). However, PD + nitrite caused a significantly larger increase in the plasma nitrite concentration compared with PDNO at the larger doses (Figure 3). The half-life of plasma nitrite in vivo was 43 ± 1 minutes. Taken together, these experiments showed that both PDNO and PD + nitrite increased plasma nitrite concentrations but that the biological effects were very different (Figure 1-3). This was evident when plotting the effects on PVR and SVR by PDNO and PD + nitrite vs the plasma nitrite concentration they caused (Figure 4). A similar differentiation between plasma nitrite and effects on FeNO, MAP, and HR was observed (data not shown).

3.1.5 | Intravenous vs left heart ventricle infusions

Before each set of infusions, FeNO (Figure 5A) and MAP (84 ± 1 mm Hg vs 86 ± 4 mm Hg) were similar. PDNO intravenously (n = 4) increased FeNO and decreased MAP dose-dependently (Figure 5), whereas the left heart ventricle infusions of PDNO (n = 4) only slightly increased FeNO and decreased MAP dose-dependently (Figure 5). At the higher intravenous doses of PDNO a significantly larger increase in FeNO was obtained, compared with the left heart ventricle infusions (Figure 5). At PDNO 400 nmol kg^{-1} min^{-1} the left heart ventricle infusion caused a larger drop in MAP than the intravenous infusion (Figure 5). The transit times from the intravenous and left heart ventricle infusion sites to the pulmonary circulation were estimated to approximately 1.5-2 seconds and 8 seconds, respectively, suggesting a relationship between transit time to target sites and effects.

3.2 | Pulmonary embolism experiments

3.2.1 | Status of the animals before acute pulmonary embolism

Before MPE all measured variables were similar in the groups (n = 6 in each group, Figure 6 and 7). Not shown in Figures, FeNO was 10 ± 1 ppb and 12 ± 1 ppb, end-tidal carbon dioxide concentration ($ETCO_2$) was 4.4 ± 0.1 % and 4.4 ± 0.1 %, HR was 254 ± 12 beats min^{-1} and 254 ± 4 beats min^{-1}, PVR was 36 ± 4 mm Hg min L^{-1} and 32 ± 3 mm Hg min L^{-1}, right ventricle RPP was 4000 ± 400 mm Hg

TABLE 1 Blood-gas values in anesthetized and ventilated rabbits subjected to infusion of organic nitrites (1,2-propanediol [PDNO]), inorganic nitrite (20 mmol L^{-1}) in 1,2-propanediol (PD + nitrite), and NO treated saline (Saline + NO)

Dose (nmol kg^{-1} min^{-1})					
Organic nitrites dose	0	50	200	800	1600
Total nitrites dose	0	100	400	1600	3200
PaO_2 (kPa)					
Saline + NO	12.4 ± 0.4	12.1 ± 0.8	12.4 ± 0.5	12.5 ± 0.5	12.1 ± 0.3
PD + nitrite	12.8 ± 0.3	12.5 ± 0.4	12.7 ± 0.4	12.6 ± 0.3	12.5 ± 0.3
PDNO	12.7 ± 0.2	13.0 ± 0.3	12.5 ± 0.4	11.9 ± 0.5[a]	11.6 ± 0.6[a]
$PaCO_2$ (kPa)					
Saline + NO	4.6 ± 0.2	4.8 ± 0.3	4.7 ± 0.2	4.7 ± 0.2	4.8 ± 0.1
PD + nitrite	4.7 ± 0.1	4.6 ± 0.2	4.6 ± 0.2	4.6 ± 0.1	4.6 ± 0.2
PDNO	4.8 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.8 ± 0.2	4.8 ± 0.2
Arterial pH					
Saline + NO	7.48 ± 0.02	7.48 ± 0.03	7.48 ± 0.02	7.48 ± 0.02	7.47 ± 0.02
PD + nitrite	7.48 ± 0.02	7.48 ± 0.03	7.48 ± 0.02	7.48 ± 0.02	7.47 ± 0.02
PDNO	7.50 ± 0.02	7.50 ± 0.02	7.50 ± 0.02	7.49 ± 0.02	7.49 ± 0.02

PaO_2, arterial partial pressure of oxygen; $PaCO_2$, arterial partial pressure of carbon dioxide.
[a]Denotes a significant difference compared to baseline in that group. N = 4-5 for each infusion.

beats min^{-1} and 4300 ± 200 mm Hg beats min^{-1}, and arterial pH was 7.50 ± 0.03 and 7.52 ± 0.02 in the PD and PDNO groups, respectively.

3.2.2 | Effects of acute pulmonary embolism

MPE infusion resulted in increased FENO (which reached statistical significance in the PD group), slightly decreased end-tidal carbon dioxide (to 4.0 ± 0.2 % and 4.1 ± 0.1 % in the PD and PDNO groups respectively), increased mPAP, increased PVR by approximately 50%, increased right ventricle RPP, and decreased MAP (Figure 6A–D and 7A). PaO_2 decreased (Figure 7B) and arterial pH slightly decreased (to 7.46 ± 0.03 and 7.45 ± 0.04 in the PD and PDNO groups, respectively). The arterial partial pressure of carbon dioxide ($PaCO_2$), physiological dead space, and venous admixture increased (Figure 7C–E). Cardiac output, HR, LAP, and methemoglobin were not affected by the MPE infusion (Figure 6E–F and 7F and data not shown).

3.2.3 | Effects of drug infusion in acute pulmonary embolism

PDNO counteracted essentially all the detrimental hemodynamic effects of acute PE. PDNO increased FENO, decreased and normalized PVR, and right ventricle RPP compared to PD ($P < 0.05$, Figure 6A,C–D). PDNO also decreased mPAP compared to values before start of drug infusion ($P < 0.05$, Figure 6B). The difference in mPAP between PDNO and PD during drug infusion did not reach statistical significance ($P = 0.26$ after 40 minutes of drug infusion) which might be explained by the relatively large scatter in the PD group, which did not exhibit the decrease in mPAP seen in the PDNO group. The effects of PDNO on mPAP, PVR, and right ventricle RPP were sustained during infusion and quickly reverted on cessation of drug infusion, without rebound effect (Supplementary Figure S3). PD did not affect mPAP, PVR, and right ventricle RPP and they remained elevated throughout the observation time (Figure 6B–D). MAP decreased compared to baseline during PDNO infusion ($P < 0.05$, Figure 7A) but was not significantly lower than during PD infusion (5 mm Hg difference, $P = 0.54$ after 40 minutes of drug infusion). This drop in MAP was quickly reversed without a rebound effect when stopping the PDNO infusion (Supplementary Figure S3). PDNO and PD did not change LAP, cardiac output, HR, blood-gas values (except $PaCO_2$ in the PD group), physiological dead space and venous admixture (Figure 6E–F and 7B–E and data not shown). Methemoglobin (Figure 7F) was very modestly increased (by ~0.3%) at the end of PDNO infusion, compared to PD and baseline. After stopping the PDNO infusion the methemoglobin started to decrease toward normal values (data not shown) and all animals had methemoglobin <1% during the study period.

4 | DISCUSSION

This study showed pulmonary vasodilatory effects of the new organic nitrite NO donor PDNO in pulmonary hypertension of acute PE, without any severe adverse effects. Methemoglobin was <1%, that is well below 2.5% which has been considered safe for inhaled NO,[27] and it was recently shown that infusion of PDNO for more than 6 hours in sheep did not significantly increase the fraction of methemoglobin.[28] Mean systemic arterial blood pressure was approximately 5 mm Hg lower (not significant) in the PDNO group compared with the PD placebo group. Systemic hypotension in acute

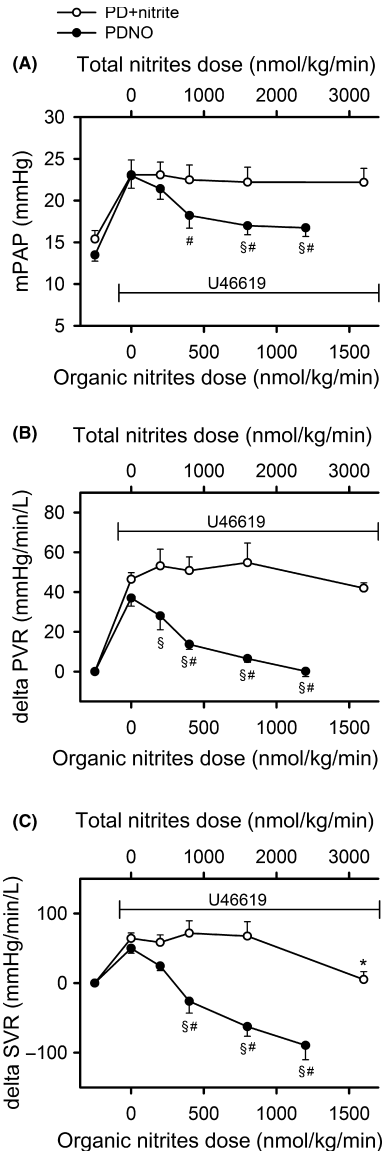

FIGURE 2 Effects of organic and inorganic nitrite infusions on pulmonary (mean pulmonary arterial pressure, mPAP, panel A; pulmonary vascular resistance, PVR, panel B) and systemic hemodynamics (systemic vascular resistance, SVR, panel C) during induced pulmonary hypertension in anesthetized and ventilated rabbits. The thromboxane A_2-mimetic U46619 was continuously administered intravenously to induce pulmonary hypertension (increase of pulmonary vascular resistance, PVR, by 100-200%). Thereafter, the organic mononitrites (1,2-propanediol (PDNO), n = 4) or 1,2-propanediol with 20 mmol L^{-1} inorganic nitrite (PD + nitrite, n = 3) were delivered intravenously in increasing doses. The lower and upper *x*-axis in each panel show the dose of organic nitrite (for the PDNO) and the total dose of nitrites (organic nitrites + inorganic nitrite) of the infusions, respectively. The circles below zero on the *x*-axis show baseline values that is before U46619 infusion. * and #indicate a significant difference when comparing the effects of PD + nitrite and PDNO, respectively, at the specific doses with only U46619 infusion (nitrites doses of 0). §denotes a significant difference between the two groups at the actual dose. Although the largest doses were not identical in the two groups they were still included for comparison in the analysis

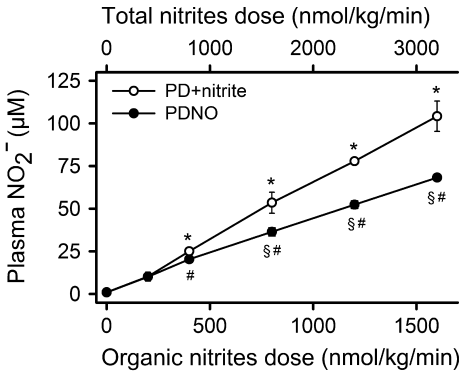

FIGURE 3 The effects on plasma nitrite concentration in anesthetized and ventilated rabbits by 10 minutes intravenous infusions at cumulatively increasing dose rates of the organic mononitrites of 1,2-propanediol (1,2-propanediol (PDNO), n = 3) and 1,2-propanediol with 20 mmol L^{-1} inorganic nitrite (PD + nitrite, n = 3). The lower and upper *x*-axis show the dose of organic nitrite (for the PDNO) and the total dose of nitrites (organic nitrites + inorganic nitrite) of the infusions, respectively. * and #indicate a significant difference when comparing the effects of PD + nitrite and PDNO, respectively, with baseline. §denotes a significant difference between the two groups at the respective corresponding dose rates

PE would be detrimental resulting in decreased perfusion of the myocardium,[29] and PDNO in this respect also seems to have promising characteristics. The effects of PDNO were short-lived and quickly reversed when stopping the infusion showing the high controllability of this NO donor. The relatively larger effect in the pulmonary vs the systemic circulations when administered intravenously was probably due to a very fast disappearance of PDNO from the circulation, evidenced by the comparison of intravenous vs left heart ventricle infusions. A short half-life of a NO donor in the circulation confines the vasodilatory effects to the pulmonary circulation when administered intravenously.[30,31]

The initial dose–response experiments with PDNO and inorganic nitrite showed that PDNO was an efficient vasodilator both in the pulmonary and systemic circulations, and that PDNO released NO as shown by the increase in F_ENO. In contrast, inorganic nitrite, at the doses used in this study, was a weak systemic vasodilator without capacity of affecting the pulmonary hypertension induced by a thromboxane A_2 analogue (U46619). Both intravenous infusions of PDNO and inorganic nitrite increased the plasma nitrite concentration. By correlating the vasodilator properties of PDNO and inorganic nitrite with the plasma nitrite levels caused by the respective infusion, it can be concluded that the major part of the vasodilatory actions of PDNO was not via increments in plasma inorganic nitrite. Furthermore, the short half-life of the vasodilation by PDNO (a few minutes) and the large difference between the intravenous and left heart ventricle infusions suggesting fast disappearance of PDNO from the circulation strengthened that the active species was not inorganic nitrite. In contrast, the half-life of inorganic nitrite was estimated to 43 minutes which is in the same range previously determined in rabbits[32] and in humans.[33] Likely, the pulmonary

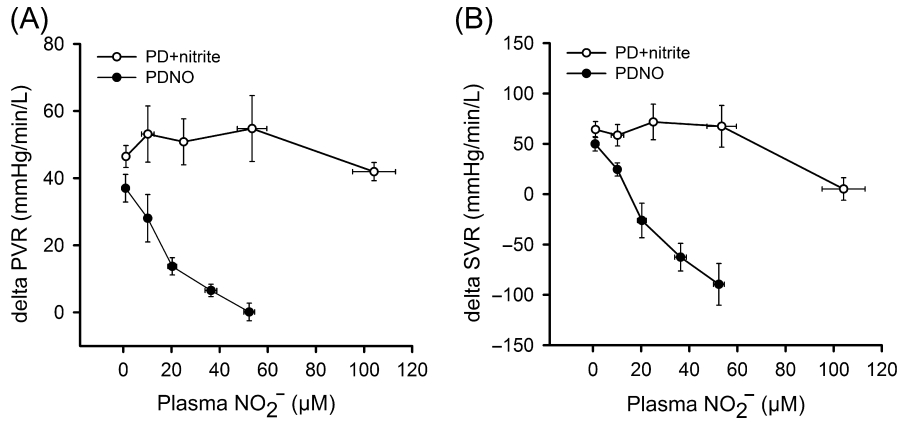

FIGURE 4 Anesthetized and ventilated rabbits. The relation between the effects on pulmonary vascular resistance (PVR, panel A) and systemic vascular resistance (SVR, panel B) during pulmonary hypertension (induced by intravenous infusion of a thromboxane A_2-mimetic, U44169) and on the plasma nitrite concentration by intravenous infusion of the organic mononitrites of 1,2-propanediol (1,2-propanediol (PDNO) at doses 200, 400, 800, 1200 nmol kg^{-1} min^{-1}) and 1,2-propanediol with 20 mmol L^{-1} inorganic nitrite (PD + nitrite at doses corresponding to PDNO 200, 400, 800, 1600 nmol kg^{-1} min^{-1})

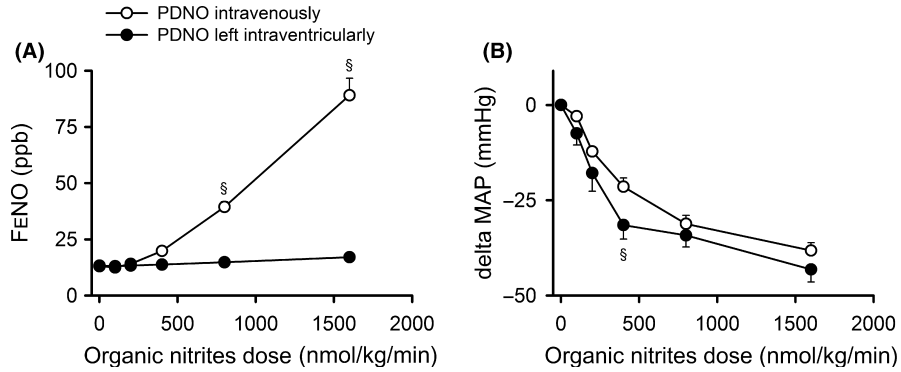

FIGURE 5 Intravenous (n = 4) vs left heart ventricle (n = 4) infusions of the organic mononitrites of 1,2-propanediol (PDNO) at increasing cumulative doses in anesthetized and ventilated rabbits, showing the response in exhaled NO (FENO, panel A) and the change in mean systemic arterial blood pressure (MAP, panel B). §denotes a statistical difference between the two infusion modes at the particular dose of PDNO

vasodilation by PDNO in the acute PE experiments and in the dose–response experiments was via release of NO or a similar active species.

The low vasodilatory efficacy of inorganic nitrite in the pulmonary circulation in this study is coherent with studies in rats[34] and newborn lambs.[35-37] In particular, the classic NO donor sodium nitroprusside was more than 100 times more potent than inorganic nitrite in rats with pulmonary hypertension.[34] Intravenous inorganic nitrite given in large bolus doses at 10-100 μmol kg^{-1} and 145 μmol kg^{-1} counteracted the pulmonary hypertension induced by a thromboxane A_2 analogue (U46619) in rats[34] and hypoxic pulmonary vasoconstriction in newborn lambs, respectively.[35] In newborn lambs, nebulized inorganic nitrite at doses of 4.3-43 μmol kg^{-1} min^{-1} had pulmonary vasodilatory effects in models of pulmonary hypertension.[36,37] The nebulized inorganic nitrite in these studies also slightly increased FENO,[36,37] which might have arisen from the ventilator circuit and not the animal,[36] whereas intravenous infusions of inorganic nitrite did not affect FENO.[35] In contrast, dogs seem to be more sensitive to intravenous inorganic nitrite since inorganic nitrite intravenously at 450 nmol kg^{-1} min^{-1} for 15 minutes followed by 280 nmol kg^{-1} min^{-1} for 105 minutes attenuated approximately half of the PVR increase in acute PE.[9] Dogs also seem more sensitive to acute systemic blood pressure lowering effects of inorganic nitrite,[9] whereas other species including humans,[33,38,39] rabbits,[32] sheep[35], and rats[34] are less sensitive, and larger doses of inorganic nitrite were needed. Importantly, doses of inorganic nitrite required to acutely lower pulmonary and systemic blood pressures also increased the methemoglobin level.[33-35,38,39] The interaction of inorganic nitrite with hemoglobin in red blood cells provides a convenient explanation for the formation of methemoglobin observed,[38] but the major part of the NO generated from inorganic nitrite in hypoxia arise from other tissues,[40] which might explain the potential protective effects of inorganic nitrite in ischemia-reperfusion injuries.[41]

The beneficial effects of NO in acute PE may be several. First, NO act as a vasodilator in the pulmonary circulation, thus

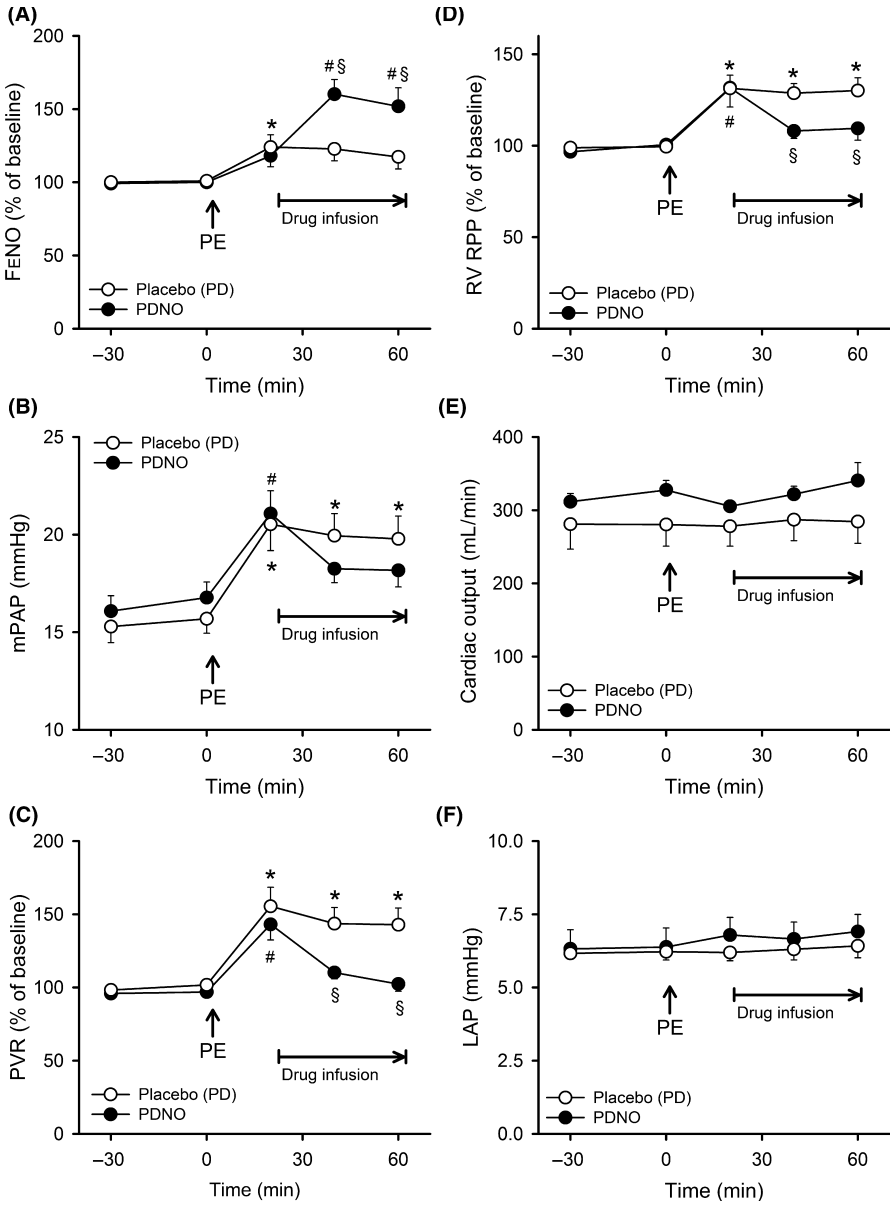

FIGURE 6 Exhaled nitric oxide (FENO, panel A), mean pulmonary arterial pressure (mPAP, panel B), pulmonary vascular resistance (PVR, panel C), right ventricle rate-pressure products (RV RPP, panel D), cardiac output (panel E), and mean left atrial pressure (LAP, panel F) in ventilated and anesthetized rabbits subjected to pulmonary embolism (PE) induced at time 0 minutes and to drug infusion started at 20 minutes. The two groups received placebo (1,2-propanediol, PD) or 200 nmol kg^{-1} min^{-1} of 1,2-propanediol mononitrites (PDNO). * and #denote a significant difference compared to baseline in the placebo group and in the PDNO group, respectively. §denotes a significant difference between the two groups at the actual time point. n = 6 in each group

counteracting the pulmonary hypertension that is due to the release of vasoactive mediators and endothelial dysfunction in the pulmonary vasculature.[2,42] Pulmonary vasodilation leads to decreased work load on the right heart and thus lowered oxygen demand of the right heart, which is beneficial in conditions associated with hypoxemia. Evidence in this study for such an effect was that PDNO decreased PVR and right ventricular RPP, which is a good estimate of oxygen consumption of the right heart.[43] Second, NO is an important regulator of coronary blood flow to the right ventricle at rest, in global hypoxia (and thus hypoxemia), and in pulmonary hypertension.[43,44] This effect may be important in a human suffering from acute PE and especially those with risk factors for atherosclerosis where the endothelial NO production in the coronary circulation is diminished.[45,46] Third, NO donors and authentic NO inhibit platelet aggregation,[47,48] which may diminish thrombin-induced Ca^{2+} mobilization and the subsequent release of vasoconstrictive agents (eg serotonin) from activated platelets[49] thus reducing pulmonary hypertension. Furthermore, inorganic nitrite in acute PE has been shown to reduce oxidation and attenuate matrix metalloproteinase 9 release.[10,11] Anti-oxidant effects by inhibition of NAPDH activity with a high dose of orally ingested inorganic nitrite was shown in a rat model of systemic hypertension.[50]

Our model of acute PE has the important signs that accompany human PE: hypoxemia due to increased venous admixture because of mismatch of ventilation and perfusion[51] and increased resistance in the pulmonary circulation.[52] Two features that are normally not present in human PE are anesthesia with pentobarbital and constant mechanical ventilation. As almost all anesthetics, pentobarbital depresses the autonomic system and therefore has impact on cardiovascular regulation.[53] Constant mechanical ventilation during PE leads to accumulation of carbon dioxide in blood in contrast to hyperventilation in PE in humans but will probably not affect the main findings in this study since the increase in $PaCO_2$ and decrease in pH were relatively small.

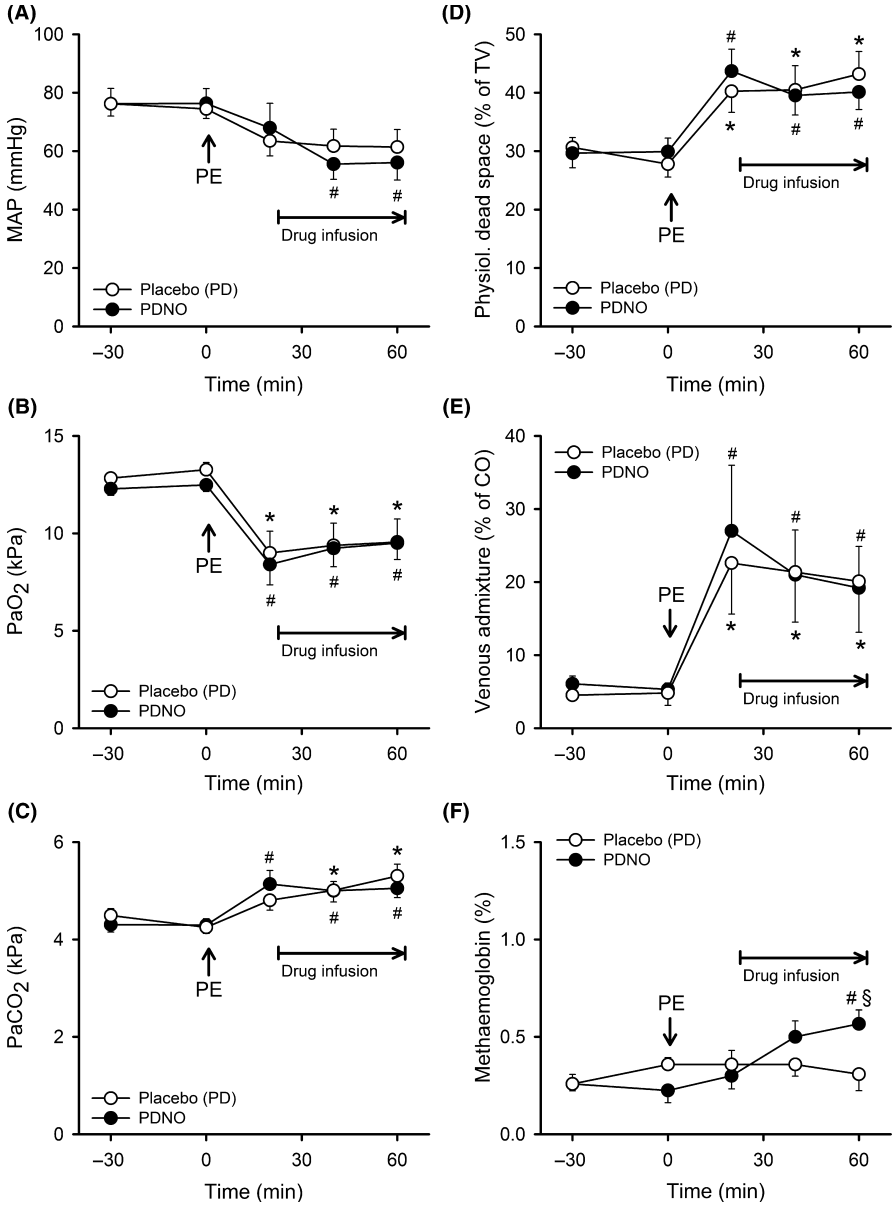

FIGURE 7 Mean systemic arterial blood pressure (MAP, panel A), arterial partial pressure of oxygen (PaO_2, panel B) and carbon dioxide ($PaCO_2$, panel C), physiological dead space (Physiol. dead space, panel D), venous admixture (panel E), and methemoglobin (panel F) in ventilated and anesthetized rabbits subjected to pulmonary embolism (PE) induced at time 0 minutes and to drug infusion started at 20 minutes. The two groups received placebo (1,2-propanediol, PD) or 200 nmol kg^{-1} min^{-1} of 1,2-propanediol mononitrites (PDNO). * and #denote a significant difference compared to baseline in the placebo group and in the PDNO group, respectively. §denotes a significant difference between the two groups at the actual time point. n = 6 in each group. CO, cardiac output; TV, tidal volume

In humans, 1,2-propanediol is used as an additive in pharmaceutical intravenous formulations of lipophilic drugs (eg lorazepam and diazepam) and 1 g kg^{-1} day^{-1} is considered safe.[54,55] In the PE experiments, the delivery of 1,2-propanediol was approximately 320 mg kg^{-1} h^{-1}, making treatment feasible over several hours. It is also important to consider the total load of organic and inorganic nitrites, since dose-limiting toxicity of long-term intravenous inorganic nitrite infusion in humans was estimated at 108 nmol kg^{-1} min^{-1}.[56] Future work on PDNO should aim to increase the concentration of PDNO while minimizing the content of inorganic nitrite in the solution, thus decreasing the supply of 1,2-propanediol and total nitrite load, respectively.

Future indications for the use of organic nitrites, for example PDNO, in acute PE could be as a life-saving treatment, to relieve the work load of the right heart by pulmonary vasodilation, in addition to current conventional and non-conventional treatments: heparin, thrombolysis, embolectomy, or extracorpeal life support.[57-59]

NO donors of the organic nitrite type have been used for more than a century for anginal pain, but their effects have never been investigated in PE as far as the present authors know. We conclude that the new organic nitrite compounds exerted beneficial effects, that is decreased pulmonary vascular resistance and decreased right ventricle pressure product, in experimental acute PE. The organic nitrites used were free from adverse methemoglobin formation, systemic hypotension, disturbed gas exchange, and tolerance development. Thus, organic nitrites with short half-life emerge as a new tentative life saving treatment in acute PE. The potency of organic nitrite to exert hemodynamic effects is superior to inorganic nitrite and hemodynamic effects of inorganic nitrite in rabbits were associated with methemoglobin formation. This study shows that it is very important to monitor methemoglobin when using inorganic and organic nitrites, as this may be a serious adverse effect. In future studies, the effects of the new organic nitrite should be tested in other relevant clinical conditions associated with pulmonary hypertension.

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AUTHORS' CONTRIBUTIONS

K. F. N. and L. E. G. designed the study, performed the research, analyzed the data, wrote the manuscript, and approved the final manuscript.

DISCLOSURES

The authors wish to declare potential competing financial interests in that they are co-inventors in international patents and co-owners of a company, pertaining to the current subject matter.

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