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OPEN

Changes in Pulmonary Vascular Resistance and Obstruction Score Following Acute Pulmonary Embolism in Pigs

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OBJECTIVES: To investigate the contribution of mechanical obstruction and pulmonary vasoconstriction to pulmonary vascular resistance (PVR) in acute pulmonary embolism (PE) in pigs.

DESIGN: Controlled, animal study.

SETTING: Tertiary university hospital, animal research laboratory.

SUBJECTS: Female Danish slaughter pigs ($n = 12$, ~60 kg).

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: PE was induced by infusion of autologous blood clots in pigs. CT pulmonary angiograms were performed at baseline, after PE (first experimental day [PEd0]) and the following 2 days (second experimental day [PEd1] and third experimental day [PEd2]), and clot burden quantified by a modified Qanadli Obstruction Score. Hemodynamics were evaluated with left and right heart catheterization and systemic invasive pressures each day before, under, and after treatment with the pulmonary vasodilators sildenafil (0.1 mg/kg) and oxygen (FIO_2 40%). PE increased PVR (baseline vs. PEd0: 178 ± 54 vs. 526 ± 160 dynes; $p < 0.0001$) and obstruction score (baseline vs. PEd0: 0% vs. 45% ± 13%; $p < 0.0001$). PVR decreased toward baseline at day 1 (baseline vs. PEd1: 178 ± 54 vs. 219 ± 48; $p = 0.16$) and day 2 (baseline vs. PEd2: 178 ± 54 vs. 201 ± 50; $p = 0.51$). Obstruction score decreased only slightly at day 1 (PEd0 vs. PEd1: 45% ± 12% vs. 43% ± 14%; $p = 0.04$) and remained elevated throughout the study (PEd1 vs. PEd2: 43% ± 14% vs. 42% ± 17%; $p = 0.74$). Sildenafil and oxygen in combination decreased PVR at day 0 (−284 ± 154 dynes; $p = 0.0064$) but had no effects at day 1 (−8 ± 27 dynes; $p = 0.4827$) or day 2 (−18 ± 32 dynes; $p = 0.0923$).

CONCLUSIONS: Pulmonary vasoconstriction, and not mechanical obstruction, was the predominant cause of increased PVR in acute PE in pigs. PVR rapidly declined over the first 2 days after onset despite a persistent mechanical obstruction of the pulmonary circulation from emboli. The findings suggest that treatment with pulmonary vasodilators might only be effective in the acute phase of PE thereby limiting the window for such therapy.

KEYWORDS: computed tomography pulmonary angiography; porcine model; pulmonary embolism; pulmonary vasoconstriction; vasodilatation

Acute pulmonary embolism (PE) is the third most common cause of cardiovascular death in Europe (1). In intermediate- and high-risk PE, the estimated 30-day mortality is 15–30%, contributing to an overall 450,000 deaths per year in Europe alone. Despite growing attention, treatment of acute PE remains largely unchanged (2). During PE, the pulmonary vascular resistance (PVR) increases, causing an increased strain of the right ventricle (RV) (1, 3). A severe increase in PVR leads to failure of the RV, systemic hypotension, shock, and death.

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KEY POINTS

Question: Is mechanical obstruction or pulmonary vasoconstriction the predominant contributor to the increased pulmonary vascular resistance (PVR) in the acute phase of pulmonary embolism (PE) in pigs?

Findings: In a porcine study, autologous PE caused 45% CT pulmonary angiography-verified obstruction of the pulmonary arteries and threefold increase in PVR, which could be attenuated by the vasodilators oxygen and sildenafil in the acute phase. The mechanical obstruction remained elevated throughout the 2 days of the study, while the PVR decreased toward baseline levels after 1 day and remained low.

Meaning: Pulmonary vasoconstriction, not mechanical obstruction, was the predominant cause of increased PVR in acute PE in pigs, but decreases rapidly, hereby limiting the possible therapeutic window for pulmonary vasodilators.

The two contributing factors to the increased PVR in acute PE are mechanical obstruction by emboli and pulmonary vasoconstriction (4–8). Attenuation of adverse pulmonary vasoconstriction has shown promise as a treatment in experimental studies of acute PE (6, 9). However, randomized clinical trials have failed to replicate these beneficial effects (10–12).

Time may be a factor in this discrepancy. In experimental studies, PVR increases abruptly after acute PE and decreases within hours to a stabile plateau (6, 9). Accordingly, attenuation of vasoconstriction seems effective in the first hours after PE (6, 7, 9, 13–16). However, the mean time from onset of acute PE to inclusion in clinical trials is days, at which time PVR may already be decreased by endogenous mechanisms (10–12). Whether the decrease in PVR in the first hours after acute PE is due to reduced mechanical obstruction or attenuation of adverse pulmonary vasoconstriction is unknown.

We aimed to investigate the comparative contribution of mechanical obstruction and pulmonary vasoconstriction to PVR in a period of 2 days following acute PE in pigs.

MATERIALS AND METHODS

Ethics

This study was approved by the Danish Animal Research Inspectorate (Number: 2018-15-0201-01521). Pigs were handled according to Danish law and standards of animal care. We followed the Animal Research: Reporting of In Vivo Experiments guidelines 2.0 (17) and animals served as their own controls to the decrease number of experimental subjects according to the 3Rs (reduction, replacement, and refinement) (18). We used female Danish slaughter pigs (crossbred of Danish Landrace/Yorkshire and Duroc, ~60 kg) corresponding to 5–6 months old. Pigs followed a specific pathogen-free program, with no genetic modification.

Before and between experimental days, all animals were monitored by trained veterinary staff to ensure no signs of distress exceeded those mentioned in the license.

Study Design

Pigs (n = 12) were included. Animals were anesthetized before baseline, hemodynamics, respiratory parameters, and CT pulmonary angiography (CTPA) scans were obtained, followed by administration of PE as previously described (16, 19). All evaluations were repeated at the same day and the following 2 days (first experimental day [PEd0], second experimental day [PEd1], and third experimental day [PEd2]) (**Fig. 1**). Vasodilation included testing of oxygen, sildenafil and the combination of sildenafil and oxygen, respectively.

In between days, pigs were awakened, relocated to the research farm, and returned the subsequent morning.

To evaluate the pulmonary vasoconstriction, pigs were divided into two subgroups. One where pigs received treatment with the vasodilators sildenafil and oxygen, alone and in combination, all days (n = 6, vasodilator). To ensure that there was no prolonged effects of sildenafil by repeated treatments, the second subgroup received sildenafil only at day 2 (n = 6, control). After final measurements day 2, pigs were euthanized with infusion of pentobarbitalnatrium (Richter Pharma AG, Wels, Austria).

Inclusion and exclusion criteria were determined a priori. For inclusion, animals needed to double mean pulmonary artery pressure (mPAP) from baseline or

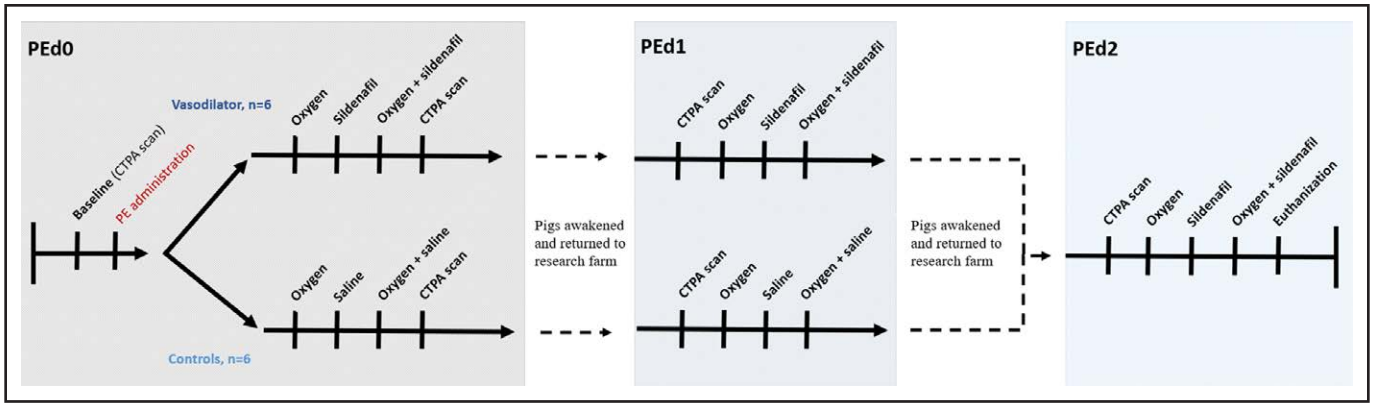

Figure 1. Study design. Animals were divided into either vasodilator or control group. Vasodilator received sildenafil all 3 d, whereas controls received saline first experimental day (PEd0) and second experimental day (PEd1), and sildenafil third experimental day (PEd2). To all timepoints, hemodynamics and respiratory parameters were evaluated. PE = pulmonary embolism, CTPA = CT angiography scan.

receive all coagulated emboli. For exclusion, animals were excluded if they showed any signs of illness (fever or signs of distress) or had a mPAP less than 25 mm Hg at baseline.

Anesthesia

Before transportation to the research facility, all animals were pre-anesthetized using an intramuscular injection of Zoletil mix (0.1 mL/kg; tiletamine 25 mg/mL, zolazepam 25 mg/mL, butorphanol 10 mg/mL, ketaminol 100 mg/mL, and xylazine 20 mg/mL [Virbac Danmark, Kolding, Denmark]). Approximately 1 hour after, general anesthesia was induced and maintained for the duration of the study by continuous venous infusion of propofol (4.0 mg/kg/hr [Fresenius Kabi, Copenhagen, Denmark]) and fentanyl (12.5 μg/kg/hr [B. Braun Medical, Frederiksberg, Denmark]). Pigs were intubated and mechanically ventilated (Datex-Ohmeda S/5 Avance; GE Healthcare, USA) with a F_{IO_2} at 21% and pressure-controlled volume gated modus. Positive end-expiratory pressure was set to 5cm H_2O to reduce atelectasis. Tidal volume was 500 mL and respiratory rate adjusted to an end-tidal Co_2 between 5 and 5.5 kPa, both settings remained unchanged throughout the experiment. Additional monitoring included peripheral oximetry, electrocardiogram, and rectal temperature that were kept constant at 38°C with a Bair Hugger (3M, Aarhus, Denmark). One and a half liters of isotone saline bolus was giving upon arrival on day 0, followed by a continuous infusion of 4 mL/kg/hr. During PE administration, bolus injections of norepinephrine 0.1 mg (Macure Pharma, Copenhagen, Denmark) were used to acutely stabilize the animal if mean arterial blood pressure (MAP) decreased below 40 mm Hg after PE. Hemodynamics was evaluated 30 minutes after injection.

Hemodynamic Measures

Vascular access was obtained using a minimally invasive ultrasound-guided technique. All vascular inserts were continuously flushed with isotonic saline to prevent coagulation. A 6-Fr sheath in left femoral artery was used for serial blood gas samplings and continuous MAP measurement. Another 6-Fr sheath was inserted in the left femoral vein for blood samples and administration of sildenafil. An 8-Fr sheath was inserted in the right femoral artery for left heart catheterization. Finally, an 8-Fr sheath was inserted in the right jugular external vein for drawing blood to create autologous emboli. Later, this was exchanged for a 26-Fr sheath (Dryseal; Gore Medical, USA) for administration of autologous emboli and right heart catheterization (Swan-Ganz thermodilution catheter 7.5-Fr; Combo; Edwards Lifesciences, USA). All catheterizations were performed under fluoroscopic guidance. Biventricular catheterization enabled measurements of left ventricle end-diastolic pressure (LVEDP), mPAP, and central venous pressure (CVP). Cardiac output (CO) was obtained using thermodilution injecting a bolus of 10 mL, 5°C 5% glucose, and three times (mean of three curves). Stroke volume (SV) was derived from CO and heart rate (HR).

PVR, systemic vascular resistance (SVR), and PVR/SVR ratio were calculated using following equations:

$$SVR = \left(\frac{MAP - CVP}{CO}\right) \times 80$$

$$PVR = \left(\frac{mPAP - LVEDP}{CO}\right) \times 80$$

Before the animals were awakened and transported back to the research farm all catheters and sheaths were removed and hemostasis secured by suture for veins or closure device for arteries (Angio-Seal VIP; Terumo).

Emboli

Our in vivo porcine model of acute intermediate-risk PE has previously been described in detail (16, 19). Venous blood was drawn from the animal and placed vertically in six separate nonheparin-coated plastic tubes each containing 30 mL at room temperature for 3–4 hours. Fully coagulated emboli were administrated sequentially, with flow of isotonic saline, through the 26-Fr sheath in the right jugular vein. Administration was ceased once baseline mPAP had doubled or all six emboli were introduced. The emboli were weighted to quantify the total mass of injected emboli.

CT Pulmonary Angiography

CTPA was performed using a 160-slice Canon Aquilion Prime SP 160 slice (Canon Medical Systems) CT scanner during inspirational breath-hold and contrast series performed with bolus tracking technique. Scans were evaluated by an observer blinded to both the source and intervention, measuring RV/left ventricle (LV) ratio, pulmonary artery (PA) diameter, and thrombus burden quantified by the Qanadli CT obstruction score (20). The Qanadli CT obstruction score was modified to pigs as they have a total of 37 lung segments (19 in left lobe, 18 in right lobe) (21, 22). Each segment appointed a score: 0 = no obstruction, 1 = partial obstruction, or 2 = complete obstruction. A segment with a proximal complete obstruction is appointed two points for all distal segments. The obstruction percentage is calculated with following equation:

$$\sum (n.d) / 74 \times 100$$

Where n = number of segment distal to the emboli and d = degree of obstruction.

Pulmonary Vasodilators

Two pulmonary vasodilators were used in this study, sildenafil (Sigma-Aldrich, St. Louis, MO) and oxygen. Throughout the study, an FIO_2 of 21% was maintained mimicking atmospheric concentrations. To induce vasodilation with oxygen, FIO_2 was increased to 40% for 15 minutes and maintained elevated, as shown previously (14). Furthermore, sildenafil in a dose of 0.1 mg/kg, dissolved in isotonic saline (0.1 mg/mL) was infused over the course of 6 minutes and measurements 30 minutes after. Sildenafil in doses of 0.3 and 0.1 mg/kg were evaluated in a pilot study, and the lower dose was observed to have comparable pulmonary effects with lesser systemic adverse effects (**Supplementary table 1**, http://links.lww.com/CCX/B302). The vasodilator group received both 0.1 mg/kg sildenafil and 40% oxygen at PEd0, PEd1, and PEd2. Controls received 40% oxygen all 3 days. However, at PEd0, PEd1 they received a saline infusion, with sildenafil only being administered at the end of PEd2.

Blood Samples

Arterial and mixed venous blood drawn at each time point were analyzed for partial gas pressures (ABL90 FLEX PLUS; Radiometer Medical, Aarhus, Denmark). Dead space and ventilation/perfusion mismatch were calculated (23).

Statistics

Based on previous studies in the model evaluating sildenafil in acute PE, a sample size calculation was performed. We aimed to detect a reduction in PVR 60 dynes by a paired *t* test. The SD of PVR in the model is approximately 35 dynes. To obtain a power of 0.80 and alpha-value of 0.05 based on the above, a sample size of six pigs was needed. To match the six pigs receiving sildenafil a similar sized control group of six pigs was chosen.

Our design involved repeated measures within subjects over time. Animals served as their own control. Normal distribution was evaluated by quantile-quantile plots and Shapiro-Wilk test. Variables were tested between time points with *t* test or Wilcoxon signed-rank test as appropriate. A *p* value of less than 0.05 was considered statistically significant.

The effects of oxygen and sildenafil were investigated at PEd0, PEd1, and PEd2 by comparing delta values before and during intervention using *t* test or Wilcoxon signed-rank test as appropriate. A *p* value of less than 0.05 was considered significant. GraphPad PRISM v. 9.0 (GraphPad Software, San Diego, CA) was used for analysis.

RESULTS

We enrolled 12 pigs; each was administered 5 ± 1 emboli weighting 79 ± 14 g (**Fig. 2**). One pig died after inclusion in the control group (cardiac arrest following PE). Four pigs (two in each group) received a bolus of 0.1 mg norepinephrine. Only minor complications, such as self-limiting bleeding, occurred during the instrumentation.

Hemodynamics

PE induced an increase in PVR (**Fig. 3*A***), HR, mPAP, PVR/SVR, and a decrease in SV compared with baseline (**Table 1**). There was no change in systemic hemodynamic parameters such as MAP, CO, and LVEDP as shown in Table 1. At PEd1, HR and PVR/SVR remained elevated and LVEDP lowered compared with baseline (Table 1). At PEd2, HR remained elevated while LVEDP and CVP were lowered compared with baseline (Table 1).

CT Pulmonary Angiography

Evaluated by CTPA, there was no PE at baseline in any of the pigs. PE increased the obstruction score at PEd0. The obstruction score decreased slightly from PEd0 to PEd1, but unaltered from PEd1 to PEd2 (**Fig. 3*B***). PA diameter increased after PE and remained dilated throughout the protocol (Table 1). A nonsignificant increase in RV/LV ratio was observed after PE, it was increased at PEd1 and unaltered from PEd1 to PEd2 compared (Table 1). Nine of 12 pigs had pulmonary infarctions after PE.

Figure 2. Autologous emboli.

Ventilation

PE increased $Paco_2$, physiologic dead space and shunt fraction and decreased arterial saturation and Pao_2 (Table 1). At PEd1 arterial saturation increased, shunt fraction decreased and physiologic dead space and $Paco_2$ remained elevated (Table 1). At PEd2, only physiologic dead space remained elevated compared with baseline (Table 1).

Effects of Pulmonary Vasodilators

Sildenafil decreased PVR on PEd0, but not PEd1 and PEd2 (**Table 2**). Sildenafil decreased MAP at all days (Table 2), but most pronounced on PEd1 and PEd2. Oxygen (Fio_2 40%) decreased PVR at PEd0 and PEd1, but not PEd2 (Table 2). Combination of sildenafil and oxygen decreased PVR on PEd0, but not PEd1 and PEd2 (Fig. 1), with an additive effect of oxygen on sildenafil at PEd0 (Table 2). Controls showed similar response to PVR with oxygen and no response to saline infusion at PEd0, PEd1, and PEd2.

DISCUSSION

PE caused a CTPA-verified obstruction of the pulmonary arteries (Fig. 4) and an increase in PVR in the acute phase. PVR decreased toward baseline levels already day 1 and remained normalized at day 2. Obstruction score remained elevated throughout the study period of 2 days. PVR could be attenuated by pulmonary vasodilation with sildenafil and oxygen in the acute phase, but not at days 1 and 2.

Autologous pulmonary emboli caused a three-fold increase in PVR. RV strain was increased as indicated by elevated in RV/LV ratio and a decreased SV and CO. We observed no changes in MAP suggesting that there was no systemic impairment making the phenotype comparable to the European Society of Cardiology's definition of acute intermediate-risk PE (1). These findings are comparable to previous studies of the current model (6, 7, 9, 13, 14, 16) and others (24–27).

Norepinephrine was administered in four of 12 pigs, two in each group, after emboli infusion. This may have increased the RV afterload initially, but at

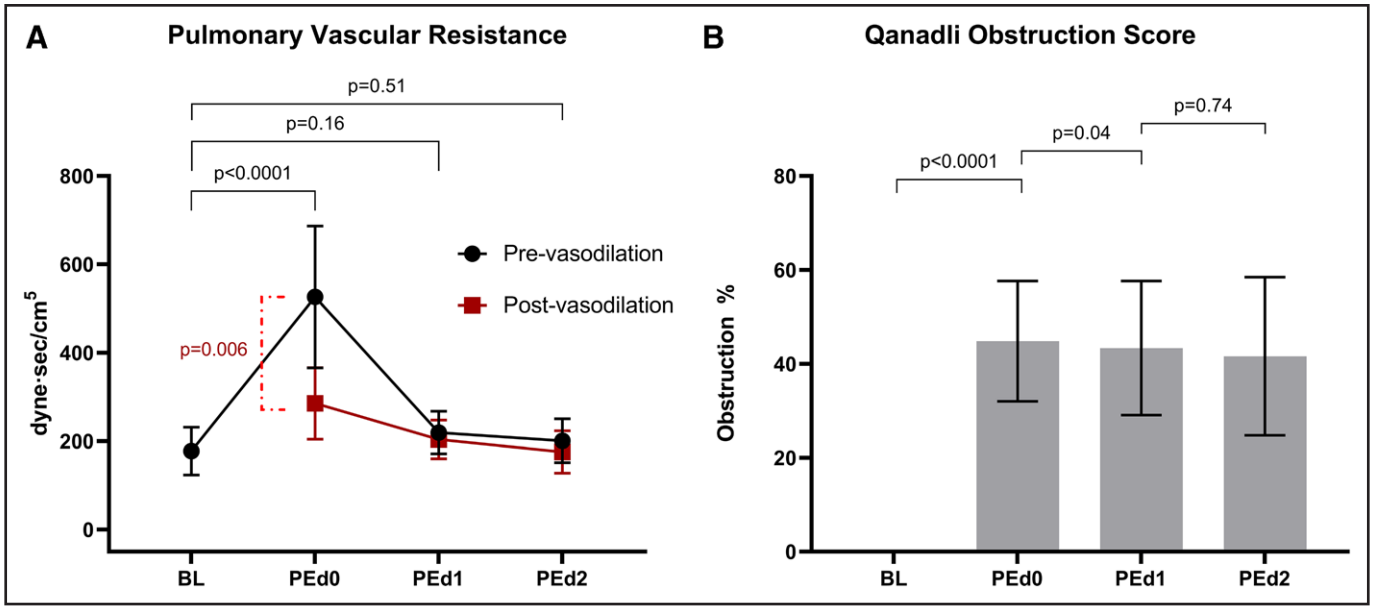

Figure 3. Results. **A**, Changes in pulmonary vascular resistance (PVR) after acute pulmonary embolism (PE) and the effects of vasodilation over days. *Black graph* (pre-vasodilation) illustrating PVR development over days for all pigs. *Red graph* (post-vasodilation) illustrating PVR following vasodilation with combined sildenafil and oxygen in the vasodilator group ($n = 6$) at first experimental day (PEd0), second experimental day (PEd1), and third experimental day (PEd2). Significant attenuation of PVR following vasodilation with combined sildenafil and oxygen at PEd0. No significant decrease in PVR on PEd1 and PEd2 following vasodilation with the combination of sildenafil and oxygen. **B**, Changes in obstruction percentage after acute PE and over days. Data are presented as mean ± SD. BL = baseline.Insert Alt Text here Results

measurements 30 minutes after infusion, the effect is expected to be negligible due to the short half-life of norepinephrine (28).

Already 1 day following PE most hemodynamic measures (**Supplementary table 2**, http://links.lww.com/CCX/B302), including PVR, returned to baseline levels. In previous studies of this model, PVR decreased autologously, but remained at an elevated plateau, 11 hours following PE (6, 9). The present study is the first in an animal model of acute PE with more prolonged follow-up for days after PE.

We observed that HR, RV/LV ratio and PA diameter all remained elevated throughout the study, indicating prolonged RV strain (29, 30). This is somewhat surprising in the face of the normalized PVR, as one would expect RV strain to be attenuated as well. However, the same discrepancy was found in an explorative trial in PE patients, where invasive PVR measurements were near normal but RV/LV were increased (10). Patients were included 2.8 days after onset of symptoms similar to the length of our study.

Obstruction of the pulmonary vessels was 45% and remained high throughout the study. This level of obstruction is comparable to patients with PE (20, 31, 32). The obstruction decreased only slightly between day 0 and 1. This may be a sign of early blood clot resolution; however, no further resolution was seen at day 2. Studies have suggested that blood clot resolution may begin as soon as days (33, 34) or within a week in humans (33–35). This rate might be comparable to what we found in our study, as we know that pigs have an increased endogenous fibrinolytic rate (36–39), which may make them comparable to patients on anti-coagulation treatment.

Despite a substantial amount of remaining clot burden, PVR near-normalized already after 1 day. This indicates that adverse pulmonary vasoconstriction is the major contributor to increased PVR in the hours following PE and that this raised vasoconstrictor tone is attenuated already in the first days after PE by endogenous mechanisms. It also highlights the pronounced compensatory reserve of the pulmonary circulation (5, 40), as PVR was normal despite a 40% pulmonary obstruction and it might explain why clot burden correlates poorly to adverse outcomes in patients with acute PE (41, 42).

Pulmonary vasodilation by sildenafil and oxygen was able to near-normalize PVR after PE. This is in accordance with previous studies in the model and further suggests that pulmonary vasoconstriction is the major contributor to PVR in the acute phase (7, 14). These

TABLE 1.
Effects of Pulmonary Embolism

Variable	Baseline ($n = 12$)	First Experimental Day ($n = 12$)	Second Experimental Day ($n = 11$)	Third Experimental Day ($n = 11$)
Hemodynamics				
Heart rate, beats/min	50 ± 11	60 ± 14[a]	66 ± 17[a]	57 ± 13[a]
Mean arterial pressure, mm Hg	75 ± 10	74 ± 11	76 ± 11	81 ± 7
Cardiac output, L/min	4.769 ± 0.721	4.125 ± 0.953	5.239 ± 0.7276	5.082 ± 0.8693
Stroke volume, mL	95 ± 16	73 ± 22[c]	84 ± 21	91 ± 15
Mean pulmonary artery pressure, mm Hg	17 ± 3	32 ± 4[d]	19 ± 4	17 ± 3
Central venous pressure, mm Hg	3 (3, 4)	4 ± 1	2 ± 2	2 ± 1[a]
Left ventricle end-diastolic pressure, mm Hg	7 ± 2	7 ± 2	5 ± 2[a]	4 ± 2[b]
SVR, dynes/s/cm^5	1229 ± 241	1395 ± 315	1184 (1103–1199)	1285 ± 278
Pulmonary vascular resistance/SVR	0.129 (0.117–0.165)	0.376 ± 0.056[c]	0.195 ± 0.046[a]	0.157 ± 0.023
Ventilation				
Arterial saturation, %	97 ± 2	84 ± 6[d]	96 (96–98)	100 (98–100)
Respiration rate	14	14	14	14
Pao_2, kPa	12.39 ± 1.22	7.85 ± 1.01[d]	10.44 ± 1.55[b]	11.68 ± 1.31
End-tidal Co_2, kPa	5.36 ± 0.20	5.20 ± 0.49	5.12 ± 0.34	5.16 ± 0.44
$Paco_2$, kPa	5.57 ± 0.31	6.62 ± 0.51[d]	5.79 ± 0.34[a]	5.59 ± 0.46
Physiologic dead space, mL	18.3 ± 16.1	106.7 ± 29.5[d]	57.1 ± 29.2[b]	37.7 ± 20.2[a]
Arterial o_2 content, mL/dL	13.02 ± 1.34	12.16 ± 1.15[b]	12.06 ± 1.14[a]	11.40 ± 0.66[b]
o_2 content mixed venous	8.04 ± 1.38	4.81 ± 0.97[d]	7.05 ± 1.36	6.54 ± 0.72[b]
Shunt fraction, %	0.013 (–0.025 to 0.049)	0.228 ± 0.086[c]	0.0628 (0.016–0.077)	–0.050 (–0.055 to 0.014)
CT pulmonary angiography				
Pulmonary artery diameter, mm	19.8 ± 1.7	22.8 ± 2.2[c]	21.9 ± 2.5[a]	21.5 ± 1.4[a]
Right ventricle/left ventricle ratio	0.79 ± 0.11	0.94 ± 0.16	0.99 ± 0.21[a]	0.93 ± 0.13

SVR = systemic vascular resistance.
[a]$p < 0.05$ compared with baseline.
[b]$p < 0.01$ compared with baseline.
[c]$p < 0.001$ compared with baseline.
[d]$p < 0.0001$ compared with baseline.
$p < 0.05$ were considered significant.
Data are presented with mean ± SD if data were normal distributed and median (interquartile range) where appropriate.

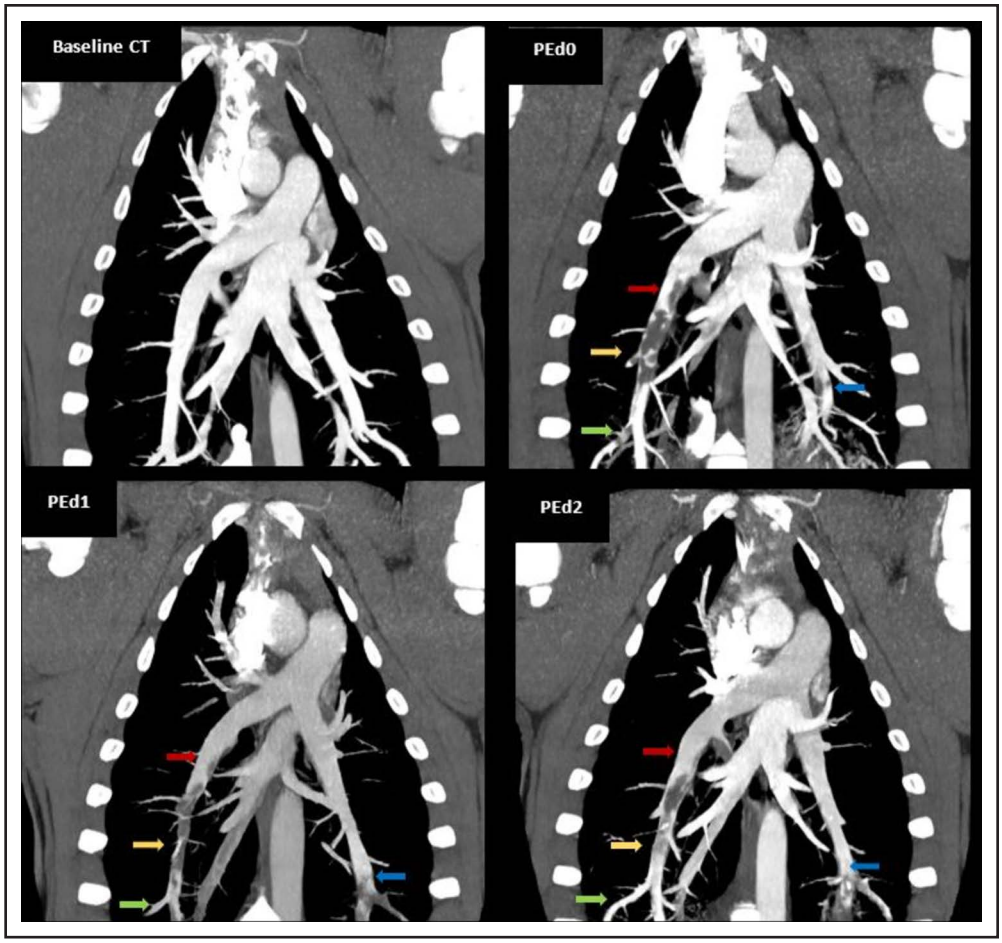

Figure 4. Repeated CT pulmonary angiographies with pulmonary emboli present at first experimental day (PEd0), second experimental day (PEd1), and third experimental day (PEd2).

findings have contributed to the hypothesis of clinical trials investigating pulmonary vasodilators as a treatment in acute PE. However, randomized clinical trials have failed to replicate the beneficial effects (10–12). Our data represents an explanation for this discrepancy as, the mean time from onset of acute PE to inclusion in clinical trials is days, at which time PVR may already be normalized endogenously. Accordingly, treatment with sildenafil and oxygen had no further vasodilatory effects from 1 day after PE and onwards in the present study.

Several pathways have been suggested to be responsible for the vasoconstriction in PE (8). We used two pulmonary vasodilators, sildenafil and oxygen, to test for residual pulmonary vasoconstriction. Both have been shown to attenuate pulmonary vasoconstriction in the acute phase of PE (7, 14, 24, 43). We found both sildenafil and oxygen to reduce PVR, with an additive effect. This may suggest involvement of multiple pathways in pulmonary vasoconstriction, given that sildenafil uses the cyclic guanosine monophosphate pathway (44) and oxygen counteracts hypoxic vasoconstriction (45).

Oxygen did not cause any adverse hemodynamic changes throughout the study and was considered safe, as previous suggested (14). Sildenafil had adverse effects with a decrease in MAP at all days. This was compensated at day 0 with increased HR and unchanged CO. When administering sildenafil at day 1 and day 2 with PVR being near-normalized, HR was actually decreased hindering compensation of its systemic vasodilator effect resulting in a decreased CO (**Supplementary table 3**, http://links.lww.com/CCX/B302). This observation is important, as sildenafil may be beneficial in the early phase, but may have adverse effects in the latter.

A similar decrease in MAP, but with a stationary HR and CO, was seen in the only randomized clinical trial treating PE patients with sildenafil, where PVR was also near normal (10). In this study, patients were treated 2.8 days after onset of PE symptoms, which is similar to the length of the present study and to the median time at which patients usually are admitted days after onset of symptoms (46). At this time, treatment with pulmonary vasodilators with concurrent systemic vasodilation may not only have limited pulmonary effect but also adverse systemic effect. This warrants caution in treatment and further studies of pulmonary vasodilators in acute PE.

LIMITATIONS

This is an experimental animal study limiting translation of findings to patients with acute PE.

Even though propofol was titrated to a minimum level anesthesia, positive pressure ventilation for 3 consecutive days might have influenced the hemodynamic

TABLE 2.
Effects of Pulmonary Vasodilation

Sub-groups	Oxygen	Sildenafil	Sildenafil + Oxygen	Additional Effect of Oxygen
Pulmonary vascular resistance, dynes/s/cm^5				
PEd0				
Vasodilator	−125 (−262 to −105)[b]	−213 ± 119[c]	−284 ± 154[c]	−61 (−123 to −27)[b]
Control	−142 ± 78[c]	−53 ± 45[a]	−191 ± 132[a,b]	−138 ± 89[b]
PEd1				
Vasodilator	−48 ± 30[b]	12 ± 56	−8 ± 27	−20 ± 36
Control	−25 (−72 to −23)[b]	4 (−2 to 24)[a]	−9 ± 23[a]	−19 ± 10[b]
PEd2				
Vasodilator	−33 ± 34	−2 ± 27	−2 ± 29	1 ± 29
Control	−26 ± 21	−23 ± 50	−38 ± 25[b]	−15 ± 27
Mean pulmonary artery pressure, mm Hg				
PEd0				
Vasodilator	−8.2 ± 3.1[c]	−7.2 ± 2.8[c]	−11.7 ± 4.7[c]	−4.5 ± 2.4[c]
Control	−7.0 ± 3.0[c]	−2.8 ± 1.8[a,b]	−10.0 (−12.0 to −10.0)[a,e]	−8.0 ± 0.9[c]
PEd1				
Vasodilator	−2.8 ± 1.3[c]	−3.5 (−4.0 to −1.0)[b]	−3.3 ± 1.4[c]	−0.5 ± 1.2
Control	−3.0 (−5.0 to −2.0)[c]	−1.0 (−1.0 to 1.0)[a]	−2.2 ± 1.3[a,b]	−1.8 ± 1.1[b]
PEd2				
Vasodilator	−2.7 ± 1.8[b]	−2.3 ± 1.0[c]	−2.0 (−2.5 to −1.8)[b]	0.2 ± 0.8
Control	−2.6 ± 1.1[c]	−2.8 ± 1.9	−4.4 ± 1.7[c]	−1.6 ± 1.3
Mean arterial pressure, mm Hg				
PEd0				
Vasodilator	−3 ± 5	−4 ± 2[c]	−3 ± 4	1 ± 3
Control	1 ± 4	0 ± 5[a]	−3 ± 6[a]	−3 ± 2[c]
PEd1				
Vasodilator	−3 ± 2[c]	−21 ± 12[c]	−17 ± 9[c]	4 ± 4[b]
Control	−2 ± 4	−6 ± 3[a,b]	−6 ± 5[a]	−1 ± 3
PEd2				
Vasodilator	−4 (−3 to 6)[b]	−18 ± 9[c]	−13 ± 8[b]	6 ± 5[b]
Control	−4 ± 2[c]	−18 ± 13[b]	−16 ± 15	2 ± 4

PEd0 = first experimental day, PEd1 = second experimental day, PEd2 = third experimental day.
[a]Control group did not receive sildenafil on PEd0 and PEd1 to these timepoints.
[b]$p < 0.05$ compared if values differed from 0.
[c]$p < 0.01$ compared if values differed from 0.
[d]$p < 0.0001$ compared if values differed from 0.
$p < 0.05$ were considered significant.
Vasodilator ($n = 6$) and controls ($n = 5$). Data are presented as delta values from pulmonary embolism timepoint to timepoint with intervention. Final column represents the additional change adding oxygen to sildenafil infusion. Presented with mean ± SD if data were normal distributed and median (interquartile range) where appropriate.

measurements to PE and the responses to the pulmonary vasodilators used (47).

One animal died after PE and, therefore, the number of animals in the control group was reduced from 6 to 5. We however find the risk type II errors minor as the SDs in the control group was lower than in the vasodilator group.

The investigator was not blinded to intervention, but timepoints for measurements were predetermined and CTPA scans were analyzed post hoc by a third part blinded to the interventions. CTPA scans were analyzed using a semi-quantitative method based on individual estimates; however, the method is well established and verified similar to other quantification scores (48).

Sildenafil has a half-life expectancy of approximately 4 hours (49), why a prolonged effect in between days is theoretically possible. As PVR also normalized at day 1 in the control group only receiving sildenafil at day 2, prolonged effects of sildenafil, if any, may be sparse.

CONCLUSIONS

Pulmonary vasoconstriction, and not mechanical obstruction, was the predominant cause of increased PVR in acute PE in pigs. PVR rapidly declined over the first 2 days after onset despite a persistent mechanical obstruction of the pulmonary circulation from emboli. The findings suggest that treatment with pulmonary vasodilators might only be effective in the acute phase of PE thereby limiting the window for such therapy.

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Dr. Merit (corresponding author) was the primary investigator. Ms. Kirk, Dr. Schultz, Dr. Hansen, Dr. Lyhne, and Dr. Nielsen-Kudsk all contributed during both experimental phase collecting data and the writing process. Mrs. Pedersen oversaw experimental phase with CT scans as Drs. Karout and Kalra were in charge of analyzing the CT scans.

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