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Inhalation of prostacyclin (PGI_2) for 8 hours does not produce signs of acute pulmonary toxicity in healthy lambs

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Abstract *Objective:* To study the potential side effects and toxicity of inhaling prostacyclin (PGI_2) aerosol for 8 h.
Design: In a prospective, randomized study 14 healthy lambs received either PGI_2 ($n = 7$) or 0.9% NaCl ($n = 7$) as an aerosol for 8 h.
Setting: Institute for Surgical Research of the Ludwig-Maximilians-University of Munich.
Interventions: All animals were studied under general anesthesia in a prone position. They were first intubated endotracheally and later tracheotomized. PGI_2 solution (median dose 28 ng/kg per min) or 0.9% NaCl was administered with a jet nebulizer (delivery rate 4–10 ml/h; mass median diameter of aerosol particles 3.1 μm). Bronchoalveolar lavage was performed before and after the inhalation period to collect epithelial lining fluid of alveoli.
Measurements and results: Hemodynamic and respiratory parameters, systemic resorption (plasma levels of 6-keto-prostaglandin-F1α), in vitro bleeding time, collagen-induced platelet aggregation and global biochemical and cellular composition of the epithelial lining fluid were examined in order to assess the side effects and signs of acute pulmonary toxicity induced by inhaled PGI_2. No statistically significant differences were found between the PGI_2 and the control groups for any of the parameters examined.
Conclusion: Inhalation of PGI_2 (28 ng/kg per min) over a period of 8 h in healthy lambs does not produce major side effects or acute pulmonary toxicity.

Key words Aerosols · Epoprostenol · Toxicity · Lung

Introduction

The reduction of increased pulmonary artery pressure is of major importance in acute pulmonary hypertension of various origins. Pulmonary hypertension may impair right ventricular function, increase the effective pulmonary capillary filtration pressure and enhance pulmonary edema [1, 2]. Unfortunately, systemic administration of potent vasodilators such as prostacyclin (PGI_2) and prostaglandin E_1 is hampered because of their lack of selectivity for the pulmonary vasculature and hence by a dose-dependent reduction in systemic vascular resistance. Furthermore, vasodilation in poorly ventilated areas of the impaired lung increases intrapulmonary shunt and worsens arterial oxygenation [3, 4].

Welte et al. [5] first were first to administer PGI_2 as an aerosol to dogs in an experimental model of hypoxia-induced pulmonary hypertension. They demonstrated that PGI_2 is able to reduce pulmonary hypertension without eliciting systemic vasodilation. These experimental findings have recently been confirmed by studies in patients with acute respiratory failure in whom a reduction in pulmonary artery pressure and intrapulmonary

shunt and an increase in partial oxygen pressure (PO_2) have been achieved with inhalation of PGI_2 [6–14].

Side effects from inhaled PGI_2 have been reported by only a few investigators. Some found inhibition of adenosine 5′-diphosphate-induced platelet aggregation [15, 16]; others reported a slight accentuation of bronchospasm after inhalation of PGI_2 aerosol in asthmatic patients [17, 18]. These clinical studies described effects only for short-term use of PGI_2 aerosol, but the effects of long-term inhalation of PGI_2 have not been investigated. Since the treatment of pulmonary hypertension with PGI_2 aerosol would require repeated inhalation for longer periods of time, the present study, in pentobarbital anesthetized ventilated lambs, was designed to investigate the effects of long-term inhalation of PGI_2 solution on cardiorespiratory parameters, platelet function and global biochemical and cellular composition of the alveolar epithelial lining fluid.

Materials and methods

Subjects

The study was performed in 14 healthy Merino-Country lambs (aged 4 months ± 3 weeks) of both sexes weighing between 28.5 and 48.5 kg. The lungs of all animals were histologically free of pneumonia. The study was approved by the Institutional Review Board for the care of animal subjects and the local animal care and use committee. All animals received care in compliance with the "Guide for the Care and Use of Laboratory Animals" (NIH publication no. 85–23, revised 1985).

Experimental groups

The animals were randomly assigned to two groups. In the PGI_2 group ($n = 7$), PGI_2 aerosol was administered over 8 h. In the control group ($n = 7$), 0.9% NaCl was aerosolized for the same duration. Otherwise, the experimental procedure was identical for both groups.

Animal preparation

After intramuscular premedication with xylazine (0.1 mg/kg) and atropine sulfate (20 µg/kg), anesthesia was induced by intravenous injection of ketamine (5–8 mg/kg) and pancuronium bromide (0.25 mg/kg), and maintained by continuous infusion of pentobarbital (5–10 mg/kg per h). Muscular paralysis was achieved by administering intravenous pancuronium bromide (0.1 mg/kg) every 2 h. Fluid losses were replaced by intravenous infusion of Ringer's solution (15 ml/kg/h) throughout the experiment. Additional Ringer's solution was infused, when necessary, to maintain pulmonary artery occlusion pressure (PAOP) at baseline level (median 10 mmHg). A warming pad was used to keep minimum core body temperature above 37 °C. When the body temperature exceeded 40 °C, cooling was performed by positioning ice packs in the groin.

The animals were first intubated endotracheally and later tracheotomized to facilitate bronchoscopy. Mechanical ventilation was performed at a rate of 12 cycles/min with fractional inspired oxygen (FIO_2) of 0.4 and positive end-expiratory pressure of 10 cm H_2O (Servo 900B, Siemens-Elema, Solna, Sweden). Inspiratory time (T_{ins}) was set to 1.66 s and tidal volume (V_T) was adjusted in order to maintain normocapnia. During inhalation, V_T and total inspiratory flow rate were kept constant by reducing the inspiratory flow from the ventilator. FIO_2 and end-expiratory CO_2-partial pressure were continuously monitored.

Fluid-filled catheters were positioned in the descending aorta and in the superior vena cava to measure arterial pressure (AP) and central venous pressure (CVP), respectively. A Swan-Ganz catheter was inserted into the pulmonary artery to determine pulmonary artery pressure (PAP), PAOP, cardiac output (CO), right ventricular ejection fraction (EF) and right ventricular end-diastolic volume.

After preparation for surgery heparin was administered continuously at a low dose of 200 IU/h i.v. to prevent catheter clotting.

Measurements

All measurements were performed with the lambs placed in a prone position. AP, PAP and CVP were recorded using Statham P 23 Db transducers referenced to atmospheric pressure at the level of the right atrium. CO was averaged from triplicate thermodilution measurements at end-expiration. Cardiac index, systemic and pulmonary vascular resistance indices (SVRI, PVRI) were calculated using body surface area (BSA) determined according to Holt et al. [19]:

$$BSA = k \cdot BW^{2/3}$$

where BW = body weight and $k = 9.8$.

Alveolar-arterial PO_2 difference (A-aDO_2) and intrapulmonary shunt (Q_s/Q_t) were calculated by standard formulas. Hemoglobin concentration was measured by absorbance spectrophotometry (Sysmex microcellcounter, model F-800, Toa medical electronics, Hamburg, Germany); hemoglobin saturation was estimated from the Kelman nomogram [20].

Peak and plateau airway pressures (P_{peak}, P_{plat}) and static complicance of lung and chest wall (C_{tot}) were assessed using an electronic lung mechanics calculator (Model 940, Siemens-Elema, Solna, Sweden) connected to the ventilator.

Administration of PGI_2 aerosol

A jet nebulizer (Servo Nebulizer 945, Siemens, FRG) was connected to a Siemens 900B ventilator. A modified nebulizer chamber (Cirrus, Intersurgical LTD., Twickenham, UK), which allowed refilling without disconnection of the nebulizer system, was attached to the endotracheal tube. The pressure supplied to the nebulizer chamber was monitored with an electronic manometer. The driving pressure of the nebulizer decreased during inspiration from 3.39 ± 0.04 bar to 2.05 ± 0.03 bar and, consequently, the flow rate of the nebulizer fell from about 15 to 9 l/min. Flow rates above 8 l/min were chosen to create a theoretical particle size of less than 2 µm. Prior to the experiments, the particle size of the aerosol was measured by aerosolizing ^{99m}Tc-labeled PGI_2 solution. The chosen nebulizer and respirator settings used in the experiments produced aerosol particles with a mass median aerodynamic diameter of 3.1 µm (geometric standard deviation = 1.9 µm).

PGI_2 was supplied as the sodium salt of epoprostenol (Flolan, Wellcome, London, UK) dissolved in 50 ml glycine buffer at pH 10.5. Before starting the experiment, the time necessary for nebulizing 5 ml of 0.9% NaCl was noted. After extrapolation of this value, PGI_2 was diluted to obtain a target dose of 30 ng/kg per min. This resulted in a median dosage of 28 (20/36) ng/kg per min delivered to the animal over 8 h.

Efficacy of PGI_2 aerosol

In order to be sure that the PGI_2 aerosol reached distal airways in the present experiment, the efficacy of inhaled PGI_2 was studied in three additional animals (body weight 32, 35 and 36 kg). The animals were ventilated with a hypoxic gas mixture (FIO_2 0.16) resulting in an increase of PVRI of 15, 120 and 138%, respectively, compared to baseline values. Inhalation of PGI_2 aerosol in a dose of 19, 21 and 25 ng/kg per min lowered increased PVRI by 23, 39, and 29%, respectively. In two animals SVRI increased by 8 and 9% during PGI_2 inhalation and in one animal SVRI decreased by 3%. Figure 1 shows the original aortic and pulmonary artery pressure recordings during hypoxic ventilation and inhalation of PGI_2 aerosol in one animal.

Platelet function

Blood samples

For preparation of platelet-rich plasma (PRP), 36 ml of venous blood was anticoagulated with 3.8% trisodium citrate and centrifuged at 162 *g* for 15 min. PRP was kept at room temperature for the duration of the measurement.

Another 8 ml of blood was drawn into two preformed blood-collecting devices (Primavette Coagulation, B. Braun Melsungen AG, Melsungen, Germany) that contained 0.43 ml citrate solution (0.106 mol/l). For examination of in vitro bleeding time, 4 ml of blood was transferred into four Eppendorf reaction caps (Eppendorf, Hamburg, Germany); the rest was centrifuged at 1459 *g* for 10 min in order to prepare platelet-poor plasma (PPP).

Platelet aggregation

If platelet counts in PRP were above 50000/mm^3, studies on platelet aggregation were carried out on a four-channel platelet aggregation profiler (Platelet aggregation profiler, model PAP-4, Bio Data Corp., Horsham, Pa., USA). The device was zeroed with PPP. Then 200 µl PRP was incubated at 37 °C for exactly 4 min in siliconized glass cuvettes. Collagen reagent (Kollagenreagens Horm, Nycomed Arzneimittel, Munich, Germany) was prepared in a number of dilutions (using isotone glucose solution, pH 2.7 – 2.9) in order to reach end-concentrations of collagen in a range of 0.625 to 50.0 µg/ml PRP. After incubation of PRP, 10 µl of collagen dilution was transferred into the test tubes. The different collagen solutions were used to achieve 50% platelet aggregation to allow comparison between experiments. Platelet function was plotted until maximum aggregation on each channel was reached.

In vitro bleeding time

In vitro bleeding time was examined with a semi-automatic measuring device (Thrombostat 4000, System Kratzer+Born, VDG Von der Goltz, Seeon, Germany).

Bronchoalveolar lavage (BAL)

In all animals, epithelial lining fluid (ELF) was obtained before and 8 h after starting inhalation by segmental BAL of two different segments of the right caudal lobe using a 60 cm flexible fiberoptic bronchoscope (inner diameter 6.0 mm). An adapter on the tracheostoma allowed simultaneous mechanical ventilation of the animal during BAL. Three 50-ml aliquots of sterile, non-pyrogenic, body-warm (37 °C) 0.9% NaCl were infused into the same subsegmental bronchus and withdrawn by hand suction. The duration of each lavage cycle was kept constant at 55 s. The returns from the three lavage cycles were pooled, filtered through sterile gauze and centrifuged for 10 min at 318 *g* to separate cells from the supernatant. The supernatant was centrifuged again at 1459 *g* for 10 min and then stored at −70 °C until measurement of the biochemical parameters. The cells were resuspended in 40 ml phosphate-buffered 0.9% NaCl (PBS) and centrifuged again at 318 *g* for 10 min. The supernatant was discarded and the cell pellet was resuspended in PBS.

Analysis of BAL fluid supernatant

The activities of lactate dehydrogenase (LDH) and alkaline phosphatase were determined using diagnostic kits (LDH opt. and ALP opt., Boehringer Mannheim, Mannheim, Germany) and a Beckman spectrophotometer DU 7500 (Beckman Instruments Inc., Fullerton, Calif., USA). Urea nitrogen was determined with an enzymatic endpoint procedure (Sigma no. 66-UV, Sigma Chemie, Deisenhofen, Germany) and a Beckman spectrophotometer DU 7500. Total protein (after denaturation with trichloric acid) and fibronectin were measured with a laser nephelometer (BN-100, Behring, Marburg, Germany) using specific anti-fibronectin antibodies (peroxidase

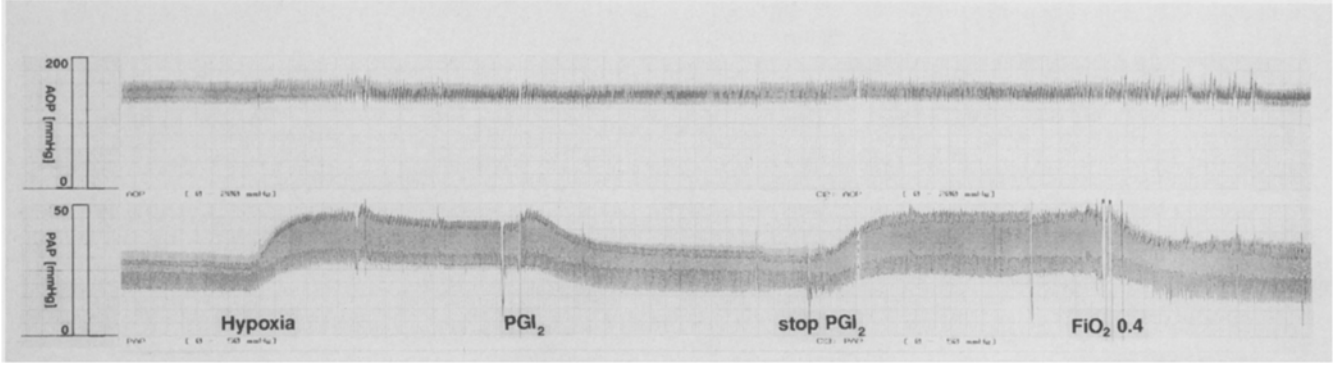

Fig. 1 Original recording of aortic pressure (*AOP*) and pulmonary arterial pressure (*PAP*) during hypoxic ventilation (FIO_2 0.16) and inhalation of PGI_2. Chart speed 5 mm/min

conjugated goat IgG, Boehringer Mannheim, Mannheim, Germany).

Analysis of BAL cells

The total cell number of BAL cells was determined in a standard hemocytometer. Differential cell counts were determined by microscopic examination of 400 cells after May-Grünwald-Giemsa staining of air-dried cytospin preparations (22.4 *g* for 5 min, Shandon cytospin 2 centrifuge, Southern Instruments, Sewickly, Pa., USA). The cell viability was measured by trypan blue exclusion.

Blood samples

Plasma urea nitrogen was determined using the same method as in BAL. A stable metabolite of PGI_2, 6-keto-prostaglandin-F 1α, was measured by radioimmunoassay in unextracted arterial plasma before and during administration of PGI_2 aerosol (6-keto-prostaglandin-F 1α, H-RIA-Kit, Advanced Magnetic, Cambridge, Mass., USA).

Arterial and mixed venous blood gases were determined in a blood gas analyzer (ABL 300, Radiometer, Copenhagen, Denmark).

Calculation of ELF

ELF was calculated by the following equation [21]:

$$[(\text{Urea})_{BAL} : (\text{Urea})_{Plasma}] \cdot \text{Volume}_{BAL} = \text{ELF ml} \ .$$

Statistics

Shapiro-Wilks statistic indicated non-normal distribution of data; therefore, non-parametric tests were used to detect significant differences between the time points "before inhalation" and "after 8 h of inhalation" within each group (Friedmann's test, Wilcoxon's test), as well as between the two groups (rANOVA, Mann-Whitney U-test). rANOVA and Friedmann's test were used prior to testing individual measurements in order to avoid increasing alpha error by multiple testing [22]. Results are presented as median, 1st and 3rd quartile.

Results

Hemodynamic parameters

The hemodynamic parameters are listed in Table 1. The only parameter that changed significantly over the 8-h period of inhalation was mean arterial pressure in control animals. No statistically significant differences for other parameters could be detected between control and PGI_2 animals before and after the 8-h inhalation.

Lung function

Respiratory parameters are summarized in Table 2. No statistically significant differences were found within a group or between the two groups before and after the 8-h inhalation.

BAL parameters

Median BAL recovery was 70 ml (57/83), median cell viability 75% (68/81) (Table 3). All BAL parameters were referenced to the ELF. Median ELF was 4.4 ml (3.2/6.2). The global biochemical and cellular composition of ELF is summarized in Table 4. There were no statistically significant differences before and after the 8-h inhalation of PGI_2 solution or 0.9% NaCl.

6-keto-prostaglandin-F 1α

The median plasma concentrations of 6-keto-prostaglandin-F 1α were below the level of detection (27 pg/ml) and remained unchanged in both groups during inhalation.

Platelet function

One animal in the control group was excluded from the analysis of platelet function because the control platelet count in PRP was below 50000/mm^3. In the control group, the median platelet count in PRP was $246 \cdot 10^3$/mm^3 (80/456) before inhalation compared to $249 \cdot 10^3$/mm^3 (140/284) after 8-h inhalation of 0.9% NaCl; in the PGI_2 group, the platelet count was $100 \cdot 10^3$/mm^3 (64/339) and $320 \cdot 10^3$/mm^3 (168/376), respectively. In the control group, the median collagen concentration required to induce a 50% aggregation of platelets in PRP was 3.25 µg/ml (2.5/10.0) before inhalation and 4.5 µg/ml (2.5/5) after inhalation of 0.9% NaCl. In the PGI_2 group, the median collagen concentrations were 10 µg/ml (2.5/20) before and 5 µg/ml (2/5) after inhalation. Median bleeding time increased in both groups over time: 115 s (90/150) to 300 s (150/360) in the control group and 90 s (60/90) to 140 s (105/360) in the PGI_2 group. The increase in the PGI_2 group was statistically significant. There were no statistically significant differences between the two groups for collagen-induced platelet aggregation and bleeding time before and after inhalation.

Discussion

The objective of our study was to identify potential side effects and acute signs of toxicity of inhaled PGI_2. We

Table 1 Hemodynamics during 8-h inhalation. Values are median (1st quartile/3rd quartile) (*MAP* mean arterial pressure, *MPAP* mean pulmonary artery pressure, *SVRI* systemic vascular resistance index, *PVRI* pulmonary vascular resistance index, *CI* cardiac index, *RVEF* right ventricular ejection fraction, *RVEDVI* right ventricular end-diastolic volume index)

Group	Inhalation time (h)	MAP (mmHg)	MPAP (mmHg)	SVRI ($dyn \cdot s \cdot cm^{-5} \cdot m^{-2}$)	PVRI ($dyn \cdot s \cdot cm^{-5} \cdot m^{-2}$)	CI ($l \cdot min^{-1} \cdot m^{-2}$)	RVEF (%)	RVEDVI ($ml \cdot m^{-2}$)
NaCl	0	135 (123/140)	23 (22/24)	2058 (1819/2612)	257 (151/312)	5.2 (3.8/5.8)	43 (34/52)	75 (71/96)
PGI_2	0	122 (111/132)	23 (21/25)	2064 (1828/2678)	215 (180/253)	4.8 (3.7/5.1)	49 (34/52)	83 (63/99)
NaCl	2	124 (108/137)	25 (21/27)	1725 (1519/2128)	173 (156/224)	5.6 (4.5/6.5)	46 (39/52)	90 (74/93)
PGI_2	2	115 (78/126)	24 (21/25)	1699 (1408/2246)	216 (150/237)	4.6 (3.3/5.3)	42 (39/51)	82 (69/108)
NaCl	4	106 (93/130)	25 (21/27)	1710 (1027/1960)	141 (136/209)	5.8 (4.9/7.3)	52 (43/58)	101 (76/124)
PGI_2	4	119 (89/130)	25 (23/29)	1356 (1232/1715)	210 (132/233)	5.7 (4.4/6.9)	42 (38/51)	88 (75/116)
NaCl	6	111 (86/123)	26 (21/27)	1798 (1040/1851)	142 (113/215)	5.5 (5.0/6.5)	47 (38/51)	96 (82/109)
PGI_2	6	107 (90/124)	22.9 (22/29)	1233 (1089/2040)	147 (100/247)	5.1 (3.5/6.2)	47 (40/49)	78 (63/180)
NaCl	8	108* (86/115)	26 (23/29)	1465 (1046/1833)	189 (113/211)	5.4 (4.4/6.4)	49 (44/54)	92 (83/115)
PGI_2	8	106 (96/130)	24 (24/27)	1234 (1192/2406)	171 (123/227)	6.1 (3.3/6.4)	47 (41/52)	87 (54/142)

*$p < 0.05$; baseline values vs after 8-h inhalation; $n = 14$

Table 2 Lung function during 8-h inhalation. Values are median (1st quartile/3rd quartile). P_aO_2 Arterial partial pressure of oxygen, P_aCO_2 arterial partial pressure of carbon dioxide, *A-aDO*$_2$ alveolar-arterial oxygen difference, pH_a arterial pH value, Q_SQ_t intrapulmonary shunt flow, P_{peak} peak inspiratory airway pressure, C_{tot} total thoracic compliance. $n = 14$

Group	Inhalation time (h)	P_aO_2 (kPa)	P_aCO_2 (kPa)	A-aDO_2 (kPa)	pH_a	Q_sQ_t (%)	P_{peak} (cmH_2O)	C_{tot} (ml/cmH_2O)
NaCl	0	25.1 (18.3/25.6)	5.2 (4.3/6)	17.3 (15.7/24.5)	7.44 (7.36/7.46)	21 (13/29)	27 (23/31)	26 (23/34)
PGI_2	0	24.5 (18.1/24.7)	5.3 (4.3/6.7)	18.8 (15.3/24.1)	7.34 (7.31/7.45)	21 (19/24)	26 (22/28)	36 (29/44)
NaCl	2	27.3 (20.3/29.7)	5.3 (4.3/5.7)	14.5 (13.2/22.4)	7.41 (7.33/7.49)		24 (24/27)	37 (26/44)
PGI_2	2	27.2 (26.1/28.7)	5.2 (4.7/5.7)	14.4 (14/16)	7.36 (7.30/7.46)		24 (23/27)	36 (31/49)
NaCl	4	28.5 (23.9/29.5)	4.7 (4.3/5.6)	14.3 (12.7/19.2)	7.41 (7.35/7.44)	17 (12/23)	26 (23/27)	32 (24/38)
PGI_2	4	27.7 (22.3/27.9)	5.3 (5.1/5.5)	15.5 (13.1/19.7)	7.34 (7.29/7.39)	18 (17/31)	26 (24/28)	35 (29/40)
NaCl	6	27.3 (15.7/27.7)	4.8 (4/5.5)	14.7 (14/27.7)	7.37 (7.33/7.46)		24 (23/27)	34 (24/39)
PGI_2	6	25.2 (24.8/27.3)	5.2 (4.4/5.3)	16.9 (14.7/18.3)	7.29 (7.28/7.37)		25 (23/30)	31 (30/38)
NaCl	8	27.6 (17.2/28.7)	5.3 (4/6)	14.0 (13.2/26.3)	7.39 (7.32/7.44)	18 (11/26)	26 (24/27)	32 (24/44)
PGI_2	8	25.3 (21.6/27.6)	5.2 (5.1/5.7)	16.3 (14.1/20.5)	7.33 (7.29/7.36)	20 (16/35)	26 (24/30)	33 (28/35)

used healthy lambs to ensure that changes observed during long-term inhalation were not confounded by underlying disease or by the experimental model of pulmonary hypertension.

The evaluation of potential acute toxic effects of PGI_2 aerosol focused on two aspects: detection of pharmacological side effects of the vasoactive substance PGI_2 itself and detection of potential tissue damage caused by the alkaline PGI_2 aerosol.

Table 3 Cell viability and counts in BAL samples obtained from lungs before and after the inhalation period. Values are median (1st quartile/3rd quartile). *PMNL* Polymorphonuclear neutrophil leukocytes. $n = 14$

Group	Cell viability (%)	Total cell count ($\cdot 10^5$)	Differential cell count		
			PMNL (%)	Alveolar macrophages (%)	Lymphocytes (%)
Before inhalation					
NaCl	81 (69/91)	60 (38/100)	2 (1/12)	88 (84/94)	4 (2/6)
PGI_2	74 (51/74)	120 (93/180)	2 (1/8)	88 (83/89)	6 (4/10)
After inhalation					
NaCl	75 (66/86)	40 (24/64)	4 (2/8)	87 (71/95)	3 (3/5)
PGI_2	76 (63/81)	116 (15/168)	13 (5/20)	69 (66/79)	6 (3/8)

Table 4 Global biochemical composition in BAL samples obtained from lungs before and after the inhalation period. Values are median (1st quartile/3rd quartile). *ELF* Epithelial lining fluid, *LDH* lactate dehydrogenase, *AP* alkaline phosphatase; $n = 14$. Data are adjusted to ELF as described in material and methods

Group	ELF-LDH (U/l)	ELF-AP (U/l)	ELF-protein (mg/ml)	ELF-fibronectin (ng/ml)
Before inhalation				
NaCl	2185 (1505/3009)	46 (37/352)	4.8 (3.3/9.7)	41.8 (14.9/61.6)
PGI_2	917 (764/2034)	172 (153/480)	9.3 (2.9/21.7)	69.3 (47.8/220.7)
After inhalation				
NaCl	725 (379/2669)	132 (67/138)	4.9 (4.4/13.7)	49.8 (34.6/80.6)
PGI_2	794 (447/2785)	164 (88/213)	9.3 (3.4/12.7)	77.5 (40.2/255.1)

Pharmacological side effects

Since PGI_2 is not metabolized in the bronchoalveolar system [23], it may accumulate locally, allowing for uncontrolled systemic resorption, and thus systemic vasodilation. In our study, we chose a median PGI_2 dose of 28 (20/36) ng/kg per min, corresponding to a total of 500 (320/650) µg PGI_2 administered over 8 h. Compared to doses known to reduce pulmonary hypertension in patients (2–50 ng/kg per min) [6–14], this represents a medium dose. The pulmonary deposition fraction of inhaled PGI_2 has not been directly assessed in our experiments. Nevertheless, in three animals with hypoxia-induced pulmonary hypertension, increased PVRI was selectively reduced by the inhaled PGI_2 aerosol, indicating that in our experiments a sufficient amount of PGI_2 must have reached the alveoli.

The absence of significant changes in arterial pressure and systemic vascular resistance indicates that systemic vasodilation did not occur during PGI_2 inhalation. This is in accordance with the absence of the non-enzymatically formed PGI_2 metabolite 6-keto-PGF 1α in plasma, ruling out significant resorption of inhaled PGI_2. Thus, it was not surprising that important changes in in vitro bleeding time and collagen-induced platelet aggregation were not observed after 8 h of PGI_2 inhalation. In the two studies suggesting impaired platelet function in humans after inhalation of PGI_2, the dose of inhaled PGI_2 was higher than in our protocol: 250±50 µg inhaled over 5 min [15], 400 µg over 3 min and 200 µg over 15 to 20 s [16]. In both studies a decrease in systemic vascular resistance occurred during inhalation, suggesting that systemic resorption of PGI_2 caused impairment of platelet function. It should be noted that the plasma concentration of 6-keto-PGF 1α was not assessed in these studies.

Other investigators [17, 18] have reported mild deterioration of pre-existing bronchospasm after the inhalation of similar high doses of PGI_2 (up to 500 µg within 5 min) in asthmatic patients. Possible reasons for such effects might be airway narrowing through mucosal engorgement due to vasodilation [18], or vagal C-fiber triggered reflex responses of airway smooth muscles [24]. In our study, at constant ventilator settings (T_{ins}, V_T and respiratory rate) no changes in peak and plateau airway pressures were recorded during PGI_2 inhalation, indicating that there was no major bronchoconstrictor effect from the PGI_2 aerosol.

Since both arterial PO_2 and alveolar-arterial oxygen gradient remained unchanged during PGI_2 inhalation, serious deterioration in pulmonary function can be ex-

cluded. The intrapulmonary shunt fraction was not influenced by PGI_2 inhalation, indicating the absence of important ventilation–perfusion mismatches.

In summary, we observed no significant changes in cardiorespiratory parameters after 8 h of PGI_2 inhalation. The inhaled PGI_2 was not systemically absorbed from the respiratory tract. Platelet function remained unchanged.

Tissue damage

Changes in both biochemical and cellular ELF composition and in the morphology of the respiratory system have been described after exposure of different species to inhaled materials such as metal salts [25], alpha quartz [26] or various kinds of smoke [27].

BAL is a well-established technique for evaluating the cellular and molecular components of ELF of the alveoli [28]. In our model, we used urea as a marker of dilution to quantify the ELF volume. This method is based on the fact that urea is freely diffusable through most body compartments including the lung. Thus, the concentration of urea in ELF is assumed to be equal to that in plasma [29, 30] and the total volume of ELF can be calculated from a simple dilution principle [21]. Once this volume is known, any cellular and biochemical BAL component can be referenced to it. The major drawback of this method is that irregular dwell times of the lavage fluid lead to an irregular diffusion of plasma urea into the fluid and therefore to different values for calculated ELF volumes. To be able to compare cellular and acellular concentrations in ELF, our lavage protocol was standardized, each cycle (50 ml aliquot) being limited to exactly 55 s, keeping dwell time during each lavage procedure constant and minimizing the differences in urea diffusion between the measurements. Moreover, determining the effects of the PGI_2 aerosol on the biochemical composition of ELF does not require the determination of absolute values of these components but the comparison of changes in concentration between the time-points "before" and "after 8 hours of inhalation." This was achieved by standardizing the BAL procedure.

The lack of statistically significant differences between the two groups of animals indicates the absence of acute toxic effects from the nebulized PGI_2 solution on the biochemical and cellular composition of ELF. Lactate dehydrogenase and alkaline phosphatase, two main markers of nonspecific cell injury and death [31], did not increase significantly in the PGI_2 group, nor did fibronectin, a serum opsonin and cell surface protein, which has been shown to be synthesized in the alveolus in tissue injury [31]. Stable ELF protein concentrations indicated that there was no major edema formation in the alveolar region [26]. The differential cell count showed no statistically significant changes in any cell species in either group or between the two groups. The slight increase in the median percentage of polymorphonuclear leukocytes in both groups can be explained by the normal neutrophil recruitment after BAL, which has been described elsewhere [32].

In summary, in healthy lambs, 8-h inhalation of PGI_2 in a clinically relevant dose did not elicit cardiorespiratory effects and produced no signs of acute pulmonary toxicity. The absence of systemic resorption of PGI_2 explains the lack of major changes in platelet function. Inhalation of PGI_2 over a time period of 8 h therefore appears safe in lambs and might be applied without harm to patients.

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