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Zardaverine and aerosolised iloprost in a model of acute respiratory failure

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ABSTRACT: In this study, the impact of aerosolised prostacyclin (PGI_2) and iloprost in the absence or presence of subthreshold intravascular doses of the dual-selective phosphodiesterase-3/4 inhibitor zardaverine was investigated in an experimental model of acute respiratory failure.

In perfused rabbit lungs, continuous infusion of the thromboxane-A_2-mimetic U46619 provoked pulmonary hypertension, accompanied by progressive lung oedema formation and severe ventilation-perfusion mismatch with predominance of shunt flow (increasing from ~2 to 58%, as assessed by the multiple inert gas elimination technique). Aerosolisation of PGI_2 (in total 1.05 $\mu g \cdot kg^{-1}$) for 15 min caused a decrease in pulmonary artery pressure (P_{pa}) and a limitation of maximum shunt flow to ~37%. When nebulised PGI_2 was combined with subthreshold intravascular zardaverine, which did not affect pulmonary haemodynamics *per se*, the duration of the PGI_2 effect was increased. Aerosolisation of 3 $\mu g \cdot kg^{-1}$ PGI_2 resulted in a transient decrease in Ppa and a reduction in shunt flow. In the presence of subthreshold zardaverine, the effects of this PGI_2 dose were only marginally increased. Aerosolisation of iloprost (in total 0.7 $\mu g \cdot kg^{-1}$) for 15 min caused a more sustained decrease in P_{pa}, some enhanced reduction of oedema formation as compared with PGI_2 and a decrease in shunt flow to ~32%. Most impressively, when combined with subthreshold zardaverine, iloprost suppressed oedema formation to <15% and shunt flow to ~8%.

In conclusion, combined use of aerosolised iloprost and subthreshold systemic phosphodiesterase-3/4 inhibitor may result in selective intrapulmonary vasodilation, a reduction in oedema formation and an improvement in ventilation-perfusion matching in acute respiratory failure.

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Increased pulmonary artery pressure (Ppa), lung microvascular leakage and ventilation-perfusion mismatch with predominance of shunt flow represent the key pathophysiological events of acute respiratory distress syndrome (ARDS) in adults [1, 2]. However, intravenous vasodilator administration, such as infusion of prostanoids, may reduce pulmonary vascular pressure at the expense of an increase in shunt flow and thereby a decrease in arterial oxygenation due to interference with hypoxic pulmonary vasoconstriction [3, 4]. Conversely, almitrine, an agent that enhances the hypoxic pulmonary vasoconstriction, improves arterial oxygenation but at the same time increases Ppa and may provoke right ventricular failure [5–7].

Inhalation of nitric oxide [8] and aerosolisation of prostacyclin (PGI_2) [3, 9] have both been suggested as alternatives to help avoid the problems described above. As both agents are distributed *via* air flow, they cause selective or preferential vasodilation in well-ventilated lung regions, with a redistribution of blood flow to these areas and a subsequent improvement in ventilation-perfusion matching. Indeed, in ARDS patients, both approaches have been shown to decrease Ppa and improve arterial oxygenation due to a reduction of shunt flow. However, due to the short half-life of both agents, continuous inhalative administration is mandatory for maintenance of this effect. Therefore, the stable PGI_2 analogue iloprost may represent an interesting alternative to PGI_2, as it is stable in aqueous solution and has a >10-fold longer half-life [10, 11]. Indeed, when applied *via* the inhalative route in patients with severe chronic pulmonary hypertension, one short-term aerosolisation manoeuvre of iloprost was found to cause a pulmonary vasodilatory response lasting for 30–90 min [12, 13].

Another strategy to prolong the pulmonary vasodilatory effect of inhaled prostanoids may be the co-administration of phosphodiesterase (PDE)-inhibitors [14, 15]. Different PDE isoenzymes regulate the intracellular levels of the nucleotides cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) [16, 17]. The PDE families 1, 3, 4 and 5 have been identified in human pulmonary artery tissue [18]. These isoenzymes differ in their substrates. PDE-3 hydrolyses cAMP and cGMP, usually with a higher affinity for cAMP [16, 17], and PDE-4 enzymes are characterised by their high affinity for cAMP. PDE-3 and -4 are therefore particularly important in the regulation of cAMP levels in the

pulmonary vasculature and their inhibition may thus have a major impact on the half-life of prostanoid effects in the lung circulation. In the present study, a dual-selective PDE-3/4 inhibitor, zardaverine, was employed to investigate this in a model of acute pulmonary hypertension, oedema formation and respiratory failure in perfused rabbit lungs, induced by infusion of the stable thromboxane (TX)A_2-mimetic U46619. The combination of subthreshold systemic doses of zardaverine with short-term iloprost inhalation was found to be most effective at achieving prolonged pulmonary vasodilation with markedly reduced shunt flow and oedema formation.

Methods

Isolated lung model

The perfused lung model has been described previously in detail [19]. Briefly, rabbits of either sex, weighing 2.2–2.9 kg, were anticoagulated with heparin (1000 $U \cdot kg^{-1}$) and anaesthetised with *i.v.* ketamine (Pharmacia and Upjohn, Erlangen, Germany)/xylazine (Bayer, Leverkusen, Germany). Tracheotomy was performed and the animals were ventilated with room air *via* a Harvard respirator (tidal volume 9–13 $mL \cdot kg^{-1}$, frequency 10 $breaths \cdot min^{-1}$, positive end-expiratory pressure 1 mmHg; Hugo Sachs Elektronik, March Hugstetten, Germany). After midsternal thoracotomy, catheters were placed into the pulmonary artery and left atrium, and they were perfused with sterile Krebs-Henseleit hydroxyethylamylopectine buffer (120 mM NaCl, 4.3 mM KCl, 1.1 mM KH_2PO_4, 23 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 1.3 mM $MgPO_4$, 2.4 $g \cdot L^{-1}$ glucose and 5% (weight/volume) hydroxyethylamylopectine (mol weight 200,000; Serag Wiesner, Naila, Germany) as an oncotic agent). The lungs were perfused at a constant flow rate of 120 $mL \cdot min^{-1}$. Left atrial pressure was set at 1.2 mmHg in all experiments and room air, supplemented with 4% carbon dioxide, was used for ventilation during artificial perfusion. Lungs were freely suspended from a force transducer so that organ weight could be monitored. P_{pa}, and pressure in the left atrium and trachea were also measured (zero point at the hilum). Perfusate samples (total perfusate volume 500 mL) were taken from both the arterial and venous parts of the system. Gas samples were taken from the outlet of an expiration gas mixing box. The whole system was heated to 37°C.

Aerosolisation

PGI_2 (Flolan®; Wellcome, London, UK) and iloprost (Ilomedin®; Schering AG, Berlin, Germany) were aerosolised with an ultrasonic device (Pulmo Sonic 5500; DeVilbiss Medizinische Produkte GmbH, Langen, Germany). The nebuliser produces an aerosol with a mass median aerodynamic diameter of 4.5 μm and a geometric SD of 2.6, as measured with a laser diffractometer (HELOS; Sympatec, Clausthal-Zellerfeld, Germany). The nebuliser was located between the ventilator and the lung, so that the inspiration gas would pass through it. The nebulisation system has been described previously [20]. For a given ventilator setting, an absolute deposition fraction of 0.25±0.02 was determined by laser photometric technique [21].

Ventilation-perfusion determination in isolated lungs

The ventilation-perfusion (V'_A/Q') distributions were determined by the multiple inert gas elimination technique as described by WAGNER *et al.* [22]. This technique has been adapted to blood-free perfused rabbit lungs [20]. An indication of an acceptable V'_A/Q' distribution is a residual sum of squares (RSS) of $\leqslant 5.348$ in half of the experimental runs (50th percentile) or $\leqslant 10.645$ in 90% of the experimental runs (90th percentile) [23]. In the present study 68.5% of RSS were <5.348 and 97.3% were <10.645.

Experimental protocols

As described previously [15, 24], a sustained increase in P_{pa} from ~8 to 34 mmHg was achieved by continuous infusion of 70–160 $pmol \cdot kg^{-1} \cdot min^{-1}$ of U46619 (Paesel-Lorei, Frankfurt, Germany). Individual titration was performed.

The efficacy of the dual 3/4 PDE inhibitor zardaverine (Altana Pharma, Constance, Germany) was assessed in dose/response curves. The PDE inhibitor was bolus injected into the recirculating buffer fluid. In separate experiments, a subthreshold dose of zardaverine, which was found to cause no changes in haemodynamic parameters, lung weight gain or ventilation/perfusion parameters over an observation period of 150 min, was followed by aerosolisation of PGI_2 or iloprost. The experimental groups were as follows.

1) Control lungs (n=6): after termination of the steady-state period, V'_A/Q' measurements were performed at 30, 45, 60, 90, 120 and 150 min; no interventions were undertaken.

2) U46619 lungs (n=6): after termination of the steady-state period, U46619 was continuously infused over 150 min to provoke an increase of P_{pa} to ~34 mmHg; V'_A/Q' measurements were performed at 30, 45, 60, 90, 120 and 150 min after initiation of U46619 infusion.

3) Dose/response curve for zardaverine (n=4): after establishing stable pulmonary hypertension *via* U46619 infusion, as described above, increasing doses of the PDE inhibitor, zardaverine, were added to the recirculating buffer fluid in an incremental manner (0.2, 2 and 20 μM).

4) PGI_2 inhalation (n=6, low dose): 30 min after the initiation of U46619 infusion, PGI_2 (~70 $ng \cdot kg^{-1} \cdot min^{-1}$) was aerosolised for 15 min; V'_A/Q' measurements were performed at 30, 45, 60, 90, 120 and 150 min.

5) PGI_2 inhalation (n=6, high dose): 30 min after the initiation of U46619 infusion, PGI_2 (~200 $ng \cdot kg^{-1} \cdot min^{-1}$) was aerosolised for 15 min; V'_A/Q' measurements were performed at 30, 45, 60, 90, 120 and 150 min.

6) Iloprost inhalation (n=6): 30 min after the initiation of U46619 infusion, iloprost (~70 $ng \cdot kg^{-1} \cdot min^{-1}$) was aerosolised for 15 min; V'_A/Q' measurements were performed at 30, 45, 60, 90, 120 and 150 min.

7) PGI_2 inhalation combined with zardaverine (n=6, low dose): 30 min after the initiation of U46619 infusion, the subthreshold dose of 0.2 μM zardaverine was added to the recirculating buffer fluid and PGI_2 (~70 $ng \cdot kg^{-1} \cdot min^{-1}$) was aerosolised for 15 min; V'_A/Q' measurements were performed at 30, 45, 60, 90, 120 and 150 min.

8) PGI_2 inhalation combined with zardaverine (n=6, high dose): 30 min after the initiation of U46619 infusion, zardaverine (0.2 μM) was added to the recirculating buffer fluid and PGI_2 (~200 $ng \cdot kg^{-1} \cdot min^{-1}$) was aerosolised for 15 min; V'_A/Q' measurements were performed at 30, 45, 60, 90, 120 and 150 min.

9) Iloprost inhalation combined with zardaverine (n=6): after establishing stable pulmonary hypertension, zardaverine was added to the buffer fluid (0.2 μM) and iloprost (~70 $ng \cdot kg^{-1} \cdot min^{-1}$) was aerosolised for 15 min; V'_A/Q' measurements were performed at 30, 45, 60, 90, 120 and 150 min.

Data analysis

All values are presented as mean±SEM. For comparison of statistical differences between groups, two-factorial analysis of variance (factors: inhaled prostanoid and *i.v.* zardaverine) with the Bonferroni correction was performed. Comparisons of one time-point after the application of the inhaled prostanoid (45 min), as well as comparisons of the end-points for the shunt flow, weight gain, normal $V'A/Q'$ and the area under the curve (AUC), were performed. Significance was assumed when $p \leqslant 0.05$.

Results

Baseline conditions

After termination of the steady-state period, all lungs displayed Ppa values of 7–10 mmHg. Baseline $V'A/Q'$ measurements revealed a unimodal narrow distribution of perfusion and ventilation to midrange $V'A/Q'$ ($0.1<V'A/Q'<10$) areas throughout the lung (table 1). Shunt flow ($V'A/Q'<0.005$) and perfusion flow to poorly ventilated areas ($0.005<V'A/Q'<0.1$) were extremely low, and there was no perfusion flow to high $V'A/Q'$ regions ($10<V'A/Q'<100$). Dead space ($V'A/Q'>100$) was 48.6±3.6% at the beginning and 50.3±3.6% at the end of the experiments.

U46619-induced pulmonary hypertension and gas exchange abnormalities

Continuous infusion of U46619 provoked an increase in Ppa to 33.6±1 mmHg within 15 min, followed by a plateau (figs 1 and 2). The rise in Ppa was accompanied by a progressive increase in shunt flow to 58.4±5.8% of total perfusion flow after 150 min (table 1, figs 3 and 4), with a concomitant decrease in perfusion of normal $V'A/Q'$ areas. Dead space increased from 55.3 to 62.5%. Marked broadening of the flow dispersion (Log SDQ') and ventilation distribution (Log SD$V'A$) in the midrange $V'A/Q'$ regions was noted under these conditions (not shown in detail). Lung weight increased continuously, with a total weight gain at the end of experiments of 17.1±2.2 g.

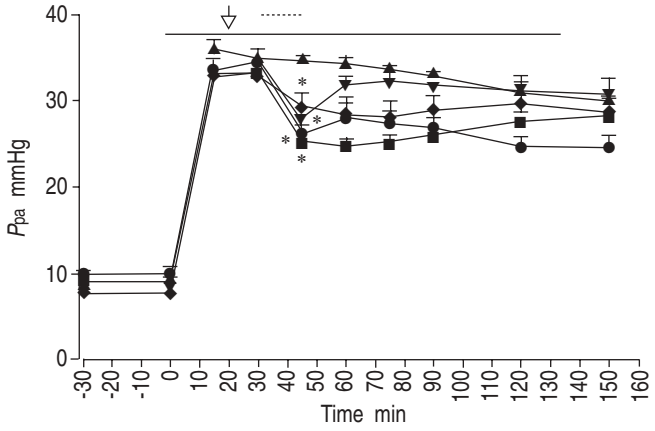

Fig. 1. – Influence of prostacyclin (PGI_2; ----) nebulisation with and without prior intravascular administration of subthreshold doses of zardaverine (arrow) on U46619 (—)-elicited pulmonary hypertension. Ppa: pulmonary artery pressure. ▲: U46619 alone; ▼: U46619 and PGI_2 (low dose); ◆: U46619, PGI_2 (low dose) and zardaverine; ●: U46619 and PGI_2 (high dose); ■: U46619, PGI_2 (high dose) and zardaverine. Data are presented as mean+SEM of six independent experiments. *: $p<0.05$ as compared with U46619 alone.

Dose/response curves for zardaverine

Increasing doses of 0.2, 2 and 20 μM zardaverine were administered under conditions of stable U46619-induced pulmonary hypertension. The 0.2 μM dose did not cause a significant alteration in Ppa, whereas 2 μM and 20 μM caused a dose-dependant Ppa decline (fig. 5).

Nebulisation of prostacyclin (low dose)

Inhalation of 70 $ng \cdot kg^{-1} \cdot min^{-1}$ PGI_2 for 15 min resulted in a significant decrease in U46619-induced pulmonary hypertension, with Ppa values decreasing by a maximum of 6.5 mmHg (~19.5%; fig. 1). Immediately after stopping the aerosol application, Ppa started to rise again and prenebulisation values of Ppa were reached within 15 min. The development of intrapulmonary shunt flow was moderately lowered to 37.8% in response to PGI_2 aerosolisation (fig. 3). The calculated AUC was 13.0±3.4 $mmHg \cdot min^{-1}$ (fig. 6). Total lung weight gain was 15.4±1.4 g.

Table 1. – Gas exchange variables U46619-induced pulmonary hypertension: response to prostacyclin (PGI_2) or iloprost inhalation

	Shunt %Q' $V'A/Q'<0.005$		Normal $V'A/Q'$ %Q' $0.1<V'A/Q'<10$		Weight g
	0 min	150 min	0 min	150 min	150 min
Control	1.5±0.5	1.8±0.9	98.5±1.6	98.2±0.9	<2
U46619	2.5±1.6	56.5±6.2	97.5±1.6	42.0±7.8	17.1±2.2
U46619/PGI_2 low	1.8±0.5	37.8±3.6*	98.2±0.6	61.6±3.2*	15.4±1.4
U46619/PGI_2 low/zarda.	1.4±0.3	35.6±4.0*	98.6±0.3	62.4±4.8*	14.3±1.3
U46619/PGI_2 high	1.5±0.5	28.2±5.2*	98.5±0.6	65.6±6.2*	12.1±0.4
U46619/PGI_2 high/zarda.	1.1±0.3	33.6±7.8*	98.9±0.3	66.4±7.8*	8.6±1.8
U46619/zarda.	2.7±0.6	54.9±3.9	97.2±0.7	42.8±4.0	14.6±1.7
U46619/ilo.	1.2±0.6	31.9±8.6*	98.2±0.6	63.4±6.8*	10.8±2.1
U46619/ilo./zarda.	2.0±1.3	8.0±2.4*,#,¶	98±1.4	90.3±2.1*,#,¶	2.3±1.0*,#,¶

Data are presented as mean±SEM. All data were obtained by multiple inert gas elimination technique. Q': perfusion; $V'A$: alveolar ventilation; weight: weight gain at the end of the experiments; PGI_2 low: 70 $ng \cdot kg^{-1}$ min PGI_2 nebulisation; PGI_2 high: 200 $ng \cdot kg^{-1}$ min PGI_2 nebulisation; zarda.: 0.2 μM zardaverine; ilo.: 70 $ng \cdot kg^{-1}$ min iloprost nebulisation. *: $p<0.05$ as compared with U46619 alone; #: $p<0.05$ as compared with U46619 and iloprost; ¶: $p<0.05$ as compared with U46619 and PGI_2.

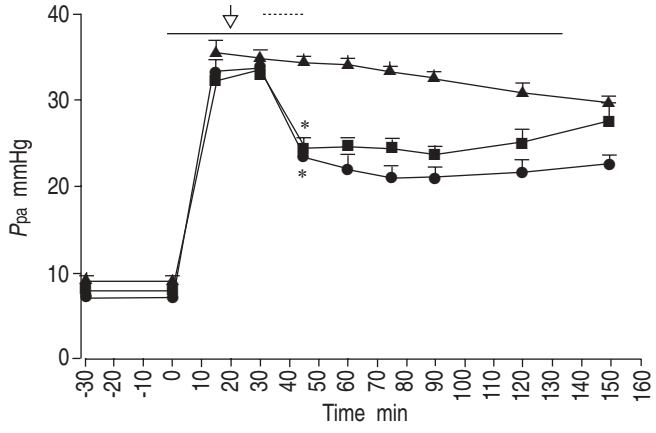

Fig. 2. – Influence of iloprost (----) nebulisation with and without prior intravascular administration of subthreshold doses of zardaverine (arrow) on U46619 (—)-elicited pulmonary hypertension. P_{pa}: pulmonary artery pressure. ▲: U46619 alone; ■: U46619 and iloprost; ●: U46619, iloprost and zardaverine. Data are presented as mean+SEM of six independent experiments. *: $p<0.05$ as compared with U46619 alone.

Nebulisation of prostacyclin (high dose)

Inhalation of aerosolised PGI_2 (200 $ng \cdot kg^{-1} \cdot min^{-1}$) for 15 min resulted in a significant reduction of U46619-induced pulmonary hypertension, with P_{pa} values decreasing by a maximum of 9.2 mmHg (~28%; fig. 1). After stopping the nebulisation, some minor rise of P_{pa} was noted. In addition, aerosolised PGI_2 caused a significant reduction in shunt flow as compared with the nontreated U46619 controls (28.2±5.2% of total perfusion flow after 150 min), with higher percentages of perfusion being distributed to normal V'_A/Q' areas. Dead space was 62.6% at the end of the experiments and an AUC of 40.9±5.6 $mmHg \cdot min^{-1}$ was calculated. The total weight gain was 12.1±0.4 g.

Nebulisation of iloprost

As depicted in figure 2, inhalation of iloprost resulted in a significant decrease in P_{pa} of 9.9 mmHg (28.8%). The P_{pa} values did not fully return to the prenebulisation level within 75 min. Shunt flow was markedly reduced in the iloprost-treated lungs and perfusion of normal V'_A/Q' areas was preserved accordingly. The calculated AUC was 49.4±3.7 $mmHg \cdot min^{-1}$. Total lung weight gain was 10.8±2.1 g.

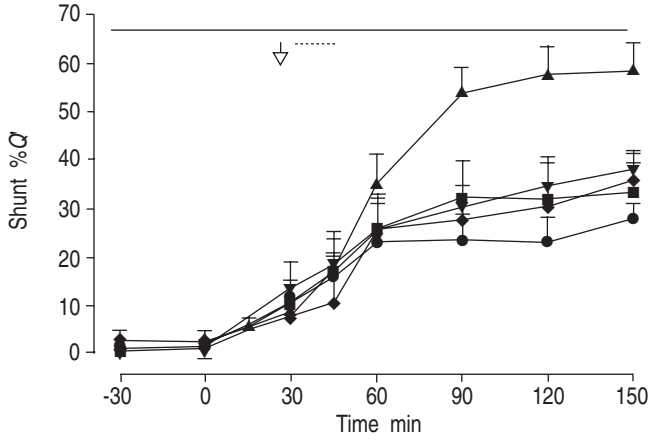

Fig. 3. – Influence of prostacyclin (PGI_2; ----) nebulisation and in combination with subthreshold doses of zardaverine (arrow) on U46619 (—)-induced intrapulmonary shunt flow. The shunt flow gives the percentage of perfusion (Q') of nonventilated areas (ventilation/perfusion ratio <0.005). ▲: U46619 alone; ▼: U46619 and PGI_2 (low dose); ◆: U46619, PGI_2 (low dose) and zardaverine; ●: U46619 and PGI_2 (high dose); ■: U46619, PGI_2 (high dose) and zardaverine. Data are presented as mean+SEM of six independent experiments.

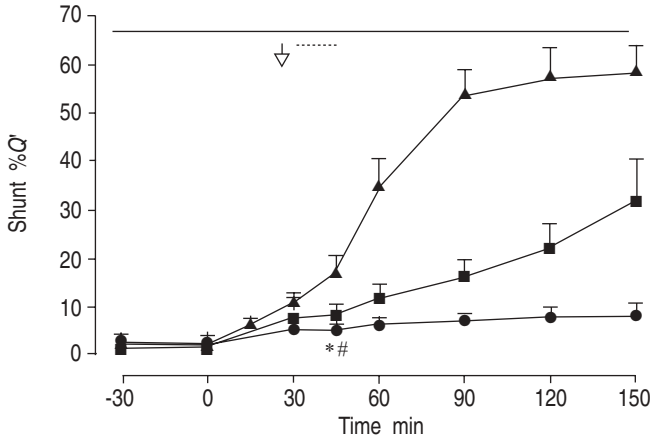

Fig. 4. – Influence of iloprost (----) nebulisation and in combination with subthreshold doses of zardaverine (arrow) on U46619 (—)-induced intrapulmonary shunt flow. The shunt flow gives the percentage of perfusion (Q') of nonventilated areas (ventilation/perfusion ratio <0.005). ▲: U46619 alone; ■: U46619 and iloprost; ●: U46619, iloprost and zardaverine. Data are presented as mean+SEM of six independent experiments. *: $p<0.05$, as compared with U46619 infusion; #: $p<0.05$, as compared with prostacyclin (PGI_2) inhalation (low dose).

Combined subthreshold application of zardaverine and inhaled prostacyclin (low dose)

A significant prolongation of the PGI_2-induced P_{pa} decline was measured in the presence of zardaverine. The AUC increased from 13.0±3.4 to 27.8±4.3 $mmHg \cdot min^{-1}$. As compared with the PGI_2 group, no significant changes in shunt flow (35.6±4.0%) and perfusion of normal V'_A/Q' areas (62.4±4.8%) were measured. Weight gain was 14.3±1.3 g at the end of the perfusion period.

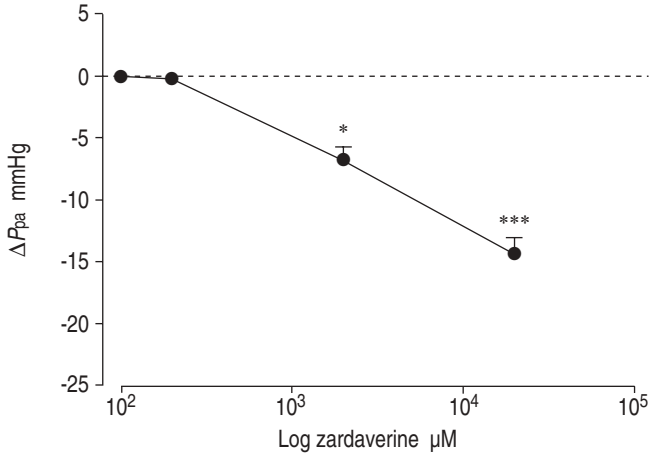

Fig. 5. – Dose/effect curves of intravascular cumulative doses of zardaverine in 20-min time steps on U46619-elicted pulmonary hypertension. The relative decrease in pulmonary artery pressure (P_{pa}) in response to each phosphodiesterase dose is given (mean+SEM, n=4). *: $p<0.05$; ***: $p<0.001$.

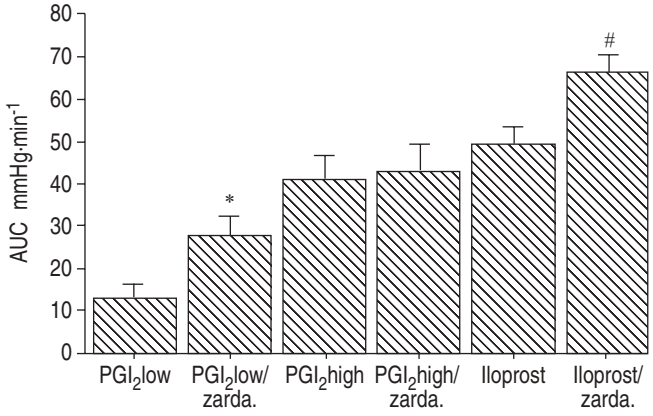

Fig. 6. – Influence of prostacyclin (PGI_2) and iloprost nebulisation with and without prior intravascular administration of subthreshold doses of zardaverine (zarda.) on the area under the curve (AUC) of the pressure response. Measurements were performed from onset of PGI_2 or iloprost nebulisation until 120 min post-aerosolisation. Data are presented as mean+SEM of six independent experiments. *: $p<0.05$, as compared with low-dose PGI_2 inhalation; #: $p<0.05$, as compared with iloprost inhalation.

Combined subthreshold application of zardaverine and inhaled prostacyclin (high dose)

In the presence of zardaverine, *P*pa values decreased to approximately the same extent as observed in the PGI_2 group, but some prolongation of the PGI_2-induced *P*pa decline was noted. Shunt flow increased and perfusion of normal $V'A/Q'$ areas decreased more slowly as compared with the PGI_2 group. As compared with the PGI_2 group, no further increase in AUC was noted (42.5±6.5 mmHg·min^{-1}). The total weight gain was 8.6±1.8 g at the end of the perfusion period. Dead space increased from 45.2 to 63.8% at the end of the observation period.

Combined subthreshold application of zardaverine and inhalation of iloprost

Co-application of subthreshold zardaverine and iloprost aerosol resulted in a decrease in *P*pa of ~12 mmHg (36.7%), which lasted until the end of the perfusion period. In parallel, a far-reaching suppression of shunt increase was noted, with maximum values of shunt flow <10%. Accordingly, perfusion of normal $V'A/Q'$ areas was largely maintained. Development of lung oedema was virtually completely avoided (2.3±1 g; $p<0.05$). As compared with iloprost inhalation alone, a significant increase in AUC was noted (66.5±3.5 mmHg·min^{-1}).

Discussion

Continuous infusion of the TXA_2 mimetic U46619 in isolated rabbit lungs has previously been described to cause predominant precapillary vasoconstriction and severe gas exchange abnormalities, with increased shunt flow and oedema formation [15, 24]. According to these observations, marked pulmonary hypertension, progressive oedema formation and a dramatic increase in shunt flow to >50% was observed in response to the present protocol of U46619 infusion. Short-term inhalation of PGI_2 exerted a rapidly transient pulmonary vasodilatory response, concomitant with some reduction of shunt flow and lung oedema formation. This response profile was only modestly influenced by co-administration of subthreshold doses of zardaverine. In the presence of zardaverine, the duration of the low-dose PGI_2 effect was increased, as shown by the AUC of the vasodilatation. One possible explanation for the persistent effect of the higher dose of PGI_2 is a spill-over of the drug into the recirculating buffer.

Inhaled iloprost was clearly more effective than PGI_2 in decreasing shunt flow. Most impressively, the combination of subthreshold zardaverine and aerosolised iloprost nearly fully blocked the appearance of shunt flow and the development of lung oedema formation, although the overall pulmonary vasodilatory response only slightly surpassed that induced by iloprost alone.

Zardaverine is a dual selective PDE-3/4 inhibitor with median inhibitory concentration values of 0.6 and 0.2 μM, respectively [25]. It has been shown to relax isolated guinea pig tracheas that were precontracted with a variety of spasmogens (*e.g.* histamine, ovalbumin, U46619 and LTC_4) [26]. Furthermore, oral zardaverine (3–30 μmol·kg^{-1}) shows bronchodilator activity in the rat [27]. In a model of isolated rat lungs, zardaverine inhibited low-phase reaction-induced bronchoconstriction and TXA_2 release into the recirculating buffer [28]. However, clinical trials showed the typical side-effects of the first generation PDE-4 inhibitors, *e.g.* nausea and vomiting, and therefore clinical development was discontinued. Against this background, the recent observation that very low doses of zardaverine, which do not exert any haemodynamic effect *per se*, enhance the efficacy of inhaled PGI_2 to cause acute pulmonary vasodilation in intact rabbits with pulmonary hypertension [14] is very interesting. This strategy might thus allow the beneficial effects of this PDE inhibitor on the pulmonary circulation while avoiding disadvantageous systemic effects. Future studies have to address this aspect in more detail. However, the most impressive finding of the present study was the fact that the co-administration of subthreshold zardaverine and inhaled iloprost nearly fully suppressed the gas exchange abnormalities and the lung oedema formation in the U46619 model. Three mechanisms may underlie this cooperative effect between low dose systemic zardaverine and inhaled iloprost, as follows.

1) The combined application of both agents resulted in a reduction in *P*pa and previous studies of the gas exchange abnormalities in the present model have demonstrated that the strength of the pulmonary hypertensive response is correlated with the severity of the $V'A/Q'$ mismatch, and in particular the extent of shunt flow, occurring even before onset of marked lung oedema formation [15, 24].

2) The PDE inhibitor may have its effects by strengthening lung barrier properties and thereby limiting pulmonary oedema formation in combination with aerosolised iloprost. At a dose of 10 μM, zardaverine has been previously reported to decrease oedema formation and endothelial permeability in H_2O_2-challenged isolated rabbit lungs [29]. The potential of zardaverine to act in a synergistic fashion with prostanoids was demonstrated in a porcine pulmonary artery endothelial cell monolayer, where the combined administration of this PDE inhibitor and prostaglandin-E_1, but neither agent alone, completely suppressed H_2O_2-induced leakage [30]. The present study extends these previous observations in showing that even subthreshold systemic doses of zardaverine synergise with inhaled iloprost to protect the vascular barrier function at the "meeting point" of these agents, the pulmonary microcirculation, under conditions of U46619 challenge.

3) The combined administration of infused zardaverine and inhaled iloprost might improve ventilation-perfusion matching *via* selective pulmonary vasodilation in well-ventilated lung areas. This interpretation suggests that combining aerosol-driven distribution of the vasodilatory prostanoid with a

subthreshold systemic PDE inhibitor for second messenger stabilisation is an efficient approach to restrict the vasodilatory response to aerosol-accessible, *i.e.* well-ventilated, lung areas, with preferred distribution of flow to these lung regions. This view is supported by the multiple inert gas elimination technique data, demonstrating enhanced perfusion of normal $V'A/Q'$ regions in parallel with reduced perfusion of shunt areas.

In conclusion, in a model of U46619-induced acute respiratory failure with pulmonary hypertension, progressive oedema formation and a dramatic increase in shunt flow, short-term inhalation of iloprost was noted to be more effective than inhalation of prostacyclin in limiting these abnormalities. Whereas the response profile to aerosolised prostacyclin was only marginally influenced by co-administration of subthreshold doses of intravascular zardaverine, the phosphodiesterase inhibitor strongly amplified the effects of iloprost. Combined use of aerosolised iloprost and subthreshold systemic phosphodiesterase-3/4 inhibitor may thus offer provide selective pulmonary vasodilation, reduction of oedema formation and improvement of ventilation-perfusion matching in acute respiratory failure.

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