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Effects of nebulised iloprost on pulmonary function and gas exchange in severe pulmonary hypertension

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Summary Nebulised iloprost is established therapy of severe pulmonary hypertension; however, the effects on the bronchoalveolar compartment have not been investigated so far. We studied the short- and long-term effects of nebulised iloprost on pulmonary function tests and gas exchange in 63 patients with severe pulmonary hypertension (idiopathic $n = 17$, chronic thromboembolism $n = 15$, connective tissue disease $n = 12$, congenital heart disease $n = 11$, respiratory diseases $n = 8$). Patients received iloprost in increasing dose up to 140 μg iloprost/24 h via an ultrasonic nebuliser.

Short-term effects were assessed before and after every nebulisation: peak expiration flow decreased in mean by 1.9% (423 ± 98 to 415 ± 98) and percutaneous oxygen saturation increased in mean by 0.7% (90 ± 6 to 91 ± 5) post-nebulisation. There were no significant differences concerning underlying diagnosis or dose of nebulised iloprost. Within 3 months, 9 patients stopped treatment due to non-compliance with frequent nebulisations ($n = 3$), or severe side effects ($n = 4$); 2 patients with additional obstructive lung disease developed bronchoconstriction.

Long-term effects were assessed by pulmonary function tests and gas exchange parameters at baseline and after 3 months treatment. There were no significant differences after 3 months therapy neither in FEV_1, FVC, TLC, residual volume nor in diffusions capacity, SO_2 at rest and during 6 min walking test, also in respect of the underlying diseases. However, there was a significant increase in 6 min walking distance (6 MWD) after 3 months (246 ± 113 to 294 ± 115 m, $P < 0.05$).

In conclusion, treatment with nebulised iloprost leads to functional improvement in severe pulmonary hypertension without systematic adverse short- and long-term effects on pulmonary function test or gas exchange. Patients with additional

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obstructive lung disease might develop bronchoconstriction. Severe side effects leading to discontinuation of treatment occurred in 9% of patients.

Introduction

Nebulised iloprost is established in the therapy of pulmonary hypertension of different origin leading to a significant symptomatic, functional and haemodynamic improvement.[1] The nebulised application of prostanoid has the advantage of pulmonary selectivity leading to fewer systemic side effects and improved ventilation–perfusion ratio in the lung, in particular important in patients with underlying pulmonary parenchymal disorder.[2,3] However, possible intrabronchial effects during nebulisation have not been investigated so far. Patients with pulmonary hypertension have an impaired gas exchange at rest and during exercise leading to an impaired oxygenation.[4] Furthermore, bronchial changes with peripheral airways obstruction have been described in patients with primary pulmonary hypertension.[5]

Previous in vitro and in vivo studies of prostanoids in the bronchial compartment showed variable results. While some authors describe a bronchodilating activity of iloprost and prostacyclin in vivo and in vitro,[6,7] other describe opposite results of inhaled prostacyclin in vivo,[8] and one report described bronchoconstrictive effects of inhaled prostacyclin in asthmatics.[9]

Published studies on treatment with nebulised iloprost, using a range of 37.5–130 μg daily iloprost dose and different nebulisers, reported typical side effect as nausea, cough, flush, chest pain, and jaw pain. However, in none of these studies bronchoalveolar side effects have been further analysed.[1,10–13]

Therefore, we evaluated the short- and long-term effects of nebulised iloprost on pulmonary function tests and pulmonary gas exchange in patients, who were newly commenced on nebulised iloprost therapy for treatment of severe pulmonary hypertension of different origin.

Patients and methods

We included 63 patients (28 male, 37 female, mean age 48 (16–83) years) with severe pulmonary hypertension or different origin. In total, 17 patients suffered from idiopathic pulmonary arterial hypertension (IPAH); 15 patients had chronic thromboembolic pulmonary hypertension (CTEPH), among them 6 patients with proximal vascular obstructions amenable for pulmonary thrombendarterectomy. In 12 patients, pulmonary arterial hypertension has been associated with connective tissue disease (CTD), and in 11 patients pulmonary arterial hypertension developed due to congenital heart disease (CHD). In 8 patients, pulmonary hypertension was associated with respiratory diseases (RD), among them 3 patients with idiopathic pulmonary fibrosis on chronic immuno-modulating therapy requiring long-term oxygen therapy, and 5 patients with COPD and emphysema in GOLD IV due to right heart failure despite treatment with oxygen supplementation, inhaled bronchodilators and diuretics.[14]

Patients underwent pulmonary function tests, 6 min walking test with measurement of percutaneous oxygen saturation, and right heart catheterisation with Swanz-Ganz Catheter including measurement of cardiac output by thermodilution method at baseline and after 3 months therapy. At the follow-up 6 min walking test (6 MWT) and haemodynamic measurements and were performed at least 2 h after last iloprost inhalation.

After baseline examinations, all patients were newly started on treatment with nebulised iloprost using an ultrasonic jet nebuliser (Multisonic compact®, Schill Company, Germany) with an efficacy of 86%.[10]

A dose of 20 μg iloprost was dissolved in 3 mls normal saline. This stock has been further diluted to obtain fluid samples of 3 ml containing approximately 3.3, 6.6, 9.9, 13.2, 16.5 or 20 μg iloprost.

Patients nebulised the iloprost solution over 5–8 min every 3 h with a night time break. The dose was gradually increased from approximately 3.3 μg up to a maximum dose of 20 μg/nebulisation over a period of 1 week in respect to the patients symptoms.

Finally, all patients received 7 nebulisations per day with a total dose of 140 μg/24 h consistent with a mouthpiece dose of 120 μg/24 h.

Short-term effects were assessed using peak expiration flow (PEF) (best of 3 readings) and percutaneous oxygen saturation (SO_2) measured before and after every nebulisation. Results were analysed as the mean change of either PEF or SO_2 after nebulisation in respect to the underlying disease and the dose of iloprost nebulised.

Long-term effects on pulmonary function and gas exchange were assessed by using spirometry and

bodyplethysmography (Jaeger®, Wuerzburg, Germany) with analysis of forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), total lung capacity (TLC), and residual volume (RV). The ratio between RV and TLC has been used as surrogate parameter for small airway function.[15,16]

Values are expressed as percent predicted using the reference values of the European Society for Coal and Steal (ESCS).

Gas exchange was assessed by measurement of diffusion capacity (Keogh factor) using the CO-single breath method corrected for haemoglobin and alveolar volume (Jaeger®, Wuerzburg, Germany). Furthermore, we measured SO_2 at rest and minimum SO_2 during 6 min walking test (6 MWT) as a sensitive parameter for exercise induced disturbance in gas exchange.[17] Results were expressed as mean change after 3 months from baseline and analysed concerning underlying disease.

As data were normally distributed, results are expressed in mean ($\pm$standard deviation). Analysis for statistically significant differences was performed using paired *t*-test.

Results

Short-term effects were analysed in all 65 patients with a mean pre- nebulisation PEF of 423 ± 98 and mean post nebulisation of PEF 415 ± 98 with a mean change of -1.9%. There were no clinically or statistically significant differences concerning the underlying diagnoses or the iloprost dosage (Tables 1 and 2). Mean SO_2 before nebulisation was $90 \pm 6\%$ and after nebulisation $91 \pm 5\%$ with a mean change of +0.7% without clinically or statistically significant differences concerning the underlying diagnoses or the iloprost dosages (Tables 1 and 2).

Among the study population, 23 patients presented with an obstructive pattern in pulmonary function tests displayed by an FEV_1/FVC ratio $<70\%$ according to the GOLD criteria.[14] In these patients, mean PEF decreased by -1.7% from 336 (81) to 331 (84), whereas SO_2 changed by +2.0% from 87 (7) to 89 (5)%. During initiation of therapy the majority of patients experienced typical side effects including flushing, headaches, jaw pain, nausea, and leg cramps, which disappeared during continuous therapy.

Within the first 3 months of treatment, 15 patients stopped treatment with nebulised iloprost, among them 4 patients due to persistent and severe side effects, especially headaches and nausea during initiation phase of treatment despite dose reduction. Three patients were non-compliant with frequent nebulisations. Two patients developed severe dyspnoea and wheezing due to bronchoconstriction after 2 and 4 weeks after

Table 1 Mean PEF before and after nebulisation in respect of the underlying diagnoses in mean (SD).

	PEF (pre)	PEF (post)	Change PEF (%)	SO_2 (pre)	SO_2 (post)	Change SO_2 (%)
Total ($n = 63$)	423 (98)	415 (98)	−1.9	90 (6)	91 (5)	+0.7
IPAH ($n = 17$)	406 (89)	400 (89)	−1.6	92 (5)	92 (6)	+0.6
CTEPH ($n = 15$)	406 (90)	397 (95)	−2.2	93 (2)	92 (2)	−0.9
CTD ($n = 12$)	412 (102)	407 (103)	−1.3	89 (5)	90 (4)	+1.9
CHD ($n = 11$)	409 (103)	397 (103)	−2.7	84 (7)	87 (5)	+3.0
RD ($n = 8$)	411 (113)	413 (112)	+0.4	88 (5)	89 (5)	+0.9

For abbreviations see text.

Table 2 Mean PEF before and after nebulisation in respect of the dose of the iloprost dose in mean (SD).

Dose per nebulisation (μg)	PEF (pre)	PEF (post)	Change PEF (%)	SO_2 (pre)	SO_2 (post)	Change SO_2 (%)
3.3	412 (108)	407 (103)	−1.3	89 (7)	90 (6)	−1.3
6.6	423 (100)	415 (102)	−1.8	89 (6)	90 (5)	−0.8
9.9	438 (97)	427 (101)	−2.5	90 (6)	91 (5)	−0.8
13.2	429 (101)	417 (101)	−2.7	90 (7)	91 (5)	−0.6
15	420 (100)	414 (100)	−1.4	90 (6)	91 (5)	−0.9
20	415 (80)	407 (80)	−1.8	91 (5)	91 (4)	−0.1

For abbreviations see text.

initiation of therapy. They recovered immediately after stop of nebulisations. Six patients underwent pulmonary thromboendarterectomy for CTEPH and received nebulised iloprost as bridging to surgery.

Forty-eight patients completed the 3 months follow up including repeat PFT, 6 MWT and right heart catheterisation. There was a significant increase in 6 min walking distance from 246 ± 113 to 294 ± 115 m; however no significant change in resting haemodynamics. The increase in functional capacity was detected in all subgroups of patients. Data are shown in Tables 3 and 7.

Table 3 Functional and haemodynamic change on treatment with nebulised iloprost in the subset of 48 patients completing the 3 months followup mean (SD).

	Baseline	3 months
RAP (mmHg)	9 (6)	10 (7)
mPAP (mmHg)	56 (13)	55 (14)
CI (l/min/qm)	1.9 (0.6)	2.1 (0.5)
TPR (dyn s cm^5)	1225 (348)	1124 (349)
$CVSO_2$ (%)	62 (8)	60 (8)
6 MWD (m)	246 (113)*	294 (115)*

RAP—right atrial pressure, mPAP—mean pulmonary artery pressure, CI—cardiac index, TPR—total pulmonary resistance, $CVSO_2$—central venous saturation, 6 MWD—6 min walking distance.
*Paired *t*-test $P<0.05$.

At baseline, mean FEV_1 and FCV were reduced with 74 ± ±20% pred. and 86 ± 24% pred., respectively. The mean FEV_1/FVC ratio was 86%. Mean TLC was marginally reduced with 93 ± 18% pred., whereas the RV was increased by 114 ± 24% pred. After 3 months treatment, there was a change of mean FEV_1 of −0.2%, mean FVC of +1.3%, mean TLC of +2.5%, and mean RV of +3.9%. Ratio of RV/TLC as surrogate parameter for small airway dysfunction was normal with 40% at baseline with minimal change after 3 months treatment.

There were no statistically significant differences in the whole study population as well as the different diagnoses groups. Details are shown in Table 4.

Concerning gas exchange, Keogh factor at baseline was reduced at 70 ± 23% pred., as well as SO_2 at rest with 92 ± 6%, and minimum. SO_2 during 6 MWT with 83 ± 12%. After 3 months treatment there was a mean change in Keogh factor by −1.9%. SO_2 at rest changed in mean by +1.7%. Assessing the minimum SO_2 during 6 MWT, there was a mean change of +0.1% after 3 months. Details are shown in Table 5. There were no statistically significant differences in the whole study population as well as the different diagnoses groups comparing baseline and 3 months follow-up parameters.

Table 4 PFT at baseline (BL) as % predicted, and change after 3 months treatment with nebulised iloprost in 48 patients, mean (SD).

	FEV_1 (BL)	Change (%)	FVC (BL)	Change (%)	TLC (BL)	Change (%)	RV (BL)	Change (%)	RV/TLC (BL)	Change (%)
Total ($n=48$)	74 (20)	−0.2	86 (24)	+1.3	93 (18)	+2.5	114 (24)	+3.9	41 (8)	+2.4
PPH ($n=17$)	76 (17)	+2.4	90 (24)	+2.4	93 (17)	+4.1	113 (20)	+6.0	39 (7)	+3.4
CTEPH ($n=7$)	79 (17)	+4.5	92 (18)	+9.1	93 (18)	−4.3	107 (28)	+6.3	40 (9)	+3.5
CTD ($n=12$)	78 (24)	−0.8	81 (24)	+1.3	90 (18)	−6.4	109 (25)	−3.3	41 (8)	+4.1
CHD ($n=8$)	72 (20)	−1.2	83 (23)	+0.9	94 (19)	+5.0	123 (22)	+4.4	43 (11)	−2.1
RD ($n=4$)	60 (27)	+4.4	80 (41)	+6.8	90 (25)	+4.2	114 (27)	+4.6	46 (9)	−4.0

For abbreviations see text.

Table 5 Gas exchange at baseline (BL) as % predicted, and change after 3 months treatment with nebulised iloprost in 48 patients, mean (SD).

	KCO (BL)	Change (%)	Rest SO_2 (BL)	Change (%)	Walk SO_2 (BL)	Change (%)
Total ($n=48$)	70 (23)	−1.9	92 (6)	+1.7	83 (12)	+0.1
PPH ($n=17$)	68 (19)	−1.0	94 (4)	+0.2	86 (11)	−0.6
CTEPH ($n=7$)	87 (16)	+7.2	94 (3)	+2.5	90 (4)	+0.7
CTD ($n=12$)	57 (20)	−3.4	94 (3)	+0.3	85 (9)	+1.1
CHD ($n=8$)	84 (19)	−3.4	87 (7)	+4.6	73 (13)	+1.3
RD ($n=4$)	41 (5)	−4.1	90 (5)	+5.1	80 (13)	+7.6

KCO—Keogh factor, rest SO_2—resting SO_2, walk SO_2—minimum SO_2 during 6 min walk test, for other abbreviations see text.

Discussion

In this study, we found neither short- nor long-term systematic adverse effects on pulmonary function test or gas exchange in patients with pulmonary hypertension of different origin. Two patients with underlying obstructive lung disease developed broncoconstriction on treatment with nebulised iloprost with recovery after cessation of therapy, accounting for about 9% of patients with underlying obstructive lung disease in our series. As any inhaled compound may provoke bronchial irritant effects, especially in these patients a routine pulmonary function testing should be performed at follow-up.

At baseline patients presented with a slight reduction in lung volumes, consistent with a recent report about impaired peripheral airway function in patients with primary pulmonary hypertension.[5] In long-term follow-up, there were no significant changes in FEV_1, FVC, TLC and RV indicating no significant obstructive or restrictive changes, or hyperinflation.[18,19]

For assessment of gas exchange, we used minimum SO_2 before and after inhalation for easy and rapid assessment of short-term effects on oxygenation. For long-term changes the CO-diffusion capacity corrected for alveolar volume and haemoglobin was used as the most sensitive and comprehensive parameter. Furthermore, minimum SO_2 during 6 MWT reflects oxygenation status during exercise.[17,18] Our data indicate that nebulised iloprost is not associated with short- or long-term adverse effect on pulmonary gas exchange in patients with pulmonary hypertension of different origin. There was a tendency toward better SO_2 at rest and during exercise in the RD group, however this was not statistically significant (Table 5). Only in one study in patients with pulmonary hypertension due to lung fibrosis there was an insignificant reduction of SaO_2 after acute inhalation of prostacyclin.[3]

In our study, 3 months treatment resulted in a significant increase in 6 MWD of 48 m in the whole study population, comparable to the result of the randomised controlled trial on nebulised iloprost in pulmonary hypertension with a mean increase in 6 MWD of 36 m after 3 months treatment.[1]

Treatment with nebulised iloprost demands a commitment, as 7 nebulisations per day are required, and compliance problems are inevitable.[18] So far, data concerning compliance with treatment and severity of side effects in chronic use of nebulised iloprost are sparse. Typically, side effects occur pronounced during initiation phase of therapy.[1,11,12,20]

Table 6 Subgroup analysis of 8 patients with pH associated with respiratory disease.

Gender, age	Diagnosis	FEV_1,%pred	FVC, %pred	KCO, %pred	Baseline			3 months follow up			
					6 MWT, m	MPAP, mmHg	CI, L/min/qm	6 MWT, m	MPAP, mmHg	CI, l/min/qm	Comments
M, 70	COPD	46	53	40	177	57	1.8				Stopped
M, 61	Emphysema	83	113	43	180	36	1.9				Stopped
F, 76	Emphysema	99	146	37	120	61	1.7				Stopped
F, 53	COPD	78	99	41	140	57	1.8				Stopped
M, 67	IPF	88	85	42	180	45	1.5	300	40	1.8	Continued
M, 54	IPF	67	66	37	180	42	1.1	240	39	2.,1	Continued
M, 52	COPD	41	72	40	240	57	1.6	180	48	2.5	Continued
F, 59	IPF	40	39	n.p.	280	52	2.5	320	46	2.7	Continued
Mean (SD)					220 (49)	49 (7)	1.7 (0.6)	260 (63)	43 (4)	2.3 (0.4)	

Table 7 Change in 6 min walking distance after 3 month's treatment according to origin of pulmonary hypertension (only patient who completed followup are enclosed).

	Baseline	3 months
IPAH ($n = 17$)	300 (105)	333 (133)
CTEPH ($n = 7$)	227 (130)	282 (96)
CTD ($n = 12$)	220 (81)	274 (67)
CHD ($n = 8$)	272 (110)	300 (124)
RD ($n = 4$)	220 (49)	260 (63)
Total ($n = 48$)	246 (113)	294 (115)

In our study, 3 patients stopped treatment due to non-compliance (4%). Furthermore, 4 patients stopped treatment due to severity of side effects during initiation of therapy (6%). This might be contributed by the relatively high dose of iloprost used, although appropriate dose reduction has been attempted in these patients. Concerning subgroup analysis, 50% of patients in the RD group stopped treatment due to side effects, and all of them had underlying COPD. Therefore, side effects due to probable airway irritation should be considered in these patients. (Tables 6 and 7).

In total, 9 patients stopped treatment due to iloprost related side effects or non-compliance with frequent nebulisations (14%).

In conclusion, nebulised iloprost is an effective treatment in pulmonary hypertension of different origin without systematic adverse effects on pulmonary function test and gas exchange. A bronchoconstrictive effect might develop in patients with underlying obstructive lung disease. In our study, 14% of patients stopped treatment due to side effects or non-compliance.

References

1. Olschewski H, Simonneau G, Galie N, Higenbottam T, Naeije R, Rubin LJ, et al. Inhaled iloprost for severe pulmonary hypertension. *N Engl J Med* 2002;**347**:322–9.
2. Walmrath D, Schneider T, Pilch J, Schermuly R, Grimminger F, Seeger W. Effects of aerosolized prostacyclin in severe pneumonia. Impact of fibrosis. *Am J Respir Crit Care Med* 1995;**151**:724–30.
3. Olschewski H, Ghofrani HA, Walmrath D, Schermuly R, Temmesfeld-Wollbruck B, Grimminger F, et al. Inhaled prostacyclin and iloprost in severe pulmonary hypertension secondary to lung fibrosis. *Am J Respir Crit Care Med* 1999; **160**:600–7.
4. Steenhuis LH, Groen HJ, Koeter GH, van der Mark TW. Diffusion capacity and haemodynamics in primary and chronic thromboembolic pulmonary hypertension. *Eur Respir J* 2000;**16**:276–81.
5. Meyer FJ, Ewert R, Hoeper MM, Olschewski H, Behr J, Winkler J, et al. Peripheral airway obstruction in primary pulmonary hypertension. *Thorax* 2002;**57**:473–6.
6. Norel X, Walch L, Labat C, Gascard JP, Dulmet E, Brink C. Prostanoid receptors involved in the relaxation of human bronchial preparations. *Br J Pharmacol* 1999;**126**:867–72.
7. Spannhake EW, Levin JL, Mellion BT, Gruetter CA, Hyman AL, Kadowitz PJ. Reversal of 5HT-induced bronchoconstriction by PGI2: distribution of central and peripheral actions. *J Appl Physiol* 1980;**49**:521–7.
8. Lammers JW, Kioumis I, McCusker M, Nichol GM, Barnes PJ, Chung KF. Effects of prostacyclin on bronchoconstriction and neutropenia induced by inhaled platelet-activating factor in man. *J Allergy Clin Immunol* 1990;**85**:763–9.
9. Hardy CC, Bradding P, Robinson C, Holgate ST. Bronchoconstrictor and antibronchoconstrictor properties of inhaled prostacyclin in asthma. *J Appl Physiol* 1988;**64**:1567–74.
10. Gessler T, Schmehl T, Hoeper MM, Rose F, Ghofrani HA, Olschewski H, et al. Ultrasonic versus jet nebulization of iloprost in severe pulmonary hypertension. *Eur Respir J* 2001;**17**:14–9.
11. Hoeper MM, Schwarze M, Ehlerding S, Adler-Schuermeyer A, Spiekerkoetter E, Niedermeyer J, et al. Long-term treatment of primary pulmonary hypertension with aerosolized iloprost, a prostacyclin analogue. *N Engl J Med* 2000;**342**: 1866–70.
12. Olschewski H, Ghofrani A, Schmehl T, Winkler J, Wilkens H, Hoeper MM, et al. Inhaled iloprost to treat severe pulmonary hypertension. *Ann Intern Med* 2000;**132**:435–43.
13. Machherndl S, Kneussl M, Baumgartner H, Schneider B, Petkov V, Schenk P, et al. Long-term treatment of pulmonary hypertension with aerosolized iloprost. *Eur Respir J* 2001; **17**:8–13.
14. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. *NHBLI/WHO Workshop Report*, National Heart, Lung, and Blood Institute, National Institute of Health; April 2001, NIH Publication 2701.
15. O'Donnell RA, Peebles C, Ward JA, Daraker A, Angco G, Broberg P, et al. Relationship between peripheral airway dysfunction, airway obstruction, and neutrophilic inflammation in COPD. *Thorax* 2004;**59**:837–42.
16. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *NEJM* 2004.
17. Paciocco G, Martinez FJ, Bossone E, Pielsticker E, Gillespie B, Rubenfire M. Oxygen desaturation on the 6-min walk test and mortality in untreated primary pulmonary hypertension. *Eur Respir J* 2001;**17**:647–52.
18. American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard technique—1995 update. *Am J Respir Crit Care Med* 1995;**152**:2185–98.
19. Stocks J, Quanjer PH. Reference values for residual volume, functional residual capacity and total lung capacity. ATS workshop on lung volume measurements. Official Statement of The European Respiratory Society. *Eur Respir J* 1995;**8**: 492–506.
20. Hoeper MM, Galie N, Simonneau G, Rubin LJ. New treatments for pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2002;**165**:1209–16.