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Morphologic Changes in Explanted Lungs after Prostacyclin Therapy for Pulmonary Hypertension

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Abstract
Prostacyclin (PGI_2) causes vasodilation and inhibition of platelet aggregation *in vivo*. PGI_2 is also postulated to affect pulmonary vascular remodeling, at least partly through anti-proliferative effect via PGI_2 receptor (PGIR). However, the mechanism(s) of action by which (PGI_2) exerts its therapeutic effect is still not clear despite clear clinical benefit seen in severe pulmonary hypertension (PH) patients. We performed a histopathologic and morphometric study on the explanted lung tissues from PGI_2-treated patients prior to lung transplantation (n = 9), in an attempt to elucidate morphologic changes associated with PGI_2 treatment. Explanted lungs from PH patients without PGI_2 treatment were examined as the control (n = 11). We also studied the possible differences in PGIR expression between the treated and untreated groups by immunohistochemical method. Seven out of 9 treated patients showed moderate to severe bronchial and perivascular inflammation, as opposed to only 1 such case in the control group. Five out of 9 treated cases showed moderate to severe alveolar edema with or without evidence of old hemorrhage, in contrast to only 1 case showing moderate alveolar edema in control patients. Morphometry did not reveal any significant difference between the two groups either in the % thickness of intima, media, or adventitia or in the density of plexiform lesions. Immunostain also failed to demonstrate any notable difference in PGIR expression. In conclusion, PGI_2-treated cases revealed more pronounced pulmonary alveolar edema and inflammation, but no morphological evidence of altered vascular remodeling or PGIR expression after PGI_2 therapy.

Key words: pulmonary hypertension, prostacyclin, PGI_2, Flolan, vascular remodeling, PGI_2 receptor immunohistochemistry, morphometry

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Introduction

Continuous intravenous prostacyclin (PGI_2) therapy was first introduced to treat severe pulmonary hypertension (PH) in the early 1980s and was approved by the Food and Drug Administration (FDA) in 1996 for primary PH (PPH) and later for some secondary PH cases as well [1]. PGI_2 therapy has been shown to improve exercise capacity, hemodynamics, and survival in a short-term randomized controlled trial in PPH, though only limited data are available for the long-term survival [2-5].

PGI_2 probably works *in vivo* at least partly via its action as a vasodilator and as an inhibitor of platelet aggregation [6]. PGI_2 may also affects pulmonary vascular remodeling via alternative mechanisms such as antiproliferative effect mediated by PGI_2 receptor (PGIR) [7]. A previous experimental study suggested antiinflammatory actions of PGI_2 that may also be of therapeutic value [8]. A potential importance of interference with other vasoactive substances such as VEGF and endothelin by PGI_2 has been reported [9-10].

Commonly observed side effects of PGI_2 are often mild and dose-related, which include flushing, headache, jaw pain, leg pain, diarrhea and nausea [11]. More serious complications are related to the complex delivery system requiring central venous access with the increased risk of serious infection or catheter related thrombosis [11]. It is yet to be seen, however, if any long-term adverse effects of PGI_2 will develop as more PH cases are exposed to the long-term PGI_2 therapy.

In the present study, we sought to study morphologic changes associated with PGI_2 therapy in a series of severe PH patients by comparing the histopathology, degree of vascular remodeling and PGIR expression in the explanted lung tissues with and without PGI_2 treatment prior to lung transplantation.

Material and Methods

Case Selection and Histological Review: All the cases in the study were identified from the surgical pathology file at the University of California San Diego Medical Center from 1992 to 2001. The study was approved by

the human subject committee at University of California San Diego. Medical records and surgical pathology reports were reviewed to verify the clinical diagnosis, treatment history, and other pertinent clinical and laboratory data. Representative lung histologic sections with routine hematoxylin and eosin (H&E) and trichrome-elastin stains were reviewed in each case. Histopathologic review was done without the knowledge of prior PGI_2 treatment. The degrees of alveolar edema, inflammation and hemorrhage were graded based on the extent of the changes found in the sections as none, mild, moderate and severe with the following criteria: None- little or no changes in all sections, Mild-occasional foci of changes filling 10x objective microscopic fields (low power fields), Moderate-frequent, readily recognizable foci of changes filling low power fields, Severe-patchy but widespread changes filling low power fields.

Pulmonary Artery Morphometry: Computer-assisted image analysis was performed on 5-um-thick section slides of selected blocks from each case (average 3.6 ± 1.2 slides/case) with combined trichrome and elastin stain. Ten pulmonary arteries of all sizes per slide were identified and the shortest diameter across the lumen, intima, media and adventitia were measured. The measurements for each pulmonary artery component were grouped into four categories as the followings: 1. PPH with PGI_2 therapy, 2. PPH without PGI_2 therapy, 3. EISEN with PGI_2 therapy, 4. EISEN without PGI_2 therapy. The cumulative data for % thicknesses of intima, media, and adventitia against the total wall thickness (the sum of intima, media and adventitia) were calculated in each group from the measured parameters. All the plexiform lesions per slide were counted and the density of plexiform lesion was calculated from the number of lesions per cm^2 of the assessed lung tissue. Images were obtained with a Zeiss Axiocam-HRC digital camera connected to a TEK PC. Image analysis and measurements were performed using Zeiss KS 300 3.0 Image software (Hallbergmoos, Germany).

Immunohistochemistry: Immunohistochemical staining was performed with the antibody to PGIR (kind gift of Mark Geraci, M.D., University of Colorado, Denver, Colorado) on a representative paraffin block from each case, using a standard avidin-biotin-peroxidase complex (dilution 1:50) using the Ventana automatic immunostainer.

Statistical Method: Data were displayed as the mean ± standard error (SE). Statistical significance of the differences between the patients with PGI_2 and without PGI_2 therapy were calculated separately in PPH and EISEN groups by using the unpaired Student's t-test. Statistical difference was accepted at $p < 0.05$.

RESULTS

Clinical Findings: The treated group (n = 9) received continuous intravenous infusion of PGI_2 (Epoprostenol Sodium; Glaxo Wellcome Inc. Research Triangle Park, NC) administered by a portable infusion pump from 17 to 62 months (mean: 39.5 ± 4.5 months). In the treated group, there were 6 PPH and 3 Eisenmenger patients. In the control group, there were 5 PPH and 6 Eisenmenger patients. The details of these cases are summarized in Table 1.

Pulmonary inflammation and edema: Seven out of 9 pretreated patients showed moderate to severe bronchial, and perivascular inflammation, as opposed to only 1 such case in control group. Some cases also reveal interstitial inflammatory infiltrates, focally reminiscent of non-specific interstitial pneumonia (NSIP) (Fig. 1A). Five out of 9 pretreated cases showed moderate to severe alveolar edema with or without evidence of old hemorrhage, in contrast to only 1 case showing moderate alveolar edema in control patients (Fig. 1B-D).

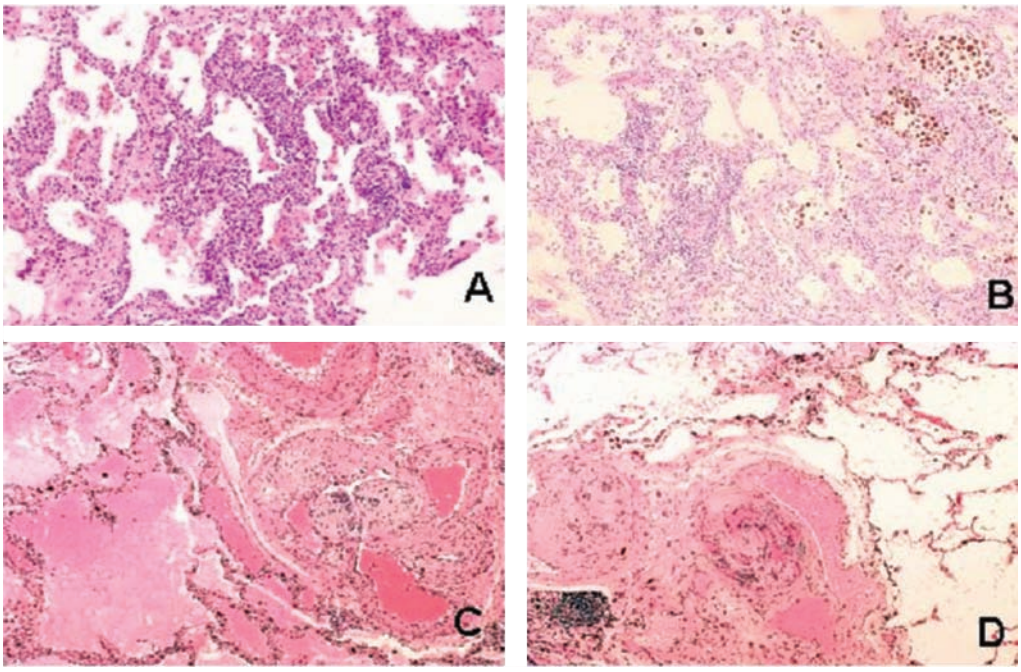

Fig. 1. Increased pulmonary interstitial inflammation (A) and increased pulmonary edema and hemorrhage in treated patients (B&C) and normal appearing parenchyma in untreated patients (D) (HE stain, 100x original magnification).

Table 1. Clinical Data.

Case No.	age/sex	Diagnosis	PAP s/d/m*	Duration of PGI_2 Tx
1	43/F	PPH	88/34/51	17 months
2	49/F	PPH	99/45/63	38 months
3	50/F	EISEN	160/76/104	62 months
4	31/F	EISEN	126/46/71	24 months
5	21/M	PPH	64/36/47	29 months
6	40/F	EISEN	102/50/70	20 months
7	54/F	PPH	125/45/65	39 months
8	37/F	PPH	80/49/58	27 months
9	31/M	PPH	84/40/53	27 months
10	45/M	EISEN	N/A	none
11	46/F	PPH	N/A	none
12	29/F	EISEN	115/50/70	none
13	32/F	EISEN	135/74/94	none
14	27/F	PPH	81/43/56	none
15	39/F	EISEN	135/71/99	none
16	29/F	PPH	101/49/67	none
17	42/F	PPH	150/90/100	none
18	43/F	EISEN	101/25/50	none
19	38/F	PPH	N/A	none
20	50/F	EISEN	N/A	none

PAP = pulmonary artery pressure; s/d/m = systolic/diastolic/mean

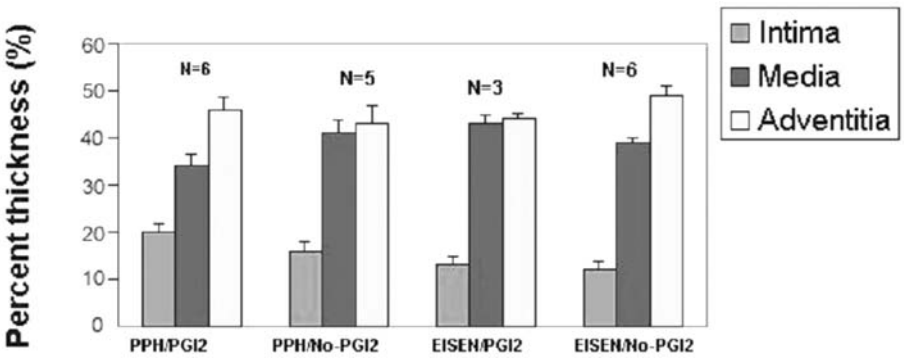

Fig. 2. No significant difference in the % thicknesses of intima, media, and adventitial between treated and untreated patients for PPH and EISEN.

Vascular remodeling: There were no significant differences in the % thicknesses of intima, media, or adventitia between the PGI_2–treated and -untreated groups in both PPH and EISEN patients ($p>0.05$) (Fig. 2). No significant difference in the density of plexiform lesions was detected ($p>0.05$) (Fig. 3).

PGIR expression: Pulmonary arteries of all sizes with or without remodeling reveal variable degrees of positive PGIR immunoreactivity predominantly in smooth muscle cells and also in some myointimal or endothelial cells. There was no apparent difference in the immunoreactivity between the two groups.

Discussion

We sought to identify the morphologic changes associated with PGI_2 therapy in a series of severe precapillary PH cases including PPH and EISEN using explanted lungs. It is, to our knowledge, the largest series studying the morphologic changes associated with PGI_2 therapy in clinical PH cases. The PGI_2 therapy appeared to induce pulmonary edema and inflammation, but did not result in any morphologically recognizable differences in vascular remodeling or in PGIR expression in our cases.

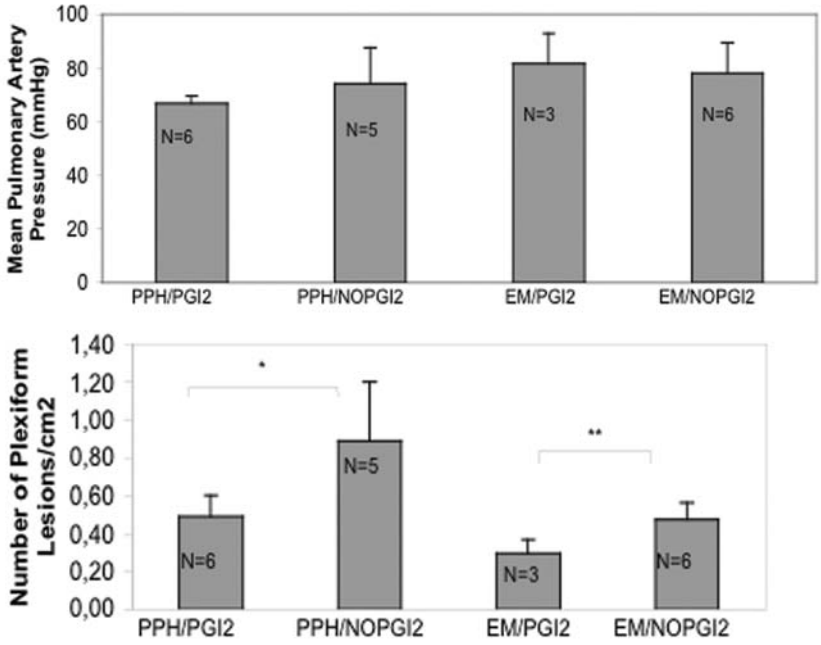

Fig. 3. Pulmonary artery mean pressure A- and the density of plexiform lesions in treated with epoprostenol and not treated patients with PPH and Eisenmenger physiology. Data are expressed as Mean ± SE, *p=0.23 and **p=0.22. There was only a trend for a smaller lesion density in the treated PPH patients.

It would be ideal to examine serial lung biopsies in a cohort of PGI_2 treated patients to assess the full spectrum of morphologic changes associated with the therapy. Since it is not practically feasible to do, we reviewed explanted lungs from the patients who underwent lung transplantation after PGI_2 treatment. All patients in the treated group received continuous PGI_2 infusion until the transplantation was performed with the explantation of native lungs. Though PGI_2-treated patients included in this study may represent a group that responded to PGI_2 therapy less favorably, they all reported an initial improvement or stabilization of symptoms after receiving the drug. Pulmonary artery pressure and the severity of disease in these PGI_2-treated patients were comparable to those in the untreated control patients used in our study.

Previous studies have reported marked pulmonary edema as a complication of PGI_2 treatment in the postcapillary PH patients including pulmonary venoocclusive disease (PVOD) and primary capillary hemangiomatosis (PCH), presumably by increasing pulmonary capillary hydrostatic pressure [12-14]. Accordingly, PGI_2 is now generally regarded as a contraindication for PVOD and PCH patients. Our study demonstrated that pulmonary edema developed even without fixed postcapillary obstruction in our cases of precapillary PH cases (i.e. PPH and EISEN) as opposed to little or no edema in the untreated control PH patients. Clinically, however, it was not a dose-limiting side effect in our patients.

There was mild degree of pulmonary inflammation even in the untreated PH cases, which has been well described to be present in association with vascular remodeling in PH cases. However, it was more pronounced in the PGI_2 treated patients. Mechanism for this increased inflammation in PGI_2 treated patients is not clear. It appears even somewhat paradoxical, given that PGI_2 is supposedly an anti-inflammatory agent [15]. A previous case report documented that a PPH patient developed interstitial lung disease with nonspecific interstitial pneumonia (NSIP)-like histopathology after a long-term PGI_2 treatment over 5 years [16]. Increased inflammation seen in our cases with PGI_2 treatment was mostly perivascular and peribronchiolar (but not prominent in the interstitial location), which is generally not sufficient for a diagnosis of NSIP. Average duration of the prostacyclin treatment in our study was 31.4 months (range 17-62 months), which is shorter than the case in the previous report. Thus, whether these cases eventually develop NSIP-like picture is yet to be seen.

It has been widely hoped that prostacyclin halts or reverses the vascular remodeling in PH cases. The reversibility of some vascular changes in PH has been an area of controversy. Despite the experimental evidence for anti-proliferative effects of PGI_2 [17-19], we could not demonstrate any morphometric evidence of difference between the treated and un-treated PH patients. Expression of PGIR has been reported to be reduced in the remodeled pulmonary arterial smooth muscle in severe PH and to play an important role in the regulation of hypoxia-induced pulmonary vascular remodeling [20]. We could not demonstrate any apparent difference in PGIR expression between the two groups in pulmonary vasculature.

In conclusion, our morphological study using explanted lungs demonstrated increased pulmonary edema and inflammation, but no apparent changes in pulmonary vascular remodeling or PGIR expression after the PGI_2 therapy in severe PH patients.

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