Prostaglandins & other Lipid Mediators 107 (2013) 48–55

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

Original research article

A comparative study of PGI_2 mimetics used clinically on the vasorelaxation of human pulmonary arteries and veins, role of the DP-receptor

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ARTICLE INFO

Article history:
Received 21 December 2012
Received in revised form 18 May 2013
Accepted 2 July 2013
Available online 12 July 2013

Keywords:
Prostanoid receptors
Prostacyclin mimetic
Vasorelaxation
Human pulmonary artery
Human pulmonary vein
Pulmonary hypertension

ABSTRACT

Prostacyclin (PGI_2) and its mimetics (iloprost, treprostinil, beraprost and MRE-269) are potent vasodilators (*via* IP-receptor activation) and a major therapeutic intervention for pulmonary hypertension (PH).

These PGI_2 mimetics have anti-proliferative and potent vasodilator effects on pulmonary vessels. We compared the relaxant effects induced by these recognized IP-agonists in isolated human pulmonary arteries (HPA) and veins (HPV). In addition, using selective antagonists, the possible activation of other prostanoid relaxant receptors (DP, EP_4) was investigated.

Iloprost and treprostinil were the more potent relaxant agonists when both vessels were analyzed. HPA were significantly more sensitive to iloprost than to treprostinil, pEC_{50} values: 7.94 ± 0.06 ($n = 23$) and 6.73 ± 0.08 ($n = 33$), respectively. In contrast, in HPV these agonists were equipotent. The relaxations induced by treprostinil were completely or partially inhibited by IP-antagonists in HPA or HPV, respectively. The effects of the IP-agonists were not significantly modified by the EP_4 antagonist. Finally, DP-antagonists inhibited the relaxations induced by treprostinil in HPV, suggesting that the DP-receptor plays a role in treprostinil-induced relaxation in the HPV.

These data suggest that iloprost and treprostinil should be the most effective clinically available agonists to decrease pulmonary vascular resistance and to prevent oedema formation (by similar decrease in HPA and HPV resistance) in PH patients.

1. Introduction

Pulmonary arterial hypertension (PAH), group 1 of the pulmonary hypertension classification [1] is a progressive vascular disease characterized by pulmonary vasoconstriction, arterial remodelling and impaired right ventricular function [2,3]. Among the different PAH therapies, treatment with prostacyclin (PGI_2, epoprostenol) or PGI_2 mimetics improves survival in patients with severe PAH awaiting lung transplantation [4]. However, the indication of epoprostenol for other groups of pulmonary hypertension like pulmonary veno-occlusive disease (PVOD) or pulmonary capillary hemangiomatosis (PCH) is controversial. Whereas cautious application of epoprostenol can be considered as a therapeutic option in such patients [5,6], in several studies, this treatment was associated with pulmonary oedema [7,8] through yet unknown mechanisms.

Epoprostenol is a potent relaxant of human pulmonary arteries [9–11]. Most of the clinical trials in PAH patients have been performed with continuous intravenous administration of PGI_2 because of its short half-life (less than 3 min) in human plasma [12]. For these reasons, more stable PGI_2 mimetics and formulations have been developed and are approved for clinical use: intravenous (epoprostenol, iloprost, treprostinil), oral (beraprost), inhaled (iloprost and treprostinil) and subcutaneous injection (treprostinil) [13]. Unlike epoprostenol and iloprost, treprostinil has a long half-life, allowing its administration by continuous subcutaneous

Abbreviations: HPA, human pulmonary artery; HPV, human pulmonary vein; PAH, pulmonary arterial hypertension; PVOD, pulmonary veno-occlusive disease; PCH, pulmonary capillary hemangiomatosis.

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http://dx.doi.org/10.1016/j.prostaglandins.2013.07.001

injection, thus avoiding the risk of infection related to continuous long term intravenous administration [14,15]. Beraprost is the first PGI_2 analogue available as an oral formulation [16]. Currently, treprostinil and selexipag (and its metabolite MRE-269 = ACT-333679) are other orally available PGI_2 mimetics in clinical development (Phase III) for the treatment of PAH [17,18]. MRE-269 is not a PGI_2 chemical analogues but this compound has high affinity and appears selective for the IP receptor [52].

Although data from numerous clinical studies are available for PGI_2 and its mimetics, their integrated *ex vivo* or *in vitro* pharmacological and biochemical characterization is incomplete. PGI_2 is an arachidonic acid metabolite synthesized sequentially *via* cyclooxygenase and prostacyclin synthase (PGIS). In general, activation of IP-receptors present on smooth muscle cells by PGI_2 or its mimetics will induce vasodilation and inhibit cell proliferation [19]. Treprostinil is one of the most potent PGI_2 analogues to inhibit proliferation of human pulmonary artery smooth muscle cells in culture. This effect is mediated by increased cAMP synthesis [20]. However, this effect on cell proliferation could be mediated by the IP receptor and/or by peroxisome proliferator activated receptor γ (PPARγ) activation [21].

In vitro physiological studies performed with an organ bath system have shown that PGI_2 and at least two of its analogues (iloprost, cicaprost) induce vasorelaxation of healthy human pulmonary arteries (HPA) and veins (HPV) *via* activation of IP-receptor [10,11]. The presence of other prostanoid receptors involved in the control of vascular tone has been also described in HPA (EP_3, TP) [22,23] and in HPV (DP, EP_4, EP_1 and TP) [24,25]. The different PGI_2 mimetics could have pharmacologically- and clinically-relevant affinities for other prostanoid receptors; therefore, the vasodilations induced by PGI_2 mimetics may also be modulated by activation of these prostanoid receptors expressed in the human pulmonary vessels [3,13].

The pulmonary vein is not just a simple conduit, but rather is an important component involved in the regulation of pulmonary circulation. Depending of the animal species and (patho) physiologic conditions, up to a third of the total pulmonary vascular resistance can be attributed to the veins [26]. Given the reported side effects of PGI_2 mimetics in some forms of pulmonary hypertension [7,8], it is therefore essential to understand whether each individual compound has the capacity to vasodilate both the arterial and venous compartments with the same efficiency. For this reason, in our experimental approach, *in vitro* studies were performed in parallel using freshly isolated HPA and HPV derived from the same patients.

The goal of the present study was to compare the relaxant effects of PGI_2 and its mimetics used clinically (iloprost, treprostinil and beraprost) or in development (MRE-269) on paired vascular preparations (HPA/HPV). In addition, we have investigated the potential role of prostanoid receptors other than IP on the vasodilation induced by PGI_2 and its mimetics. In particular, we have assessed which relaxant receptors (DP or EP_4) could be involved by using selective antagonists for these receptors.

2. Methods

2.1. Isolated vascular preparations

All research programmes involving the use of human tissue were approved and supported by the AP - HP (Assistance Public - Hôpitaux de Paris), Ethics Committee (Institutional Review Board No. IRB00006477), agreement (No. 11-045). Human lung tissues were obtained from patients (after written consent) who had undergone surgery for lung carcinoma (vessels were dissected in the macroscopically healthy part of the lung). The mean age of the patients was 64 ± 01 years. Human pulmonary arteries ($n = 40$) and veins ($n = 35$); females $n = 18$ and males $n = 31$. All preparations were used within 1–12 h post-surgery. These preparations were used with intact endothelium. Pulmonary arterial and venous preparations (3–6 mm internal diameter) were cut as rings (177 preparations) and set up in 10 ml organ baths containing Tyrode's solution (concentration mM: NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5) gassed with 5% CO_2 and 95% O_2, pH 7.4 and maintained at 37 °C. Each ring was initially stretched to an optimal load (1.5 g). Changes in force were recorded by isometric force displacement transducer (F-60 Narco, Houston, USA) and physiographs (Linseis, Selb, Germany). The data acquisition was done on a computer by using IOX software (version 1.8.9.4, Emka, Paris, France). Subsequently, preparations were allowed to equilibrate for 90 min with bath fluid changes taking place every 10 min.

2.2. Study on the vasorelaxation induced by PGI_2 mimetics in human pulmonary vessels

After the equilibration period, a submaximal pre-contraction was induced with norepinephrine (NE; 10 μM, [27,28]); when the contraction reached a plateau, cumulative concentrations of PGI_2 or its mimetics were added to the baths every 3–6 min over approximately 30 min. In some protocols, the preparations were incubated (30 min) in the presence or the absence of one of the following prostanoid receptor antagonists: GW627368X (EP_4-, TP- antagonist); AH6809 (EP_1-, EP_2-, DP- antagonist); L-877499 [29], BWA868C or ONO-AE3-237 (DP-antagonists); CAY10441 (RO1138452, [30]) or RO3244019 (AGN230933 [31]) (IP antagonists) just before NE pre-contraction and prostacyclin mimetics concentration–response curve. Each ring was exposed only to one concentration–response curve and NE pre-contractions were not significantly modified by the antagonist incubations.

2.3. Measurement of the expression of the prostanoid receptors, Western blots analysis

Arterial and venous pulmonary preparations were ground using a porcelain mortar with liquid nitrogen and homogenized in RIPA solution (Tris–HCl buffer in mM: Tris: 50, NaCl: 150, EDTA: 5, pH: 8; Triton X-100: 1%; sodium desoxycholate 1%; SDS 0.1%) with a protease inhibitor cocktail 1% (Sigma–Aldrich, St. Louis, MO, USA). The protein concentrations were quantified using a bicinchoninic acid (BCA) protein assay kit (ThermoScientific, Rockford, IL, USA). Approximately 50 μg of protein sample were loaded on a 13% sodium dodecyl sulfate (SDS)-polyacrylamide gel. Proteins were transferred on to nitrocellulose membranes (Amersham Biosciences, Glattbrugg, Switzerland). The rat brain and pulmonary artery smooth muscle cell were used as standards. The membranes were blocked for 1 h in TBS (20 mM Tris, pH 8, 300 mM NaCl, 0.1% Tween-20, 5% non-fat dry milk) and incubated overnight at 4 °C with one of the following antibodies against: DP (polyclonal 1/200, Cayman, Ann Arbour, MI, USA) and IP (polyclonal 1/500, was supplied by Dr Lucie Clapp, University College London, UK). Subsequently, the membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (Jackson, West, Chester, PA, USA). Bands were visualized using the ECL plus or prime luminescence system (Amersham Biosciences). The membranes were rehybridized with anti-α-actin polyclonal antibody (1:6000, Dako, Vancouver, Canada) and with the appropriate secondary antibody (anti-Mouse from Sigma–Aldrich) for normalization.

2.4. Data analyses

The effects induced by the different agonists were expressed in grams or normalized (%) with respect to the NE pre-contraction. The data are positive for the contractions and negative for the relaxations. Where possible, a four parameter logistic equation of the form:

$$E = \frac{E_{max}[A]^{nH}}{EC_{50}^{nH} + [A]^{nH}}$$

was fitted to data obtained from each organ bath protocols to provide estimates of the maximal relaxation (E_{max}) induced by the PGI_2 mimetics [A], the half-maximum effective concentration values (EC_{50}), as well as Hill slope (nH) parameters. All results were analyzed using SigmaPlot® version 12.0 (Systat Software, San Jose, CA, USA). The pEC_{50} values were calculated as the negative log of EC_{50} values and represent the sensitivity of a preparation to an agonist. For these calculations, the contractions obtained with the highest concentrations of agonist were omitted. The equilibrium dissociation constant for the antagonist (K_B) was calculated using the following equation: $K_B = [B]/(DR - 1)$, where $[B]$ is the concentration of the antagonist and DR (dose ratio) is the EC_{50} of agonist in the presence and absence of antagonist. The (pK_B) was calculated as the negative log of the K_B value. For specific protein content measured by Western blot, the levels of protein expression were normalized by the α-actin content. Scion Image software (National Institute of Health, USA) was used to calculate the optical density of each band and the noise of the membrane was cut off. Subsequent normalization was done by dividing the intensity of the band of interest by the value of the α-actin in the same well.

Statistical analyses were performed using the programme SigmaStat® (Systat Software, Point Richmond, CA, USA). All data are means ± standard error of the mean (s.e.mean) derived from (n) lung samples and statistical analyses on the curves, pEC_{50} and E_{max} values were performed using Two-way Repeated Measures (RM) ANOVA followed by Bonferroni *post hoc* test; Student's paired t test were used for receptor content comparison. A P-value <0.05 was considered statistically significant.

2.5. Compounds

Iloprost, beraprost, treprostinil, PGI_2, MRE-269, GW627368X, BWA868C, CAY10441 and AH6809 were purchased from Cayman Chemical Company (Ann Arbour, MI, USA). Treprostinil was also obtained from United Therapeutics Corporation (Silver Spring, MD, USA). RO3244019 (AGN230933) was a gift from Allergan, Inc. (Irvine, CA, USA). L-877499 [9-(4-chloro-benzyl)-8-methanesulfinyl-2,3,4,9-tetrahydro-1H-carbazol-1-yl]-acetic acid was a gift from Merck (Kirkland, Canada). ONO-AE3-237 (N-(p-alkoxy) benzoyl-2-methylindole-4-acetic acid) was a gift from Ono Pharmaceutical Co. (Osaka, Japan), UK. All these compounds were dissolved in ethanol at 0.1 mM and subsequent dilution were made in Tyrode's solution. Norepinephrine was purchased from Sigma Chemical Co.

3. Results

3.1. Comparison of the vasorelaxation induced by PGI_2 and its mimetics in human pulmonary vessels

The norepinephrine- (NE, 10 μM) induced contractions were similar in isolated HPA (1.07 ± 0.09 g, n = 40) and veins (1.44 ± 0.18 g, n = 35) derived from control lungs. Concentration-dependent relaxation curves of HPA and HPV preparations produced by PGI_2 and its mimetics (iloprost, treprostinil, beraprost and MRE-269) are shown in Fig. 1A and 1B. The pharmacological values derived from these curves with the statistical analyses are presented in Table 1. The relaxations induced by iloprost and treprostinil in both human pulmonary vessels are greater when compared to the other PGI_2 mimetics (Fig. 1A, 1B). Beraprost induced potent relaxations in HPA but exhibited contractile effects in HPV at concentrations higher than 0.1 μM. The vasorelaxations of HPA induced by iloprost and treprostinil were significantly different (Fig. 1A). The sensitivity (pEC_{50}) of HPA to iloprost was significantly higher (16 fold) than that of treprostinil (Table 1). However, this difference between iloprost and treprostinil sensitivities was significantly reduced in HPV (4 fold only; Table 1). On the other hand, the sensitivity of HPA to treprostinil was significantly lower than in HPV (see pEC_{50}, Table 1) and this difference in HPA *versus* HPV was not observed with iloprost.

3.2. Effects of IP antagonists on the relaxation induced by the PGI_2 mimetics

In HPA, the relaxations induced by treprostinil were completely inhibited (90–100%, n = 3; results not shown) in the presence of CAY10441 (1 μM). In contrast, incubation of HPV either with CAY10441 or RO3244019 (1 μM) only partially inhibited (29–32%) the relaxations induced by treprostinil (n = 2–4, data not shown).

3.3. Effects of DP antagonists on the relaxation induced by the PGI_2 mimetics

The relaxations induced by PGI_2 analogues (iloprost and treprostinil) in HPA were not affected by the presence of a DP antagonist (L-877499, 10 μM; Fig. 2A). The E_{max} and pEC_{50} values obtained with iloprost and treprostinil in the presence of L-877499 or BWA868C (another DP antagonist) were not significantly different from the respective control values (Table 2). In contrast, in HPV, the presence of L-877499 (10 μM) caused a concentration-related rightward shift of the treprostinil concentration–relaxation curves (Fig. 2B); the pEC_{50} and E_{max} values were significantly increased (Table 2). For this reason, a pK_B value (5.46 ± 0.18; n = 7) was calculated for L-877499 (10 μM) when the relaxations were induced by treprostinil. However, the relaxation induced by iloprost was unaffected in presence of this DP antagonist (Fig. 2B). In addition, lower relaxations induced by treprostinil were measured in presence of other DP-receptor antagonists BWA868C (1 μM, Table 2) or ONO-AE3-237 (10 μM, n = 2 data not shown) in HPV. Finally, when HPV were co-incubated with both RO3244019 and L-877499 a greater inhibition (60%, n = 2) of the maximal relaxation induced by treprostinil was detected in comparison to each treatment alone.

3.4. Effect of an EP_4 antagonist on the relaxation induced by the PGI_2 mimetics

The relaxations induced by the PGI_2 mimetics were not modified in the presence of the EP_4 antagonist GW627368X (1 μM; Fig. 2B) for both types of human pulmonary vessels. The pEC_{50} and E_{max} values obtained in the presence of GW627368X were not statistically different from the respective control values (see Table 2) obtained in arterial and venous preparations derived from the same human lung sample.

3.5. Western blot analyses

The presence of the different relaxant prostanoid receptors (IP and DP) was detected in both types of human pulmonary vessels by Western blot analyses (Fig. 3A and B). The protein content of each receptor was not statistically different in HPA as compared to HPV.

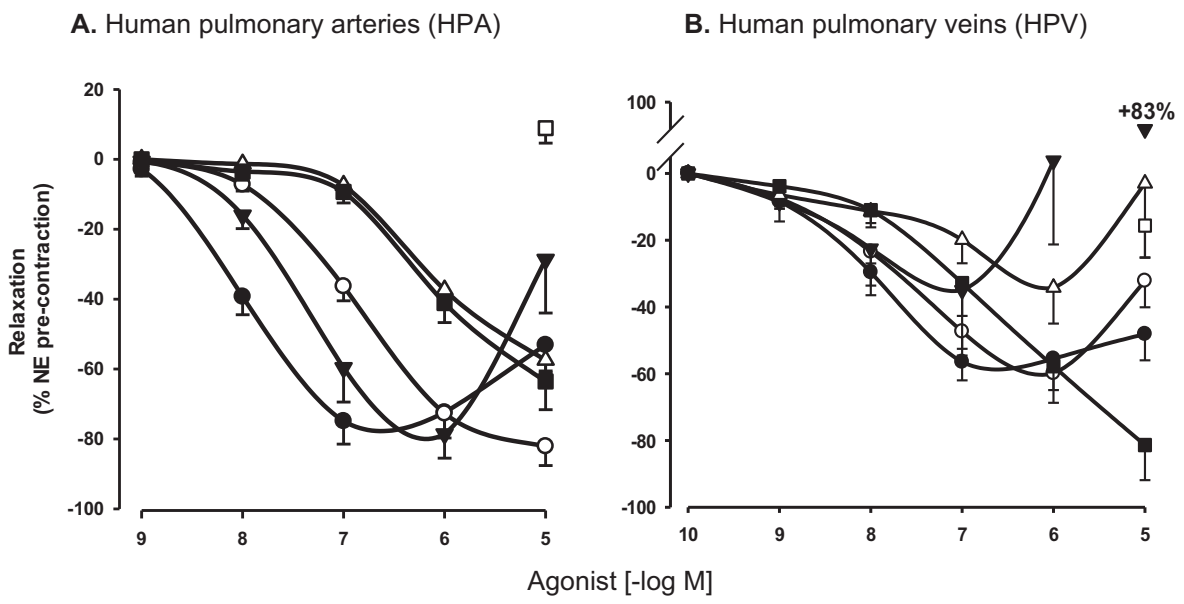

Fig. 1. Cumulative concentration–response curves induced by PGI_2 and its mimetics in human pulmonary arteries (A) and veins (B). The preparations were pre-contracted with norepinephrine (NE, 10 μM) and cumulative concentrations of PGI_2 and its mimetics (iloprost, treprostinil, MRE-269 and beraprost) were added into the organ baths. Responses are expressed as a percent of the NE (10 μM) induced contraction and values are means ± s.e.mean. (*n*) indicates the number of lung samples assessed. pEC_{50} and E_{max} values are indicated in Table 1 and in Section 3.

Table 1
Pharmacological values derived from the relaxation curves induced by PGI_2 and its mimetics in human pulmonary arteries and veins.

IP agonists	Human pulmonary arteries (HPA)			Human pulmonary veins (HPV)		
	pEC_{50} values	E_{max} (%NE)	(*n*)	pEC_{50} values	E_{max} (%NE)	(*n*)
Iloprost	7.94 ± 0.06	−83 ± 07	23	7.96 ± 0.11	−68 ± 04	19
Treprostinil	6.73 ± 0.08a	−88 ± 05	33	7.49 ± 0.10a,*	−67 ± 05*	28
Beraprost	7.13 ± 0.18a	−80 ± 07	7	7.93 ± 0.31a,*	−38 ± 18	5
PGI_2	<6.19 ± 0.12a,b	<−60 ± 03	5	6.86 ± 0.15a,b,*	−46 ± 14	4
MRE-269	<6.19 ± 0.09a,b,c	<−69 ± 07	15	<6.41 ± 0.22a,b,c	<−82 ± 13	9
Time control		+10 ± 05	7		−16 ± 10	3

The maximal responses (E_{max}) are expressed as percent of the norepinephrine (NE, 10 μM)-induced contraction and the half-maximum effective concentration (EC_{50}) values are presented. Values are means ± s.e.mean, (*n*) indicates the number of lung samples assessed. In a same type of vessel, a–c indicate values significantly different from the corresponding values obtained with iloprost, beraprost and treprostinil, respectively. *Indicates value significantly different from the corresponding value obtained in human pulmonary arteries. Significance is defined as $P < 0.05$, Two-way ANOVA.

Table 2
Effects of EP_4 and DP antagonists on the relaxations induced by PGI_2 mimetics in human pulmonary arteries and veins.

PGI_2 mimetics	Antagonist treatments	Human pulmonary arteries (HPA)			Human pulmonary veins (HPV)		
		pEC_{50}	E_{max} (%)	*n*	pEC_{50}	E_{max} (%)	*n*
Iloprost	Control	7.97 ± 0.08	−65 ± 04	8	7.99 ± 0.19	−69 ± 09	7
	GW627368X	7.61 ± 0.11	−75 ± 13	8	7.95 ± 0.18	−73 ± 11	7
	Control	8.09 ± 0.04	−101 ± 27	5	8.06 ± 0.28	−58 ± 06	4
	L-877499	7.94 ± 0.05	−75 ± 08	5	7.94 ± 0.08	−45 ± 10	4
Treprostinil	Control	6.67 ± 0.16	−84 ± 09	7	7.40 ± 0.17	−54 ± 11	7
	GW627368X	6.63 ± 0.17	−84 ± 10	7	7.56 ± 0.14	−56 ± 10	7
	Control	6.33 ± 0.26	−69 ± 13	5	7.47 ± 0.19	−67 ± 05	7
	L-877499	6.46 ± 0.31	−101 ± 11*	5	6.84 ± 0.20*	−51 ± 08	7
	Control				7.74 ± 0.35	−67 ± 08	5
	BWA868C				6.47 ± 0.37*	−52 ± 11	5
MRE-269	Control	<6.28 ± 0.19	<−67 ± 09	6	<6.42 ± 0.29	<−88 ± 22	5
	GW627368X	<6.17 ± 0.18	<−75 ± 18	6	<6.39 ± 0.38	<−131 ± 32	5

The human pulmonary vascular preparations were treated for 30 min with an EP4 antagonist: GW627368X (1 μM) or a DP antagonist: L-877499 (10 μM) or BWA868C (1 μM). The maximal relaxations (E_{max}) induced by the IP agonists (treprostinil, iloprost and MRE-269) are expressed as percent of the norepinephrine NE (10 μM), induced pre-contraction. The half-maximum effective concentration (EC50) values are presented. Values are means ± s.e.mean, obtained with paired preparations (control versus treated), (*n*) indicates the number of lung samples assessed. *Data significantly different ($P < 0.05$) from respective control values (Two-way RM ANOVA).

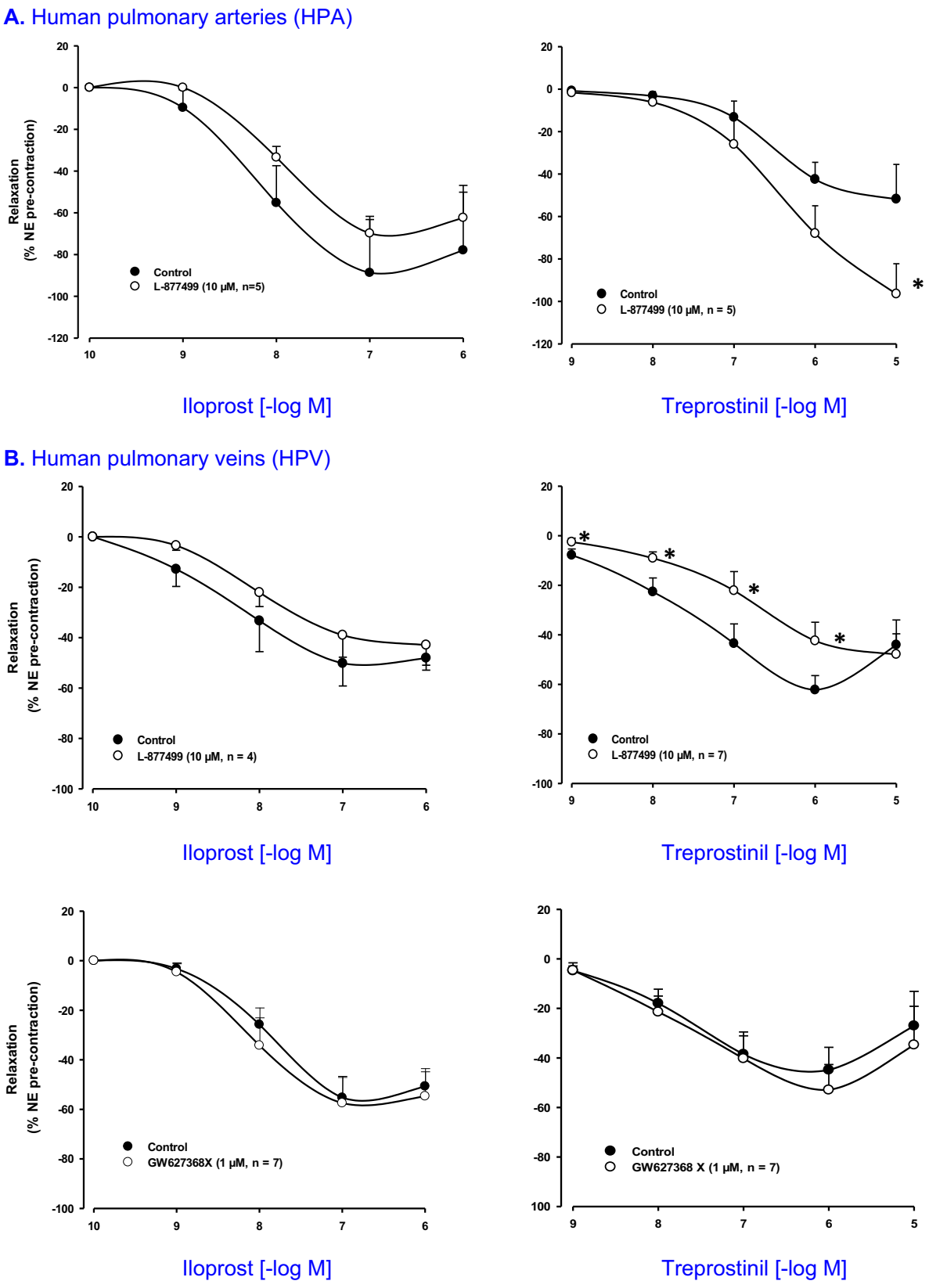

Fig. 2. Effect of the DP (L-877499; 10 µM) or EP_4 (GW627368X; 1 µM) antagonists on the relaxation induced by iloprost or treprostinil in human pulmonary arteries (A) and veins (B). Concentration–response curves induced by the indicated PGI_2 analogues were performed after an incubation period (30 min) with or without one antagonist. Responses are expressed as percent of the norepinephrine (NE, 10 µM)-induced pre-contraction. Values are means ± s.e.mean. (*n*) indicates the number of lung samples assessed. pEC_{50}, E_{max} and pK_B values are indicated in Table 2 or in Section 3. *Data significantly different from control values (Two-way RM ANOVA).

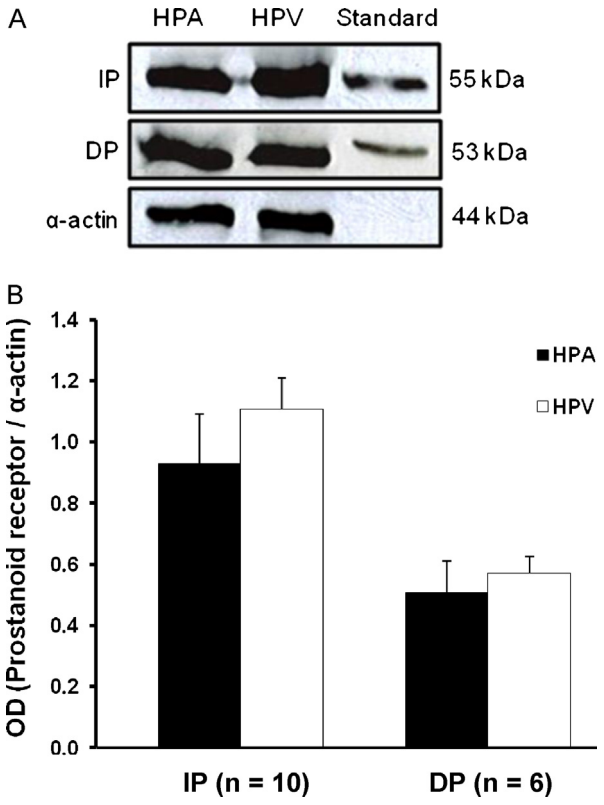

Fig. 3. IP- and DP- receptor expressions in human pulmonary arteries and veins. (A) Representative photograph of Western blot of IP and DP receptors. (B) Prostanoid receptors (IP and DP) density in human pulmonary arteries (HPA, $n = 6–10$) and veins (HPV, $n = 6–10$). Optical density values (OD, arbitrary units) are expressed as means ± s.e.mean.

4. Discussion

The results of this *in vitro* study demonstrate that iloprost and treprostinil are more potent vasorelaxant agonists in comparison to PGI_2, MRE-269 and beraprost in both types of human pulmonary vessels. The HPA are more sensitive (16 fold) to iloprost than to treprostinil where only the IP-receptor could be activated. In contrast, treprostinil and iloprost relaxed HPV to a similar extent, which is likely related to the additional effect of treprostinil activating the DP-receptor in this tissue.

In the present report, the different relaxations observed between iloprost and treprostinil in HPA could be explained mostly by the binding affinity and efficacy of these compounds for the IP-receptor. Whittle and collaborators [32] using human recombinant IP-receptor expressed in HEK-293 and CHO cells measured a greater binding affinity (8 fold) and a greater potency (5 fold) in cAMP assays with iloprost in comparison to treprostinil. This interpretation of the different relaxations observed between iloprost and treprostinil in HPA is quite simple, since previous studies have shown the IP-receptor to be the only prostanoid receptors implicated in the vasodilation of the HPA [10,11].

Our data on the vasodilator effect of treprostinil and iloprost in human pulmonary vessels could be compared with the study of Orie et al. (2013, POLM, same issue) performed in rat pulmonary vasculature. Their study showed that in rat pulmonary arteries, treprostinil is a more potent agonist than iloprost. However, a reversed potency of these agonists was found in our HPA. This discrepancy could be explained by the different vasoconstrictor agonists used in human (norepinephrine) and rat (U46619, TP receptor agonist) pulmonary arteries. In pulmonary veins (rat or human), both agonists (iloprost and treprostinil) were equipotent to induce vasorelaxation; however, the HPV are more sensitive to these compounds than rat pulmonary veins.

In the human pulmonary vessels, the expression of different prostanoid (EP_1-, EP_3-, EP_4-) receptors has been previously been evaluated by immunohistochemistry [24,33]. In the present report, the presence of IP- and DP- receptors was evaluated by Western blot analyses, and no significant difference between HPA and HPV was observed. PGI_2 and its mimetics (other than selexipag) do not act only on the IP-receptor, but rather, they have affinities for other prostanoid receptors [32,34–36]. The receptor selectivity of PGI_2 and its mimetics could greatly impact its control of human pulmonary vascular tone. In theory, activation of EP_4 and/or DP in the pulmonary veins could potentiate the vasorelaxation induced by IP-receptor activation. Whereas, activation of EP_1 and/or TP in veins, or EP_3 and/or TP in arteries, could counterbalance the vasorelaxation induced by activating the IP-receptor [13,19]. For example, that is the case in HPV, we have already shown that the relaxant effects of PGI_2 and PGE_1 are reduced by activation of EP_1-receptors present on smooth muscle cells [27,37]. That is also the case with iloprost which is able to induce EP_1 mediated contraction of HPV [25], in consequence, the HPV relaxations induced by iloprost are greater in presence of an EP_1 antagonist (AH6809; [11]) as compared to those induced in absence (present report). These results are supported by the high affinity values (1–4 nM) found for iloprost in binding studies with the human IP- and EP_1-receptors in comparison to the other prostanoid receptors subtypes [32,34].

In the present report, the DP-receptor antagonist (L-877499) significantly inhibited the vasorelaxation induced by treprostinil in HPV while the relaxation induced by iloprost was unaffected. Similarly, two other DP-receptor antagonists (BWA868C and ONO-AE3-237) inhibited the relaxation of HPV induced by treprostinil. The DP-receptor antagonists had no effect in HPA, where the concentration–response curves induced by treprostinil or iloprost were not modified in their presence. In a previous study we have described vasodilations mediated by DP-receptor activation in HPV while PGD_2 and a DP agonist (BW245C) did not relax the HPA [11]. Thus it would appear that the DP receptor found by Western blot analyses in HPA is not functional. Together, these results support the involvement of the IP- and DP- receptor subtypes during the vasodilations induced by treprostinil in HPV. In addition, our data are in accordance with a recent binding study of Whittle and collaborators [32]. This study showed that treprostinil had a greater affinity for the DP-receptor (230 fold) than iloprost with K_i values = 4.4 nM and 1016 nM, respectively. For this reason, in pulmonary veins, the similar vasorelaxations observed with iloprost and treprostinil illustrate the consequence of the greater binding affinity of treprostinil for the DP-receptor in comparison to iloprost [32]. This effect compensates for the lower affinity of treprostinil for the IP-receptor in comparison to iloprost.

In this study, the complete antagonism in HPA and the partial antagonism of the relaxations induced by treprostinil observed in presence of the IP antagonists (CAY10441 and RO3244019) support again a role for the DP-receptor in the relaxation induced by treprostinil in HPV. In a study conducted on isolated piglet saphenous veins, an EP_4-receptor antagonist (GW627368X, 10 μM) could inhibit (90%) the relaxation induced by iloprost and PGI_2 [38]. In addition, previous binding studies [32,34] showed that iloprost has a moderate affinity for the human EP_4-receptor (K_i = 212 nM). In the present report, the relaxations induced by iloprost in HPV (which contain a functional EP_4-receptor [24]) were not modified after incubation with GW627368X. Similar results were obtained using treprostinil and MRE-269 as relaxant agonists. These data suggest the absence of the EP_4-receptor activation by iloprost, treprostinil or MRE-269 in the HPV and do not support the hypothesis developed by Lai and collaborators [39] on the role of the prostanoid

EP_4-receptor in iloprost-mediated vasodilation during therapeutic use in human PAH.

The involvement of the EP_3-receptor subtype during the vasorelaxations induced by PGI_2 mimetics has been recently suggested [40–42]. Iloprost, beraprost and treprostinil (but not MRE-269) activate the "contractile" EP_3-receptor and reduce their vasodilatory capacity. These experiments have been performed in rat isolated arteries derived from tail or lung. Considering the moderate affinity value of iloprost (200 nM) and low affinity value of treprostinil (2500 nM) for the human EP_3-receptor, we can hypothesize that these inhibitory effects could be not detectable in studies on HPA relaxations. In addition, in isolated human vascular preparations (intercostal and mammary arteries [43,44]), where a functional (contractile) EP_3 receptor has been characterized, iloprost induced only dose-dependent relaxations.

The results (present report) obtained either with PGI_2 or iloprost on pre-contracted human pulmonary vessels are in accordance with those presented in previous studies from our group [11,27]. The pulmonary vessels were less sensitive to PGI_2 in comparison to iloprost, an effect probably due to the short half-life of PGI_2 which is more unstable than the other compounds and the high oxygen partial pressure in organ baths that may accelerate its degradation. Furthermore, the pEC_{50} values obtained in HPA with PGI_2 or iloprost are similar for each agonist in the presence (previous studies) or absence (present report) of a TP-antagonist. This comparison suggests that TP-receptors are not activated during the vasorelaxations induced by these agonists. On the contrary, the potent contractions induced by beraprost (concentrations greater than 0.1 μM) in human pulmonary vessels could be explained by activation of the TP-receptors. In one experiment these contractions were blocked by a TP antagonist (Bay u3405, data not shown). This TP-mediated effect of beraprost has also been previously demonstrated in isolated canine femoral veins and in guinea pig left atria [45,46].

The relaxations induced by PGI_2 presented in Fig. 1 and in our previous study [27] are significantly lower in the pulmonary veins as compared to the arteries. These results could account for the detrimental effect observed with epoprostenol in PVOD or PCH patients, where oedema formation is frequently observed [7,8]. One major cause of pulmonary oedema is the venous contraction and obstruction; for this reason oedema therapy during heart failure includes the use of pulmonary venous or mixed (arterial/venous) vasodilators like nitroprusside, nitroglycerin, nesiritide or sildenafil [47–50]. In this context, the potent vasorelaxations induced by iloprost and treprostinil in both types of pulmonary vessels could be a strong argument in favour of the use of these PGI_2 analogues in PVOD or PCH treatment and pulmonary oedema prevention. One case report from Hsu et al. [51], with iloprost-treatment of lung oedema after pulmonary thromboendarterectomy supports this hypothesis.

In conclusion, beside the anti-proliferative effects of these PGI_2 mimetics, they have potent vasodilator effects on isolated human pulmonary vessels. Our study provides strong evidence for the involvement of the DP-receptor in treprostinil-induced relaxation of the human pulmonary veins. Our data and a binding study [52] suggest that MRE-269 seems to be more selective for the human IP-receptor than the other prostanoid receptors but not a very potent relaxant agonist in our system. On the contrary, iloprost (due to its greater activity at the IP receptor) and treprostinil (due to its activity at both the IP and DP receptors in pulmonary veins) should be the most effective agonists to facilitate the pulmonary blood circulation in pulmonary arterial hypertensive patients, since these two agonists are able to potently relax both types of human pulmonary vessels, and consequently, to prevent pulmonary oedema formation.

Conflict of interest

This work was supported by a grant from United Therapeutics.

Acknowledgements

The authors would like to thank Dr. Takayuki Maruyama for providing the ONO compounds, Dr David Woodward for the gift of RO3244019 (AGN230933), Merck for L-877499 compound. We thank Elisabeth Brunet and Amina El Hilali from the Anapathology laboratory, CHU X. Bichat for their help.

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