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Association between Leflunomide and Pulmonary Hypertension

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Abstract

Rationale: Pulmonary hypertension (PH) has been described in patients treated with leflunomide.

Objectives: To assess the association between leflunomide and PH.

Methods: We identified incident cases of PH in patients treated with leflunomide from the French PH Registry and through the pharmacovigilance in Pulmonary Arterial Hypertension (VIGIAPATH) program between September 1999 to December 2019. PH etiology, clinical, functional, radiologic, and hemodynamic characteristics were reviewed at baseline and follow-up. A pharmacovigilance disproportionality analysis using the World Health Organization's global database was conducted. We then investigated the effect of leflunomide on human pulmonary endothelial cells. Data are expressed as median (min–max).

Results: Twenty-eight patients treated with leflunomide before PH diagnosis was identified. A total of 21 (75%) had another risk factor for PH and 2 had two risk factors. The median time between leflunomide

initiation and PH diagnosis was 32 months (1–120). Right heart catheterization confirmed precapillary PH with a cardiac index of $2.37 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ (1.19–3.1) and elevated pulmonary vascular resistance at 9.63 Wood Units (3.6–22.1) without nitric oxide reversibility. Five patients (17.9%) had no other risk factor for PH besides exposure to leflunomide. No significant hemodynamic improvement was observed after leflunomide withdrawal. The pharmacovigilance disproportionality analysis using the World Health Organization's database revealed a significant overrepresentation of leflunomide among reported pulmonary arterial hypertension–adverse drug reactions. *In vitro* studies showed the dose-dependent toxicity of leflunomide on human pulmonary endothelial cells.

Conclusions: PH associated with leflunomide is rare and usually associated with other risk factors. The pharmacovigilance analysis suggests an association reinforced by experimental data.

Keywords: translational medical research; antirheumatic agents; pharmacovigilance; pulmonary hypertension

Pulmonary hypertension (PH) is defined by an increase in mean pulmonary arterial pressure (mPAP) ≥ 25 mm Hg assessed by right heart catheterization (RHC) (1). PH is classified into five groups according to pathomechanisms and clinical management. Group 1 (pulmonary arterial hypertension [PAH]) includes precapillary PH with a normal pulmonary artery wedge pressure (PAWP) ≤ 15 mm Hg and pulmonary vascular resistance (PVR) > 3 wood units (WU). PAH can be idiopathic, heritable, drug induced, toxin induced, or associated with various conditions. The list of agents suspected of causing drug-induced PAH is evolving. Establishing causality of drug-induced PAH is a challenge for several reasons, including 1) variability in the temporal association of exposure (e.g., the median delay between exposure and PAH diagnosis for anorexigen agents ranges from a few months with fenfluramines to more than 10 years with benfluorex) (2, 3), 2) low incidence among exposed patients (e.g., methamphetamines and anorexigens) (4), 3) nonspecific clinical presentations with histologic lesions similar to idiopathic PAH (5), and 4) absent or incomplete resolution of PAH after medication cessation. Identification of medications that cause PAH can provide insights into new pathophysiological pathways (6) and can lead to the development of new models that improve our understanding of PAH (7). The classification of drugs and toxins associated with PAH was updated in the sixth World Symposium, with two categories describing the probability of causality: definite and possible (8). Leflunomide, suspected to cause drug-induced PAH on the basis of a few case reports and a case series, has been listed as a possible cause of PAH (8–11). Leflunomide is a synthetic disease-modifying antirheumatic

drug (12) used to treat rheumatoid arthritis (RA) for more than 20 years. It can be a first-line therapy if methotrexate is contraindicated or can be used as an adjunctive therapy if the disease activity remains high despite methotrexate (13, 14). Leflunomide is a prodrug metabolized to an immunoregulatory and antiproliferative molecule A77 1726 (also called teriflunomide), which is a noncompetitive inhibitor of dihydroorotate dehydrogenase. It has been shown to inhibit prostaglandin E2 through direct inhibition of cyclooxygenase-2 (15) and has a tyrosine kinase inhibitor (TKI) effect on sarcoma (src) at high doses (16). Some TKIs have been reported to induce or worsen PAH, as demonstrated with dasatinib and bosutinib (17–21). This study aims to describe all cases of PH associated with leflunomide to enlighten its potential role as a cause of PH and to explore potential toxicity on human pulmonary endothelial cells (h-PECs).

Methods

Patients

We reviewed all cases of precapillary PH in patients from the French PH Registry and the pharmacoVIGILance in Pulmonary Arterial Hypertension (VIGIAPATH) program who were treated with leflunomide. Precapillary PH was defined as mPAP ≥ 25 mm Hg (as recommended before the sixth World Symposium) with a PVR > 3 WU. Patients with isolated postcapillary PH (defined as mPAP ≥ 25 mm Hg, PAWP > 15 mm Hg, PVR < 3 WU) were excluded. The registry was established in accordance with French bioethics laws (Commission Nationale de l'Informatique et des Libertés #842063), and all patients gave their informed consent. During

this study, the French pharmacovigilance organization dedicated to drug-induced PH (VIGIAPATH) was consulted regarding two other cases from Canada and the United Kingdom, respectively, which were included in the analysis, with expressed patient consent.

Clinical, Functional, and Hemodynamic Evaluation

Clinical, functional, and hemodynamic evaluation, as well as screening for risk factors for PH, were assessed as previously described (22) and are detailed in METHODS E1.

All data concerning patient evaluations are recorded in the PH French registry. Evaluation of the two patients outside the French PH Network (United Kingdom and Canada) was done according to each center's standard of care. Effects of leflunomide cessation were assessed based on routine functional, clinical, and hemodynamic follow-up. We defined hemodynamic improvement as a reduction in PVR of at least 20% from the previous hemodynamic assessment (23). The items addressed in this study were chosen to comply with the latest Strengthening the Reporting of Observational Studies in Epidemiology statement (24).

Pharmacovigilance Disproportionality Analysis

We conducted a pharmacovigilance disproportionality analysis using the World Health Organization's (WHO) global database of individual case safety reports (ICSRs) (VigiBase), which includes spontaneously reported cases of adverse drug reactions from 130 countries. We evaluated the association between PAH and leflunomide and teriflunomide using the information component (IC). IC is a Bayesian disproportionality measure that compares

*These authors contributed equally to this work.

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observed and expected values of an adverse drug reaction to find an association with a drug. A lower boundary of the IC 95% credibility interval (IC_{0.25}) greater than zero is deemed significant (25).

In Vitro Evaluation of Leflunomide Effects on Human Pulmonary Endothelial Cells: Cell Culture MTS Assay and iCELLigence

h-PECs were cultured as previously described (26) and were used for the study between passages 3 and 6. All patients gave informed consent before this study. Cell viability was quantified using a tetrazolium reduction-based assay (MTS, CellTiter 96 Aqueous Nonradioactive Cell Proliferation Assay; Promega). After treatment for 1–48 hours, the MTS assay was performed. Cell index was acquired by iCELLigence system (ACEA Biosciences) with the real time cell-monitoring analysis system. At confluence, each well was treated with different concentrations of leflunomide or teriflunomide or dimethyl sulfoxide (DMSO) control. Complete methods are detailed in METHODS E1.

Statistical Analysis

Statistical analysis was performed using GraphPad (GraphPad) software. The results are expressed as a frequency and percentage for binary variables and median (min–max) for continuous variables. Before and after leflunomide cessation changes in continuous variables were compared using Wilcoxon signed-ranked test and functional class using the Friedman test. Multiple groups were compared using analysis of variance and then groups were compared using Tukey's multiple comparison test. A *P* value <0.05 was considered statistically significant.

Results

Patients Description

From approval of leflunomide in France in September 1999 to December 31, 2019, precapillary PH was diagnosed in 26 patients who had a previous or current history of treatment with leflunomide from the PH registry. Four patients were part of a previously published review (11) and two patients from Canada and the United Kingdom were added to this cohort.

Thus, 28 patients were included in this study, and their characteristics at diagnosis are shown in Table 1.

Table 1. Clinical, functional, hemodynamic, and biological data of pulmonary hypertension at diagnosis in 28 patients who were treated with leflunomide

	Number of Patients	Value
Demographics		
Age, yr, median (range)	28	58.5 (22–78)
Females, <i>n</i> (%)	28	14 (50)
Leflunomide exposure, median (range) (unless otherwise stated)		
Delay between initiation of leflunomide and PH diagnosis, mo	25	32 (1–120)
Dose of leflunomide, mg	21	20 (10–20)
Indication for leflunomide, <i>n</i> (%)		
Rheumatoid arthritis	—	22 (78.6)
Psoriatic rheumatism	—	2 (7.1)
Undifferentiated CTD	—	2 (7.1)
Sarcoidosis	—	1 (3.6)
Unspecified polyarthritis	—	1 (3.6)
Functional and biological parameters, <i>n</i> (%) (unless otherwise stated)		
NYHA functional class, I–II	28	5 (17.9)
NYHA functional class, III–IV	—	23 (82.1)
Increased BNP, >100 pg·ml ⁻¹ or NT-proBNP, >300 pg·ml ⁻¹	22	19 (86.4)
6MWD, m, median (range)	22	246.5 (0–522)
PaO ₂ in room air, mm Hg, median (range)	21	68.7 (42–89)
Pulmonary functional tests, median (range)		
FEV ₁ , % pred	27	78 (45–129)
FVC, % pred	25	87 (43–136)
FEV ₁ /FVC	24	0.74 (0.43–0.91)
TLC, % pred	25	77 (41–117)
DL _{CO} , % pred	23	41 (14–77)
Hemodynamic parameters, median (range) (unless otherwise stated)		
mPAP, mm Hg	28	44 (28–79)
PAWP, mm Hg	28	8.5 (2–23)
RAP, mm Hg	27	8 (0–28)
CI, L·min ⁻¹ ·m ⁻²	28	2.37 (1.19–3.1)
PVR, wood units	28	9.63 (3.64–22.08)
TPR, wood units	28	11 (4.5–23.9)
Acute vasodilator response, <i>n</i> (%)	20	0

Definition of abbreviations: % pred = percentage of predicted value; 6MWD = 6-minute-walk distance; BNP = brain natriuretic peptide; CI = cardiac index; CTD = connective tissue disease; DL_{CO} = diffusing capacity for carbon monoxide corrected for hemoglobin concentration; FEV₁ = forced expiratory volume in 1 second; FVC = forced vital capacity; mPAP = mean pulmonary artery pressure; NT-proBNP = N-terminal probrain natriuretic peptide; NYHA = New York Heart Association; PaO₂ = partial arterial pressure of oxygen in blood; PAWP = pulmonary artery wedge pressure; PH = pulmonary hypertension; PVR = pulmonary vascular resistance; RAP = right atrial pressure; TLC = total lung capacity; TPR = total pulmonary resistance.

Twenty-three patients (82.1%) had at least one other risk factor for PH, with two having more than one risk factor for PH. Nine patients had a disease associated with PAH (1 patient had a congenital heart disease [1 cm atrial septal defect], two had portal hypertension, four had connective tissue disease [CTD], and two were exposed to another medication definitely associated with PAH). One patient had mixed precapillary and postcapillary PH. Nine patients had fibrosing interstitial lung disease (ILD). Three had chronic thromboembolic PH (CTEPH) and

three had diseases from group 5 (one sickle cell disease, one microscopic polyangiitis, and one sarcoidosis). Fifteen patients (53.6%) had a sign of interstitial pulmonary disease—10 patients with RA (9 patients classified as group III PH associated with ILD and 1 with PH associated with microscopic polyangiitis), 4 patients treated with leflunomide for another etiology (1 for sarcoidosis and 3 with CTD) and one patients without risk factors, who had debuting interstitial disease deemed insufficient to explain PH.

Table 2. Clinical, functional, and hemodynamic data at diagnosis of five patients without risk factors for pulmonary hypertension

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Demographics and treatment exposure					
Age at diagnosis, yr	40	71	63	69	65
Sex	F	F	F	F	M
Delay between initiation of leflunomide and PH diagnosis, mo	35	116	79	24	13
Delay between initiation of leflunomide and first symptoms, mo	33	113	70	7	13
Functional and biological parameters					
NYHA functional class	3	4	4	3	4
NT-proBNP, ng·L ⁻¹	1,642	4,109	890	319	23,442
6MWD, m	408	132	0	300	0
Hemodynamic parameters					
RAP, mm Hg	6	0	3	7	15
mPAP, mm Hg	52	43	44	45	47
PAWP, mm Hg	8	5	6	9	12
CI, L·min ⁻¹ ·m ⁻²	1.83	1.6	1.2	1.19	1.4
PVR, wood units	12.1	14.5	20.5	17	12.9
Acute vasodilator response	No	No	No	No	—
Pulmonary function tests					
FEV ₁ , % pred	83	83	85	86	68
FEV ₁ /FVC	0.79	0.50	0.72	0.63	0.43
FVC, % pred	87	87	—	108	120
TLC, % pred	77	—	—	100	117
D _{LCO} , % pred	73	77	—	67	14
Treatment initiated					
PAH-specific therapy	ERA + PDE5i	ERA + PDE5i	PDE5i + IVP	PDE5i	IVP*

Definition of abbreviations: % pred = percentage of predicted value; 6MWD = 6-minute-walk distance; CI = cardiac index; D_{LCO} = diffusing capacity for carbon monoxide; ERA = endothelin receptor antagonist; F = female; FEV₁ = forced expiratory volume in 1 second; FVC = forced vital capacity; IVP = intravenous prostacyclin; M = male; mPAP = mean pulmonary artery pressure; NT-proBNP = N-terminal probrain natriuretic peptide; NYHA = New York Heart Association; PAWP = pulmonary artery wedge pressure; PDE5i = phosphodiesterase type 5 inhibitors; PH = pulmonary hypertension; PVR = pulmonary vascular resistance; RAP = right atrial pressure; TLC = total lung capacity.

*Patient 5 developed pulmonary edema in the context of suspected pulmonary venoocclusive disease after initiation of oral dual therapy.

These risk factors are presented in Table E1 in the online supplement. Four of the five patients without a risk factor for PH were females with a median age of 65 years (40–71) (Table 2).

Leflunomide Exposure

Leflunomide was indicated mainly for RA with a daily dose of 20 mg for 85.7% with a median delay between initiation of leflunomide to PH diagnosis of 31.5 months (1–120) (Table 1). Three of the 28 patients had symptoms of PH preceding the initiation of leflunomide, all of whom had another risk factor for PH. For the patients who developed symptoms after leflunomide exposure, the median delay between initiation of treatment and symptoms was 33.5 months (2.5–112).

Other Medication Exposures

The older and concurrent treatments received for the same indication as leflunomide were recorded for 24 patients as well as treatment received after leflunomide's cessation (Table E2). Cyclophosphamide, as an alkylating agent, was the only treatment considered a possible drug associated with PAH (27). It was

used in two patients for ILD treatment several years before the PH diagnosis and then used as a treatment for the associated CTD in one patient. No worsening of PH after cyclophosphamide treatment was recorded in this patient.

Characteristics of PH at First Evaluation

At diagnosis, almost all patients had marked functional impairment with New York Heart Association (NYHA) functional class of II, III, and IV in 5, 12, and 11 patients, respectively. Hemodynamic parameters of patients are presented in Table 1, showing severe impairment with a median cardiac index of 2.37 L·min⁻¹·m⁻² (1.19–3.1). A nitric oxide reversibility test was performed on 20 of 28 patients and none were responders (Table 1). Among the 23 patients with risk factors for PH, 17 were treated with PAH-specific medication including 12 patients with monotherapy, 3 with dual oral therapy, and 2 with triple therapy.

The five patients without risk factors for PH had severe functional impairment as all were either NYHA class III or IV. They

presented with severe PAH with a markedly reduced cardiac index of 1.4 L·min⁻¹·m⁻² (1.19–1.83) and a high PVR of 14.5 WU (11.1–20.5) and were all treated with PAH-specific medications (Table 2).

Follow-Up and Outcome

Follow-up data were available for all patients. As of December 31, 2019, the median follow-up was 46.9 months (2–146.8) and 12 of 28 patients had died. The causes of death were refractory heart failure in seven, cancer in one, mesenteric ischemia in one, and an unknown cause in three. Two of the five patients with no risk factors for PH died of refractory right heart failure despite leflunomide cessation and PAH vasodilator therapy including an intravenous prostacyclin. The estimated survival rates were 85.7%, 95% confidence interval (CI) [94.4; 66.3] and 77.5%, 95% CI [86.1; 50.7] at 1 and 3 years respectively (Figure 1).

Evolution after Leflunomide Cessation

There were data for 14 patients who had leflunomide stopped either at diagnosis or during the follow-up. For six patients, leflunomide cessation occurred together with

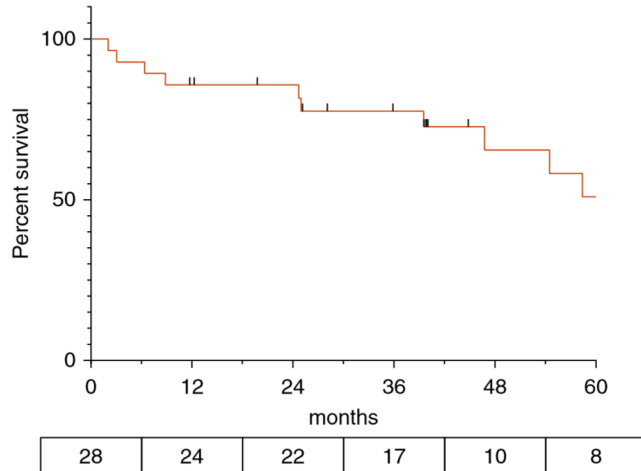


Figure 1. Survival curve of 28 patients with pulmonary hypertension who were treated with leflunomide. The line represents the survival curve: each step down is an event of mortality and each dot on the line represents a censored subject without event. The numbers below the x-axis represent the number of patients at risk.

the addition of PAH therapy. Therefore, we analyzed the functional and hemodynamic effects of leflunomide cessation in the remaining eight patients who did not receive PAH-specific treatment (Table 3). Despite a reduction of more than 20% in PVR for four patients, this improvement was not significant in the untreated subgroup of eight patients (Figure 2A). There was no difference in NYHA functional class after leflunomide cessation (Figure 2B). Four patients had a 6-minute walk

distance test measured before and after leflunomide cessation without significant improvement after leflunomide cessation (Figure 2C).

In patients without other PH risk factors ($n = 5$), two had hemodynamic improvement. One had a 65% reduction in PVR with dual oral therapy initiation, which is consistent with the improvement seen in other studies (28). The other had a 28% reduction in PVR after leflunomide cessation on stable dual therapy.

Results of Pharmacovigilance Disproportionality Analysis

Among the 18,521,083 ICSRs reported in VigiBase in January 2019, 7,294 PAH cases were reported. Thirty cases, of which 83% were issued from France, were related to leflunomide and one was related to teriflunomide. Pharmacovigilance disproportionality signals were significant for leflunomide ($IC_{025} = 1.35$) but not for teriflunomide ($IC_{025} = -6.31$). Twenty-one of the reported patients with PAH were female and eight were male (two had missing sex specification); median age was 57 years (22–71). The leflunomide dose was 20 mg per day in 19 cases, 10 mg per day in 5 cases (6 were missing), and teriflunomide dosage was 14 mg per day. The median time to PAH onset was 55.9 months (2.45–178.9) after drug introduction. Seventeen patients recovered from PAH, nine did not, and one patient died (three were missing outcome data). Reports are detailed in Table E3.

Effect of Leflunomide on Human Pulmonary Endothelial Cells

Using an MTS cell toxicity assay, we demonstrated that leflunomide had a direct toxic effect on h-PECs at a concentration of 500 μM (Figure 3A), highlighted by the reduction in cell metabolism 1 hour and 6 hours after incubation compared with DMSO control and other concentrations ($P < 0.001$).

Table 3. Clinical, functional, and hemodynamic data of eight patients before and after leflunomide cessation

	Number of Patients	Before Leflunomide Cessation	After Leflunomide Cessation	P Value
Time between assessments, mo, median (range)	8	10.5 (4.7–22.3)		—
Functional and biological parameters				
NYHA functional class, n (%)	8	—		0.56
I–II	—	5 (62.5)	6 (75)	—
III–IV	—	3 (37.5)	2 (25)	—
6MWD, m, median (range)	4	479 (348–490)	478 (270–538)	0.87
NT-proBNP $\text{ng}\cdot\text{L}^{-1}$, median (range)	6	128.5 (55–3,600)	79.5 (39–3,577)	0.06
Hemodynamic parameters, median (range)				
RAP, mm Hg	7	4.5 (3–12)	8 (0–18)	0.07
PAWP, mm Hg	8	8.5 (4–14)	8.5 (5–14)	0.88
mPAP, mm Hg	8	41.5 (28–59)	39.5 (24–59)	0.84
CI, $\text{L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$	8	2.58 (1.81–4.97)	3.1 (1.92–4)	0.61
PVR, wood units	8	6.82 (2.79–12)	5.55 (3.2–12.2)	0.31

Definition of abbreviations: 6MWD = 6-minute-walk distance; CI = cardiac index; mPAP = mean pulmonary artery pressure; NT-proBNP = N-terminal probrain natriuretic peptide; NYHA = New York Heart Association; PAWP = pulmonary artery wedge pressure; PVR = pulmonary vascular resistance; RAP = right atrial pressure.

Comparisons between groups were analyzed by the Wilcoxon signed-rank test for continuous variables and the Friedman test for NYHA functional class.

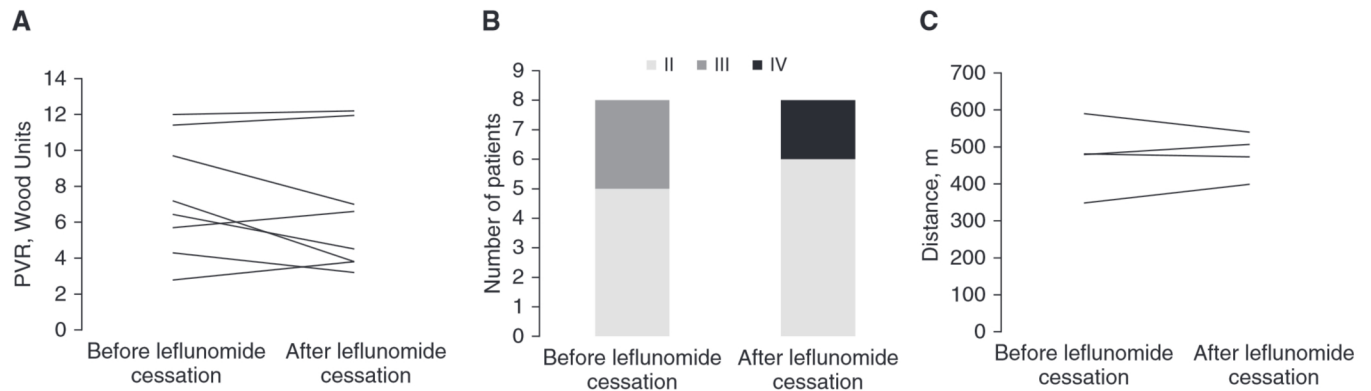


Figure 2. Changes in pulmonary vascular resistance (PVR), functional class, and 6-minute-walk distance test after leflunomide cessation in patients who did not receive pulmonary arterial hypertension therapies. (A) An invasive hemodynamic evaluation after cessation of leflunomide was available for eight patients after a median delay of 10.5 months (min–max, 4.7–22.3 mo), with no significant improvement observed in PVR ($P=0.31$). (B) Functional capacity changes after a median delay of 10.5 months (min–max, 4.7–22.3 mo) after leflunomide cessation. No improvement in New York Heart Association functional class was observed ($P=0.56$). Two patients went from functional class III to IV and one from III to II. The five patients in functional class II remained stable. (C) Evolution in 6-minute-walk distance was available in four of the eight patients after leflunomide discontinuation and showed no significant improvement in comparison with baseline values ($P=0.87$).

This effect of leflunomide on h-PECs remained at 24 and 48 hours and was seen at the lower concentrations, revealing either a toxic or antiproliferative effect of leflunomide. Teriflunomide, however, induced significant cell toxicity at 1 hour only at a concentration of 500 μM ($P < 0.01$) compared with DMSO control. Its toxic or antiproliferative effect at 24 and 48 hours remained only for the highest concentration of teriflunomide (Figure 3B).

Using the iCELLigence RTCA system, we showed that 1 hour after incubation leflunomide induced a significant concentration-dependent decrease in h-PEC cell index (defined by relative impedance measured between the wells) at a concentration of 500 μM and 125 μM compared with 5 μM ($P < 0.05$) and DMSO controls ($P < 0.01$) (Figure 3C). Teriflunomide induced significant toxicity 5 hours after treatment at a concentration of 500 μM compared with DMSO and teriflunomide 5 μM and 125 μM ($P < 0.05$). There was no significant difference between teriflunomide 5 μM and DMSO (Figure 3D).

Discussion

Through the French PH Registry and VIGIAPATH program, we identified 28 patients with PH related to leflunomide. We found another risk factor for PH in 82.1% of our study population and idiopathic PAH associated with leflunomide was rare (17.9%). These data suggest that any link between leflunomide and PAH is either low risk or

fortuitous. However, despite the presence of another risk factor for PH in most patients, we suspect a potential pulmonary vascular toxicity of leflunomide in patients with preexisting disease, as has been shown with interferon-associated PAH in patients with portal hypertension (23). In favor of this hypothesis, our analysis of the VigiBase pharmacovigilance database found a significant disproportionality signal for leflunomide, suggesting an overrepresentation of leflunomide in patients with PH. We observed a trend toward hemodynamic improvement in a small subgroup of patients after leflunomide discontinuation, which is suggestive, although not strongly supportive, of a causal role. Finally, we demonstrated preliminary results showing a direct toxic effect of leflunomide *in vitro* on pulmonary endothelial cells using two complementary approaches.

Leflunomide is a multipotent drug that could lead to PAH through multiple pathways (Figure 4). First, it has TKI activity, mainly through inhibition of the src pathway (29). Src is implicated in the pathogenesis of PAH through modulation of potassium channels that mediate control of pulmonary vascular tone (30) and seems to play a central role in TKI-related PAH (31). Several TKIs such as bosutinib, ponatinib, and dasatinib have been reported to cause PAH (17, 18, 21, 32, 33). Dasatinib was categorized as a definite cause of PAH in the sixth World PAH Symposium after being associated with a minimum incidence of PAH of 0.45% in dasatinib-treated patients, and with hemodynamic

reversibility in almost two-thirds of patients after its cessation (8, 32). We also observed partial reversibility in PVR for 50% of patients who had leflunomide cessation alone. However, this was not statistically significant, potentially due to lack of power. Second, leflunomide inhibits COX-2 (cyclooxygenase-2) through its active metabolite A77 17126, which, when knocked down, has been shown to enhance hypoxia-induced vasoconstriction and pulmonary vascular remodeling in mice (34). Third, we demonstrated that leflunomide had dose-dependent toxicity against h-PECs. It has previously been demonstrated that teriflunomide has an antiproliferative effect on cultured human umbilical vein endothelial cells (35). The mechanism of this toxicity is not fully understood. In hepatocytes, leflunomide (and to a lesser extent its active metabolite) has dose-dependent cellular toxicity mediated by endoplasmic reticulum stress, a pathway related to EIF2 α (Eukaryotic translation initiation factor 2 subunit α) (36), and direct mitochondrial toxicity (37). The high variability in leflunomide concentrations, from 1.9 mg/L to 156.6 mg/L (or 7–580 μM) (38), might favor this pathway of toxicity in PH and potentially explain the scarcity of events. This also links the potential pathogenesis of leflunomide-associated PAH to dasatinib-associated PAH through mitochondrial dysfunction (19).

Of note, the majority of patients had been treated with leflunomide for RA, a CTD noted in the European Respiratory Society/European Society of Cardiology 2015 guidelines as a cause of PAH associated with

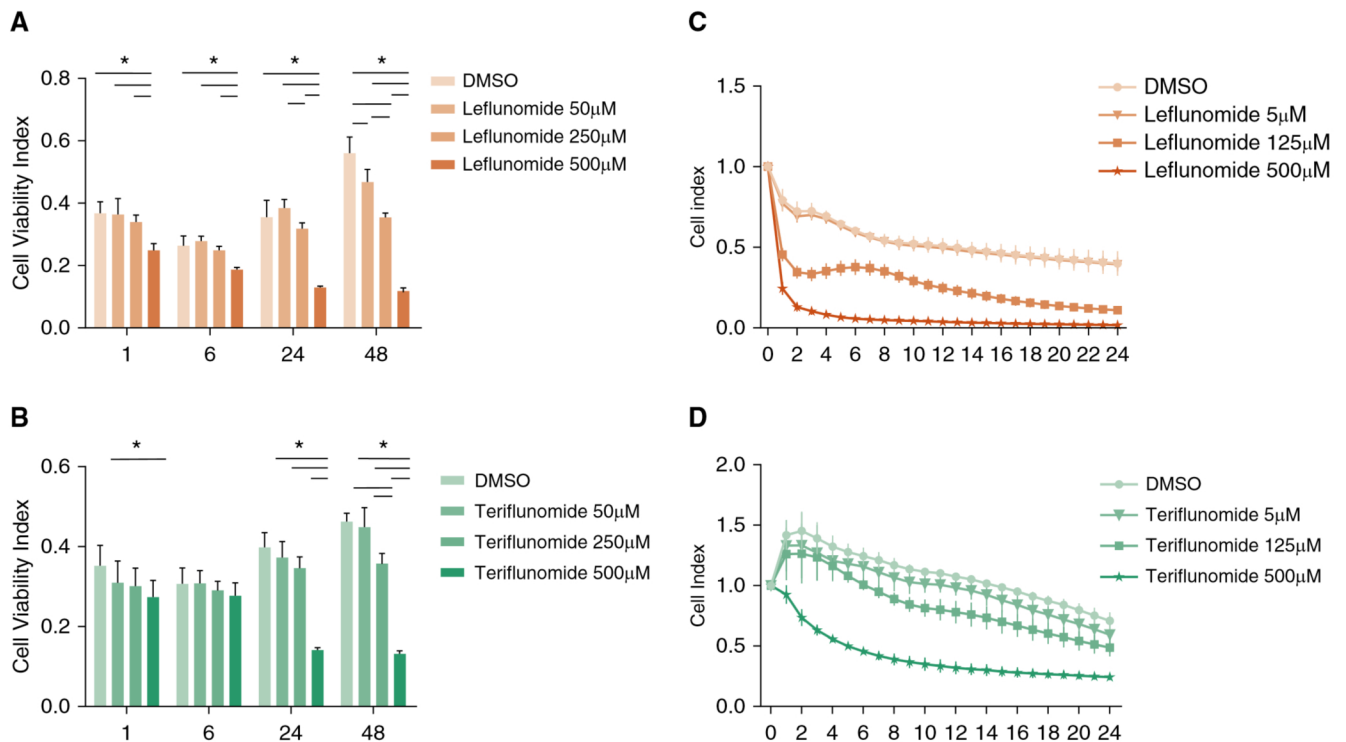


Figure 3. Leflunomide and teriflunomide induce human pulmonary endothelial cell toxicity. (A and B) Cell viability index measured by MTS reduction assay showing dose-dependent toxicity of leflunomide and teriflunomide against $n=6$ cultures of human pulmonary endothelial cells. (C) Cell viability measured using real-time cell analysis showing dose-dependent toxicity of leflunomide at 1 hour after incubation of treatment compared with dimethyl sulfoxide (DMSO) ($n=4$ for each condition). (D) Cell viability measured using real-time cell analysis showing dose-dependent toxicity of teriflunomide at 5 hours after incubation of treatment compared with DMSO ($n=3$ for DMSO and $5\ \mu\text{M}$; $n=4$ for $125\ \mu\text{M}$ and $500\ \mu\text{M}$). $*P<0.05$ between the groups on each side of the horizontal bars. MTS = (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium).

CTD, although it is not regarded as a common cause of group 1 PAH (1). An elevation of systolic pulmonary artery pressure was found in 10–31% of patients with RA; however, these measures were noninvasive and thus strongly limited by the lack of a gold-standard definition of PH requiring RHC (39, 40). In a large population of patients ($>5,000$) with precapillary PH confirmed by RHC from the French PH Registry, the prevalence of RA was 0.35% (95% CI [0.23–0.54]) in the PH population and 0.58% (95% CI [0.30–1.11]) in idiopathic PAH, comparable to the prevalence of RA in the general population (22). These data do not support a strong and specific association between precapillary PH and RA. Based on this last study, we chose not to consider RA as a definite risk factor for PH. However, its participation in PH in our patients cannot be excluded and leflunomide could play a role as a second trigger in the population at risk. The presence of ILD may play an aggravating role of PH associated with leflunomide because 53.7% of patients had signs of ILD on computed tomography scan. The COX-2 inhibition of leflunomide may

favor the association between PH and ILD through enhanced vascular remodeling.

The estimated survival rate was 85.7% (95% CI [94.4; 66.3]) and 77.5% (95% CI [86.1; 50.7]) at 1 and 3 years, respectively, seemingly altered compared with 93% at 1 year in a Canadian Cohort of PAH associated with RA (41), and approaches the 87% and 76% at 1 and 3 years, respectively, for incident idiopathic, heritable, and anorexigen-associated PAH (42). The increased mortality in our study compared with the Canadian cohort may be partially explained by more severe PH at diagnosis with 82.1% WHO class III/IV versus 39% in their cohort and more altered median 6-minute walk distance of 256 m versus 440 m in their study. This estimation is based on few prevalent cases, which tend to bias the result (42), and is not only composed of group I PAH.

Concerning the antecedent drugs received by patients, none are known to be associated with PAH other than cyclophosphamide. Adalimumab and infliximab are not associated with PAH but can be linked to PAH through ANCA-related vasculitis or systemic lupus erythematosus

(43–45). Rituximab and tocilizumab are currently under investigation as treatment of PAH (46). Hydroxychloroquine has been known to induce cardiac toxicity; however, its relation to PAH has never been established. A recent literature review described PH related to hydroxychloroquine toxicity in 3.9% of cases (47). These five cases were predominantly associated with risk factors for postcapillary PH and all had marked left heart dysfunction except for one case of precapillary PH with confounding factors (48–51).

Despite the multiple plausible mechanisms of leflunomide-associated PAH, cases remain rare for a drug with a market authorization for over 20 years and have been widely prescribed in a relatively common disease. In cases with confirmed precapillary PH, leflunomide should be stopped with close clinical and hemodynamic follow-up and the addition of PAH treatment should be discussed in the most severe cases. This strategy may help elucidate the potential reversibility of PH. Lack of PH reversibility on discontinuation of leflunomide would not discount a causal role, as this has been seen in

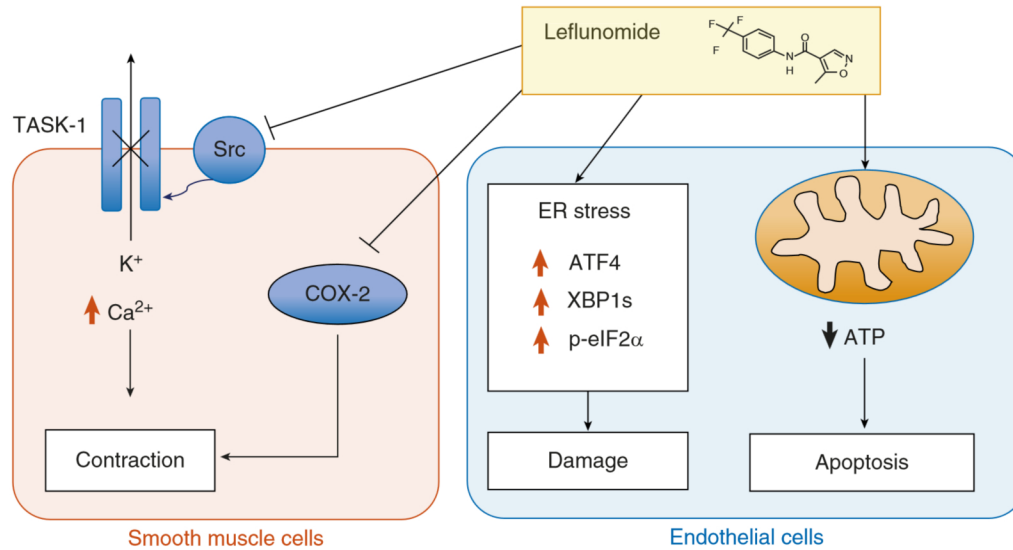


Figure 4. Potential pathophysiological mechanism for leflunomide-associated pulmonary arterial hypertension. Adapted from data from References 17, 19, 29, 36, and 37. ATF4 = activating transcription factor 4; ATP = adenosine triphosphate; Ca^{2+} = calcium; COX-2 = cyclooxygenase-2; ER = endoplasmic reticulum; K^+ = Potassium; p-eIF2 α = phosphorylated eukaryotic translation initiation factor 2 subunit 1; Src = Sarcome; TASK-1 = TWIK-related acid sensitive potassium channel-1; XBP1s = spliced X-box binding protein-1.

anorexigen drugs (52), nor does it exclude an aggravating role. However, the presence of reversibility would favor either a causal or aggravating role in PH pathogenesis. A potential aggravating role of leflunomide in PH, such as that demonstrated with interferon related PH (23), could not be investigated by our study. Only one patient in the registry had leflunomide initiated after their PH diagnosis and was not included in our study.

Concerning teriflunomide, only one patient was identified in the French registry with a clear risk factor (BMPR2 mutation).

The main limitation to this study is the pharmacovigilance-driven and retrospective nature, which carries the risk of underreporting. This bias would tend to underestimate the association between leflunomide and PH due to preferential reporting of the most severe and unusual cases in the pharmacovigilance program. Another potential bias is the lack of systematic hemodynamic evaluation after leflunomide cessation alone. In addition, the concurrent use of PAH-specific treatments could

confound the effect of leflunomide cessation on objective parameters.

Conclusions

The majority of patients exposed to leflunomide had other identified risk factors for PH. The association of PAH with leflunomide is rare, which favors a low-risk or fortuitous association. However, its potential role as a secondary trigger of PH via multiple possible pathways is plausible, based on our pharmacovigilance database analysis and demonstration of its toxic effect on human pulmonary endothelial cells. Furthermore, the observation that a subgroup of patients had trends to hemodynamic improvement with discontinuation alone favors the hypothesis that leflunomide could have direct pulmonary vascular toxicity. In patients with suspected PH on leflunomide, we recommend RHC to confirm the diagnosis and hemodynamic profile together with a systematic workup to exclude other risk factors for PH. Withdrawal of leflunomide should be considered to reinforce the diagnosis, but this should be done

in parallel with PAH therapies in severe cases. Close case follow-up with drug level monitoring and further experimental data may be of interest to understand the underlying mechanisms of leflunomide-associated PAH.

Author disclosures are available with the text of this article at www.atsjournals.org.

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