Retrospective Review of Combined Sirolimus and Simvastatin Therapy in Lymphangioleiomyomatosis

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BACKGROUND: Combined simvastatin and sirolimus therapy reduces tuberous sclerosis complex 2-null lesions and alveolar destruction in a mouse model of lymphangioleiomyomatosis (LAM), suggesting that therapy with both drugs may benefit patients with LAM.

METHODS: To determine whether simvastatin changed the prevalence of adverse events or altered the therapeutic effects of sirolimus, we recorded adverse events and changes in lung function in patients with LAM treated with simvastatin plus sirolimus ($n = 14$), sirolimus alone ($n = 44$), or simvastatin alone ($n = 20$).

RESULTS: Sirolimus-related adverse events in the simvastatin plus sirolimus and sirolimus-only groups were 64% and 66% for stomatitis, 50% and 52% for diarrhea, 50% and 45% for peripheral edema, 36% and 61% for acne, 36% and 30% for hypertension, 29% and 27% for proteinuria, 29% and 27% for leukopenia, and 21% and 27% for hypercholesterolemia. The frequency of simvastatin-related adverse events in the simvastatin-only and simvastatin plus sirolimus groups were 60% and 50% for arthralgias and 35% and 36% for myopathy. Before simvastatin plus sirolimus therapy, FEV_1 and diffusing capacity of the lung for carbon monoxide (DLCO) yearly rates of change were, respectively, -1.4 ± 0.2 and $-1.8 \pm 0.2\%$ predicted. After simvastatin plus sirolimus therapy, these rates changed to $+1.2 \pm 0.5$ ($P = .635$) and $+0.3 \pm 0.4\%$ predicted ($P = .412$), respectively. In 44 patients treated with sirolimus alone, FEV_1 and DLCO rates of change were -1.7 ± 0.1 and $-2.2 \pm 0.1\%$ predicted before treatment and $+1.7 \pm 0.3$ and $+0.7 \pm 0.3\%$ predicted after treatment ($P < .001$).

CONCLUSIONS: Therapy with sirolimus and simvastatin does not increase the prevalence of drug adverse events or alter the therapeutic effects of sirolimus.

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ABBREVIATIONS: DLCO = diffusing capacity of the lung for carbon monoxide; GTP = guanosine triphosphate; GTPase = guanosine triphosphatase; LAM = lymphangioleiomyomatosis; mTOR = mechanistic target of rapamycin; Rheb = Ras homolog enriched in brain; TSC = tuberous sclerosis complex

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Lymphangiomyomatosis (LAM) is a multisystem disease affecting predominantly women and is characterized by cystic lung destruction, abdominal angiomyolipomas, and lymphatic tumors (eg, lymphangiomyomas).¹⁻³ The clinical and pathologic features of LAM result from proliferation of a neoplastic LAM cell that has characteristics of both smooth muscle cells and melanocytes.^{3,4} LAM associated with tuberous sclerosis complex (TSC) and the sporadic form of LAM are both caused by mutations in the *TSC1* or *TSC2* suppressor genes.⁵⁻⁷ *TSC1* and *TSC2* encode hamartin and tuberin, two proteins that form a cytosolic complex acting upstream of the intracellular serine/threonine kinase mechanistic target of rapamycin (mTOR), which mediates growth factor, energy, and stress signaling and regulates cell growth and proliferation.⁸⁻¹⁰ Two complexes involving mTOR have been described¹¹: mTORC1, which contains raptor (regulatory-associated protein of mTOR), and mTORC2, which contains rictor (rapamycin-insensitive companion of mTOR).^{11,12} Regarding regulation of mTORC1, tuberin acts as a GAP (guanine triphosphatase [GTPase]-activating protein) for the guanine nucleotide-binding protein Ras homolog enriched in brain (Rheb), promoting the formation of inactive Rheb-guanosine diphosphate from active Rheb-guanosine triphosphate (GTP).^{9,10} Inhibition of *TSC1/2* by growth factor stimulation inhibits GAP activity and promotes accumulation of active Rheb-GTP.^{9,10} Rheb-GTP stimulates mTORC1, which phosphorylates ribosomal S6 kinase and eukaryotic initiation factor 4E-binding protein, leading to enhanced translation and protein synthesis.^{9,10,13}

Sirolimus and everolimus, two immunosuppressant compounds, form a complex with FK506-binding protein-12, which binds and inhibits mTORC1.^{14,15} In clinical studies, sirolimus and everolimus have been shown to be effective in decreasing the size of renal angiomyolipomas in patients with TSC and sporadic LAM,¹⁶⁻¹⁸ improving and stabilizing lung function while reducing the size of chylous effusions and abdominal lymphangiomyomas in patients with LAM^{19,20} and reducing the size of giant cell astrocytomas in patients with TSC.^{21,22} Although mTORC1 is acutely sensitive to sirolimus, in some cases, mTORC2 is only sensitive to prolonged sirolimus exposure.^{23,24} Experimental data, however, have shown that both mTORC1 and mTORC2 are necessary for TSC2-dependent cell proliferation and survival.²⁵ In TSC-null and human LAM-derived cells, RhoGTPase activity was required for cell proliferation and survival.²⁵ In the absence of TSC2, RhoA GTPase

activity was increased, resulting in enhanced cell survival.²⁵ Downregulation of RhoA in TSC2-deficient rat-derived TSC2-null Eker leiomyoma/myosarcoma tumor-derived 3 cells increased apoptosis, suggesting that pharmacologic inhibition of RhoA in TSC2-null cells may impair their survival.²⁵ Because sirolimus and everolimus only suppress mTORC1, there is a rationale for new therapies targeting mTORC2 signaling.^{25,26}

HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme-A) reductase is essential for cholesterol metabolism and geranylgeranylation of RhoA GTPase, which is necessary for its attachment to cell membranes.²⁷ Statins are HMG-CoA reductase inhibitors,²⁷ which inhibit geranylgeranylation of RhoGTPases and farnesylation of the small GTPases Ras and Rheb.²⁷ In agreement, atorvastatin was reported to inhibit the growth of *Tsc2*^{-/-} Eker leiomyoma/myosarcoma tumor-derived 3 and mouse embryonic fibroblast cells while impairing Rheb-GTPase activity and function.²⁸ In another study, simvastatin was shown to inhibit RhoA GTPase activity and proliferation of TSC-null cells and TSC2-null tumor growth in mice and to promote apoptosis.²⁵ Treatment with sirolimus and simvastatin prevented recurrence of the tumors even after discontinuation of both drugs, an effect that required administration of both sirolimus and simvastatin.²⁵ This effect appeared to be specific for simvastatin because atorvastatin failed to reduce the size of liver and renal tumors in a mouse model of TSC.²⁹ In a mouse model of LAM carrying *Tsc2*-null lesions that showed α smooth muscle-actin expression, mTORC1 activation, vascular endothelial growth factor D expression, increased lymphangiogenesis, and lung cystic destruction, simvastatin prevented alveolar space enlargement and, together with sirolimus, blocked matrix metalloproteinase upregulation and alveolar destruction.³⁰

Preliminary clinical observations suggested no correlation between statin use and angiomyolipoma response to sirolimus in patients with TSC or sporadic LAM.¹⁶ In a retrospective study, the rate of decline in lung diffusion capacity for patients with LAM treated with statins for hypercholesterolemia was greater than that of matched off-statin control patients.³¹ However, the number of patients on simvastatin was small, and the patients were not given an mTOR inhibitor. For several years, we have followed a cohort of patients with LAM being treated with sirolimus; some of these patients also are being treated with simvastatin for hypercholesterolemia. We reviewed the clinical and physiologic characteristics,

rates of disease progression, and drug-related adverse events of patients treated with sirolimus and simvastatin and compared these data with those from patients treated with either sirolimus or simvastatin alone. The principal aim of this study was to determine whether

the toxicity of sirolimus was affected by simultaneous administration of simvastatin. The second aim was to examine the combined effect of both drugs on progression of lung disease and compare these findings with data from patients treated with sirolimus alone.

Materials and Methods

Patient Population

Patients were referred to the National Institutes of Health for participation in an LAM natural history and pathogenesis protocol, which was approved by the National Heart, Lung, and Blood Institute Institutional Review Board (NHLBI Protocol 95-H-0186). In addition to self-referral

and referral by individual physicians, patients were informed of the studies by the LAM Foundation and the Tuberous Sclerosis Alliance. All patients gave written informed consent before enrollment. Clinical, radiologic, and functional data, including history and physical examination, laboratory tests, CT scans of the thorax and abdomen, pulmonary function tests (including postbronchodilator studies), and cardiopulmonary exercise testing, were obtained.

TABLE 1 Demographic and Clinical Data of 78 Patients With LAM

Demographic	Simvastatin and Sirolimus	Sirolimus	Simvastatin
No. patients	14	44	20
Race/ethnicity			
White	11	39	19
Black	1	0	1
Hispanic	1	4	0
Asian	1	1	0
Age at LAM diagnosis	41.5 ± 7.7	37.0 ± 9.4 ^a	45.2 ± 7.7
Range	28-50	22-59	27-58
Age, first symptom	35.8 ± 8.0	34.2 ± 8.9	39.7 ± 11.4
Range	23-49	19-54	12-58
Age, first visit	45.4 ± 6.2	39.1 ± 9.5 ^a	47.6 ± 7.4
Range	35-52	23-60	30-58
Deaths during study	0	0	0
Oxygen therapy	11 (79)	25 (57)	5 (4)
Lung transplantation	0	0	0
Initial symptoms			
Dyspnea	5 (36)	23 (52)	7 (35)
Pneumothorax	5 (36)	15 (34)	3 (15)
Hemoptysis	2 (14)	2 (4.5)	0
Abdominal pain	3 (21)	4 (9)	4 (20)
No respiratory symptoms	2 (14)	0	3 (15)
Extrapulmonary findings			
Lymphangioliomyomas	8 (57)	28 (64)	6 (30)
Angiomyolipomas	7 (50)	16 (36)	10 (50)
Mode of diagnosis of LAM			
CT scan	4 (29)	12 (27)	5 (25)
Chylous effusions	0	5 (11)	0
Open lung biopsy	5 (36)	16 (36)	4 (20)
Abdominopelvic mass biopsy	2 (14)	5 (11)	6 (30)
Transbronchial lung biopsy	2 (14)	5 (11)	0
Biopsy of angiomyolipoma	1 (7)	1 (2)	5 (25)

Data are presented as counts, mean ± SD, or No. (%) unless otherwise indicated. LAM = lymphangioliomyomatosis.

^aStatistically significantly different from the other two groups. *P* < .05.

Statistical Methods

Available data included clinical information and multiple pulmonary function and exercise measurements. Rates of change in lung function per year were estimated as previously reported.³² In patients with at least 11 months of follow-up, the estimated yearly rate of change (slope) was calculated from a linear regression for pulmonary function data. An adjusted analysis was performed using mixed-effects models. A time-dependent group indicator and adjustment for baseline lung function tests were used in all models.

Both unadjusted and adjusted analyses account for inpatient correlation. All data, except the estimated yearly rate of change in lung function, are shown as mean $\pm$ SD. Rates of lung function change are expressed as mean $\pm$ SEM, which is a more precise estimate of the variability because it takes into consideration the number of observations used, a function of the number of subjects and the number of visits for each subject. Because the number of subjects available for each outcome also varies, SEM is better for comparing variability between outcomes.

Results

Patients Characteristics

Fourteen patients were treated with sirolimus (mean dose, 2.7 ± 0.9 mg/d) and simvastatin (mean dose, 23.3 ± 11.6 mg/d), 20 received simvastatin alone (mean dose, 32.0 ± 16.1 mg/d), and 44 were treated with sirolimus alone (mean dose, 2.4 ± 0.8 mg/d). The initial prevalence of hypercholesterolemia in the simvastatin plus sirolimus group was 64%. Simvastatin was prescribed for the treatment of preexisting hypercholesterolemia or hypercholesterolemia that worsened or developed after beginning therapy with sirolimus. Sixty-one percent of the sirolimus group patients also presented with hypercholesterolemia. These patients either were treated with atorvastatin instead of simvastatin or were placed on a low-fat diet and food supplements, such as red yeast rice. All patients enrolled in the simvastatin group had hypercholesterolemia. Demographic data for the three groups

of patients are shown in Table 1. Of note, patients treated with sirolimus were younger than those treated with sirolimus and simvastatin or simvastatin alone. At the time of enrollment, no significant difference was observed in pulmonary function between patients treated with simvastatin and sirolimus and those treated only with sirolimus (Table 2). The group of patients treated with simvastatin alone had milder lung disease, with mean FEV₁ and diffusing capacity of the lung for carbon monoxide (DLCO) being within normal limits (Table 2).

Adverse Events Related to Drugs

Table 3 lists the adverse events observed in the three groups of patients. Acne, rashes, decreased wound healing, hypertension, peripheral edema, hypercholesterolemia, diarrhea, stomatitis, leukopenia, thrombocytopenia, proteinuria, urinary tract infections, and upper respiratory tract infections occurred with similar frequency in the sirolimus plus simvastatin and

TABLE 2 Initial Pulmonary Function of 78 Patients With LAM

Drug Therapy	Simvastatin and Sirolimus	Sirolimus	Simvastatin
No. patients	14	44	20
TLC, L	5.1 ± 0.8	5.2 ± 1.1	4.8 ± 0.8
TLC, % predicted	99.5 ± 15.7	95.1 ± 18.8	92.8 ± 16.0
FRC, L	2.9 ± 0.6	2.9 ± 0.7	2.4 ± 0.6
FRC, % predicted	106.4 ± 21.6	99.5 ± 21.0	88.3 ± 20.2
RV, L	1.8 ± 0.5	1.8 ± 0.5	1.6 ± 0.4
RV, % predicted	110.9 ± 37.2	105.5 ± 27.3	93.0 ± 21.2
RV/TLC, %	36.5 ± 6.5	35.0 ± 6.8	34.0 ± 6.0
FVC, L	3.0 ± 0.4	3.3 ± 0.7	3.1 ± 0.6
FVC, % predicted	88.7 ± 14.6	89.7 ± 19.2	93.8 ± 17.6
FEV ₁ , L	1.7 ± 0.5	2.1 ± 0.7	2.1 ± 0.6
FEV ₁ , % predicted	64.6 ± 21.3	71.4 ± 22.2	82.7 ± 25.1
FEV ₁ /FVC, %	70.4 ± 36.9	63.6 ± 17.0	70.2 ± 12.7
DLCO, mL/min/mm Hg	12.1 ± 3.4^a	13.6 ± 5.0^a	17.4 ± 4.5
DLCO, % predicted	55.5 ± 16.4^a	61.7 ± 21.6^a	85.6 ± 21.6

Data are presented as mean $\pm$ SD unless otherwise indicated. DLCO = diffusing capacity of the lung for carbon monoxide; FRC = functional residual capacity; RV = residual volume; TLC = total lung capacity. See Table 1 legend for expansion of other abbreviation.

^aStatistically significantly different from the simvastatin group, $P < .01$.

TABLE 3] Adverse Events Associated With Sirolimus and Simvastatin

Drug Therapy	Simvastatin and Sirolimus	Sirolimus	Simvastatin
No. patients	14	44	20
Endocrine/metabolic			
Hypercholesterolemia	3 (21)	12 (27)	...
Hypertriglyceridemia	1 (7.1)	12 (27.3)	5 (25)
Elevated glucose	1 (7.1)	10 (22.7)	3 (15)
Increased hemoglobin A1C	3 (21.4)	1 (2.3)	2 (10)
GI			
Abdominal pain	1 (7.1)	6 (13.6)	1 (5)
Constipation	6 (42.8)	3 (6.8)	1 (5)
Diarrhea	7 (50)	23 (52.3)	2 (10)
Nausea	2 (14.3)	4 (9.1)	1 (5)
Stomatitis	9 (64.3)	29 (65.9)	0
Vomiting	1 (7.1)	6 (13.6)	1 (5)
Gastritis	5 (35.7)	3 (6.8)	2 (10)
Increased liver enzymes	3 (21.4)	12 (27.3)	6 (30)
Dermatologic			
Acne	5 (35.7)	27 (61.4)	0
Rash	7 (50)	11 (25)	2 (10)
Delayed wound healing	3 (21.4)	9 (20.4)	0
Skin infection	2 (14.3)	0	0
Squamous cell carcinoma	1 (7.1)	0	0
Hematologic			
Anemia	1 (7.1)	0	1 (5)
Thrombocytopenia	3 (21.4)	7 (15.9)	2 (10)
Neutropenia/leukopenia	4 (28.6)	12 (27.3)	1 (5)
Musculoskeletal			
Arthralgia	7 (50)	5 (11.4)	12 (60)
Myopathy	5 (35.7)	4 (9.1)	7 (35)
Neurologic			
Headache	5 (35.7)	19 (43.2)	10 (50)
Confusion	0	0	1 (5)
Memory impairment	1 (7.1)	1 (2.3)	4 (20)
Tremor	3 (21.4)	2 (4.5)	1 (5)
Renal			
Elevated serum creatinine	3 (21.4)	0	0
Urinary tract infection	3 (21.4)	7 (15.9)	4 (20)
Proteinuria	4 (28.6)	12 (27.3)	6 (30)
Respiratory			
Epistaxis	1 (7.1)	1 (2.3)	1 (5)
Upper respiratory infection	8 (57.1)	25 (56.8)	11 (55)
Fungal infection	0	1 (2.3)	0
Cardiovascular			
Peripheral edema	7 (50)	20 (45.4)	3 (15)
Hypertension	5 (35.7)	13 (29.5)	6 (30)

(Continued)

TABLE 3] (continued)

Drug Therapy	Simvastatin and Sirolimus	Sirolimus	Simvastatin
Other			
Fever	0	0	0
Pain	1 (7.1)	16 (36.4)	3 (15)
Cancer	0	1 (2.3)	0
Weakness	2 (14.3)	0	1 (5)

Data are presented as No. (%) unless otherwise indicated.

sirolimus-only groups. Among the patients treated with sirolimus plus simvastatin, the frequency of gastritis, constipation, myopathy, arthralgias, and increased serum creatinine level was greater than in the sirolimus-only group. Arthralgias and myopathy occurred more frequently in the patients who received only simvastatin.

Rates of Decline in Lung Function

We estimated the rate of change in lung function before (3.4 ± 2.1 years) and after (2.7 ± 2.3 years) treatment with sirolimus plus simvastatin, before (5.4 ± 4.9 years) and after (2.8 ± 1.9 years) treatment with sirolimus alone, and during (7.2 ± 3.6 years) treatment with simvastatin. In the simvastatin plus sirolimus group, yearly changes in FEV₁ before and after therapy were, respectively, $-1.4\% \pm 0.2\%$ predicted (-55 ± 4 mL) and $1.2\% \pm 0.5\%$ predicted (26 ± 13 mL, $P = .635$) (Fig 1A). Corresponding changes in DLCO were $-1.8\% \pm 0.2\%$ predicted (-0.45 ± 0.03 mL/min/mm Hg) and $0.3\% \pm 0.3\%$ predicted (0.02 ± 0.07 mL/min/mm Hg, $P = .412$) (Fig 1A). For the patients treated only with sirolimus, changes in FEV₁ before and after therapy were, respectively, $-1.7\% \pm 0.1\%$ predicted (-60 ± 4 mL) and $1.6\% \pm 0.3\%$ predicted (39 ± 1 mL, $P < .001$) (Fig 1B). Corresponding changes in DLCO were $-2.1\% \pm 0.1\%$ predicted (-51 ± 0.02 mL/min/mm Hg)

and $0.7\% \pm 0.3\%$ predicted (0.1 ± 0.06 mL/min/mm Hg, $P < .001$) (Fig 1B). In the group of 20 patients treated with simvastatin, yearly changes in FEV₁ and DLCO were, respectively, $-1\% \pm 2.2\%$ predicted (-45 ± 45 mL) and $-2.2\% \pm 2.8\%$ predicted (-0.52 ± 0.55 mL/min/mm Hg).

Discussion

Because mTORC1, mTORC2, and RhoGTPase activity are all required for cell proliferation and survival,²⁵ inhibition of mTORC1, mTORC2, and RhoGTPase may be more effective in abrogating LAM cell growth and proliferation than mTORC1 blockade with mTOR inhibitors alone.^{25,30} In the only human study evaluating changes in lung function in patients with LAM treated with statins, the rate of decline in lung diffusion capacity was found to be greater than that of matched off-statin-therapy control patients; however, the number of patients on simvastatin was small.³¹ The current study, undertaken in patients treated for several years with sirolimus plus simvastatin, shows no increase in the prevalence of adverse events beyond those anticipated from the use of simvastatin or sirolimus together. The data suggest that no major unexpected adverse events should be anticipated from therapy with sirolimus plus simvastatin in the setting of a clinical trial or for the treatment of LAM and hypercholesterolemia.

Figure 1 – A and B, Rates of change in FEV₁ and DLCO expressed as % predicted/y in patients treated with sirolimus plus simvastatin (A) and sirolimus alone (B). Following treatment with sirolimus and simvastatin (A), FEV₁ and DLCO increased. However, because of the small number of patients, there was no statistically significant difference in changes in lung function before and after treatment. During treatment with sirolimus alone (B), FEV₁ and DLCO increased significantly. Data are presented as mean $\pm$ SEM. * $P < .001$. DLCO = diffusing capacity of the lung for carbon monoxide.

The second aim of this study was to examine the combined effect of simvastatin and sirolimus on progression of lung disease and to compare these findings with data from a cohort of patients treated with sirolimus alone. As is the case with the sirolimus therapy, we found that treatment with sirolimus plus simvastatin resulted in a stabilization of lung function.

The results of this study should be interpreted with caution. The study was not controlled, and the assignment to simvastatin therapy was either because of preexisting hypercholesterolemia that was worsened by sirolimus or because of a new onset of hypercholesterolemia. In addition, the study comprised a relatively small number of patients who had less-severe lung disease and lower rates of FEV₁ decline than patients who participated in the Multicenter International LAM Efficacy of Sirolimus (MILES) trial.¹⁹ The lower rate of functional decline combined with the small number of patients may have resulted in the statistical analysis lacking power to detect significant differences between presirolium and postsirolium rates of functional decline.

The trend, however, was for stabilization or small improvement in lung function.

Conclusions

Combined therapy with sirolimus plus simvastatin does not result in a greater prevalence of adverse events beyond those expected from the use of each drug alone. Within the limitations of this study and because of the small numbers of patients treated with sirolimus plus simvastatin, we found no evidence that simvastatin enhances or diminishes the beneficial effects of sirolimus therapy in LAM. Because there is good evidence that simvastatin and sirolimus act synergistically to abrogate the development of lung cystic lesions in animal models of LAM, a study testing the effect of sirolimus and simvastatin in patients with LAM is probably warranted. This study, reporting for the first time to our knowledge the combined effect of both drugs in patients with LAM, provides information that may be useful in designing such a drug trial.

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Authors contributions: A. M. T.-D. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. A. M. T.-D. and J. M. contributed to the study design, data analysis, and writing of the manuscript; A. M. J. and P. A. J.-W. contributed to the data collection and review; M. S. contributed to the statistical analysis; and A. M. T.-D., A. M. J., P. A. J.-W., M. S., and J. M. reviewed and approved the manuscript.

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