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ORIGINAL ARTICLE

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Phosphodiesterase type 5 inhibitors improve microvascular dysfunction markers in pulmonary arterial hypertension associated with congenital heart disease

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Abstract

Background: Ideally, vasodilator therapies for pulmonary arterial hypertension (PAH) should have a favorable impact on markers of vascular dysfunction, in addition to their known effects on hemodynamics, cardiac function, and patient's physical capacity.

Methods: We analyzed circulating (plasma) markers of endothelial and platelet activation/dysfunction (enzyme-linked immunoassays) in the specific setting of advanced PAH associated with congenital heart disease, during the course of sildenafil and tadalafil therapies. Thirty-one patients were enrolled (age 10-54 years), most of them with chronic hypoxemia and elevated hematocrit. Drugs were administered orally for 6 months (sildenafil [n = 16], 20 mg t.i.d.; tadalafil [n = 15], single daily dose of 40 mg). Measurements were performed at baseline, and 90 and 180 days.

Results: Compared to controls, patients had elevated baseline β-thromboglobulin (β-TG, P = .002), P-selectin (P = .027), tissue-type plasminogen activator (t-PA, P = .009), and von Willebrand factor antigen (VWF:Ag, P = .010). Thrombomodulin was importantly reduced (TM, P < .001), while soluble CD40 Ligand was not changed (P = .320). Tadalafil administration was associated with improvement of β-TG (P = .004), t-PA (P = .003) and TM (P = .046) levels, while P-selectin was improved by sildenafil treatment only (P = .034). VWF:Ag improved transiently in the sildenafil group (P = .019). Both therapies were associated with improvement of the physical capacity (functional class and distance walked during the 6-minute test, P < .05), hematocrit and hemoglobin level (P < .05), and health-related quality of life (physical and mental components, P < .05).

Conclusion: In PAH associated with congenital heart disease, phosphodiesterase 5 inhibitors seem to have beneficial actions at microcirculatory level, beyond the proposed effects as vasodilators.

KEYWORDS

biomarkers, congenital heart disease, endothelial dysfunction, phosphodiesterase 5 inhibitors, platelets, pulmonary hypertension

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1 | INTRODUCTION

The efficacy of treatments for pulmonary arterial hypertension (PAH) has been investigated in several ways. Drugs primarily developed to act as vasodilators have been tested for their ability to promote improvement of the physical capacity, pulmonary hemodynamics, cardiac dysfunction, and survival.[1] More recently, outcomes based on morbidity and mortality have been considered in clinical trials.[2] It is equally important to investigate how these treatments modify parameters related to vascular biology and microcirculation, as they may eventually reflect disease stability or the onset and progression of microvascular and macrovascular complications. Attempts have been made to analyze the effects of PAH drugs on markers of vascular dysfunction,[3-7] with potential impact on future therapeutic strategies.[8,9]

Pulmonary vascular disease is a known complication in unoperated patients with large congenital cardiac communications. Advanced disease (the so-called Eisenmenger syndrome) is associated with right-to-left shunting across the cardiac communications, systemic hypoxemia, blood hyperviscosity due to secondary erythrocytosis, microvascular dysfunction involving endothelial cells, platelets, and other circulating elements, tendency to intravascular coagulation, pulmonary, and systemic thrombosis, bleeding, and a number of other multiorgan abnormalities.[10-13] Although PAH drugs have been proved beneficial in patients with advanced pulmonary vasculopathy associated with congenital heart disease,[10,14,15] their effects on microvascular dysfunction markers have not been fully investigated in this particular setting.

Phosphodiesterase type 5 (PDE5) inhibitors sildenafil, tadalafil, and vardenafil have been largely used in the management of PAH in general, and PAH associated with congenital heart disease in particular.[16] Although the rationale for their use is the induction of vasodilation via increase in cyclic guanosine monophosphate level in pulmonary vascular smooth muscle cells, a number of additional effects have been reported with impact on the behavior of several cell types, hemorheology, inflammation, angiogenesis, and cancer.[17-20] However, there are differences among PDE5 inhibitors in terms of their actions in vascular cells,[21,22] in addition to the known differences in pharmacokinetic properties. The present study was planned to comparatively investigate the chronic effects of oral sildenafil and tadalafil administration on markers of microvascular dysfunction in patients with advanced PAH associated with congenital heart disease. We also analyzed drug effects on the physical capacity, systemic oxygen saturation, hematocrit, hemoglobin level, and health-related quality of life.

2 | PATIENTS AND METHODS

Patients were from the Heart Institute (InCor), University of São Paulo School of Medicine, São Paulo, Brazil. Adolescents and

TABLE 1 Cellular origin and biological actions of proteins investigated in the study

Proteins/types	Expressed in/secreted by…	Biological actions
β-thromboglobulin Chemokine-like protein (C-X-C motif)	Alpha-granules of platelets	Marker of platelet activation and release reaction. Chemoattractant for fibroblasts and neutrophils. Stimulator of mitogenesis and extracellular matrix synthesis. Affects megakaryocyte maturation
CD40 ligand Transmembrane homologue of tumor necrosis factor alpha Soluble form is also detectable	Activated platelets and T cells, monocytes, macrophages, and dendritic cells	Interacts with cellular membrane CD40 (cell-to-cell interaction) initiating cytokine synthesis and release. Induces an inflammatory reaction of CD40-bearing endothelial cells
Thrombomodulin Transmembrane chondroitin sulfate proteoglycan	Endothelial cells, vascular smooth muscle cells, fibroblasts, monocytes, macrophages, neutrophils, and other cell types	Interacts with thrombin, thus activating protein C and inactivating coagulation factors V and VIII. Induces antiproliferative cellular signaling. Acts as an anti-inflammatory and cytoprotective factor
Tissue-type plasminogen activator Serine protease	Endothelial cells, vascular smooth muscle cells, fibroblasts, mast cells, macrophages, and several tumor cell lines	Catalyses the cleavage of plasminogen to plasmin. Releases growth factors from extracellular matrix deposits. Contributes to plaque destabilization in atherosclerosis. Is involved in tumorigenesis and metastasis
P-selectin Adhesion molecule with high affinity for sialylated ligands (sialyL—Lewis X)	Mainly platelets and endothelial cells	Mediates platelet-leukocyte and endothelial cell-leukocyte interactions. Mediates neutrophil recruitment under hypoxic conditions. Mediates aggregation of platelets with cancer cells, thus facilitating metastasis
von Willebrand factor Multimeric glycoprotein (adhesion molecule)	Endothelial cells, megakaryocytes and platelets	Present in circulation as a carrier for coagulation factor VIII. Mediates endothelial cell anchorage to the sub endothelium. Promotes platelet adhesion and aggregation. Released under hypoxic, inflammatory, and thrombotic conditions

adults with advanced pulmonary hypertension associated with congenital heart disease were enrolled if they were naïve in terms of previous PAH-specific therapies. They entered the study consecutively from October 2013 to March 2017. Patients had cardiac catheterization and cardiac magnetic resonance data obtained at different times prior to the study that helped establishing the diagnosis of congenital heart disease-associated PAH. Systolic pulmonary arterial pressure (echocardiography) was updated at the beginning of the study. Additional diagnostic procedures were used to exclude other etiologies of PAH. Only inoperable patients were enrolled. Updated cardiac catheterization was not considered as necessary for decision about operability in cyanotic patients with clear clinical features of Eisenmenger syndrome. It was performed in a few patients that were acyanotic at rest with systemic oxygen desaturation only on exertion. Clinically unstable and hospitalized patients were not included. A written informed consent was necessary for inclusion. The study was approved by the local Ethics Committee of University of São Paulo School of Medicine (CAPPesq 9324/12).

2.1 | Administration of PDE5 inhibitors and assessment of effects

Randomization to treatment groups (sildenafil and tadalafil) was not possible because tadalafil was available in the institution for a short time and a limited number of patients. We therefore planned to start with the tadalafil group and use sildenafil subsequently. Sildenafil and tadalafil were administered orally for 6 months. The respective daily doses were 60 mg (20 t.i.d.) and 40 mg (single dose). Treatment efficacy was determined by registering the 6-minute walked distance (6MWD, American Thoracic Society protocol) and assessing health-related quality of life using the SF-36 generic questionnaire.[23] Measurements were performed at baseline, and 90 and 180 days of treatment. Additional parameters included blood pressure, systemic oxygen saturation (pulse oximetry), hemoglobin level, hematocrit, and platelet count. We also report echocardiographic Tei index of right ventricular performance[24] that was obtained before and just after the end of the study. Once patients were started on PDE5 inhibitors, no further changes in medication were made for 180 days.

TABLE 2 Descriptive analysis of the study population

Age, years	28.0 (18.1-41.7)
Gender, M:F, *n*	10:21
Diagnosis	
Pretricuspid shunts, *n* (%)	6 (19.3)
Post-tricuspid shunts except for atrioventricular canal, *n* (%)	16 (51.6)
Atrioventricular canal, *n* (%)	3 (9.7)
Conotruncal defects, *n* (%)	2 (6.5)
Complex anomalies, *n* (%)	4 (12.9)
Functional class, I/II:III/IV, *n*[a]	5:26
Pulmonary arterial pressure (systolic), mm Hg[b]	93 (80-116)
Myocardial performance (Tei) index[c]	0.58 (0.54-0.60)
Systemic arterial pressure, mm Hg	
Systolic	127 (120-139)
Diastolic	86 (78-93)
Mean	100 (95-105)
Six-minute walked distance, m[d]	480 (420-516)
Peripheral oxygen saturation, %	
Resting	87 (80-92)
Peak exercise	64 (54-70)
Hematocrit, %	55 (51-64)
Hemoglobin level, g/dL	17.8 (16.6-20.2)
Platelet count, $\times 10^9$/L	210 (180-254)
Brain natriuretic peptide, pg/mL[e]	47.0 (26.0-86.0)
Chronic warfarin use, *n* (%)	18 (58.1)

Numeric variables are presented as median with interquartile range.
[a]World Health Organization classification for pulmonary hypertension.
[b]Assessed by transthoracic echocardiography based on the velocity of the tricuspid regurgitant jet.
[c]Assessed by echocardiography according to reference 24 (normal values, 0.28 ± 0.04, mean with SD).
[d]Six-minute walk test according to the American Thoracic Society protocol.
[e]Reference normal values <100 pg/mL.

The eventual need for changing any treatments during the study period (conventional or PAH-specific therapy) would be considered as a criterion for patient's exclusion from final analysis.

2.2 | Microvascular dysfunction markers

Circulating (plasma) levels of P-selectin, tissue-type plasminogen activator (t-PA), thrombomodulin, and von Willebrand factor antigen (VWF:Ag) were measured to assess endothelial dysfunction.[25] Plasma β-thromboglobulin was used as an index of endogenous platelet activation. Soluble (plasma) CD40 ligand (sCD40L) was measured as a marker of interaction between platelets and endothelial cells.[25] Table 1 provides information about the cellular origin of these proteins, their targets, and principal biological and pathobiological actions. Plasma samples were processed in duplicate using commercially available enzyme-linked immunoassay kits: Diagnostica Stago (Asnières, France) for t-PA, β-thromboglobulin, and VWF:Ag, and R&D Systems (Minneapolis, Minnesota) for P-selectin, thrombomodulin, and sCD40L. Absorbance was measured at 450 nm (SpectraMax Paradigm, Molecular Devices, Wals, Austria). Gender matched healthy blood donors with age range of 18-50 years were used as controls. These subjects came from the same geographic area as the patients, and had the same genetic background.

2.3 | Statistical analysis

All variables were tested for closeness to the normal distribution. Unless otherwise specified, numeric variables are presented as median with interquartile range. Differences between groups were tested using the Student's *t* test or the Mann-Whitney test (numeric variables), or the chi-square family of tests (categorical variables). Numeric data computed at baseline, and 90 and 180 days of treatment were analyzed using repeated measures ANOVA or the Friedman's test (with appropriate post hoc tests for multiple comparisons). Categorical variables (same subject) were tested using the McNemar's test. In all procedures, .05 was assumed as significance level. Analyses were carried out using the IBM-SPSS statistical software, version 24 (IBM, Armonk, New York).

3 | RESULTS

Thirty-one patients were enrolled, with age of 10-54 years. Demographic and diagnostic data are depicted in Table 2. Data obtained during cardiac catheterization prior to the study period showed a hemodynamic profile compatible with the diagnosis of PAH. The mean pulmonary arterial pressure was 67 (49-82) mm Hg and pulmonary wedge pressure was 9 (7-13) mm Hg. The majority of patients were cyanotic, with resting oxygen saturation lower than 90% (typical Eisenmenger presentation), but none of them were on supplemental oxygen therapy during the study period. Four patients had oxygen saturation of >93% at rest, with a rapid decrease during the walk test. They had elevated hematocrit (49%-61%) and hemoglobin level (16.4-20.7 g/dL), confirming the presence of chronic, although not sustained hypoxemia. Despite the decreased physical capacity (median 6-minute

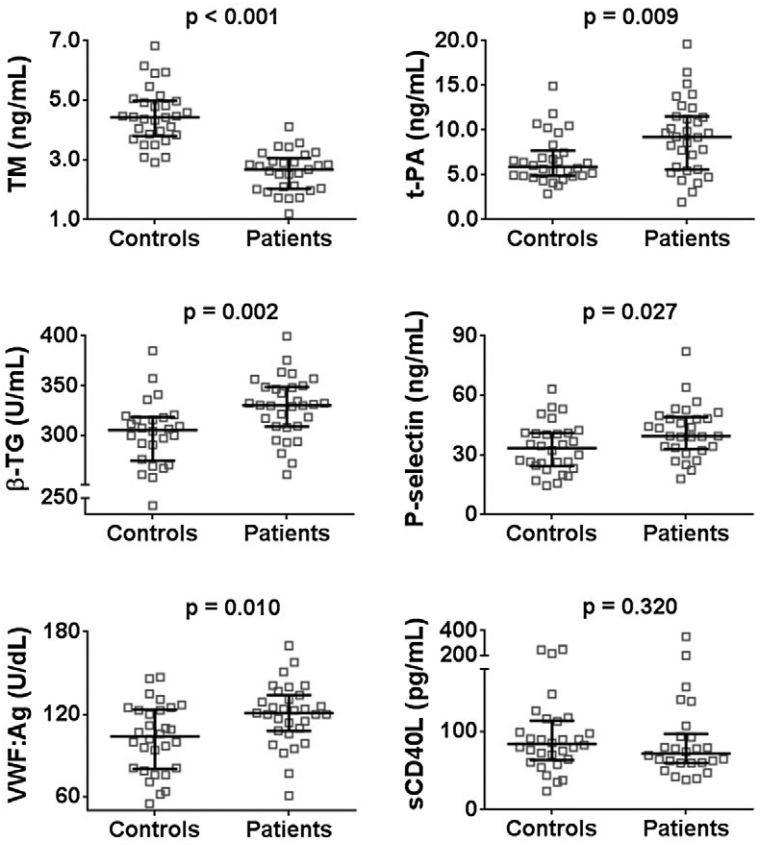

FIGURE 1 Baseline plasma levels of microvascular dysfunction markers. TM, t-PA, β-TG, VWF:Ag, and sCD40L, respectively, thrombomodulin, tissue-type plasminogen activator, β-thromboglobulin, von Willebrand factor antigen, and soluble CD40 ligand. In all graphs, bars represent the median value with interquartile range. All differences were analyzed using the Mann-Whitney test

walked distance of 480 m), a stable cardiac function was suggested by normal circulating levels of BNP. Elevated right ventricular myocardial performance index was interpreted as diastolic dysfunction.

Altered baseline levels of five microvascular dysfunction markers were observed (Figure 1). Plasma t-PA, β-thromboglobulin, P-selectin, and VWF:Ag were increased, while thrombomodulin was impressively decreased. Plasma sCD40L was not significantly changed.

Despite the lack of randomization, Table 3 shows that treatment groups were similar at baseline, except for lower peripheral oxygen saturation in the tadalafil group (at rest and peak exercise). In both groups, there were patients on warfarin use at enrollment. This routine was kept unchanged all over the study. Plasma levels of biomarkers were not influenced by warfarin therapy. In particular, levels of thrombomodulin (a vitamin-K dependent factor) in warfarin users and nonusers, respectively, were 2.52 (2.00-2.90) ng/mL and 2.83 (2.52-3.25) ng/mL (P = .192).

Administration of PDE5 inhibitors was not associated with relevant adverse effects in any patients. Transient nasal congestion or obstruction was eventually reported. Skeletal muscle pain, particularly in the abdomen or lower extremities was eventually observed at the beginning of tadalafil administration. It was promptly relieved by dose reduction to 20 mg/day for a few days. In the

TABLE 3 Baseline clinical and laboratory parameters in the study groups

	Sildenafil group (n = 16)	Tadalafil group (n = 15)	P value
Age, years	26.6 (17.4-40.0)	30.3 (19.7-43.1)	.826
Gender, M:F, n	7:9	3:12	.252
Diagnosis			
Pretricuspid shunts, n (%)	2 (12.5)	4 (26.7)	.312
Post-tricuspid shunts except for atrioventricular canal, n (%)	10 (62.5)	6 (40.0)	
Atrioventricular canal, n (%)	2 (12.5)	1 (6.7)	
Conotruncal defects, n (%)	0 (0.0)	2 (13.3)	
Complex anomalies, n (%)	2 (12.5)	2 (13.3)	
Functional class, I/II:III/IV, n[a]	2:14	3:12	.654
Systolic PAP, mm Hg	91 (83-105)	100 (80-128)	.168
Myocardial performance (Tei) index[b]	0.54 (0.39-0.65)	0.60 (0.56-0.65)	.289
Mean SAP, mm Hg	99 (86-106)	101 (97-105)	.333
Peripheral oxygen saturation, %			
Resting	90 (86-93)	81 (77-89)	.008
Peak exercise	69 (59-72)	56 (52-65)	.047
Six-minute walked distance, m[c]	484 (410-523)	480 (462-510)	.892
Hematocrit, %	51 (48-61)	56 (54-71)	.064
Hemoglobin level, g/dL	17.2 (16.4-19.9)	19.1 (17.4-20.8)	.093
Platelet count, $\times 10^9$/L	201 (183-259)	217 (158-254)	.808
Thrombomodulin, ng/mL	2.85 (2.11-3.28)	2.52 (1.93-2.84)	.223
t-PA, ng/mL	8.89 (5.32-11.45)	9.66 (5.89-12.47)	.953
β-Thromboglobulin, U/mL	326 (294-348)	332 (319-350)	.423
P-selectin, ng/mL	39.2 (33.3-48.6)	43.5 (27.2-51.5)	.559
VWF:Ag, U/dL	120 (98-138)	124 (110-134)	.505
sCD40L, pg/mL	69.5 (57.5-93.2)	74.1 (60.7-131.2)	.631
Chronic warfarin use, n (%)	8 (50)	10 (66.7)	.565

Abbreviations: PAP, pulmonary arterial pressure assessed noninvasively by echocardiography; SAP, systemic arterial pressure; t-PA, plasma tissue-type plasminogen activator; VWF:Ag, plasma von Willebrand factor antigen; sCD40L, plasma soluble CD40 ligand.
Numeric variables are presented as median with interquartile range.
[a]World Health Organization classification for pulmonary hypertension.
[b]Assessed by transthoracic echocardiography according to reference [24].
[c]Six-minute walk test according to the American Thoracic Society protocol.

TABLE 4 Effects of treatments on functional and general laboratory parameters

		Baseline	3 months	6 months	*P* value
Functional class, I/II:III/IV, *n*[*]	S	2:14[a]	4:12[b]	8:8[b]	.041
	T	3:12	5:10	7:8	.221
Resting SAP, mm Hg	S	99 (86-106)	98 (90-111)	97 (92-109)	.705
	T	101 (97-105)[a]	98 (86-105)[ab]	92 (91-96)[b]	.024
Resting O_2 sat., %	S	90 (86-93)	90 (86-93)	90 (86-94)	.999
	T	81 (77-89)[a]	88 (81-92)[b]	86 (81-90)[ab]	.012
Peak exercise SAP, mm Hg	S	108 (98-119)	110 (100-114)	105 (90-114)	.060
	T	105 (93-114)	109 (104-114)	107 (94-133)	.687
Peak exercise O_2 sat., %	S	69 (59-72)	70 (60-78)	66 (54-72)	.051
	T	56 (52-65)	62 (53-70)	63 (51-72)	.081
6MWD, m	S	484 (410-523)[a]	521 (493-557)[b]	521 (484-559)[ab]	.007
	T	480 (462-510)[a]	539 (491-561)[b]	540 (516-582)[b]	<.001
Dyspnea, Borg score	S	6 (5-6)[a]	5 (4-6)[b]	6 (5-6)[a]	.041
	T	6 (5-7)[a]	5 (5-6)[b]	5 (4-6)[b]	.016
Hematocrit, %	S	51 (48-61)[a]	51 (46-58)[ab]	49 (47-56)[b]	.046
	T	56 (54-71)[a]	53 (51-62)[b]	53 (51-58)[b]	<.001
Hemoglobin, g/dL	S	17.2 (16.4-19.9)[a]	17.5 (15.8-19.2)[ab]	17.0 (16.0-19.0)[b]	.020
	T	19.1 (17.4-20.8)[a]	17.3 (16.4-20.3)[ab]	17.3 (16.7-19.2)[b]	.011
Platelet count, $\times 10^9$/L	S	201 (183-259)[a]	193 (177-213)[b]	194 (167-227)[b]	.018
	T	217 (158-254)	191 (153-242)	209 (191-230)	.091

Abbreviations: SAP, mean systemic pressure; O_2 sat., peripheral oxygen saturation; 6MWD, distance walked during the six-minute walk test (American Thoracic Society protocol). The intensity of dyspnea during the test was graded according to the Borg classification,[26] were grade 1 and grade 10 correspond to the lowest and highest intensity, respectively.

Numeric variables are presented as median with interquartile range. Letters are used to highlight differences identified by *post hoc* tests following repeated measures ANOVA or Friedman's test. Values not sharing the same letter are significantly different. S and T correspond to sildenafil and tadalafil treatment, respectively.

[*]World Health Organization classification for pulmonary hypertension.

present series, this symptom was not observed in any patients receiving sildenafil.

Changes in clinical and laboratory parameters as a result of PDE5 inhibitor administration are shown in Table 4. Treatments were associated with improvement of dyspnea and physical capacity (6-minute walked distance). These effects seemed to be more consistent in the tadalafil group. However, sildenafil use was associated with a consistent improvement of the functional class. Patients on tadalafil use had lower systemic pressure at 6 months compared to baseline, with no detrimental effect on oxygen saturation. Both, treatments influenced hematocrit and hemoglobin levels, which were decreased at 6 months compared to baseline. Platelet count decreased mildly but significantly during sildenafil administration. There were no significant changes in right ventricular myocardial performance index. Respective values before and after treatment were 0.54 (0.39-0.65) and 0.60 (0.48-0.64) in the sildenafil group ($P = .345$), and 0.60 (0.56-0.65) and 0.46 (0.42-0.65) in the tadalafil group ($P = .500$).

Physical and mental aspects of health-related quality of life were positively influenced by PDE5 inhibitor administration. Beneficial effects were observed with both drugs at 3 and 6 months of treatment. Summary scores for the physical and mental domains of the SF-36 questionnaire are shown in Figure 2.

Sildenafil and tadalafil influenced the levels of biomarkers in different ways. Tadalafil administration was associated with a consistent improvement of thrombomodulin, t-PA, and β-thromboglobulin plasma levels, while P-selectin decreased progressively during sildenafil use (Figure 3). Both treatments tended to provoke a transient decrease in VWF:Ag (it was significant for subjects receiving sildenafil), with a subsequent increase toward pretreatment levels. Plasma sCD40L did not change with any of the drugs. Respective levels at baseline, 3 and 6 months were 69.5 (59.9-92.9) pg/mL, 64.9 (50.2-116.1) pg/mL, and 66.8 (49.4-101.5) pg/mL in the sildenafil group ($P = .607$), and 74.1 (60.0-139.1) pg/mL, 72.3 (57.8-106.3) pg/mL, and 93.7 (50.9-177.3) pg/mL in the tadalafil group ($P = .779$). Improvement of circulating biomarkers was not related to any changes in the physical capacity, oxygen saturation, or hematological parameters.

4 | DISCUSSION

In addition to improving the physical capacity and health-related quality of life, PDE5 inhibitors had favorable effects on circulating levels of endothelial and platelet dysfunction markers in the specific

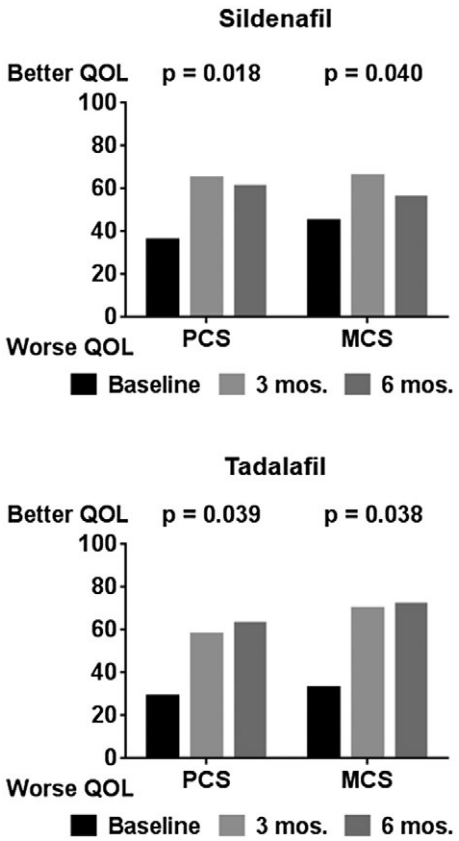

FIGURE 2 Effects of treatments on health-related quality of life (QOL). Summary scores are provided for the physical and mental domains of the SF-36 questionnaire. PCS and MCS, physical component score and mental component score, respectively

setting of advanced PAH associated with congenital heart disease. Although the study was not randomized to allow for a direct comparison of efficacy between the drugs, the effects of tadalafil administration seemed to be more consistent across the investigated biomarkers, with improvement of thrombomodulin, t-PA, and β-thromboglobulin plasma levels. However, sildenafil administration was followed by a decrease in plasma P-selectin, an effect that was not seen in the tadalafil group. Importantly, not all of the effects observed at 3 months were sustained beyond this time (this was the case of VWF:Ag decrease), thus emphasizing the need for longer periods of observation in this particular disorder.

Vasodilation with improvement of flow conditions at microcirculatory level is probably a very simplistic explanation for the changes observed in microvascular dysfunction markers as a result of treatments. In fact, PDE5 inhibitors have been demonstrated to have numerous additional actions in several cell types. Some of the effects that have been reported in endothelial and other vascular cells, and platelets are enhancement of chemokine CXCR4 expression,[19] induction of prostacyclin synthesis with reduction of thromboxane and fibrinogen levels,[20] inhibition of proinflammatory cytokine expression (tumor necrosis factor alpha and interleukin-1 beta),[21] reduction of oxidative activity (monocytes),[27] and inhibition of intracellular Ca^{2+} release with changes in other selected cellular functions (platelets).[28] The differences that we observed in effects between sildenafil and tadalafil reinforce the assumption that the drugs did not act simply as vasodilators.

P-selectin and von Willebrand factor are stored in endothelial Weibel-Palade bodies and released by several stimuli including hypoxia, thrombin, vasoactive substances, and cytokines[29]; t-PA can also be detected albeit very poorly. It is not surprising that baseline levels of these proteins were similar between our study groups despite the difference in oxygen saturation (Table 3). These patients usually have a rapid decrease in oxygen saturation on mild exertion, and probably remain importantly hypoxemic during the majority of their daily tasks. On the other hand, t-PA organelle and the type-2 chemokine-containing organelle represent a single endothelial compartment responsible for unstimulated secretion of t-PA or interleukin-1 β-stimulated secretion of chemokines. This compartment lacks detectable von Willebrand factor and P-selectin, and in contrast with Weibel-Palade bodies, is very poorly responsive to Ca^{2+}-elevating secretagogues.[30] In our study, it was interesting that sildenafil provoked a decreased in P-selectin and VWF:Ag but not t-PA, while tadalafil administration was followed by a reduction of t-PA level without affecting P-selectin or VWF:Ag. Platelet α-granules also contain P-selectin and von Willebrand factor, and account for ~20% of VWF:Ag present in platelet-rich plasma.[31] We observed a progressive decrease in plasma β-thromboglobulin (a marker of platelet secretion phase) during tadalafil administration, suggesting deceleration of endogenous platelet activation. The lack of concomitant decrease in circulating P-selectin and VWF:Ag points toward the endothelium as the principal source of these protein in the present disorder. The lack of changes in sCD40L (another marker of platelet activation) is not surprising. Proteolysis of platelet membrane CD40L which generates sCD40L is a very complex process. It depends not only on reactions on platelet surface but also engagement of intracellular structures even during the solubilization phase.[32]

Thrombomodulin is a transmembrane proteoglycan present in endothelial cells and leukocytes. It acts as a natural thrombin-dependent anticoagulant that promotes the inactivation of coagulation factors V and VIII via protein C activation. Hypoxia severely depresses endothelial thrombomodulin expression at transcriptional level.[33] Thrombomodulin-deficient mice develop extensive intravascular fibrin deposition when exposed to hypoxia.[34] In our patients, circulating thrombomodulin was critically reduced at baseline, but increased slightly but consistently during tadalafil administration. This slight increase may be relevant considering that the plasma fraction corresponds to proteolytic fragments of membrane thrombomodulin. Furthermore, circulating thrombomodulin is biologically active and recombinant thrombomodulin has been given consideration for the management of inflammatory and thrombotic disorders.[35] Finally, thrombomodulin has been shown to have antiproliferative effects acting as a "second thrombin receptor."[36] This may have implications for the management of pulmonary vascular disease.

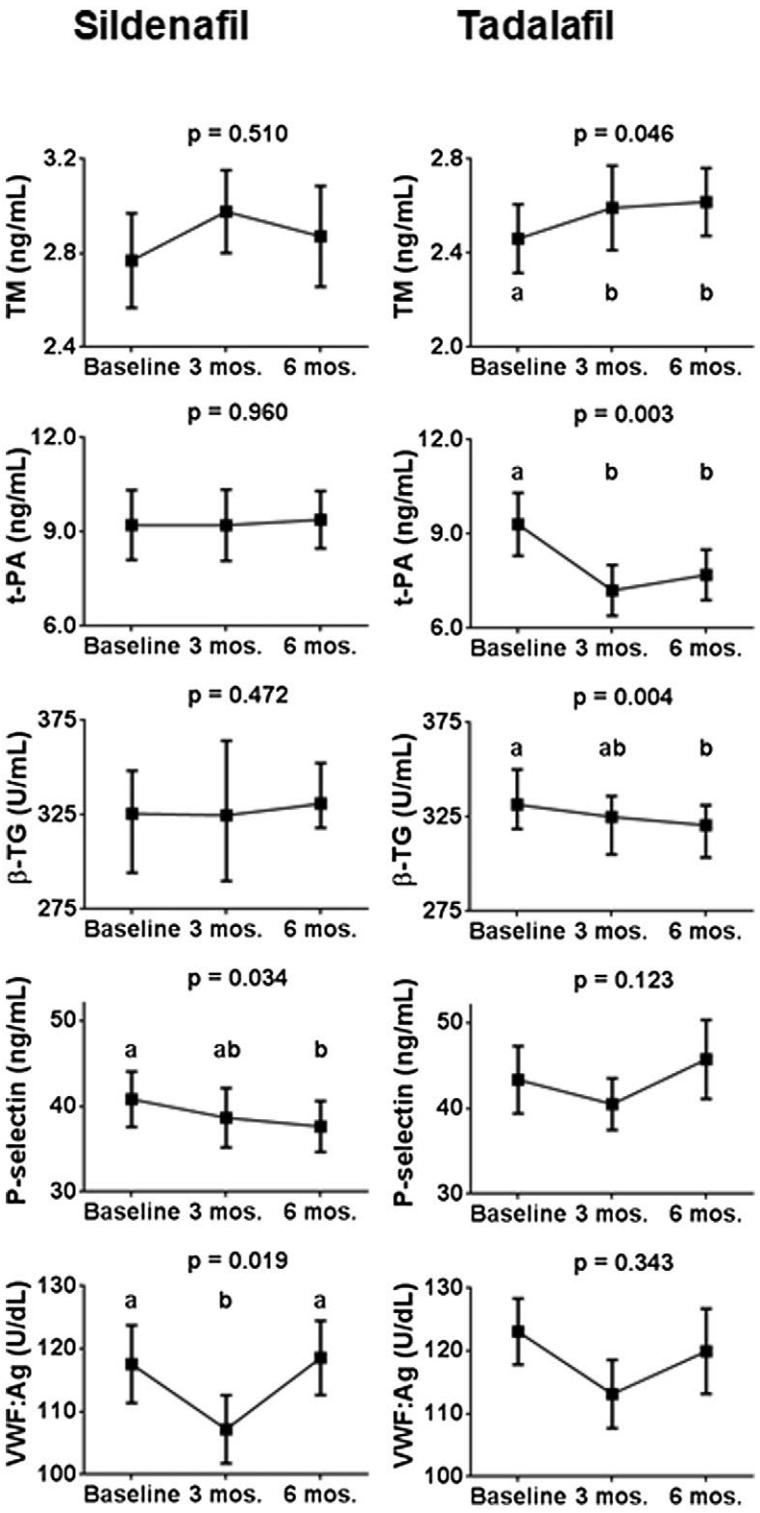

FIGURE 3 Effects of treatments on plasma levels of microvascular dysfunction markers. TM, t-PA, β-TG, and VWF:Ag, respectively, thrombomodulin, tissue-type plasminogen activator, β-thromboglobulin, and von Willebrand factor antigen. Results are shown as mean value with standard error (TM, t-PA, P-selectin, and VWF:Ag) or median with interquartile range (β-TG). Values not sharing the same letter are significantly different by post hoc tests following repeated measures ANOVA or Friedman's test

In addition to the small number of patients, the impossibility of randomizing them to treatment groups constituted a limitation of the study, making it difficult to directly compare effects between sildenafil and tadalafil. It seemed to us that tadalafil was more effective than sildenafil in improving biomarkers. However, we used only the recommended dose of sildenafil (20 mg t.i.d.). We therefore speculate that additional effects might be obtained with higher doses that are sometimes used in PAH (eg, up to 80 mg t.i.d.).

5 | CONCLUSION

In conclusion, PDE5 inhibitor administration improved circulating levels of endothelial cell and platelet activation markers, in addition to improving the physical capacity and health-related quality of life in advanced pulmonary hypertension associated with congenital heart disease. Further studies are needed for a better understanding of the mechanisms and pathways

involved, as the drugs seem to act beyond the level of simple vasodilation.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

M. M. Clavé manager, data collection, laboratory analyses, and manuscript preparation; N. Y. Maeda laboratory senior, supervision of all analyses, and manuscript preparation; A. M. Thomaz clinical parameter registration, adherence to medication, and safety procedures; S. P. Bydlowski study development and manuscript preparation, A. A. Lopes senior author, study design, statistical analysis, and manuscript preparation. All the authors have reviewed the final manuscript and approved the submission to this journal.

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