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Efficacy of Mammalian Target of Rapamycin Inhibitors in Antiphospholipid Syndrome and Antiphospholipid Syndrome Nephropathy

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ABSTRACT

Antiphospholipid syndrome (APS) is a potentially devastating clinical condition that is usually associated with recurrent arterial or venous thrombotic events and/or pregnancy morbidity. This condition can also affect the kidneys. APS nephropathy (APSN) is an increasingly recognized and studied renal small vessel vasculopathy that is characterized by non-inflammatory occlusion of renal blood vessels. Renin angiotensin aldosterone blockers and anticoagulants are frequently used to treat APS nephropathy. In 2014, the study by Canaud et al, demonstrated the interaction of antiphospholipid antibodies with endothelial cells through mammalian target of rapamycin (mTOR) pathway and the relationship between activation of mTOR complex and proliferation of endothelial cells, suggesting a possible mechanism for the efficacy of mTOR inhibitors in APSN. This study provided a new possible pathogenetic mechanism and a new possible therapeutic avenue for APSN. This review aims to to cover the increasing number of animal studies, in vitro studies and clinical studies on the role of mTOR pathyway in APS and the available clinical data on the efficacy of mTOR inhibitors in APSN.

Introduction

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by recurrent thrombotic events and/ or pregnancy morbidity associated with the presence of antiphospholipid antibodies (aPL)[1]. Until the introduction of 2023 EULAR/ACR classification criteria[2], numerous cardiac, pulmonary, hematological, renal manifestations of APS have been defined as "non criteria" clinical features of APS, as they were not included in the Sapporo classification criteria[3]. One of these manifestations, the renal involvement is a well recognized feature of APS, which includes renal artery thrombosis or stenosis, renal vein thrombosis and a renal small vessel vasculopathy, known as "APS nephropathy" (APSN)[4]. APS nephropathy is characterized by non-inflammatory occlusion of renal blood vessels. Acute lesions take the form of thrombotic microangiopathy, and chronic lesions are characterized by arteriosclerosis of arteries and arterioles, fibrous intimal hyperplasia, fibrous obliteration of arteries and arterioles and focal cortical atrophy[1]. Controlled studies for the management of APS nephropathy are lacking[3]. Renin angiotensin aldosterone system blockers are frequently used to lower blood pressure, reduce proteinuria and slow the progression of chronic kidney disease[5]. Despite conflicting data about its benefits on kidney outcomes , APSN patients are generally anticoagulated as most of them also suffer from macrovascular thrombotic events[5]. Some studies demonstrated that rituximab, plasmapheresis and eculizumab can be effective on a case level[6-12].

The study by Canaud et al, demonstrating the interaction of antiphospholipid antibodies with endothelial cells through mammalian target of rapamycin (mTOR) pathway and the relationship between activation of mTOR complex and proliferation of endothelial cells has suggested a possible mechanism for the efficacy of mTOR inhibitors in APSN[13]. This study provided a new possible pathogenetic mechanism and a new possible therapeutic avenue for APSN[13]. However there is also data demonstrating that the mTOR inhibitor rapamycin could activate platelets, therefore creating concern for the procoagulant effect of rapamycin in liver and renal transplant recipients[14]. This review aims to cover the expanding data on the role of mTOR pathway and mTOR inhibitors in APS and the available clinical data on the efficacy of mTOR inhibitors in APSN.

Animal Studies and In Vitro Studies Assessing the Role of Mammalian Target of Rapamycin Pathway in Antiphospholipid Syndrome

Animal studies and in vitro studies assessing the role of mTOR pathway in APS are listed in Table-1.

Table-1: Animal studies and in vitro studies assessing the role of mammalian target of rapamycin pathway in antiphospholipid syndrome

Study (Date)	Summary of the Studies
Oaks et al (2016)	Animal study demonstrating that antiphospholipid antibody production precedes disease onset and responds to rapamycin in lupus-prone mice
Xia et al (2017)	In vitro study demonstrating the mTOR involvement in regulating anti-β2GPI/β2GPI-induced expression of tissue factor and interleukin-8, that is responsive to rapamycin
Müller-Calleja et al (2017)	In vitro study demonstrating that antiphospholipid antibodies activate two major proinflammatory signal transduction pathways (endosomal NADPH oxidase and monocyte LDL receptor-related protein 8) depending on their epitope specificity and that hydroxychloroquine and rapamycin, alone or in combination could completely suppress these processes
Hollerbach et al (2019)	In vitro study demonstrating that anti-β2GPI aPL-induced platelet activation depended on interaction of aPL with the low affinity Fcγ-receptor IIa on the platelet surface. When rapamycin or everolimus were added to the platelet aggregation assay, they inhibited the platelet aggregation that was induced by anti-β2GPI antibodies
Mu et al (2020)	Animal study demonstrating that RapaLink (a third generation mTOR inhibitor) decreased the area of chronic vascular lesions in the animal model of thrombosis by prevenitng apoptosis and enhancing autophagy of macrophages. In addition Rapa-Link decreased the aPL antibodies in the sera of the mice.
Wei et al (2020)	In vitro study demonstrating the effect of anticardiolipin in inducing human umbilical vein endothelial cells injury by inhibiting autophagy and activiating mTOR/S6K pathway. Hyperoside reduced anticardiolipin induced secretion of proinflammatory cytokines and endothelial adhesion cytokines, activated autophagy and suppressed mTOR/S6K and TLR4/Myd88/NF-κB pathways.
Wei et al (2020)	Animal study demonstrating the rat model of pregnancy loss induced by the anticardiolipin IgG fractions. Hyperoside treatment improved pregnancy outcomes by enhancing autophagy and inhibiting inflammation, demonstated by downregulation of the expressions of phosphorylated mTOR, phosphorylated p70S6 kinase and inhibition of the expressions of TLR4, MyD88 and NF-κB p-p65 pathways.
Rodríguez et al (2021)	In vitro study demonstrating the impact of aPL from different patient populations on endothelial cell mitochondrial function, activation of the mTOR pathway, autophagy and cellular growth. Antibodies from patients with pregnancy morbidity and vascular

Study (Date)	Summary of the Studies
	thrombosis increased activation of the mTOR and autophagic pathways and induced cellular stress which was supported by mitochondrial hyperpolarization.
Zhang et al (2021)	In vitro study demonstrating that oxidized LDL/β2GPI/anti-β2GPI complex could induce the foam cell formation of macrophages and vascular smooth muscle cells and the expression of inflammatory cytokines in endothelial cells, that results in the formation of atherosclerotic plaques with APS background. The mTOR inhibitor rapamycin attenuated the oxidizded LDL/β2GPI/anti-β2GPI complex induced endothelial inflammation, oxidative stress and apoptosis.
Winans et al (2023)	Animal study demonstrating that inactivation of transaldolase-aldose reductase axis results in metabolic stress, which is characterized by reduced mitophagy, enhanced overall autophagy, activation of the mTOR pathway, diminished glycosylation of paraoxonase 1, production of antiphospholipid antibodies, loss of CD161+ NK cells and expansion of CD38+ Ito cells, which are responsive to treatment with rapamycin in vivo.

Abbreviations: Anti-β2GPI: anti beta 2 glycoprotein-1, aPL: antiphospholipid antibody, LDL: low density lipoprotein, mTOR: mammalian target of rapamycin, NADPH: nicotinamide adenine dinucleotide phosphate hydrogen

The animal study by Oaks et al demonstrated that anticardiolipin and anti-beta-2-glycoprotein I (β2GPI) levels were elevated preceding the development of nephritis in 4-week-old MRL, C57BL/6.lpr and MRL/lpr mice. Treatment with rapamycin selectively blocked mTORC1 activation and aPL production in lupus-prone mice. This study demonstrated that mTORC1 dependent mitochondrial dysfunction contributes to aPL generation, which can be blocked by mTOR inhibitors The beneficial effect of rapamycin on aPL may be attributed, at least partially, to selective blockade of mTORC1 in the immune system[15]. In their discussion the authors discuss the possible benefits of inhibition aPL production in APS patients, citing the increased life expectancy that was attributed to rapamycin in an animal study[16]. They also underline the necessity of close monitorization for thrombosis during rapamycin therapy[15].

In their in vitro study, Xia et al investigated whether mTOR was involved in anti-beta2 glycoprotein1/beta2 glycoprotein1 (anti-β2GPI/β2GPI) complex induced expression of tissue factor and interleukin-8 (IL-8/CXCL8) in monocytes and explored the relationship among toll like receptor (TLR4), mTOR MAPKs and NF-κB in this process. This study demonstrated that anti-β2GPI/β2GPI complex markedly induces mTOR activation as well as expression of tissue factor and interleukin-8 in THP-1 cells or primary monocytes. The mTOR inhibitor rapamycin could attenuate the elevated TF and IL-8 expression. Rapamycin also decreased the phosphorylation of p38, ERK1/2 and NF-κB p65 stimulated by anti-β2GPI/β2GPI or APS-IgG/ β2GPI complex but it had no effect of JNK. Anti-β2GPI/β2GPI or APS-IgG/ β2GPI complex-induced phosphorylation of mTOR in THP-1 cells was down-regulated through inhibition of p38 or ERK rather than inhibition of JNK or NF-κB. In addition, mTOR activation could also be affected by exposure to TLR inhibitor. They concluded that mTOR was involved in regulating anti-β2GPI/β2GPI-induced expression of TF and IL-8, maybe through the phosphorylation of p38, ERK1/2 and NF-κB, in monocytes and that mTOR pathway inhibition might be beneficial for treatment of aP-mediated thrombosis and inflammation in APS patients[17].

In their in vitro study, Müller-Calleja et al analyzed the effect of three human monoclonal aPLs with different epitope specificities. Expression of tumor necrosis factor-α mRNA by mouse and human monocytes was assessed. Analysis included cells from genetically modified mice and the use of specific inhibitors in monocytes. They validated the data with IgG isolated from 20 APS patients. Cofactor independent anticardiolipin aPLs activated monocytes by induction of endosomal NADPH oxidase, which could be blocked by hydroxychloroquine. Anti-β2GPI aPL activated monocytes by interacting with LDL receptor-related protein 8 (LRP8), which could be blocked by rapamycin. Analysis of 20 APS patients' IgG demonstrated that all IgG fractions activated the same two pathways as the monoclonal aPL, depending on their epitope patterns as determined by ELISA. Monocyte activation by APS IgG could be blocked by hydroxychloroquine and/or rapamycin, which suggests that in most APS patients there is no other relevant signaling pathway. They concluded that aPLs activate two major proinflammatory signal transduction pathways, depending on their epitope specificity and that hydroxychloroquine and rapamycin, alone or in combination could completely suppress signaling by APS IgG[18].

The in vitro study of Hollerbach et al analyzed the ability of three human monoclonal aPL with distinctly different antigenic specificities to activate platelets in vitro. They demonstrated that a co-factor-independent human monoclonal anti cardiolipin aPL had no discernible effect on human platelets. Two monoclonal aPL reactive against β2GPI induced platelet aggregation, integrin αIIbβ3 activation and P-selectin surface expression. These data were confirmed with APS patient IgG fractions which could only induce aggregation, if they had anti-β2GPI activity. Anti-β2GPI aPL-induced platelet activation depended on interaction of aPL with the low affinity Fcγ-receptor IIa on the platelet surface. When the mTOR inhibitors rapamycin or everolimus was added to the platelet aggregation assay, they inhibited that platelet aggregation that was induced by β2GPI antibodies but it did not affect ADP-induced platelet aggregation. The authors discussed that the reported effects of mTOR inhibitors on platelet aggregation are variable and appear to depent on the experimental conditions, concluding that the role of mTOR signaling in platelets is explored incompletely and requires further research [14].

In the study by Mu et al, BALB/c mice were injected with monoclonal anti-β2GPI antibodies to induce APS in vivo and $FeCl_3$ to induce thrombosis in carotid artery of mice. Analyses of the total aorta revealed arterial thrombus plaque in APS mice, and the quantitative data showed decreased area of plaque when RapaLink-1 (a third generation mTOR inhibitor with superior inhibitory effect on mTOR complex 1) is administered, compared with APS mice that were fed with normal diet. Anti- β2GPI and anticardiolipin activities in the mice sera was partially reduced by Rapalink-1. Immunostaining protocols demonstrated that RapaLink-1 inhibited plaque initiation and progression, decreased the extent of macrophage infiltration and enhanced the autophagy process. In vitro cultured human monocyte cell line THP-1 macrophages were initially exposed to oxidized low-density lipoprotein and then treated with Rapalink-1. Administration of Rapalink-1 prevented cell apoptosis and enhanced autophagy of macrophages, which was indicated by the increased expression of autophagy-related proteins under electron microscopy. This study demonstrated evidence that mTORC pathway is a potential target in chronic vascular lesions associated with APS. As a therapeutic agent, mTOR inhibitor Rapalink-1 has a potential to inhibit the formation of thrombus plaque in APS and these effects were dependent on facilitating cell autophagy both in vivo and in vitro conditions[19].

In their in vitro study, Wei et al demonstrated that anticardiolipin antibodies (obtained from the blood of 16 patients) induced human umbilical vein endothelial cells (HUVEC) injury by inhibiting autophagy and activiating mTOR/S6K pathway. Hyperoside, which is a flavonoid extracted from medicinal plants traditionally used in Chinese medicines, reduced anticardiolipin induced secretion of proinflammatory cytokines interleukin-1 beta and interleukin-8 and endothelial adhesion cytokines such as E-selectin, tissue factor, intercellular cell adhesion molecule-1 and vascular cell adhesion-1 in HUVECs. In addition, hyperoside activated autophagy and suppressed mTOR/S6K and TLR4/Myd88/NF-κB signal transduction pathways in anticardiolipin induced HUVECs. This study links the effect of mTOR pathway and autophagy on anticardiolipin induced injury and demonstrates that the inhibition of mTOR signaling is crucial for activation of autophagy by hyperoside. In the same study the authors demonstrated that the mTOR inhibitor rapamycin, just like hyperoside was effective in alleviating anticardiolipin induced injury of HUVECs by activiating autophagy. They also demonstrated that 3-methyladenine (3-MA), which is a widely used autophagy inhibitor, reversed the inhibitory effects of hyperoside on anticardiolipin induced inflammation in HUVECs[20].

Wei et al also evaluated the rat model of pregnancy loss that was induced by the anticardiolipin IgG fractions obtained from the serum of APS patients and the effects of hyperoside on this model. The fetuses were counted, and the placentas were weighed and the protein expressions of inflammation and autophagy were measured by western blot analysis. Hyperoside treatment improved pregnancy outcome, increasing the weight of fetuses and decreasing the fetal resorption rate. Hyperoside exhibits this protective effect through enhancing autophagy and inhibiting inflammation which is demonstated by downregulation of the expressions of phosphorylated mTOR, phosphorylated p70S6 kinase and inhibition of the expressions of TLR4, MyD88 and NF-κB p65 in pregnancy loss animal model. The authors claim that hyperoside may provide a potential drug candidate for treatment of recurrent pregnancy loss[21].

In their study Rodríguez et al investigated the impact of aPL from different patient populations on endothelial cell mitochondrial function, activation of the mTOR pathway, autophagy and cellular growth. They used an in vitro model where HUVECs were treated with polyclonal immunoglobulin G purified from the serum of women with both pregnancy morbidity and vascular thrombosis (PM/VT), patients with only vascular thrombosis (VT) or with pregnancy morbidity and non-criteria aPL (seronegative-obstetric, SN-OAPS). There were two control groups: women with pregnancy morbidity and without aPL, and healthy women with uncomplicated pregnancies. Mitochondrial function, mTOR activation, autophagy and cell proliferation were evaluated by Western blot, flow cytometry and functional assays. They demonstrated that IgG from PM/VT patients increased HUVEC mitochondrial hyperpolarization and activation of the mTOR and autophagic pathways. IgG from VT patients induced endothelial autophagy and cell proliferation, without elevated mTOR activity or mitochondrial dysfunction. IgG from SN-OAPS group had no effect on any of these HUVEC responses. This study underscores the heterogeneity of aPL antibodies in patients with different clinical features. The authors concluded that aPL from PM/VT patients increased activation of the mTOR and autophagic pathways and induced cellular stress which was supported by mitochondrial hyperpolarization. Rodríguez et al hypothesized that these mechanisms may play a role in the pathogenesis of obstetric APS. Authors underlined that there was a complex relationship between mTOR and autophagic pathways: classically mTOR pathway activation was associated with inhibition of autophagy, however in some cases such as tumor growth and in their own findings (in this study), mTOR and autophagy activation can coexist[22].

Zhang et al demonstrated that oxidized low density lipoprotein (oxLDL)/β2GPI/anti-β2GPI complex could induce the foam cell formation of macrophages and vascular smooth muscle cells and the expression of inflammatory cytokines in endothelial cells, that results in the formation of atherosclerotic plaques with APS background. In order to assess the effect of this complex on autophagy and explore potential mechanisms, they used HUVECs and mouse brain endothelial cell line as models of vascular endothelial cells. They showed that oxLDL/ β2GPI/anti-β2GPI complex suppressed the autophagy. This suppression was associated with PI3K/AKT/mTOR and eNOS pathways. The mTOR inhibitor rapamycin attenuated the oxLDL/β2GPI/anti-β2GPI complex induced endothelial inflammation, oxidative stress and apoptosis. The autophagy inhibitor

3-MA alone induced endothelial injury. In this study, the authors provided a novel mechanism for vascular endothelial injury in atherosclerotic patients with APS background while underlining the potential role of mTOR inhibitors in alleviating that damage[23].

In their study Winans et al demonstrated that in transaldolase deficient mice, inactivation of transaldolase-aldose reductase axis results in metabolic stress, which is characterized by reduced mitophagy, enhanced overall autophagy, activation of the mTOR pathway, diminished glycosylation of paraoxonase 1, production of antiphospholipid antibodies, loss of CD161+ NK cells and expansion of CD38+ Ito cells, which are responsive to treatment with rapamycin in vivo. Rapamycin restores paraoxonase 1 secretion and blocks aPL production[24].

Clinical Studies Assessing the Role of Mammalian Target of Rapamycin Pathway in Non-Renal Manifestations of Antiphospholipid Syndrome

Clinical studies assessing the role of mTOR pathway in non-renal manifestations of APS are listed in Table-2.

Table-2: Clinical studies assessing the role of mammalian target of rapamycin pathway in non-renal manifestations of antiphospholipid syndrome

Study (Date)	Summary
Mora-Ramírez et al (2016)	Case report demonstrating the efficacy of sirolimus eluting stents in preventing stent stenosis, thrombosis or neointimal hyperplasia in an antiphospholipid syndrome patient
Sartorelli et al (2019)	Case report demonstrating that efficacy of sirolimus treatment enabling dramatic clinical, echocardiographic and laboratory improvements in cardiac function in an antiphospholipid syndrome patient with cardiac microangiopathy.
Rodríguez-García et al (2019)	Case report demonstrating a patient with Smith-Kingsmore syndrome and antiphospholipid syndrome that had a gain-of-function variant, treated with sirolimus
Sevim et al (2022)	Study investigating the mTOR activation in the skin of aPL-positive patients with livedo. This study demonstrated increased mTOR activity in livedoid lesions of aPL-positive patients with or without SLE compared to aPL-negative patients with SLE.

Abbreviations: aPL: antiphospholipid antibody, mTOR: mammalian target of rapamycin

In a case report, Mora-Ramírez et al report a 47 year old female patient with antiphospholipid syndrome and acute myocardial infarction who was initially treated with a paclitaxel eluting stent. Thirty days later the stent was occluded and this time she was treated with sirolimus-eluting stent. She was treated with double antiplatelet agents and warfarin. She remained asymptomatic for 3 years. Then she presented with angina and coronary angiography revealed critical ischemia proximal to the stent. Two more sirolimus eluting stents were implanted. One year later coronary CT angiography demonstrated absence of in-stent stenosis, thrombosis or neointimal hyperplasia. This study the success of "local administration" of sirolimus in preventing in-stent neointimal hyperplasia and neoangiogenesis in this patient[25].

Sartorelli et al report a 43 year old male patient with antiphospholipid syndrome that presented with cardiac microangiopathy, confirmed by cardiac magnetic resonance imaging, cardiac positron emission tomography and endomyocardial biopsy. He was on hydroxychloroquine and warfarin treatment. After initiation of glucocorticoids and sirolimus patient demonstrated a dramatic clinical, echocardiographic and laboratory improvement in cardiac function. Authors conclude that this case supports the pivotal role of mTOR pathway in the pathogenesis of APS cardiomyopathy and underlines the efficacy of mTOR inhibitors in APS[26].

Rodríguez-García report a 13 year old girl with Smith-Kingsmore syndrome (a rare disorder that causes intellectual disability, developmental delay, megalencephaly and seizures) and antiphospholipid syndrome (chronic ischemic lesions in cortical and subcortical areas suggestive of infarcts of small vessels in brain and antiphospholipid antibody positivity) who had a novel de novo mTOR gain-of-function variant. To further avoid brain damage, compassionate use of mTOR inhibitor sirolimus was started in this patient[27].

Sevim et al examined mTOR activation in the skin of aPL-positive patients with livedo. They studied three patient groups (a total of 10 patients) with livedo: 4 persistently aPL-positive patients with systemic lupus erythematosus (SLE), 4 persistently aPL-positive patients without SLE and 2 aPL-negative SLE patients. In all aPL positive patients, epidermal p-AKT and p-S6RP (mTOR activity markers) staining were significantly increased in both peripheral and central skin samples when compared to aPL-negative SLE: both were more pronounced in the lower basal layers of epidermis. This study demonstrated mTOR activity in livedoid lesions of aPL-positive patients with or without SLE compared to aPL-negative patients with SLE. They concluded that this study serves as a basis for further investigating the role of mTOR pathway in aPL-positive patients[28].

Clinical Studies Assessing the Role of Mammalian Target of Rapamycin Pathway in Antiphospholipid Syndrome Nephropathy

Clinical studies assessing the role of mTOR pathway in APS nephropathy are listed in Table-3.

Table-3: Clinical studies assessing the role of mammalian target of rapamycin pathway in antiphospholipid syndrome nephropathy

Study (Date)	Summary
Canaud et al (2014)	The pivotal study demonstrating the activation of mTORC pathway in vascular endothelium of proliferating intrarenal vessels from APSN patients .In cultured vascular endothelial cells, IgG antibodies from patients with APS stimulated mTORC through the phosphatidylinositol 3-kinase-AKT pathway. Patients with APSN who required transplantation and were receiving sirolimus had no recurrence of vascular lesions and had decreased vascular proliferation on biopsy compared to patients with aPL antibodies who were not receiving sirolimus. Activation of mTORC was also found in the vessels of autopsy specimens from CAPS patients.
Canaud et al (2015)	Editorial underlining the importance of the previous study and identifying mTORC pathway as a novel molecular pathway critically involved in the development of intimal hyperplasia which accompanies the most severe variants of APS. This editorial is also presenting the novel therapeutic strategy of mTOR inhibition as a potential treatment that can prevent kidney loss.
Lai et al (2015)	Study examining the mitochondrial mass, superoxide production, mTOR and FoxP3 expression in 72 SLE patients (12 of them had accompanying APS) and 54 healthy controls by flow cytometry. There was a similar mTOR activity between SLE patients with or without APS. Authors concluded that oxidative stress and Treg depletion rather than mTOR activation underlie APS in patients with SLE. Activation of mTOR pathway may not differentiate lupus patients with APS from those without APS and that mTOR blockade may be equally beneficial for both patient groups due to the underlying SLE
Dufour et al (2020)	Case report demonstrating an APS patient with thrombotic microangiopathy lesions in native kidney biopsy that were characteristic of APS nephropathy. After demonstration of endothelial mTORC activation at the molecular level, this patient was successfully treated with sirolimus that was added to anticoagulant and angiotensin converting enzym inhibitor therapy
Zhang (2022)	Case report demonstrating a patient with SLE and APS who had class III lupus nephritis. This patient was refractory to cyclophosphamide and mycophenolate. Immunofluorescence of the kidney biopsy showed the activation of the mTOR pathway. Sirolimus was added to warfarin. Patient had complete remission in the sixth month, discontinued sirolimus 1 year later and remained in remission even after discontinuation of sirolimus for another 3.5 years.

Abbreviations: aPL: antiphospholipid antibodies, APS: antiphospholipid syndrome, APSN: antiphospholipid syndrome nephropathy, mTOR: mammalian target of rapamycin, mTORC: mammalian target of rapamycin complex, SLE: systemic lupus erythematosus

In their pivotal study, Canaud et al analyzed the mammalian target of rapamycin complex (mTORC) pathway in renal APS. They used double immunostaining to evaluate pathway activation in the mTORC and the nature of cell proliferation in the vessels of patients with primary of secondary APSN. They also evaluated autopsy specimens from persons who had catastrophic antiphospholipid syndrome (CAPS). The molecular pathways through which aPL antibodies modulate the mTORC pathway were evaluated in vitro and potential pharmacologic inhibitors were also tested in vitro. They studied the effect of sirolimus in kidney-transplant recipients with APS. This study demonstrated that the vascular endothelium of proliferating intrarenal vessels from patients with APSN showed indications of activation of the mTORC pathway. In cultured vascular endothelial cells, IgG antibodies from patients with APS stimulated mTORC through the phosphatidylinositol 3-kinase (PI3K)-AKT pathway. Patients with APSN who required transplantation and were receiving sirolimus had no recurrence of vascular lesions and had decreased vascular proliferation on biopsy compared to patients with aPL antibodies who were not receiving sirolimus. Seven of 10 patients had a functioning renal allograft 144 months after transplantation versus 3 of 27 untreated patients. Activation of mTORC was also found in the vessels of autopsy specimens from CAPS patients. Combining all these findings Canaud et al concluded that mTORC pathway was involved in the vascular lesions associated with APS[13].

In their editorial, Canaud et al shared their observation that the development intimal hyperplasia in APS vasculopathy is associated with the activation of both mTORC1 and mTORC2 in the endothelial cells. This activation lead to the endothelial cell proliferation and the proliferation of the surrounding vascular smooth muscle cells, with the strongest activation in CAPS autopsy cases. They also provided the first evidence that AKT/mTORC pathway inhibition protects kidneys from deterioration and prevents graft loss by preventing intimal hyperplasia. They underlined that they identified mTORC pathway as a novel molecular pathway critically involved in the development of intimal hyperplasia which accompanies the most severe variants of APS. This discovery also presented the novel therapeutic strategy of mTOR inhibition which can block this process and prevent kidney loss [13,29].

Since mTOR is a sensor of oxidative stress, Lai et al examined mitochondrial mass, superoxide production, mTOR and FoxP3 expression in 72 SLE patients (12 of them had accompanying APS) and 54 healthy controls by flow cytometry. In this study, oxidative stress generating mitochondrial mass was increased in CD4-CD8- double negative T cells of SLE patients with APS in comparison to

those without APS. Superoxide production was increased in all lymphocyte subsets of APS patients. FoxP3+ cells were depleted within CD4+CD25+ Tregs in APS patients compared to those without APS. There was a similar mTOR activity between SLE patients with or without APS. They concluded that oxidative stress and Treg depletion rather than mTOR activation underlie APS in patients with SLE. These findings may suggest that activation of mTOR pathway does not differentiate lupus patients with APS from those without APS and that mTOR blockade may be equally beneficial for both patient groups due to the underlying SLE[30]. The authors underlined that 16 of the 28 APS patients in the initial study of Canaud[13] had concomitant SLE[30].

Dufour et al reported a 36 year old APS patient with thrombotic microangiopathy lesions in native kidney biopsy that were characteristic of APS nephropathy. In this patient endothelial mTORC activation was substantiated at the molecular level. Treatment with sirolimus was added to anticoagulant and angiotensin converting enzyme inhibitor. After this treatment, serum creatinine reduced from 1.84 to 1.30 mg/dl, repeat kidney biopsy demonstrated resolution of microangiopathy. Immunofluorescence showed inactivation of AKT/mTORC pathway in endothelial cells. Sirolimus was well tolerated with no side effects. This study supported the potential of a precision medicine for patients with APS nephropathy and the use of mTORC activation as a biomarker of disease activity and as a therapeutic target[31].

Zhang et al reported a 44 year old patient with SLE and APS who had a history of autoimmune hemolytic anemia, deep vein thrombosis, and later developed nephrotic range proteinuria and renal failure. Renal biopsy revealed class III lupus nephritis which was complicated by acute tubular injury. This patient was treated with cyclophosphamide and mycophenolate before biopsy could be performed (due to receiving concomitant anticoagulant therapy) and was refractory to these agents. Immunofluorescence showed the activation of the mTOR pathway. Sirolimus 2 mg/day was added to warfarin. Complete remission was achieved in 6 months. After 1 year of treatment, sirolimus was reduced to 1 mg/day and 6 months later sirolimus was stopped. Lupus nephritis remained in remission with a low dose of prednisone even after discontinuation of sirolimus for another 3.5 years. During the period of sirolimus, she had two courses of upper respiratory infection, and no serious adverse events were reported[32].

Conclusion

The accumulating data from animal studies, in vitro studies and clinical studies indicate that mTOR pathway may play a significant role in the pathogenesis of pregnancy morbidities and vasculopathy in APS, while also providing a rationale for use of mTOR inhibitors for the treatment of patients that have APS nephropathy and other clinical findings of APS such as cardiac involvement and livedoid skin lesions. Use of mTOR inhibitors as an add on treatment to anticoagulation will also offset the possible concerns about the procoagulant effects of mTOR inhibitors. Prospective randomized controlled studies that will include a large number of patients with APS nephropathy will improve our understanding of the role of mTOR pathway in APS and APSN pathogenesis and clarify its place in the future within treatment algorithms.

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