Rapamycin Limits the Growth of Established Experimental Abdominal Aortic Aneurysms

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WHAT THIS PAPER ADDS

Abdominal aortic aneurysms generally enlarge and rupture unless resected or repaired. To date, pharmacological strategy has proven ineffective in preventing disease progression. In this study, rapamycin proved remarkably effective in preventing progression of established experimental aneurysms. Despite beginning therapy after aneurysm initiation, rapamycin preserved aortic architecture, and attenuated aortic mural angiogenesis and macrophage accumulation. This study adds to the growing body of evidence supporting the use of rapamycin for medical abdominal aortic aneurysm disease management.

Objectives: Abdominal aortic aneurysm (AAA) is a chronic inflammatory disease affecting 4—8% of men older than 60 years. No pharmacologic strategies limit disease progression, aneurysm rupture, or aneurysm-related death. We examined the ability of rapamycin to limit the progression of established experimental AAAs.

Methods: AAAs were created in 10—12-week-old male C57BL/6J mice via the porcine pancreatic elastase (PPE) infusion method. Beginning 4 days after PPE infusion, mice were treated with rapamycin (5 mg/kg/day) or an equal volume of vehicle for 10 days. AAA progression was monitored by serial ultrasound examination. Aortae were harvested for histological analyses at sacrifice.

Results: Three days after PPE infusion, prior to vehicle or rapamycin treatment, aneurysms were enlarging at an equal rate between groups. In the rapamycin group, treatment reduced aortic enlargement by 38%, and 53% at 3 and 10 days, respectively. On histological analysis, medial elastin and smooth muscle cell populations were relatively preserved in the rapamycin group. Rapamycin treatment also reduced mural macrophage density and neoangiogenesis.

Conclusion: Rapamycin limits the progression of established experimental aneurysms, increasing the translational potential of mechanistic target of rapamycin-related AAA inhibition strategies.

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INTRODUCTION

Abdominal aortic aneurysm (AAA) is a lethal, age- and gender-related chronic inflammatory disease. Approximately one million Americans aged 50—84 years[1] are at risk for premature, AAA-related death. Key pathological features present in AAA disease include mural leukocyte infiltration, neoangiogenesis, smooth muscle cell (SMC) depletion, extracellular matrix degradation, and progressive intraluminal laminar thrombus accumulation.[2] Although multiple pharmacological inhibition strategies have succeeded in experimental aneurysm models,[3—10] none have been successfully translated to clinical practice. Therefore, surgical repair, limited to patients suffering from advanced disease, remains the only available option.[11—13]

Rapamycin is approved by the US Food and Drug Administration for preventing rejection of transplant allografts, as well as coronary artery restenosis following transluminal angioplasty and stenting.[14,15] Mechanistically, rapamycin disrupts cellular signaling through mechanistic/mammalian target of rapamycin (mTOR), a serine/threonine kinase modulating numerous biological processes, including cell growth, metabolism, aging, angiogenesis, and inflammation.[16] In experimental atherosclerosis, rapamycin treatment increases plaque stability and limits disease progression.[17—19] Rapamycin is effective in limiting experimental aneurysm progression when administered prior to AAA initiation.[20,21] However, of critical relevance to translational potential, the ability of rapamycin to limit existing aneurysm progression has not been examined to date.

In this study, rapamycin therapy was initiated 4 days after intra-aortic infusion of porcine pancreatic elastase (PPE), at

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which point the aneurysmal degeneration has been initiated. Rapamycin is remarkably effective in limiting progression in this construct, further highlighting the translational potential of this therapeutic strategy.

MATERIALS AND METHODS

Animals

Male C57BL/6J mice aged 10—12 weeks were used for all experiments. All animal protocols were in compliance with the Laboratory Animal Care Guidelines of Stanford University, and reviewed and approved by the Stanford University Administrative Panel on Laboratory Animal Care.

Aneurysm creation and follow-up

AAAs were created via intra-aortic PPE infusion as previously described.[8,22] Briefly, under inhaled anesthesia and under operative magnification, 30 μL of type 1 PPE (1.5 U/mL in saline, Catalog # 098K7008; Sigma-Aldrich, St. Louis, MO, USA) was infused for 5 minutes into an isolated segment of infrarenal aorta. At days 3, 7, and 14 after the infusion procedure, the aortic diameter was measured serially using the Vevo 770 ultrasound system (40 MHz RMV-704 Scanhead with a resolution of 30 μm; VisualSonics, Toronto, ON, Canada).[8,22] Two investigators performed all measurements independently, without knowledge of study group assignment.

Rapamycin treatment

Rapamycin was purchased from the LC Laboratories (Woburn, MA, USA) and prepared in 0.2% carboxy-methylcellulose immediately prior to use. Mice were treated daily with 5 mg/kg rapamycin or an equal volume of vehicle alone via oral gavage, depending on study group assignment, beginning 4 days after PPE infusion and continuing for 10 days. This dose was chosen based on a literature review of published work in atherosclerosis models.[17—19]

Histological analyses

Aortae were harvested 14 days after PPE infusion, embedded in optimal cutting compound media, sectioned (6 μm), and fixed with cold acetone. Elastin integrity was evaluated using Verhoeff's Van Gieson (EVG) stain. SMCs, macrophages, and angiogenesis were stained with antibodies against SMC α actin, CD68, and CD31, respectively, using a standard three-step biotin—streptavidin—peroxidase immunostain.[8,22] Based on EVG and SMC α actin staining, destruction of medial elastin and SMCs were graded as I (mild) to IV (severe) if present:[8,22] (I): elastin break/degradation or SMC loss limited to one outer medial elastin layer; (II): elastin degradation or SMC loss involving more than two medial elastin layers, or entire medial elastin layers, but limited to less than one-quarter of the aortic circumference; (III): elastin degradation or SMC loss involving entire medial elastin layers, but limited to less than half the aortic circumference; and (IV): elastin degradation or SMC loss involving entire medial elastin layers and expanded to more than three-quarters of aortic circumference. Mural macrophages and angiogenesis were quantified as $CD68^+$ cells and $CD31^+$ blood vessels, respectively, per aortic cross section (ACS).

Statistical analysis

All data are presented as mean and standard deviation (SD), and were analyzed using GraphPad Prism (version 5a; GraphPad Software, La Jolla, CA, USA). Two-way ANOVA (using the Bonferroni correction for multiple comparisons) or the non-parametric Mann—Whitney test was used to identify differences between groups. Significance was assumed at $p < .05$.

RESULTS

Effects of rapamycin treatment on further progression of established aneurysms

In our prior experience with this model, most mice develop characteristic histological features and > 50.0% infrarenal diameter enlargement consistent with AAA formation within 3—4 days of PPE infusion.[8] To evaluate the ability of rapamycin to limit progression of established AAAs, mice were treated with 5 mg/kg/d rapamycin via oral gavage, beginning 4 days after the PPE infusion and continuing for 10 days in total. Prior to drug or vehicle treatment (3 days after PPE infusion), five and six mice in the vehicle and rapamycin groups, respectively, developed AAAs as defined by a > 50.0% increase in aortic diameters over the baseline levels. As demonstrated in Fig. 1, rapamycin treatment was associated with a small, but significant, loss of weight during the course of the experiment (103.8 ± 4.9% and 97.2 ± 3.6% of baseline levels in vehicle and rapamycin groups, respectively; $p < .05$). Fig. 2 demonstrates the time-dependent progression of aortic diameter, as determined by ultrasound imaging. In vehicle-treated mice, aortic diameters increased by an average of 0.13 and 0.36 mm between days 3 and 10, respectively. In contrast, treatment with rapamycin resulted in remarkable attenuation of

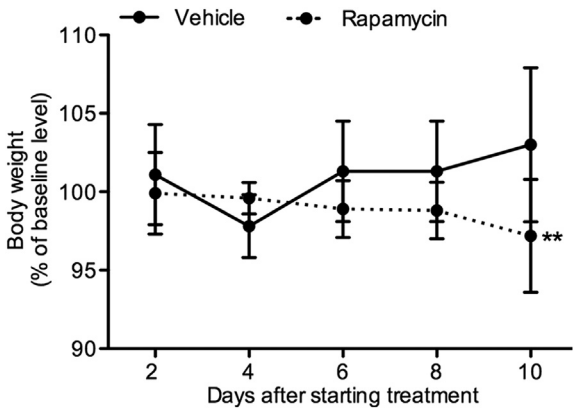

Figure 1. Effect of rapamycin treatment on body weight. Data on body weight are presented as the percentage of baseline levels (prior to vehicle or rapamycin treatment). Two-way analysis of variance with the Bonerroni correction, $p < .01$ between two groups; $n = 7—8$ mice/group.