Inhibition of mTOR Signaling With Rapamycin Regresses Established Cardiac Hypertrophy Induced by Pressure Overload

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Background—Rapamycin is a specific inhibitor of the mammalian target of rapamycin (mTOR). We recently reported that administration of rapamycin before exposure to ascending aortic constriction significantly attenuated the load-induced increase in heart weight by ≈70%.

Methods and Results—To examine whether rapamycin can regress established cardiac hypertrophy, mice were subjected to pressure overload (ascending aortic constriction) for 1 week, echocardiography was performed to verify an increase in ventricular wall thickness, and mice were given rapamycin (2 mg · kg⁻¹ · d⁻¹) for 1 week. After 1 week of pressure overload (before treatment), 2 distinct groups of animals became apparent: (1) mice with compensated cardiac hypertrophy (normal function) and (2) mice with decompensated hypertrophy (dilated with depressed function). Rapamycin regressed the pressure overload–induced increase in heart weight/body weight (HW/BW) ratio by 68% in mice with compensated hypertrophy and 41% in mice with decompensated hypertrophy. Rapamycin improved left ventricular end-systolic dimensions, fractional shortening, and ejection fraction in mice with decompensated cardiac hypertrophy. Rapamycin also altered the expression of some fetal genes, reversing, in part, changes in α-myosin heavy chain and sarcoplasmic reticulum Ca²⁺ ATPase.

Conclusions—Rapamycin may be a therapeutic tool to regress established cardiac hypertrophy and improve cardiac function. (Circulation. 2004;109:3050-3055.)

Key Words: hypertrophy ■ heart failure ■ signal transduction

In response to growth factors and amino acids, the mammalian target of rapamycin (mTOR) controls the mammalian translational machinery. Rapamycin, a lipophilic macrolide, inhibits growth by forming a gain-of-function inhibitory complex with FKBP12 (FK506-binding protein, with a MW of 12 kD 1,2). This complex binds to mTOR, preventing activation of mTOR targets such as the 40S ribosomal S6 protein, an important regulator of protein synthesis. Rapamycin was shown to inhibit angiotensin II– and phenylephrine-induced increases in protein synthesis in cardiac myocytes in vitro. Recently, we reported that rapamycin attenuated pressure overload–induced cardiac hypertrophy in mice. In that study, rapamycin was administered to mice before they were subjected to pressure overload. The aim of the present study was to examine whether rapamycin can regress established cardiac hypertrophy.

Methods

Generation of Mice With Aortic Constriction

For aortic-banding experiments, ascending aortic constriction was performed in 12-week-old male FVB/N mice as described. Animal care and experimentation were approved by the Institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Center.

Administration of Rapamycin

Rapamycin (2 mg · kg⁻¹ · d⁻¹; gift from Wyeth-Ayerst) or vehicle was administered intraperitoneally to aortic-banded or sham-operated mice as previously described.

Echocardiography

Echocardiography was performed as previously described, except that 2,2,2-tribromoethanol (Aldrich; 0.4 mg/g) was used for anesthesia. Echocardiography was performed 1 week after aortic banding or the sham operation and 1 week after administration of rapamycin or vehicle. To evaluate the degree of stenosis (1 week after aortic banding), the pressure gradient across the constriction was assessed by Doppler echocardiography. A nonimaging Doppler pencil transducer (continuous wave) was placed at the apex and oriented toward the proximal ascending aorta. The peak velocity (m/s) was measured, and the maximum instantaneous gradient (mm Hg) was calculated by use of the Bernoulli equation: pressure gradient = 4 × (velocity)².
thickness in the left ventricular (LV) walls of aortic-banded mice. Mice were randomly divided into 4 groups: (1) sham vehicle, (2) band vehicle, (3) sham rapamycin, and (4) band rapamycin. Mice received vehicle or rapamycin for 1 week, and echocardiography was performed before they were killed.

**LV Dry Weight**
The LV was dissected and the dry weight obtained as described previously. In brief, the LV dry weight was obtained by desiccation in an oven at 93°C for 48 hours.

**Measurement of the Ribosomal S6 Protein**
S6 phosphorylation was measured as described previously.

**Northern Blot Analysis**
Northern blot analysis was performed as described previously. Total RNA (10.0 μg) was electrophoresed in 1.3% denaturing formaldehyde agarose gels and blotted onto Hybond N membranes (Amer). Membranes were probed with atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), β-myosin heavy chain (MHC), α-MHC, α-skeletal actin, sarcoplasmic reticulum Ca²⁺ ATPase (SERCA)2a, and GAPDH radiolabeled probes. The probes for ANP, BNP, α-skeletal actin, SERCA2a, and GAPDH have been described previously. Rat β-MHC 3' untranslated region cDNA and mouse α-MHC 3' untranslated region cDNA were kind gifts from M. Buckingham (Department of Developmental Biology, Pasteur Institute, France).

**Statistical Analysis**
Data are presented as mean ± SEM. For comparisons of parameters within the same time point, ie, pretreatment or posttreatment, 1-way ANOVA was used to test for overall significance, followed by the Tukey post hoc test. Paired t test and ANOVA with repeated measures were used to test for significance of the same parameter, within the same group, at 2 and 3 different time points, respectively. A value of P < 0.05 was considered significant.

**Results**
Hypertrophy in response to a pathological stress is an adaptive response to an increase in cardiac load. Initially, the increase in heart mass serves to normalize wall stress, and the heart can function normally at rest. This adaptation is referred to as compensated hypertrophy. However, if the stimulus for pathological hypertrophy is sufficiently intense or prolonged, the ventricle dilates, cardiac function diminishes, and the heart fails (decompensated hypertrophy). On the basis of the echocardiography results measured 1 week after aortic constriction (before administration of rapamycin or vehicle), 2 distinct groups of mice became apparent: (1) mice with compensated cardiac hypertrophy (increase in LV wall thickness, normal function: fractional shortening ≥ 45%) and (2) mice with decompensated hypertrophy (increase in LV thickness, dilation, depressed function: fractional shortening < 45%). Cardiac function was considered normal if the fractional shortening was at least 45%, because all sham-operated mice had a fractional shortening of 45% or greater, and in our hands, “normal” mice (nonoperated, nontransgenic mice) have fractional shortenings of 45% or greater. Aortic-banded mice were subsequently further subdivided: compensated band-vehicle, compensated band-rapamycin, decompensated band-vehicle, and decompensated band-rapamycin.

**Assessment of Cardiac Hypertrophy**
In this study, it was critical to demonstrate that exposure to pressure overload for 1 week induced significant cardiac hypertrophy. Before administration of rapamycin or vehicle, echocardiography was performed on each group of mice. Mice in each of the aortic-banded groups displayed significantly greater LV wall thicknesses compared with sham-operated mice (Table 1, Pretreatment: interventricular septum thickness [IVS] and LV diastolic posterior wall thickness [LVPW]). To verify that this reflected a significant increase in heart mass, 3 groups of mice were killed 1 week after the operation without receiving vehicle or rapamycin (Table 1, Sham No Treatment, Band Compensated Hypertrophy No Treatment, Band Decompensated Hypertrophy No Treatment). As previously reported, mice develop significant hypertrophy after being subjected to aortic banding for 1 week (HW/BW ratio increased by > 35% compared with sham, Table 1).

**Compensated Cardiac Hypertrophy Versus Decompensated Cardiac Hypertrophy**
Before treatment with rapamycin or vehicle, mice with decompensated cardiac hypertrophy had significantly larger LV end-systolic dimensions, lower fractional shortenings, and lower ejection fractions than mice with compensated hypertrophy (Table 1). Aortic-banded mice within the compensated and decompensated groups were randomly divided to receive rapamycin or vehicle. Importantly, before administration of rapamycin or vehicle, mice were indistinguishable within their groupings on the basis of the echocardiography results. Thus, before treatment, mice designated to receive vehicle or rapamycin within the compensated group were considered to have similar HW/BW ratios, as was the case with the decompensated group.

**Rapamycin Regresses Established Cardiac Hypertrophy**

**Compensated Cardiac Hypertrophy**
The HW/BW ratio of aortic-banded mice receiving vehicle for 1 week was not different from that attained in the group of mice killed 1 week after banding (without receiving treatment, Table 1: Band Compensated Hypertrophy No Treatment.). By contrast, the HW/BW ratio of aortic-banded mice receiving rapamycin for 1 week was significantly lower. Rapamycin regressed the increase in heart size by 68% (Figure, A). In aortic-banded mice treated with rapamycin, the LV wall thicknesses were not different from those of vehicle-treated mice or compared with the values measured in the same mice before treatment. However, there was a significant decrease in the LV end-diastolic dimension. This finding is consistent with our previous study. Thus, rapamycin regressed cardiac hypertrophy, and this was associated with a reduction in chamber size.

**Decompensated Cardiac Hypertrophy**
As observed in mice with compensated hypertrophy, the HW/BW ratio of aortic-banded mice receiving vehicle for 1 week was not different from that attained in the group of mice killed 1 week after banding (without receiving treatment,
Rapamycin also regressed cardiac hypertrophy in the group of mice with compensated hypertrophy. However, in contrast to the 68% regression demonstrated in the compensated group, rapamycin regressed hypertrophy by 41% in the decompensated group (Figure A). Interestingly, rapamycin significantly reduced the LV end-systolic dimension in mice with decompensated hypertrophy and improved fractional shortening and ejection fraction (Table 1). The reduction in LV end-systolic dimension translated into a reduction in LV end-systolic volume (before treatment, 15.2±3.6 mm³; after treatment, 9.2±2.9 mm³, P<0.05). By contrast, there was no change in LV end-systolic volume in vehicle-treated mice with decompensated hypertrophy (before treatment, 14.2±4.0 mm³; after treatment, 14.8±3.4 mm³). Mice receiving rapamycin also tended to have a lower lung weight/BW ratio compared with the decompensated group receiving vehicle.

**Effect of Rapamycin on Body Weight and Mortality**

Injection of vehicle or rapamycin did not cause mortality in any group. Furthermore, rapamycin did not cause body weight loss (Table 1) or a change in other organ weights (kidney, spleen, liver; data not shown).

**Effect of Rapamycin on the Ribosomal S6 Protein**

The 40S ribosomal S6 protein is a target of mTOR, which regulates protein synthesis. We previously reported that S6 phosphorylation increased in the heart in response to pressure overload. In the present study, S6 phosphorylation was significantly elevated in hearts of vehicle-treated aortic-banded mice with compensated hypertrophy compared with...
A, Rapamycin significantly regressed pressure overload–induced cardiac hypertrophy. Percent change in HW/BW ratio of sham-operated mice (receiving vehicle or rapamycin), mice killed 1 week after aortic banding without receiving treatment, mice receiving vehicle or rapamycin for 1 week after previous subjection to aortic banding for 1 week, ie, killed 2 weeks after aortic banding. All HW/BW ratio data were collected at time of death. Percent change in HW/BW ratio of sham-operated mice given vehicle was normalized to 0%. Other groups are expressed relative to sham vehicle. On the basis of fractional shortening values, aortic-banded mice were divided into 2 groups: compensated hypertrophy and decompensated hypertrophy. For all analysis in this figure (A–C), 1-way ANOVA was used to test for overall significance, followed by Tukey’s post hoc test. *P<0.05. Numbers of mice in each group are the same as those in Table 1. B, Effect of rapamycin on ribosomal S6 protein. Phosphorylation of ribosomal S6 protein (pS6) in heart lysates of sham and aortic-banded mice 1 week after treatment. pS6 levels were normalized by expressing them relative to GAPDH. Representative blots (left). SV indicates sham-operated mice receiving vehicle; SR, sham-operated mice receiving rapamycin; BV, aortic-banded mice receiving vehicle; BR, aortic-banded mice receiving rapamycin; Comp., compensated hypertrophy; Decomp., decompensated hypertrophy. Quantitative analysis (right). Mean values for sham vehicle were normalized to 1, n=3 in each group. *P<0.05 vs sham vehicle. †P<0.05 vs sham rapamycin. ‡P<0.05 vs band vehicle. C, Effect of rapamycin on fetal gene expression in mice with compensated hypertrophy. Expression levels of ANP, BNP, α-skeletal (α-sk) actin, β-MHC, α-MHC, and SERCA2a were examined by Northern hybridization (left). Loading of RNA was normalized by reprobing the membrane with GAPDH. Right, Quantitative analysis of Northern blots. Mean values for sham vehicle were normalized to 1, n=3 in each group. *P<0.05 vs sham vehicle. †P<0.05 vs sham rapamycin. ‡P<0.05 vs band vehicle.
rapamycin caused a significant increase in the expression of these genes between mice with compensated hypertrophy and aortic-banded mice (Figure, B). A similar trend was apparent in banded mice with decompensated hypertrophy. Rapamycin significantly attenuated S6 phosphorylation in sham-operated mice and aortic-banded mice (Figure, B).

**Effect of Rapamycin on Fetal Gene Expression**
Pathological cardiac hypertrophy is usually associated with reexpression of the fetal gene program. In aortic-banded mice receiving vehicle, hypertrophy was associated with an increase in expression levels of ANP, BNP, α-skeletal actin, and β-MHC compared with sham. By contrast, α-MHC and SERCA2a levels were depressed (Figure, C). We detected no significant changes in the expression of these genes between mice with compensated and decompensated hypertrophy (data not shown). Interestingly, rapamycin caused a significant increase in the expression of β-MHC and α-MHC in sham-operated mice. Furthermore, in aortic-banded mice with compensated hypertrophy, rapamycin reversed the fall in SERCA2a and enhanced the expression of β-MHC and α-MHC compared with vehicle-treated banded mice (Figure, C).

**Wet and Dry Weight Analysis of the LV**
Some immunosuppressants have been associated with changes in hydration state. To exclude the possibility that the heart weight data were confounded to some degree by differential edema, the wet/dry weight ratio of the LV was measured. In aortic-banded mice, rapamycin treatment was associated with a significant reduction in the LV wet weight/BW and LV dry weight/BW ratio compared with vehicle (Table 2). Aortic-banded mice had a small but significantly greater wet/dry weight ratio compared with sham-operated mice. Importantly, rapamycin was not associated with a change in the wet/dry weight ratio in sham or aortic-banded mice (Table 2).

**Discussion**
In the present study, we have demonstrated that rapamycin can significantly regress cardiac hypertrophy induced by pressure overload, in the absence of deleterious effects on cardiac function or mortality. Unexpectedly, in response to pressure overload for 1 week, 2 groups of mice became apparent: (1) mice with compensated cardiac hypertrophy and (2) mice with decompensated hypertrophy. The reason for obtaining these 2 groups is not immediately obvious; however, mice with decompensated hypertrophy tended to have more lobulated masses of brown fat around their aortas, and this was an additional complication during surgery that may have resulted in a tighter band. In support of this suggestion, these mice were exposed to a slightly greater load as measured by the aortic gradient by echocardiography (P=0.054).

Rapamycin was effective in regressing compensated hypertrophy as well as decompensated hypertrophy, although it is worth noting that rapamycin was less effective in regressing hypertrophy in mice with decompensated hypertrophy than compensated hypertrophy (~40% and 70% regression, respectively). We previously reported that rapamycin attenuated load-induced hypertrophy via an mTOR-p70 ribosomal S6 kinase (S6K1)-S6 protein–dependent mechanism, and rapamycin significantly blocked the phosphorylation of S6 in the present study. One possible explanation for the above finding is that the mTOR-S6K1-S6 signaling cascade is the major pathway activated during compensated hypertrophy, but an mTOR-S6K1-S6–independent pathway is activated when the transition to decompensated hypertrophy occurs. Consistent with this working hypothesis, S6 phosphorylation was attenuated to a similar degree in mice with compensated and decompensated hypertrophy, and S6K1 knockout mice develop cardiac hypertrophy in response to aortic banding for 1 week.

Rapamycin administration resulted in a number of significant changes in fetal gene expression. In response to aortic banding, ANP, BNP, α-skeletal actin, and β-MHC expression levels increased in mice receiving vehicle, whereas α-MHC and SERCA2a levels fell. Rapamycin, in part, reversed the changes in α-MHC and SERCA2a. Perhaps the most interesting change was the reversal of the fall in SERCA2a gene expression. Studies have suggested that a decrease in SERCA2a expression and/or activity is a major defect responsible for impaired cardiac contractility in the failing heart.

It remains unclear whether current therapeutics can prevent the transition from compensated to decompensated hypertrophy or whether they only delay the transition. In the group of mice with decompensated hypertrophy, rapamycin appeared to reverse, at least in part, parameters characteristic of decompensated hypertrophy, ie, increased LV end-systolic dimension, depressed fractional shortening, and ejection fraction. The term ventricular remodeling has been applied to pathological states associated with altered ventricular vol-

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<th>TABLE 2. Wet and Dry Weight Analysis of the Left Ventricle From Aortic Banded Mice With Compensated Hypertrophy</th>
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BW indicates body weight; HW, heart weight; and LV, left ventricle. One-way ANOVA was used to test for overall significance, followed by the Tukey post hoc test.

*P<0.05 vs sham vehicle and sham rapamycin; †P<0.05 vs band vehicle.
volume, wall thickness, and shape. Rapamycin significantly decreased LV end-systolic dimensions in aortic-banded mice with decompensated hypertrophy, and this translated into a reduction in end-systolic volume by \( \approx 40\% \). For clinical trials, it has been suggested that a 10% reduction in LV end-diastolic or end-systolic volume can be considered a clear benefit of remodeling and can be used as sufficiently supportive of a directionally favorable but otherwise inconclusive data set with regard to morbidity and mortality outcomes,\(^\text{15}\) although it is also important to distinguish a drug’s effect on cardiac load from a true impact on the remodeling process.\(^\text{15}\) Additional studies would be required to determine whether rapamycin has a true impact on remodeling and whether this translates into a reduction in mortality in the pressure-overload model.

Rapamycin has been used clinically for the treatment of transplant rejection without signs of end-organ toxicity;\(^\text{16}\) thus, rapamycin may be a therapeutic tool to regress established cardiac hypertrophy and improve cardiac function. Further studies are required to examine whether long-term administration of rapamycin can completely prevent or reverse the transition from compensated to decompensated hypertrophy and whether it ultimately affects mortality.

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**References**

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