Dietary rapamycin selectively improves arterial function in old mice

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ABSTRACT

Dietary rapamycin inhibits mTOR, reduces age-related pathologies and extends lifespan, but it is unknown if it reverses age-related arterial dysfunction. The effects of mTOR inhibition on endothelial function and arterial stiffness were evaluated in young (Y: 6±2 mo) and old (O: 30±2 mo) male B6D2F1 mice fed control (C) or rapamycin-supplemented diet (RAP: 14mg/kg encapsulated chow) ad libitum for 6 weeks. Aortic mTOR signaling, assessed by S6 Kinase (S6K) Western blot, was higher in aged mice (P<0.05) and lower in rapamycin-treated old mice (P<0.05). Rapamycin selectively increased NO bioavailability (OC v. ORAP, P<0.05) and augmented endothelium-dependent dilation (EDD) to acetylcholine (YC: 96±2% v. OC: 73± 4%, P<0.05; v. ORAP: 95±1%, P<0.05) in carotid arteries of old mice. RAP also reduced aortic stiffness, assessed by pulse wave velocity, in old animals (Post-Pre: -43±19 cm/s, P<0.05). These improvements coincided with lower aortic nitrotryosine (YC: 1.0±0.2 v. OC: 4.7 ±1.6, P<0.05; v ORAP: 1.5±0.3, P<0.05) and collagen expression (YC: 37±9% v. OC: 78±5%, P<0.05; v. ORAP: 50±9%, P<0.05). In young mice, RAP impaired endothelial function (P<0.05) and did not affect arterial stiffness. In conclusion, rapamycin improves arterial function in old mice with enhanced mTOR activity, suggesting that mTOR may be an effective therapeutic target in older adults.

BACKGROUND

- Advancing age is one of the major risk factors for developing cardiovascular disease (CVD) and is associated with endothelial dysfunction and arterial stiffening in men and women in the absence of clinical disease[2-6].
- We have previously demonstrated the mammalian target of rapamycin (mTOR) signaling is elevated in arteries from old mice and is associated with endothelial dysfunction and arterial stiffening
- Rapamycin given to aged mice slows multiple signs of aging and extends lifespan[9].
- Currently, it is unknown if rapamycin treatment and mTOR inhibition can reverse vascular aging.

HYPOTHESIS

We hypothesize that inhibition of mTOR signaling with dietary rapamycin will significantly reverse age-associated arterial stiffening and endothelial dysfunction.

RESULTS

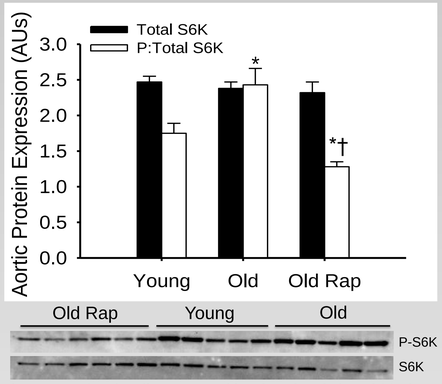


Figure 1: mTOR signaling assessed by phosphorylation of S6 Kinase (S6K) is increased with advancing age and is reduced by dietary rapamycin. Total (black bars) and phosphorylated (white bars) S6k , expressed as a ratio of total to phosphorylated , protein expression in the aortas of young , old and old rapamycin treated (RAP) B6D2F1 mice. Values are means±SEM. * Denotes p<0.05 vs young control

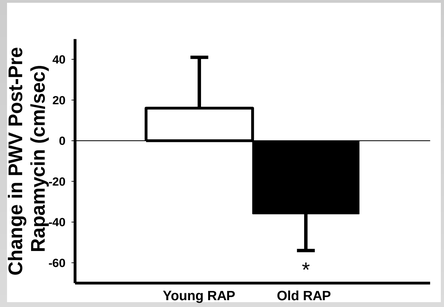


Figure 2: Arterial Stiffening, analyzed via aortic pulse wave velocity (PWV), was reduced after dietary rapamycin treatment in old but not young mice. Old mice had a significantly greater PWV than young mice (Old 390 ± 11 vs. Young 306 ± 10 P<0.05, not shown). PWV decreased in old mice (black bar) after rapamycin treatment, whereas there was no effect of rapamycin on PWV in young (white bar) mice. Values are means±SEM.
* Denotes p<0.05 vs pre-treatment

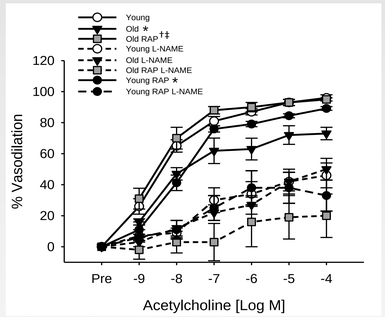


Figure 3: Dietary rapamycin ameliorates age-associated reductions in endothelium dependent dilation (EDD) in old, but blunts EDD in young mice. Carotid artery EDD was lower in old (black triangles) compared with young (white circles) mice. Rapamycin treatment reduced EDD in young (black circles), but improved EDD in old (grey squares) mice. Incubation with L-NAME, a NOS inhibitor, reduced EDD in all groups and after L-NAME, EDD was lower in old rapamycin-treated mice compared to old mice. Values are means±SEM. * Denotes p<0.05 vs Young, † Denotes p<0.05 vs Old, ‡ Denotes p<0.05 vs Y-RAP

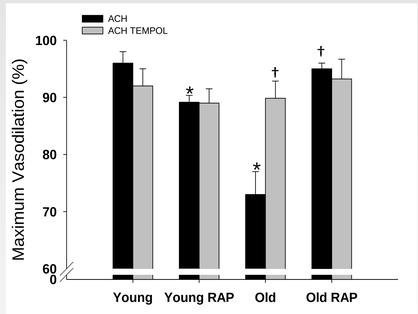


Figure 4: In vitro treatment with the superoxide dismutase mimetic, TEMPOL, restores endothelium dependent dilation (EDD) in carotid arteries from old mice but was without effect on arteries from young or old rapamycin treated mice. Maximal vasodilation to acetylcholine (ACh) in the absence (black bars) or presence (grey bars) of TEMPOL in carotid arteries from young and old control and rapamycin (RAP) treated mice. Values are means±SEM.* Denotes p<0.05 vs Young ACh, † Denotes p<0.05 vs Old ACh

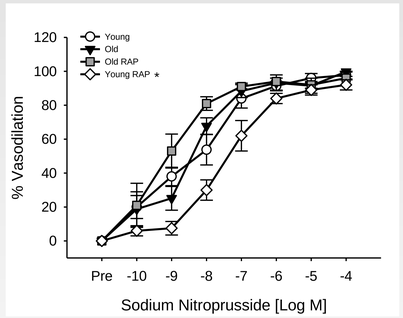
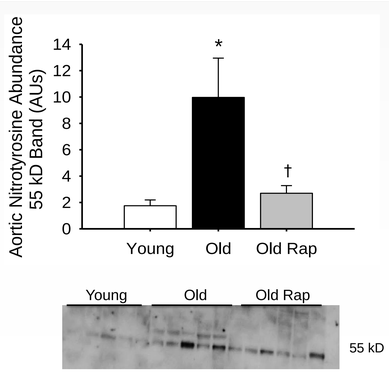


Figure 5: Although blunted in young mice by dietary rapamycin, endothelium independent dilation (EID) to sodium nitroprusside (SNP) was not altered by aging or rapamycin treatment in old mice. Dose response curves to the cumulative addition of SNP in carotid arteries from young (white circles), old (black triangles), young rapamycin (white diamonds) and old rapamycin (grey squares) treated mice. Values are means±SEM. * Denotes p<0.05 vs young control

Figure 6. Age-associated increases in arterial oxidative stress are ameliorated by rapamycin treatment. Aortic nitrotyrosine abundance, an oxidative stress marker, in young (white bar), old (black bar) and old rapamycin (grey bar) treated (RAP) B6D2F1 mice. Values are means±SEM. * Denotes p< 0.05 vs. Young, † denotes p<0.05 vs. Old Control.



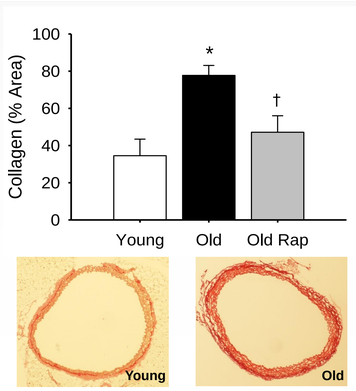


Figure 7. Age-associated increases in arterial collagen content are ameliorated by rapamycin treatment. Aortic collagen content, assessed by picrosirius red staining, in young (white bar), old (black bar) and old rapamycin (grey bar) treated (RAP) B6D2F1 mice. Values are means±SEM. * Denotes p< 0.05 vs. Young, † denotes p<0.05 vs. Old Control.

METHODS

Animal Model

- Mice were purchased from aged rodent colonies maintained at Charles River Inc., and studied at 4-6 mo (young: Y) and 29-31 mo (old: O) of age.
- Animals were assigned to one of four experimental groups: young control, young rapamycin-treated, old control, and old rapamycin-treated.
- The mice were housed with a 12:12 light-dark cycle, fed standard chow or standard chow supplemented with rapamycin and water ad libitum for six weeks (Rapamycin dose at 14 mg/kg of standard chow[9]).
- Body weight and food/water consumption were monitored weekly before, during, and after 6 weeks of treatment.
- Non-terminal measures were performed pre- and post-rapamycin.

Vascular Function

Aortic Pulse Wave Velocity

- Aortic pulse wave velocity (PWV) was measured on anesthetized mice before and after treatments by the use of two Doppler probes, one placed externally next to the aortic arch and the other next to the abdominal aorta.
- Distance between the two probes divided by the time required for a bolus of blood to travel from one probe to the other were used to compute the velocity of the pulse. Stiffening of the aorta reduces compliance, thus increasing the speed of the pulse wave.[10]

Endothelium In-/Dependent Dilation

- Carotid arteries were incubated in warm physiological saline solution at 37°C, pressurized to 50 mmHg, and allowed to equilibrate for 60 minutes. Vessels were pre-constricted with phenylephrine (2μM).
- Lumen diameter was measured in response to cumulative addition of acetylcholine (ACh: 10^{-9}-10^{-4} M), in the absence or presence of the nitric oxide synthase inhibitor, L-NAME (0.1mM).
- ACh responses were repeated in the contra-lateral vessel after incubation with the superoxide dismutase mimetic, TEMPOL (1mM).
- Endothelium-independent dilation (EID) was determined in response to cumulative addition of sodium nitroprusside (SNP: 10^{-9}-10^{-4} M).

Oxidative Stress

- Western blots for nitrotyrosine, a marker of oxidative stress, were performed on aortic lysates

Collagen Content

- Aortic tissue was preserved in OCT compound, then cut into 10μm sections.
- Histochemistry was performed on aortic sections with picrosirius red, which stains for collagen I and collagen III.

Statistics: Differences in dose responses were determined via Repeated Measures ANOVA and Mean Arterial Pressure/Pulse Wave Velocity via Student's T-test. P≤0.05, with a Bonferonni correction for multiple comparison, P<0.013.

CONCLUSIONS

- Reducing mTOR signaling via six weeks of dietary rapamycin supplementation improved endothelial function and NO bioavailability by lowering oxidative stress in old mice
- Rapamycin treatment in old mice resulted in blunted aortic arterial stiffness and was associated with lower collagen content
- Rapamycin treatment blunted arterial vasoreactivity in young mice suggesting that improvements in arterial function may be limited to arteries with augmented mTOR signalling

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