Rapamycin Ameliorates Age-Associated Intervertebral Disc Degeneration In Male Marmosets

Becca Kritschil

NO DISCLOSURES
- Cells age over time, and accumulated damage accelerates this process
- Over time, the accuracy of repair mechanisms for molecular damage decreases
- Impaired mechanisms for repair contribute to more cell aging and perpetuate the aging cycle, causing more accumulation of molecular damage
Intracellular degradation mechanism

Activated in times of cellular stress, i.e., starvation and hypoxia

Removes damaged proteins, and organelles through fusion with lysosome

Shown to be decreased with age and in some age-related degenerative diseases such as Parkinson’s
• Rapamycin has been shown in several animal models, including mammalian, to increase lifespan and decrease aging and age-associated pathologies

• Rapamycin inhibits mTOR, which is a negative regulator of autophagy

**Question:** Does rapamycin mitigate disc aging through inhibiting mTOR, leading to upregulation of autophagy?

**Goal of study:** Determine the effect of acute rapamycin treatment on age-associated IDD in marmosets
METHODS

Used: common marmoset

Rapamycin capsule in yogurt

- **AGE**
  - **< 5 years**
  - **1X/day**
  - **1 YEAR**
  - **OLD + Rapa**
  - **> 10 years**

- **AGE**
  - **> 10 years**

- **AGE**
  - **YOUNG**
  - **< 5 years**

**Outcome measures**

- mTOR inhibition western blot
- Disc matrix anabolism (aggrecan IHC, DMMB assay)
- Disc histological features (H&E)
- Disc matrix catabolism (aggrecan fragmentation, MMP & ADAMTS protein)
- Disc autophagy markers
RESULTS

mTOR inhibition

A. mTOR western blot. To confirm rapamycin treatment inhibited mTOR in disc cells, active phosphorylated mTOR was probed for in disc cells compared to total mTOR. B. There is a decrease in active phosphorylated mTOR in the old marmosets given rapamycin.

Histology

C. Lumbar NP GAG content significantly increased in old marmosets given rapamycin. D-E. Aggrecan IHC staining showed older marmosets given rapamycin had aggrecan staining in red compared to old control marmosets.

D. Aggrecan IHC

E. Aggrecan IHC

F., G. Histology photos and quantification. F. NP and AF images shown for 3 independent animals per group. G. Older marmosets had a significantly higher histological grade compared to young marmosets and rapamycin treatment given to old marmosets significantly decreased histological grade.
RESULTS

A., B. Aggrecan fragmentation. A. Young marmosets’ aggrecan fragmentation mediated by MMPs and ADAMTS’ compared to old marmosets. There was a significant increase in ADAMTS- mediated aggrecan fragmentation in the old marmosets compared to you. B. Old marmosets’ aggrecan fragmentation compared to rapamycin treated old marmosets. There was a trend in old + rapamycin marmosets to have decreased MMP and ADAMTS mediated fragmentation.

C., D. Catabolic protein expression. MMP13 and ADAMTS-4 protein expression was measured via western blot. C. Quantified protein expression in young, old, old + rapamycin marmosets shows a significant decrease in ADAMTS-4 expression in old + rapamycin marmosets compared to old marmosets.
RESULTS

**Autophagy markers**

A., B. Autophagy markers protein expression. Five known protein markers were probed and quantified. A. LC3-II expression significantly increased in old marmosets compared to young marmosets. p62 expression approached a significant decrease in rapamycin treated old marmosets compared to old marmosets. B. Western blots for each protein, normalized to b-actin.
- Rapamycin treatment significantly restored proteoglycan homeostasis and significantly improved histological score in the disc of aged marmosets

  ❖ *Implication:* one-year daily rapamycin treatment delayed IDD progression in older marmosets

- Autophagy protein markers do not conclusively show autophagy regulation with rapamycin treatment in old marmosets compared to old marmosets not given rapamycin

  ❖ *Implication:* rapamycin treatment may regulate other mTOR pathways that lead to the observed beneficial effects in old marmosets

- **Novel study** of the *in vivo* effects of rapamycin treatment on age-associated IDD in marmosets
  ❖ Some beneficial outcomes on disc aging were reported

- First study to use a **nonhuman primate model** to study age-associated IDD and rapamycin treatment
Acknowledgements

Ferguson Laboratory
Dong Wang
Qing Dong
Chao-Ming Zhao

Gwendolyn Sowa
Joon Lee
Kevin Bell
Nam Vo

The Southwest National Primate Research Center (SNPRC) and the San Antonio Claude D. Pepper Older Americans Independence Center (Pepper Center) at the University of Texas Health Science Center at San Antonio

Department of Orthopaedic Surgery
Ferguson Grant