The Effect of Rapamycin Dosing on A Murine Model of Congenital Muscular Dystrophy Over a Trial of Six Months

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Introduction

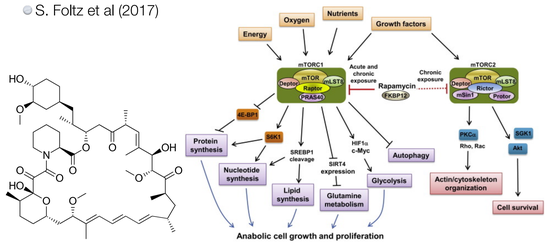
- Muscular dystrophy (MD) is a group of genetic diseases that are most known for causing the progressive weakening and loss of function of the skeletal muscle.
  - Can also negatively impact other organ systems
  - Has been known to cause a variety of cardiopulmonary complications
  - Can also impact the brain, leading to nervous system malformations and side effects such as lissencephaly, seizures, and hydrocephalus
  - Can kill via breathing issues, meaning that many patients must use ventilators in the late stages of the disease
- Prognosis is extremely dependent on the type of MD and the individual patient themselves (8)
- The process through which MD is inherited is currently not well-understood
  - Different subsets of MD exhibit unique inheritance patterns
  - Can be autosomal, sex-linked, recessive, or dominant, depending on the specific subtype
  - Can also present in people with no family history of the disease
- Duchenne muscular dystrophy is caused by the absence of the protein dystrophin, which assists in the cohesion of myocytes (12)
  - The absence of dystrophin is caused by a mutation in the DMD gene, which is usually inherited, but may also occur spontaneously
- Recessive mutation myodystrophy (MYD) mice are a murine model of muscular dystrophy in which dystroglycanopathy is caused by a mutation on chromosome 8 that results in abnormal glycosylation of the protein α-dystroglycan (15) T
  - This causes a variety of symptoms, such as progressively deteriorating muscular weakness, fatigue, a smaller adult size, reduced growth, and early fatality. (13)
- The drug rapamycin (also known as sirolimus) may be able to decrease myopathy in people with muscular dystrophy (5)
  - Induces autophagy, a process by which old or unhealthy cells are destroyed

Healthy Tissue

MD Tissue

Literature Review

- Duchenne muscular dystrophy is a severe form of muscular dystrophy causing progressive myopathy, scoliosis, intellectual disability, and eventual immobility.
  - C. Angelini et all (2017)
- Because the heart and diaphragm are both muscles, muscular dystrophy can negatively affect them, resulting in problems with breathing and eventual cardiac arrest which can result in death.
  - J. Bach et al (1997) and I. Seigal et al (1972)
- Duchenne muscular dystrophy is caused by a mutation in the dystrophin encoding gene
  - K. Campbell et all (1989), A Anh et al (1993), and P Spitali (2012)
- This occurs due to improper glycolysation of alpha dystroglycanase
  - P Grewal et al (2001)
- Rapamycin limited muscular dystrophy development and improved symptoms in fukutin-deficient mice when dosed over a four week period, and additional manipulations with the mTOR (mammalian target of rapamycin) pathway could have potential therapeutic applications in dystroglycanopathy patients
  - S. Foltz et al (2017)



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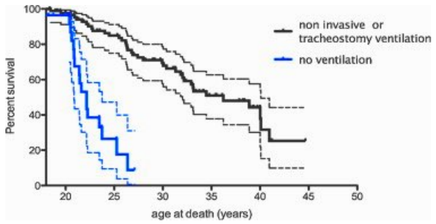
Hypothesis

Treatment with rapamycin three times per week will improve muscular dystrophy symptoms in MYD mice over a period of six months.

Gap in the Research



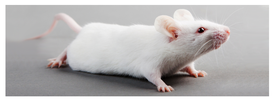
There is currently no cure for muscular dystrophy.

https://www.sciencedirect.com/science/article/pii/S1877065713000869

Methodology

Mice

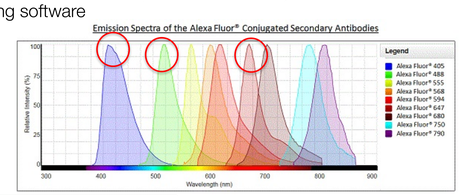
- Knockout, MD prone mice obtained from Jackson Labs and maintained at SUNY Binghamton
- Earclips taken at birth for genotyping purposes
- Tested for a variety of criteria (weight, torque, ALT & BUN)



Conditional dystrophin knockouts

Microscopy

- Iliopsoas muscle used
  - Small and easy to access, multiple can fit on a single slide
- Sectioned with microtome cryostat and stained with H&E
- Stained with Alexa Fluor far-red, blue, and green dyes for fluorescence microscopy
- Completed slides were placed underneath an X71 inverted epifluorescent microscope with camera
- Section maps were compiled both manually and with the use of imaging software

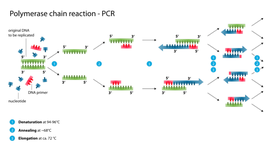


Data Analysis

- AlphaView 3.0 software (Protein Simple) and Prism 5 (GraphPad)

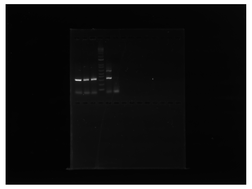
PCR

- Identify incomplete knockouts, complete knockouts, and controls

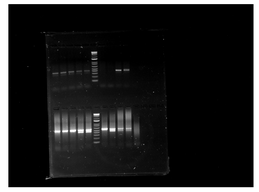


https://www.abcam.com/goat-rabbit-igg-hl-alexa-fluor-488-ab150077.html

Results

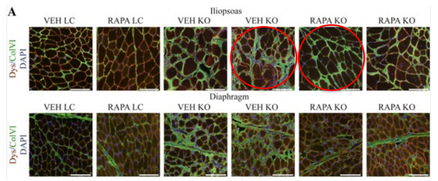


Rosa



DT Cre & DT Flox

- Early behavioral observations indicate improvement in muscular dystrophy mice who have received treatment
- Initial 4 weeks showed decreased fibrosis (blue) in dosed mice

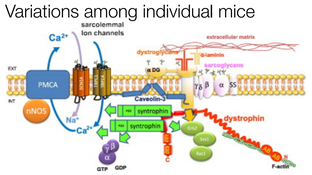


Discussion

- Four-week daily dosing results of the initial study showed a marked improvement in myopathy in dosed animals
  - Fiber size was increased, fibrosis and inflammation were reduced, and torque was higher in experimental group
- Significant correlation between treated animals and decreased dystroglycanopathy progression
  - Indicate that mTOR inhibition for five days per week results in disease feature improvement in murine dystroglycanopathy models
- Adverse effects of daily treatment were numerous, and certain mice did not improve
  - Side effects of rapamycin negatively impacted kidney and liver health
  - Toxicity of 5 times/week dosage led to other damage even in healthy mice
- Dose needs to be decreased

Limitations

- Incomplete dystrophin knockouts
  - Knockout is embryonic lethal, so researchers set it to be knocked out under certain conditions only so mice don't die in utero
  - Possibility that some of the healthier mice were incomplete knockouts
- Variations among individual mice



Dystrophin can't be knocked out completely or mice will die in utero, so a conditional knockout is made

https://www.sciencedirect.com/science/article/pii/S0005273613003027

Conclusion

- Ongoing study
- Limitations created by incomplete knockouts
- Rapamycin is effective, but high doses can cause unwanted side effects
- Ultimately, more research is needed before this treatment progresses to human trials

Applications

- Despite rapamycin's toxicity in large doses, it still has potential as a treatment for muscular dystrophy
  - Rapamycin is already approved by the FDA, so it wouldn't need to go through such an extensive approval process all over again for muscular dystrophy treatment
- Decreased muscular atrophy can mean individuals affected by muscular dystrophy can have a higher quality of life
  - Reduction of wheelchair usage
  - Increased participation in athletic activities
  - Decreased need for hospitalization
  - Longer lifespan

Future Research

- Monitoring of rapamycin's side effects
  - Effect on kidneys and liver in particular
- Determining ideal dosing schedule for maximum efficiency and minimum side effects
- How to create fewer incomplete knockouts

Sirolimus is another name for rapamycin

https://www.drugs.com/sfx/sirolimus-side-effects.html

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