Induction of Apoptosis with Staurosporine in Tendon Explants

Lizaveta Slootskaya[1,2], Emma Stowe[2], Brianne Connizzo[2]
[1]Saint John Vianney High School, Holmdel, NJ, [2]Boston University, Boston, MA

Introduction

Background:

- Tendon injuries are a prevalent problem among the aging population
- Senescent cells, which are apoptosis resistant, accumulate in aged tendons, and increase risk of injury [1]
- It is unknown whether aged and senescent tendons are resistant to apoptosis *in vitro*

Goal:

- Develop a model of late-stage apoptosis in tendon explants with staurosporine (SP)

Hypothesis:

- Increased SP treatment will increase apoptosis, leading to increased cell death and TUNEL staining

Methods

Sample Preparation:

- 20 FDL tendons explants were harvested from young male mice [3]
- Explants were cultured in standard media conditions for 7-days in stress-deprivation

Apoptosis Induction:

- Explants were treated with 1uM SP for a 48, 36, and 24-hours at D7

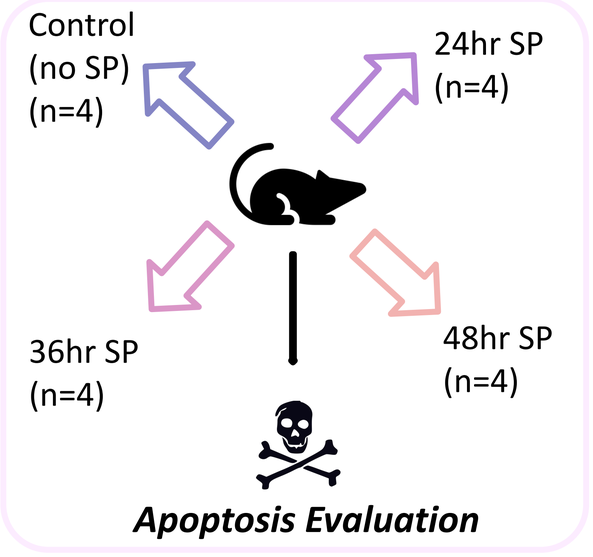

Figure 1. Study design

Cell Metabolism:

- A resazurin reduction assay was performed at day 7

Apoptosis Staining:

- Tendons were stained with TUNEL and imaged

Statistics:

- One-way ANOVAs with post-hoc corrected t-tests (*p≤0.05)

Results

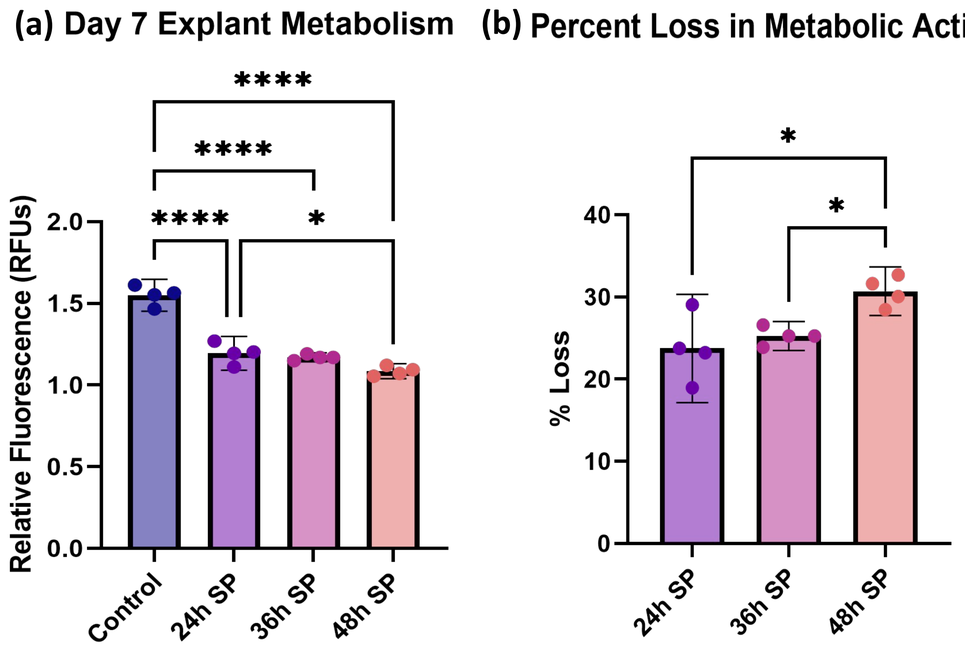

Figure 2. (a) D7 explant metabolism (b) calculated loss in metabolism due to SP treatment, *p<0.05.

- There is a significant difference between the control group and all the treatment groups and between the 24 and 48-hour treatment in cell metabolism, an indicator of explant health
- The 24 and 36-hour SP treatment and the 36 and 48-hour SP treatment are significantly different in calculated loss in metabolism

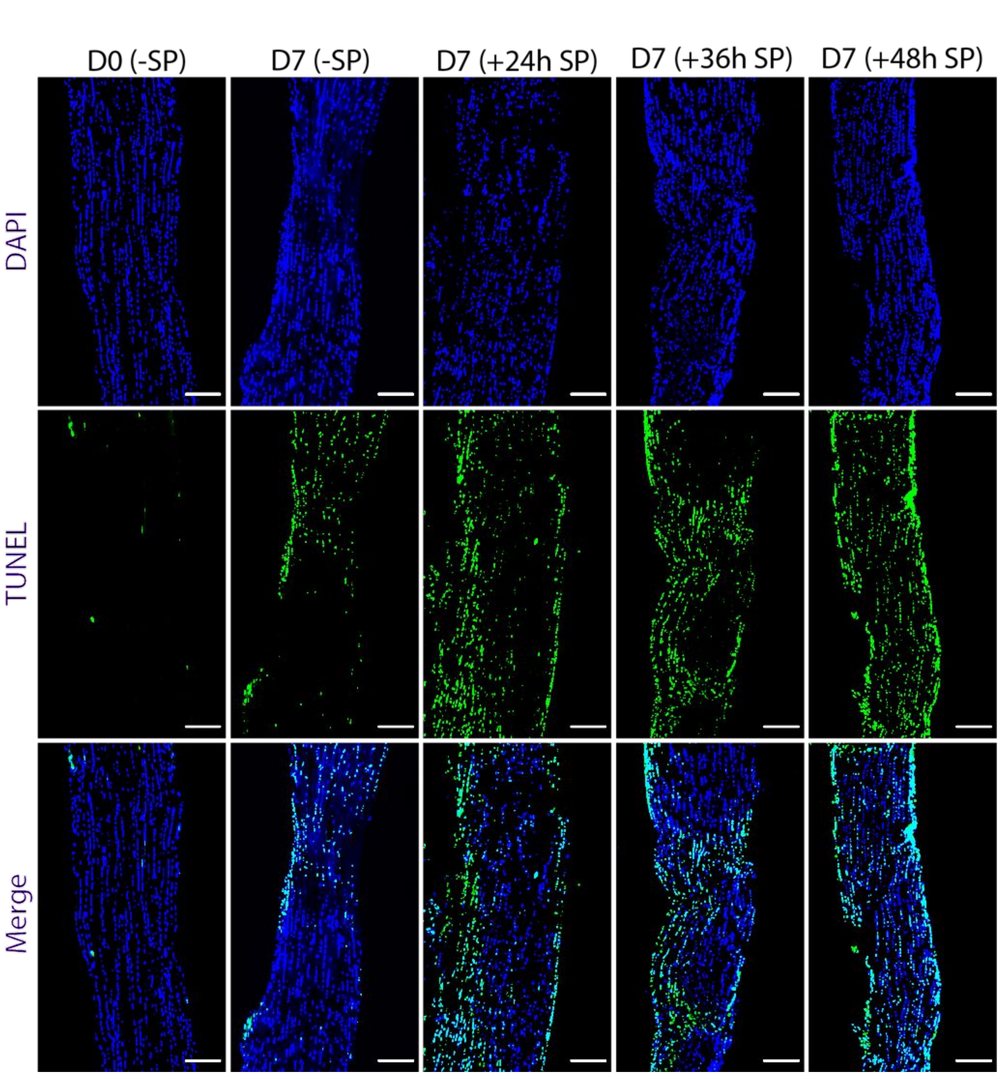

Figure 3. Representative Images showing TUNEL activity at D0 and D7 (20x, 200μm scale bars).

- Representative images show a trend of increased TUNEL staining suggesting more late-stage apoptosis in groups with longer SP treatment.
- Image quantification shows that the D0 control is 0.47% apoptotic, the D7 is 15.57% apoptotic, and all three of the treatment groups are between 65-75% apoptotic (n=1/group).

Conclusions

Discussion:

- 24 hours to 48 hours of SP treatment induces apoptosis in tendon explants, shown through explant metabolism and TUNEL staining
- Data supports the hypothesis that increased treatment time in SP increases apoptosis

	24hr SP	36hr SP	48hr SP
Metabolism	⇩	⇩	⇩ ⇩
TUNEL	⇧	⇧	⇧

Table 1. Summary of key results

Future Directions:

- Performing TUNEL staining on a larger sample size
- Monitoring the apoptotic pathway through caspase activations (qPCR)
- Exploring apoptosis resistance in aged and senescent tendons

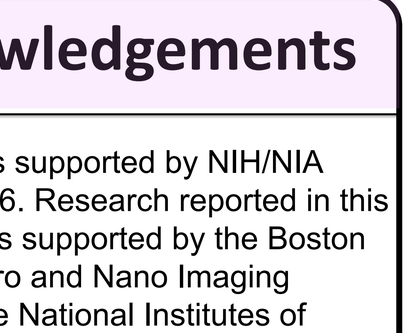

Figure 4. Senescence vs. apoptosis. Recreated from [2].

References

[1] Hernandez-Segura+2018, *Trends in cell bio.*, 28.6: 436-453.
[2] Soto-Gamez+2019, *Journal of molecular bio.,* 431.15: 2629-2643.
[3] Connizzo+2019, *Connect Tissue Res.*, 61(1):48-62.

Acknowledgements

This study was supported by NIH/NIA R00-AG063896. Research reported in this publication was supported by the Boston University Micro and Nano Imaging Facility and the National Institutes of Health (S10OD024993).