

Induction of Apoptosis with Staurosporine in Tendon Explants

Introduction

Background:

- Apoptosis is a mechanism of programmed cell death.
- Cell death results in the degradation of the cellular components through the activation of caspases.
- Staurosporine (SP) is an antibiotic and well-known to induce apoptosis.

Goal: Monitor the exact timing and mechanism of caspase upregulation leading to apoptosis induced by staurosporine.

Hypothesis: After 6 hours of staurosporine treatment, caspase-8 would be upregulated.

Methods

Sample Preparation:

20 Flexor digitorum longus tendon explants were harvested from young (12-week) male mice. [3]

Apoptosis Induction: 1 μ M of SP was added to media for a 2, 4, 6 and 12-hour treatment.

Gene Expression: The gene expression of Caspase-3, Caspase-8, and Caspase-9 were quantified using quantitative reverse transcription polymerase chain reaction (RT-qPCR).

Statistics: One-way ANOVAs with post-hoc corrected t-tests (significance level, * $p < 0.05$)

Results

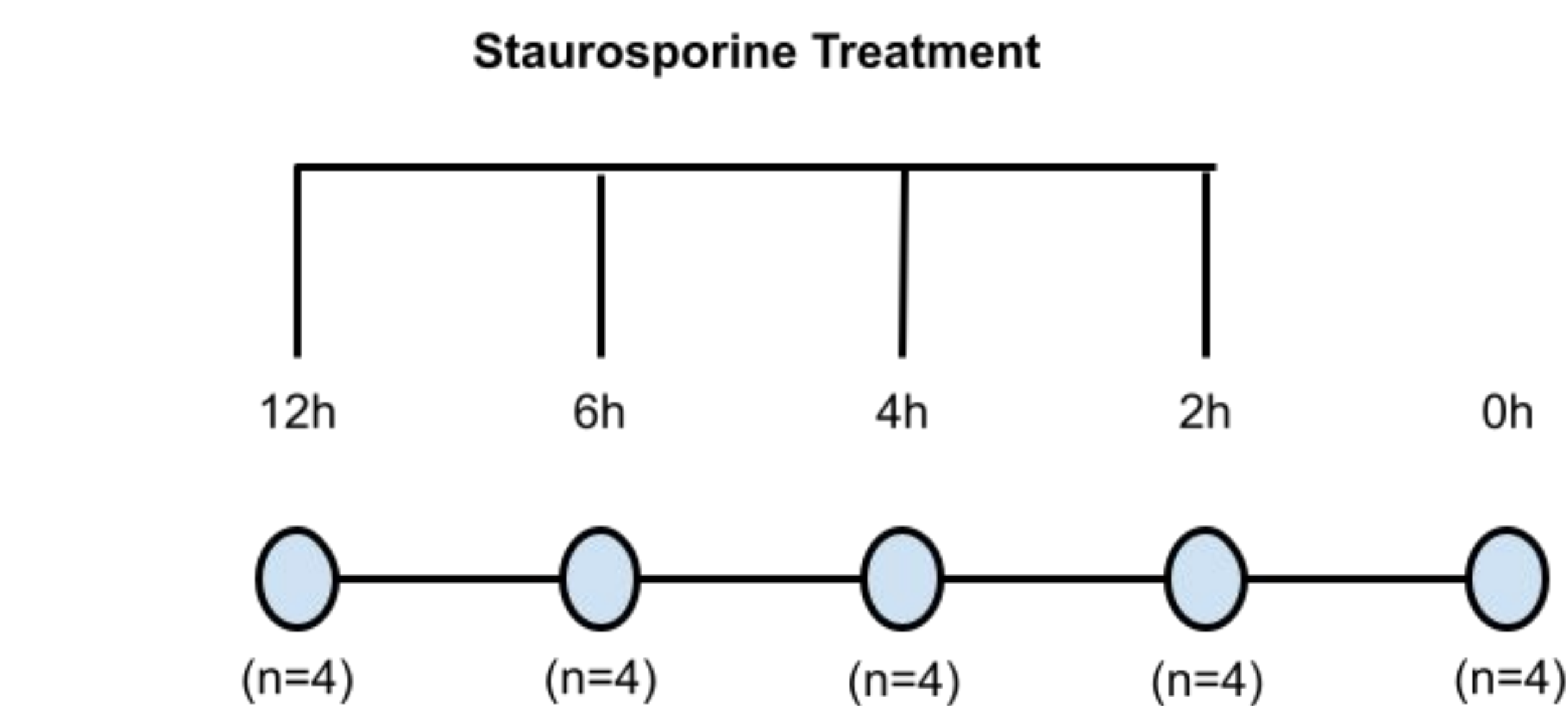


Figure 1. Apoptosis Induction Timeline

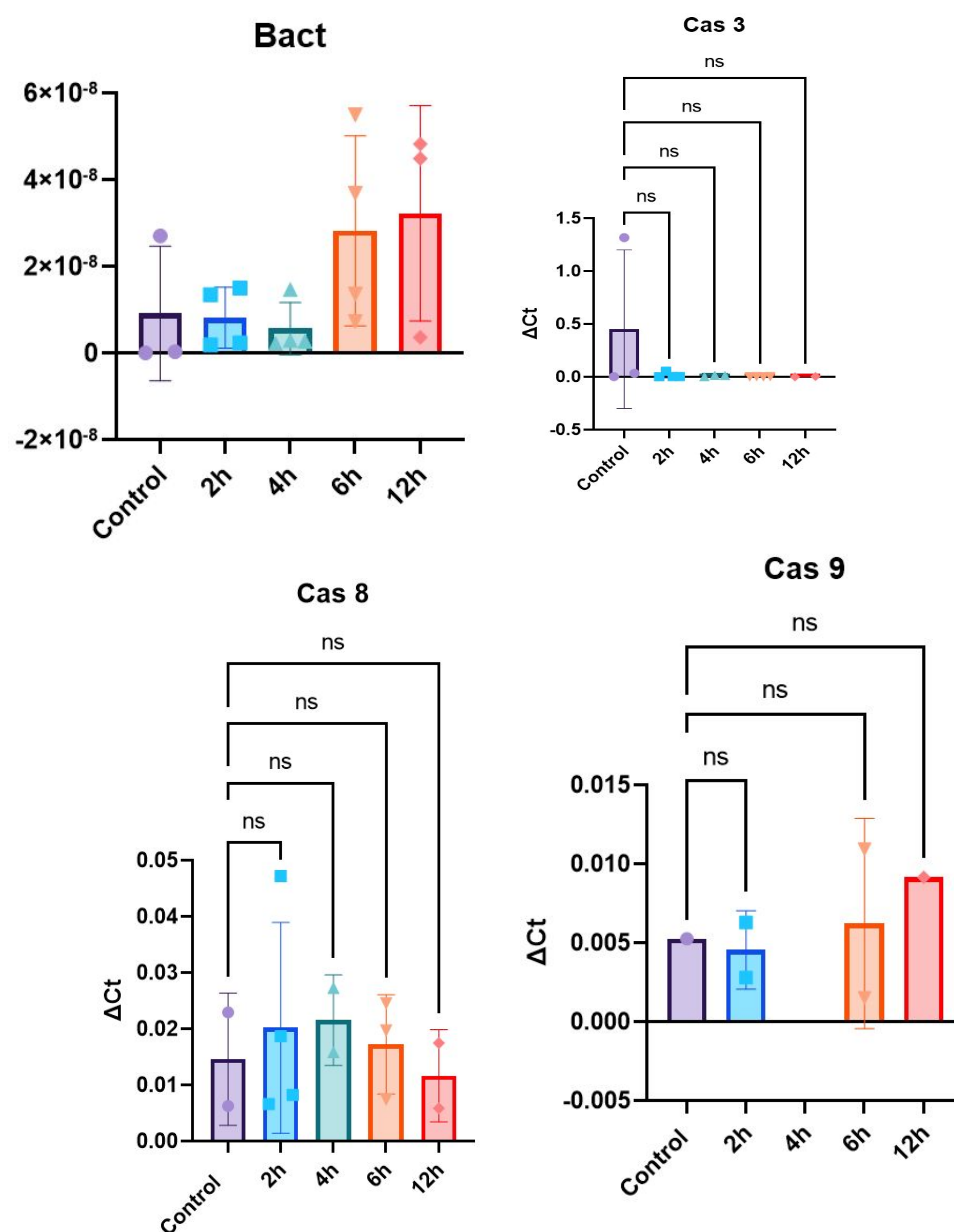


Figure 2. Gene expression data for (A) Bact, (B) Cas-3, (C) Cas-8, and (D) Cas-9. Data is presented as mean \pm standard error with significance bars spanning between groups where * $p < 0.05$.

- At 4-hour after staurosporine treatment, caspase-8 appears to be upregulated (no significant difference statistically).
- At 12-hour after staurosporine treatment, caspase-9 appears to be upregulated (no significant difference statistically).

Conclusions

Discussion:

- There is no significant difference in upregulation of caspases at any time point in comparison to control.
- Data refuted the hypothesis of caspase-8 being upregulated after 6 hours of staurosporine treatment.

Future Directions:

- Increasing sample size
- Exploring apoptosis resistance in aged tendons
- Performing assays for late-stage markers of apoptosis (TUNEL, Annexin V)
- Upregulation of caspases using other agents (e.g. TNF- α)

References

- [1] Hernandez-Segur+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

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