ACHILLES TENDON HEALING IN INOS KNOCKOUT MICE

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Introduction:

Nitric oxide (NO), a diatomic free radical, regulates the functions of many mammalian cells. We have previously reported that nitric oxide synthases (iNOS, eNOS and bNOS) are induced during rat tendon healing and inhibition of nitric oxide synthases (NOS) inhibited rat tendon healing [1]. The aim of this study was to evaluate the effects of deleting the iNOS gene on Achilles tendon healing in mice.

Methods:

1. Experimental groups:
Twenty-one 8–12 weeks old male iNOS gene knockout (iNOS -/-) mice (129/SvEv) and eight their wild-type (iNOS +/-) (C57BL/6) mice were utilized for this experiments: group 1: iNOS +/- mice (n = 8); group 2: iNOS -/- mice (n = 11) and group 3: iNOS -/- mice treated with a systemic NOS inhibitor (aminoguanidine in a mini-osmotic pump) (n = 10).

2. Animal model:
All procedures and protocols were approved by the Animal Care and Ethics Committee of the University of New South Wales, Sydney, Australia. The right Achilles tendon was transected in all mice, in a similar fashion to that previously outlined for the rat [2]. No operation was performed on the left uninjured hind limb. Group 3 mice were treated with aminoguanidine (AG), 500 g/kg/day, via an intraperitoneal mini-osmotic pump for seven days. The Achilles tendons were harvested on day 7 after surgery by sacrificing the mice with CO2 inhalation.

3. Measurement of tendon’s cross-sectional area:
A constant gap was created between two glass slides using standard shims placed at each end (0.27 mm for uninjured tendon, 0.53 mm for injured tendon). The tendon was placed between the two slides causing the tissue to flatten. The constrained tendon created a shadow when viewed through a transmission microscope and this was used to determine the tendon width. The cross-sectional area was calculated by multiplying the shim thickness by the tendon width.

4. Biomechanical tests:
Biomechanical assessment was carried out on an in-house mini tensile testing system. The muscle and intramuscular tendinous fibers were compressed between one set of serrated aluminium grips that were attached to the lead screw of the testing system. A block of dry ice was placed against the side of the grips to freeze the compressed tissue. The calcaneus was fixed to a second pairs of grips (set 7.5mm from the first grips), that were attached to a 50N load cell. The specimens were kept moist throughout the entire testing procedure by constant irrigation with 0.9% saline. Each specimen underwent a constant velocity ramp to failure (2.5mm/sec). Force and displacement data was collected using a dedicated computer and software.

5. Statistical analysis:
All data are presented as mean ± SE. Differences among experimental groups were assessed using unpaired two-tailed Student’s t-tests and analysis of variance (ANOVA). The level of statistical significance was accepted at P < 0.05.

Results:

1. Cross-sectional areas:
A significant reduction in cross-sectional area of the healing Achilles tendon was observed in iNOS -/- mice treated with the nitric oxide synthase inhibitor, AG. No significant difference was found between iNOS +/- group and iNOS -/- group (Fig 1).

Discussion and Conclusion:

Systemic NOS inhibition resulted in a reduction in cross-sectional area of the healing Achilles tendon, which is consistent with healing Achilles tendons in rats. Deletion of iNOS gene (iNOS -/-) did not affect tendon cross-sectional area or biomechanical properties. Our experimental results support the hypothesis that NO is important in tendon healing, but that the iNOS gene is not solely responsible for the beneficial effects of NO on tendon healing [3,4].

References:


Fig 1. The cross-sectional area data (mean ± SE) of mice Achilles tendon are shown. iNOS +/-: n = 8; iNOS -/-: n = 11 and iNOS -/- + AG: n = 10. **: P < 0.01.

Fig 2. Biomechanical properties of healing Achilles tendons are shown. A. Failure load (N) and B. Stress (N/mm2) measurement of mice healing Achilles tendon. (mean ± SE). iNOS +/-: n = 8; iNOS -/-: n = 11; iNOS -/- + AG: n = 10.