Nicotine Alters the Material Properties of the Normal Supraspinatus Tendon in a Rat Model

INTRODUCTION:
Several studies have shown that nicotine has a detrimental effect on the rotator cuff tendon. However, little has been known about its mechanism. We hypothesized that nicotine administration would affect the material properties of the normal tendon tissue. The purpose of this study was to evaluate the effect of nicotine on the material properties of the supraspinatus tendon in a rat model.

METHODS:
Animal models

Twelve-week-old male Sprague-Dawley rats (weight, 350–400 g) were used in this study. Osmotic pumps, which were designed to allow the continuous infusion of test drugs over a period of four weeks, were implanted subcutaneously along the spine. Before implantation, the pumps were filled with nicotine at a concentration of 45 ng/ml or 22.5 ng/ml in saline solution for the experimental groups, and with saline solution for the control group. Twenty-seven rats were randomly allocated to the following three groups (nine rats each): high-dose nicotine (HN), low-dose nicotine (LN), and control (C) groups.

Under general anesthesia with intraperitoneal injection of sodium pentobarbital (30 mg/kg), an osmotic pump was implanted under the skin on the back of each rat. The implanted pumps were replaced twice every four weeks; thus, the total experimental period was twelve weeks. One rat in the HN group died while anesthetized for pump implant surgery; another rat in the LN group developed skin necrosis at the site of pump insertion. These two rats were excluded from further analyses. After completion of the twelve-week treatment, all rats were euthanized with pentobarbital (30 mg/kg), an osmotic pump was implanted under the skin on the back of each rat. The implanted pumps were replaced twice every four weeks; thus, the total experimental period was twelve weeks. One rat in the HN group died while anesthetized for pump implant surgery; another rat in the LN group developed skin necrosis at the site of pump insertion. These two rats were excluded from further analyses. After completion of the twelve-week treatment, all rats were euthanized with an overdose of sodium pentobarbital.

Quantification of serum cotinine levels

Level of serum cotinine, a breakdown product of nicotine, was evaluated by enzyme-linked immunosorbent assay (ELISA) using commercially available kits.

Biomechanical testing

To assess the mechanical properties of the supraspinatus tendon, tensile testing was performed on specimens of the supraspinatus tendons harvested from the left shoulder of each animal in the HN (N = 8), LN (N = 8), and C (N = 9) groups. Two specimens (one each from the NH group and LN groups) were excluded because of technical failure during the tensile testing. Therefore, seven specimens from the HN group, seven from the LN group, and nine from the C groups were used for further assessments. Specimens of the supraspinatus tendon-humerus complex were prepared and stored at −20°C until testing. Specimens were gradually thawed at room temperature and the supraspinatus muscle belly was removed from the intramuscular tendon. For biomechanical testing, the supraspinatus tendon strip was prepared with a custom-made razor blade device. This device consisted of two parallel blades fixed at an interval of 0.8 mm. The thickness of the tendon strip was measured with an area micrometer. The cross-sectional area of the tendon strip was calculated as the thickness of the specimen multiplied by its width (0.8 mm). The block of bismuth alloy into which the humeral shaft was embedded was clamped, and the proximal end of the supraspinatus tendon strip was held with fine-grit sandpaper lined by silicone rubber to prevent slippage. The tendon specimen was tested along its longitudinal axis in uniaxial tension with a tensile testing machine at a constant elongation rate of 3 mm/min until failure occurred. The total specimen strain from grip-to-grip was measured optically with a video camera and digital image recorder. The load-displacement curve was obtained by this system automatically. To calculate strain, the displacement between grips was divided by the initial distance at no load. The stress was then determined using the data from the cross-sectional area. The elastic modulus was calculated using linear regression from the near-linear region of the stress-strain curve.

Statistical analysis

All data values were expressed as means ± SD (standard deviation). Differences between groups were examined for statistical significance using Student’s t-test with Welch’s correction. Statistical analysis was performed using the Prism 5 software package (Version 5.0a for Mac OS X). The level of statistical significance was set at P < 0.05.

RESULTS:

Serum cotinine levels

The mean cotinine levels in the HN and LN groups were 1739.9 ± 121.5 ng/ml and 700.2 ± 51.9 ng/ml, respectively. On the other hand, the presence of cotinine was not detectable in the C group. There were significant differences among the three groups (P < 0.001). Serum cotinine levels in these groups, which demonstrated the systemic release of nicotine, were successfully controlled in the present study.

Biomechanical testing

The maximum loads in the HN, LN, and C groups were 8.6 ± 2.1 N, 7.1 ± 0.8 N, and 5.3 ± 1.1 N, respectively. The maximum stresses in the HN, LN, and C groups were 30.8 ± 8.8 MPa, 27.0 ± 3.7 MPa, and 17.8 ± 3.9 MPa, respectively. Both biomechanical measurements showed no significant differences among the three groups.

The elastic moduli (Fig. 1) of the nicotine groups (HN and LN) were higher than that of the control group (C). A statistically significant difference was observed between the LN group and the C group (p = 0.042).

Figure 1. Elastic modulus. * P<0.05 versus control.

DISCUSSION:

Nicotine increased the modulus of elasticity of the supraspinatus tendon in a rat model. In the clinical setting, it is widely believed that stretching exercise reduces the risk of tendon injury. This prevention of tendon injury may be related to decreased elasticity of the tendon. On the other hand, the elastic modulus is known to be significantly greater in the anterior portion of the supraspinatus tendon, where rotator cuff tears most frequently occur. Increased elastic modulus due to nicotine administration might be associated with the development of rupture of the supraspinatus tendon.

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