INTRODUCTION

It has been hypothesized that there is a reversible bond that allows the sliding of collagen fibrils past one another. Releasing these interfibrillar bonds is a potential method of tendon lengthening. The pentapeptide NKISK has been reported to inhibit the binding of decorin, a proteoglycan on the surface of collagen fibrils, to fibronectin, a tissue adhesion molecule and has produced in vivo tendon lengthening. Our lab has previously demonstrated a dose-response curve of tendon lengthening in response to NKISK, and has also demonstrated the specificity of the NKISK molecule by comparing its effect to a "nonsense" molecule of similar weight and charge. The study is an extension of our prior investigations of the in vivo effect of NKISK in a rat patellar tendon model. We hypothesized that NKISK may offer a potential nonsurgical alternative to tendon-lengthening procedures that currently must be performed in an operating room. This study’s aim is to examine clinically plausible injection volumes, concentrations, and dosing schedules. We increased the concentrations we have previously used by ten times and also varied the volume of material injected. In some cases, we decreased our volume of administration by one-tenth. Moreover, we varied the administration time to simulate a convenient schedule for patients. Many of our experimental schedules involved a once-weekly injection. In our previous studies we sacrificed animals from 1 to 7 days following the last administration of NKISK. In this study we extended our observations of some groups to 28 days to determine if the effects of NKISK administration persisted. We also included one group of daily NKISK administration for 4 weeks to determine if there were any adverse long-term effects of administration.

METHODS

NKISK was synthesized by the Peptide Synthesis Facility at the University of North Carolina at Chapel Hill. Experimental solutions of NKISK were prepared in sterile phosphate buffered saline (PBS). The control solution was sterile 1X PBS. Male Sprague-Dawley rats, 450-600 grams, were divided into eight groups (n = 7). The rats’ knees were clipped and then the patellar tendons were percutaneously injected superficially and deep using a 27g tuberculin needle with varying concentrations and volumes of NKISK or PBS. Each day after injection the rats were placed in a water bath for five minutes of swimming to facilitate distribution of the injected solution and increase tensile loading of the tendon. Rats were sacrificed from 24 hours to 1 month following their final injection. The origins and insertions of the patellar tendons were measured three times under 1.5X magnification with a caliper accurate to 0.02 mm. The lengths of the experimental tendons were compared to the contralateral controls using a paired t-test. Using the force-deformation data, the maximum load, the energy to yield, structural stiffness (in linear region between load limits of 25-75% max. load), elastic modulus, displacement and strain at maximum load were calculated, and the experimental tendons were compared to the control tendons using a one way analysis of variance.

ESSENTIAL RESULTS

A single 1 cc injection of 50 mM NKISK yielded a significant increase in tendon length of 7% ± 2.5% (Figure 1). Carried out in a 1 cc 50 mM weekly dose over the course of 4 weeks, this regimen increased tendon length 20% ± 3.5% (also significant) (Figure 2). To determine if the effects of percutaneous NKISK administration lasted beyond 24 or 48 hours, one group received a 1cc injection of 5mM NKISK daily for 7 days, then was sacrificed at 28 days. There was significant lengthening of 4.7% ± 2% but this was less than some of the other treatment groups. To determine if long-term administration of NKISK had adverse biomechanical effects on tendons, we gave one group a daily 1 cc injection of 5 mM for 4 weeks and did not find significant differences in biomechanical testing. The animals in this group did have significant lengthening of 13% ± 2%. The 50 mM one-time injection did not have adverse biomechanical effects, even when repeated over 4 weeks. Although we did not show statistically significant differences in biomechanical properties, on examination one of the 50mM injections had a healing partial tendon rupture and several specimens had small hematomas around the injection site.