BIOMECHANICAL EFFECTS OF THREE DIFFERENT PERIODS OF GaAs LASER PHOTOSTIMULATION ON TENOTOMIZED TENDONS

Chukuka S. Enwemeka, Elyssa Cohen-Kornberg, Eileen P. Duswalt, Denise M. Weber and Ivette M. Rodriguez

1Department of Physical Therapy Education, University of Kansas Medical Center, Kansas City. 2Division of Physical Therapy, Department of Orthopedics and Rehabilitation, University of Miami School of Medicine, Florida; and 3Veterans Affairs Medical Center, Miami, Florida, USA

The calcaneal tendons of 31 rabbits were tenotomized, repaired and immobilized in order to determine the effects of treatment intervention time on tensile strength, tensile stress, energy absorption capacity, and stress-strain characteristics of regenerating tendons treated with laser. Healing tendons were treated transcutaneously with 0.5 J/cm² GaAs laser either on postoperative days 1-14 (group one), 1-7 (group two), or 8-14 (group three). Control tendons (group four) were similarly tenotomized and repaired but not treated with laser. Using initial body weight and change in body weight as covariables, the tensile strength, tensile stress, energy absorption capacity, and modules of elasticity of the tendons were compared via multivariate analysis of covariance (MANCOVA). Laser treatment induced a significant increase in tensile strength (p = 0.03), tensile stress (p = 0.004), energy absorption capacity (p = 0.003), and modulus of elasticity (p < 0.002) of the tendons. Specifically, the (mean ± SE) tensile strength was 80.62 ± 9.87 N for group one, 65.12 ± 11.34 N for group two, 87.50 ± 8.14 N for group three, and 56.45 ± 4.38 N for controls. Similarly, mean tensile stress values were 243.42 ± 21.25 N/cm², 191.54 ± 27.12 N/cm², 252.54 ± 24.86 N/cm², and 153.59 ± 12.57 N/cm², respectively; the corresponding energy absorption capacity values were 512.8 ± 91.0 mJ, 373.7 ± 77.7 mJ, 580.7 ± 124.6 mJ, and 257.8 ± 47.9 mJ; while the modules of elasticity were 475.45 ± 50.32, 391.22 ± 68.12, 487.84 ± 56.74, and 178.77 ± 32.92. Posthoc analyses showed that treatment with 0.5 J/cm² GaAs laser significantly augments the healing strength, tensile stress, energy absorption capacity, and modules of elasticity of rabbit calcaneal tendons especially during postoperative days 8-14 (t < 0.05). Although healing of rabbit tendons may differ from healing of human tendons, our findings suggest that similar beneficial effects may be obtained if human tendon ruptures are treated with 0.5 J/cm² GaAs laser.

Key words: Tendon healing; laser biostimulation; collagen synthesis

Introduction

Tendons and other dense connective tissues do not heal quickly. In addition to basic cell proliferation and vascularization, tendons must synthesize large quantities of collagen, in order to withstand the forces generated by muscles. Consequently, a prolonged period of protective immobilization is required after surgical repair of ruptured tendons. Prolonged immobilization causes muscle atrophy, osteoarthritis, atrophy and ulceration of joint cartilage, skin necrosis, infection, tendocutaneous adhesion and thrombophlebitis, all of which complicate post-operative care and the functional outcome of tendon repair. It is estimated that 17 million Americans sustain connective tissue injuries each year. This high incidence of injury reveals the need to find a mode of treatment which facilitates the healing process of tendons and other collagenous tissues.

The use of physical agents to facilitate the healing process of tendons and hence minimize complications, has been the focus of many investigations. It has been shown that physical activity, ultrasound, electrical stimulation and other forms of physical therapy can accelerate the healing process of dense connective tissues. Furthermore, evidence indicates that photostimulation with low incident power densities of laser energy accelerates fibroplasia and collagen biosynthesis.

Address for Correspondence: Prof. Chukuka Enwemeka Ph.D., FACS. Department of Physical Therapy Education, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA.

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thesis in skin wounds, \(^{(30,33,37,39)}\) increases the pool of type I and type III procollagen specific mRNA levels in pig skin wounds, \(^{(50)}\) and promotes ATS synthesis in cultured mitochondria. \(^{(49)}\)

Because tendons and skin require the same basic processes of inflammation, fibroplasia, and collagen biosynthesis, the effects of 1-5 mJ/cm\(^2\) He:Ne laser on the healing process of denotomized rabbit calcaneal tendons was examined recently. \(^{(41)}\) Laser treated tendons were found to have collagen fibrils which were more aligned along the longitudinal axis of the tendon and had a higher collagen fibril density than control non-treated tendons. Subsequent research has shown that daily application of 1.5 J/cm\(^2\) doses of He:Ne or GaAs laser energy does not promote tendon healing as much as daily doses of 1.0 J/cm\(^2\). \(^{(42)}\) Although lower doses (1, 2, 3, 4, and 5 mJ/cm\(^2\)) modulate collagen synthesis, \(^{(41)}\) they do not significantly increase the tensile strength of tendons as 1.0 and 1.5 J/cm\(^2\) doses do. \(^{(42)}\)

The effects of laser photostimulation may depend not only on dosages but also on timing of treatment intervention. Studies have addressed the importance of timing treatment intervention when using physical modalities to promote tendon healing. \(^{(44-49)}\) It has been shown that the regenerating denotomized tendon undergoes different stages of healing. Each stage involves a different set of ultrastructural events, suggesting that therapeutic strategies should be modified according to the specific events at each stage. \(^{(43)}\) The inflammatory stage lasts at least five days post-injury, and fibroplasia and fibrillogenesis begin at about the seventh day. The stage of remodelling which overlaps fibrillogenesis becomes noticeable by the twenty-first day of healing. \(^{(43)}\) Because laser photostimulation may influence one or two stages of tendon healing more than the other, optimal effects may be achieved by targeting a specific period of healing.

Consequently, the purpose of this study was to determine the effects of three specific periods of laser treatment intervention on regenerating tendons. Specifically, our aims were to compare the tensile strength, tensile stress, energy absorption capacity, and modulus of elasticity of tendons treated at three different time periods with control values.

**Methods**

**Subjects**

Thirty-one New Zealand rabbits, weighing 1.9-3.0 kg (mean \(\pm\) sd = 2.39 \(\pm\) 0.23 kg) and randomly assigned to four groups, three treatment groups and one control group, were used for this study. The animals were housed individually in standard 30.5 x 71 x 51 cm rabbit cages, in a light controlled room maintained at 22 \(\pm\) 1°C, fed high fiber rabbit chow and water *ad libitum*, and were under veterinary care throughout the study.

**Surgery**

Each rabbit was weighed and anesthetized with a mixture of 20 mg/ml xylazine, 100 mg/ml ketamine and 10 mg/ml acepromazine by intramuscular injection of 1 ml/kg body weight. The right hind limb was shaved and cleaned, and a longitudinal incision was made slightly medial to the visible outline of the calcaneal tendon. Following exposure, the right calcaneal tendon was severed transversely 2 cm above its insertion. Three loops of 3.0 absorbable polyglycolic acid suture were used to approximate the ends of the severed tendon. Subsequently, the skin incision was closed and the hind limb was immobilized in a plaster cast with the knee flexed at 90 degrees, and the ankle fully plantarflexed.

**Laser Photostimulation**

A GaAs laser unit Model 2400 (Respond Systems, Inc., Madison, CT) of 904 nm wavelength was used to deliver a daily dose of 0.5 J/cm\(^2\) to the immediate 5.0 cm\(^2\) area overlying the site of each denotomized calcaneal tendon. A laser power meter Model SE51L (United Detector Technology, Hawthorne, CA) was used to verify the power output of the laser device. This power meter was specially calibrated for light sources ranging from 400-1000 nm wavelength. Subsequently, the energy density (ED) applied was calculated from the following formula:

\[
ED = \frac{AP \times t}{a} \quad (\text{J/cm}^2)
\]

where \(AP\) = average power in watts, \(t = \text{time in seconds},\) and \(a = \text{irradiated area in square centimetres.}\)

Following surgery, the tendons were treated daily as follows: Group one was treated with GaAs laser on post-operative days 1-14, Group two on post-operative days 1-7, Group three on post-operative days 8-14; and Group four served as untreated controls (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Design of the experiment.</th>
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<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
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<td>2</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>4</td>
</tr>
</tbody>
</table>

**Tendon Excision**

On the fourteenth post-operative day, each rabbit was weighed and anesthetized as previously described. After removing the plaster cast and shaving and cleaning the skin, the incision was carefully reopened and the calcaneal tendon was freed from the surrounding tis-
sues. To excise the tendon, sharp transverse cuts were made below the musculotendinous junction and above the calcaneal insertion. Each rabbit was then sacrificed by administering an overdose of nembutal.

The 31 excised tendons were then frozen in 0.09% NaCl solution and stored at -30°C until the biomechanical tests were performed. Evidence indicates that this method of preservation does not affect biomechanical test results.50) Before biomechanical testing the cross-sectional areas (i.e., volume/length) of the tendons were determined by measuring the length and volume of each tendon. Thereafter, a pneumatic clamp was used to attach the tendon to the cross-heads of an Instron Materials Testing System Model 1000 (Instron Inc, Canton, MA). Using a load cell of 500 N each tendon was pulled to rupture at a cross-head speed of 500 mm/min. The elongation, tensile strength, tensile stress, Young's modulus of elasticity, and total energy absorbed were determined from the Load-Deformation curve recorded by the Instron and the cross-sectional area previously obtained.

Data Analysis
Using the initial body weight and change in body weight of the rabbits as covariates, multivariate analysis of covariance (MANCOVA) was used to determine the effects of laser treatment on the tensile strength, tensile stress, energy absorption capacity and modulus of elasticity of the tendons. Tensile strain and tensile stress were calculated using the following formulas:

\[
\text{Tensile strain} = \frac{\text{change in length}}{\text{original length}} \times 100 \%
\]

\[
\text{Tensile stress} = \frac{\text{tensile strength}}{\text{area}} \text{ (N/cm}^2\text{)}
\]

Young's modulus of elasticity (stress/strain) was then calculated from the values obtained.

Results
The highest mean tensile strength 87.50 ± 8.12 N was observed in Group three tendons that were treated on days 8-14. The mean tensile strength for Group one (1-14 day treated group), Group two (1-7 day treated group), and control (Group four) tendons was 80.62 ± 9.87 N, 65.12 ± 11.34 N, and 56.43 ± 4.38 N, respectively (Figure 1). Similarly, the highest tensile stress, 252.54 ± 24.86 N/cm², was observed in group three. The mean tensile stress for Groups one, two, and four was 243.42 ± 21.25 N/cm², 191.54 ± 27.12 N/cm² and 153.59 ± 12.57 N/cm², respectively (Figure 2). Group three also had the highest mean value

Fig. 1: Tensile strength developed by laser photostimulated and control tendons. The mean tensile strength values of laser photostimulated tendons were significantly higher than that of the control tendons (P = 0.03).

Fig. 2: Tensile stress developed by laser photostimulated and control tendons. The mean tensile stress values of laser photostimulated tendons were significantly higher than that of the control tendons (P = 0.004).
for energy absorption capacity, 580.07 ± 126.4 mJ. The energy absorption capacity for Groups one, two and four was 512.8 ± 91.0 mJ, 373 ± 77.7 mJ and 257.8 ± 47.9 mJ, respectively (Figure 3). Similarly, the highest modulus of elasticity, 487.84 ± 56.74, was recorded for Group three. The corresponding value for Groups one, two, and control Group 4 tendons was 475.45 ± 50.32, 391.22 ± 68.12, and 178.77 ± 32.92, respectively (Figure 4).

Multivariate analysis of covariance (MANCOVA) revealed a significant effect of laser treatment on tensile strength (p < 0.004), tensile stress (p < 0.004), energy absorption capacity (p < 0.003), and Young's modulus of elasticity (p < 0.002) of the tendons (Tables 2-5). Specifically, tendons treated from day 1 to 14 and those treated during the last 7 days of healing had significantly higher tensile strength, tensile stress, energy and modulus of elasticity than controls (p > 0.05) compared to the other Groups. Similar statistically significant differences were not found between controls and tendons treated during the first 7 days of healing (p < 0.05).

Discussion

Our findings warrant the conclusion that 0.5 J/cm² of GaAs laser irradiation is effective in augmenting the tensile strength, tensile stress, energy absorption capacity, and modulus of elasticity of regenerating rabbit calcaneal tendons. These findings suggest that 0.5 J/cm² GaAs laser photostimulation can facilitate healing of tendons and therefore minimize the complications associated with immobilization. Following cast immobilization, the initial rate of muscle atrophy is very rapid with up to 50% of the eventual atrophy occurring in the first week alone.51-53 By limiting immobilization to the shortest period of time, laser photostimulation may limit muscle atrophy.
Table 2: Multivariable analysis of covariance: effects of treatment on the tensile strength of tendons, using initial body weight (IBW) and change in body weight (CBW) as covariates.

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<th>Source</th>
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<th>P</th>
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<td>1918.75</td>
<td>4.83</td>
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<td>Corrected Total</td>
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<td>3</td>
<td>3888.78</td>
<td>1296.26</td>
<td>3.26</td>
<td>0.38</td>
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<td>3888.78</td>
<td>1296.26</td>
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<td>0.38</td>
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</table>

df = Degree of freedom; F Value = Fisher's distribution value; P = Probability of rejecting H0; IBW = Initial body weight; CBW = Change in body weight; CV = Coefficient of variation; SS = Sum of squares; MSE = Mean square error; R-Square = Sequence of correlation coefficient (predictive power)

Table 3: Multivariable analysis of covariance: effects of treatment on the tensile stress of tendons, using initial body weight (IBW) and change in body weight (CBW) as covariates.

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<tr>
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<td>14357.91</td>
<td>4.88</td>
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<table>
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<th>Mean</th>
<th>Mean Square</th>
<th>F Value</th>
<th>P</th>
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<td>211.33</td>
<td>0.07</td>
<td>0.790</td>
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</tr>
<tr>
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<td>6.72</td>
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<tr>
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<td>3</td>
<td>43073.73</td>
<td>14357.91</td>
<td>4.88</td>
<td>0.008</td>
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</table>

The group means indicate that tendons that were treated on post-operative days 8-14 attained the highest values for tensile strength, tensile stress, energy absorption capacity, and modulus of elasticity. This time period coincides with the period of fibroblast proliferation and collagen synthesis. It had been shown that tendons undergo three stages of healing, which are overlapping but related to each other: (1) inflammation, (2) fibroblast proliferation and synthesis of collagen fibrils, and (3) alignment of fibrils with the long axis of the tendon. During the first five days post-injury, there is massive inflammation. Approximately seven days post injury, fibroplasia and collagen synthesis begin. Beginning from this time fibroblasts increase in size and number. Simultaneously, an increasing amount of collagen accumulates in the ex-
tracellular compartment, suggesting that fibroblasts are engaged in collagen synthesis. Treating the tendons on postoperative days 8-14 produced a significantly better result than treating the tendons on post-operative days 1-7. Therefore, it is likely that treatment during the former time interval promotes fibroblast proliferation and collagen synthesis, i.e., the same processes that begin at about 7 days after tenotomy. It is likely that GaAs laser treatment does not influence the healing events that occur during post-operative days 1-7 to the same extent as it affects the events that occur thereafter. There is no strong evidence that the process of inflammation is significantly affected by laser photostimulation, although Mester et al. have reported changes in the levels of prostaglandins following laser photostimulation of healing wounds.

For key, see Table 4 above.

Table 4: Multivariable analysis of covariance: effects of treatment on the energy absorption capacity of tendons, using initial body weight (IBW) and change in body weight (CBW) as covariates.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
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R-Square C.V. Root MSE ENERGY Mean
0.48 45.92 20.06 43.69

Source df Type I SS Mean Square F Value p
IBW 1 216.9 216.69 0.54 0.470
CBW 1 6160.62 6160.62 15.31 0.001
RXGroup 3 3853.11 1284.37 3.19 0.041

Source df Type III SS Mean Square F Value p
IBW 1 0.45 0.46 0.00 0.973
CBW 2 5536.75 5536.75 13.76 0.001
RXGroup 3 3853.11 1284.37 3.19 0.041

df = Degrees of freedom; F Value = Fisher's distribution value; p = Probability of rejecting H0; IBW = Initial body weight; CBW = Change in body weight; CV = Coefficient of variation; SS = Sum of squares; MSE = Mean square error; R-Square = Sequence of correlation coefficient (predictive power).

Table 5: Multivariable analysis of covariance: effects of treatment on the value of Young's modulus, using initial body weight (IBW) and change in body weight (CBW) as covariates.

<table>
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<th>Source</th>
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R-Square C.V. Root MSE YM0D Mean
0.52 35.43 138.16 389.92

Source df Type I SS Mean Square F Value p
IBW 1 37345.89 37345.89 1.96 0.174
CBW 1 90613.92 90613.92 4.75 0.039
RXGroup 3 388178.43 129392.81 6.78 0.002

Source df Type III SS Mean Square F Value p
IBW 1 96.25 96.25 0.00 0.944
CBW 1 68818.88 68818.88 63.61 0.069
RXGroup 3 388178.43 129392.81 6.78 0.002

For key, see Table 4 above.
whereas ruptured human tendons are subjected to the forces of abrupt rupture, resulting in shredding of the tendon ends. In addition, the interval between injury and repair are different for tenotomized and ruptured tendons. Tenotomized tendons are sutured immediately, permitting healing to begin right after surgery. In contrast, a longer time may elapse before a patient with a tendon rupture sees a physician, in which case the tendon would have begun to degenerate. Other factors that hinder the application of this model to clinical situations include the anatomical and biomechanical differences between rabbits and humans, and the restrictive environment in which the animals were housed. These limitations must be considered in extrapolating our findings to treatment of human tendon ruptures.

On the basis of this biomechanical study, we conclude that the application of a daily dose of 0.5 J/cm² of GaAs laser photostimulation augments tensile strength, tensile stress, energy absorption capacity, and elasticity of healing rabbit calcaneal tendons. This effect is greater when the treatment is applied on post-operative days 8-14. Although healing in rabbits may not accurately reflect healing in humans, our findings suggest that surgically repaired human calcaneal tendons may heal faster with 0.5 J/cm² GaAs laser photostimulation, especially during post-operative days 8-14.

Acknowledgement

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