THE BIOLOGICAL EFFECTS OF LASER THERAPY AND OTHER PHYSICAL MODALITIES ON CONNECTIVE TISSUE REPAIR PROCESSES

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Connective tissue injuries, such as tendon rupture and ligamentous strains, are common. Unlike most soft tissues that require 7-10 days to heal, primary healing of tendons and other dense connective tissues take as much as 6 - 8 weeks during which they are inevitably protected in immobilization casts to avoid re-injury. Such long periods of immobilization impair functional rehabilitation and predispose a multitude of complications that could be minimized if healing is quickened and the duration of cast immobilization reduced. In separate studies, we tested the hypothesis that early function, ultrasound, 632.8 nm He-Ne laser, and 904 nm Ga-As laser, when used singly or in combination, promote healing of experimentally severed and repaired rabbit Achilles tendons as evidenced by biochemical, biomechanical, and morphological indices of healing. Our results demonstrate that: (1) appropriate doses of each modality, i.e., early functional activities, ultrasound, He-Ne and Ga-As laser therapy augment collagen synthesis, modulate maturation of newly synthesized collagen, and overall, enhance the biomechanical characteristics of the repaired tendons. (2) Combinations of either of the two lasers with early function and either ultrasound or electrical stimulation further promote collagen synthesis when compared to functional activities alone. However, the biomechanical effects measured in tendons receiving the multi-therapy were similar, i.e., not better than the earlier single modality trials. Although tissue repair processes in humans may differ from that of rabbits, these findings suggest that human cases of connective tissue injuries, e.g., Achilles tendon rupture, may benefit from appropriate doses of He-Ne laser, Ga-As laser, and other therapeutic modalities, when used singly or in combination. Our recent meta-analysis of the laser therapy literature further corroborate these findings.

Key Words: Laser therapy, Therapeutic ultrasound, Electrical stimulation, Tissue Repair, meta-analysis.

Introduction

Connective tissue injuries, such as tendon rupture and ligamentous strains, are common. Unlike most soft tissues that require 7-10 days to heal, primary healing of tendons is believed to take at least six weeks during which they are protected in immobilization cast [1-3]. Such long periods of immobilization predispose muscle atrophy [4, 5], joint stiffness [6], atrophy and ulceration of joint cartilage [7, 8], osteoarthritis [9, 10], skin necrosis [11, 12], tendo-cutaneous adhesion [12], re-rupture [13-15] and thrombophlebitis [14, 15]. These complications retard post-operative rehabilitation, because in addition to pursuing the functional gains for which surgery was intended, it is necessary to (a) physically recondition the patient, (b) recover muscle strength, (c) regain joint range of motion, (d) release tendocutaneous adhesions, (e) reverse cartilage ulceration and atrophy, and (f) improve skin condition. Several months commonly elapse before these complications are overcome. Consequently, the cost of treatment increases simultaneously as the anticipated functional gains are retarded.

Overcoming the complications associated with tendon repair has been the focus of our research during the last sixteen years. As a part of this effort, we have examined the effects of several modalities that can potentially enhance healing of connective tissues. The evidence that these modalities, e.g., functional activities, electrical stimulation, ultrasound, and laser photostimulation, can facilitate healing of connective tissues may be summarized as follows.

Effect of Functional Loading

Roux's "law of functional adaptation" suggests that tendons and other connective tissues adapt themselves structurally to functional demands. The sensitivity of tendons to load enhancement or load deprivation is exemplified by the fact that intact and healing tendons are strengthened by physical activities and weakened by inactivity [16, 17]. For example, patellar tendons, when surgically transplanted to replace torn anterior cruciate ligaments, gradually assume the structural, biochemical and functional characteristics of ligaments in response to their new loading conditions as ligaments [18]. These and other findings [4, 19-22] indicate

that repaired tendons may heal faster when subjected to functional loads. Therefore, earlier in our studies, we implemented minimal (short duration) cast immobilization to facilitate functional loading and rapid repair of experimentally tenotomized rabbit Achilles tendons.

Effects of Therapeutic Ultrasound

Accumulating evidence suggests that therapeutic ultrasound facilitates fibroplasia and collagen synthesis [23-26]. Our first series of experiments [27] with this modality revealed that these specific effects of ultrasound engender faster healing in our experimental model when 1 MHz continuous wave ultrasound is applied daily at 1.0 W cm^{-2}. Previous studies [24] suggest that sonication at lower intensities, i.e., < 0.5 W cm^{-2} may be more beneficial than treatment with higher intensities. Consequently, we used this lower range of intensity in the experiments reported in this paper.

Effects of Laser Photostimulation

The initial impulse which prompted the use of low intensity lasers to promote the healing process of soft tissues arose from the works of Mester et al. [28] and Hardy and associates [29]. In this work, Hardy et al. [29] reported a five-fold increase in the number of fibroblasts exposed to four 10.0 J cm^{-2} doses of pulsed ruby laser when compared to controls that were not treated with laser light. Subsequent studies have since shown that the increased proliferation of fibroblasts results in increased collagen synthesis in vitro as well as in vivo [30-38]. For example, a total of 4.0 joules laser energy applied on experimentally-induced skin incisions [39] or skin defects in rats [30], produces faster collagen synthesis as judged by assaying ^{14}C glycine and ^{3H}proline or ^{3H}hydroxyproline [30, 39]. Similarly, fractures induced in the radii of 44 New Zealand rabbits, irradiated daily with 236 mW cm^{-2} CO_2 laser for 10 minutes on 10 successive days have been shown to heal faster than controls [40] as do mice tibial fractures irradiated with low intensity He-Ne laser [41].

Although the precise mechanisms of action remain unclear, in vitro studies suggest that photostimulation promotes nucleic acid synthesis and cell division in cultures of human fibroblasts [30-32, 42, 43] and augment the pools of type I and type III procollagen mRNA in pig skin wounds. A similar increase in ATP production has been found in rat liver mitochondria after photostimulation with He-Ne laser in vitro [44, 45] These metabolic effects result in increased tensile strength of healing skin wounds, as demonstrated by Lam et al. [46] and Kovacs et al. [47].

Tendon Repair Studies

Compared to studies on skin wound healing, works concerning the effects of laser photostimulation on tendon and ligament repair are still in their infancy. Virtually all the studies in this area have been conducted in our laboratory. In the works summarized below, we used the experimentally severed and repaired Achilles tendons of rabbits as a model to explore the effects of various intensities of laser phototherapy on tendon healing. Our initial results which constitute the bases of some of the studies compiled in this paper may be summarized as follows. A dose of 1 - 5 mJ cm^{-2} He-Ne laser augments the force per unit area (tensile stress) acquired by the tendons after 21 days of healing, albeit without significantly increasing the ultimate tensile strength or energy absorption capacity of the tissue [48]. No dose dependent effects were observed within this dose range. Similarly, 1 - 5 mJ cm^{-2} 632.8 nm laser modulates collagen synthesis as evidenced by altered collagen fibril morphometry, and promotes the formation of membrane-bound intracytoplasmic collagen fibrils in tendon fibroblast and myofibroblasts [49, 50], as does 1 - 5 mJ cm^{-2} of 904 nm laser (unpublished data).

In contrast to the biomechanical effects of the lower dose range, i.e. 1 - 5 mJ cm^{-2}, 632.8 nm He-Ne laser of 0.5 or 1.0 J cm^{-2} induces a significant 30 - 40% increase in the ultimate tensile strength, and tensile stress of the tendons after 14 days of healing. These beneficial effects are slightly better with the 1.0 J cm^{-2} dose than with other dose range [51]. Similarly, 904 nm Ga-As laser of 0.5, 1.0 or 1.5 J cm^{-2} significantly increases the ultimate tensile strength and tensile stress of the tendons by as much as 40% after 14 days of healing. As with the visible 632.8 nm laser study, these beneficial effects are slightly better with the 1.0 J cm^{-2} dose than other dose levels [52]. With both wavelengths of laser, in this experimental model, photostimulation in the continuous mode produces a slightly better effect than stimulation in the pulsed mode [51, 52]. And photostimulation of repaired tendons during the last 7 days of healing yields a better result than irradiation during the first 7 days of healing. This may relate to the modulating effects of light on specific events during these two phases of tendon repair i.e., the inflammation phase and the collagen synthesis phase. Our data indicate that the period of intervention may be as critical with 904 nm light as it is with the 632.8 nm wavelength. In summary, these initial findings suggest that appropriate doses and wavelengths of visible red and near infra-red light, can accelerate the repair processes of tendons.

Effects of Electrical Stimulation

Functional loading, ultrasound and laser therapy are not the only potent accelerator of tendon repair. In situations where tendocutaneous adhesion is a major problem, electric stimulation has been used to effectively promote the gliding function and the healing strength of surgically

repaired tendons [53, 54]. The rationale for this approach is that electric stimulation of the muscle will cause the muscle to contract and hence exert mechanical force on the healing tendon. Our own preliminary results indicate that electric stimulation of the triceps surae significantly reduces atrophy of the soleus and plantaris muscles after tenotomy and repair of the rabbit Achilles tendon [55]. Therefore, stimulation-induced loading will not only strengthen the healing tendon, but facilitate gliding and minimize the muscle atrophy that invariably accompanies cast immobilization.

Given these findings, it would seem that a combination of functional loading, laser photostimulation and electrical stimulation will further accelerate healing of tenotomized tendons. As detailed in the following sections of this paper, in a series of experiments, we tested the hypothesis that early function, ultrasound, He-Ne laser, and Ga-As laser, when used singly or in combination, accelerate the healing process of experimentally tenotomized and repaired rabbit Achilles tendons, as evidenced by biochemical, biomechanical, and morphological indices of healing.

Overview of Experimental Methods

Animals:

In each of the experiments reported here, New Zealand rabbits, aged 10 -12 weeks, were used. The animals were housed in standard 30.5 X 71 X 51 cm rabbit cages, maintained at 22ºC, and fed rabbit chow and water *ad libitum*. Following surgery, the animals were randomly assigned to groups, with one group serving as non-treated controls.

Surgical Tenotomy and Repair of The Achilles Tendon:

In these experiments, the right Achilles tendon was severed, repaired and used as described below. On the day of surgery each rabbit is weighed, and anesthetized with an intra muscular injection of 3mg/kg body weight Xylazene and 35 mg/kg body weight Ketamine. Subsequently, the skin overlying the tendon was shaved scrubbed and anaesthetized locally with 2mg/kg body weight lidocaine. Following anesthesia, the right Achilles tendon of each rabbit was tenotomized and repaired as detailed in previous publications [49, 56, 57] Briefly, a longitudinal incision was made lateral to the visible outline of the tendon. By blunt dissection, the tendon was isolated from adjoining tissue and transected approximately 1.5cm above its calcaneal attachment. The severed ends were then approximated and sutured with absorbable suture.

Following skin closure, the surgical limb was immobilized using either a plaster cast, as for example, in experiments performed before 1995, or a lighter weight custom designed pre-molded polyurethane splint, as in later experiment. To promote rapid recovery from anesthesia, an injection of 0.2mg/kg body weight Yohimbine was given. Subsequently, the rabbits were kept warm in an oxygen chamber, and observed until they regained consciousness.

Laser Treatment

In each of the experiments detailed below, the laser beam was delivered to the target tissue transcutaneously, i.e, through the overlying skin. Each treatment session was timed to yield the desired energy density, taking the area treated and the average power of each laser into consideration.

Tendon Excision

After a designated post-surgical period as detailed for each experiment below, the animals were euthanized with pentobarbital sodium (Nembutal), and the surgical incision reopened. The two Achilles tendons of each animal were excised as follows. The skin overlying the Achilles tendon was shaved, and a longitudinal incision made lateral to the visible outline of the tendon. After separating the tendon from the surrounding tissue, sharp transverse cuts were made below the musculotendinous junction and above the calcaneal insertion of the tendon. Tendon specimens used for biomechanical and biochemical analysis were snap frozen in liquid nitrogen, and stored at -70ºC until tests were performed. For electron microscopy, tendons were fixed *in situ* before excision.

Biomechanical analysis

The cross-sectional area of each tendon was measured with a customized caliper as described by An et al. [58]. A pneumatic clamp was used to attach each tendon to the cross heads of an Instron Materials Testing System (Instron Inc., Canton, MA). Using a 500N load cell, each clamped tendon was pulled to rupture at a cross head speed of 250mm/min. A load deformation curve and other biomechanical parameters, including tensile strength, stress, strain, Young's modulus of elasticity, toughness, and energy absorption capacity were automatically computed and stored in the computer.

Biochemical Analysis

Immediately after performing the biomechanical tests, the ruptured tendons were collected and used for biochemical analysis. To process regenerating tendons for this analysis, the neotendon, i.e., newly formed connective tissue at the site of rupture, was carefully dissected out using a Nikkon microscope. The new tendon tissue was easily identifiable because of its higher cellular content and lighter color compared to mature tendon.

Total collagen was determined by measuring the concen-

tration of hydroxyproline in each tissue specimen as described in our previous report [59]. The solubility profile of collagen in the tendons were examined by sequential extractions in neutral salt buffer, acetate buffer, and acetate buffer containing pepsin as described in our previous studies [56, 57].

The hydroxypyridinium cross-links of the tendons were determined as previously reported [56, 57, 60] using a high performance liquid chromatography (HPLC), (Shimadzu, Kyoto, Japan).

Transmission Electron Microscopic Studies

Tendon specimens were fixed for two hours in 2.5% glutaraldehyde (pH 7.4), buffer washed, and post-fixed for another two hours in 1% aqueous solution of osmium tetroxide (pH 7.4). Each specimen was then washed with dH_20 and dehydrated in graded alcohol before final dehydration in propylene oxide. After gradual infiltration with a mixture of propylene oxide and resin, each specimen was embedded in EMBED 812 resin and kept in an oven at 60oC for 65 hours. Each resin embedded specimen was then trimmed, sectioned transversely at 70-80nm to obtain representative silver/silver gray sections that were mounted on a grid. Finally, grid mounted sections were stained with both uranyl acetate and lead citrate for 5 minutes each before they were studied under the electron microscope. From each grid, electron micrographs of several fields of collagen fibrils were taken using a JEOL 1200EX scanning transmission electron microscope.

Effects of Laser Photostimulation

In our earlier studies, the therapeutic effects of laser photostimulation were studied by analyzing the morphometric, ultrastructural and biomechanical changes in the tendons. Considering the role of connective tissue collagen in tissue repair, we examined the effects of laser photostimulation on objective measures of collagen production in the healing rabbit Achilles tendons of 24 rabbits.

Following tenotomy, an equal number of rabbits was randomly assigned to the control and the treatment group. Treated tendons received 1.0 J cm^{-2} He:Ne laser treatment (632.8 nm) transcutaneously, beginning from day one and continuing for 14 days. In addition, to prevent muscle atrophy, the triceps surae muscle complex of each repaired tendon was stimulated using interrupted galvanic current during the first five days of immobilization. On the fifth postoperative day, immobilization casts were removed from the surgical limbs of the treated and control groups of rabbits to permit free movement of the animals within their cages. Two weeks after surgery, the tendons were excised, and compared biochemically as detailed above.

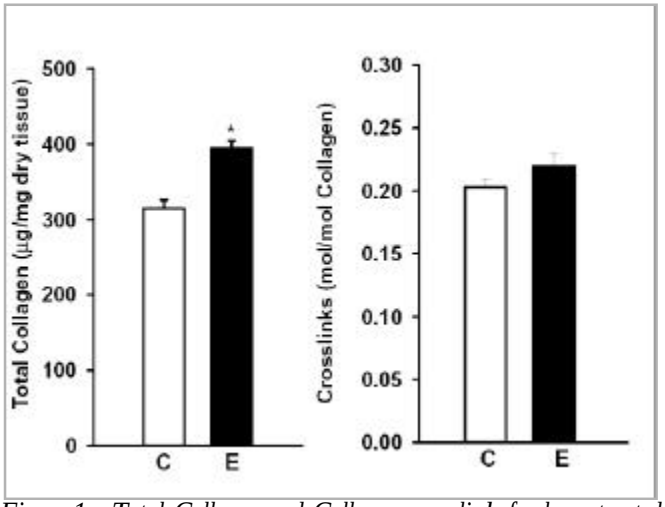
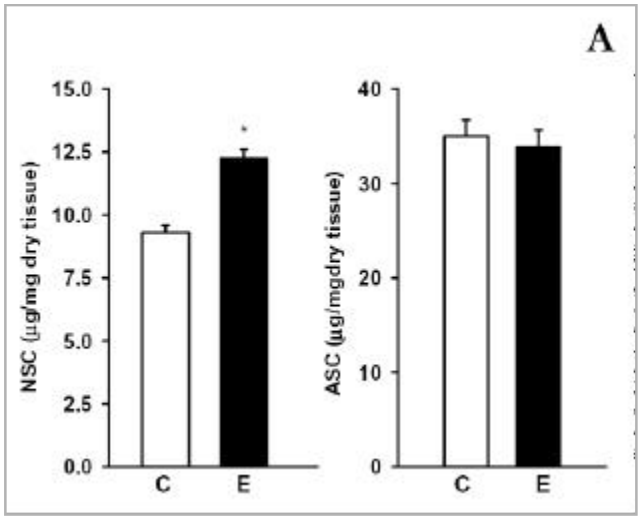


Figure 1: Total Collagen and Collagen cross-link for laser treated tendons (E) and control tendons (C).

The analysis revealed significant differences in the collagen content, and the collagen solubility profile between control and laser treated tendons. The total collagen for laser treated tendons was significantly higher compared to the control tendons, 395.15 + 9.5 mg versus 314.85 + 11.1 mg/mg dry tissue (Fig. 1; $p<0.01$). In contrast to the collagen levels, the hydroxypyridinium cross-links were not influenced by laser photostimulation. The cross-link density was 0.203 + 0.006 moles/mole collagen for laser treated and 0.220 + 0.01 moles/mole collagen for control tendons (Fig. 1; $p> 0.05$). With the exception of acid soluble collagen (ASC), sequential extraction of collagen from both samples indicated that the proportions of neutral salt soluble collagen (NSC), pepsin soluble collagen (PSC), and insoluble collagen (ISC) were significantly different for treated and control tendons (Fig. 2, $p < .05$). These experiments yielded 12.25 $\pm$ 0.36 and 9.30 $\pm$ 0.29 micrograms of NSC, 33.87 $\pm$ 1.80 and 35.03 $\pm$ 1.70 micrograms of ASC, 191.28 $\pm$ 5.6 and 208.16 $\pm$ 4.10 micrograms of PSC, and 157.75 $\pm$ 7.0 and 118.59 $\pm$ 5.6 micrograms of ISC for treated and control tendons respectively.



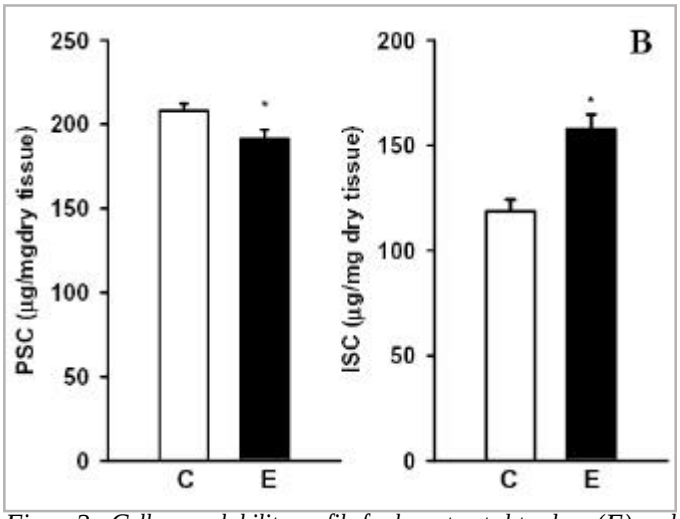


Figure 2: Collagen solubility profile for laser treated tendons (E) and control tendons (C).

The relative amounts of the distribution of soluble collagen in relation to total tissue collagen indicates that approximately 3.1% and 2.58% of NSC was extractable in the neutral salt soluble fraction from laser treated tendons and control tendons respectively. Using 0.5M acetic acid, about 8.57% and 9.5% of ASC was extracted from laser treated tendons and control tendons, respectively. The results of pepsin digested samples showed that a large amount of PSC was solubilized in both groups of tendons (48.3% laser treated compared to control tendons, 55.66%). The percent of insoluble collagen (ISC) was appreciably higher in laser treated tendons (39.94%) compared to control tendons (32.85%). Statistical analysis of the solubility profile revealed a significant increase in NSC and ISC and an appreciable decrease in PSC contents in laser treated tendons compared to control tendons. However, no statistical differences were observed in the ASC content between laser treated and control tendons. The sequential extraction and distribution studies of soluble collagen collectively suggest rapid collagen remodeling in the laser treated tendons compared to the control tendons.

Effects of Combining Laser Photostimulation, Electrical Stimulation and Functional Loading.

Given our previous studies which revealed that early functional loading, and laser photostimulation independently promote tendon healing, we tested the hypothesis that a combination of mechanical loading and laser photostimulation would further accelerate healing of experimentally tenotomized and repaired rabbit Achilles tendons. Following surgical tenotomy and repair, experimental and control tendons were immobilized for 5 days during which they were mechanically loaded via electrical stimulation-induced contraction of the triceps surae. In addition, all through the 14 day period of the study, the experimental tendons were treated with 1J cm^{-2} 632.8 nm He-Ne laser.

The combination of laser photostimulation and mechanical load increased the maximal stress, maximal strain, and Young's modulus of elasticity by 30, 13, and 33%, respectively. However, multivariate analysis of variance (MANOVA) revealed no statistically significant differences in these biomechanical indices of repair of control and experimental tendons (Table 1). Biochemical assays showed a 32% increase in collagen levels ($p < 0.05$), and no significant differences were found in collagen cross-links between treated and control tendons ($p > 0.05$). Similarly, electron microscopy and computer morphometry revealed no significant differences in the morphometry of the collagen fibers, and no visible differences in the ultrastructure of the cellular and matrical components of control and experimental tendons.

Table 1: Biomechanical effects of combined 632.8 nm laser, and mechanical laod on tendon repair.

Biomechanical Index of Healing	Treated	Control	*p*-Value
Maximum load (N)	138.3 ± 19.5	120.4 ± 11.7	0.21
Maximum stress (Mpa)	2.62 ± 0.39	2.02 ± 0.2	0.19
Maximum strain (% change)	58.0 ± 3.0	51.0 ± 3.0	0.40
Young's modulus of elasticity	10.7 ± 2.1	7.5 ± 1.2	0.08
Energy absorbed at failure (J)	0.36 ± 0.05	0.38 ± 0.06	0.64

The positive effects of combined mechanical load and laser therapy was more evident in the biochemical indices of healing than in the biomechanical measurements. The outcome of the biochemical analysis indicates that collagen production was appreciably enhanced by the combined therapy. However, trifunctional hydroxypyridinium cross-links of collagen in the modality-treated group was decreased, based on dry weight measurements. Relative to the molar amounts of collagen, the quantities of mature cross-links decreased significantly in the modality-treated group; a finding which coincides well with the observed increase in type III procollagen mRNA. Therefore, the combined therapy enhanced tendon repair but seemed to produce changes at the molecular level that were yet to develop into gross tissue changes, such as increases in the biomechanical indices of repair, 15 days after surgery. Therefore, our findings warrant the conclusion that at 15 days post surgery, the combination of laser therapy and early mechanical loading of tendons increases collagen production, with marginal biomechanical effects on repaired tendons.

Effects of Combined Laser Therapy, Electrical Stimulation, Ultrasound & Functional Load

As an extension of the previous study, we examined the combined effects of four modalities; laser therapy, electri-

cal stimulation, ultrasound and functional loading, using 63 rabbits. The set up of this experiment was essentially the same except that a similar dose of 904 nm Ga-As laser, i.e., 1 J cm^{-2} , was used instead of the 632.8 nm laser. The combined treatment produced a 14% increase in maximum strength, a 20% increase in maximum stress, and a 31% increase in Young's modulus of elasticity. Biochemical analysis showed an increase of 23% in the total amount of collagen, and as in the previous study, fewer mature cross-links (6% less). This biochemical evidence is consistent with the conclusion that more new (relatively less mature) collagen was formed in response to the treatment combination used in this study. Our measurement of procollagen mRNA levels in the tissue samples of the two groups revealed no significant differences. However, the larger variation between samples in the level of procollagen mRNA debars us from drawing conclusions concerning gene expression.

No electron microscopic or morphometric differences were observed between the experimental and the control groups. And consistent with the previous multiple modality study, these findings indicate that our combination of 904 nm laser therapy, ultrasound, electrical stimulation, and mechanical force, enhance collagen synthesis with moderate to minimal improvements in the biomechanical indices of healing.

Overall Effect of Laser Therapy on Tissue Repair Processes - Outcomes of Statistical Meta-Analysis

In two separate studies, we used the technique of statistical meta-analysis to determine the overall effect of laser therapy on tissue repair processes. Following an exhaustive review of the literature, we applied stringent inclusion and exclusion criteria to identify appropriate studies from which reliable statistical treatment effect sizes could be calculated. Effect sizes were calculated using Cohen's *d* statistic. Cohen's *d* is the standardized difference between the means of the experimental group and the comparison group divided by a standard deviation of the comparison group, conceptually expressed as:

$$d = \frac{x_1 - x_2}{SD_{comparison}}$$

In this formula *d* = effect size, x_1 = mean value for the laser group, x_2 = the mean value for the comparison group, and $SD_{comparison}$ = the standard deviation of the comparison group.

According to Cohen, the values of 0.2, 0.5, 0.8 indicate a small, medium, and large average effect size respectively. Where means or standard deviations were not reported but percentages were reported; then, d was calculated as follows:

$$t = \frac{(P_2 - P_1)}{\{(P_2)(1 - P_2) + (P_1)(1 - P_1)\}}$$

$$\frac{\qquad\qquad}{N_2} \qquad \frac{\qquad\qquad}{N_2}$$

In this formula P_2 is the population of the laser group, P_1 is the population of the comparison group, N_2 is the number of subjects in the laser group, and N_1 is the number of subjects in the comparison group. Once a t-value was calculated, then it was converted to d as follows:

$$d = \frac{2t}{df}$$

In this formula d = Effect Size, t = t-value, and *df* = degrees of freedom.

Once calculated, the effect size was assigned a positive or negative value. Positive values were assigned to experiments whose data support the use of laser therapy for tissue repair. Negative values were assigned to those whose data do not support the use of laser therapy in this regard. Once the effect size of each independent study was calculated, the average of all effect sizes was calculated. All of the effect sizes were summed and the total was divided by the total number of effect sizes:

$$d_{average} = \frac{d}{N}$$

In this formula $d_{average}$ = the mean effect size; d = the sum of the effect sizes; N = the total number of effect sizes.

Multiple effect sizes were calculated in some studies. Therefore, to avoid violating the assumption of independence, no more than two effect sizes were used in theses studies. Moreover, given the probability that we did not obtain every available study concerning the effects of laser therapy on tissue repair, a statistical fail safe number (N) was calculated. The fail-safe number is defined as the number of additional studies with effect sizes below a set criterion value that would have to be included in the meta-analysis to change the findings. In this study a set criterion of 0.10, less than the small effect size of 0.2, was utilized, and the following formula used to calculate the fail-safe number:

$$N_{fs} = \frac{N (\bar{d} - d_c)}{d_c}$$

Where N_{fs} = the fail-safe number; $\bar{d}$ = the average effect size of all the studies; and dc = the criterion value utilized. In one of our studies, 24 studies with a total of thirty-one effect sizes met the inclusion and exclusion criteria; in the other study 34 articles met all the criteria, and were used to calculate 46 effect sizes.

Whereas according to Cohen, the values of 0.2, 0.5, 0.8 indicate a small, medium, and large average effect size respectively, we calculated an overall mean effect size of +2.22in one study (n = 24) and 1.81 in the other (n = 34). These large effect sizes mandate the conclusion that laser therapy is an effective therapeutic modality for promoting tissue repair.

Conclusions

First, appropriate doses of laser therapy, ultrasound, electrical stimulation, and functional loading promote collagen synthesis, modulate the maturation of newly synthesized collagen, and overall, enhance the biomechanical characteristics of repaired tendons. Second, combining either visible 632.8 nm or near infra-red 904 nm laser with functional loading and either electrical stimulation or ultrasound and electrical stimulation, further promote collagen synthesis when compared to functional loading alone. However, the biomechanical effects of these combinations of therapies are similar to the single modality treatment, i.e., not better. Third, when the literature concerning the effects of laser therapy on tissue repair is aggregated and subjected to statistical meta-analysis, the results reveal a strong overall positive effects of laser therapy on tissue repair. This finding, and the outcomes of our series of experiments mandate the overall conclusion that laser therapy effectively promotes collagen synthesis and tissue repair.

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