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## Effects of Infra-Red Lasers on denervated muscle: A preliminary study in the rats.

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- Objective** : *To study the effect of InfraRed Laser on the denervated muscle in comparison with electrical stimulating treatment.*
- Setting** : *Department of Anatomy, Faculty of Medicine, Chulalongkorn University.*
- Design** : *Experimental study*
- Experimental animals** : *25 adult Wistar rats were divided into five groups: one control and four denervated groups included no treatment, electrical stimulation (ES), laser and ES plus laser groups*
- Methods** : *Four days after cutting the right sciatic nerve, the tibialis anterior muscle of that side was treated according to its group. The circumference and weight of the muscle were measured. Cryostat sections of the muscle were stained with H & E, and ATPase for differentiation of muscle fiber types and morphometry.*
- Results** : *Type-I muscle fiber was totally disappeared in the denervated muscle without any treatment, 3% of type-I fiber in the laser treated group, 16.9% in the ES group, 14.8% in ES plus laser*

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group and 57% in normal (control) group. There were significant differences in the weight, circumference and diameter of muscle fibers between control vs denervated group and laser group, denervated vs ES group and ES plus laser group, ES vs laser group and laser vs ES plus laser group. There were no significant differences between the control VS ES, and ES plus laser, denervated vs laser and ES vs ES plus laser groups.

**Conclusions** : The results indicate that Infra - Red laser treatment at a dosage of  $4 \text{ j/cm}^2$  1mw 2.13 minute every other day for 90 days cannot significantly retard denervation atrophy .

**Key words** : Infra-Red Laser, Denervated muscle, Sciatic nerve denervation, Low-power laser, Muscular atrophy.

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วิไล ชินธเนศ, วียะดา ไรวา, มีชัย ศรีใส, วิษณุ สะอาดสุด, ผลของอินฟรา-เรด เลเซอร์ ต่อกล้ามเนื้อที่ขาดเส้นประสาทมาเลี้ยง : การศึกษาเบื้องต้นในหนู. จุฬาลงกรณ์เวชสาร 2539 พ.ย; 40(11): 921-32

- วัตถุประสงค์** : เพื่อศึกษาผลของการใช้อินฟรา เรด เลเซอร์ ต่อกล้ามเนื้อที่ขาดเส้นประสาทมาเลี้ยง เพื่อเปรียบเทียบกับ การรักษาด้วยการกระตุ้นด้วยไฟฟ้า
- สถานที่ทำการศึกษา** : ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
- รูปแบบการวิจัย** : การทดลอง
- สัตว์ทดลอง** : หนูพันธุ์วิสตาจำนวน 25 ตัว แบ่งเป็น 5 กลุ่ม ได้แก่ กลุ่มควบคุม กลุ่มตัดเส้นประสาทออก 4 กลุ่ม แบ่งเป็นกลุ่มที่ไม่ได้รับการรักษา กลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้า กลุ่มที่ได้รับการฉายแสงเลเซอร์ และกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้าร่วมกับฉายแสงเลเซอร์
- วิธีการวิจัย** : สี่วันหลังจากตัดเส้นประสาทไขาดิคข้างขวา กล้ามเนื้อที่เบียดิส แอน-ทีเรียร์ข้างนั้นก็ได้รับการรักษาโดยกระตุ้นด้วยไฟฟ้าและหรือฉายแสงเลเซอร์ (ตามกลุ่ม) เส้นรอบวงและน้ำหนักของกล้ามเนื้อได้รับการบันทึกไว้ ตัดกล้ามเนื้อเป็นแผ่นด้วยเครื่องตัดแช่แข็งแล้วย้อมด้วยสี H&E และ ATPase เพื่อแยกชนิดของเส้นใยกล้ามเนื้อ รวมทั้งวัดขนาดของเส้นใยกล้ามเนื้อ
- ผลการวิจัย** : เส้นใยกล้ามเนื้อชนิด Type I ในกลุ่มที่ตัดเส้นประสาทแล้วไม่ได้รับการรักษา จะหายไปหมด พบ 3% ในกลุ่มที่ฉายแสงเลเซอร์ 16.9% ในกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้า 14.8% ในกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้าร่วมกับการฉายแสงเลเซอร์และ 5.7 % ในกลุ่มควบคุม เมื่อเปรียบเทียบน้ำหนัก เส้นรอบวงของกล้ามเนื้อทั้งมัด และ เส้นผ่าศูนย์กลางของเส้นใยกล้ามเนื้อ พบว่ามีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่มควบคุมกับกลุ่มที่ตัดเส้นใยประสาท และกลุ่มเลเซอร์ กลุ่มที่ตัดเส้นประสาทกับกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้า และกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้าร่วมกับการฉายแสงเลเซอร์ กลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้ากับกลุ่มเลเซอร์ และกลุ่มเลเซอร์กับกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้าร่วมกับเลเซอร์ ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่มควบคุมกับกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้า และกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้าร่วมกับเลเซอร์ กลุ่มที่ตัดเส้นประสาทกับกลุ่มเลเซอร์ และกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้ากับกลุ่มที่ได้รับการกระตุ้นด้วยไฟฟ้าร่วมกับเลเซอร์

**วิจารณ์และสรุป** : ผลการวิจัยชี้ให้เห็นว่าแสงอินฟรา-เรด เลเซอร์ ในขนาด 4 จูล/ตารางเซนติเมตร 1 มิลลิวัตต์ เวลา 2.13 นาที วันเว้นวัน เป็นเวลา 90 วัน ไม่สามารถลดอัตราการฝ่อของกล้ามเนื้อที่ตัดเส้นประสาทออกได้อย่างมีนัยสำคัญทางสถิติ

Denervation of muscle causes various alterations, including biochemical, mechanical, electrical and morphological changes. It has been found that the electrical properties of the membrane become altered,<sup>(1)</sup> the fibers become sensitive to acetylcholine over their entire length instead of only at the endplate,<sup>(2)</sup> the neuromuscular junctions may begin to degenerate,<sup>(3)</sup> the fiber organelles undergo alterations, and the fibers become atrophic.<sup>(4)</sup> The belief that inactivity might be responsible for these changes has led to attempts to arrest or inhibit the rate of muscle degeneration by electrical stimulation therapy. Electrical stimulation has been shown to be beneficial in retarding denervation atrophy for both types of muscle fiber.<sup>(5)</sup> Contraction of denervated muscle by electrical stimulation prevents the loss of oxidative enzymes<sup>(6)</sup> and the atrophy associated with denervation.

In recent years, laser technology has been applied to many aspects of society. Laser beams have been used in medicine for surgical purposes to reduce destructive lesions, especially in ophthalmology, neurosurgery and general surgery. Over the past 20 years many reports have appeared on the use of weaker lasers which include low power and low level lasers. The definition of a low level laser is one in which energy output is low enough so that the temperature of the treated tissue does not rise above 36.5°C. Although the medical application of low power lasers remains controversial, the clinical use of these devices for a variety of analgesic and wound healing applications is steadily increasing. Numerous biological and physiological effects have been reported. These include the contraction of

denervated muscle by electrical stimulation which prevents the loss of oxidative enzymes.<sup>(6)</sup> Low level lasers have also been proven to increase the cyclic AMP level in Chinese hamster fibroblast, DNA synthesis in Hela cells and protein synthesis in E.coli.<sup>(7)</sup> The stimulatory effect of low dose lasers on the injured sciatic nerves of rats has also been investigated.<sup>(8)</sup> It causes a significant increase in the amplitude of the action potential recorded in the corresponding gastrocnemius relative to the action potential of injured but untreated nerves. This evidence led to the hypothesis that low power lasers may have some physiological effects on the denervated muscle fiber which may retard its atrophy. Treatment by laser is completely painless and aseptic. Therefore, it is very convenient and does not produce unpleasant feelings in the patient as does treatment with electrical stimulation.

The objectives in our study were: to study the effect of an Infrared Laser (wavelength 830 nm) on denervated muscle in comparison with electrical stimulation. This was determined by examining the morphological changes in the muscle fibers, including measuring their diameter in type-I and II fibers under a light microscope.

## Materials and Methods

Twenty-five male Wistar rats, body weight 300-400 g, were divided into 5 groups of 5 rats per group. The 5 groups were the control group, those denervated without any treatment (denervated group), those denervated with electrical stimulation (ES group), those denervated with laser treatment (Laser group) and those denervated with ES and

laser treatment (ES and laser group). Four days after cutting the right sciatic nerve in the ES group, the tibialis anterior muscle was treated with electrical stimulation by interrupted direct current. The intensity of stimulation was 10 mA with a 10 msec pulse width and a 50 msec pulse interval. One hundred dorsiflexions of the foot were recommended for each treatment. The schedule of stimulation was every other day for 45 treatments. In the laser group, 2.13 minutes of  $4 \text{ j/cm}^2$  1 mW infrared laser in continuous mode was applied to the tibialis anterior muscle with the same schedule as in the ES group. In the ES and Laser group, both ES and laser were applied to the tibialis anterior muscle with the same intensity and duration as in the above groups.

After 90 days, the tibialis anterior muscle of the right hindlimb was excised and its circumference and weight were measured. The middle third of each muscle was cut into 6-8  $\mu\text{m}$  thicknesses with a cryostat, and stained with hematoxyline and eosine for morphological examination. The tissue sections were also processed by the myosin ATPase technique for differentiation of types of muscle fiber. Photomicrographs were made from all quadrants of the sections. Random fascicles were chosen and the muscle fiber diameter was measured according to the method of Brooke and Engel.<sup>(9)</sup> Sufficient photographs were taken to include at least 200 fibers. In order to measure these, a transparent ruler was construct from a photograph, at the same magnification, of a micrometer slide graduated at 10  $\mu$  intervals. The fibers were measured across their smallest diameter. In practice, the "smallest diameter" is the greatest distance between the opposite side of the narrowest aspect of the fiber. The

significance of the differences in each parameter between groups was determined by unpaired t-test.

## Results

### Muscle weight and circumference

The various weights and circumferences of the tibialis anterior muscle were recorded as shown in Table 1. The mean weight of this muscle in the control group was 0.44 g. It was much reduced in the denervated laser treatment groups (0.17g). The weight of the muscle was less reduced in the ES and ES plus laser groups (0.40 for both). Similar results were obtained for the circumference of the muscle (Table 1). There was no significant difference between the denervated and laser groups, but a significant difference occurred between the denervated and ES group.

### Histology and type of muscle fiber

**H & E staining.** The normal shape of the muscle cells in the transverse sections was polygonal and they were closely packed with no intercellular space (Figure 1A). In the denervated group, the shape of the muscle cells was round and more intercellular space was seen. Most fibers were reduced in size (Figure 1B). In the ES group, the shape and size of the muscle fibers were quite similar to those of the control group (Figure 1C). Some atrophic muscle fibers appeared in the periphery of the muscle mass (Figure 1D). Transverse sections of muscle in the laser group showed small and round muscle fibers (Figure 1E), similar to those of the denervated group. In the last group (ES plus laser), the morphology of the muscle fiber was very well preserved. The shape and size were close to the control and ES groups (Figure 1F).

**ATPase pH 9.5 staining.** Using this technique, the muscle fibers can be differentiated into two types: light staining (Type-I) and dark staining (Type-II) (Figure 2A). The numbers of type-I and type-II muscle fibers were counted and converted to percentages. In the control group there were 57% type-I and 43% type-II. Type-I muscle fibers were absent in the denervated group (Figure 2B), and the fibers were rather small and stained darkly. In the ES group, 16.9% of type-I fibers were preserved (Figure 2C), and in the ES plus laser group 14.8% of type-I fibers were found (Figure 2D). In the laser group, only 3% of type-I fibers were present (Figure 2 E, F).

### Size of muscle fibers

The diameters of the muscle fibers in each sample were measured and the means are shown in Table 2. Type-II fibers were considerably smaller than the type-I fibers. There were no type-I fibers in the denervated group (Figure 2B). In the ES group, type-I fibers were preserved (Figure 2C) but their size was smaller than in the controls. Both types of muscle fiber were much reduced in size in the laser group (Figure 2E, F). In the last group, type I-fiber was quite close in size to those of the control group. There was no significant difference between the denervated and laser groups but a significant difference occurred between the denervated and ES groups.

**Table 1.** Effects of electrical stimulation and laser treatment on the weight and circumference of tibialis anterior muscle.

| Treatment    | Weight (g)               | Circumference (cm)       |
|--------------|--------------------------|--------------------------|
| Control      | 0.44±0.02                | 2.52±0.13                |
| Denervated   |                          |                          |
| No treatment | 0.17±0.01*               | 0.98±0.08*               |
| ES           | 0.40±0.02 <sup>1</sup>   | 2.16±0.16 <sup>1</sup>   |
| Laser        | 0.17±0.01 <sup>*.2</sup> | 1.02±0.04 <sup>*.2</sup> |
| ES+Laser     | 0.40±0.01 <sup>1,3</sup> | 2.21±0.08 <sup>1,3</sup> |

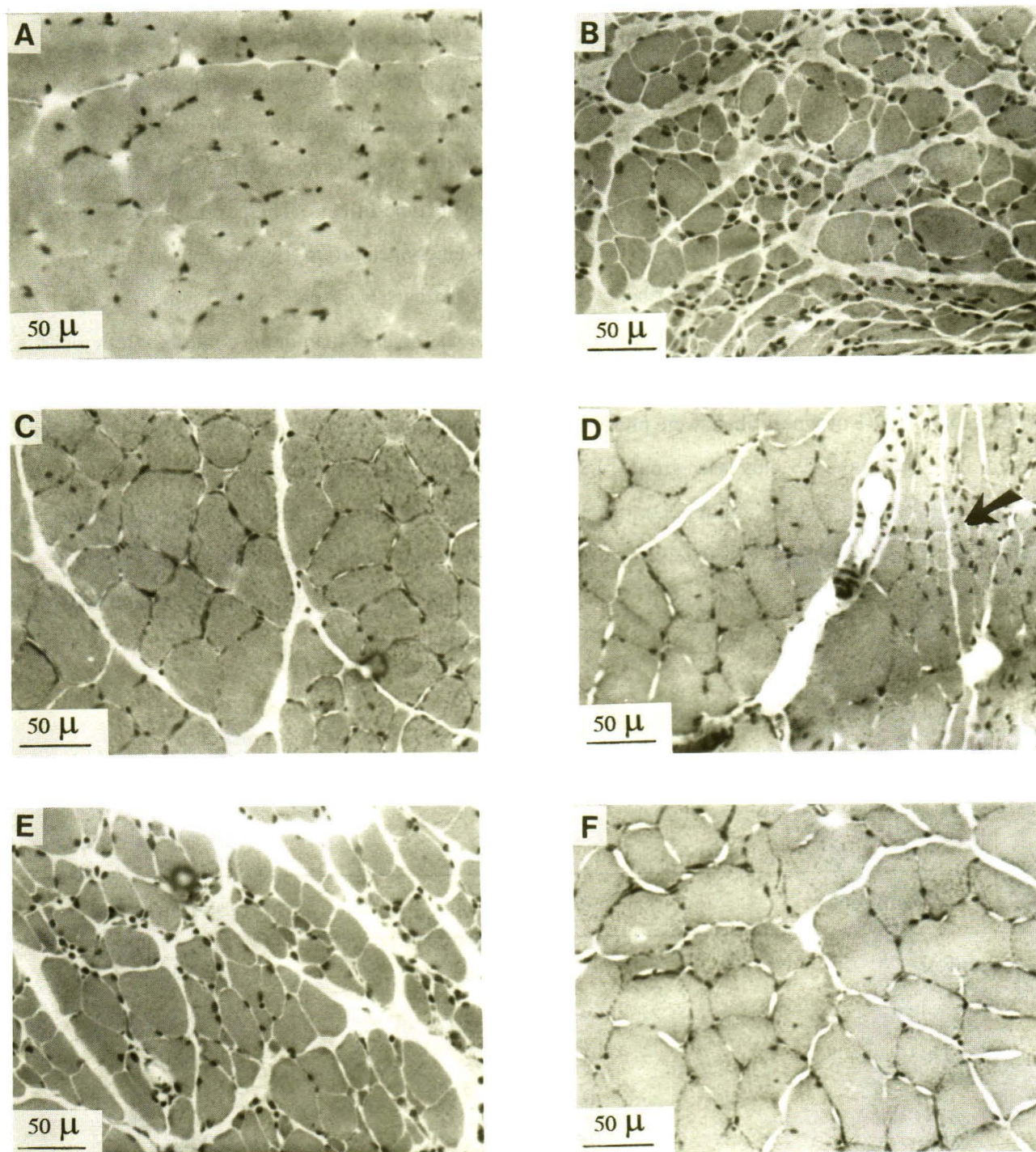
\* Significantly different from controls      at 0.05 level

<sup>1</sup> Significantly different from denervated      at 0.05 level

<sup>2</sup> Significantly different from ES      at 0.05 level

<sup>3</sup> Significantly different from laser      at 0.05 level

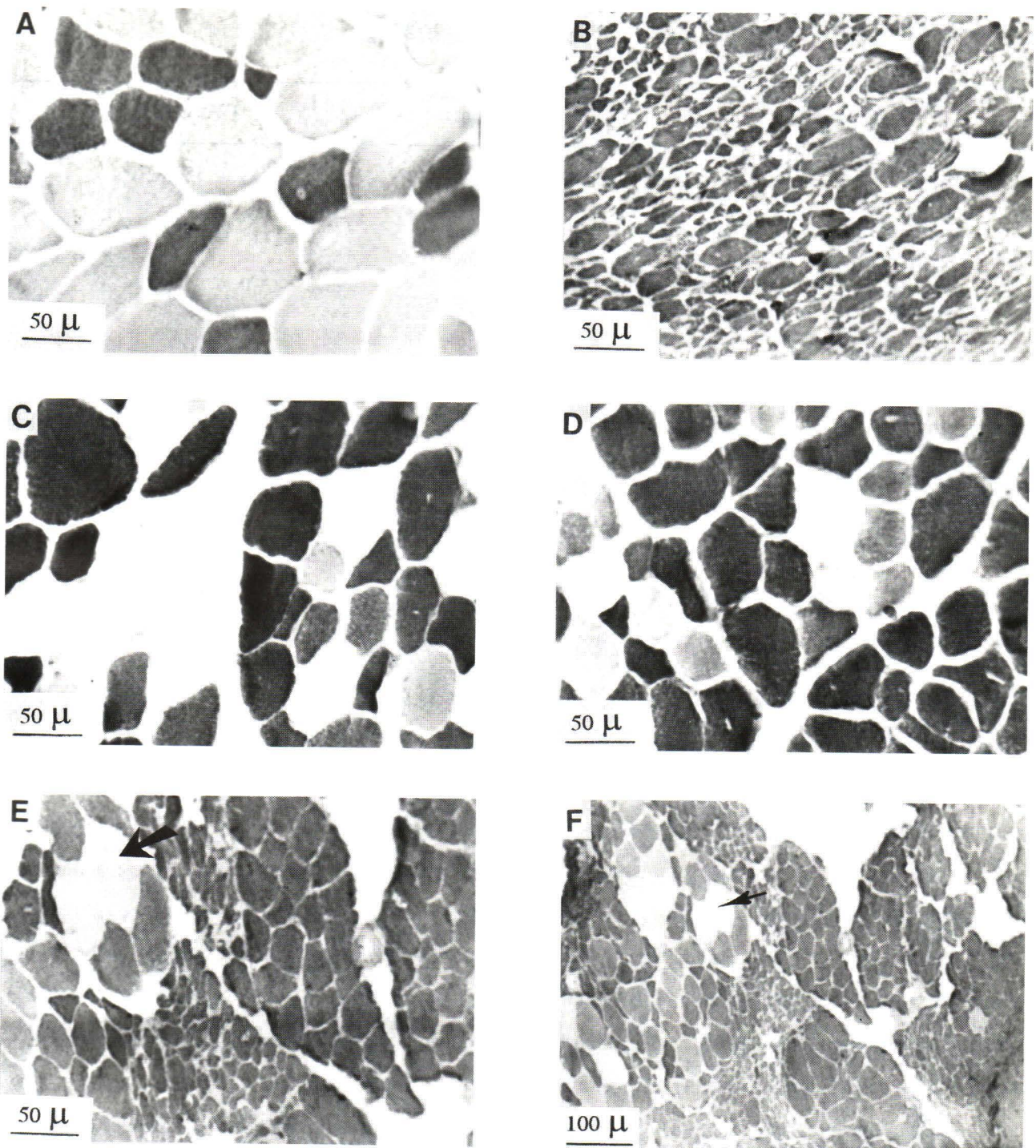




**Figure 1.** Transverse section of tibialis anterior muscle with H & E staining

- A = Control group
- B = Dernervated without treatment group
- C = ES group
- D = ES group with some atrophic area (arrow)
- E = Laser group
- F = ES + laser group





**Figure 2.** Transverse section of tibialis anterior muscle with ATPase pH 9.5 staining

- A = Control group
- B = Denervated without treatment group
- C = ES group
- D = ES + laser group
- E, F = Laser group, few type I muscle fiber is seen (arrow)

**Table 2.** Mean diameters of type-I and II muscle fibers in each group.

| GROUP        | TYPE I (μm)                 | TYPE II (μm)                |
|--------------|-----------------------------|-----------------------------|
| Control      | 59.41 ± 3.65                | 34.35 ± 2.16                |
| Denervated   |                             |                             |
| No treatment | -                           | 9.4 ± 2.16*                 |
| ES           | 30.97 ± 14.76*              | 34.68 ± 3.98 <sup>1</sup>   |
| Laser        | 15.91 ± 1.88 <sup>*,2</sup> | 11.10 ± 0.85 <sup>*,2</sup> |
| ES+Laser     | 29.09 ± 2.7 <sup>*,3</sup>  | 33.25 ± 2.79 <sup>1,3</sup> |

\* Significantly different from controls at 0.05 level

<sup>1</sup> Significantly different from denervated at 0.05 level

<sup>2</sup> Significantly different from ES at 0.05 level

<sup>3</sup> Significantly different from laser at 0.05 level

## Discussion

The normal proportions of type I-and type-II muscle fibers of the tibialis anterior muscle of the rat are 57% and 43%, respectively. The mean diameters of type-I and type-II muscle fibers are 59.41 μm and 34.35 μm, respectively. Following denervation, the fiber organelles undergo alteration and the fibers become atrophic. In this experiment, type-I muscle fiber was more vulnerable to denervation atrophy than type-II fiber. This is not in agreement with previous studies<sup>(5,10,11)</sup> which described a so-called “preferential type-II atrophy” after denervation which implies that type-I muscle fibers are affected by atrophy to a much lesser extent than type-II fibers. The difference between our experiment and the others was in the type of muscle selected and the duration of the treatment. Jaweed et al. (1975) suggested that the denervation atrophy of muscle fiber depended on the individual muscle and the duration of denervation.<sup>(12)</sup> We chose the tibialis anterior muscle instead of the extensor

digitorum longus muscle because the tibialis anterior muscle layed superficially and was easier to stimulate and treat with the laser. The duration of our experiment was 90 days but in Pachter, et al (1982), it was only 24 days. The appropriate dose and duration of treatment with low power lasers is of vital importance if optimum results are to achieved.<sup>(13)</sup>

It is recommended that the dosage be sufficient to produce a reaction which is an improvement in the condition either during or immediately following the first treatment session. In our experiment, we could not observe the treatment reaction so we followed the recommended dosages for treatment of wounds, which are 1-4 j/cm<sup>2</sup> in continuous mode. Our schedule was every other day for a period of 90 days. This dosage and schedule may not be sufficient to slow down the atrophic process. However, ours was a preliminary study of the effects of the infrared laser on denervated muscle and further investigation is needed. There are several existing reports of the effect of lowpower lasers on the peri-

peral nervous system.<sup>(8,14)</sup> An He Ne laser increased the action potential, prevented scar formation and accelerated the degeneration process of crushed sciatic nerve. The dosage used was a 17 mW current wave for 20 consecutive days (7 minutes per treatment). Although infrared lasers seem to have no retarding effect on denervated muscular atrophy, one striking finding was the presence of Type-I muscle fiber in the laser treatment group. We believe that the low power laser must have had some effect on the denervated muscle and suggest that further experiments be carried out to determine the effects of changing the dosage, wave length, mode and duration of treatment. Morphological studies should also include both light and electron microscopy for detection of minute structural changes. Histochemical and biochemical studies of oxidative enzymes, cAMP levels and rates of DNA and RNA synthesis should also be considered.

## Conclusion

It may be concluded that infrared laser treatments at a dosage of  $4 \text{ j/cm}^2$  1mW for 2.13 minutes every other day for 90 days cannot prevent muscular atrophy after denervation. Some type-I muscle fibers were preserved, but the effect was not statistically significant.

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