EFFECT OF PULSED ULTRASONIC THERAPY ON SKELETAL MUSCLE INJURY IN RABBITS

By

El-Ghaweet A. and Helal N

From

The Departments Of Physical Medicine and Pathology.

Faculty Of Medicine, Mansoura University.

Received For Publication : 10/4/1990.

INTRODUCTION

Skeletal muscle injury which comprises, a high proportion of sport injury, is associated with inflammation, transudation and fibrin deposition (Murray - Leslie, 1986). The injured muscle fibres, when relatively small, have the capacity for regeneration and repair and are again capable of contraction. With larger injury and/or presence of systemic diseases, there is a reduced capacity for regeneration and repair and the muscle fibers can ultimately be replaced by fat and/or fibrous tissue (Ambrosia, 1986). A modality able to enhance healing of such injury may facilitate restoration of the prior level of function. Low dose pulsed ultrasonic therapy was proved to be more effective than radiant heat, short-wave diathermy or paraffin baths in helping patients with sprained ankles to return to work (Middlemast, 1978).Various other researches including topics as surgical wounds, varicose ulcers and pressure sores, were in favor of the healing effect of pulsed ultrasound and support such an idea (McDiarmid et al.,1985 and Hong et al., 1988). Pulsed ultrasound can be applied safly during the early stages of healing without fear of bleeding due to its minimal thermal effect in comparison with the non thermal one (Dyson & Suckling, 1978; Partridge, 1987 and Lehman & DeLateur, 1989). Other contradictory results proved that low dosage of pulsed ultrasound impair healing when applied on freshly healing tendons in rabbits (Roberts, 1982). Despite these numerous experiments and the importance of early recovery after skeletal muscle injury, no study has been reported that examines the effect of ultrasound on the healing of muscle in-

jury. This was therefore the basic thrust of this study.

MATERIAL AND METHODS

Twenty-four young rabbits, weighing 980 to 1350 gm., were used in this experiment. They were 18 males and 6 females. The rabbits were prepared for muscle injury according to the method of Foster and Carlson (1980). A small area of the skin over the belly of the right and left gastrocnemus muscle of each rabbit was shaved. The center of each muscle was gently squeezed between 2 fingers and injected with one cc. of 2 % Xylocaine, a myotoxic drug, directly into the center of the muscle belly. The injection was given at a depth of 0.4 cm., and the site was marked with indelible ink for later identification. Second and third injections were administered at the same site 5 and 12 hours, respectively, after the first injection. Twenty-four hours after the first injection, the right gastrocnemus of each rabbit was treated with pulsed ultrasound applied by micromassage technique using a Siemens sonostat 733. The usual dosage in clinical use was used, being 0.5 W/ cm2 for 5 minutes, every other day. The left gastrocnemus of each rabbit was mock treated with pulsed ultrasound for 5 minutes, every other day, but with the transducers turned off. Half of the rats (n = 12) were sacrificed after one week of treatment, while the other half (n = 12) were sacrificed after two weeks of treatment.

The central part of each gastrocnemus muscle then was removed and fixed in 10 % neutral buffered formalin solution. Formalin-fixed tissues were embedded in paraffin by a standard procedure, sectioned and stained with hematoxylin and eosin (Lillie and Fullmer, 1977). Between 20-25 slides were produced for the belly of each gastrocnemus muscle to evaluate the degree of muscle fibre degeneration and regeneration (Brown and Barker, 1987). Muscle fibres undergoing degeneration exhibited cellular infiltrations and their cells displayed pyknotic or missing nuclei, altered cytoplasmic staining or a loss of cytoplasm (Fig. 1 & 2). The cells of regenerating muscle fibres, although small, possessed cytoplasm and normal appearing nuclei that were placed centrally (Fig. 2). The degree of muscle regeneration was determined according to Foster and Carlson (1980) and Brown & Baker (1987). Mild regeneration (grade I) = the proportion of the regenerating fi-

bres was lesser than the degenerating fibres. Moderate regeneration (grade 2) = the proportion of the regenerating fibres was equal to the degenerating one . Marked regeneration (grade 3) = a few small patches of degenerated fibers existed, while the regenerated fibres with central nuclei occupied the major portion of the lesion. Excellent regeneration (grade 4) = the regenerating fibres occupied the whole site of lesion. The cytoplasmic staining and the size of the regenerating fibres were nearly the same as unaffected fibres (Fig. 4).

A t-test was performed to compare between the degree of improvement after the first and second week of treatment in both the insonated and mock-insonated groups. A probability level of 0.05 was considered to be significant.

RESULTS

- After one weak of ultrasonic irradiation (Table 1), the insonated muscles ,showed a more rapid grade of healing than of the contrtol group. The insonated muscles showed mild regeneration in 3 muscles, moderate and marked regeneration in 7 and 2 muscles respectively. In the mock - insonated muscles mild and moderate regeneration were demonstrated in 8 and 4 muscles respectively while no marked regeneration was observed in any muscles (Fig,I, 2 and 3). The level of significance between the degree of improved regeneration in both the insonated and mock-insonated muscles reached a significant level ($P<0.025$).

- After two weeks of ultrasonic irradiation (Table 2),better degree of healing was observed in the insonated muscles in comparison with the mock insonated ones. Mild and moderate degrees of regeneration were demonstrated in no muscles & only in one muscle respectively in the insonated muscles, while they were demonstrated in 2 and 4 muscles respectively in the mock insonated muscles. Marked and excellent improvement in the tissues from insonated muscles was observed in 6 and 5 muscles respectively in comparison to 5 and one muscles in the mock-insonated group (Fig. 1, 2, 3 and 4). The level of significance between the degree of improved regeneration in both the insonated

and mock-insonated muscles after two weeks of treatment reached a more significant level ($P < 0.01$).

DISCUSSION

The process of skeletal muscle degeneration and regeneration has been studied for the past two decades. Various methods have been used to induce muscle injury including removing, mincing and replacing a muscle in situ; occluding the circulation to a muscle; transplanting whole muscles, and injecting a muscle with a myotoxic drug (Hall-Craggs, 1974; Foster & Carlson, 1980 and Allbrook, 1981). Each of these methods was found to be an effective means for producing muscle injury in animals. The severity and extent of injury differs according to the method used (greatest injury secondary to ischemia), but the process of regeneration is similar irrespective to the cause of injury (Hall Craggs, 1974 and Allbrook, 1981). After muscle injury secondary to Xylocaine injection, a regeneration of muscle fiber procceds optimally along intact cylinders of the basal lamina (Brown and Barker,1987). Assisting the process of healing whenever possible, can improve the final outcome and therefore decrease complications. Methods to enhance healing of such injury could be used as an aid in the repair of relatively larger muscle injury. Previous studies have shown that ultrasonic irradiation can serve to alter the course of tissue response to injury (El-Batouty et al., 1986). For example , studies of hard tissues have demonstrated beneficial effects of ultrasound in the repair of nonunited bone fracture (Cochran et al., 1985). In soft tissue repair, much intrest has been given to the stimulating effect of ultrasound when given to skin wounds (El-Batouty et al., 1986), and tendon lesions (Enwemeka, 1989). There is no avialable study demonstrating such an effect on muscle injury.

Piersol et al., (1952) and Carstensen et al.,(1953) have demonstrated that ultrasonic absorption occurs primarily in the tissue protein. Huter and Bolt (1951) has shown that attenuation of ultrasound in tissue depends on whether or not the ultrasonic beam is parallel to myofascial interfaces, thus demonstrating a selective absorption at interfaces that can be explained on the basis of scattering, which, in turn, results in an increased absorption at irregular surfaces. Prop-

er application and low dosage can produce the therapeutic effects of ultrasound inside the treated muscles, without any side effects (Lehman and DeLateur, 983).

Throughout the present study, it was found that the insonated muscles healed more rapidly than those of the mock-insonated muscles, as manifested by better grades of healing at any time during the regeneration process. This difference in the rate of healing between the insonated and non-insonated muscles was statistically significant after the first and second weeks of treatment ($P< 0.025$ and 0.01 respectively). The mechanism by which pulsed ultrasound stimulates healing is mostly due to the nonthermal effects and,to a lesser extent , to the thermal effect as well (Dyson & Pond, 1970 and Dyson, 1987). Of the nonthermal effects of ultrasound , cyclic and acoust streaming , it is the latter which seems to be most important (Madden & Smith, 1970). Cyclic effects relate to oscillatory movements accompanied by waves of pressure which are repeated at each wave cycle. They may induce a kind of "micromassage" which could facilitate tissue repair by reducing edema, but effect on tissue growth, per se, is not considered significant because the maximum displacement velocity produced at the ultrasound dosage giving maximal growth stimulation is too low to bring about the molecular interactions involved in growth (Madden and Smith, 1970).The acoustic streaming phenomena is observed mainly at interfaces such as the membrane of cells and organelles and it might be responsible for tissue repair (Webster et al., 1980). The acoustic streaming is thought to produce changes in diffusion rates and membrane permeability which might accelerate the rate of protein synthesis (Frieder et al., 1988). The mild degree of temperature elevation which may be produced as a result of pulsed ultrasound may enhance the healing brought about by non-thermal effects (Dyson, 1987). In this study, no complications as hemorrhage or progressive damage were noted during the period of treatment. Fortunately, if the proper equipment is used and a therapeutic dosage is applied with the proper technique, the destructive effect due to cavitation should not be observed under therapeutic conditions. Also there is no available study evaluating the healing effects of the the therapeutic ultrasound on muscle injury, the results of the present study support the effec-

tiveness of ultrasound in promoting tissue regeneration in other soft tissue lesions.

In conclusion, application of pulsed ultrasound at an intensity of 0.5 W/cm. for 5 minutes every other day for two weeks augments the repair process after skeletal muscle injury in rats with no complications. These results provide a foundation for the clinical therapeutic use of low - dose pulsed ultrasound during the early stage of skeletal muscle healing with resultant improved outcome.

SUMMARY

The purpose of this study was to determine whether or not low dose pulsed ultrasound would enhance healing of skeletal muscle injury. Twenty-four rabbits were the subjects used in this study. The muscle injury was induced by giving all 24 rabbits three local injections of a myotoxic drug (Xylocaine) into the right and left gastrocnemius muscle. The right gastrocnemius of each rabbit was irradiated with pulsed ultrasound every other day. The left gastrocnemius in all rabbits was mock insonated and acted as controls. Half of the rabbits (n = 12) were sacrificed after one week of treatment, while the other half (n = 12) were sacrificed after two weeks of treatment. The results revealed that the insonated muscles healed more rapidly and significantly than that of the mock-insonated ones after the first and second weeks of treatment ($P < 0.025$ and < 0.01 , respectively). These results provide a foundation for the clinical therapeutic use of low-dose pulsed ultrasound during the early stage of skeletal muscle healing with expected improved outcome.

REFERENCES

Allbrook D. (1981) : Muscle Nerve, 4 : 234 : 245.

Ambrosia R. D. (1986) : Musculoskeletal disorders . Sec. ed. J B. Lippincott Company . New York P. 145.

Brown M. and Baker R. (1987) : Phys. Therapy. 67: 208-214.

Carstenen E.; LiK.and Schwan (1953) : J. Acoust Soc. Am., 25: 286 - 289.

Cochran V.; Johnson M.:

M. and Palmieri R. (1985) J. Orth. Res., 3: 508 - 513.

Dyson M and Pond J. (1970) : Physiotherapy, 56 : 136 - 142.

Dyson M. and Suckl ing (1978) : Physiotherapy, 64 : 105 - 108.

Dyson M (1987) : Physiotherapy, 73 : 116 - 120.

El-Batouty M.; El-Gindy M.; El-Shawaf I.; El-Ghaweet A. and El-Emam (1986) : Scand J Rheumat., 15 : 381 - 386.

Enwemeka C. (1989) : Am. J. Phys. Med., 68 : 283 - 287.

Foster A.and Carlson B. (1980) : Anesth Analg, 58: 727 - 736.

Frieder S.; Joseph W.; Fleming B. and Stank A. (1988) : J. Orthop. Sports. Phys. Therapy, 10 : 39 - 46.

Hal l-Craggs E. (1974) : Exp. Neurol, 43 : 349 - 355.

Hong C.; Harrison H. and Jen Y (1988) : Arch. Phys. Med. Rehabil., 69: 410 - 414.

Hulter T. and Bolt F. (1951) : J. Acoust Soc. Am., 23: 160 - 167.

Lehman J. F. and De-Lateur B. J. (1989) : Therapeutic heat and cold 4th ed, Baltimore, Williams & Wikins. P. 218.

Lillie R. D. and Fullmer H. M. (1977) : Histopathologic technic and partical histochemistry, ed 4. New York. Mc Graw-Hill Inc, P. 123.

Madden J. W. and Smith H. C. (1970) : Surg. Gynecol .Obstet. 130 487 - 492.

Mc Diarmid T.; Burns P.; Lewith T. and Machin D (1985) : Physiotherapy, 71 : 66 - 70.

Murray-Leslie C. F. (1986) : Recent advances in rheumatology. Vol. 4 Chur-

chi11. Livingstone, London. New York. P. 185.

Partridge C. (1987) : Physiotherapy, 73: 166 - 168.

Piersol G.; Schwan H.; Pennell R. and Carstenen E. (1952) : Arch Phys. Med., 33 : 327 - 332.

Roberts M.; Rutherford and Harris D. (1982) : The Hand, 14 : 17-20.

Webster D. R.; Harvery W. and Dyson M (1980) : Ultrasonics, 17 : 34 - 37.

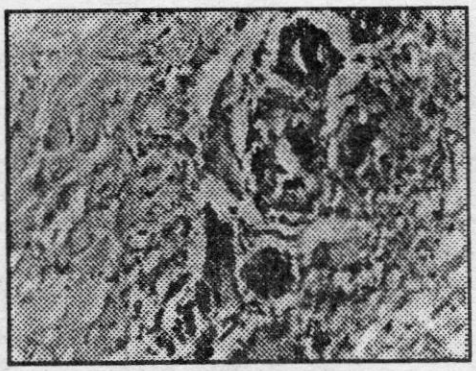

Fig. 1 : Section of a muscle after one week of mock-ultrasonic irradiation showing muscle cellular infiltration, their cells displayed missing nuclei and altered cytoplasmic staining Hx. & E. stain X 100).

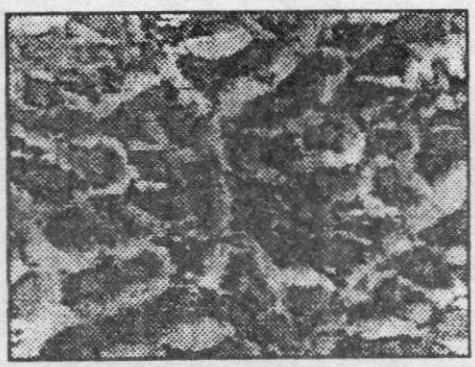

Fig. 2 : Section of a muscle after one week of ultrasonic irradiation showing less cellular infiltration, with appearance of newly formed muscle fibers with central nuclei (Hx. & E. stain X 100).

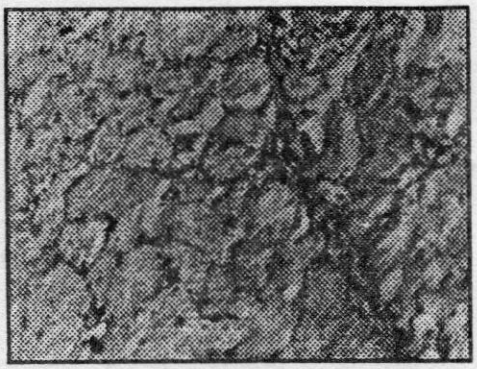

Fig. 3 : Section of a muscle after two weeks of mock-ultrasonic irrad -iation showing some cellular infiltration, their cells dis played missing nuclei and altered cytoplasmic staining with appearance of newly formed muscle fibres with central nuclei (Hx. & E. stain X 100).

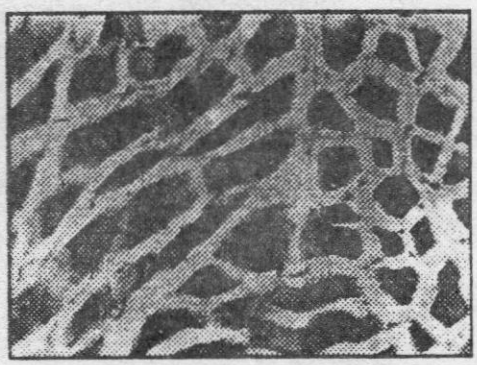

Fig. 4 : Section of a muscle after two weeks of ultrasonic irradiation showing no cellular infiltration, with appearance of normal peripheral nuclei and cytoplasmic staining (Hx. & E. stain X 100).

Table 1 : The reported degree of regeneration after one week in the mock-insonated and insonated gastrocnemus mscles.

Degree of Improvement	Number of Muscles	
	Mock-inson.	Insonated
Mild	8	3
Moderate	4	7
Marked	-	2
Mean improv.	1.333	1.916
S. D.	0.492	0.668
P	< 0.025	

P = Statistical level of significance between the degree of regeneration in the mock-insonated and insonated muscles.

الملخص العربى

تأثير العلاج بالموجات فوق الصوتية المتقطعة على اصابات العضلات الهيكلية فى الأرانب

د. عاطف الغويط د. نجوى هلال

يهدف هذا البحث إلى دراسة تأثير الموجات فوق الصوتية المتقطعة على التئام إصابات العضلات الهيكلية فى الأرانب، حيث تم أحداث الاصابات بحقن دواء ضار بالعضلة (زيلوكايين) فى منتصف العضلة ذات البطن للساق الأيمن والأيسر لأربعة وعشرين أرنباً، وتم علاج الاصابة فى العضلة اليمنى بواسطة الموجات فوق الصوتية المتقطعة (٠ر٥ واط/سم٢) يوم بعد يوم بينما تركت العضلة اليسرى بدون علاج فعال لغرض المقارنة، وبعد أسبوع واحد من العلاج تم فحص هستوباثولوچى للعضلات المصابة فى نصف الأرانب (عدد ١٢ أرنب) وتم الفحص فى نصف الأرانب الثانى بعد اسبوعين.

أوضح الفحص الهستوباثولوچى للعضلات ذات البطن بعد أسبوع واسبوعين من العلاج أن الموجات فوق الصوتية المتقطعة قد ساعد على سرعة التئام إصابات العضلات بدرجة ذات دلالة أحصائية أكثر من تلك التى تركت بدون علاج فعال. وعلى ذلك يوصى باستخدام هذه الموجات فى علاج اصابات العضلات الهيكلية.