Effects of hypertonic dextrose on injured rat skeletal muscles

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ABSTRACT

Objective: Histological examination of proliferative therapy effects on the healing process of muscular injury.

Methods: We performed this study between March and August 2002 at Ankara University, School of Medicine, Laboratory of Animal Experiments, Ankara, Turkey. We used an experimental animal model by conducting a standardized cut injury of the gastrocnemius muscle in 30 adult male albino rats, which we divided into 2 groups; proliferative therapy group and control group. We evaluated the injured rat muscles by light microscopy on the fifth, eight, and twelfth day of injury.

Results: The muscular regeneration process began at day 5 in both the control and proliferative therapy groups. The proliferative therapy group revealed a prominent inflammatory reaction, fibroblast migration, and necrosis with accompanying regeneration and excessive connective tissue formation.

Conclusion: We cannot consider proliferative therapy an appropriate treatment modality for muscular injuries, unless there is evidence of normal muscle physiology and biomechanics post traumatically.

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A vast amount of data is available on the pathophysiology of bony and ligamentous injuries in orthopedic and sports medicine research. Musculotendinous injuries account for approximately 90% of all sports-related injuries.[1,2] There are studies on muscle strain injuries,[3,4] contusions,[5,6] myotendinous junction tears,[7-9] and crush injuries.[10] After the initial injury, reinjury, muscle atrophy, and contractures can occur with significant morbidity regarding time lost to training and competition.[1,11,12] Although there are efforts made to decrease such morbidity, the best treatment of muscle injury has not yet been defined.[1] Treatment of muscular injuries is largely based on empirical findings. Rest, stretching, strengthening, non-steroidal anti-inflammatory medication and steroids; both corticosteroids and anabolic steroids, were emphasized in the treatment issue.[1,2,11-19] Evidence supporting the use of other pharmaceutical interventions is scarce. Non steroidal anti-inflammatory drugs are often used in the clinical setting, although data regarding their benefits are conflicting.[13,16] Corticosteroids and anabolic steroids continue to be used clinically into the site of injury, both to relieve the pain and promote the healing, and to expedite the player's return to active status.[14] However, the International Olympic Committee (IOC) and World Anti Doping Agency (WADA) institute more and more stringent rules and tests in many sports organizations, and these drugs used for muscle injuries are regarded as doping agents. Therefore, new agents are sought for clinical use in muscular injuries. Proliferative therapy involves the injection of a dextrose solution, often mixed with glycine and local anesthetic, into soft tissues for the management of injury and pain.[15,20,21] In this report we aim to study the proliferative therapy effects on the healing process of muscular injury by histological examination of an animal model.

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Methods. We performed this study between March and August 2002 at Ankara University, School of Medicine, Laboratory of Animal Experiments, Ankara, Turkey. Thirty male adult albino rats were used in the experiments. The experimental protocol was approved by the Ethical Committee of Ankara University Faculty of Medicine. They were anesthetized with intramuscular ketamine injection and received a cut injury across the mid belly of the gastrocnemius muscle of the right hind limbs. The skin of the right leg was opened, and a small perpendicular incision was made with a No. 11 surgical blade. The cut was 5 mm deep and 5 mm long. A suture was placed at either end of the cut for future identification of the lesion site. The skin wound overlying the lesion was closed and sutured under sterile conditions. The rats were divided into 2 groups in equal numbers as proliferative therapy group (PT) and control group (C). The mixture of proliferative therapy injection included 10% dextrose, 1.5% glycine, and 0.05% lidocaine solutions with equal volumes. Using insulin syringes, 0.02 ml of this compound was injected into the injured sites of the PT group before suturing while the laceration was visible. The solution was prepared daily and sterilized before injection. The same amount of saline injection was administered to the C rats. All the rats were then allowed to move freely in their cages. At 5, 8, and 12 days postinjury, the injured muscles of the rats were evaluated histologically by light microscopy. Five days after the injury, 5 rats from both PT and C groups were randomly selected and anesthetized. After the intracardiac fixation, all of the animals were killed by cervical dislocation. The injured muscle was totally removed, and 6-8 mm serial sections were obtained for microscopical assessment. The same procedure was conducted on day 8 and day 12 to 5 rats from each group. For paraffin sections, the removed tissues were fixed in phosphate buffered 10% formaldehyde for 2 days, dehydrated in alcohol and embedded in paraffin. Four to 6 micrometer thick sections were obtained by Leitz-1512 microtome (Leitz, Oberkochen, Germany), stained with hematoxylin-eosin. For semithin sections, the removed tissues were fixed in 2.5% glutaraldehyde in a phosphate buffer for 24 hours and postfixed in 1% osmium tetroxide. The materials were dehydrated in graded alcohol, embedded in Araldite CY 212, sectioned with a Leica Ultracut R Ultratome (Leica, Solms, Germany). Semithin sections were stained with Toluidine blue-Azure II. Sections were photographed by Zeiss Axioscope photomicroscope (Carl Zeiss, D-7082 Oberkochen, Germany).

Results. ***Control group (C).*** At day 5, affected muscle tissue due to injury was seen in the samples. In this area, there were a few fibroblasts and sparse connective tissue elements. The new myotubes of various sizes were scattered in the periphery of the involved area. The skeletal muscle fibers of the undamaged area were seen normal with euchromatin nuclei and cross striations (**Figure 1**). At day 8, the connective tissue elements were more abundant than those of the 5th day. At day 12, the affected area was invaded by loose connective tissue with fibroblasts and randomly oriented thin collagen bundles within the regenerated muscle tissue (**Figure 2**).

Proliferative therapy group (PT). At day 5, in contrast to the control group, there were large

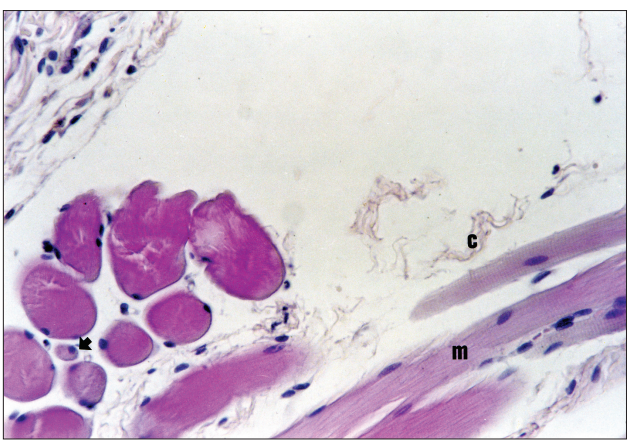

Figure 1 - Day 5 control group. Loose connective tissue is seen in the affected area. C - collagen bundles, M - muscle fiber, arrow - myotube. Hematoxylin-eosin X100. The affected area is invaded by loose connective tissue with fibroblasts and randomly oriented thin collagen bundles within the regenerated muscle tissue.

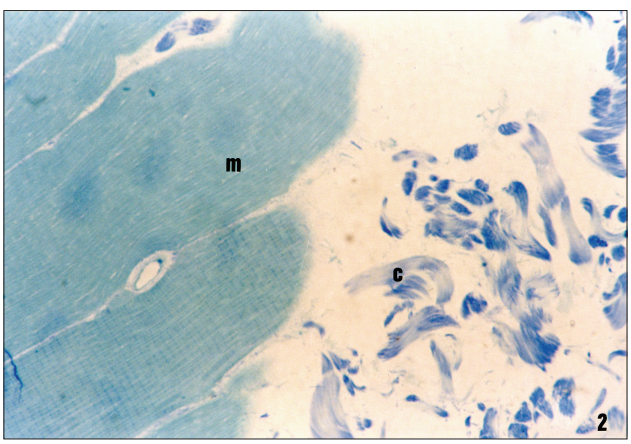

Figure 2 - Day 12 control group. Loose connective tissue is seen in the affected area. C - collagen bundles, M - muscle fiber. Toluidine blue-Azure II X250. The affected area is invaded by loose connective tissue with fibroblasts and randomly oriented thin collagen bundles.

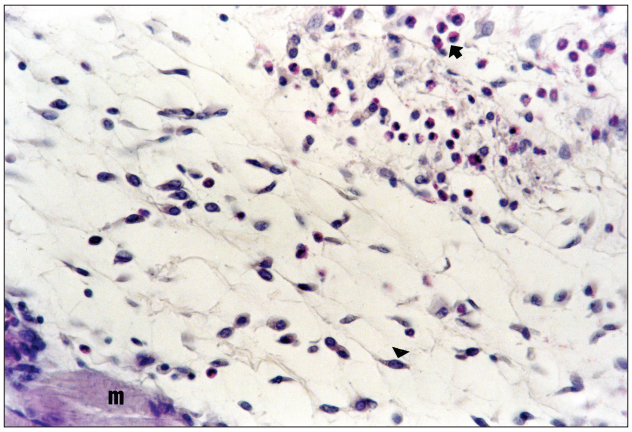

Figure 3 - Day 5 proliferative therapy group. Fibroblast (arrow head) and inflammatory cells, especially eosinophils (arrow), are seen in the affected area. M - muscle fiber. Hematoxylin-eosin X100. There are large quantities of fibroblasts and inflammatory cells, especially eosinophils in the affected area.

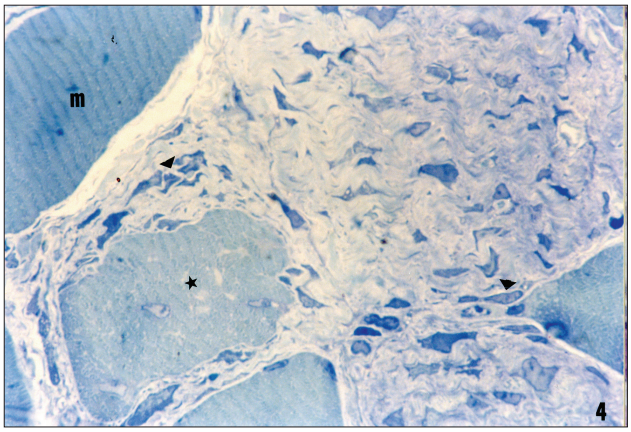

Figure 4 - Day 12 proliferative therapy group. A lot of fibroblasts (arrow heads) and dense collagen bundles are seen in the damaged area. M - muscle fiber, *degenerated muscle fiber. Toluidine blue-Azure II X250. Large quantities of fibroblasts and newly formed thick collagen bundles are present in the section.

quantities of fibroblasts and inflammatory cells, especially eosinophils, in the affected area (**Figure 3**). The skeletal muscle fibers and new forming myotubes were seen in the peripheral tissue. Capillary proliferation and congestion were present in the connective tissue among the muscle fibers. At day 8 in the affected area, inflammatory cell infiltration and fibroblasts were more prominent than those of the 5th day. Capillary proliferation and congestion were also present in this group. Degenerative changes with varying severity were observed in some of the peripheral muscle fibers, and some were necrotic. At day 12, the affected area was invaded by mature, dense connective tissue. Different from the control group, the obtained sections had large quantities of fibroblasts and newly formed thick collagen bundles (**Figure 4**). Besides the increased amount of necrotic ones, the newly regenerated muscle fibers were also present.

Discussion. When skeletal muscle is injured, the damaged muscle fibers are usually replaced by newly formed muscle cells; myotubes. Regeneration of new muscle results from an initial proliferation of mononuclear muscle precursors; potential myoblasts. Many, possibly all these precursors, are derived from satellite cells of muscles that survive injury. Within 5 days of injury post-mitotic cells, myoblasts have fused with damaged muscle fibers or with other myoblasts to form multinucleated myotubes that subsequently mature into new muscle fibers.[22] The precise time of initiation and the duration of muscle precursor proliferation after different types of injury is not known. One of the major problems in this determination is that early presumptive myoblasts cannot be distinguished from other mononuclear cells present in the wound area, by either light or electron microscopy.[22]

Local response to injury includes the stimulation of cell proliferation and the synthesis of extracellular matrix components. During the formation and evolution of granulation tissue, cells produce collagen very actively.[18] During the repair of injured muscles, 2 processes are competing: the regeneration of disrupted muscle and the production of connective tissue scar. Collagen participates in muscle regeneration by forming a sheath and a framework around fusing myoblasts during myotube formation.[23] Although collagen is thought to be necessary for muscle regeneration excessive scarring may inhibit the complete regeneration of muscle fibers by excessive formation of granulation tissue.[18] In this study, the dense connective tissue formed through the injured area at the PT group was striking. Although muscle regeneration was not delayed, compared to the C group, the amount of connective tissue was beyond being only a supporting framework.

Administration of dextrose to injured ligaments and tendon tissues resulted in enhanced repair with fibrosis and new collagen formation.[15,20,21] In this study, a cut injury was applied to rat muscle and dextrose was concomitantly administered to the injury site to investigate its effects on injured muscle tissue. At day 5 of injury, the muscular regeneration process was distinguished from the materials taken from both C and PT group rats in our study. This finding was consistent with several studies.[1,13,18,22,24] Different from the control group, sections of PT group revealed large quantities of fibroblasts and inflammatory cells, especially eosinophils in the involved area. Muscle

tissue damage triggers an initial inflammatory phase followed by subsequent phases of tissue healing, repair, and remodeling.[16] The hypertonic content of proliferative therapy injection caused tissue necrosis. This might have invited the inflammatory cells into the lesion site much more evidently than the C group. In the present study, an inflammatory reaction with concomitant fibroblast migration was more evident in the PT group. This was followed by prominent connective tissue and thick collagen bundle formation observed both with electron and light microscopical examinations, indicating tissue fibrosis.

In a study of McGeachie and Grounds, it has been indicated that muscle precursor proliferation was more advanced in the cut compared with crush injury.[22] In our study, the cut injury was more extensive than the injury created in their study. Their results showed that myogenesis in both cut and crush injuries could start 30 hours after injury. In our study, the lesions were larger and there were areas showing excellent regeneration with myotubes, but the injured region was fibrotic and contained necrotic muscle fibers and prominent connective tissue formation in the PT group.

Studies investigating the effects of different treatment modalities on the healing of muscle must start with a standard injury in respect to size and location. Although most contusion and strain injuries comprise approximately 90% of sports related injuries, it is quite difficult to convey these injury mechanisms into standardized experimental studies.[1,2] Therefore, in our study, all the rats were injured at the mid-belly of the gastrocnemius muscles by conduction of a standard incisional approach. Clearly the histological approach is objective and visible to examine the wound healing process, but is insufficient to explain the functional status of injured muscles. Excessive connective tissue formation concomitant with muscle regeneration in the PT group, raises the question of whether proliferative therapy is clinically beneficial or harmful. We suggest conduction of electrophysiological studies for testing the force-generating capacities and biomechanical investigations to detect the failure-load of traumatized muscle tissues to determine the functional capacities possibly affected by administered treatment agents, besides the structural analysis.

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