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ARAŞTIRMA MAKALESİ/**RESEARCH ARTICLE**

THE ROLE OF SATELLITE CELLS IN CRUSH INJURY OF RAT SKELETON MUSCLE

Dilek BURUKOĞLU[1*], M. Caner ÖZER[1], Mustafa ÇELİK[1], Cengiz BAYÇU[1]

ABSTRACT

The crush type of injury in rat skeletal muscle is often used in tissue degeneration and regeneration. After crush injury muscle tissue begins to regenerate. In this process, it is accepted that satellite cells play an important role which are very sensitive to muscle injury. The aim of this microscopic study was to examine role of satellite cells in muscle regeneration in crush injury. This research was done the department of Histology&Embryology in Eskişehir Osmangazi University in 2008. Ethic approval of this study has been received. During the study, the whole essential and ethics conditions have been done. In the study 36 Spraque-Dawley rats were used. The rats were separated into 5 groups as test and control groups. Crush type of injury has been applied on muscles of right hind extremities of testing group rats by applying 3.5 kg of weight for 6 hours. In according to testing periods rats were anaesthetized intraperitoneally with ketamine 30mg/kg + xylazine 10mg/kg and sacrificied 3, 7, 14 and 21-day intervals. After crush injury, increased satellite cells were particularly observed on day 7. Also significant increased of satellite cells and regenerated myofibrils were detected on day 14. However, satellite cells were seen on day-21 were similar to control group. In crush injuries, number of satellite cells were markedly increased and actively involved into regeneration process of the skeleton muscle.

Key Words: Satellite cell, Cush injury, Skeleton muscle, Rat, Microscopy

SIÇANLARDA İSKELET KASININ CRUSH (EZİLME) YARALANMASINDA SATELLİT HÜCRELERİNİN ROLÜ

ÖZ

İskelet kasında doku dejenerasyonu ve rejenerasyonunun incelenmesinde ezilme (crush) tipi yaralanma oldukça sık kullanılmaktadır. Ezilme yaralanmasının ardından kas hemen rejenerasyona başlar.Bu süreçte kas hasarına oldukça duyarlı olan satellit hücrelerinin önemli rol üstlendiği düşünülmektedir.Bu çalışmada sıçanların iskelet kasında oluşturulan ezilme yaralanmasında satellit hücrelerin kas rejenerasyonu sürecinde nasıl bir rol üstlendiği mikroskobik düzeyde araştırılmıştır. Bu araştırmada toplam 36 adet Spraque-Dawley türü sıçan kullanıldı. Sıçanlar kontrol grubu ve 3 günlük, 7 günlük, 14 günlük ve 21 günlük deney grupları olacak şekilde 5 gruba ayrıldı. Deney grubundaki sıçanların sağ arka ekstremitelerine 6 saat süreyle 3,5kg'lık ağırlık konularak kaslar üzerinde ezilme tipi yaralanma oluşturuldu. Deney sürelerine göre sıçanlar ketamin (30mg/kg) + xylazine (10mg/kg) anestezi ile uyutularak sakrifiye edildiler ve mikroskobik incelemeler için kas doku örnekleri alındı. Crush yaralanmasından sonra özellikle 7.günde satellit hücrelerin yaralı bölgede sayıca artmış olduğu görüldü. Aynı şekilde 14. günde satellit hücrelerin fazlalığı ve rejenere miyofibrillerin bulunması dikkat çekti. 21. günde ise satellit hücreleri kontrol grubuna benzer şekilde gözlendi. Satellit hücrelerinin ezilme yaralanmasında iskelet kasının rejenerasyonuna aktif bir şekilde katıldıkları mikroskobik düzeyde gözlendi.

Anahtar Kelimeler :Satellit hücresi, Crush (ezilme) yaralanması, İskelet kası, Sıçan, Mikroskop

1 Department of Histology and Embryology, Faculty of Medicine, Eskişehir Osmangazi University, Eskişehir-TURKEY.
* Corresponding author: e-mail:dburukoglu@yahoo.com
e-mail: mc.ozer@mynet.com
e-mail: mustafacelik1982@gmail.com
e-mail:cbaycu@ogu.edu.tr

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1.INTRODUCTION

In tissue degeneration and regeneration in skeleton muscle, crush type of injury is widely used (Akimau et al. 2005; Better et al. 1990; Haugk et al. 1995). Muscle crush injury resulted over extremities with a weight applied to one part of the body more than 30-40 minutes. However, in this type of injury, damaged could not be observed externally but seen in subcutaneous tissues (Haugk et al. 1995; Huard et al. 2002). Crush injury is a frequent complication that many factors like earthquake or traffic accident may cause crush type injuries over extremities and various vital organs (Akimau et al. 2005; Better et al. 1990; Haugk et al. 1995). If the injury is more acute, then it is defined as "Crush syndrome". (Akimau et al. 2005; Better et al. 1990). Crush syndrome (also traumatic rhabdomyolysis) is a serious medical condition characterized by major shock and renal failure following a crushing injury to skeletal muscle. Distribution of muscle cells metabolism, breakdown of electrolyte and acid-base balance, hypovolemia and acute renal failure were also seen of rescued people after disasters (Better et al. 1990). Muscle damage is one of the most important problems in traumatology (Better et al. 1990; Cooke 2005; Haugk et al. 1995, Holterman and Rudnicki, 2005). The recovery of damaged muscle cell is very slow and incomplete. Damaged muscle tissue begins to regenerate at once. However, recovery of injured muscle is frequently inhibited and become ineffective by the formation scar tissue (Better et al. 1990). By using various biological methods after muscle damage, muscle fibrositis blockage muscle regeneration (Fisher 2006; Shi and Garry 2006). Mechanic compression over the muscle engenders extracellular calcium entry with integrity breakdown of myofibres plasma membrane and basal lamina. Damaged myofiber undergoes necrosis with self–contained digestion by the mediation of intrinsic protease. Following the damage, rapid swelling and hematoma is followed by increased muscle degeneration. Necrosis area is encircled with blood vessels and rich in mononuclear cells, macrophage and T lymphocyte. As lymphocytes activate cytokines are secreted simultaneously. Secretion of the elements like adhesion molecules (P-selectin, L-selectin and E-selectin) and cytokines (IL8, IL6, IL1, TNFα) affects local blood flow, vascular permeability and inflammatory response acceleration (Haugk et al. 1995, Mohri et al. 2006). Release of various growth factors like IGF-1, HGF etc. from the damaged tissue regulates myoblast proliferation and differentiation in muscle regeneration and reflection (Hill et al. 2003; Mohri et al. 2006; Zammit et al. 2004). Muscle cells do not undergo mitosis and therefore they have limited regeneration ability (Hill et al. 2003). However, regeneration process provide by satellite cells in muscle. These are mononuclear cells and located between basal lamina and plasma membrane of the muscle cell which are very sensitive to damage and play key role in regeneration (Adams 2006; Hawke and Garry 2001; Jejurikar et al. 2003). Due to their localizations these cells are named as satellite cells and also myoblasts which have their own replication and differentiation capacity (Bischoff 1990; Morgan et al. 2003). Normally satellite cells are quiescent and they neither differentiate nor undergo mitosis. When muscle cells undergo injury they activate, released from basement membrane, divide and differentiate as myoblasts to form new myotubes (Cooke 2005; Hill et al. 2003; Takagi et al. 2010).In this study, crush type muscle injury has been applied on skeleton muscle and role of satellite cells at muscle regeneration examined microscopically.

2.METHODS

This research was done the department of Histology&Embryology in Eskişehir Osmangazi University in 2008. Ethic approval of this study has been received. During the study, the whole essential and ethics conditions have been done. In this study 36 male Spraque-Dawley rats were used. The rats were housed at room temperature (24 ± 2 C°), fed with standard pellet add libitum and water. They were divided as test and control groups. The animals were anaesthetized under general anesthesia with ketamine 30mg/kg + xylazine 10mg/kg intraperitoneally and crush type of injury was performed by applying a weight of 3,5 kg over shaved right hind legs for six hours. Test and control groups were sacrificed at 3, 7, 14 and 21-day intervals. Muscle tissue samples were cut at the damaged area and transferred into 10% neutral formaline fixation solution for 24 hours. After routine histological methods, tissues were embedded in paraffin. 5µ cross-cut sections were stained with hematoxylin and eosin. Additionally, semi–thin sections were taken to better observation of the satellite cells. For this purpose, muscle samples were placed in 2,5% glutaraldehide fixative solution for 5 hours and postfixed with osmium tetraoxide for two hours. After routine electron microscopic methods they were embedded into Araltide CY 212. Semi-thin sections were stained with Toluidine blue. Both paraffin and semithin sections were examined using Olympus BH–2 light microscope and photographed with Olympus DP–70 camera.

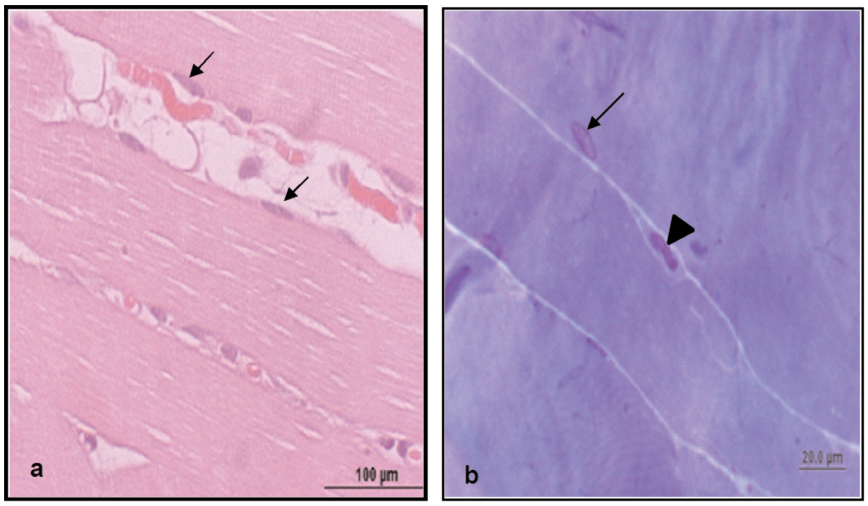

Figure 1: Control group: Cellular organization of muscle and dark and light bands were observed normally in paraffin (a) and semithin section (b). Myonucleus (arrow), satellite cell (arrow head).

Figure 1: Control group

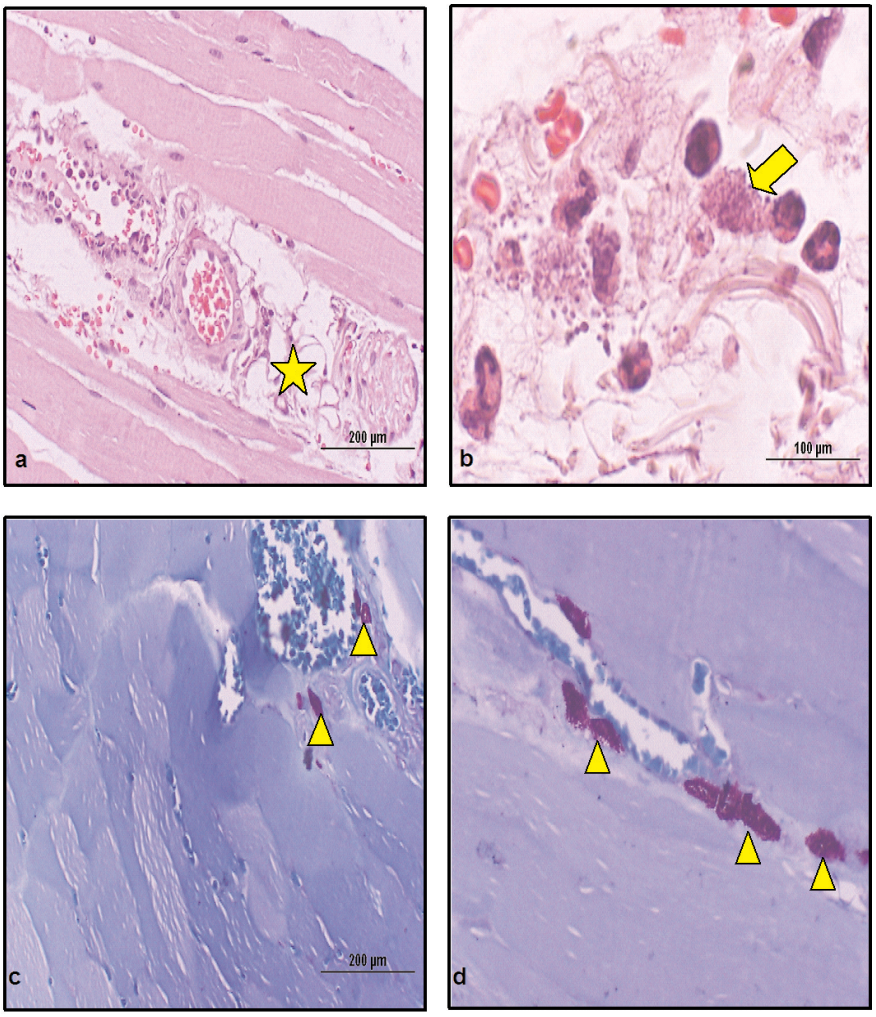

Figure 2: 3st day: In third day after the injury sporadic rupture and degeneration of myofibrils were observed in paraffin (a,b) and semithin sections (c,d). Endomysial edema (a),numerous macrophages (b), mast cells around vessels (c,d) and fibroblasts were present in interstitium. Edema (*), macrophage (arrow), mast cell (arrow head).

Figure 2: 3st day

3.RESULTS

Control group: In paraffin and semithin sections cellular organization of muscle and dark and light bands were observed normally (Figure 1).

3st day: In third day after the injury sporadic rupture and degeneration of myofibrils were observed. Endomysial edema, numerous macrophages, mast cells around vessels and fibroblasts were present in interstitium (Figure 2).

7rd day: In paraffin section and semithin sections in rat skeleton muscle significant increase in the number of satellite cells in injured area was observed on day-7 (Figure 3).

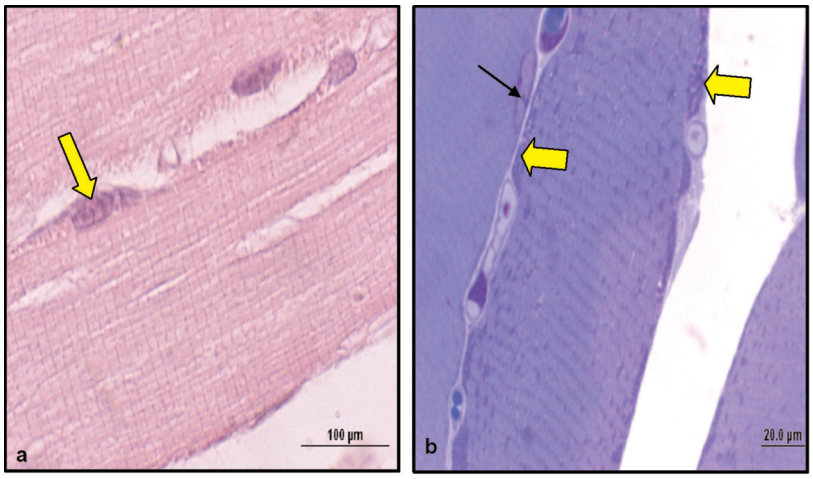

Figure 3: 7rd day

Figure 3: 7rd day: Significant increase in the number of satellite cells in injured area was observed in paraffin section (a) and semithin section (b). Satellite cell (thick arrow), myonucleus (thin arrow).

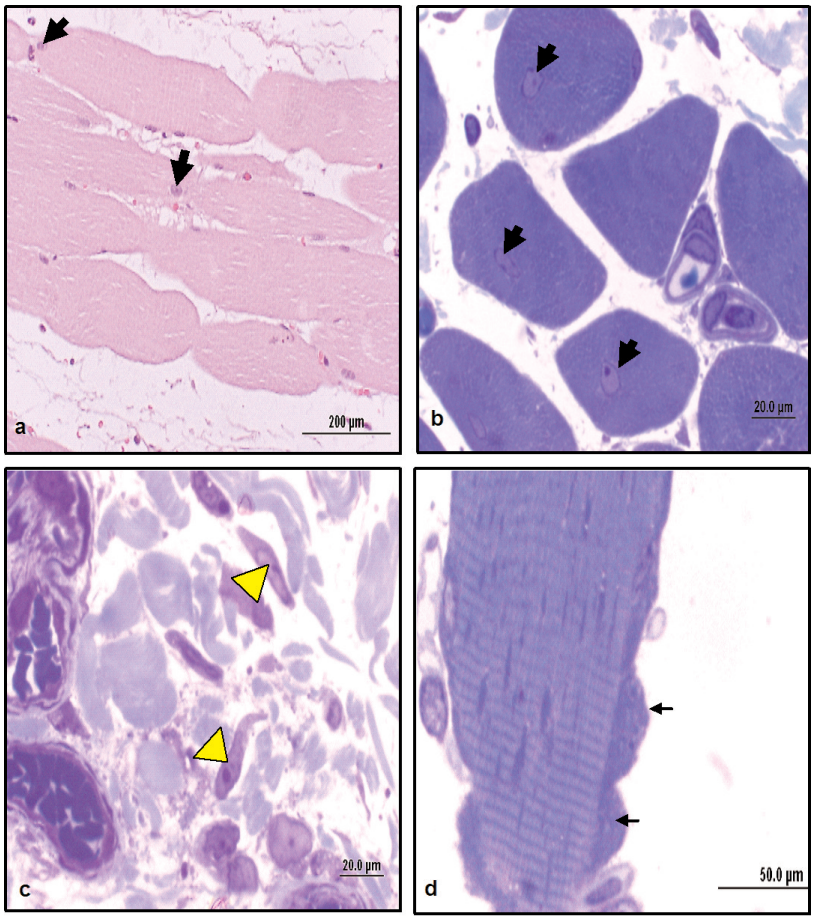

Figure 4: 14th dayla montana (C) seeds in different treatments

Figure 4: 14th day: Regenerated muscle cells were determined with their centrally located nuclei in paraffin section (a) and semithin section (b). Numerous extracellular fibroblasts also seen in semithin section (c). Increased number of satellite cells in injured area was observed in semithin section (d). Satellite cell (thin arrow), fibroblast cell (arrow head), myonucleus (thick arrow).

14th day: In this group, marked regeneration process was observed in crushed area. Regenerated muscle cells were determined with their centrally located nuclei. Numerous extracellular fibroblasts also seen in the same group muscles. Increased number of satellite cells was also noted on day-14 (Figure 4).

21th day: Dark and light bands of muscle cells and cellular organization were intact but minimal fibrosis was observed on day 21. In this group, regenerated muscles were observed with their centrally located nuclei. Also increased number of satellite cells were also seen on day-21 (Figure 5).

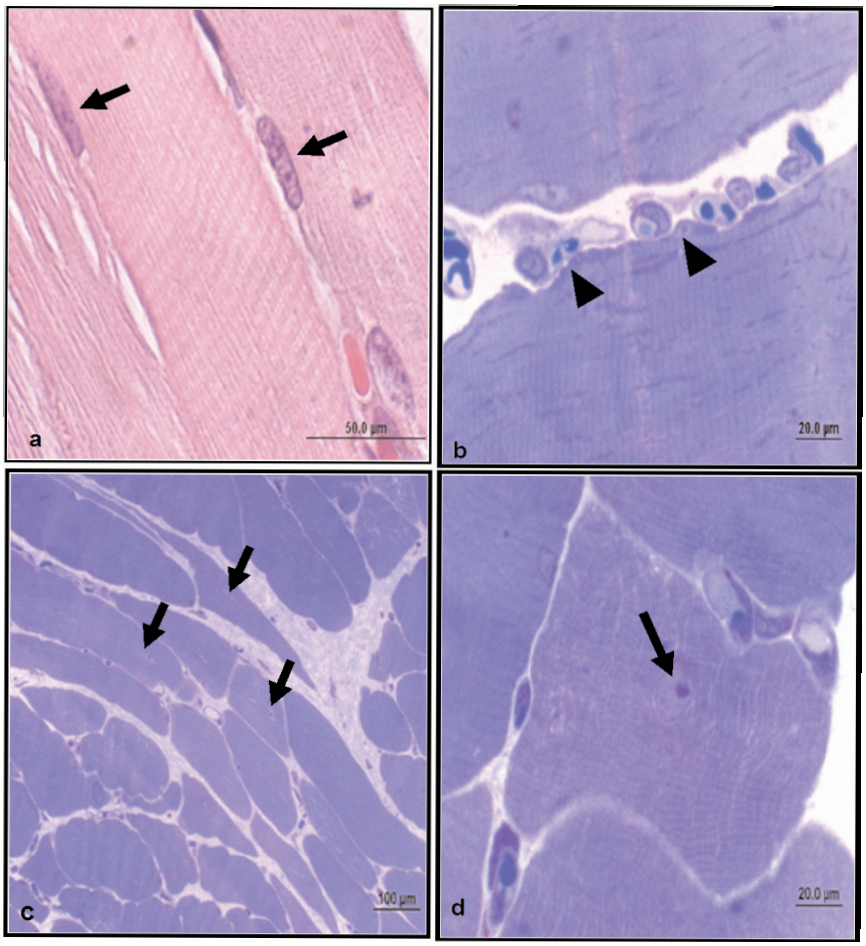

Figure 5: 21th day: Dark and light bands of muscle cells and cellular organization were intact in paraffin section. (a). Increased number of satellite cells in injured area was observed in semithin section (b). In semithin section, regenerated muscles were observed with their centrally located nuclei (c,d). Satellite cell (arrow head), myonucleus (arrow).

Figure 5: 21th day

4.DISCUSSION

Muscle tissue is a very active tissue and easily tends to injury such as crush injury, ischemia and etc. throughout our lives. After the damage, the muscle regenerates and returns to its normal physiologic activity. Improving muscle regeneration is important for hastening the repair and restoration of the muscle function. The cellular and molecular mechanisms of muscle regeneration after injury and degeneration have been described extensively (Hawke and Garry 2001; Huard et al. 2002; Politi et al. 2006). Unfortunately, all evidence indicates that once muscles are damaged the muscle repair regeneration process is not always complete and can often be slowed or complicated by fibrotic infiltration and scarring. Incomplete and slow repair may result in disability or handicap. Injury over the skeleton muscle may cause with various mechanisms such as chemical, mechanical or physical (Akimau et al. 2005; Better et al. 1990; Cooke 2005; Hill et al. 2003; Huard et al. 2002). In this study crush type of injury was used which is often encountered in our daily life. The crush injuries, caused by vehicle accidents, firearm injuries, falling or squashing under heavy objects, are acute traumas which threaten our lives (Akimau et al. 2005; Better et al. 1990). If the trauma is more acute then it is defined as "crush syndrome" which disrupts muscle cell nutrition, damages capillary and causes severe muscle damage (Better et al. 1990; Cooke 2005).

Physiological and biochemical studies have shown that after crush injury, intracellular pressure increases over 240mm Hg (Better et al. 1990; Takagi et al. 2010). In crush syndrome, medical treatment in acute traumatic deaths is insufficient.

Therefore knowledge of medical treatment is the most important point to minimize the loss of life at post disasters in crush injuries / syndrome. Skeletal muscle has a remarkable regenerative capacity in response to an extensive injury. Muscle regeneration includes necrosis of the damaged tissue, inflammation, activation of myogenic stem cells and, as a result of this activation, formation of new myofibers and reconstitution of a functional contractile apparatus. This highly synchronized process, which requires satellite cell activation, proliferation, migration and terminal differentiation, is activated and controlled by a complex network of signaling pathways and requires collaboration of different cell types such as satellite cells (Schmalbruch 2006; Zammit et al. 2004).After crush injury, satellite cells become activated and proliferate in muscle tissue. Some of the satellite cells will re-establish a quiescent satellite cell pool through a process of self-renewal. Satellite cells migrate to the damaged region and depending on the severity of the injury, fuse to the existing myofiber or align and fuse to produce a new myofiber. In the regenerated myofiber, the newly fused satellite cell nuclei will be initially centralized but will later migrate to more peripheral location (Hawke and Garry 2001). Besides, the nuclei of satellite cells contain more heterochromatin than nuclei of myotubes (myonuclei). In our study, increased number of satellite cells was observed injured muscles between days 3, 7 and 14. In the present study, nuclei of regenerated myofibrils were located centrally on day 3 and 7. We suggest that new muscle cells are formed during regeneration and our findings were in concordance with previous studies (Fisher and Rathgaber 2006; Huard et al. 2002; Jejurikar et al. 2003).Studies have shown that several molecular factors play a role in muscle regeneration (Hawke and Garry 2001; Mohri et al. 2006). MyoD, is a transcription factor, induces satellite cell proliferation in crush injury. Galactin-1 is a new factor succeeding the muscle injury by increasing both axonal growth and myoblast coalescence and as the result regulates myotube growth in muscle regeneration which is not well explained. In healthy adult skeleton muscle galactin-1 is related with basal membrane of myofibril. It is shown that Galactin-1 immunoreactivity increases in the cytoplasms of satellite cells after muscle injury. c-Met, the receptor for hepatocyte growth factor (HGF), is a marker of quiescent satellite cells (Kierzszenbaum 2006). Satellite cell membranes carry receptors encoded with proto-onchogen c-Met. Besides, HGF-c-Met linkage regulates signal range and satellite cells proliferation and myoblast stereospecificity that express Myf5 and MyoD (Haugk et al. 1995; Huard et al. 2002; Holterman and Rudnicki, 2005). Pluripotential nature of satellite cells increases the stem cell treatment application probability in a range of degenerative disease like muscular dystrophy.

5. CONCLUSION

Determination of muscle injury, repair period and behavioral characters of the satellite cells that participate in regeneration process will be utmost important matter. Satellite cells may play a role in this process and are also key factor in genetic myopathies treatment or natural disasters as earthquakes. Pluripotential nature of satellite cells increases the stem cell treatment application probability in a range of degenerative disease like muscular dystropy. In this study, number of satellite cells were markedly increased and actively involved into regeneration process of the skeleton muscle. As a conclusion, it is suggested that satellite cells may play an important role in muscle regeneration.

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