The effect of cerebrolysin treatment on skeletal muscle healing in adult albino rat: a histological and immunohistochemical study

Gamal H. Mohamed, Mohamed H. Shaaban

Department of Anatomy and Embryology, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence to Gamal H. Mohamed, MD, Assisted Professor of Anatomy and Embryology Faculty of Medecine Cairo University, Shoubra, Cairo, Egypt
Tel: +20 100 520 3558;
e-mail: drgamalhosny2012@hotmail.com

Received 19 April 2016
Accepted 10 July 2016

Kasr Al Ainy Medical Journal
2016, 22:91–98

Objective
The aim of this study was to evaluate the role of Cerebrolysin in muscle healing in vivo.

Materials and methods
Twenty adult albino rats were used in this study. Both right and left gastrocnemius muscles of the albino rats were cut at 60% of their length from their distal insertion through 75% of their width and 50% of their thickness and then sutured with simple sutures using absorbable catgut sutures. The gastrocnemius muscles of all albino rats were injected directly in the lesion with 100 ng/ml/kg of cerebrolysin at 1, 3, and 5 days after laceration. The sham control muscles were subjected to the same treatment but were injected with the same volume of physiological normal saline. One week after the injury the animals were killed and the gastrocnemius muscles were removed and processed for paraffin sectioning and stained with hematoxylin and eosin, *Bcl-2* (B-cell lymphoma 2), and *P53* (tumor suppressor gene) immune peroxidase stains.

Results
There was a significant increase in the mean myonuclei/myofiber diameter ratio and the size of the regenerating myofibers as an index of muscle regeneration in the cerebrolysin-injected group compared with the control. It is anticipated that cerebrolysin is a potent stimulator of muscle regeneration in vivo. It has also been found that regenerating myofibers were located both in the deep and the superficial areas of the injured site of muscles only when treated with cerebrolysin, thereby demonstrating greater initial healing when the injured muscle is treated with cerebrolysin. Cerebrolysin retarded apoptosis in neutrophils, macrophages, and other inflammatory cells by activating the anti-apoptotic *Bcl-2* (B-cell lymphoma 2) protein leading to accumulation of these inflammatory cells.

Conclusion
This study has shown that injections of cerebrolysin into an injured muscle improved muscle healing in vivo. Also, documents the occurrence of a protective *Bcl-2* in inflammatory neutrophils and macrophages, which were expressed in cerebrolysin-treated muscles, leading to enhancement of the effect of these inflammatory cells. We have also found that Inflammatory cells play a role in satellite cell activation and proliferation but, the tumor suppressor gene p53, which is recognized as a central regulator of cell cycle and apoptosis, had no role in muscle regeneration in this in-vivo study.

Keywords:
Bcl-2, cerebrolysin, muscle regeneration, muscle trauma, p53

Kasr Al Ainy Med J 22:91–98

1687-4625

Introduction

Skeletal muscle regeneration after injury is both rapid and efficient; in the rodent, muscle patterning and contractile ability are regained within days of myonecrotic injury, with complete recovery of mass and strength within 21 days. 'This process is dependent on satellite cells, the resident stem cell of skeletal muscle. When stimulated by factors released by damaged muscle, satellite cells rapidly exit the quiescent state and proliferate extensively to build up a supply of adult myoblasts, which will subsequently differentiate and fuse either with each other or with existing myofibers to repair local damage [1].

Satou *et al.* [2] showed that 'in the normal state, these satellite cells remain in a nonproliferative quiescent state. On the other hand, in response to stimuli such as

 DOI: 10.4103/1687-4625.195888

cerebrolysin injection, remodeling, denervation, or muscle injury, satellite cells become activated and stimulated to enter the cell cycle. They appear as a swelling on the myofibers with cytoplasmic processes extending from one or both poles of the cell'. 'Satellite cells proliferate and express myogenic markers (now termed myoblasts). Ultimately, during regeneration of damaged skeletal muscle, these cells fuse to existing muscle fibers or fuse together to form new myofibers' [3].

'During regeneration after cerebrolysin injection, macrophages are essential in the repair process as they secrete a collection of cytokine factors that regulate the satellite cell pool' [4]. 'Importantly, in the presence of an enhanced macrophage response, there is an increase in satellite cell proliferation and differentiation' [5]. Madison *et al.* [6] suggest that regulation of macrophage apoptosis may be pivotal in the outcome of an inflammatory response. The cytokines, which are a unique family of growth factors, might either promote or prevent macrophage apoptosis. This process is effectively reversed by the addition of growth stimuli, suggesting that apoptosis may represent the mechanism for eliminating activated macrophages that are no longer necessary as inflammation wanes.

'Neurotrophic factors are known to play a crucial role in neural growth and differentiation. Neurotrophic factors are produced by various cell types including vascular smooth muscle cells and fibroblasts following wounding' [7]. 'They might play an accessory role in the regulation of immune and inflammatory reactions through stimulation of chemotactic migration of human polymorphonuclear leukocytes both *in vitro* and *in vivo*. They also have an additional role in promotion of the proliferation, growth, and differentiation of other blood elements' [8]. Gharaibeh *et al.* [9] observed that 'neurotrophic factors are a potent stimulator of myoblast proliferation and fusion *in vitro*. However, their role and effect on muscle healing following trauma need further investigation. Therefore, the present work was carried out to study the effect of cerebrolysin on the early events of muscle regeneration following mechanical injury *in vivo*'.

Materials and methods

Animals

Twenty adult male albino rats obtained from the Animal House, Faculty of Medicine, Cairo University, were used in this study. Their ages varied from 2 to 3 months (birth date recorded on the cage), and they weighed 200–300 g. They had free access to standard diet and drinking water. The animals were housed in polypropylene cages and maintained under standard laboratory conditions (temperature 25±2°C, 12:12 h; light–dark cycle and 50±5% relative humidity with free access to food and water). They were acclimatized to laboratory conditions before the test (5 min daily for 1 week). All experiments were carried out during the light period (08:00–16:00 h). This study was carried out according to the international ethics standard for animal experiments and was approved by the Kasr Al-Ainy Experimental Ethics Committee.

Chemicals

Cerebrolysin (100 ng/ml) injected directly into the lesion was obtained from Arzneimittel Pharmaceutical Laboratories, Unterbach, Austria, Europe.

Experimental design

The animals were randomly divided into two equal groups (10 rats each):

Group I (controls): In this group two plain control rats were not subjected to any procedure and eight sham control rats were anesthetized with ether inhalation. This method of anesthesia was suitable as the surgical procedure required only a few minutes. Injury to skeletal muscle was induced by subjecting gastrocnemius muscles to a cut injury as described by Menetrey *et al.* [10]. Under sterile precautions both right and left gastrocnemius muscles of the albino rats were cut at 60% of their length from their distal insertion, through 75% of their width and 50% of their thickness, and then sutured with simple sutures using absorbable catgut sutures (6-0) with rounded nontraumatic needles. The severity of the lesion sustained by the muscle had been previously determined as a grade III injury according to the classification of Buckwalter *et al.* [11]. A nonabsorbable suture such as prolene was used for skin sutures and served as a mark for injection. All albino rats were injected directly in the lesion of the gastrocnemius muscles with the same volume of physiological normal saline at 1, 3, and 5 days after injury using a microsyringe.

Group II: In this group, as in sham controls, the gastrocnemius muscles of all albino rats were injected directly in the lesion with 100 ng/ml/kg of cerebrolysin at 1, 3, and 5 days after injury using a microsyringe. Animals were killed with an overdose of ether inhalation on the seventh day after the injury, and the gastrocnemius muscles were removed and processed for histological examination.

Histological study: The isolated gastrocnemius muscles of both sides were fixed in 10% neutral formalin and

processed for paraffin sectioning. For histological analyses, 4–6 mm cross and longitudinal sections were collected along the entire length of the muscle and at the site of the lesion to evaluate muscle regeneration. The sections were stained with hematoxylin and eosin.

Immunohistochemical study: It was performed using streptavidin–biotin peroxidase complex [12] using monoantibodies for *Bcl-2* (B-cell lymphoma 2: Ab-3 1 : 501; Lab Vision, Fremont, California, USA) and *P53* (tumor suppressor gene: Do-7 prediluted IgG, Kappa; Dako AS, Copenhagen, Denmark) and counterstained with hematoxylin. All selected sections were examined and photographed using an Olympus microscope, Olympus Corporation, Shinjuku, Tokyo, Japan.

Quantitative study: Sections containing the largest area of damage were selected for analysis. An image of the core of the damaged area, defined as the region that was least regenerated, was taken. For analysis of control muscles, sections were selected from the same anatomical region as those from injured muscles. The diameter of the regenerating myofibers in both group II and sham control muscles was measured from photomicrographs. Centronucleated cells were considered as regenerating myofibers. All the centrally nucleated myofibers within this field were counted and their diameters were measured and compared with those of the sham control muscle. Nuclei at the periphery of the myofibers (satellite cells nuclei and myonuclei) were counted and the mean number and ratio of their diameter compared with myofiber diameter. Nucleocytoplasmic ratio was calculated and measured in group II and sham control muscles [13].

Statistical analysis: The diameters of the regenerating muscle fibers and the nucleocytoplasmic ratio were compared in all groups using a paired Student *t*-test. The satellite/myonuclear number was compared in all groups using the Mann–Whitney test. All values are expressed as means±SD. Significant difference was considered at *P* value less than 0.05.

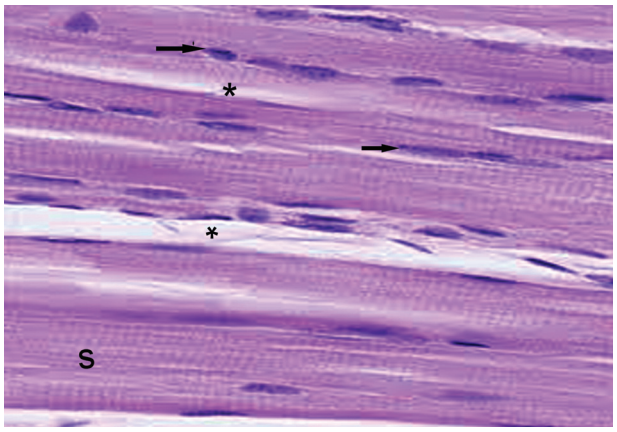
Results

Group I

Histological examination of group I

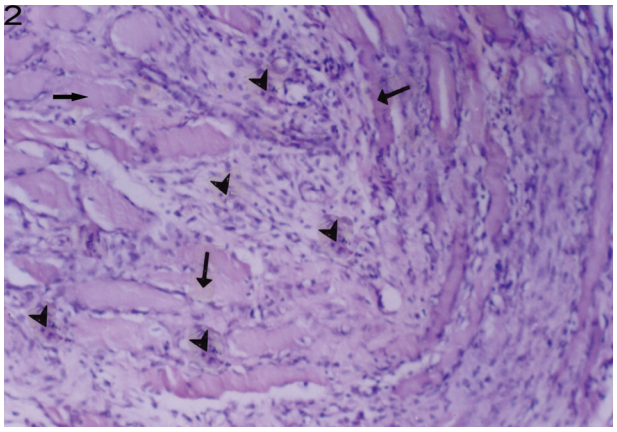
In plain controls the muscle was seen covered in a layer of connective tissue known as the epimysium. The muscle was arranged in bundles of fibers, known as fasciculi, which were seen surrounded by another connective tissue, called the perimysium. Endomysium, which insulates each muscle fiber, was also seen. Muscle fibers or myofibers appeared as multinucleated syncytium with a striated appearance resulting from the regular pattern of sarcomeres within the cells (Fig. 1). Sham control muscles showed signs of degeneration and acute inflammation. There was invasion of the site of laceration by inflammatory cells seen perimysial and endomysial. Some degenerated muscle fibers appeared pale, and others with interstitial edema (Fig. 2). In longitudinal sections, degenerated muscle fibers appeared pale and contracted and were invaded by phagocytic inflammatory cells (Fig. 3). New regenerating myofibers identified by their centrally located nuclei

Figure 1



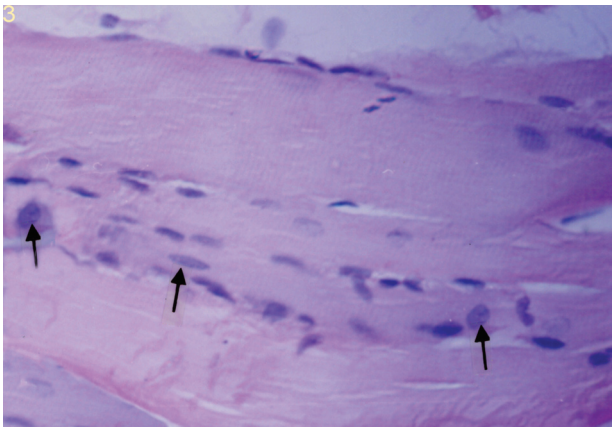
Photomicrograph of a longitudinal section of a plain control muscle showing muscle fibers or myofibers appearing as multinucleated (arrows) syncytium with striated appearance (s), resulting from the regular pattern of sarcomeres within the cells. Endomysium, which insulates each muscle fiber, was also seen (*) (hematoxylin & eosin, ×400).

Figure 2



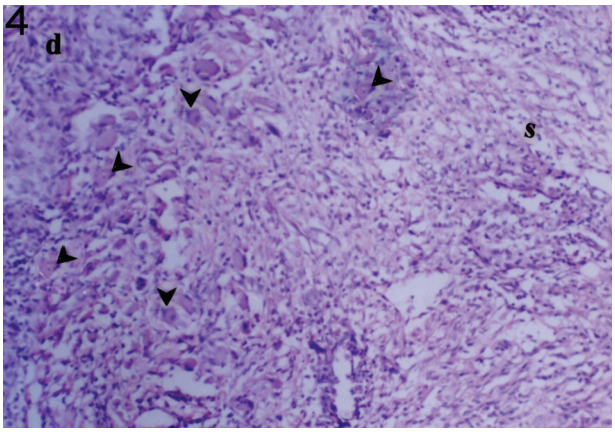
Photomicrograph of a transverse section of a sham control muscle 1 week after injury at the site of the lesion showing degenerated muscle fibers (arrows) and infiltration of the area by inflammatory cells (arrowheads) (hematoxylin & eosin, ×100).

Figure 3



Photomicrograph of a longitudinal section of a sham control muscle 1 week after injury at the site of the lesion showing pale contracted muscle fibers invaded by inflammatory cells (arrows) (hematoxylin & eosin, ×400).

Figure 4



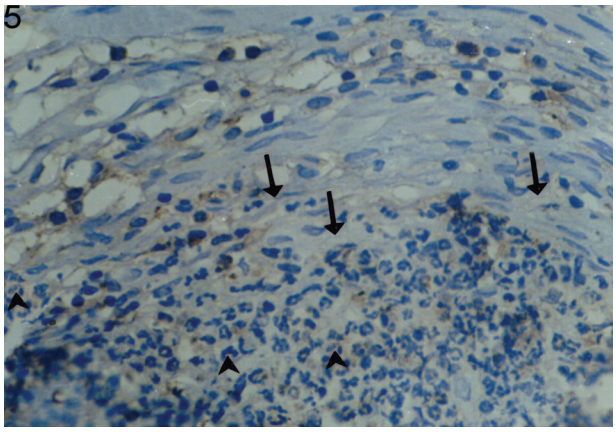
Photomicrograph of a transverse section of a sham control muscle 1 week after injury at the site of the lesion showing a few regenerated myofibers (arrowheads) in the deep part of the section (d). The superficial part of the section (s) infiltrated by inflammatory cells (hematoxylin & eosin, ×100).

and basophilic sarcoplasm were found deep to the site of laceration, whereas the superficial part was infiltrated by inflammatory cells (Fig. 4).

Immunohistochemical examination of group I

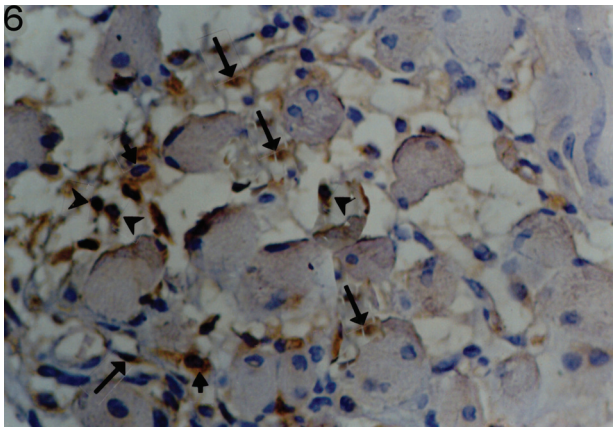
In the sham control sections stained with immunoperoxidase with anti-*Bcl-2* monoclonal antibodies, there was an invasion of the injury site by inflammatory cells, mainly granulocytes, which were negatively expressing *Bcl-2* (Fig. 5). Fibroblasts, fibrocytes, and lymphocytes were seen among the regenerating myofibers and were positively stained with *Bcl-2* (Fig. 6). The sham control sections stained with immunoperoxidase with anti-p53 monoclonal antibody showed that p53 protein was not expressed in the nuclei of

Figure 5



Photomicrograph of a longitudinal section of the sham control muscle 1 week after injury at the site of the lesion showing degenerated muscle fibers (arrows) and infiltration of the area by negatively stained (blue) inflammatory cells, mainly granulocytes (arrowheads) (*Bcl-2* immunoperoxidase stain, ×100).

Figure 6



Photomicrograph of a transverse section of a sham control muscle 1 week after injury at the site of the lesion showing positively stained (brown) fibrocytes (arrows) and inflammatory cells, both lymphocytes (arrowhead) and macrophages (short arrows) (*Bcl-2* immunoperoxidase stain, ×400).

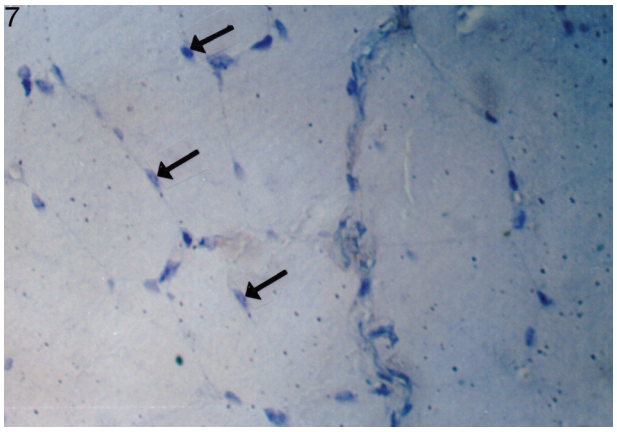
the satellite cells (peripheral nuclei) in the quiescent (Fig. 7), active, or differentiated stages (Fig. 8).

Group II

Histological examination of group II

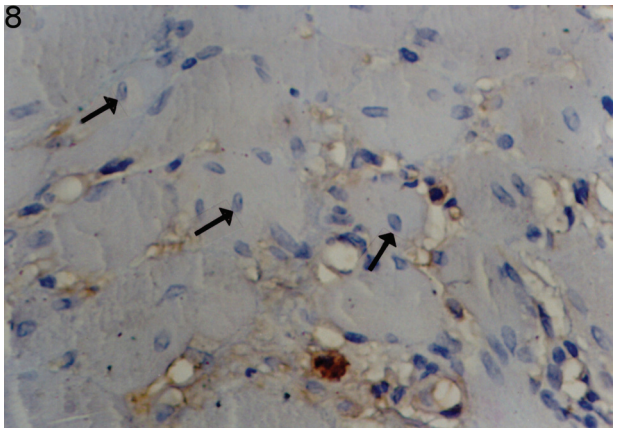
Muscles showed signs of degeneration and acute inflammation. There was invasion of the site of laceration by inflammatory cells seen perimysial and endomysial with degenerated muscle fibers appearing pale (Fig. 9). In longitudinal sections, degenerated muscle fibers appeared pale and contracted and were invaded by phagocytic inflammatory cells (Fig. 10). The muscles showed many regenerating myofibers uniformly distributed

Figure 7



Photomicrograph of a transverse section of a sham control muscle 1 week after injury away from the site of the lesion showing negatively stained nuclei (blue) of the quiescent satellite (peripheral) cells (arrows) (*P53* immunoperoxidase stain, ×400).

Figure 8



Photomicrograph of a transverse section of sham control muscle 1 week after injury at the site of the lesion showing negatively stained nuclei (blue) of the satellite cells and myonuclei (arrows) in the regenerated myofibers (*P53* immunoperoxidase stain, ×400).

both in the superficial and the deep parts of the laceration (Fig. 11).

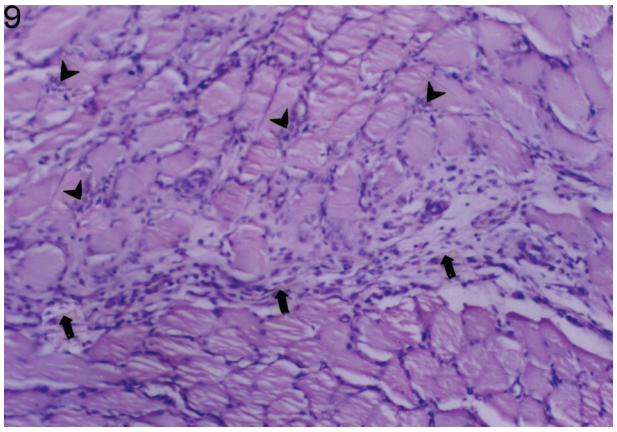
Immunohistochemical examination of group II

Bcl-2 protein was strongly positive (dark brown) in the cytoplasm of inflammatory cells. The positive cells were mainly lymphocytes and macrophages; macrophages showed typical kidney-shaped nuclei (Figs 12 and 13). Sections stained with immunoperoxidase with anti-p53 monoclonal antibody showed that p53 protein was not expressed in the nuclei of the satellite cells (peripheral nuclei) either in the quiescent (Fig. 7), active, or differentiated stages (Fig. 8).

Quantitative histological examination

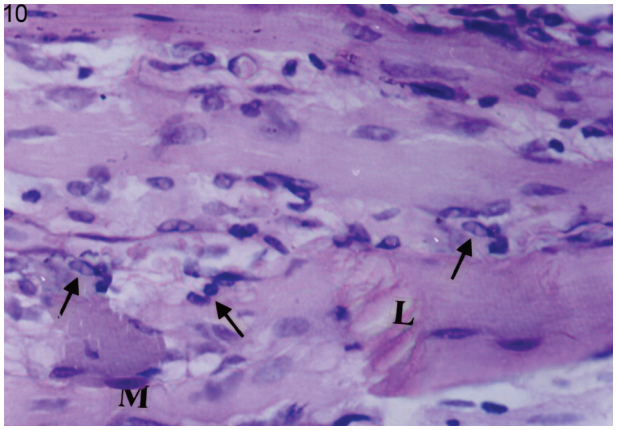
Table 1 summarizes the mean diameter±SD of the regenerating myofibers, the mean number±SD of the

Figure 9



Photomicrograph of a transverse section of group II muscle 1 week after injury at the site of the lesion showing degenerated muscle fibers with interstitial edema infiltration of the area by inflammatory cells both seen perimysial (arrows) and endomysial (arrowheads) (hematoxylin & eosin, ×100).

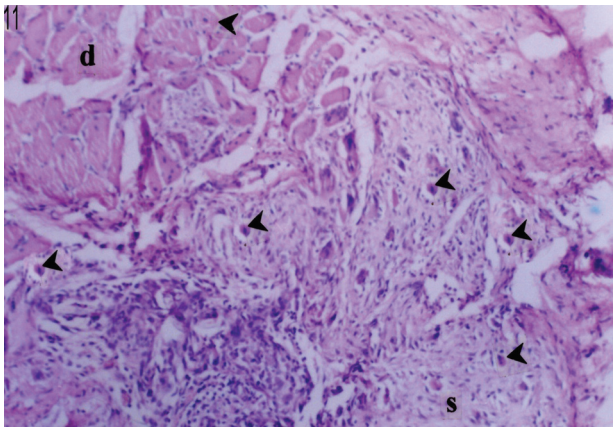
Figure 10



Photomicrograph of a longitudinal section of group II muscle 1 week after injury at the site of the lesion showing pale necrotic (M) and lacerated (L) muscle fibers invaded by inflammatory cells (arrows) (hematoxylin & eosin, ×400).

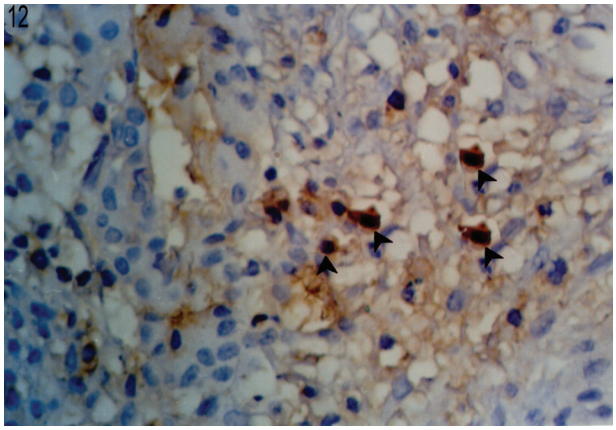
satellite myonuclear cells, and the mean ratio of the myonuclear/myofiber diameter±SD in both group I and group II. The mean diameter of the regenerating myofibers was 28.1±7.6 in the sham control and 41.3±12.3 in group II. The mean ratio of the myonuclear/myofiber diameter was 0.07±0.02 in the sham control and 0.001±0.03 in group II. The mean number of satellite myonuclei cells was 35.7±8.4 in the sham control and 45.1±15.6 in group II. The mean diameter of the regenerating myofibers and the mean ratio of the myonuclear/myofiber diameter were significantly higher in group II compared with the sham control, whereas the mean number of satellite myonuclei cells in group II was not significantly different from that of the sham control.

Figure 11



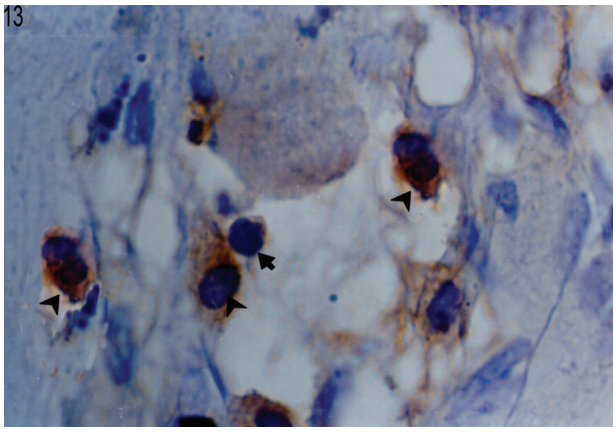
Photomicrograph of a transverse section of group II muscle 1 week after injury at the site of the lesion showing regenerated myofibers (arrowheads) in the deep (d) and superficial (s) parts of the section (hematoxylin & eosin, ×100).

Figure 12



Photomicrograph of a transverse section of group II muscle 1 week after injury at the site of the lesion showing positively stained (dark brown) cytoplasm in the inflammatory cell (arrowheads) (*Bcl-2* immunoperoxidase stain, ×400).

Figure 13



Photomicrograph of a transverse section of group II muscle 1 week after injury at the site of the lesion showing positively stained cytoplasm of the inflammatory cells, lymphocytes (short arrow), and macrophages with kidney-shaped nuclei (arrowheads) (*Bcl-2* immunoperoxidase stain, ×1000).

Table 1 The mean diameter of regenerated myofibers and the mean number of satellite/myonuclear cells and the mean myonuclear/cytoplasmic ratio

Groups	Group I (sham control) (mean±SD)	Group II (mean±SD)	*P* value
Satellite cell/ myonuclear number	35.7±8.4	45.1±15.6	0.33
Myonuclear/ cytoplasmic ratio	0.07±0.02	0.001±0.03	0.001*
Regenerated myofibers diameter (μm)	28.1±7.6	41.3±12.3	0.001*

*Highly significant.

Discussion

The development of the use of growth factors, which will give quicker and more complete recovery, may significantly affect the downtime after injury. Muscle regeneration following injury depends primarily upon satellite cells and upon other cellular processes involving nonmuscle cells. One of the earliest of these is the inflammatory response, which facilitates myogenesis through phagocytosis of cellular debris and the release of growth factors.

In the present study it was found that when muscle fibers are damaged by cut injury, several overlapping cellular processes, including inflammation and myogenesis, take place. These initial data confirm those of Seale *et al.* [14]. Skeletal muscle regeneration, initiated by muscle injury, involves the degeneration of damaged myofibers, formation of a hematoma necrosis of muscle tissue, and an inflammatory cell response. Fukushima *et al.* [15] reported that 'neutrophils and macrophages accumulate at the injury zone as early as 30 min after injury. Neutrophils prepare the site for further events in muscle regeneration by releasing compounds that break down both foreign and cellular materials, thus decreasing the likelihood of the wound becoming a route for systemic infection'. They then undergo apoptosis (programmed cell death). 'Macrophages migrate to the damaged area through the blood stream and from the muscle and surrounding tissue by direct chemotaxis under the effect of soluble signals from tissue destruction' [16]. Macrophages are thought to be responsible for phagocytosis of the bulk of the macromolecules resulting from damaged myofibers, extracellular matrix, and apoptotic neutrophils. They can also localize the immune responses in the injury zone by producing a large variety of soluble factors [17]. Gharaibeh *et al.* [18]

'showed that release factors such as platelet-derived growth factor and an undefined satellite cell-specific factor stimulate satellite cell proliferation in the injury zone'.

In this study there was a significant increase in the mean myonuclei/myofiber diameter ratio and the size of the regenerating myofibers as an index of muscle regeneration in the cerebrolysin-injected group compared with the control. It is anticipated that cerebrolysin is a potent stimulator of muscle regeneration *in vivo*. It has also been found that regenerating myofibers were located both in the deep and the superficial areas of the injured site of muscles only when treated with cerebrolysin, thereby demonstrating greater initial healing when the injured muscle is treated with cerebrolysin. This agreed with the results of Hodgetts *et al.* [19] and Chazaud *et al.* [20], who showed 'a direct effect of cerebrolysin upon satellite cells or indirectly through upregulation of macrophages, which clean the site of injury by initiating phagocytosis of tissue debris'. Siegel *et al.* [21] postulated that 'during the preparation for the healing process macrophages produce factors that influencing satellite cell proliferation and differentiation through stimulation of its migration and delay its apoptosis by activation of death inhibiting proteins'. Skeletal muscle repair includes activation of satellite cells [22]. 'When a muscle is damaged, these cells are activated within 18 h of the injury' [23]. 'Quiescent satellite cells are activated by various growth factors or myogenic regulatory factors, 'wound hormones', which are released from the damaged myofibers and the extracellular matrix surrounding them. This is done through interaction with growth factor receptors on the surface of these cells. Once these satellite cells have undergone differentiation they can fuse to existing muscle cells' [24]. Musaro *et al.* [25] showed that 'satellite cells fusion allows donating needed myonuclei to the wounded or developing muscle cell. It also appears that these cells are able to migrate, and may move from healthy areas of the muscle to the injured area in response to chemical stimuli and they fuse together to form myoblasts, which in turn fuse to form myotubes'. 'One of the hallmarks of regenerating myofibers is the centrally located position of the myonuclei. Finally, the regenerated muscle matures and contracts with reorganization of the scar tissue. Upon maturing, muscle fiber nuclei are located along the cell periphery' [26].

Inflammation, on the other hand, results in an early and maintained elevation in the levels of cerebrolysin in injured tissue [27]. Li and Huard [28] postulated that 'Neutralization of the action of the increased cerebrolysin with specific anti-cerebrolysin antibodies decreases inflammatory hypersensitivity, indicating that this neurotrophin is very important in the production of inflammatory pain'. The systematic and local application of exogenous cerebrolysin has been shown to produce a rapid and prolonged behavioral hyperalgesia in both animals and humans [29]. Such behavior was observed in rats injected with cerebrolysin in this study as they frequently bit their sutures after the injection.

In this study it was found that cerebrolysin retarded apoptosis in neutrophils, macrophages, and other inflammatory cells by activating the anti-apoptotic *Bcl-2* (B-cell lymphoma 2) protein leading to accumulation of these inflammatory cells. This was in agreement with the results of Zammit and Beauchamp [30]. Cerebrolysin, on the other hand, has been shown to regulate the life span of both granulocytes and macrophages *in vitro* [31].

Tiangco *et al.* [32] strongly supported the role of p53 (tumor suppressor gene) in myogenesis *in vitro*, as expression of p53 increases during differentiation and fusion of skeletal muscle cells. In contrast, myotube formation *in vitro* is impaired in myogenic cells expressing a dominant-negative p53 protein and in primary myoblasts derived from p53 muscle [33]. This role was not manifested in this in-vivo study. This finding is supported by Foster *et al.* [34], who indicated that p53 is not required for the regulation of myoblast proliferation and differentiation and myotube formation *in vivo* during myogenesis of adult skeletal muscle. This could be explained by the possibility that genes responsible for myoblast differentiation could be upregulated in a way independent of p53 *in vivo*.

Conclusion

This study has shown that injections of cerebrolysin into an injured muscle improved muscle healing *in vivo*. The current study also shows the occurrence of a protective *Bcl-2* in inflammatory neutrophils and macrophages, which were expressed in cerebrolysin-treated muscles. It is also concluded that inflammatory cells play a role in satellite cell activation and proliferation. We have also found that the tumor suppressor gene p53, which is recognized as a central regulator of cell cycle had no role in muscle regeneration in this study.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Hawke TJ, Garry DJ. Myogenic satellite cells: physiology to molecular biology. J Appl Physiol (1985) 2001; 91:534–551.

2 Satou T, Itoh T, Tamai Y, Ohde H, Anderson AJ, Hashimoto S. Neurotrophic effects of FPF-1070 (Cerebrolysin) on cultured neurons from chicken embryo dorsal root ganglia, ciliary ganglia, and sympathetic trunks. J Neural Transm (Vienna) 2000; 107:1253–1262.

3 Haninec P, Houst'ava L, Stejskal L, Dubový P. Rescue of rat spinal motoneurons from avulsion-induced cell death by intrathecal administration of IGF-I and Cerebrolysin. Ann Anat 2003; 185:233–238.

4 Haninec P, Dubový P, Sámal F, Houstava L, Stejskal L. Reinnervation of the rat musculocutaneous nerve stump after its direct reconnection with the C5 spinal cord segment by the nerve graft following avulsion of the ventral spinal roots: a comparison of intrathecal administration of brain-derived neurotrophic factor and Cerebrolysin. Exp Brain Res 2004; 159:425–432.

5 Lavasani M, Lu A, Peng H, Cummins J, Huard J Nerve growth factor improves the muscle regeneration capacity of muscle stem cells in dystrophic muscle. Hum Gene Ther 2006; 17:180–192.

6 Madison RD, Sofroniew MV, Robinson GA. Schwann cell influence on motor neuron regeneration accuracy. Neuroscience 2009; 163:213–221.

7 Grosheva M, Guntinas-Lichius O, Angelova SK, Kuerten S, Alvanou A, Streppel M, *et al.* Local stabilization of microtubule assembly improves recovery of facial nerve function after repair. Exp Neurol 2008; 209:131–144.

8 Fanbin M, Jianghai C, Juan L, Yang W, Yuxiong W, Yanhua C, *et al.* Role of transforming growth factor-β1 in the process of fibrosis of denervated skeletal muscle. J Huazhong Univ Sci Technolog Med Sci 2011; 31:77–82.

9 Gharaibeh B, Lavasani M, Cummins JH, Huard J. Terminal differentiation is not a major determinant for the success of stem cell therapy – cross-talk between muscle-derived stem cells and host cells. Stem Cell Res Ther 2011; 2:31.

10 Menetrey J, Kasemkijwattana C, Day CS, Bosch P, Vogt M, Fu FH, *et al.* Growth factors improve muscle healing in vivo. J Bone Joint Surg Br 2000; 82:131–137.

11 Buckwalter JA, Einhorn TA, Cruess RL. Healing of the musculoskeletal tissues. In: Rockwood CA, Green DP, Heck-man JD, editors. *Fractures in adults*. 4th ed. Philadelphia: Lippincott Raven; 1996. 261–304.

12 Bhatavdekar JM, Patel DD, Chikhilkar RR. Over expression of CD44: a useful independent predictor of prognosis in patient with colorectal carcinomas. Ann Surg Oncol 1998; 5:495–501.

13 Auer RN. Automated nerve fibre size and myelin sheath measurement using microcomputer-based digital image analysis: theory, method and results. J Neurosci Methods 1994; 51:229–238.

14 Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA. Pax7 is required for the specification of myogenic satellite cells. Cell 2000; 102:777–786.

15 Fukushima K, Badlani N, Usas A, Riano F, Fu F, Huard J. The use of an antifibrosis agent to improve muscle recovery after laceration. Am J Sports Med 2001; 29:394–402.

16 Huard J, Li Y, Fu FH. Muscle injuries and repair: current trends in research. J Bone Joint Surg Am 2002; 84-A:822–832.

17 Shen W, Prisk V, Li Y, Foster W, Huard J. Inhibited skeletal muscle healing in cyclooxygenase-2 gene-deficient mice: the role of PGE2 and PGF2alpha. J Appl Physiol (1985) 2006; 101:1215–1221.

18 Gharaibeh B, Lu A, Tebbets J, Zheng B, Feduska J, Crisan M, *et al.* Isolation of a slowly adhering cell fraction containing stem cells from murine skeletal muscle by the preplate technique. Nat Protoc 2008; 3:1501–1509.

19 Hodgetts S, Beilharz M, Scalzo A, Grounds M. Why do cultured transplanted myoblasts die in vivo? DNA quantification shows enhanced survival of donor male myoblasts in host mice depleted of CD4+ and CD8+ cells or Nk1.1+ cells. Cell Transplant 2000; 9:489–502.

20 Chazaud B, Sonnet C, Lafuste P, Bassez G, Rimaniol AC, Poron F, *et al.* Satellite cells attract monocytes and use macrophages as a support to escape apoptosis and enhance muscle growth. J Cell Biol 2003; 163:1133–1143.

21 Siegel SG, Patton B, English AW. Ciliary neurotrophic factor is required for motoneuron sprouting. Exp Neurol 2000; 166:205–212.

22 Hill M, Wernig A, Goldspink G. Muscle satellite (stem) cell activation during local tissue injury and repair. J Anat 2003; 203:89–99.

23 Huard J, Cao B, Qu-Petersen Z. Muscle-derived stem cells: potential for muscle regeneration. Birth Defects Res C Embryo Today 2003; 69:230–237.

24 Sato K, Li Y, Foster W, Fukushima K, Badlani N, Adachi N, *et al.* Improvement of muscle healing through enhancement of muscle regeneration and prevention of fibrosis. Muscle Nerve 2003; 28:365–372.

25 Musarò A, Giacinti C, Borsellino G, Dobrowolny G, Pelosi L, Cairns L, *et al.* Stem cell-mediated muscle regeneration is enhanced by local isoform of insulin-like growth factor 1. Proc Natl Acad Sci USA 2004; 101:1206–1210.

26 Vleggeert-Lankamp CL, de Ruiter GC, Wolfs JF, Pêgo AP, Feirabend HK, Lakke EA, Malessy MJ. Type grouping in skeletal muscles after experimental reinnervation: another explanation. Eur J Neurosci 2005; 21:1249–1256.

27 Streppel M, Azzolin N, Dohm S, Guntinas-Lichius O, Haas C, Grothe C, *et al.* Focal application of neutralizing antibodies to soluble neurotrophic factors reduces collateral axonal branching after peripheral nerve lesion. Eur J Neurosci 2002; 15:1327–1342.

28 Li Y, Huard J Differentiation of muscle-derived cells into myofibroblasts in injured skeletal muscle. Am J Pathol 2002; 161:895–907.

29 Gu X, Ding F, Yang Y, Liu J. Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration. Prog Neurobiol 2011; 93:204–230.

30 Zammit P, Beauchamp J. The skeletal muscle satellite cell: stem cell or son of stem cell? Differentiation 2001; 68:193–204.

31 Wieteska-Skrzeczyńska W, Grzelkowska-Kowalczyk K, Tokarska J, Grabiec K. Growth factor and cytokine interactions in myogenesis. Part I. The effect of TNF-alpha and IFN-gamma on IGF-I-dependent differentiation in mouse C2C12 myogenic cells. Pol J Vet Sci 2011; 14:417–424.

32 Tiangco DA, Papakonstantinou KC, Mullinax KA, Terzis JK. IGF-I and end-to-side nerve repair: a dose-response study. J Reconstr Microsurg 2001; 17:247–256.

33 Ota S, Uehara K, Nozaki M, Kobayashi T, Terada S, Tobita K, *et al.* Intramuscular transplantation of muscle-derived stem cells accelerates skeletal muscle healing after contusion injury via enhancement of angiogenesis. Am J Sports Med 2011; 39:1912–1922.

34 Foster W, Li Y, Usas A, Somogyi G, Huard J. Gamma interferon as an antifibrosis agent in skeletal muscle. J Orthop Res 2003; 21:798–804.