RESEARCH CONCERNING THE REGENERATIVE EFFECT OF A SILVER NANODISPERSION IN WATER-OIL EMULSION ON SKELETAL MUSCLE

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Abstract

Experimentally, on 18 Wistar male rats, divided into three equal groups, we performed a closed injury, the crushing of the gastrocnemius muscle. Before that, the muscle that would be harmed was injected with a silver nanodispersion in water-oil emulsion (group II) and with an aqueous silver nanodispersion (group III); in the experiment we used a control group (I) that has not undergone any treatment. After 7 days animals were sacrificed and macroscopic and histological examinations of muscle that was injured showed a more advanced stage of healing, nearest resembling normal, for the goup treated with a silver nanodispersion in water-oil emulsion. Microscopic analysis suggests a pro-inflammatory effect of silver nanoparticles, muscle regeneration taking place probably by NF-kappaB activation and proliferation of satellite cells.

key words: skeletal muscle, injury, nanoparticular silver

Introduction

Repair, regeneration and striated muscle growth requires activation, proliferation and terminal differentiation of muscle satellite cells [1], which could be obtained from mesenchymal stem cells derived from adipose tissue [2]. Given that silver nanoparticles to 2.5 ppm concentration stimulates mesenchymal stem cells and cytotoxic effect is manifested at more than 5 ppm [3] we want to pursue the direct effect of these nanoparticles on striated muscle wound healing. The literature describes the occurrence of a muscle fibers regeneration after three days in rats soleus muscle after an minimum extent experimental lesion reproducing stretch muscle lesion that occurs in sports [4]. Because after an acute mechanical injury striated muscle is quickly invaded by neutrophils, which in turn additional harm the muscle fibers [1] (in humans after damage caused by eccentric contractions the neutrophils were present after 5 days [5]), we considered suitable for study the regenerative effect of silver nanoparticles the macroscopic and histologic evaluation of closed injuries caused on a striated muscle previously injected with a silver nanodispersion after 7 days, using rats as experimental animal. We consider necessary to study the effect of contact time with muscle tissue of silver nanoparticles by comparing the effects of a silver nanodispersion in water-oil emulsion with an aqueous silver nanodispersion with same concentration, given that depot injections have oily base.

Materials and methods

The study was conducted on 18 Wistar male rats aged 6 weeks, which after a prior locoregional anesthesia (right sciatic nerve block) with 0.1 ml lidocaine 1% [6] were caused mechanically, by crushing with haemostatic forceps, a gastrocnemius closed injuries (fig. 1). An hour before, right gastrocnemius muscle were injected with 0.1 ml oily emulsion of silver nanoparticles with a maximum of 0.5 ppm to 6 rats (group II) and with same amount of aqueous silver nanodispersion containing about the same proportion of nanoparticles the other 6 rats (group III) – fig. 2.

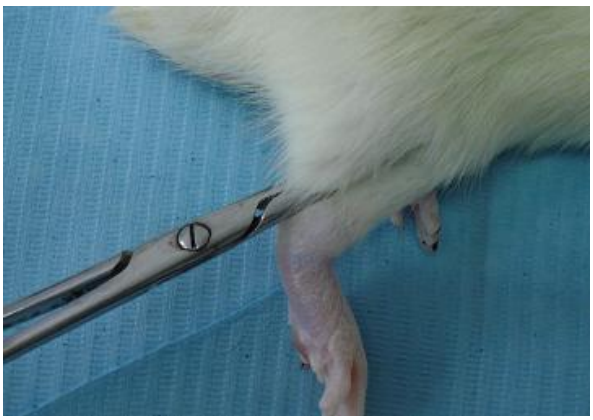

Fig. 1. Gastrocnemius muscle crushing with hemostatic forceps

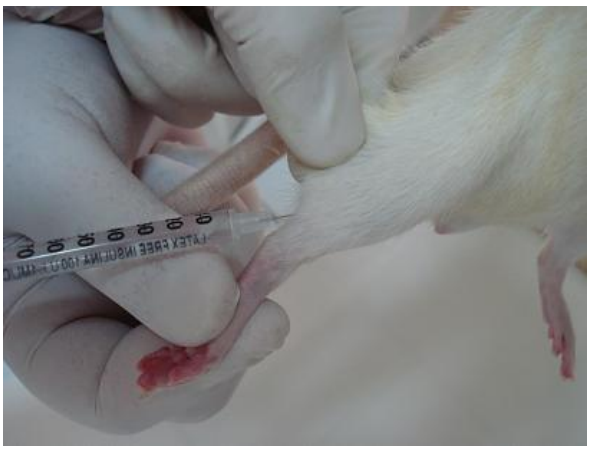

Fig. 2. Intramuscular injection with silver nanodispersion (group II and III).

Both nanodispersions were made using commercial product NANO SILVER (aqueous solution containing 20-25 ppm silver nanoparticles) and neutralized and sterilized sunflower oil, respectively distilled water. The remaining rats were constitute the control group (group I). During the experiment, animals were given acetaminophen in drinking water, concentration of 0,25 mg / ml, according to the method described Tachi et al [7]. After 7 days the animals were killed by ether overdose and injured gastrocnemius muscle was taken for histological analysis. Fixing muscle fragments was done in neutral formalin, 15% concentration, following processing by inclusion in paraffin and cut in microtome sections of 4 mm. Staining was performed by hematoxylin-eosin method (HE) and Van Gieson method (VG). Histological examination and image acquisition were performed with an Olympus microscope.

Results

Macroscopically was obviously a more advanced stage of healing for the group previously treated with the silver nanodispersion in water-oil emulsion (group II), compared with group I and group III (fig. 3, 4, 5). Histological analysis confirmed this fact: in group II were highlighted in injured gastrocnemius intense eosinophilic areas separated by areas with normal appearance by a band of connective tissue and homogenized look of muscle fibers (fig. 8, 9); these elements are present in samples from group III, but without uniform appearance (fig. 10, 11). In addition, in the muscles treated with aqueous silver nanodispersion there is a deep intertwining of normal muscle fibers and intense eosinophilic fibers without a clear boundary demarcation (fig. 10). In the case of injured muscles from animals in the control group histological examination revealed muscle fibers with varying orientation and with dense connective tissue in their thickness (fig. 6), which suggests that healing occurs anarchy. Some muscle fibers have the appearance of multinucleated cells, suggesting regeneration of young muscle (fig. 7). The trichromic staining revealed normal muscle areas separate from the damaged areas by a dense connective tissue (fig. 8). Trichromic coloration also showed a most abundant connective tissue that delineates modified areas from those of normal muscle in control group compared with histological preparations from rats treated with silver nanodispersions (fig. 7, 9, 11). Is found the rare inflammatory foci in the muscle interstitium.

Inflammatory infiltration is reduced in the control group and the most abundant (consisting predominantly of macrophages and with aspects of destruction of muscle fibers) in the group pretreated with silver oily nanodispersion (fig. 12, 13, 14). For group pretreated with aqueous silver nanodispersion capillary neoformation can be identified (fig. 14).

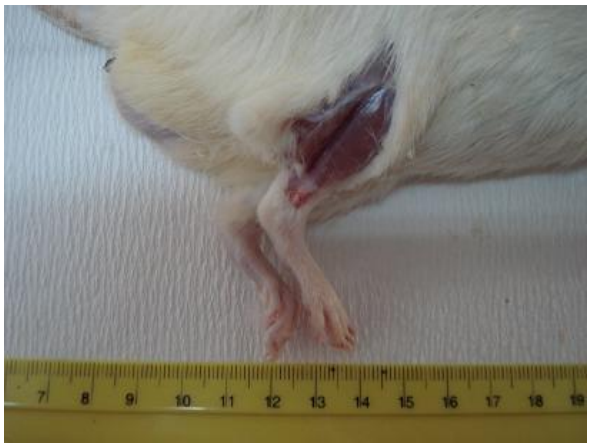

Fig. 3. Macroscopic aspect of the gastrocnemius muscle, 7 days after experimental injury (group I).

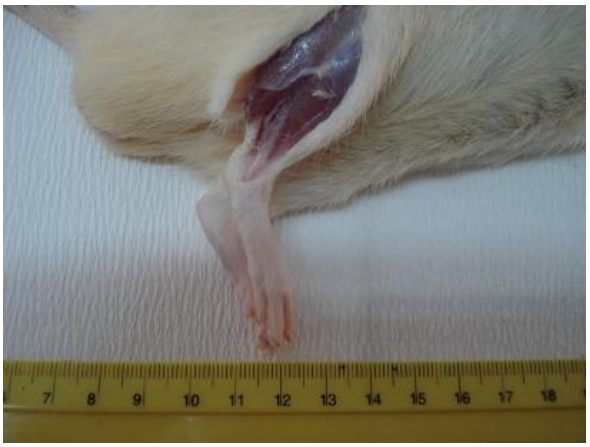

Fig. 4. Macroscopic aspect of the gastrocnemius muscle, 7 days after experimental injury, group II, pretreated with a silver nanodispersion in water-oil emulsion.

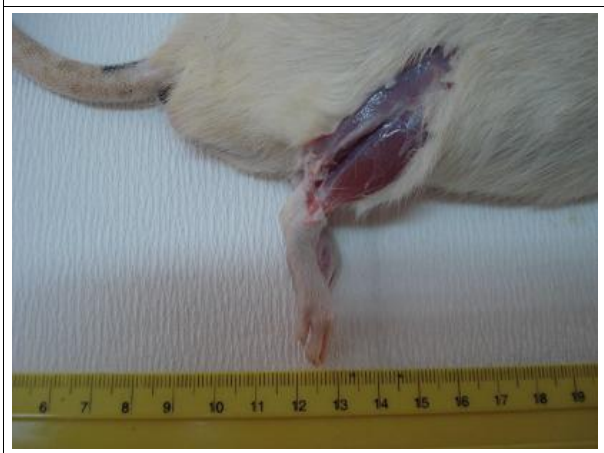

Fig. 5. Macroscopic aspect of the gastrocnemius muscle, 7 days after experimental injury, group III, pretreated with an aqueous silver nanodispersion.

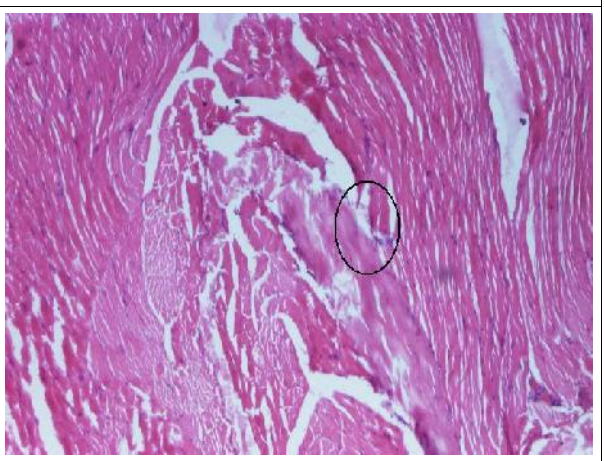

Fig. 6. Gastrocnemius muscles, 7 days after experimental injury, group I (control). In bounded area are observed best anarchic aspect of healing, with dense connective tissue in muscle fiber thickness. Histological section (HEx40).

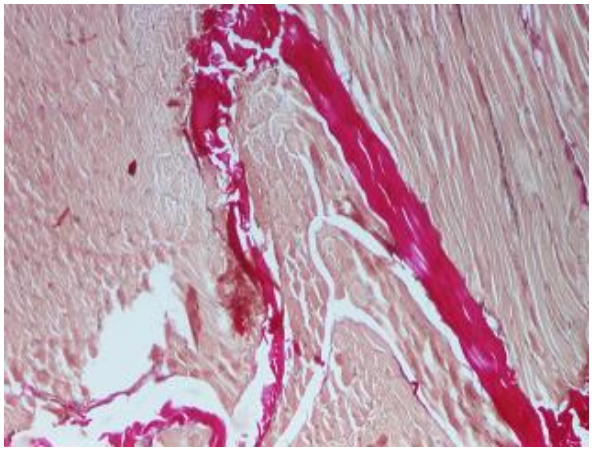

Fig. 7. Gastrocnemius muscles, 7 days after experimental injury, group I (control). Histological section (VGx40).

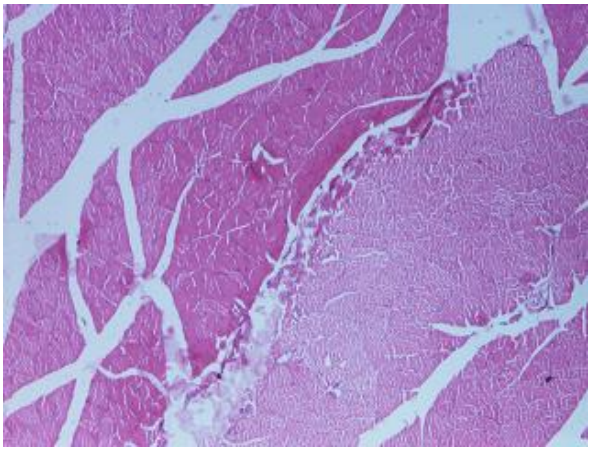

Fig. 8. Gastrocnemius muscles, 7 days after experimental injury, group II (pretreated with a silver nanodispersion in water-oil emulsion). Histological section (HEx40).

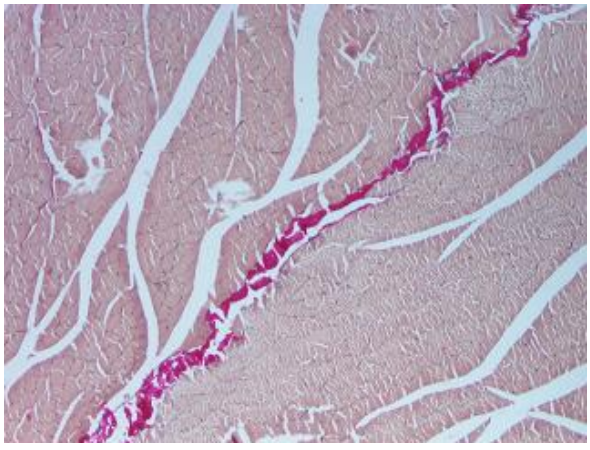

Fig. 9. Gastrocnemius muscles, 7 days after experimental injury, group II (pretreated with a silver nanodispersion in water-oil emulsion). Histological section (VGx40).

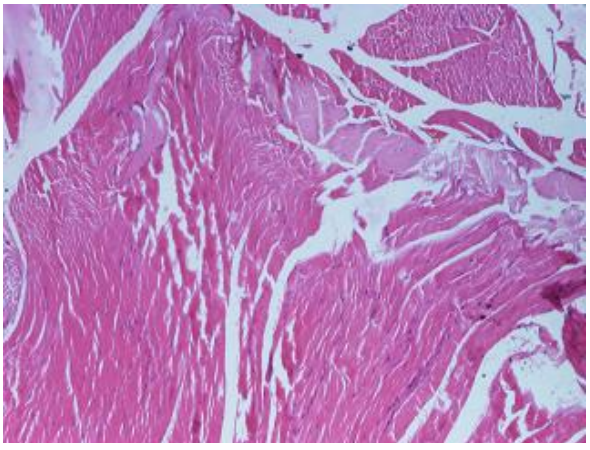

Fig. 10. Gastrocnemius muscles, 7 days after experimental injury, group III (pretreated with an aqueous silver nanodispersion). Histological section (Hex40).

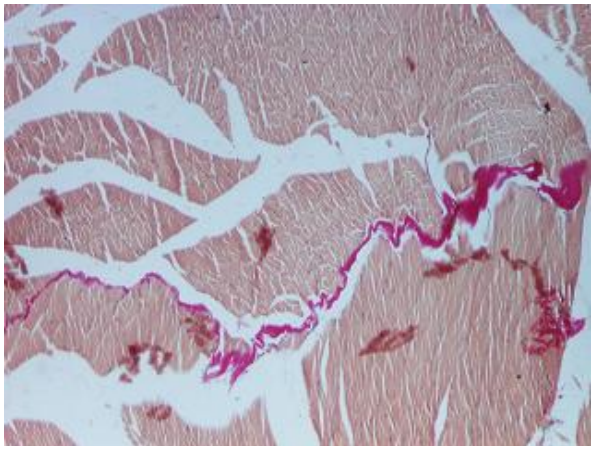

Fig. 11. Gastrocnemius muscles, 7 days after

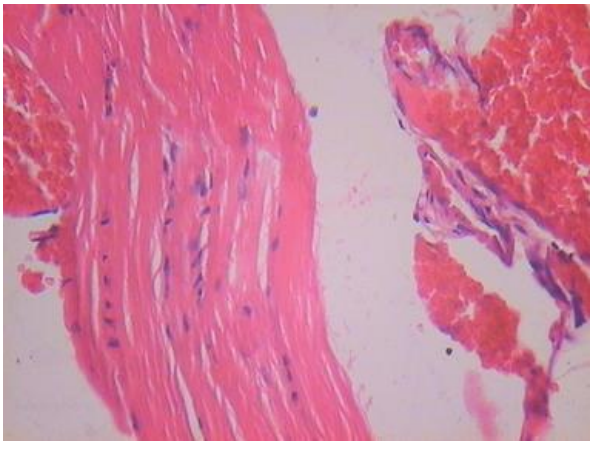

Fig. 12. Inflammatory infiltrate in gastrocnemius

experimental injury, group III (pretreated with with an aqueous silver nanodispersion). Histological section (VGx40).	muscle, 7 days after experimental injury, group I (control). Histological section (HEx200).
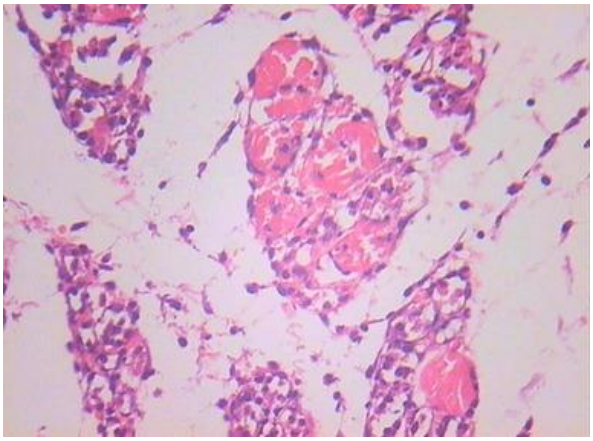	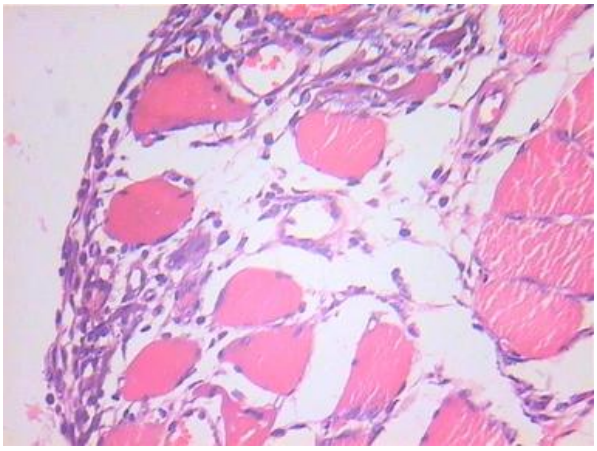
Fig. 13. Inflammatory infiltrate in gastrocnemius muscle, 7 days after experimental injury, group II (pretreated with a silver nanodispersion in water-oil emulsion). Histological section (HEx200).	Fig. 14. Inflammatory infiltrate in gastrocnemius muscle,7 days after experimental injury, group III (pretreated with an aqueous silver nanodispersion). Histological section (Hex200).

Discussions

Histological analysis suggests that pre-injection with silver nanodispersion induces a faster healing process, which dominated the regenerative process to conjunctive repair. Oily emulsion containing nanoparticular silver has the ability to generate a high quality healing, in addition to lower volume of scar tissue muscle bundles having a homogenous appearance, suggesting functional and aesthetic benefits. In vitro studies have shown that nanoparticular silver concentration up to 2.5 ppm stimulates mesenchymal stem cells, as evidenced by inducing expression of interleukin 8 (IL 8) [3]; is experimentally demonstrated that silver nanoparticles with a hydrodynamic diameter of 80 nm, polyvinylpyrrolidone (PVP) coated, are sequestered in mesenchymal stem cells endolysosomes by endocytosis and macropinocytosis [8]. Literature data allow partial explanation of the mechanism by which mesenchymal stem cells are activated by silver nanoparticles: mesenchymal stem cells maintained in a more acidic pH secrete increased quantities of interleukin 8 (IL 8), which is an angiogenic factor, which result from nuclear factor kappa B cells (NF-kappaB) activating [9]; low intracellular pH may be produced through the endocytolysis necessary for nanoparticles phagocytosis, which requires early endosomes maturation to lysosomes [10]. The role of NF-kappaB activation in stimulating differentiation of mesenchymal stem cells has been shown for osteogenic differentiation of mesenchymal stem cells derived from human adipose tissue [11], also a study on mice showed they can differentiate into muscle satellite cells [2]. The results indicates that is very likely a NF-kappaB activation of mesenchymal stem cells as a result of endocytosis of silver nanoparticles, which resulted in the differentiation of these stem cells in muscle satellite cells responsible for accelerated and superior quality healing of injured muscle. Stimulation of NF-kappaB can explain more intense inflammatory reaction and presence of capillary neoformation in groups pretreated with silver nanodispersions. IL-6 can induce proliferation of satellite cells [12], activation of its gene expression taking place through NF-kappaB transcription factor [13]. This study suggests the perspective to use of depot injections containing up to 5 ppm of silver nanoparticles in the treatment of muscle dystrophies or preoperative preparation of tissues.

Conclusions

1. Macroscopic examination revealed an advanced stage of healing of rat gastrocnemius

muscle at 7 days after the creation of a closed crush injury if pretreatment with a silver nanodispersion in water-oil emulsion compared with pretreatment with an aqueous silver nanodispersion or with control group.

2. Microscopic analysis confirmed the macroscopic aspects, histological examination revealing that pretreatment with silver nanodispersions induces a high quality healing, with the predominance of the regenerative process to conjunctive repair, nearest normal appearance being obtained in group treated with silver nanodispersion in water-oil emulsion. The presence of inflammatory foci with abundant infiltrated for group pretreated with oily silver nanodispersion and the capillary neoformation shown at group pretreated with aqueous silver nanodispersion suggests a pro-inflammatory effect of silver nanoparticles, muscle regeneration taking place probably by NF-kappaB activation and proliferation of satellite cells.

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