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Effects of microcurrent therapy on excisional elastic cartilage defects in young rats

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ABSTRACT

The effects of microcurrent application on the elastic cartilage defects in the outer ear of young animals were analyzed. Sixty male Wistar rats were divided into a control (CG) and a treated group (TG). An excisional lesion was created in the right outer ear of each animal. Daily treatment was started after 24 h and consisted of the application of a low-intensity (20 μA) continuous electrical current to the site of injury for 5 min. The animals were euthanized after 7, 14 and 28 days of injury and the samples were submitted to analyses. In CG, areas of newly formed cartilage and intense basophilia were seen at 28 days, while in TG the same observations were made already at 14 days. The percentage of birefringent collagen fibers was higher in CG at 28 days. The number of connective tissue cells and granulocytes was significantly higher in TG. Ultrastructural analysis revealed the presence of chondrocytes in TG at 14 days, while these cells were observed in CG only at 28 days. Cuprolinic blue staining and the amount of glycosaminoglycans were significantly higher in TG at 14 days and 28 days. The amount of hydroxyproline was significantly higher in TG at all time points studied. The active isoform of MMP-2 was higher activity in TG at 14 days. Immunoblotting for type II collagen and decorin was positive in both groups and at all time points. The treatment stimulated the proliferation and differentiation of connective tissue cells, the deposition of glycosaminoglycans and collagen, and the structural reorganization of these elements during elastic cartilage repair.

1. Introduction

Elastic cartilage is a highly specialized connective tissue that is derived from the embryonic mesenchyme. The tissue is avascular and contains nerve endings in its stroma. The structural organization of elastic cartilage is characterized by a small number of cellular elements, chondrocytes, and large amounts of extracellular matrix (ECM) rich in elastin. The chondrocytes are responsible for the production, organization and renewal of ECM macromolecules that surround them, permitting a strong morphofunctional interaction between these components and with the ECM (Negri et al., 2007). Furthermore, the chondrocytes found in elastic cartilage are nourished by the diffusion of metabolites through the ECM by means of capillaries in the perichondrium (Ito et al., 2001).

The composition of cartilaginous ECM includes fibrillar elements such as collagen and elastin. The main types of collagen found in cartilage are types II, IX and XI, with type II being the most abundant. The function of collagen fibrils and fibers in the matrix is to withstand tensile forces and to sustain stromal organization (Myllyharju and Kivirikko, 2001). Proteoglycans (PGs) consist of a central protein covalently bound to extensive and different polysaccharide chains, called glycosaminoglycans (GAGs) and can be classified according to molecular weight or the type of associated GAG (Vogel, 1994; Iozzo, 1998). Elastic fibers consist of an elastin core surrounded by a mantle of microfibrils rich in molecules called fibrillins. The presence of elastic fibers in the ECM of cartilage increases tensile strength and tissue elasticity (Kielty et al., 2002).

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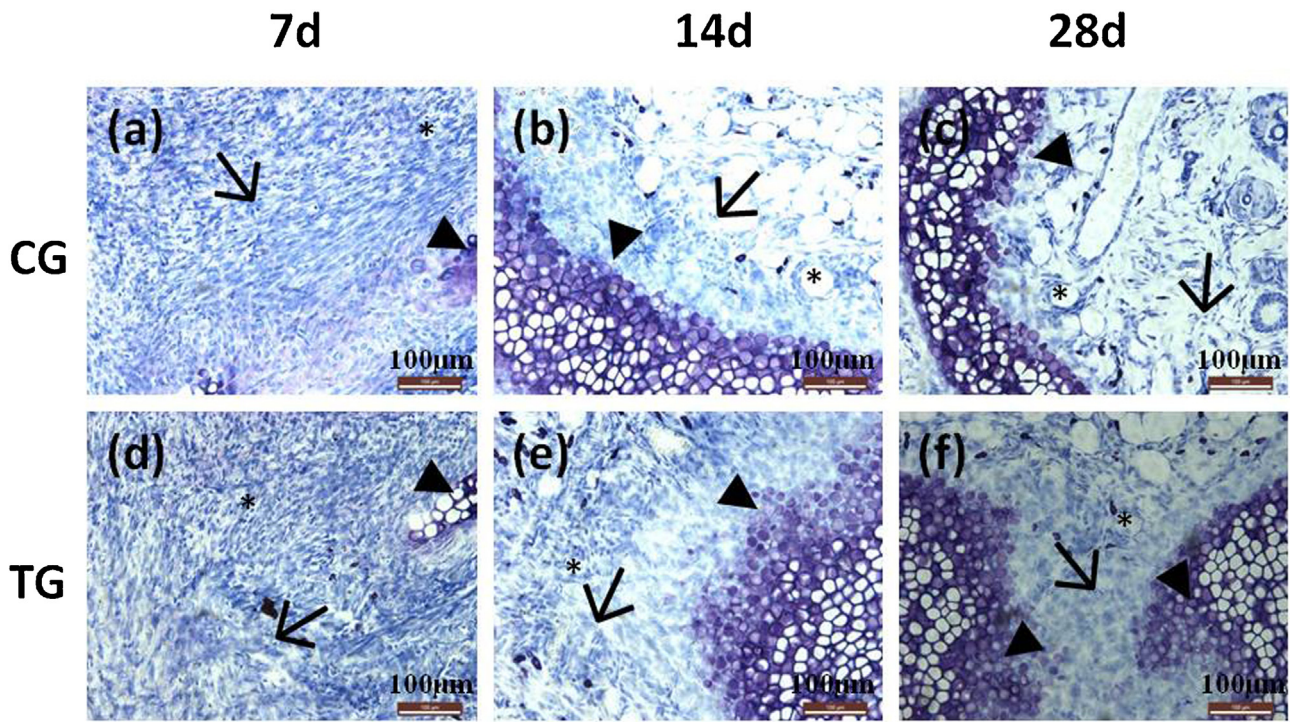

Fig. 1. Photomicrographs of longitudinal sections of the elastic cartilage defect created in the ear of rats. The sections were stained with Toluidine blue in McIlvaine buffer, pH 4. Control group (a–c). Group treated with a microcurrent (20 μA/5 min) (d–f). The treatment and observation periods were 7 (a and d), 14 (b and e) and 28 (c and f) days. (*) Blood vessels; (→) connective tissue cells; (►) cartilage areas. Bar = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The organization, composition and concentration of the main ECM components are intimately related to the functional properties of the tissue (Brighton et al., 2008). The action of mechanical forces on tissues, as well as the natural process of aging, directly influences the organization of elastic cartilage (Hyttinen et al., 2001; Carrington, 2005; Hennerbichler et al., 2008). Experimental studies have shown that small-diameter cartilage defects induced in young animals trigger cartilage repair characterized by the formation of new tissue with characteristics and properties similar to the original tissue. This fact is attributed to the proliferation and synthesis capacity of ECM promoted by the accelerated metabolism of chondrocytes (Newman, 1998; Nixon and Fortier, 2001; Anraku et al., 2009; de Campos Ciccone et al., 2013).

In view of the impact on public health investments, most studies on cartilage repair use models of osteochondral injuries that permit to monitor the incorporation of articular cartilage under conditions of compressive stress. However, these models do not permit to evaluate the characteristics of the newly formed tissue and its incorporation into the surrounding preserved tissue (Khan et al., 2008).

The tissue repair responses of non-articular cartilage differ from those of articular cartilage since the damage caused to the former are chondral and not osteochondral injuries as in the latter (Moyer et al., 2010; Rajnoch et al., 2003). Furthermore, chondrocytes isolated from non-articular cartilages contain larger amounts of lipid inclusions and glycogen due to the slower metabolism in these tissues (Stockwell, 1967; Souza et al., 2001). Finally, the structure and size of the keratan sulfate chains present in high- and low-molecular weight PGs isolated from cartilage ECM differ according to anatomical site and functional demand of the tissue (Nieduszynski et al., 1990).

Recent studies have opened various treatment options for cartilage disorders designed to improve the quality of the repaired tissue. These options include electrical and electromagnetic stimulation and autologous grafts of chondrocytes, mesenchymal cells or biocompatible tissues derived from the periosteum and perichondrium which exhibit a great chondrogenic potential (Hennerbichler et al., 2008; Khubutiva et al., 2008). However, in contrast to electrical stimulation, the other techniques require invasive procedures for the implantation of these cells and tissues at the site of injury (Johnson et al., 2004; Anraku et al., 2009).

In addition to being a noninvasive and low-risk option, the use of electrical stimuli induced by low-intensity electrical currents has been shown to be an effective treatment for cartilage repair. However, there are only few studies in the literature proposing protocols and the type of electrical current to be used in order to promote the regeneration of elastic cartilage in mammals (Snyder et al., 2002; Haddad et al., 2007).

Therefore, the objective of the present study was to evaluate the structural, ultrastructural and biochemical alterations that occur during the repair of excisional elastic cartilage defects in the outer ear of young rats after microcurrent application.

2. Materials and methods

All animal procedures described here were approved by the Ethics Committee on Animal Use of FHO, Uniararas (Protocol No. 023/2013).

2.1. Experimental groups

Sixty male Wistar rats, 45 days old and weighing on average 180 g, were obtained from the Luis Edmundo de Magalhães Center of Animal Experimentation, Centro Universitário Hermínio Ometto (FHO), Uniararas. The animals were maintained in individual cages and received commercial chow and water *ad libitum*. In view of their genetic similarity, the animals were divided into two groups of 30 animals each: a control group (CG) and a group treated with a microcurrent (TG).