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Histomorphological Evaluation of Transcutaneous Electrical Neural Stimulation in Healing of Experimentally Induced Partial Hip Joint Cartilage Defect in Rabbit

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Abstract

Objective- To determine the effect of the transcutaneous electrical neural stimulation on healing of hip joint cartilage defect in rabbit.
Design- Experimental in vivo study.
Animals- 12 adult New Zealand rabbits were used.
Procedures- Under effective the right femoral head was subluxated and the maximum accessible cartilage was denuded up to subchondral bone using dental bit in each rabbit. Then rabbits were divided into two groups of control(I) with no treatment and treatment group(II) which were subjected to Transcutaneous Electrical Neural Stimulation on 3rd day with frequency of 100 Hz and 80 µs intensity daily for 10 minutes in 14 days, having 6 rabbits each. These groups further were subdivided into 2 subgroups of 3 rabbits each with duration of one, and three months. The samples were collected for histomorphological study on day 30 and 90 days which were stained with H&E stain.
Results- The samples did not show any local reaction on denuded surface in control group; where as, the fibrous tissue in the central defect, with progression of the healing tissue to fibro cartilage as

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healing progressed up to 3 months in treated ones. Preliminary results indicate an increased stimulation of the repair tissue, as evidenced by total healing of three of the experimental defects with fibro cartilage as compared to the control specimens.
Conclusion and Clinical Relevance- TENS as a physical method of therapy is quite effective in cartilage healing and induces faster remodeling of fibro cartilage fibers.
Key Words: TENS, Cartilage, Hip Joint, Rabbit.

Introduction

The combination of relatively low cellularity, avascularity, a dense multilayer extra cellular matrix, and the unavailability of stem cells leaves cartilage with little to no reparative potential. In the immature individual, chondrocytes have shown an innate ability to replicate in the lamina splendors layer. The chondrocyte response to superficial lesions is very different from its response to deep lesions, where the subchondral bone has also been violated, thus allowing for a "fibrin clot" formation. Superficial lesions have been shown in animal models to exhibit limited regeneration potential. These are lesions that do not allow access to the blood borne nutrients and stem cells of the subchondral bone's medullary canall Meachum[1,2] showed cartilage necrosis, apoptosis, and loss of the extra cellular matrix, specifically proteoglycans, after superficial lacerations of articular cartilage in rabbits. Furthermore, D'Lima and coworkers[3] showed that 34% of cells near the margins of the laceration undergo apoptosis, and approximately 24 hours after injury, the remaining cells exhibit an increase in mitotic activity. Approximately 6 months after the injury, the bone is healed and the cartilaginous portion of the defect is filled with a mixed histologic pattern of fibro cartilage and some hyaline cartilage. This defect, however, usually fails to restore the articular surface.[4] The size and age of the patient are all factors that have been shown to affect the outcome of our body's limited ability to repair and regenerate osteochondral defects.[5,6,7] As the healing of cartilage depends upon many factors,[8,9] but the regeneration of native articular cartilage after insult has not been replicated using physical modality like transcutaneous electrical neural stimulation in the laboratory animal model, like rabbit The role of electrical stimulation in cartilage-healing is unclear, and further definition of parameters as well as testing in a wider variety of experimental and clinical settings is required.

Materials and Methods

This study was performed in accordance with the Islamic Azad University, Sciences and Research Center's law on animal experimentation and this research project was approved by the Specialized Faculty of Veterinary Sciences Research Councils. All rabbits were treated accordingly to animal welfare legal regulations. Twelve adult male New Zealand rabbits with body weights ranged from 3.5±0.45 kg and above 6 months of age were submitted to orthopedic surgery under effective anesthesia using combinations of acepromazine (1mg/kg i.m), ketamine (35 mg/kg i.m) and xylazine (5mg/kg i.m).). The right femoral head was subluxated and the maximum accessible cartilage was denuded up to subchondral bone using dental bit in each rabbit then the capsule was sutured with nylon 5/0. The animals were divided into two groups of control(I) with treatment and second treatment group(II) which were 3rd day was subjected to Transcutaneous Electrical Neural Stimulation on 3rd day with frequency of 100 Hz and 80 μs intensity daily for 10 minutes in 14 days, then further they were subdivided into 2

subgroups of 3 rabbits each with duration of 1, and 3 months. Post-operative treatment included, antibiotics (penicillin G procaine 40000 IU/kg i.m bid) dexamethasone 0.6 mg/kg, Vitamin B.Complex 0.2 mg/kg and tramadol hydrochloride (5 mg/kg i.m bid) were administered for 5 post-operative days. All rabbits were pharmacologically euthanized by direct intracardiac injection of thiopental sodium 10 % at the end of 30 and 90 days, and . The full head of right femoral bone was collected and stored in the 10% buffered solution of formalin and then stained with H & E stain.

Results

The control group samples did not show any connective tissue reaction on denuded surface on subchondral bone after 3 months, (Fig 1.) whereas the repair response in treated group consisted of fibrous tissue in the central defect, with progression of the healing tissue to fibro cartilage as healing time progressed up to to 3 months. The defect margins exhibited some cellular proliferation and matrix production, and an occasional specimen displayed encroachment of proliferating hyaline cartilage from the margins of the defect (Fig 2). The specimens treated with TENS showed an increase in marginal cellular response, as evidenced by increased proliferation and matrix production. These findings were compatible with the image created by serial sectioning of the specimens and evaluation with light microscopy. The repair response was not totally efficient, however, as revealed by the lack of total healing with articular cartilage (Fig 3).

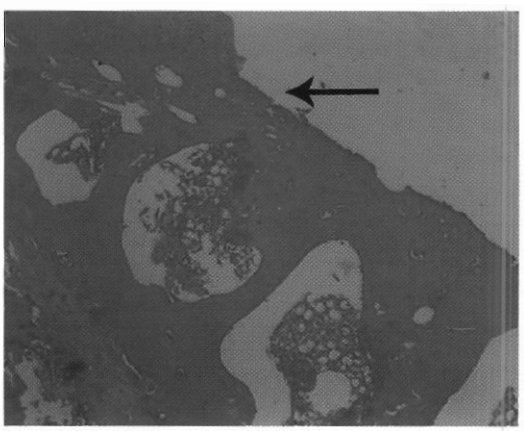

Figure 1. Partially denuded femoral head at 3 months.Osteochondral spongy bone (arrow) without hyaline cartilage with little sign of normal left over cartilage. No sign of repair was observed (H & E X160).

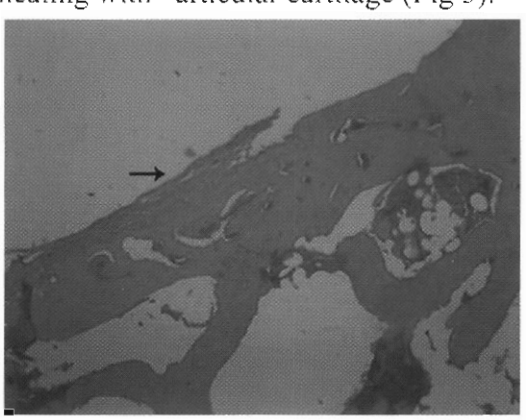

Figure 2. Treated femoral head at 3 months .Osteochondral spongy bone (arrow) covered with collagen fibers with fibroblasts cells with sign of normal hyaline cartilage left over on the subchondral bone (H & E X160).

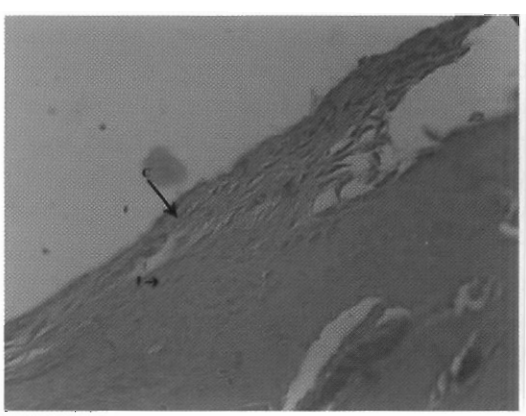

Figure 3. Treated femoral head at 3 months Osteochondral spongy bone (arrow) covered with collagen fibers (c) and with fibroblasts cells(b) on the subchondral bone (H & E X640).

Preliminary results indicate an increased stimulation of the repair tissue, as evidenced by total healing of three of the experimental defects with fibro cartilage as compared to the control specimens. Work with these TENS is currently being continued as an attempt to define electrical parameters and techniques that will allow consistent enhancement of total repair of articular cartilage defects. TENS as a physical method of therapy is quite effective in cartilage healing and induces faster remodeling of fibro cartilage fibers.

Discussion

Attempts to enhance the intrinsic healing potential of cartilage have traditionally been focused on recruiting pluripotential cells from the bone marrow by penetrating the subchondral bone or providing a mechanical, electrical, laser, or other stimulus for healing . More recently the use of bioactive agents such as growth factors and cytokines, sometimes in combination with scaffolds on which healing can be structured, has been investigated articular chondrocytes reside in an avascular environment and do not usually effect healing when damage to the joint surface is limited to the layer of cartilage.[10,11] Many investigators have attempted to stimulate cartilage-healing by drilling, abrading, or producing so-called micro fractures in the subchondral bone.[12,13,14] All of these techniques have in common the goal of recruiting pluripotential stem cells from the marrow by penetration of the subchondral bone with their own specific limitations. In this study, non- invasive method using TENS with bearable intensity recruiting of fiberblast and collagen at the site of denuded cartilage was achieved. Meacham and Roberts performed a number of perforations in each defect in twenty-one knees of adult male rabbits after the knees had been denuded of cartilage.[1,2] For as long as two years after the procedure, the cartilage never fully healed, and even complete covering of the denuded bone with noncartilaginous tissue was rarely seen ,but in this study within three months there were trace of fibrocatilage tissue covering denuded area without having any site effect using TENS, but there was no trace of fibro cartilage formation in the control group in which total femoral head cartilage was denuded .It has been reported that larger denuded area needed much more time for recruiting chondrocyte to shape hyaline cartilage provided the joint to have normal range of movement.[15,16,17] Salter and his colleagues[18] demonstrated that healing of articular cartilage was enhanced in rabbits by the postoperative use of continuous passive motion. In this trial all rabbits had free and active movement without lameness, which could have been additional micro-movement to stimulate the remaining cartilage on femoral head surface to show reaction in form of fibro cartilage or even hyaling cartilage formation. But Salter reported in one series, multiple (four) small (one-millimeter) drill-holes were made in one knee of each rabbit; after four weeks, healing with predominantly hyaline cartilage was seen in 60 percent of the forty defects in ten adolescent rabbits and in 44 percent of the forty defects in ten adult rabbits that had been treated with continuous passive motion, whereas such healing was seen in 10 percent or fewer of the defects in rabbits that had had postoperative immobilization in a cast or had been allowed free movement in their cages.[15,19] Subsequent studies showed that, although continuous passive motion enhanced cartilage-healing, the effect was much less pronounced in defects that were larger than three millimeters in diameter.[20,21] Electrical stimulation for cartilage-healing has not received as much attention as has electrical stimulation for fracture-healing.[22,23,24,25] Lippiello et al. demonstrated slightly improved healing of cartilage defects in the knees of rabbits after treatment with pulsed direct current.[26,27,28] Maximum efficacy was seen after four hours of exposure per day. Baker et

al. reported that electrical stimulation improved healing, but their sample sizes (three or fewer per group) were insufficient.[28] More importantly, the effect was not seen in the articular defects but rather in the surrounding cartilage. At present, efforts to induce healing and regeneration of cartilage are being directed toward enhancing the natural healing potential of cartilage or replacing the damaged cartilage with tissues or cells that can grow cartilage.[29,30] These approaches have shown promise, but they are still far from reliable and are not sufficiently versatile to be employed in many clinical settings. Autogenous graft, allograft, osteochondral, use of matrix, beside application of laser and electrical stimulation for repairing and regeneration of cartilage should be considered investigational until they can be proved in rigorous clinical trials, which, ideally, should be randomized, controlled, and blinded.

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چکیده

ارزیابی هیستومورفولوژیکی اثرات تحریکات الکتریکی عصبی در التیام ضایعات تجربی غضروف مفصل لگن در خرگوش

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هدف- بررسی اثرات تحریکات الکتریکی در التیام ضایعات تجربی غضروف مفصل لگن در خرگوش.
طرح مطالعه- مطالعه تجربی.
حیوانات- ۱۲ سر خرگوش بالغ سالم نر نژاد نیوزیلندی با وزن ۰.۴۵±۳.۵ کیلوگرم و میانگین سن ۶ ماه.
روش کار- خرگوش ها با استفاده از کتامین هیدروکلراید به همراه گزیلازین دیازپام و آسیرمازین بیهوش شدند. در شرایط کاملا" اسپسی با ایجاد برش بر روی مفصل لگن سمت راست و جدا سازی ماهیچه های ناحیه و تروکانتر بزرگتر و برش بر روی کپسول مفصلی جابجایی مفصل رانی-لگنی ایجاد گردید. با استفاده از فرز دندانپزشکی غضروف مفصلی تمام قطر تراسیده شد. و بعد از بخیه کردن ناحیه حیوانات به دو گروه کنترل و درمان با ۶ خرگوش در هر گروه تقسیم شدند. حیوانات در هر گروه دوباره به زیر گروه یک ماهه و سه ماهه با سه خرگوش در هر زیر گروه تقسیم شدند. حیوانات گروه درمان بعد از ۷۲ ساعت، تحت درمان روزانه تحریکات الکتریکی به مدت ۱۰ دقیقه با فرکانس 100 HZ و شدت 80 µS در طی ۲ هفته قرار گرفتند در صورتی که در گروه کنترل از هیچ روش درمانی استفاده نگردید. در پایان زمان مطالعه در ۳۰ و ۹۰ روز نمونه بافتی از محل نقیصه غضروفی تهیه گردید و بعد از تهیه مقاطع بافتی و رنگ آمیزی با روش هماتوکسیلین و ائوزین تحت ارزیابی میکروسکوپی قرار گرفتند.
نتایج- درطول دوره مطالعه هیچگونه واکنش بافتی بر روی استخوان اسفنجی سر استخوان ران مشاهده نگردید.در صورتیکه در گروه درمان با تحریکات الکتریکی حضور رشته های کلاژن همراه با سلولهای فیبروسیتی و عروق خونی مشاهده گردید. رشد پوشش کلاژنی در ناحیه نقیصه غضروفی حاکی از اثرات مستقیم تحریکات الکتریکی بوده است که در گروه کنترل مشاهده نگردید.
نتیجه گیری و کاربرد بالینی- تحریک الکتریکی به عنوان یک روش درمان فیزیکی، روشی بسیار موثر در التیام غضروف بوده و می تواند سبب تسریع در روند بازسازی الیاف فیبری غضروفی گردد.
کلید واژگان- تحریک الکتریکی، غضروف، مفصل لگنی رانی، خرگوش.