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Original article

Effect of light-emitting diode (LED) therapy on the development of osteoarthritis (OA) in a rabbit model

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ABSTRACT

Objective: The objective of this study was to evaluate whether light-emitting diodes (LEDs) could be effective in a noninvasive, therapeutic device for the treatment of osteoarthritic (OA) knee joints.
Design: Five weeks following the anterior cruciate ligament transection (ACLT) of mature New Zealand White rabbits, the animal knees were exposed to LED stimulation at intervals of 10 min/day, 5 days/week for 5 weeks in the experimental group ($n = 7$). The device used high intensity red and infrared (IR) LEDs with a total amount of energy delivered to the skin of 2.4 J/cm^2. Animals were sacrificed at 9 weeks postoperatively. Femoral surface gross morphology was evaluated with a modified Outerbridge classification and mRNA expression of catabolic and anabolic markers from femoral condyle cartilage and synovial tissue was assessed using RT-PCR. A control group was harvested 9 weeks following untreated ACLT.
Results: Gross morphometry of the control group showed four Grade II, two Grade III and one Grade IV (average 2.6) condyles macroscopically. The experimental group showed two Grade I and five Grade II (average 1.7) (Table 1). mRNA expression of aggrecan in the cartilage showed no difference between the groups, however type II collagen expression increased in the experimental group compared with control. TNF-α expression was significantly decreased in the experimental group compared to control.
Conclusions: There was general preservation of the articular surface and decreased levels of inflammation in the osteoarthritic joints with the application of LED therapy. This may provide potential application as a noninvasive treatment.

1. Introduction

Osteoarthritis (OA) is one of the most common diseases of aging, characterized as articular cartilage and underlying subchondral degeneration, with more than 20 million people affected in the United States [1]. OA causes joint pain and a limitation of joint range of motion (ROM), with the end stage resulting in decreased quality of life (QOL). For patients with advanced OA, however, surgical options are available. Osteoarthritic cartilage may be repaired by chondroplasty, drilling and microfracture or it may be replaced through autologous or allogenic osteochondral transplantations in an attempt to remodel the joints to their original morphometry [2–8]. Artificial joint replacement and arthrodesis are salvage procedures for severe osteoarthritic joints [9], and autologous chondrocyte implantation (ACI) is used as a cartilage reconstruction trial [10].

Early OA is usually treated by controlling pain and improving ROM. Conservative procedures begin with weight loss, ROM exercises, muscle stretching and strengthening, followed by medication and intra-articular injections of hyaluronic acid, analgesics and traditional NSAIDs. Long-term use of these drugs may increase gastric mucosal damage and cardiovascular disease [11]. Injection of hyaluronan has been shown to improve joint lubrication and decrease pain [12], however the effectiveness diminishes with time, and repetition can cause joint infection. A long-lasting, noninvasive procedure to provide anti-inflammation and preserve articular cartilage in early OA is therefore clinically desirable.

Light amplification by stimulated emission of radiation (LASER) treatment has been reported to improve wound healing, including increase of cell proliferation, collagen synthesis and growth factor production. The LASER does, however, have limitations in wavelength capacities and beam widths. An optimal wavelength of LASER for each tissue may be difficult for larger areas, and heat productions from LASER light could damage tissue [13].

Light-emitting diodes (LEDs) are complex semiconductors that convert electrical current into incoherent narrow-spectrum light.

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LEDs are available at wavelengths ranging from ultraviolet (UV) to visible to near infrared (NIR) bandwidths (247 to 1300 nm). The National Aeronautics and Space Administration (NASA) has reported on the acceleration of plant growth and wound healing following exposure to LEDs. This has led to investigations into nonthermal therapy, especially in dermatology, leading to its approval as a nonsignificant risk procedure for humans by the Food and Drug Administration (FDA) [14].

We hypothesize that LED treatments can penetrate into joints, stimulate the inside of the joint cavity and articular surface, and have the potential to repair OA. The objective of this study was to evaluate the effect of LED light therapy on osteoarthritic knee joints using the anterior cruciate ligament transection (ACLT) model of OA *in vivo*.

2. Materials and methods

2.1. Animals

A total of 14 skeletally mature, female New Zealand White rabbits (9–15 months old weighing 3.5 to 4.5 kg) with closed epiphyses were used for both control and experimental evaluation ($n = 7$ for each group). All procedures conformed to the guidelines of the University's Animal Subjects Committee and the American Association for Accreditation of Laboratory Animal Care.

2.2. Osteoarthritic knee joint model

The study animals were given presurgical general anesthesia injections of 35 mg/kg ketamine and 5 mg/kg xylazine intramuscularly. An anesthesia machine was attached to a facemask delivering 1–2% isoflurane for maintenance of anesthesia during surgery. The central part of the right leg was shaved and draped in sterile fashion using betadine. A 3 cm anterior midline incision was made through the skin of the knee, subcutaneous tissues were dissected, the joint capsule incised with a medial parapatellar retinacular approach, and the articular surfaces of the knee joint exposed. The ACL was then completely transected with a blade (Fig. 1). An intraoperative Lachman test was performed to verify that anterior instability had been created before closing the capsule with 2-0 Vicryl (Ethicon Inc. Somerville NJ) and the skin with 4-0 Vicryl. Previous studies have shown that this method produces a reliable and reproducible degradation of articular cartilage after 9 weeks [15,16]. All rabbits were allowed normal cage activity after the procedure in a temperature-controlled environment with a 12 h light-dark cycle. Intramuscular buprenorphine was administered for at least 72 h for postoperative pain control.

2.3. LED therapy device and treatment

Five weeks following ACLT, LED exposure commenced at intervals of 10 min per day, 5 days per week for 5 weeks, under awake and alert conditions without sedation, in the experimental group ($n = 7$). The light therapy was accomplished on the experimental knees with a very light custom-designed brace fitting comfortably over the knee and was held in place with two Velcro straps (3 M, St. Paul MN). The device applied two sets of LEDs, with wavelengths of 630 nm (red) and 870 nm (infrared: IR). LEDs mounted to the underside of the brace covered 44 cm^2, and could treat the entire area of the rabbit knee joint (Fig. 2). It alternated at high frequency between two patterns of off and on diodes. The amounts of energy delivered to the skin were ~2 J/cm^2 (red) and 2.5 J/cm^2 (IR) (Light Sciences Oncology, Inc., Snoqualmie WA).

2.4. Harvest tissues

Ten weeks following ACLT, and 3 days after the 5-week treatment, the treated animals ($n = 7$) were sacrificed with intravenous injections of 97.8 mg/kg sodium pentobarbital via a lateral ear vein following anesthesia. In the control group without any treatment ($n = 7$) the animals were sacrificed at 9 weeks following ACLT in a similar manner.

2.5. Gross morphology

The rabbit femurs were harvested and initially put in a phosphate buffered solution (PBS) with protease inhibitors: 2 mM disodium-ethylenediaminetetraacetic acid (Na2-EDTA), 1 mM phenylmethanesulphonyl fluoride (PMSF), 5 mM benzamidine hydrochloride (Benz-HCl), and 10 mM N-ethyl maleimide (NEM) for 1 h. The femoral condyles were then stained with india ink (Higgins Waterproof Drawing Ink, Sanford Co of Bellwood IL). The ink was applied to the surfaces of the femoral condyles with a small paintbrush and a quick rinse of sterile water. Images of the condyles were taken before and after staining with a digital camera (Nikon DX 5 megapixel) for analysis. These images were assessed for residual ink remaining on the cartilage surface by three blinded individuals. A modified Outerbridge classification score was used to grade each condyle. The score criteria consisted of: Grade 1 (intact surface) – surface appears normal and does not retain ink;

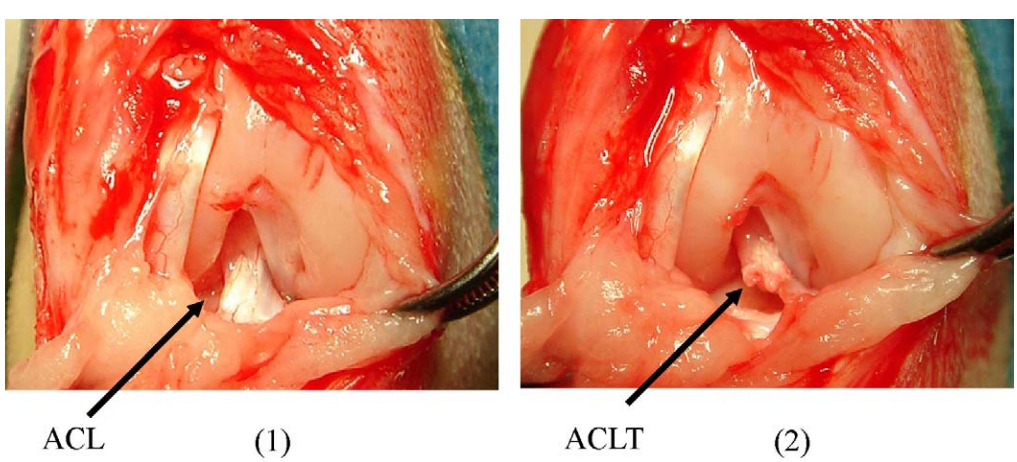

Fig. 1. (1) The ACL attachment of the lateral femoral condyle to the tibial plateau in a right knee joint. (2) The ACL is transected and the knee instability checked with a Lackman test.