Anti-inflammation Effect of Low Intensity Laser Therapy in Collagen-induced Arthritis in Rats

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Arthritis of the knee is the most common type of joint inflammatory disorder and it is associated with pain and inflammation of the joint capsule. The aim of present study was to investigate the endogenous effect of low intensity laser acupuncture on collagen-induced arthritis in rats. Forty Sprague-Dawley rats were randomly divided into normal group, arthritis group, low laser group with 10 rats in each group. Arthritis in rats was induced by subcutaneous injection of type II collagen combined with complete Freund's adjuvant. Here we investigated the effects of low intensity laser therapy in experimentally induced rat knee arthritis. To evaluate preventive and therapeutic effects of low intensity laser acupuncture on collagen-induced arthritis rats. In collagen induced arthritic rats, there was significant increase in rat paw volume and decrease in body weight increment, whereas low intensity laser therapy groups, showed significant reduction in paw volume and normal gain in body weight. The altered biochemical parameters(blood urea, serum creatinine, total proteins and acute phase proteins) in the arthritic rats were significantly brought back to near normal by the low intensity laser therapy. Therefore, low intensity laser acupuncture may be a useful treatment in the prevention and treatment of collagen-induced arthritis.

Key words : acupuncture point, arthritis, low intensity laser

Introduction

Rheumatoid arthritis(RA) is a systemic autoimmune disease of multi-factorial etiology and a systemic disorder characterized by synovium inflammation and subsequent destruction and deformity of joints[1,2]. The disease is characterized by articular inflammation and by the formation of an inflammatory and invasive tissue, rheumatoid pannus that eventually leads to the destruction of joints[3]. In particular, it was reported that the inflammatory cytokine, such as tumor necrosis factor(TNF)-α, interleukin(IL)-1β, and interleukin(IL)-6, play key roles in the inflammation and joint damages during the development of RA[4]. As most joint damage is largely irreversible, persistent damage will inevitably result in greater disability[5]. Therefore, the treatment goals in RA include prevention of joint damage and loss of joint function, a decrease of RA symptoms, prevention of disease progression[6].

The most commonly prescribed medication for RA inflammation treatment is non-steroidal anti-inflammatory drugs (NSAIDs)[7]. While these drugs effectively reduce the RA symptoms such as joint pain, swelling, tenderness, and morning stiffness. But they may cause serious side effects such as gastrointestinal ulcer and renal morbidity[8,9]. Therefore in the therapeutic strategy for RA, reducing side effect should be carefully considered as well as conventional treatment.

In the traditional medicine of many Asian countries, acupuncture is considered as one of the most effective medicinal treatments, especially for chronic diseases accompanied with severe pain, such as, osteoarthritis, rheumatoid arthritis, back pain[8]. Manual or electrical stimulation of the acupuncture point is the most popular form of acupuncture therapy[10]. Laser treatment at acupuncture, also called low intensity laser therapy, is a therapy method equivalent to needle acupuncture[11]. Therapeutic application of laser acupuncture is rapidly gaining in popularity. The stimulation of ac acupuncture point with the laser is not only painless, but also does not cause any "tingling" sensation at all[12].

Low intensity laser therapy has the many benefits, such as microcirculation enhancement, vasodilatation, tissue metabolism improvement, and analgesic effects. However, objective evaluation of its efficacy in published studies is

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difficult also because laser acupuncture is often associated with phototherapy in systematic reviews[13]. Although energy dosage is extremely low in both cases, phototherapy uses the direct effect of light on tissues, whereas in laser acupuncture the selection of acupuncture point is based on acupuncture theories[14].

In addition, the low intensity laser therapy has many potential therapeutic effect in acupuncture. But, they are not evaluated for their therapeutic activities in concrete results. In the present study, using a experimental arthritis rat models, we demonstrated that the effectiveness of 850 ㎚ gallium arsenide aluminum (Ga-As-Al) laser therapy on joint swelling, arthritis index, and inflammatory cytokine.

Materials and Methods

1. Animals

Male Sprague-Dawley rats (Orient, Seoul, Korea) weighing 225-230 g were used in this study. They were housed in standard plastic cages with controlled temperature(23 ± 2 ℃), light(12/12 hr light and dark cycle), and relative air humidity 50-60% controlled condition. All rats had free access to standard rodent pellet food (NIH#31M, Samtako, Korea) and water except when fasted before experiments. All animals received care compliant with the "Guide for the care and use of laboratory animals," published by the national institutes of health(NIH). The university of Dongshin institutional animal care and use committee approved the experimental procedures which followed the principles of laboratory animal care according to national institutes of health publications.

2. Induction of collagen-induced arthritis

Chicken type II(Sigma, St. Louis, MO, USA) collagen was dissolved in 0.01 mmol/L acetic acid and emulsified with an equal volume of Freund's incomplete adjuvant (Sigma, St. Louis, MO, USA) in 4℃ ice-bath just before use. The final concentrations of Chicken type Ⅱ collagen and BCG were both 2 g/L. 1 ㎖ aliquot of the emulsion was injected intradermally into the left hind paw and the proximal one-third of the tail. For the second immunization, the same emulsion was injected subcutaneously on day 14. The rats of the control group received an injection of an equal volume of 0.01 mmol/L acetic acid at the same location.

3. Experimental design and treatment

In our study, a total 40 rats(10 normal rat, 30 arthritis rat) used. These rats were randomly divided into four groups of 10 rats after collagen-induced arthritis(Table 1).

Table 1. Classification of experimental groups

Group(n=40)	Characteristics
Normal group(n=10)	Saline-treated non-arthritic rats
Control group(n=10)	Collagen-induced arthritis rats
Experimental group Ⅰ (n=10)	Collagen-induced arthritis rats with low intensity laser treatment at acupuncture point(ST36 Zusanli, GB39 Hyunjong)
Experimental group Ⅱ (n=10)	Collagen-induced arthritis rats with low intensity laser treatment at non-acupuncture point

The Gallium-Aluminum-Arsenide(GaAlAs, infrared laser) diode laser device (Chatta- nooga group, USA) with a wavelength of 850 ㎚, power output of 100 ㎽, continuous wave and 0.07 ㎠ spot area laser were used for the laser therapy.

The laser was applied with a dosage of 3 Joule/㎠ at acupuncture points(ST-36, GB39) for 1 minute at each point over the knee. Experimental group Ⅱ was applied in the same way but the device was applied with a dosage of 3 Joule/㎠ at peri-articular during 21 days from arthritis induction. Researcher were asked to use protective eyeglasses during laser treatment for safety.

4. Measurements of the body weight and the paw volume

Body weights were regularly measured by a digital balance after the adjuvant injection. The body weight changes were observed on every week. The paw volume of all the animal groups was measured by plethysmograph at 0, 7, 14, 21, and 28 days after low intensity laser therapy. The paw volume was blindly measured using a water displacement plethysmometer (YLS-7A, Yiyan Sci Ltd., Jinan, China). The mean values were calculated and plotted at each time point.

5. Arthritis evaluation

The clinical symptoms of arthritis in hind limbs were evaluated according to visual appearance by two observers, who were unaware of the identity of the animal groups. The scores were rated on a scale of 0–4 for each knee joint: 0 = no swelling or erythema, 1 = slight swelling and/or erythema, 2 = low to moderate edema, 3 = pronounced edema with limited joint usage, and 4 = excess edema with joint rigidity[15,16]. The mean articular index score was given for each foot of adjuvant induced arthritis rat. The total score for each animal was then calculated and used as an articular index with a maximum value of 16 point.

6. Biochemical evaluations

On 28th day, at the end of experiment, all animals were sacrificed by cervical decapitation and blood was collected in EDTA containing tubes, respectively for serum separation. The

serum and homogenized samples were subjected to biochemical examination like total protein and albumin, globulin, acute phase proteins fibrinogen, Ceruloplasmin[17-19]. Blood was allowed to clot and serum separated by centrifugation at 3,500 rpm for 15 min. The serum was assayed either immediately or stored at -20℃. The serum biochemical examination was assayed by enzyme-linked immunosorbent assay kit (ELISA, Boerhringer Mannheim, Germany).

7. Statistical analysis

All the data were expressed as mean ± standard deviation (S.D.) of three replications. Statistical calculations by SPSS version 12.0 software were carried out One-way ANOVA was applied for determining differences between oh samples. Duncan test was taken to compare the data. Values of $p<0.05$ were considered as significantly different.

Results

1. Effect of body weight and paw edema changes

Fig. 1 showed that the effect of the low intensity laser therapy on body weight and paw swelling in arthritis rats. In the present study, it is clear from the data obtained(Fig. 1A), that there is a close relationship between the extent of joint inflammation and the degree of weight loss. The low intensity laser therapy treated groups (acupuncture point or non-acupuncture point) exhibited the same progression patterns of body weights. But they exhibited low recovery of their body weights after non-acupuncture point treatment LILT, compared to the control group. There is a significant increase in rat paw volume in collagen injected arthritic control rats when compared to the normal control rats ($p<0.05$). Experimental groups Ⅰ and Ⅱ showed significant reduction in rat paw edema volume when compared with the arthritic group (Fig. 1B).

2. The changes of arthritic index

Arthritis developed about 2 weeks after the secondary injection of rats with type II collagen. The collagen induced arthritis rats randomly divided into groups respectively treated with LILT(acupuncture point or non-acupuncture point), and there were no significant ($P > 0.05$) differences in arthritis index between the experimental groups. The effects of LILT(acupuncture point or non-acupuncture point) on disease progression of collagen induced arthritis rats are shown in Fig. 2, LILT (acupuncture point; ST-36 GB39) significantly ($P < 0.05$) decreased arthritis index compared to the control between days 14 and 21 after the collagen-induced arthritis. Between these three groups, no differences in arthritis index were detected at 21 days.

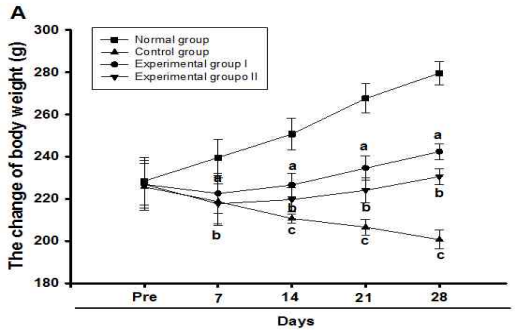

Fig. 1. Effect of low intensity laser treatment at acupuncture point on the body weight and the paw swelling in adjuvant-induced arthritis rats. (A) The body weight. (B) The edema rate. (All values are showed mean±SD. Value with different superscripts in the same column are significant($p<0.05$) by Duncan's multiple range test and compare with control group).

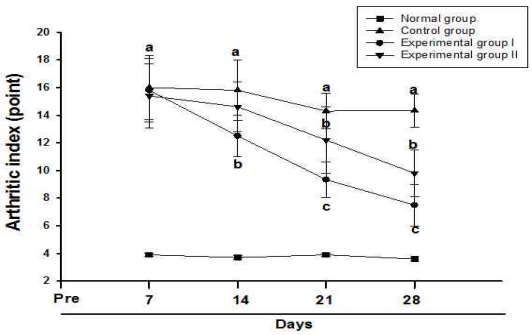

Fig. 2. Effect of low intensity laser treatment on the articular indexes in collagen-induced arthritis rats. (All values are showed mean±SD. Value with different superscripts in the same column are significant($ap<0.05$) by Duncan's multiple range test and compare with control group).

Table 2. Effect of low intensity laser therapy(acupuncture point and non-acupuncture point) on serum proteins.

Groups	Normal group	Control group	Experimental group Ⅰ	Experimental group Ⅱ
Total protein (g/dℓ)	10.54±1.25	6.04±0.90^a	7.90±1.06^b	6.68±1.02^b
Albumin (g/dℓ)	4.30±0.12	2.88±0.46^a	3.80±0.44^b	3.40±0.28^b
Globulin (g/dℓ)	1.88±0.10	3.04±0.08^a	2.32±0.06^b	2.66±0.08^b
Fibrinogen (㎎/dℓ)	25.60±6.04	58.04±8.82^a	31.18±9.00^b	46.66±10.04^c
Ceruloplasmin (㎎/dℓ)	12.30±2.65	30.82±9.08^a	19.54±2.90^b	26.42±3.26^c

(Valued are mean±SD, All values are showed mean±SD. Value with different superscripts in the same column are significant($p<0.05$) by Duncan's multiple range test and compare with control group).

3. The changes of biochemical factors

The changes in biochemical parameters in collagen induced arthritic rats are shown in Table 2. In collagen induced arthritic rats, there was significant decrease ($p<0.05$) in total protein and albumin levels but significant ($p<0.05$) increase in globulin level on comparison with the normal

group. Fibrinogen and ceruloplasmin are regarded as acute phase proteins. In arthritis-induced rats, these two acute phase markers were significantly ($p<0.05$) increased when compared with the arthritic control group (Table 3). Treatment with SPP and SPE have significantly ($p < 0.05$) decreased the levels of acute phase proteins in arthritic rats (Table 2).

Discussion

Rheumatoid arthritis (RA) is a chronic inflammation disease characterized by persistent joint synovitis, proliferation of the synovial cells, and destruction of the cartilage and bone in a symmetrical distribution[20]. Rheumatoid genetic factors are assumed to be involved in the development of disease, but the precise etiopathogenesis has not been completely elucidated[21]. Acupuncture therapy is a popular complementary treatment method in oriental medicine, and is receiving increasing interest in western medicine[22]. Also, acupuncture has been shown to be effective in pain relief suggested for treating various kinds of functional disabilities in traditional oriental medicine[23].

Low intensity laser therapy is defined as the stimulation of traditional acupuncture points with low intensity, non thermal laser irradiation. Various appliances with different intensity ranges are now available and are used in hospitals[24]. It is used for a wide variety of conditions including superficial skin sores, musculoskeletal and joint pain, and dentistry[25]. Moreover, although there is an implicit and unproven assumption that all acupuncture methods share common mechanisms, no studies have sought to elucidate the RA therapeutic mechanisms responsible for the benefits of laser therapy. Hence, we investigated the effects of low-level laser therapy in collagen-induced arthritis (CIA) rat model. The ST36 and GB39 and was used to evaluate the therapeutic effectiveness of the low intensity laser therapy.

Collagen-induced arthritis (CIA) in rats is an experimental model that shares some clinical features with human RA, such as swelling, cartilage erosion, inflammation, pannus and loss of joint function[26]. Paw swelling is an index of measuring the antiarthritic activity of various therapeutic method and it is employed here to determine the effectiveness of low intensity laser therapy. Low intensity laser therapy treatment markedly reduces the paw volume as compared with the arthritic control group. Yoshikawa et al[27] found that there was significant body weight loss, the day following the injection of the adjuvant, but thereafter continued to show normal weight gain in rats. It was previously reported that the redness and swelling of the arthritic joint and the loss of body weight usually began to appear at the onset stage of arthritis. Our result of the present study also indicates that there is a close relationship between the extent of synovial membrane inflammation and loss of body weight.

Low intensity laser therapy application mode were established according to previous results in acute inflammatory model in rats[28], myofascial pain and soft tissue edema in human[29]. We have demonstrated that the low intensity laser therapy stimulation is effective in experimental RA models of acute pain when used on acupuncture points, but is ineffective when used on surrounding joint tissue.

The Zusanli (ST36) and Xuanzhong (GB39) acupuncture point was selected on the basis of many scientific studies about its antiarthritic or analgesic effects[30,31]. In traditional oriental medicines, ST36 is one of the most frequently used acupuncture point and certainly the most intensively studied single acupuncture point in acupuncture and moxibustion therapies. Moreover, this acupuncture point has the advantage of being relatively easy to find in small animals, including rats and mouse[32].

In the present study there was a significant decrease in albumin level and increase in globulin level in arthritic rats. The collagen induced arthritis causes changes in plasma protein concentrations that are manifested as an increase in the globulin concentration and decrease in the albumin concentration[33]. In tish study treatment with low intensity laser therapy on acupuncture point significantly increased the albumin and decreased the globulin level in collagen induced arthritic rats which indicates that low intensity laser therapy might have a suppressive action on the mediators of inflammation. The measurement of acute phase proteins in plasma provides a clinically valuable information of the presence of inflammation and its degree[34]. Ceruloplasmin, a major plasma copper binding protein is produced in liver in response to tissue injury and released into circulation[35]. Thus, ceruloplasmin raised serum level during chronic arthritis might indicate long term tissue injury[36]. In the present study, the increased acute phase proteins fibrinogen and ceruloplasmin were significantly decreased by the low intensity laser therapy stimulation in acupuncture, which shows its effect in arthritic tissue repair.

Several studies have recorded the anti-inflammation effect of laser therapy[37-39]. In fact, very low laser therapy was effective in reducing edema and hyperalgesia in acute and chronic inflammation[28]. But It was ineffective in chronic rat polyarthritis, where almost complete joint destruction is found[40]. The ways in which laser treatment attenuates pain are not yet fully understood, but they are becoming more clear.

Conclusion

In the present study, we established an collagen-induced arthritis model in rats and evaluated the effects of low intensity laser therapy stimulation on the experimental arthritis. Finally there are controversial results about the efficacy of low intensity laser therapy (acupuncture point; ST36, GB39) in collagen induced arthritis. Our results showed improvements on body weight, paw edema volume, arthritic index, and inflammation serum protein collagen-induced arthritis rat. However no superiority over group II was observed.

In conclusion, these findings suggest that low intensity laser therapy on acupuncture point may offer therapeutic benefits for the treatment of collagen induced arthritis, at least partially through the reduction of arthritic swelling, pain, and inflammation serum protein. Our data also suggest that the inhibition of arthritic activity in extracellular fluids needs to be investigated as a potential strategy for therapeutic intervention in RA.

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