Controlled Animal Study

Acquiring the Optimal Time for Hyperbaric Therapy in the Rat Model of CFA Induced Arthritis

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Free full manuscript: www.painphysicianjournal.com **Background:** We previously published an article about the pressure effect using a rheumatoid animal model. Hyperbaric therapy appears to be beneficial in treating rheumatoid arthritis (RA) by reducing the inflammatory process in an animal model. In this sense, acquiring the optimal pressure-treatment time parameter for RA is important and no optimal hyperbaric therapy time has been suggested up to now.

Objective: The purpose of our study was to acquire the optimal time for hyperbaric therapy in the RA rat model.

Study Design: Controlled animal study.

Methods: Following injection of complete Freund's adjuvant (CFA) into one side of the knee joint, 32 rats were randomly assigned to 3 different time groups (1, 3, 5 hours a day) under 1.5 atmospheres absolute (ATA) hyperbaric chamber for 12 days. The pain levels were assessed daily for 2 weeks by weight bearing force (WBF) of the affected limb. In addition, the levels of gelatinase, MMP-2, and MMP-9 expression in the synovial fluids of the knees were analyzed.

Results: The reduction of WBF was high at 2 days after injection and then it was spontaneously increased up to 14 days in all 3 groups. There were significant differences of WBF between 5 hours and control during the third through twelfth days, between 3 hours and control during the third through twelfth days, and between 3 hours and 5 hours during the third through seventh days (P < 0.05). The MMP-9/MMP-2 ratio increased at 14 days after the CFA injection in all groups compared to the initial findings, however, the 3 hour group showed a smaller MMP-9/MMP-2 ratio than the control group.

Limitation: Although enough samples were used for the study to support our hypothesis, more samples will be needed to raise the validity and reliability.

Conclusion: The effect of hyperbaric treatment appears to be dependent upon the elevated therapy time under 1.5 ATA pressure for a short period of time; however, the long-term effects were similar in all pressure groups. Further study will be needed to acquire the optimal pressure-treatment parameter relationship in various conditions for clinical application.

Key words: Pressure effect, arthritic knee, arthritic pain, long-term effect of pressure, biophysiologic assessment, pain behavior assessment, arthritis treatment

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rthritis is a major cause of pain in adults and can lead to mental and social disabilities as well as physical illness (1-9). The pain condition of patients has been known to be influenced by weather changes (10-15) and pressure is also believed to treat animal models of inflammatory pain in previous studies (16,17). However, the exact mechanism of pressure has not been elucidated. We previously published the effect of pressure on arthritic knees in a rat model (18). In the previous study, we objectively measured the effect of pressure in a rat model for 2 weeks of experiment time. Following an injection of complete Freund's adjuvant (CFA) into one side of a knee joint, 32 rats were under 2.5 atmospheres absolute (ATA) in a hyperbaric chamber for 5 hours. The reduction of weight bearing (WB) of the affected limbs peaked at 2 days after the injection of CFA and then decreased spontaneously during the last day of the experiment in the control and study groups. In the high pressure group (2.5 ATA group), the WB was significantly better during the experiment. Also, the objective results for reduction of arthritis (the MMP-9/ MMP-2 ratio) decreased compared to the control group during experiment days. From the previous studies, we could observe that high atmospheric pressure improves a painful condition in the animal experiment study by reducing the inflammatory process.

However, some questions still remained. First, how long does the effect of pressure last after exposure to the high pressure? Second, how long should the pressure be given for a given pressure and what is the optimal pressure-time parameter? Third, how high should the pressure be given to the patients with arthritis for optimal therapeutic effects?

Recently we studied the first question and could observe favorable outcomes. During the 14 days of the experiment, the 3 hours of 1.5 ATA pressure effect appears to last for a given day, and the other group, pressure for 7 days and no pressure for 7 to 14 days, lasted one week. At the end of the experiment day, the effects between the 2 groups did not make a statistical difference. Also, we could acquire the expression of MMP-2/MMP-9 at 2 weeks after CFA injection in all groups. Therefore, we can assume that 3 hours of 1.5 ATA pressure effect might last for a given day and a minimum of 7 days of pressure treatment appears to be enough to give a therapeutic effect in an animal model.

The third question, how high is the optimal pressure for therapeutic effects for the animal model, should be considered in the future regarding the patient's safety and technical feasibility.

In this study, we studied the optimal time parameter that pressure should be given for a given pressure and what would be the optimal pressure for hyperbaric treatment.

Therefore, the purpose of our study was to figure out the optimal treatment time parameter of the pressure in the rat model of CFA induced arthritis.

METHODS

Experimental Animals

Experiments were performed on young adult male Sprague-Dawley rats (200 – 250 g, Hyochang Science, Daegu, Korea). The animals were housed in groups of 2 in plastic cages with soft bedding and were provided free access to food and water under a 12/12 hour reversed light-dark cycle (dark cycle: 8:00 A.M. – 8:00 P.M.). All animals were acclimated for 7 days before the experiment began. All experimental procedures were carried out according to the Animals (Scientific Procedures) Act 2008 (Korea) and complied with the recommendations of the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The studies were approved by the Ethics Committee on Animal Research of Pusan National University.

Induction of Arthritis

Experiments were done on the model of experimental mono-arthritis in the knee joint, complete Freund's adjuvant (CFA) models. The rat was anesthetized with isoflurane and CFA arthritis was induced by an intra-articular injection of 0.125 mL of CFA (Sigma, St. Louis, MO, USA) into the synovial cavity of the right knee joint. The joint was then manipulated by rapid flexion and extension movements for one minute.

Hyperbaric Chamber

A hyperbaric pressure chamber (Hyperbaric chamber, Shinhwa Medical, Korea) was used. 1.5 ATA pressure of oxygen is supplied from an oxygen generator outside (the concentration of oxygen is $7\ell/\min \pm 10\%$ and the velocity of oxygen is $70\% \pm 10\%$). Compression and decompression time can be controlled, and temperature and humidity is monitored from outside.

Test Group

Pain levels were measured at 10 hours after CFA injection and then the rats were tested after exposure to

the pressure chamber. The given pressure was increased from 1 ATA to 1.5 ATA for 30 minutes and lasted for 1, 3, and 5 hours. After exposure to each pressure, the pressure was decreased to 1 ATA for another 30 minutes. The set pressure was given daily for 14 days. After decompression each day, the pain behaviors were assessed in the whole study period for 14 days. The synovial fluid and knee joint samples were collected at 14 days after CFA injection in both groups.

ASSESSMENTS

Pain Behavior Test: Weight Bearing Measurement

To confirm that CFA-induced arthritic pain occurred in the rat knee, we measured the WB ratio using a weight-bearing device (Acculab Pocket pro 250-B, PA, USA) before and after the injection of CFA. This behavioral test is appropriate for measuring non-evoked pain behaviors. The detailed procedure to measure the WB ratio has been fully explained in a previous paper (18). The WB was converted to a WB ratio according to the following formula: (Post-injection value/pre-injection value) X 100.

Biophysiologic Assessments: Gelatin Zymography Analysis

For the gelatin zymography assay, the synovial fluids of the knee joints were obtained by inserting a 26 gauge hypodermic needle into the synovial cavity. This synovial fluid was then centrifuged at 1xg (1,000 rpm) and 4°C for 5 minutes. The protein concentrations of the supernatant were assayed using the Bradford method. Substrate gel zymography of the expressions and activities of MMP-2 and MMP-9 was performed using a previously described method (18) with some modification. Briefly, the synovial fluid supernatant was resuspended in a sample buffer and loaded (without boiling) into a 7.5% acrylamide/bisacrylamide (29.2:0.8) separating gel containing 0.1% (w/v) gelatin. Electrophoresis was carried out at 4°C. After electrophoresis, the gels were soaked in 0.25% Triton X-100 (twice for 30 minutes) at room temperature and then rinsed in distilled water. The gels were incubated at 37°C for 20 hours in an incubation buffer, stained for 30 minutes with 0.1% (w/v) Coomassie blue R-250 in 30% methanol and 10% acetic acid, and destained in the same solution without the Coomassie blue dye. The relative quantities of MMPs were measured by scanning densitometry using image analysis software (IMT i-Solution, IMT i-Solution

Inc., Vancouver, BC, Canada), and quantified by a comparison with standard MMPs.

Statistical Analysis

The data are expressed as the mean \pm standard error of mean (SEM). Statistical analyses were conducted by student t-test or one way analysis of variance (ANO-VA) followed by the Dunnett's post-hoc test. The *P*-value of less than 0.05 was considered to be significant.

RESULTS

Weight Bearing Measurement

The WB of the control groups, 1, 3, and 5 hours were depicted in Fig. 1. In all groups, the WB decreased from the first day and was lowest on the second day and then gradually increased until day 14. There were significant differences in WB between the 5 hour group and control group from the third to the twelfth days (P< 0.05). WB differences between the 3 hour and control group were significant during the third to the fifth and tenth to the twelfth days (P < 0.05). WB differences between the 3 hour group and control group were significantly different from the third to the seventh days (P < 0.05). During the thirteenth and fourteenth day, all pressure groups (1, 3, and 5 hours) did not make a statistical difference.

Gelatin Zymography Analysis

At 7 days and 14 days, 6 rats in each test group were sacrificed for analysis of gelatinase expression (Fig. 2). The synovial fluids of affected knee joints in both groups were collected. SDS-PAGE containing 0.25% gelatin of each group is shown in Fig. 2. The levels of MMP-9/MMP-2 ratio in the control group and 1 and 3 hour group activity at 14 days were 37.6 ± 4.4 , 18.0 ± 3.0 , and 10.3 ± 2.7 , respectively.

Discussion

Symptomatic treatment for arthritis has been performed clinically since most of the pain assessment was taken from pain behavior of the patients. Usually, pain and inflammation are treated with non-steroidal anti-inflammatory drugs (NSAIDS) and corticosteroids in clinical settings. There have been several possible explanations for arthritic pain, including increased sympathetic discharge, adrenergic sensitivity, and pain threshold changes.

In many reports, arthritis is believed to develop as a result of the autoimmune process in regional hypox-



Fig. 1. WB ratio of control and 1, 3, 5 hour pressure groups following the CFA injection into the knee joints. Over the entire test period, the test group showed a significant increase in the WB ratio. However at the thirteenth and fourteenth days, all pressure groups did not make a statistical difference.

CFA: Complete Freund's Adjuvant. The number of rats in each group is shown in parenthesis. ATA: Atmosphere Absolute. n: number of rats in each group.



Fig. 2. Gelatin zymographic analysis of the synovial fluid of the knee joints. Arthritis was induced by injecting CFA into the knee in 3 groups of 6 rats. The gelatinase expression ratio was reduced significantly by both of repetitive 1 hour or 3 hour exposure to 1.5 ATA at 14 days after the CFA injection (A). The ratio was more down regulated in 1.5 ATA-3 hour group than 1.5 ATA-1 hour group. The gelatin zymographic images of 14 days after the CFA injection are shown in B. Asterisks indicate values significantly different (* P < 0.05, ** P < 0.001) from the 1.0 ATA value by the one-way ANOVA followed by the Dunnett's post-hoc test. CFA: Complete Freund's Adjuvant. ATA: Atmosphere Absolute. emia (10,14,16,17). Increased oxygen demands and decreased blood flow in regionally damaged tissue could be one of the major factors causing arthritic pain. In this sense, the ability of pressure therapy to increase delivery and uptake of oxygen by tissue indicates potential therapeutic effects for arthritis.

Although several studies have been reported about the relationship between the pressure changes and arthritic pain, no study demonstrated the precise mechanism of the effect of pressure on pain.

McCarty (19) reviewed the available evidence in search for a rationale for hyperbaric treatment in the management of rheumatoid arthritis. Hypoxia of the arthritic patient is evidenced by low synovial P02 levels and increased dissoloved oxygen in the damaged tissue by increasing microcirculation of blood flow after pressure treatment.

With a biophysiological assessment, we observed that pressure treatment could be one of the major factors to decrease arthritic pain in an animal model in our previous study (18). In this study we tried to find the optimal treatment time parameter of pressure in the rat model. Under 1.5 ATA, 5 hours of hyperbaric treatment appears to be the most effective in relieving arthritis pain in the rat model by pain behavior and serologic assessments. However, the effects of each hyperbaric treatment were similar on the last day of the experiment. Although 5 hours of hyperbaric treatment is superior to 1 or 3 hours of treatment in relieving arthritic pain in the rat model, 1 or 3 hours of hyperbaric treatment also could be an alternative treatment option when it is applied to the RA patients.

There are several advantages in our study. Our study protocol consists of long pressure time during the day and slow compression and decompression time, which is believed to cause less stress for the animals.

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Also, 14 days is considered to be optimal to reveal the time course of arthritic pain in an animal model compared to the previous short one day protocol. Our study also revealed the effect of pressure on arthritis in an objective manner to reduce the possible flaws made by using a subjective assessment.

Although enough samples were used for the study to support our hypothesis, more samples will be needed to raise the validity and reliability. Further study should be aimed at investigating the precise mechanism of pressure effect on arthritis by quantitative and qualitative analysis. We expect a series of these studies to be the theoretical basis for alternative treatments options for the patients with arthritis.

CONCLUSION

The high pressure appears to be effective in relieving arthritic pain according to treatment for a given period of time. By investigating the precise mechanism of pressure effect, the high pressure might be an alternative treatment option for patients with arthritis.

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Conflict of interest: Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (i.e., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

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