

Animal Study

The Continuance Time of Pressure Effect in the Rat Model of Complete Freund's Adjuvant Induced Arthritis

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Background: We previously published on the pressure effect using a rheumatoid animal model (Pain Physician in 2013 and 2014). However, we do not know how long the pressure effect lasts after exposure to high pressure.

Objective: We evaluated the duration of the pressure effect in a day for a given study period.

Study Design: Laboratory animal study.

Methods: Following injection of Complete Freund's Adjuvant (CFA) into one side of the knee joint, 8 rats were assigned to 1.5 atmospheres absolute (ATA) hyperbaric chamber 3 hours per day for one or 2 weeks (1WPG or 2WPG). Pain levels were assessed daily for 2 weeks according to weight bearing force (WBF) of the affected limb. In addition, the levels of gelatinase, MMP-2, and MMP-9 expression in synovial fluids of the knees were analyzed.

Results: The reduction of WBF was high at one day after injection and then increased spontaneously up to 2 weeks in 1WPG and 2WPG. The pressure effects lasted for a given day and did not exceed the pressure effects of the next day in all study periods. Improvement of WBF in 2WPG was significantly greater than that of 1WPG during 8~14 days. The gelatinase expression ratio was significantly reduced in 1WPG and 2WPG, and 2WPG showed the lowest gelatinase ratio at 2 weeks.

Limitation: Although enough samples were used for the study, more samples will be needed to raise the reliability.

Conclusion: The 3 hours of 1.5 ATA pressure effect lasted for more than a day. Longer pressure exposure time appears to yield a greater therapeutic effect in an RA animal model up to a given study period. Continuous application of high pressure might be beneficial for achievement of a better therapeutic effect in 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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Arthritis is a major cause of pain in adults and can lead to mental and social disability as well as physical illness (1-9). Pain condition of the patient has been known to be influenced by weather changes (10-15), and, in particular, use of pressure in

treatment of animal models of inflammatory pain has been reported in previous studies (16,17). However, the exact mechanism of pressure has not been elucidated. We previously published on the effect of pressure on arthritic knees in a rat model (18,19). In the first study

(18), we performed an objective measurement of the effect of pressure in a rat model during an experimental period of 2 weeks. Following injection of complete Freund's adjuvant (CFA) into one side of a knee joint, 32 rats were placed in a hyperbaric chamber for 5 hours under 2.5 atmospheres absolute (ATA). In the control and study groups, reduction of weight bearing (WB) of the affected limbs peaked at 2 days after injection of CFA and then decreased spontaneously during the final day of the experiment. In the high pressure group (2.5 ATA group), significantly better WB was observed during the experiment. In addition, 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 in animal experiments, we observed that high atmospheric pressure improves a painful condition by reducing the inflammatory process.

However, there are still some questions remaining. First, how long does the effect of pressure last after exposure to high pressure? Second, how long should the pressure be applied for a given pressure and what would be the optimal pressure-time parameter? Third, how high should the pressure be for patients with arthritis in order to achieve optimal therapeutic effects?

Recently, we studied the second question and observed favorable outcomes (19). Although pain behavioral differences were observed during an experimental period of 2 weeks, no statistical differences were observed during the experiment and the final days of the experiment in the 1, 3, and 5 hours of treatment groups. In addition, we acquired the same serologic findings at 2 weeks after CFA injection in all groups. Therefore, we can assume that one hour of 1.5 ATA pressure might be more effective in the acute phase and might be sufficient to provide a therapeutic effect in an animal model up to 14 days.

The third question, how high would the optimal pressure be for achievement of the greatest therapeutic effects in a rheumatoid arthritis (RA) animal model, should be considered in future study regarding the patient's convenience and technical feasibility.

In this study, we attempted to answer the first question on how long the effect of pressure lasts after exposure to high pressure. Therefore, the purpose of our study was to determine the lasting effects of the pressure effect in a given day and to compare the pressure effect between 14 days and 7 days of pressure exposure groups in the rat model of CFA induced arthritis.

METHODS

Experimental Animals

Experiments were performed on 8 young adult male Sprague-Dawley rats (200 – 250 gram, Hyochang Science, Daegu, Korea). 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 performed 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 (PNU-2012-0041).

Induction of Arthritis

Experiments were performed on the model of experimental mono-arthritis in the knee joint, complete Freund's adjuvant (CFA) models. Rats were anesthetized with isoflurane and CFA arthritis was induced by 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 from outside (the concentration of oxygen is 7ℓ/min ± 10% and the velocity of oxygen is 70% ± 10%). Compression and decompression time and temperature can be controlled, humidity is monitored from outside.

Test Group

Pain levels were measured at 10 hours after CFA-injection and the rats were then 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 3 hours. After 3 hours at 1.5 ATA pressure, the pressure was decreased to 1 ATA for another 30 minutes. The set pressure was given daily for 14 days in the 2-week-pressure group (2WPG) and 7 days for the one-week pressure group (1WPG). Four rats each were allocated to 2WPG and 1WPG. After performance of decompression each day, pain behaviors were assessed at 0, 3, and 6 hours during the entire study period in 2WPG, and

0, 3, and 6 hours until one week and > 0 hours until 2 weeks in 1WPG. Synovial fluids were collected at 14 days after CFA injection in both groups.

ASSESSMENTS

Pain Behavior Test: Weight Bearing Measurement

To confirm the occurrence of CFA-induced arthritic pain in the rat knee, we measured the weight-bearing force (WBF) ratio using a weight-bearing device (Acculab Pocket pro 250-B, PA, USA) before and after injection of CFA. This behavioral test is appropriate for measurement of non-evoked pain behaviors. The detailed procedure for measurement of the WBF ratio has been fully explained in a previous paper (18,19). The WBF was converted to a weight bearing ratio according to the following formula: post-injection value/pre-injection value X 100. For the diurnal pain behavioral test, pain levels were assessed daily for one week according to weight bearing force (WBF) of the affected limb at 0, 3, and 6 hours of high pressure exposure. For comparison of the 1WPG and 2WPG group, the WBF were assessed immediately after exposure to high pressure for 8 to 14 days.

Gelatin Zymography Analysis

At 14 days, 4 rats from each test group were sac-

rified for analysis of gelatinase expression. Synovial fluids of the affected knee joints in both groups were collected. SDS-PAGE containing 0.25% gelatin of each group were performed. The levels of MMP-9/MMP-2 ratio in 1WPG and 2WPG activity at 14 days were acquired.

Statistical Analysis

Data are expressed as the mean \pm standard error of mean (SEM). Statistical analyses were performed using student t-test or one way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. A *P*-value of less than 0.05 was considered significant.

RESULTS

Weight Bearing Measurement

The Diurnal Pressure Lasting Effect in 2WPG

No diurnal difference of pressure effect was observed until day 14 of the experiment (Fig. 1). In addition, the pressure effect in a given day did not exceed the pressure effect of the next day. According to these results, the pressure effect appears to last more than 6 hours in a given day. The pressure effect showed a gradual increase daily and its effect lasted in a given day until day 14 of the experiment (Fig. 1).

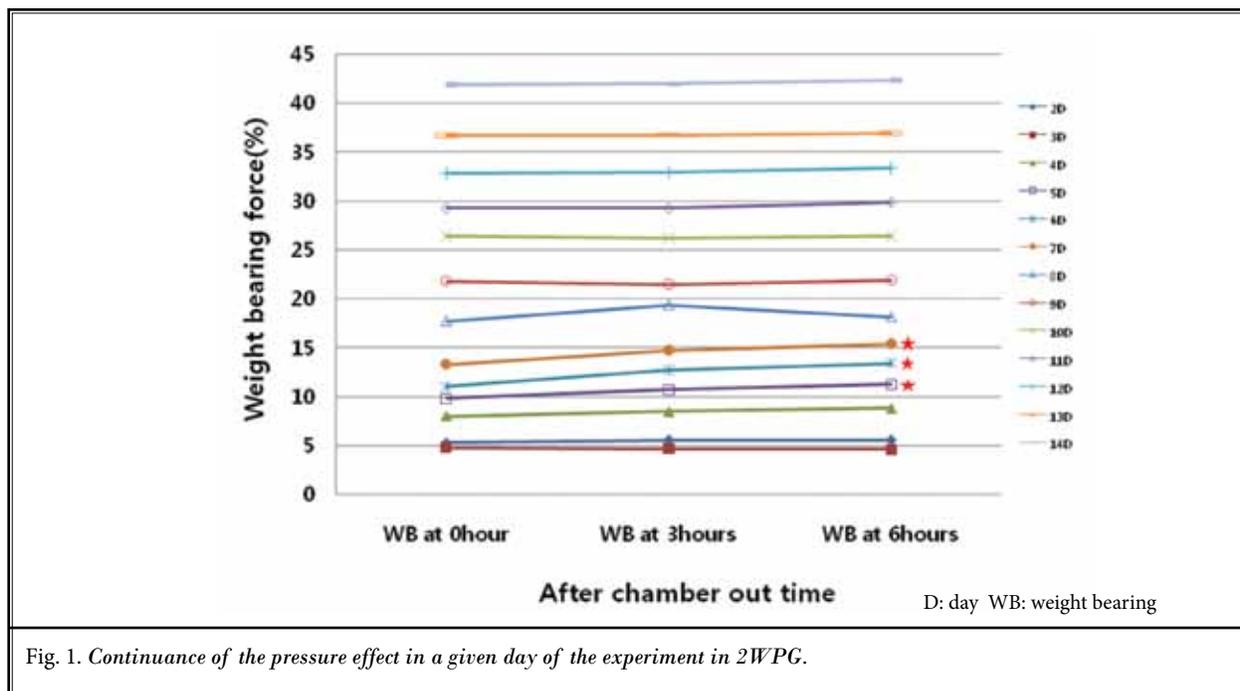


Fig. 1. Continuance of the pressure effect in a given day of the experiment in 2WPG.

The Pressure Lasting Effect between 2WPG and 1WPG

The WBFs of the control groups were decreased from the first day and were lowest on the second day in 2WPG and 1WPG (Fig. 2). From the third day, the WBFs were increased until day 14 in both groups. In 2WPG, significant differences were observed from day 8 to the end of experiments. However, 1WPG and 2WPG showed gradual diurnal improvement of WBF until day 14. There was no difference between 2WPG and 1WPG until 7 days. However, There was a significant difference from 11 days until 14 days of the experiment (Fig. 2).

Gelatin Zymographic Analysis

At 14 days, 4 rats in each test group were sacrificed for analysis of gelatinase expression (Fig. 3). Synovial fluids of the affected knee joints in both groups were collected. Results of SDS-PAGE containing 0.25% gelatin for each group are shown in Fig. 3. The levels of MMP-9/MMP-2 ratio in the control and 1WPG, 2WPG activity at 14 days were 35.6 ± 5.7 , 26.9 ± 10.6 , and 9.1 ± 2.7 , respectively.

Discussion

RA is a chronic debilitating disease, which is believed to develop as a consequence of an autoimmune

disease in regional hypoxemia (20). Several possible explanations for arthritic pain have been proposed, including increased sympathetic discharge, adrenergic sensitivity, and pain threshold changes (21-23).

Taking medication long term could be harmful and cause inconvenience to patients. Therefore, instead of treating arthritic pain and inflammation with non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, non-pharmacological treatments have attracted considerable attention. However, to date, no definite alternative therapeutic options for RA have been proposed. Weather change has been reported to have a major effect on arthritis, and, in particular, the relationship between pressure change and pain has been studied (16,17,24-27). In 1981, McCarty (28) reported a positive relationship between hyperbaric treatment and RA. However, due to a lack of controlled animal studies, long-term effects, and objective assessment, none of the previous studies addressed the objective pressure effect change on damaged tissue.

In our previous studies (18,19), we observed that exposure to hyperbaric treatment resulted in a decrease in neuropathic pain in an animal model. Increased oxygen demand and decreased blood flow in regional damaged tissue appears to be a major factor of arthritis. In this sense, the higher the pressure, the

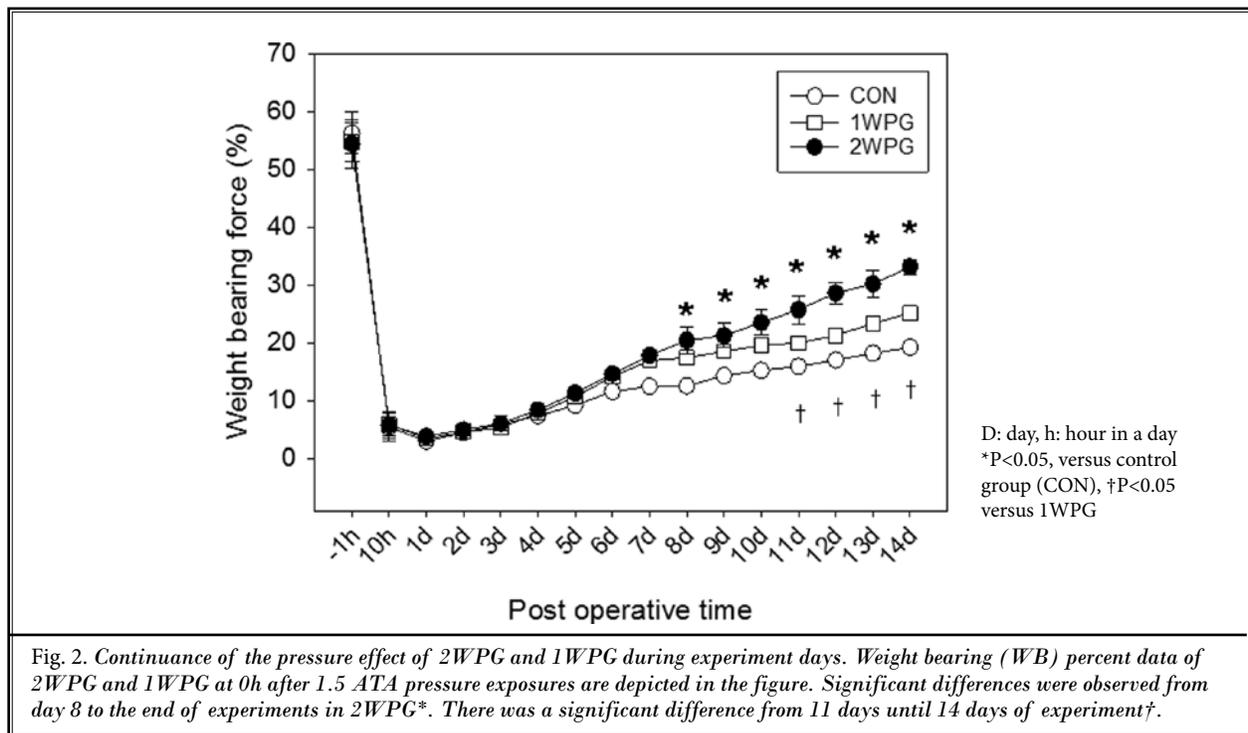
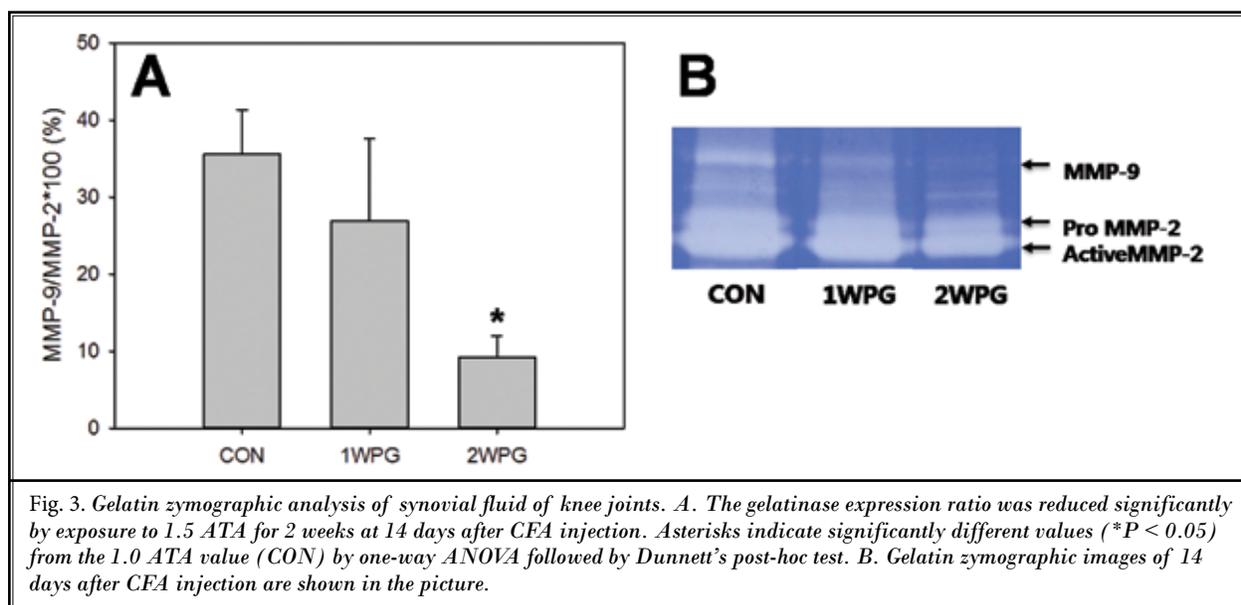


Fig. 2. Continuance of the pressure effect of 2WPG and 1WPG during experiment days. Weight bearing (WB) percent data of 2WPG and 1WPG at 0h after 1.5 ATA pressure exposures are depicted in the figure. Significant differences were observed from day 8 to the end of experiments in 2WPG*. There was a significant difference from 11 days until 14 days of experiment†.



higher the amount of oxygen uptake by tissue, suggests its potential for use in the treatment of arthritis.

However, we still do not know the exact optimal parameters of pressure for application to patients. Those parameters include duration of pressure treatment, optimal pressure treatment time, and optimal intensity of the pressure. Determining the optimal therapeutic parameter between "pressure and time" is of utmost importance to reducing the potential oxygen harm to patients and to minimizing the time for treatment.

Traditionally, hyperbaric oxygen therapy has been applied to patients with CO poisoning and air or gas embolism (29,30). In addition to the traditional use, various medical applications have been reported, including hematologic disease, vascular disease, and stroke patients (31-33). Usually, 1.5~2 ATA with 60 minutes of hyperbaric oxygen treatment appeared to be safe and no serious side effects were reported. However, chronic exposure to higher pressure might be harmful and may yield unwanted side effects. Therefore, prudential consideration should be given to a minimal application of hyperbaric oxygen treatment with the same results.

In this study, we observed the continuance time of pressure effect after exposure to hyperbaric oxygen. On each given day, the pressure effect lasted more than 6 hours and it was not greater than the effect of the next day. However, a statistical difference of the pressure effect was observed between 2WPG and 1WPG during 8 to 14 days. The effect of pressure appears to increase depending on the amount of the given pres-

sure time. Although the effect lasts more than a given day, there is no increase after cessation of pressure. In this sense, according to previous studies (17,34,35), 2 weeks of hyperbaric treatment time might be optimal for full treatment expression. In our animal study, the pressure effects could last for a given day and increased according to the given pressure time up to 2 weeks unless there was a cessation of pressure.

Our study has the advantage that the authors attempted to determine the optimal dose of time-pressure relationship for application to clinical settings. Although our results require additional consideration, they could provide basic data for a non-pharmacologic treatment option for RA and could reduce the minimal therapeutic dose of time pressure of hyperbaric treatment.

Our study has some limitations. Although a sufficient number of samples was used for the study to support our hypothesis, more samples will be needed in order to increase the validity and reliability. This is because even though a significant difference in pain behaviors during 8~14 days was observed between 1WPG and 2WPG at 1.5 ATA, it does not mean that the same results will be obtained for all different pressure groups, such as 2 ATA and 2.5 ATA. In addition, the RA condition induced by experimental animal study could not completely match the clinical RA setting in humans. Therefore, although the optimal parameters in an animal study were acquired, the results should be seriously considered before application to clinical treatment.

We expect that a series of these studies will provide a theoretical basis for alternative treatment options for patients with arthritis. Further study should be aimed at investigation of the precise mechanism of the pressure effect on arthritis by quantitative and qualitative analysis.

CONCLUSION

The 3 hours of 1.5 ATA pressure effect appears to last during a given day and increased according to the given pressure time for 2 weeks. Further study will be needed in order to determine an optimal pressure-treatment time parameter for clinical application.

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