

Animal Study

## The Effects of Pressure on Arthritic Knees in a Rat Model of CFA-Induced Arthritis

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**Background:** Pain is influenced by weather changes under certain circumstances, and inflammatory pain in animal models is ameliorated by pressure, but the underlying mechanism of atmospheric pressure has not been clearly elucidated.

**Objective:** To examine the effect of pressure on pain in an arthritic animal model.

**Study Design:** Controlled animal study.

**Setting:** Laboratory animal study.

**Methods:** Following an injection of complete Freund's adjuvant (CFA) into one side of a knee joint, 32 rats were assigned randomly to 2 groups and either placed under 1 or 2.5 atmospheres absolute (ATA) in a hyperbaric chamber for 5 hours. The pain levels were assessed daily for up to 2 weeks post-injection to determine the changes in weight bearing (WB) of the affected limbs. In addition, the levels of gelatinase, MMP-2, and MMP-9 expression in the synovial fluids of the knees were analyzed.

**Results:** After arthritis induction, the rats in the 1 ATA group showed reduced WB of the affected limbs (< 10% of normal limbs). This reduction in WB peaked at 2 days after the injection and then decreased spontaneously. Nevertheless, the pain behavior lasted for more than 2 weeks. In the 2.5 ATA group, the WB was significantly better during the experiment. The MMP-9/MMP-2 ratio increased at 7 and 14 days after the CFA injection in the 1 ATA group. However, repetitive exposure to 2.5 ATA significantly reduced this ratio in the 2.5 ATA group.

**Limitations:** Although a sufficient number of samples were used to support the hypothesis that high atmospheric pressure improves a painful condition in this study, an additional larger-scale study will be needed to confirm these findings.

**Conclusion:** Exposure to elevated pressures appears to relieve arthritic pain for extended periods by reducing the inflammatory process and should be considered as a possible alternative pain-reducing therapy.

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

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**A**rthritis is a major cause of pain in adults (1-3). Moreover, arthritic pain can lead to mental and social disabilities as well as physical illness (4-12). Furthermore, the number of patients suffering from arthritis worldwide is expected to increase from approximately 46 million in 2005 to 65 million in 2030 due to the aging population (13).

Weather has been reported to have a major effect on arthritic pain, and various relationships between weather and arthritic pain intensity have been reported (13-18). So called "weather-sensitive pain" has been attributed to changes in barometric pressure, humidity, or temperature (13-18). Weather-sensitive pain in humans has been studied using only psychological factors, which could be affected by a range of confounders, such as emotional factors, individual variability, and different pain thresholds (19). The, not surprisingly, reported results regarding the relationships between weather changes and arthritic pain are disparate (20-24). Several clinical studies have reported a consistent relationship between changes in meteorological factors and pain intensity in subjects with chronic pain (14), but other studies failed to find any relationships between pain and weather changes (25,26).

Compared to other meteorological factors, the relationship between barometric pressure changes and arthritic pain has attracted little attention. The majority of studies on the effects of pressure assessed subjective human pain using questionnaires and retrospective study designs (13,15,27,28), and failed to identify an objective relationship between pain intensity and pressure changes. Furthermore, only a few controlled animal studies have been conducted on the effects of pressure on arthritic pain (29,30).

Sato et al (29) reported the relationship between pressure changes and pain intensity using an animal model, but their study, which was conducted for one hour in one day, did not reveal a persistent effect of pressure. Furthermore, abrupt pressure changes (i.e. within one hour) could also influence pain intensity. Thompson et al (30) examined the effect of pressure over 14 days, but assessed only pain-related behavior rather than using an objective measure of the changes in pain intensity.

Accordingly, the relationship between the effect of pressure and arthritic pain has not been assessed objectively or quantitatively, and no study has examined the long-term effects of pressure or the mechanism involved. Therefore, this study examined the relationship between the long-term effects of pressure in a

rat model of arthritis using bio-physiological and pain behavioral assessments.

## **METHODS**

### **Experimental Animals**

The experiments were performed on 16 young adult male Sprague-Dawley rats (200 – 250 g, Hyochang Science, Daegu, Korea). The animals were housed in pairs in plastic cages with soft bedding and were provided access to food and water ad libitum under a reversed 12/12 hour light-dark cycle (dark cycle: 8:00 A.M. – 8:00 P.M.). All animals were acclimated for 7 days before beginning the experiments. All experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act 2008 (Korea) and all complied with the recommendations of the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The study was also approved by the Ethics Committee on Animal Research at Pusan National University (PNU-2012-0041).

### **Induction of Arthritis**

The experiments were performed using a complete Freund's adjuvant (CFA) model of experimental monoarthritis of the knee joint. Briefly, arthritis was induced as follows. A rat was anesthetized with isoflurane and then intraarticularly injected with 0.125 mL of CFA (Sigma, St. Louis, MO) in the synovial cavity of the right knee joint. The joint was then manipulated by rapid flexion and extension for one minute.

### **Hyperbaric Chamber**

A hyperbaric pressure chamber (Hyperbaric chamber, Shinhwa Medical, Korea) was used for the experiment. The chamber allows pressures ranging from one to 2.5 atmospheres. Oxygen is supplied from an external oxygen generator (the oxygen flow rate was 7L/min  $\pm$  10% and the oxygen concentration was 70%  $\pm$  10%). This unit allows the compression and decompression times to be controlled with constant temperature and humidity monitoring.

### **Test Groups**

The pain levels were measured at 10 hours post-injection and the rats were then randomized into the 2 study groups (1 and 2.5 ATA groups). For the 2.5 ATA group, the chamber pressure was increased from one ATA to 2.5 ATA over a 30 minute period and held at that pressure for 5 hours. After 5 hours, the pressure was

decreased to one ATA over another 30 minute period. As a control group, the 1 ATA group was placed in the chamber with the pressure maintained at atmospheric pressure for the same period. The pressure treatments were administered daily for 14 days. After decompression on each day, the pain-related behaviors were assessed. The synovial fluid and knee joint tissue samples were collected 7 and 14 days post-injection (Fig. 1).

## Assessments

### Pain Behavior Test: Weight Bearing Measurements

To confirm that CFA-induced arthritic pain occurred in rat knees, the weight-bearing (WB) ratios (defined as post-injection WB/pre-injection WB X 100) were measured using a weight-bearing device (Acculab Pocket pro 250-B, PA, USA) before and after CFA administration. This behavioral test is appropriate for measuring non-evoked pain behaviors (31). The detailed procedure used to measure the WB ratio is explained elsewhere (31).

### 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 (32) 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.

### Histology

Rat knee joints were fixed in 10% neutral buffered formalin for 48 hours and decalcified using ethylene diamine tetra acetic acid (EDTA) for 14 days. The knee joints were sectioned along the longitudinal axis. All samples were processed and embedded in paraffin. After paraffin embedding, 5 µm thick sections through the entire explant thicknesses were prepared using a tungsten-carbide blade on a rotary microtome. The sections were stained with hematoxylin and eosin.

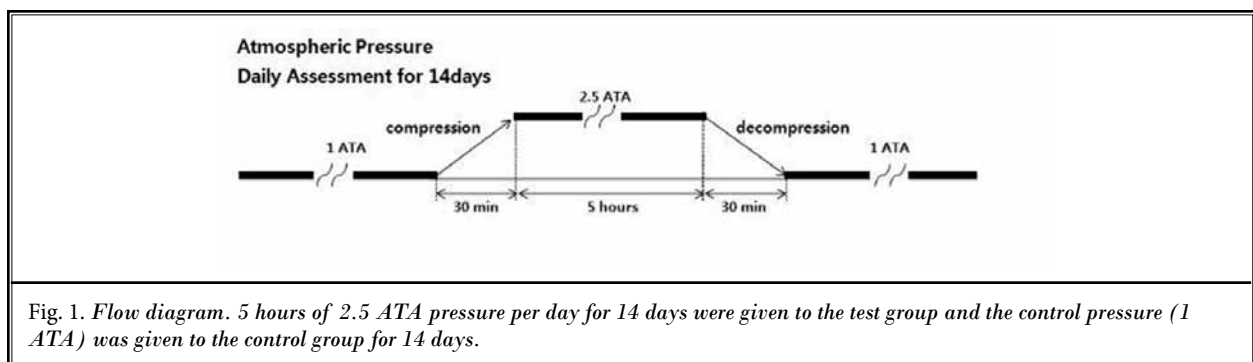
### Statistical Analysis

The data is expressed as the mean ± standard error of mean (SEM). Statistical analyses were conducted using a student's t-test or by one way analysis of variance (ANOVA) followed by a Dunnett's post-hoc test. *P* values < 0.05 were considered significant.

## RESULTS

### Weight Bearing Measurements

The WB in the control 1 ATA group decreased one day post-injection and was lowest at 2 days post-injection (Fig. 2). In contrast, the WBs in the 2.5 ATA group did not decrease until 2 days post-injection. From the



third day, the WB in both groups increased until day 14. The 2.5 ATA group showed more significant improvement in the WB than the control group throughout the test period.

### Gelatin Zymography Analysis

At 7 and 14 days post-injection, 6 rats per group were sacrificed to analyze gelatinase expression. The synovial fluids of the affected knee joints in both groups were collected. Fig. 3 presents the SDS-PAGE containing 0.25% gelatin results for the 2 groups. The MMP-9/MMP-2 ratios in the control and test group at 7 and 14 days post-injection were 26.7 and 12.71, and 18.7 and 8.7, respectively. The MMP-9/MMP-2 ratios at 14 days were lower than that at 7 days in both groups.

### Histology

In the control group, the knee joint histology was normal, with no abnormalities and a homogeneous matrix (Fig. 4A and 4D). In contrast, a section of the synovial joint of the CFA injected 1 ATA group revealed the for-

mation of pannus, invasion of the intraarticular cavity, and erosion of the articular cartilage and bone (Fig. 4B and 4E). The section of the CFA injected 2.5 ATA group revealed a reduction of the abnormal changes observed in the CFA injected 1 ATA group (Fig. 4C and 4F).

### Discussion

Symptomatic treatments for arthritis have attracted considerable attention because most pain assessments assessed pain behavior of the patients. Normally, pain and inflammation are treated with non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids in a clinical setting. Several possible explanations of arthritic pain have been proposed, which include increased sympathetic discharge, adrenergic sensitivity, and pain threshold changes (33-35).

In many reports, arthritis is believed to develop as a result of an autoimmune process in regional hypoxemia. Increased oxygen demand and decreased blood flow in the regional damaged tissue can be major factors causing arthritic pain (36). In this sense, the ability

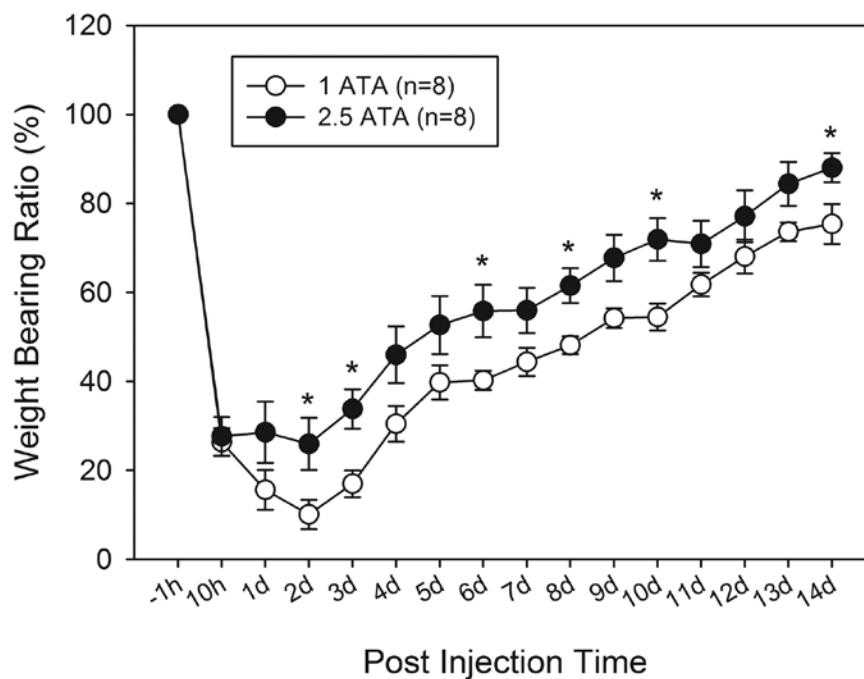
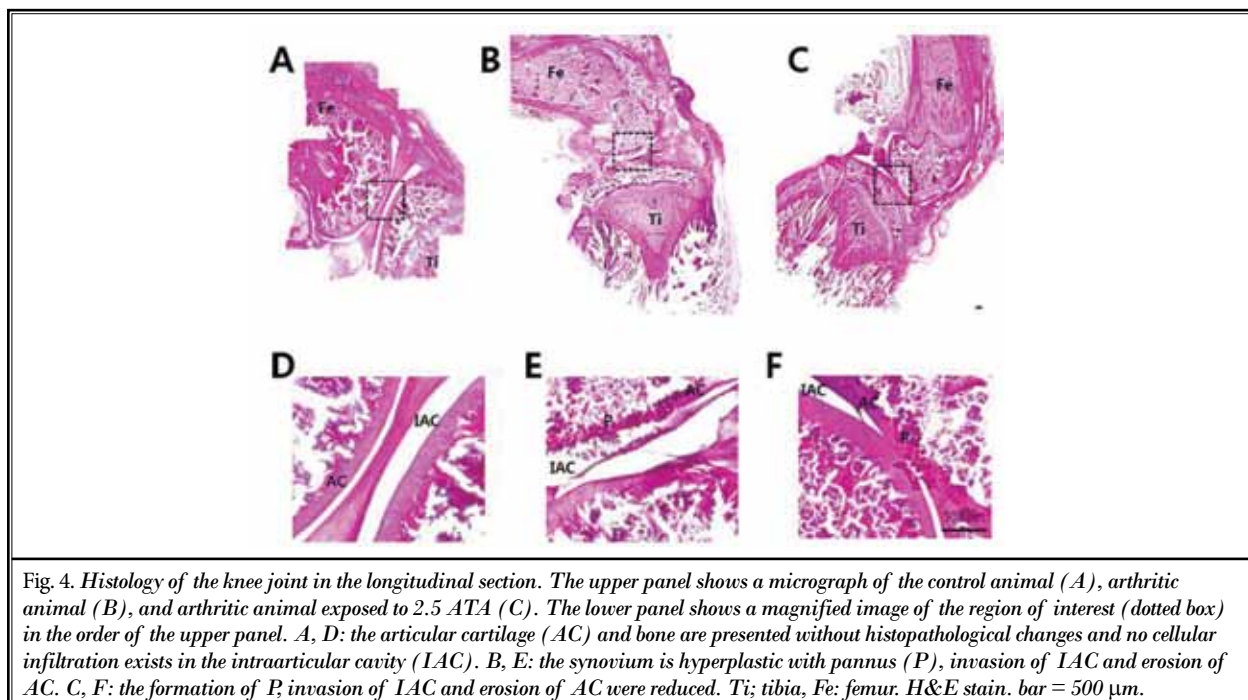
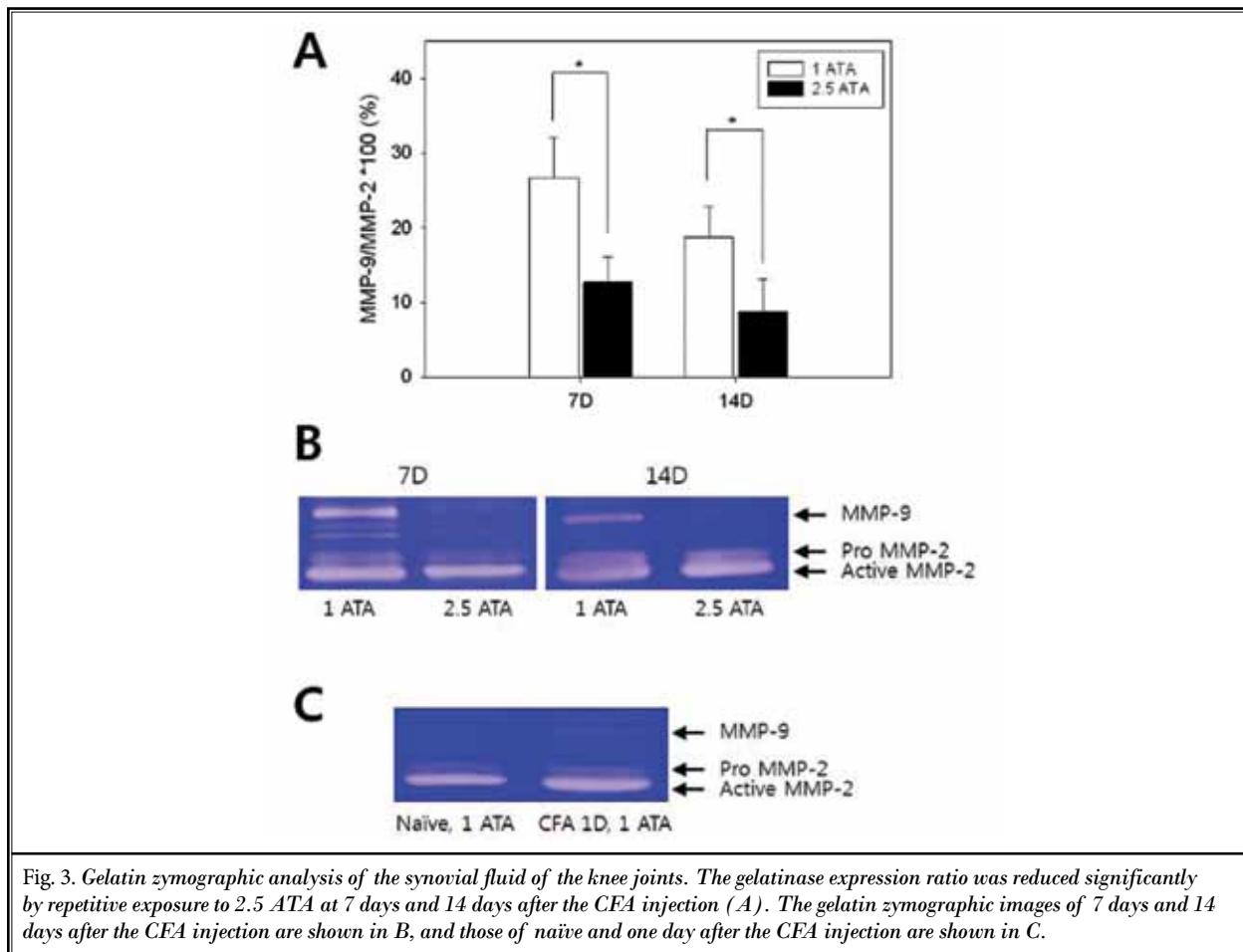


Fig. 2. Weight bearing ratio following the CFA injection into the knee joints. Over the entire test period, the test group showed a significant increase in the weight bearing ratio.

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



of pressure therapy (37) to increase the delivery and uptake of oxygen by tissue suggests that it can be a potential treatment for arthritis.

Up to now, several studies have reported the relationship between pressure changes and arthritic pain. Hyperbaric treatment — oxygen under pressure — is one of those avenues explored. On the other hand, no study has demonstrated the precise mechanism of the pressure effect on pain. McCarty (37) reviewed the available evidence in the search for a rationale for hyperbaric treatment in the management of rheumatoid arthritis (RA). Hypoxia of arthritic patients is evidenced by the low synovial PO<sub>2</sub> (partial oxygen pressure) levels but these are not specific to RA. Several studies reported (38) that local inflammation could be the key factor in arthritic pain and arthritic pain could be decreased by relieving this inflammation. Increased dissolved oxygen in the damaged tissue and increased microcirculation of blood flow could occur after a pressure treatment, and these changes can have favorable effects on the damaged tissue. Therefore, a pressure treatment by delivering oxygen to the damaged regional tissue might decrease the inflammation associated with neuropathy and decrease the level of pain.

Previous studies reported that a hyperbaric treatment decreased the neuropathic pain in an animal model but only a subjective assessment of the pain intensity was used. The change in behavior, of which the nature of pain is psychological, can also be influenced by a range of confounding factors, such as emotional, individual variability, and different pain thresholds. Subjective human studies, one day protocols (39,40), and no-controlled animal studies did not reveal a direct relationship between weather factors and pain intensity by a subjective pain behavioral assessment (29,41,42). In addition, none of these studies addressed the objective pressure effect change on damaged tissue, and most studies did not observe the long-term effects of pressure changes in an animal model.

Cartilage degradation in osteoarthritis (OA) has been considered to be mediated mainly by the matrix metalloproteinases (MMPs), which are responsible for cartilage collagen breakdown both in normal physiological processes, such as development and wound healing, and in several disease states (43). In the pathogenesis of RA and OA, degradation of the cartilage collagen matrix is primarily accomplished by

MMPs that include collagenases (MMP-1, MMP-9, and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3 and MMP-10), and membrane type MMPs (MP-MMPs). The increase of the enzyme activity of MMP-9 compared to MMP-2 is closely involved in the collagen breakdown which is considered to be the object measurement tool for arthritic activity of the affected joints. In our study, we could also observe the low enzyme activity of the MMP-9/ MMP-2 ratio in the test group. By assessing a biophysiological assessment, the pressure treatment was found to be one of the major factors decreasing arthritic pain in the animal model in this study. The pain behavior and morphological changes also showed the same results reported in previous studies.

This study had several advantages. The study protocol consisted of long pressure time during the day and a slow compression and decompression time, which is believed to cause less stress to the animals. In addition, the 14 day study period is considered optimal to reveal the time course of arthritic pain in an animal model. This study also revealed the effect of pressure on arthritis objectively to reduce the possible flaws made using a subjective assessment.

Nevertheless, although a sufficient number of samples were used in this study to support our hypothesis that high atmospheric pressure improves painful conditions, more samples will be needed to improve the validity and reliability. Further studies should examine the precise mechanism of the pressure effect on arthritis by quantitative and qualitative analysis. A series of these studies is expected to form the theoretical basis of alternative treatment options for the patients with arthritis.

## CONCLUSION

During long-term exposure in a hyperbaric chamber, the high pressure appears to be effective in relieving arthritic pain by decreasing the inflammatory process. By examining the precise mechanism of the pressure effect, high pressure might be an alternative treatment option for patients with arthritis.

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