Anti-arthritic effect of radix *Paeoniae rubra* herb-acupuncture: a behavioral evaluation on adjuvant-induced arthritis rats

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SUMMARY

Radix *Paeoniae rubra* (PR) has been widely used for recovering acute inflammation with swelling and pain in Far East Asian countries. In order to investigate the anti-arthritic effect of PR herb-acupuncture, several behavioral parameters such as body weight, knee circumference, squeaking threshold, and weight distribution ratio were investigated in an adjuvant-induced mono-arthritis rat model. We performed PR herb-acupuncture on Zusanli (ST36) of the right hind-leg once every second day for a total of 6 times from the second day of initiating arthritis with adjuvant injection, while the control group was treated with saline in the same way. In the experimental group, body weight significantly increased, knee circumference and squeaking threshold significantly decreased indicating the reduction of edema, and both the pain and inflammatory swelling of the hind paws measured by incapacitance meter decreased. Conclusively, PR herb-acupuncture has more promising effect in alleviating arthritic pain and inflammation than saline-acupuncture in rat model, hence further clinical study is required.

Key words: Radix *Paeoniae rubra*; Herb-acupuncture; Acupoint; Monoarthritis; Rat

INTRODUCTION

Arthritis is a common response of joint tissues which have undergone multiform injuries. Its causes include joint deformation, genetic inheritance, physical force, inner-cellular changes and biochemical factors. (Braunwald *et al.*, 2002) Its pathological symptoms usually result from the failure of the cartilage repairing function to keep up with cartilage breakdown followed by accompanying inflammation. Common symptoms of arthritis are pain, stiffness, limited motion of joints, swelling, and creaking of joints, and the knee joint is the most frequently affected by osteoarthritis (Bennell and Hinman, 2005). Knee osteoarthritis is found in approximately 10% of the population over the age of 65 (Krohn, 2005). Surgical approaches, several pharmacologic and non-pharmacologic methods are available, and interestingly, non-pharmacologic methods including exercise, weight loss, rest, use of canes and crutches, physical therapy, osteopathic manipulative treatment, and braces received attention. (Rubin, 2005) Besides these, acupuncture and herb-
acupuncture have been advocated for its clinical effectiveness.

Several types of acupuncture for arthritis have already been studied to some extent: acupuncture (Arichi et al., 1983; Thomas et al., 1991; Laing et al., 2002; Um et al., 2005), electro-acupuncture (Shafshak, 1995), bee venom acupuncture (Kwon et al., 2001; Lee et al., 2004; Vas et al., 2004) and herb-acupuncture (Yeom et al., 2003, 2006). Among the methods, herb-acupuncture has been used for curing various diseases such as osteoarthritis, shoulder pain, and lumbago in Korea. The method is an injection of distilled preparation of herb on acupuncture point (Yeom et al., 2003, 2006). Meanwhile, Zusanli (ST36) acupoint was used in most of those studies, and is known for spasmolytic and analgesic effects on knee joints (Camp, 2001; Kwon et al., 2001; Tillu et al., 2001; Baek et al., 2006; Yeom et al., 2006) and gastrointestinal tract (Lee et al., 2001), and homeostatic effect in the endocrine system and metabolism (Lian et al., 2000; Jang et al., 2003; Choi et al., 2004; Li et al., 2005; Tseng et al., 2005).

We were encouraged by our previous studies of herb-acupuncture and some other studies of Paeoniae rubra (PR), red peony root, which has been used to alleviate a febrile disease, to manifest blood stagnation, and to recover acute inflammation with red swelling and pain from external injury (Bensky and Gamble, 1993; Ahn, 1998; Korean Institute of Herbal Acupuncture, 2000). In this study, the anti-arthritic effects of PR herb-acupuncture were investigated in an adjuvant-induced mono-arthritis rat. To assess the arthritic symptoms of pain and inflammation, several preclinical and behavioral tests, such as body weight, knee circumference, and squeaking threshold, weight distribution ratio, were measured and their accuracy and effectiveness as an arthritic indicator were compared.

**MATERIALS AND METHODS**

**Animals**

Female Sprague-Dawley rats (Samtaco Co., Osan, Korea), weighing 120 - 140 g, were used for the experiments. Animals were given a period of 1 week to adjust to the new environment (22 ± 2°C, 45 - 60% humidity, and 12 h light/12 h darkness cycle, raising 5 - 6 animals per cages) and provided with hard food (Oriental Co., Korea) and water ad libitum. The experimental procedures were carried out according to the animal care guidelines of the NIH and the Kyung Hee University Institutional Animal Care and Use Committee.

**Arthritis induction**

In this study, a mono-arthritis inducing protocol, that is, a fixed quantity of the antigen was injected into the synovial cavity of the knee joint, was used (Neto et al., 1999; Yu et al., 2002; Bäckdahl et al., 2003). After anesthetized with halothane, the rats were injected with 80 mg of arthritis-inducing adjuvant with a disposable 1 ml-syringe (26 guage needle, Korea vaccine Co., Korea) at the synovial cavity of the right knee joint. The arthritis-inducing adjuvant was 1:1 mixture of saline and heat-killed Mycobacterium tuberculosis H37 RA (Difco Co., Detroit, MI, USA), emulsified in 0.15 ml squalene (Sigma-Aldrich Chemical Co., MO, USA). An injected region was sterilized by 70% ethanol.

**Herbal component of herb-acupuncture**

The dried herb of Radix Paeoniae rubra (PR) was kindly supplied from Kyung-Hee University Oriental Hospital (Seoul, Korea), and voucher specimens have been deposited in the Herbarium of Kyung-Hee University. The distilled water extracts of PR for herb-acupuncture treatment were manufactured in an ampoule by Korean Institute of Herbal Acupuncture (Seoul, Korea). The manufacturing processes sequentially proceeded by washing the herb by distilled water, boiling, evaporation, condensation, filtering the extract with 0.45 µm and 0.1 µm filter films, refrigerated precipitation for mineral exclusion using 0.9% NaCl, pH regulation by Na3PO4 and citric acid, and high pressure sterilization steps, as previously reported (Korean
Institute of Herbal Acupuncture; 2000; Yeom et al., 2003), which were always under the quality controls of no suspending particles using a visible spectrophotometer. The extraction was started with 200 g of dried PS and finally produced 300 ml of PS distillates for herb-acupuncture. By the regulation of Korean Institute of Herbal Acupuncture, the PS distillates were passed the germ-free, anti-histamine, and endotoxin-free tests, and thus accepted as an s.c. injection grade for human.

**Experimental groups**

Experimental animals were divided into three groups:

1) no-treated, non-arthritic animals (NOR, n = 10);
2) PR-treated/ST36 acupoint (Zusanli) arthritic animals (PR, n = 10); and 3) saline-treated/ST36 acupoint (Zusanli) arthritic animals (SAL, n = 10).

After induced, the adjuvant-induced rats were randomly divided into two groups (PR and SAL) on day 2. Degrees of redness and swelling on the joints were similar between the two groups, and rats having symptoms which were too severe or not induced were excluded.

**Herb-acupuncture therapy**

The PR herb-acupuncture treatment initiated on day 2 was the post adjuvant injection and executed to the right leg every second days for 12 consecutive days, for a total of 6 times. The 0.05 ml of the water extract of PR was subcutaneously injected into ST36 acupoint using 0.5 ml insulin syringe (30 gauges, BD Biosciences Co., CA, USA). The acupoint is located near the knee joint of the hind limb, 2 mm lateral to the anterior tubercle of the tibia. The injected region was sterilized by 70% ethanol just prior to the treatment.

**Behavioral & pre-clinical parameters**

From day 0 to day 14, body weight was regularly measured every second day by a digital balance, especially on day 1. After measuring body weight, the circumference of the knee joint was measured twice at the lateral and medial ligament region of the joint, using a flexible and elastic band. Calculating the average of results, we compared the difference between the average circumference of an arthritis-induced knee joint (right side) and that of a healthy joint circumference (left side). Then, squeaking threshold was investigated by slowly extending and flexing the arthritic-induced knee joint of a rat five times. The recorded results were then calculated. Each squeak was counted as one point, and the highest point was 10. The interval of the extension-flexion test was 5 s. A rat was placed into a specifically created sloping acrylic box, and the lower limbs were positioned onto an IITC 600 capacitance meter (IITC Life Science Inc, CA, USA). The average of the weight loaded at the each lower limb was measured for 5 s and repeated four times. Then, the average of the series was utilized. Using this method, weight distribution ratio was measured on day 0, 2, 8, 14, and was then calculated using the next formula.

\[
\text{Weight Distribution Ratio} = \frac{\text{weight borne by ipsilateral paw}}{\text{total weight borne by both paws}} \times 100
\]

**Data analysis**

The data were presented as the mean ± S.E.M. The significance of the differences between groups was assessed by a one-way analysis of variance (ANOVA), followed by the Tukey HSD post-hoc analysis. Difference was considered significant at \( P < 0.05 \).

**RESULTS**

Using an adjuvant-induced mono-arthritis rat, the therapeutic effect of PR herb-acupuncture for arthritis was investigated in this study. Behavioral tests such as body weight, knee circumference, and squeaking threshold were measured, and those results were compared with weight distribution ratio.
Body weight

Fig. 1 displays the effect of PR herb-acupuncture therapy on body weight of adjuvant-induced mono-arthritis rat. Until day 2, there was little increase in body weights of all rats involved in the experiment. From day 2, the body weight of the PR group continually increased, and during days 6 and 14, a significant increase in weight was observed in the PR group as compared to that of SAL group (day 8: \( P < 0.05 \), day 14: \( P < 0.01 \)). In terms of water and food consumption, there were no significant differences amongst the three groups (data not shown). In the case of the NOR group, the weight increase was more pronounced, as compared to those in the arthritis-induced groups (PR and SAL).

Knee circumference

For measuring the degree of edema in an arthritis-induced knee joint, the variation of knee circumference was analyzed. Fig. 2 displays the effect of PR herb-acupuncture therapy on the knee circumference of an adjuvant-induced mono-arthritis rat. A variation in the knee circumference of the NOR group was not observed during the experimental period (14 days). In the PR and SAL groups, a decrease was noticed beginning from day 2, and the circumference decreased less than 0.2 cm after day 6. However, a significant decrease between the two groups was not observed during the first 10 days.

Squeaking threshold

In the case of the NOR group, from 0 day to 14 day (conclusion of the experiment), most of the squeaking scores were zero. However, a difference of squeaking scores between the PR and SAL groups could be observed. The score of the SAL group was almost 10, and it decreased a little after day 12. On the other hand, the score of the PR group maintained a score of about 7 after day 6, which was significantly less than that of the SAL group (\( P < 0.001 \)). (Fig. 3)

Weight distribution ratio

Fig. 4 shows the effect of PR herb-acupuncture therapy on weight distribution between the paws of an adjuvant-induced mono-arthritic rat. The weight distribution ratios of the unilateral paws in the NOR group were nearly 50%. In case of the PR
group, the weight distribution ratio of the arthritis-induced paw (right side) in the early stage was 20%. However, a change in the ratio could be seen from day 8, and the change was significant (30%, \( P < 0.05 \) vs SAL group) on day 14. In comparison, the ratio of an arthritis-induced paw in the SAL group was maintained at about 30% in the early stage, however, the ratio continued to decrease during the whole experimental period, and it was at about 15% on day 14. This means that the saline injection into the acupoint did not produce a medicinal effect and the arthritic symptoms worsened as time passed.

**DISCUSSION**

Herb-acupuncture therapy is one of the newer therapies, is it widely used in Korean traditional medicine. It has adopted both the medical benefits of acupuncture and herbal therapy (Kwon et al., 2001; Yeom et al., 2003; Lee et al., 2004; Yeom et al., 2006). In this study, radix PR was selected as the material for the herbal extract. It contained paeoniflorin, albiflorin, paecn, paeonol, benzoic acid, tannin, etc. This extract has been widely used in traditional Korean and Chinese medicines for thousands of years to invigorate the blood and remove blood stasis, to clear heat and cool the blood, and to clear liver fire (Bensky and Gamble, 1993; Ahn, 1998). Concretely, it has been known to be an effective medicine in alleviating febrile disease (Genfa et al., 2005), manifesting blood stagnation such as dysmenorrhea and amenorrhea (Xie et al., 2005), dispelling abdominal pain and immobile abdominal masses, reducing skin blotches, stopping spasms, and in recovering acute inflammation with red swelling and severe pain (Ahn, 1998; Bensky and Gamble, 1993; Korean Institute of Herbal Acupuncture, 2000). Therefore, it was suitable choice as a medicinal herb for arthritis treatment.

In this study, the effect of PR herb-acupuncture therapy was investigated by using an adjuvant-induced mono-arthritic rat. The parameters such as
body weight, knee circumference, and squeaking score were measured, and the results were compared to those of the weight distribution ratio. To assess behavioral and pre-clinical arthritic symptoms of a pain animal model, the following experimental methods are generally used - body weight measuring, acetone atomizing and hot plate test, heat sensitivity measuring method of the paw, and the Von Frey hair test. For measuring the degree of edema in the inflamed region of the body, mainly the paw volume, knee circumference, squeaking threshold, and articular index are analyzed. However, just as various analgesia equipment have their merits and demerits, the experimental limits, such as over subjective inclination, measuring the degree of pain and edema, requirement of general anesthesia can also have a similar effect. Therefore more than two methods were generally used at the same time, in order to improve experimental accuracy. For overcoming certain limits, an incapacitance meter, a new type of analgesia meter which is used to measure the weight difference between both soles of the arthritic rat’s feet, was used for exclusion of subjective inclination, analyzing pain and edema at once, and the use of anesthesia was not necessary (Schott et al., 1994; Hay et al., 1997).

In the case of body weight, the weights of all groups were not increased during first 2 days. After that, the weights were begun to increase gradually, and a significant increase was observed compared to the SAL group on day 6 and 14. The animal model in this study had a short arthritis-induced and recovery period, compared to the chronic poly-arthritis animal model. In the results, the experimental schedules had to be designed within a 2 week span, in order to investigate the therapeutic effects. Also, it was difficult to observe the loss of body weight due to the local injection of adjuvant for inducing a mono-arthritis, and to observe the recovery of body weight by medical therapy in a mono-arthritic rat. For these reasons, the efficacy of the PR herb-acupuncture therapy in terms of body weight recovery was not significantly verified in this study.

For investigating the degree of edema caused by local induction of arthritic inflammation, the variation of knee circumference was measured. Between the PR and SAL groups, the decrease in the knee circumferential variation was observed on day 2, and the variation decreased lesser than 0.2 cm from day 6. After day 12, compared to the SAL group, the PR group had decreased more, but not so much that it was statistically significant. Kwon et al. (2001) was reported that edema was dramatically decreased after injecting bee venom into ST36, and Yeom et al. (2006) was also reported that inflammation decreased after injecting water extracts of *Hominis placenta* using a poly-arthritic animal model. In this study, a significant decrease of knee circumference by the PR herb-acupuncture was not observed, but the tendency to decrease was displayed. These results indicated that PR herb-acupuncture was somewhat effective for adjuvant-induced mono-arthritis, although a significant decrease was not observed. In order to obtain a more solid verification, longer-term experiments must be conducted studied.

Squeaking threshold scoring, using modified methods of Park (2001) and Yu (2002), were used for analyzing rigidity and pain of knee arthritis. In a knee joint, arthritis was the cause of sensitized pain receptors, induced rigidity, and restricted extension-flection. Consequently, squeaking was not induced in normal animals by extension-flection of knee joint, but was induced in arthritis-induced animals. In this study, after day 6, squeaking scores in the PR group were significantly different from those in the SAL group. This would indicate that PR herb-acupuncture therapy was effective for alleviating rigidity and pain as an arthritic symptom. However, a significant decrease was not observed in the knee circumference test. These results also indicated that the PR herb-acupuncture therapy was more effective for alleviating arthritic pain than for reducing inflammation and arthritic edema.

In the end, using an incapacitance meter, a paw
pain measuring unit, the efficacy of the PR herb-acupuncture therapy for treating arthritis was investigated. This device was designed to detect the difference of weight distribution between the two paws of an arthritis-induced rat, referring to Hay (1997) and Schott (1994). In this animal model, the pain only occurred at the arthritis-induced paw (right side) when body weight was placed onto both hind paws. To avoid or minimize the pain, the rats shifted their body weight onto the healthy paw (left side). Therefore, the difference of weight distribution between the two paws was attributed to an action for avoiding pain. In the results, the wider the difference in weight distribution ratio means the more painful a paw had become, due to the arthritis.

Before the arthritis induction, the weight distribution between two paws was equal, that is 5:5. After arthritis induction, the ratio rapidly changed to 8:2 on day 2. In the PR group, the efficacy of the PR herb-acupuncture therapy was observed on day 8, and the ratio improved to 7:3 on day 14. After that, the ratio nearly approached 5:5 again. In the case of the SAL group, during the experimental period (14 days), the ratio was maintained at about 8:2. It suggested that a spontaneous cure effect had not appeared in this acute-arthritis rat model during the 14 days, and that the PR herb-acupuncture therapy had been effective for pain in the arthritis-induced knee joint, at least in terms of reducing pain.

In spite of a few demerits, such as requiring an adaptation period for maintaining the standing posture of a rat, there were many advantages of this device. First of all, anesthesia is not necessary, so the arthritic symptoms, such as pain, edema, and rigidity, can be synthetically expressed. It was therefore suggested that the analyzing values of this device were sufficient as a pain measuring method.

In conclusion, for investigating the anti-arthritic effects of PR herb-acupuncture, body weight, knee circumference, squeaking threshold, and weight distribution ratio of an adjuvant-induced mono-arthritis rat model were measured, as compared to those of that had a saline injection administered to the same acupoint as a control. In regards to the arthritic model development, the adjuvant injection to the knee joint produced arthritic symptoms such as edema, pain, stiffness and rigidity. The PR herb-acupuncture therapy continually increased the body weight, and a significant increase of the body weight was observed in the PR group, when compared to SAL group. The therapy began to decrease the variation of knee circumference from day 2, but a significant decrease was not observed. In an extension-flection test, a type of pain analysis method for indicating the degree of arthritic severity, the squeaking score had decreased significantly. And the efficacy of the PR herb-acupuncture was synthetically verified in pain control by an incapacitance meter, a new type of paw pain measuring device analyzing the difference in weight distribution between two paws.

Taken together, the PR herb-acupuncture therapy was more effective for the alleviation of pain and inflammation than the simple acupuncture such as saline herb-acupuncture as previously reported (Kwon et al., 2001; Kang et al., 2002; Lee et al., 2004). And the injection of highly purified herbal distillates into the specific acupoint is essential to achieve herb-acupuncture therapy.

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