

## Original Article

## Study on Application of Static Magnetic Field for Adjuvant Arthritis Rats

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In order to examine the effectiveness of the application of static magnetic field (SMF) on pain relief, we performed a study on rats with adjuvant arthritis (AA). Sixty female Sprague–Dawley (SD) rats (age: 6 weeks, body weight: approximately 160 g) were divided into three groups [SMF-treated AA rats (Group I), non-SMF-treated AA rats (Group II) and control rats (Group III)]. The SD rats were injected in the left hind leg with 0.6 mg/0.05 ml *Mycobacterium butyrium* to induce AA. The rats were bred for 6 months as chronic pain model. Thereafter, the AA rats were or were not exposed to SMF for 12 weeks. We assessed the changes in the tail surface temperature, locomotor activity, serum inflammatory marker and bone mineral density (BMD) using thermography, a metabolism measuring system and the dual-energy X-ray absorptiometry (DEXA) method, respectively. The tail surface temperature, locomotor activity and femoral BMD of the SMF-exposed AA rats were significantly higher than those of the non-SMF-exposed AA rats, and the serum inflammatory marker was significantly lower. These findings suggest that the pain relief effects are primarily due to the increased blood circulation caused by the rise in the tail surface temperature. Moreover, the pain relief effects increased with activity and BMD of the AA rats.

**Keywords:** static magnetic field – adjuvant arthritis – thermography – locomotor activity

### Introduction

The biological response to a static magnetic field (SMF) has recently been widely studied from the perspective of possible health benefits as well as potential hazards (1–3). With respect to the possible health effects, it has been reported that local SMF application has beneficial actions in orthopedic diseases.

In Japan, SMF generated by small, cylindrical Ferrite magnets has been used to provide pain relief from neck, shoulder and lower back pain (4,5). These magnetotherapies have been found to be clinically effective.

Rat adjuvant arthritis (AA) is a model of acute periarticular proliferative synovitis that is induced by a single intradermal injection of tubercle bacillus in the rat posterior foot (6). As one

of the experimental arthritis models, AA rats have been used to evaluate the efficacy of drugs, especially anti-inflammatory drugs, at several institutions (7). In the present study, AA rats were left untreated for a prolonged period to develop chronic pain and osteopenia models (8). These model animals were exposed to SMF, and its influence on the tail surface temperature and the level of locomotor activity was assessed using a thermograph and a metabolism measuring system, respectively. The effect of the SMF on the bone mineral density (BMD) and serum inflammatory markers was also studied.

### Subjects and Methods

#### Study Animals and Environmental Conditions

Female Sprague–Dawley (SD) rats (age: 5 weeks, body weight: approximately 150 g) were purchased from Japan Crea Co., Ltd. (Shizuoka, Japan). The animals were housed individually in cages and kept in a room maintained at a temperature

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of  $23.0 \pm 1.0^\circ\text{C}$  with a relative humidity (RH) of  $55 \pm 5\%$  and a 12 h/12 h light/dark cycle. Solid rodent chow and tap water were offered *ad libitum*. After 1 week of acclimatization under these conditions, animals showing favorable growth were selected and used for further studies.

In addition, this research was approved by the animal experiment Ethics Committee of this University. The animals were sacrificed under anesthesia and the femur was extracted.

### Experimental Animals

Sixty female SD rats (age: 6 weeks, body weight: approximately 160 g) were divided into three groups. In Group I (20 animals) and Group II (20 animals), *Mycobacterium butyrium* suspended in paraffin oil (0.05 ml) was injected in the left posterior foot in a dose of 0.6 mg/0.05 ml to induce AA. These animals were then maintained for 24 weeks to develop a chronic pain model. Starting at 24 weeks, Group I was exposed to SMF for 12 weeks (up to week 36 after the onset of AA). Group II (the non-SMF-treated AA rats) was not exposed to SMF. The 20 normal rats in Group III (control rats) were maintained without any treatment for 36 weeks (Fig. 1).

### Conditions of Exposure to SMF

The SMF exposure device (length, 300 mm; height, 130 mm; width, 440 mm; Pip Fujimoto Co., Ltd., Osaka, Japan) was composed of a pair of rectangular magnetic plates, externally placed parallel to and at the shortest distance of 1 cm from both the outer sides of a transparent cage (length, 340 mm; height, 180 mm; width 400 mm) (Fig. 2A, B). The mean flux

density at the center of the cage was 30 mT (range, 20–80 mT) and the magnetic surface was 200 mT (Fig. 2C).

For Group I, the SMF exposure device was fixed on the left and right sides of the cage, and each animal was exposed to the magnetic field continuously for 12 weeks. For Group II, a 0 mT magnetic surface was similarly placed as a control.

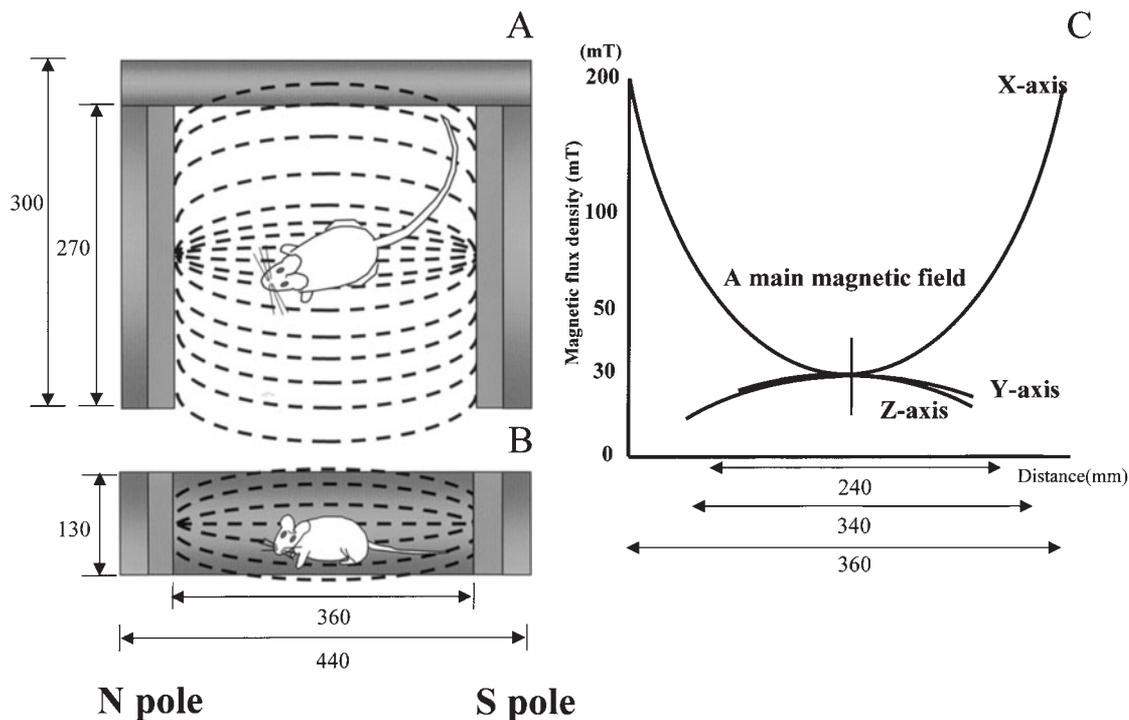
### Measurement of Parameters

#### Measurement of Tail Surface Temperature

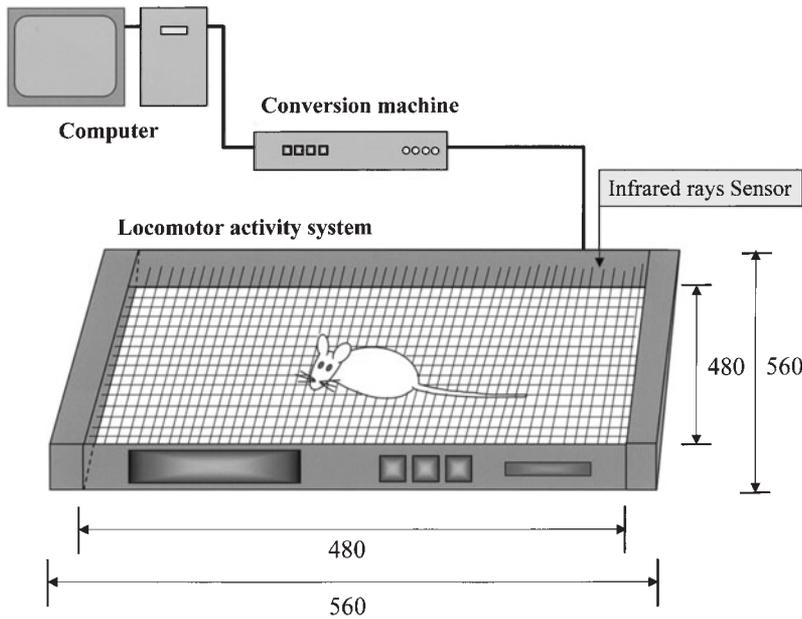
To avoid the influence of the haircoat, the tail surface temperature was used as an indicator of the peripheral circulation (9). The tail surface temperature was measured before and after 12-week exposure to SMF (at 24 weeks and 36 weeks) in Group I, and at the respective time points in Group II and Group III. The tail surface temperature was measured using a thermograph (TVS-2300 Mk IIST; Japan Abionics Co., Ltd., Tokyo, Japan). After 15-min acclimatization to the environment, the tail surface temperature was measured in conscious animals in a windless room maintained at a temperature of  $15 \pm 1^\circ\text{C}$  with 50–55% RH. In thermography, the mean skin temperature of the entire tail was determined using the software for temperature measurement (TW-SIBMW, resolution: 0.01; Japan Abionics Co., Ltd., Tokyo, Japan).

#### Measurement of the Level of Locomotor Activity Over 24 h

The level of locomotor activity over a 24-h period was measured before and after 12-week exposure to the SMF (at 24 weeks and 36 weeks) in Group I, and at the respective time points in Group II and Group III. The level of locomotor activity was measured



**Figure 1.** Schematic diagram of the magnet, showing its top (A) and front (B) plan view. The mean magnetic force at the center of the cage was 30 mT (C).



**Figure 2.** The level of locomotor activity was measured using a metabolism measuring system (SCANET MV-10; Toyo Sangyo Co., Ltd., Toyama, Japan). In this system, infrared rays are spread horizontally (lengthwise and breadthwise) at 5-mm intervals, and the number of infrared rays blocked by the animal is counted at 30-s intervals. The total locomotor activity over 24 h was measured as the daily locomotor activity.

using a metabolism measuring system (SCANET MV-10; Toyo Sangyo Co., Ltd., Toyama, Japan). In this system, infrared rays are spread horizontally (lengthwise and breadthwise) at 5-mm intervals, and the number of infrared rays blocked by the animal is counted at 30-s intervals (10). The total locomotor activity over 24 h was measured as the daily locomotor activity.

Further, the magnitude of change in the locomotor activity was calculated as the rate of increase in locomotor activity by the following equation: rate of increase in locomotor activity = (locomotor activity after exposure—locomotor activity before exposure) ÷ (locomotor activity before exposure) × 100.

#### *Measurement of the Levels of Serum Inflammatory Markers and Femoral BMD*

Each animal was sacrificed under deep ether anesthesia. Blood was drawn from the cervical vein, and the serum was isolated. The level of albumin (Ab) and sialic acid were assayed using commercial kits (Wako Pure Chemical Industries, Ltd., Wakayama, Japan). The level of acid-soluble glycoprotein (ASGP) was assayed using a Sequential Multiple Autoanalyser plus Computer (Technion, Ltd., Tokyo, Japan). Both femoral bones were obtained and their BMD was measured by dual-energy X-ray absorptiometry (DEXA; DCS-600R Dichroma scan, Aloca Co., Ltd., Tokyo, Japan). The mean BMD of the two femoral bones was calculated. The BMD of the entire femoral bone was measured using the software for the analysis of BMD (small animal mode, SYS-D 62-V 6.0; Aloca Co., Ltd., Tokyo, Japan).

#### **Data Analyses**

The data obtained in each group are expressed as the mean ± standard error. The significance of the differences among groups was assessed by the Wilcoxon sum-rank test, and the level of significance was set at  $P < 0.05$ .

## **Results**

### **Tail Surface Temperature Before and After Exposure to SMF**

Before exposure of Group I to the SMF at 24 weeks, the tail surface temperature was significantly lower in the AA rats (Group I and Group II) than in the control rats (Group III). After 12-week exposure of Group I to the SMF (at 36 weeks), the tail surface temperature of the SMF-treated AA rats (Group I) was significantly higher than that of the non-SMF-treated AA rats (Group II). No significant difference was observed in the tail surface temperature between Group I and Group III at 36 weeks (Table 1).

### **Locomotor Activity Before and After Exposure to SMF**

Before exposure of Group I to the SMF at 24 weeks, the level of daily locomotor activity was lower in the AA rats (Group I and Group II) than in the control rats (Group III) (Table 2). After 12-week exposure of Group I to the SMF, the rate of increase in locomotor activity was significantly higher in the SMF-treated AA rats (Group I) than in the non-SMF-treated AA rats (Group II).

### **Serum Ab, Sialic Acid, ASGP Levels and BMD After Exposure to SMF**

The serum Ab level was significantly lower in the AA rats (Group I and Group II) than in the control rats (Group III). A significant difference was observed in the ASGP levels between the SMF-treated AA rats (Group I) and the non-SMF-treated AA rats (Group II) (Table 3).

The BMD was significantly lower in the non-SMF-treated AA rats (Group II) than in the control rats (Group III).

However, there was a significant difference between the SMF treated AA rats (Group I) and the control rats (Group III).

## Discussion

AA has been widely studied and is used as a model of experimental arthropathy in the evaluation of drugs, especially, anti-inflammatory agents (11). The mechanism involved in the development of rat AA is autoimmunity cross-reaction, in which T lymphoids sensitized to tubercle bacillus cross-react with a protein in rat articular cartilages, causing arthritis (12). However, the pathology of this model may not be the same as that of human rheumatic arthritis, although similar symptoms develop in both (13). Briefly, although the redness and swelling in various joints worsen 2–3 weeks after the induction of AA, these symptoms resolve subsequently, leaving articular ankylosing deformity. Histologically, both chronic granulomatous inflammation and acute inflammation associated with inflammatory cell infiltration have been noted in rat AA, and the disease does not reach the chronic state (14).

We maintained AA rats without treatment for a prolonged period to utilize the animals as chronic ischemic pain and osteopenia models. Rheumatic arthritis is often accompanied

by ankylosing deformity and coldness of the joints, and it is thought that AA rats that have not been treated for a prolonged period can be used as an experimental model of abnormal cold feeling (15).

Several factors, including systemic changes due to the pain of multiple arthritis, side effects caused by treatments such as steroid preparations and osteoporosis due to a reduction in physical activity, are considered to contribute to the onset of osteopenia accompanied by rheumatic arthritis. In particular, reduced physical activity is said to cause reductions in the BMD of the lumbar and femoral bones. In fact, we have previously confirmed by computed X-ray densitometry (CXD) and DEXA that AA rats that have been kept untreated over a prolonged period can be used as an experimental osteopenia model.

In the present study, animals that had remained untreated for 6 months after the induction of AA were exposed to SMF. Since the tail surface temperature of AA animals significantly increased by an average of 1.43°C after prolonged exposure to SMF for 12 weeks, it is suggested that exposure to SMF improved the peripheral circulation. The mechanism of SMF action may be as follows: it was previously shown that exposure to a magnetic force increased the release of acetylcholine from cholinergic vasodilator nerve endings by inhibiting the effect of cholinesterase, resulting in vasodilation (16). Further, it was confirmed using a metabolism measuring system that the locomotor activity of AA rats was clearly higher (approximately 10% increase in a 24-h period) after 12 weeks of exposure to SMF than before the exposure. Furthermore, there was a significant difference in the observed levels of serum inflammatory markers (ASGP) between the SMF-treated and non-SMF-treated AA rats. Therefore, SMF appeared to have an analgesic action; a clinical study reported that SMF therapy relieved pain in patients with neck and shoulder pain and muscle fatigue due to ischemic conditions in the microcirculation (17). We have previously reported that the tail skin temperature as well as the activity of AA rats treated with moxibustion increased significantly compared with normal rats. Moxibustion has been widely employed for relieving the perception of pain (18).

Consequently, the SMF-induced increase in locomotor activity in our AA rats was presumably ascribable to the alleviation of pain rather than to the induction of stress. Physical therapy, including therapeutic exercise of bones, has been reported to be very effective for osteoporosis (19). Briefly, since the dynamic exercise of bones is considered to improve the bone structure and increase BMD, physical therapy is generally preferable to drug therapy, which may cause side effects. In

**Table 1.** Tail surface temperature of AA and normal rats before and after 12-week exposure to SMF

	Tail surface temperature (°C)	
	Before	After
Group I: SMF-treated AA rats	14.63 ± 0.26***	16.06 ± 0.26 <sup>#</sup>
Group II: Non-treated AA rats	15.18 ± 0.17***	15.17 ± 0.30*
Group III: Control rats	16.14 ± 0.19	16.34 ± 0.46

Group II and Group III were not exposed to SMF. The results are expressed as mean ± SEM. \**P* < 0.05; significantly different between before and after in Group I, <sup>#</sup>*P* < 0.05; \*\*\**P* < 0.001.

**Table 2.** Locomotor activity over a 24-h period of AA and normal rats before and after 12 week exposure to SMF

	Locomotor Activity (count)	
	Before	After
Group I: SMF-treated AA rats	68983 ± 9090****	78279 ± 8902***
Group II: Non-treated AA rats	71745 ± 8683***	72340 ± 9807***
Group III: Control rats	133358 ± 10132	145263 ± 7773

Group II and Group III were not exposed to SMF. The results are expressed as mean ± SEM. \**P* < 0.05; \*\*\*\**P* < 0.001.

**Table 3.** Serum inflammatory markers and BMD in AA and control rats after 12-week exposure to SMF

	Changes in inflammatory index substances and BMD			
	Albumin (g/dl)	Sialic acid (mg/dl)	ASGP (mg/dl)	BMD (g/cm <sup>2</sup> )
Group I: SMF-treated AA rats	4.52 ± 0.13**	124.3 ± 23.6	275.5 ± 1.9*	192.6 ± 4.2*
Group II: Non-treated AA rats	4.17 ± 0.18**	134.9 ± 16.3	281.2 ± 2.8*	179.5 ± 3.2***
Group III: Control rats	5.15 ± 0.03	110.9 ± 8.4	262.5 ± 1.9	195.5 ± 3.3**

The results are expressed as mean ± SEM. \**P* < 0.05; \*\**P* < 0.01.

Japanese individuals, it was confirmed by the quantitative ultrasound method that the BMD among people who habitually exercised was significantly higher than that among people who consumed a large quantity of nutritional food, but did not exercise (20). These results suggest that the increased BMD of AA animals upon exposure to SMF was partially ascribable to the increased locomotor activity. It was reported that 3 weeks after the implantation of a 200-mT magnet into the femoral bone in a rat osteoporosis model that had been prepared by ligating the femoral artery, there was an approximately 10% increase in the BMD, as recorded by DEXA (21).

Therefore, the possibility that SMF directly affects the bone cannot be ruled out, because the BMD also increased with the increase in locomotor activity.

Further basic research using a pathophysiological animal model is necessary to clarify the pain relieving mechanism of SMF in various painful conditions, e.g., ischemic and inflammatory pain.

## References

- Ohkubo C, Okano H. Acute effects of static magnetic fields on cutaneous microcirculation in rabbits. *Int J In vivo Res* 1997;11:221–6.
- Okano H, Ohkubo C. Biphasic effects of static magnetic fields on cutaneous microcirculation in rabbits. *Bioelectromagnetics* 1999;20:161–71.
- Kanai S, Okano H, Abe H. Efficacy of toki-shigyaku-ka-gosyuyu-syokyo-to on peripheral circulation in autonomic disorders. *Am J Chin Med* 1997;25:69–78.
- Kanai S, Taniguchi N, Susuki R, Okano H. Therapeutic effects of static magnetic fields on various painful conditions. *International Symposium on New Magneto-Science '99 Proceeding of the Third Meeting* 1999;412–22.
- Susuki R, Kanai S, Okano H. Examination of the effect of magnetic treatment for low back pain. *Symposium on New Magneto-Science '98 Proceeding of the Second Meeting* 1998;17–22.
- Pearson CM. Rheumatic manifestations of polymyositis and dermatomyositis. *Arthritis Rheum.* 1959;2:127–43.
- Kobashi O. Adjuvant arthritis. *Rheumatism* 1980;20:22–30.
- Kanai S, Taniguchi N. Comparison of measuring methods for osteopenia using two different rats. *Orthop Traumatol* 1999;48:1237–41.
- Kanai S, Taniguchi N, Umeda T, Susuki R. Mechanism of improvement by Kampo medicines for abnormal cold feeling. *J Tradit Med* 1999; 15:468–9.
- Ohki-Hamazaki H, Watase K, Yamamoto K, Ogura H, Yamano M, Yamada K, et al. Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature* 1997;390:165–9.
- Eden Van W. Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. *Proc Natl Acad Sci USA* 1985;82:5117–20.
- Kitaichi K. Effects of risperidone phencyclidine-induced behavior comparison with haloperidol and ritanserin. *Jpn J Pharmacol* 1994;166: 181–9.
- Taki M. Quality and pharmacological investigation of processed Aconiti tuber. *Nat Med* 1998;52:343–52.
- Kanai S, Susuki R, Taniguchi N, Okano H. Clinical study of adjuvant arthritis rats monitored by thermography. *Orthop Traumatol* 1998;47: 450–3.
- Kanai S, Okano H, Orita M, Abe H. Therapeutic effectiveness of static magnetic fields for low back pain monitored with thermography and deep body thermometry. *J J Society of Pain Clinics* 1997;5:5–10.
- Takeshige C, Sato M. Comparisons of pair relief mechanisms between needling to the muscles, static magnetic field, external qigong needling to the acupuncture point. *Acupuncture and Electro-Therapeutics Res Int J.* 1996;21:119–31.
- Kanai S, Okano H, Orita M, Abe H. Clinical study of neck and shoulder pain for therapeutic effectiveness with application of static magnetic field. *J J Society of Pain Clinics* 1996;3:11–7.
- Kanai S, Take A, Taniguchi N. Study of moxibustion for experimental arthritis model rat. *Oriental Medicine and the Pain Clinic* 2000;30: 16–21.
- Hayashi Y. Physical training. *Jpn J Clin Med* 1998;56:1551–6.
- Suzuki Y, Uehara R, Ide M, Ichikawa Y. Osteoporosis in rheumatoid arthritis. *Jpn J Clin Med* 1998;56:1598–603.
- Xu S, Tomita N, Ohata R, Yan Q, Ikada Y. Static magnetic field effects on bone formation of rats with an ischemic bone model. *Biomed Mater Eng* 2001;11:257–63.

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