Analgesic effects of indirect moxibustion on an experimental rat model of osteoarthritis in the knee

Noriko Uryu, Kaoru Okada, Kenji Kawakita

Abstract
Aims The analgesic effects of moxibustion on an experimental model of osteoarthritis of the knee were investigated.

Methods Male Wistar rats (n=36, 296-421g) were used. Intra-articular injection of mono-iodoacetic acid (MIA) was performed to induce knee osteoarthritis. Indirect moxibustion was applied to the lateral aspect of the knee joint every other day for 28 days (14 treatments). Weight bearing of the hind legs was measured directly by the downward pressure applied to footplates, using an Incapacitance Tester. Morphine was injected for testing the validity of weight bearing as a pain measure, and naloxone was used to examine the participation of endogenous opioids in the mechanism of moxibustion analgesia. Data were analysed by calculating the area under the curve.

Results Injection of MIA significantly reduced weight bearing. No analgesic effects of moxibustion were observed during the initial 7 days (unpaired t test, P=0.83). Continued moxibustion treatments increased weight bearing at the 14th day significantly, and this effect continued until the end of the experiment on the 28th day (P<0.05). A single moxibustion treatment had no immediate effect on weight bearing. The analgesia due to the cumulative effect of moxibustion was antagonised by naloxone injection. Morphine injection in control MIA injected rats increased weight bearing to the normal range, confirming the validity of the measurements.

Conclusion These results highlight the importance of repeated moxibustion treatments for pain relief in experimental knee osteoarthritis and suggest the existence of sustained inhibitory modulation by endogenous opioids in the moxibustion group.

Keywords Moxibustion, osteoarthritis, MIA induced arthritis, weight bearing test, endogenous analgesia mechanism.

Introduction Osteoarthritis (OA) is an age related degenerative disease and has become very common in the increasingly elderly population. The morbidity rate of OA is much higher than rheumatoid arthritis (RA),1 OA of the knee, accompanied by severe knee pain, is one of the major causes of reduced quality of life and activities of daily living among the elderly.

Recently, several large clinical trials were conducted in the USA, Germany and Spain that clearly demonstrated the effectiveness and safety of acupuncture treatment for osteoarthritis of the knee.2-6 Systematic reviews and meta-analyses have also indicated the much higher effectiveness of real acupuncture than usual care or waiting list, and a slight positive effect when compared with sham treatment. Thus, the efficacy of acupuncture for knee OA patients has been demonstrated.6 However, the mechanism of action of acupuncture on knee OA has not been determined, although the basic mechanisms of acupuncture analgesia mediated by the endogenous opioid systems and diffuse noxious inhibitory controls (DNIC) are frequently cited as its underlying mechanisms.
Moxibustion is also a well known procedure in traditional Chinese medicine, but clinical evidence regarding the effect of moxibustion on knee OA is almost non-existent. One of the reasons for the shortage of clinical trials of moxibustion therapy might be its unpopularity in Western countries. In Japan, acupuncturists frequently use moxibustion for treatment of various diseases,7-10 and self-application of indirect moxibustion used in the present study at home has also been widely performed in patients with knee pain and other pain related conditions.

It is acknowledged that the original concept of meridians in traditional Chinese medicine referred not to acupuncture, but to moxibustion.11 Therefore, it seems very relevant to investigate moxibustion. Several animal experiments have demonstrated the analgesic and anti-inflammatory effects of moxibustion in RA, but we know of no study that has used an OA model.12-14

Mono-iodoacetate (MIA) injection was introduced by Kalbhen as a method for producing an animal model of OA.15 Intra-articular injection of an MIA solution induces disorganisation of the articular cartilage by inhibition of chondrocyte metabolism, and the ensuing degenerative changes in the articular cartilage are similar to those described in human OA patients.16,17 The MIA model was first used for histological study in the 1980’s,17 and thereafter it was adopted to study arthritic pain.18 Currently, the pain behaviour of MIA animals is measured by the hind limb weight method, using an Incapacitance Tester.19,20 The weight of the individual hind paw is detected by measuring the downward pressure as the animal stands on a pair of plate transducers, and the test limb weight as a percentage of total limb weight is used as the pain index (%WTL).

The purpose of this study was to examine the analgesic effect of repeated moxibustion treatments for one month on MIA induced OA in rats, and to examine the involvement of endogenous opioid systems in moxibustion treatments.

Materials and Methods
Animals
Male Wistar rats (n=36, 9-14 weeks old, 296-421g) were used. Each rat was housed alone in a plastic box under a 12/12 hour light/dark cycle. Room temperature (23°C) and humidity (50±10%) were controlled to be constant. Food and water were available ad libitum. All rats were handled for several 10 minute sessions daily for two weeks so that they could adjust to the acrylic box containing the Incapacitance Tester and to being restrained in a cloth holder during the moxibustion stimulation.

Three linked experiments were conducted for different purposes. In the first experiment (n=26), the effects of repeated application of moxibustion were examined over 28 days. Rats were allocated to MIA control (restraint without moxibustion) group (n=14) and MIA moxibustion group (n=12). In a subgroup, on the 18th day, the immediate effect of moxibustion treatment was examined by making additional pain measurements after moxibustion or restraint: this was conducted with 10 rats, randomly selected half from the MIA moxibustion and half from the MIA control group. Two further experiments were conducted in other subgroups of rats, also on the 18th day of the first experiment. In experiment two, the antagonistic effect of naloxone on the analgesia of repeated moxibustion was examined in a subgroup of MIA moxibustion rats (n=5). In experiment three, using rats from the MIA control group (n=5), morphine was applied to examine the validity of the %WTL measurement as a pain measure.

All procedures for animal experiments were based on the guidelines of animal experiment of the Japanese Physiological Society. The protocol of the experiments was approved by the local ethics committee of Meiji University of Oriental Medicine (approval number 17-5-1).

Induction of experimental osteoarthritis
MIA (iodoacetic acid sodium, Wako Co Ltd, Kyoto, Japan) was dissolved in saline (90mg/ml). Under pentobarbital (50 mg/kg) anesthesia, 50µl of MIA solution was given by intra-articular injection through the patellar ligament of the knee using a 26G needle under anesthesia with sodium pentobarbital (Nembutal, 40mg/kg, ip).

Moxibustion
In the MIA moxibustion group, a single indirect moxibustion apparatus, as supplied commercially for clinical use (Kamaya Mogusa Co Ltd, Tokyo, Japan), was applied on the skin of the lateral side of the knee joint of the MIA injection side for 28 days.
every alternate day (a total of 14 moxibustion treatments). Moxibustion started on the day following the MIA injection. In the MIA control group, the animals were restrained for the same period but moxibustion was not applied. The stimulus temperature was continuously monitored by a thermocouple of 2.0mm in diameter with a time constant of 0.1 seconds (BAT-12, Sensortek Inc, CA, USA) applied on the skin surface just beneath the indirect moxibustion tube. The moxibustion tube was removed when the stimulus temperature exceeded 50°C to avoid tissue damage.

The indirect moxibustion apparatus consists of a cardboard tube (height 12mm, diameter 10mm), the top of which is filled with moxa (dried leaves of Artemis vulgaris). The bottom of the tube is adhesive and can be attached firmly to the skin surface. There is a fixed 11mm air gap between the moxibustion and skin surface. The average peak temperature was 50±3°C, the time to reach peak temperature was 120±30 seconds, the period of heat stimulation over 40°C was 81±33 seconds and that over 45°C was 53±28 seconds. During moxibustion, the rat was restrained in a cloth holder and both hind limbs were exposed.

Pain measure
Injection of MIA induces local inflammatory responses in the knee joint, but these responses disappear within seven days after the MIA application, so we did not use swelling as an outcome measure. In this study we used hind limb weight bearing as a pain measure. This method has been widely used as a new pain assessment for the MIA model of OA, and the validity of %WTL as a pain measure has been well established in experiments using morphine. Several other pain measures, such as pressure pain threshold of the knee joint or the von Frey test, were available but we used only the weight bearing test because it is a simple procedure and the animals received no other additional stresses during measurements. The pain assessments were performed daily in both moxibustion and restraint control group rats. In the days when moxibustion was given, assessment was done before the moxibustion stimulation.

Each rat was put into the acrylic box on the Incapacitance Tester (Columbus Instrument, Columbus, MO, USA). Both hind limb weights were measured for five minutes by separate plates with pressure transducers. The pressure on each plate, as well as a picture of the animal’s posture during measurement, were monitored and sent to a personal computer every two seconds, simultaneously (Geosense Co Ltd, Osaka, Japan).

Rats standing on the Incapacitance Tester in the plastic box tended to be calm, but both hind limbs did not always locate evenly on the two separate plates for weight measurement. However, continuous measurement of %WTL at intervals of two seconds for a period of five minutes gave stable and reproducible results, even though the rats sometimes adopted unusual postures during measurement. So the average values over a five minute period (each two second interval, a total 150 data points) were used for further analysis.

The data are shown as the weight of the hind limb of the MIA injection side as a percentage of the total weight borne by both limbs, (test limb weight/sum of both limb weights) ×100, the %WTL, an index of pain measure.

Pharmacological studies
Pharmacological tests were performed in two groups on the 18th day after the MIA injection. Naloxone hydrochloride (Wako Co Ltd, Kyoto, Japan) and morphine hydrochloride (Takeda Pharmacoeutical Co Ltd, Japan) were dissolved in distilled water. Naloxone (3mg/kg ip) was used in the MIA moxibustion rats (experiment 2, n=5), to investigate the participation of endogenous opioids in the moxibustion effect. Morphine (5mg/kg) was used to examine the validity of %WTL as pain measure in the MIA control rats (experiment 3, n=5).

Time schedule and statistical analysis
The hind limb weight assessments were done for three consecutive days before injection of MIA, for seven consecutive days after the injection, and on the 14th, 21st, 28th days after the injection. A total of 13 measurements were done. Such pain assessments were performed before every moxibustion stimulation or control, if due that day. In the pharmacological studies, pain assessment was performed before and after drug injection at intervals of five minutes.

All data are expressed as means ± SD. Statistical analysis for behavioural experiments was carried out using repeated measures of ANOVA followed by a
Figure 1 The time course of the hind limb weight bearing for 28 days after MIA injection shows persistent pain in the control (restraint only) group, and return to near baseline values in the group treated with moxibustion. The data are expressed as mean ± SD; *P<0.05 repeated measures one way ANOVA and post hoc Bonferroni/Dunn test vs baseline; NS – not significant.

Figure 2 The difference in weight bearing between the moxibustion and control (restraint without moxibustion) groups is expressed here as the area under the curve (AUC). The data are expressed as mean ± SD. The AUCs were calculated as the areas under the 50% WTL line in Figure 1; *P<0.05 unpaired t test.
Bonferroni/Dunn post hoc test. P<0.05 was set as the level of statistical significance. The areas under the curve (AUC) of %WTL in the two groups were calculated as the area below the line for 50% weight bearing, and an unpaired t test was used to assess the difference between AUCs. The paired t test was used to compare the before and after moxibustion weight bearing results. Statistical analysis was done using Stat View 5.0 (SAS Institute Japan Inc, Osaka, Japan), and SYSTAT11 (SYSTAT Software Inc, California, USA).

Results
Effect of moxibustion on pain in MIA induced arthritis
Figure 1 shows the time course of the change in %WTL induced by MIA injection. After MIA injection, %WTL in MIA control and MIA moxibustion groups was decreased significantly (compared with baseline) by a similar degree. Moxibustion had almost no effect during the initial seven days. In the control group, a significant decrease of %WTL on the MIA injection side continued for 28 days after the injection (vs baseline, P<0.05). On the other hand, the %WTL in MIA moxibustion rats recovered to near baseline values by the 14th day.

The corresponding area under the curves (AUCs) of the hind limb weights after MIA injection are shown in Figure 2. The AUC of the MIA moxibustion group was significantly smaller than that of the MIA control group (P<0.05, unpaired t test).

Figure 3 shows the immediate effects of moxibustion on %WTL, as measured on the 18th day. No significant difference was found between %WTL before and after the moxibustion stimulation. In control rats, no significant change was observed after restraint of the animals in the holder.

Pharmacological studies
In MIA moxibustion rats, administration of naloxone (experiment 2) on the 18th day reduced the accumulated effect of moxibustion treatment 10 minutes after injection (P<0.05). Moreover,
Figure 4 The effect of naloxone is to reverse the cumulative analgesic effect of moxibustion on hind limb weight bearing. These experiments were conducted on 18th day after MIA injection, and ‘baseline’ indicates the data on 18th day. The data are expressed as mean ± SD; *P<0.05 repeated measures one way ANOVA and post hoc Bonferroni/Dunn test vs baseline.

Figure 5 The use of weight bearing as a method of pain measurement was shown to be valid, as morphine injection restored normal values in a subgroup of 5 animals. The effect was declining at 20 hours. This experiment was conducted on the 18th day after MIA injection, and ‘baseline’ indicates the value on that day. The data are expressed as mean ± SD; *P<0.05 repeated measures one way ANOVA and post hoc Bonferroni/Dunn test vs baseline.
moxibustion treatment after naloxone injection did not restore the treatment effect (Figure 4).

In experiment 3, to confirm the validity of %WTL as a pain measure, morphine injection resulted in a significant increase of %WTL after 5 to 15 minutes (P<0.05). The %WTL returned to the pre-medication level 20 hours after the morphine injection (Figure 5).

Discussion
This is the first report that we are aware of that shows that repeated moxibustion treatments suppress pain related behaviour in the rat MIA induced knee OA model.

Effects of repeated vs single moxibustion treatment
Significant increases of %WTL by moxibustion were detected 14 to 28 days after the MIA injection to the knee, but the effects were detected only after seven days of treatments. On the other hand, no immediate effect of moxibustion was detected in the control MIA rats 18 days after MIA injection.

The importance of repeated interventions for the treatment of chronic pain is well recognised. Ezzo and colleagues proposed that it is proper to perform treatment at least once a week more than six times to achieve a sufficient clinical effect, and used these criteria for adequacy of the intervention in a systematic review of acupuncture for OA. Our present data support the importance of repetition of treatments in the knee OA rat model.

The recovery of %WTL induced by repeated moxibustion was clearly antagonised by naloxone, but it returned to the recovery level after 20 hours. These results suggest that a sustained endogenous opioid mediated analgesic mechanism was activated after repeated moxibustion, whereas a single application of moxibustion had no effects on %WTL. The actual reason for the lack of effect of a single moxibustion treatment is not clear, but it should be noted that a single application of morphine clearly induced recovery of the %WTL of MIA injected rats. These data suggest that the mechanism of indirect moxibustion as used in the present study is not the same as so called electroacupuncture analgesia that involves endogenous opioid mediated analgesia. The lack of effect of moxibustion during the initial seven days of treatments might be a consequence of the processes of MIA induced osteoarthritis.

Characteristics of MIA induced osteoarthritis
Pomonis and colleagues demonstrated that the reduction of weight bearing by the MIA injection was biphasic. The first phase lasted for seven days, and the second phase started on the 14th day after injection. Histological investigations suggested that acute inflammation continued for the initial three days after MIA injection, damage of articular tissue on the seventh day after injection was in cartilage, and damage progressed to subchondral bone by the 14th day. These differences in histological changes might affect the pain mechanism and offer one explanation for possibility of the lack of effect of moxibustion within the initial seven days.

Mechanisms of analgesic action of acupuncture and moxibustion
The mechanisms of electroacupuncture with various frequencies on acute nociceptive pain have been well established, but few moxibustion studies have been conducted. On the other hand, the similarity of peripheral mechanisms of acupuncture and moxibustion have been pointed out, and polymodal receptors have been proposed as a possible candidate mediating their mechanisms. These receptors are also assumed as the inputs for the development of diffuse noxious inhibitory controls. So it is plausible that similar endogenous analgesic mechanisms might be activated by acupuncture and moxibustion.

In chronic pain conditions, various changes might occur in the central nervous system. The descending modulation (inhibition and facilitation), central sensitisation and plastic changes of synaptic connections in the spinal cord and NMDA and AMPA receptors participate in such processes. To induce analgesia in such chronic pain conditions, different mechanisms might be activated by acupuncture and moxibustion, although the details are not clear.

Regarding analgesic actions of acupuncture and moxibustion on the RA model, the adjuvant induced arthritis and collagen arthritis models were widely used, and prolonged interventions produced analgesic and anti-inflammatory effects. However, no investigation of the MIA induced OA model has been reported. In a recent study of electroacupuncture
with the neuropathic model in rats, mechanical allodynia was suppressed by low frequency electroacupuncture (2 Hz) and a low dose of ketamine, an NMDA receptor antagonist, potentiated the anti-allodynia effect. On the other hand, involvement of peripheral opioid receptors in the mechanism of electroacupuncture analgesia in the inflammation induced pain condition has been suggested. These mechanisms might have contributed to the analgesic action of repeated moxibustion in the present study.

In conclusion, repeated moxibustion treatments are required for pain relief in the present rat experimental knee osteoarthritis model and the involvement of sustained inhibitory modulation mediated by endogenous opioids is suggested.

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Conflicts of interest
None declared.

Summary points
Moxibustion is an ancient therapy associated with acupuncture and widely used in Japan
So far, there is little research into the analgesic effects of moxibustion
This study tested the analgesic effects of moxibustion in an animal model of osteoarthritis
Although no immediate effect was seen, there was a significant cumulative effect during a course of moxibustion, which was reversible by naloxone

Reference list
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