Antinociceptive effects of magnetic stimulation in the rat neuropathic pain model

Kazuo Okamoto¹, Nozomi Ami¹, Yukinori Kubotera¹
Hidehiko Ooshima¹, and Hozumi Tatsuoka²

¹Research and Development Center, Terumo Corporation
²Research Center for Frontier Medical Engineering, Chiba University

Abstract
We evaluated the effect of magnetic stimulation on neuropathic pain using a unique magnetic stimulation apparatus. This system produced an alternating magnetic field of an appropriate uniform intensity that we applied to a rat model of chronic constrictive nerve injury (CCI). We performed an experiment on rats that showed reduction in the paw withdrawal threshold to mechanical stimuli, thermal hyperalgesia and cold hypersensitivity 2 weeks after nerve injury. Animals that underwent CCI were divided into three groups: non–stimulation control (NSC, n=8), low-strength magnetic stimulation (LSM, n=9), and high–strength magnetic stimulation (HSM, n=9). Alternating magnetic stimulation was delivered to the lateral sides of both femurs, operated and unoperated, near the sciatic nerves, at different intensities (ca. 25 mT and 70 mT; 50 Hz) for 30 min every 5 days. Treatment with magnetic stimulation reversed the decreased withdrawal threshold of mechanical allodynia, the effect being more significant in the HSM group as compared to the NSC group on day 5. Magnetic stimulation improved the paw withdrawal latency to thermal stimuli on day 1, though the effect was not significant. Magnetic stimulation had no obvious effect on cold hypersensitivity.

In conclusion, alternating magnetic stimulation improved neuropathic pain in CCI rats. The advantage of magnetic stimulation is that since it is non–invasive, it could be a suitable method for the non–pharmacological treatment of chronic pain.

Keywords
Magnetic stimulation; Neuropathic pain; Nerve injury model;
Mechanical allodynia; Hyperalgesia

Received: 15 November 2010
Accepted: 25 January 2011
Neuropathic pain is an intractable chronic pain that arises from functional changes in the pain sensory system after nerve degeneration, and might be difficult to control with conventional analgesics. Multimodal pain management is recommended for the treatment of chronic neuropathic pain, and several non-pharmacological treatments, such as electrical or magnetic neuromodulation therapies, especially spinal cord stimulation (SCS) and transcutaneous electric nerve stimulation (TENS), are established therapies. The efficacy of SCS, which is reportedly an effective treatment for intractable pain, has been evaluated in rat neuropathic pain models. However, it is an invasive method that requires the implantation of leads in the spinal cord. Magnetic stimulation, on the other hand, is a popular treatment method for chronic pain and is also available for home health care. The advantage of magnetic treatment is its non-invasiveness, although its effectiveness is not clear and its mechanism of action is unknown. We designed and built a unique magnetic stimulation apparatus to clarify the effects of magnetic stimulation on neuropathic pain, in which an alternating magnetic field of an appropriate uniform intensity was applied to the rat chronic constrictive nerve injury (CCI) model.

Materials and methods

Animals and surgery

All experimental procedures were performed according to the ethical guidelines of the International Association for the Study of Pain, and protocols were approved by the Committee on Animal Experiments of Terumo Corporation. Male Sprague–Dawley rats (Charles Rivers Laboratories Japan, Inc., Kanagawa, Japan) weighing 200 – 300 g at the time of nerve injury were used in the study. Animals were housed with a 12–hr light / dark cycle starting at 8 a.m., and were given food and water ad libitum. The CCI model was produced by modifying the method of Bennett. Rats were anesthetized with 40 mg/kg pentobarbital (Somnopentil®, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan) intraperitoneally. An incision was made in the skin overlying the right lateral femur, exposing the sciatic nerve. After the nerve was dissected from surrounding connective tissue, three ligatures were loosely tied around the nerve at intervals of 1 mm with 8–0 nylon sutures. Following the procedure, the muscle was sutured with absorbable 4–0 sutures and the wound was closed with metal clips. After the treatment, rats were maintained in transparent plastic cages for 2 weeks, prior to assessing paw withdrawal thresholds to mechanical stimuli.

Behavioral tests

Before behavioral tests to assess the mechanical threshold of the paw, rats were acclimated to a stainless steel grid within a Plexiglas box for more than 5 min. Mechanical allodynia was assessed using von Frey filaments (Touch–Test®, North Coast Medical Inc., Morgan Hill, CA, USA) ranging from 1.0 g to 26 g. An incremental series of monofilaments were applied to the plantar region of each hind paw with a force enough to bend the filaments. The softest filament that produced brisk withdrawal to at least two out of three stimuli at three different points on the plantar surface determined the withdrawal threshold. When no response was observed with the force of the thickest filament (26 g), 26 g was
assigned as the withdrawal threshold to prevent tissue damage that could occur with stronger stimulation. Rats with a paw withdrawal threshold of 10 g or less were used for assessing the neuropathic pain.

To assess the effects of magnetic stimulation, the rats were divided into three groups according to the strength of the magnetic stimulus applied: non–stimulation control (NSC, \(n=8\)), low–strength magnetic stimulation (LSM, \(n=9\)), and high–strength magnetic stimulation (HSM, \(n=9\)). Thermal hyperalgesia was assessed according to the method of Hargreaves et al.\(^8\). Neuropathic rats were individually placed in transparent plastic chambers with a glass floor and acclimated for 5 min. A radiant heat source (Plantar test No 37370, Ugo Basile, Comerio, VA, Italy) was aimed through the glass onto the mid–plantar area of both paws. Paw withdrawal latencies (PWLs) of both hind paws were recorded, and an automatic cut–off time of 26 sec was set to prevent tissue damage. Each paw was tested three times at 5–min intervals. The differences in withdrawal latencies (∆PWLs) between operated and unoperated sides were assessed.\(^18\) For assessing cold hypersensitivity, we used a micro syringe connected to a covered probe with a silicone tube, and spread ca. 40 µl of acetone on the plantar surface of the paw.\(^19\) Paw withdrawal frequencies (PWFs) were recorded for 2 min.

**Magnetic stimulation**

We used magnetic stimulation apparatus (Toyo Jiki Industry Co., Ltd., Saitama, Japan) that radiated the alternating magnetic force produced by a 50 Hz alternative current from an electric power supply (250 V). The apparatus consists of two pole faces (15 × 30 mm each), the distance between which can be varied according to the size of the treatment object. In our experiments, the distance between the pole faces was 60 mm.

Rats were placed approximately equidistant from both plates and the gap between the lateral side of the femur and the pole face was about 1 cm (Fig. 1). The sciatic nerves in the rat thighs on both sides, operated and unoperated, were continuously stimulated with magnetic fields of two strengths (approximately 25 mT and 70 mT, respectively). The distribution of the magnetic density was measured using a Tesla Meter (HGM–9300, ADS Ltd., Tokyo, Japan) and a 3–dimensional magnetic field analyzer (3FMA–150/150/100, Toyo Jiki Industry Co., Ltd.), and was shown to be from a distance of about 1 cm from the pole faces (Fig. 2). The apparatus was cooled with circulating tap water to prevent an increase in temperature. The rats were set in a holder (Lomir Biomedical Inc., Quebec, Canada) to restrict their movements. After setting the rats in the gap of the apparatus, the magnetic stimulus was applied for 30 min. Control rats were also set in the holder but without magnetic stimulation. All rats were subjected to this treatment for
Fig. 2 Magnetic density distribution maps of the apparatus.

The high-strength magnetic field forces were measured with a Tesla Meter and a 3-dimensional magnetic field analyzer and magnetic density distribution at a distance of about 1 cm from the pole faces was shown. Fig. 2–A shows the horizontal distribution map of the measured magnetic force. Fig. 2–B shows the 3-dimensional distribution map of the measured magnetic force.
5 days. After the treatments, each rat was placed in a separate Plexiglas box and acclimated for 5 min. The withdrawal responses to mechanical stimuli and thermal and cold responses were assessed successively. These assessments were performed on day 1 and day 5.

Statistical analysis

Results are expressed as mean ± SEM. Data analysis was performed using Prism version 5.03 (Graph Pad Software, San Diego, CA, USA), and two-way ANOVA followed by Bonferroni test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Effect on mechanical allodynia

The rats used in this study showed reduction in the paw withdrawal thresholds (PWTs) to mechanical stimuli, together with thermal hyperalgesia and cold hypersensitivity. Figure 3 shows the effects of magnetic stimulation on mechanical allodynia in the rats. On day 1, before the stimulation, PWTs in NSC, LMS and HMS groups were $6.3 \pm 0.6$ g, $6.3 \pm 0.6$ g and $7.3 \pm 0.7$ g, respectively. After 30 min of magnetic stimulation, PWTs in the LMS and HMS groups increased to $9.3 \pm 2.2$ g and $10.7 \pm 2.4$ g, respectively, while those in the NSC group were not altered. On day 5, before the stimulation, PWTs in the three groups were nearly the same as on day 1 post-treatment. After magnetic stimulation, PWTs in the LMS and HMS groups increased to $9.9 \pm 2.3$ g and $13.2 \pm 2.8$ g, respectively, while those in the control group were not altered. Magnetic stimulation was more effective in the HMS group, the increase in PWTs being statistically significant ($p<0.05$) in the HMS group as compared to the NSC group on day 5, although PWTs in the HMS group did not recover to the normal withdrawal thresholds in the uninjured paw.
Figure 4 shows the effects of magnetic stimulation on thermal hyperalgesia. There were no differences between the groups before application of the magnetic stimulation. On day 1, before the stimulation, ∆PWLs in the NSC, LMS and HMS groups were \(-5.0 \pm 0.8\) sec, \(-4.2 \pm 0.6\) sec and \(-4.4 \pm 0.6\) sec, respectively. After 30 min of magnetic stimulation, ∆PWLs in the LMS and HMS groups improved to \(-0.6 \pm 1.5\) sec and \(-0.1 \pm 1.3\) sec, although the improvement was not statistically significant. On day 5, there were no differences between the three groups before the stimulation and no beneficial effects of magnetic stimulation were obvious after the treatment.

**Thermal hyperalgesia**

Effects of magnetic stimulation on thermal hyperalgesia, represented by paw withdrawal latencies (PWLs), in rats before (Pre) and after (Post) magnetic stimulation on day 1 (A) and day 5 (B) were studied. Differences in withdrawal latencies (∆PWLs) between ipsilateral and contralateral sides are expressed as mean ± SEM in NSC (control, n=8), LMS (25 mT, n=9) and HSM (70 mT, n=9) groups.

Effects of magnetic stimulation on cold hypersensitivity, represented by paw withdrawal frequencies (PWFs), in rats before (Pre) and after (Post) magnetic stimulation on day 1 (A) and day 5 (B) were studied. Values are expressed as mean ± SEM in NSC (control, n=8), LMS (25 mT, n=9) and HSM (70 mT, n=9) groups.
Cold hypersensitivity

Figure 5 shows the PWFs to cold stimuli applied for 2 min to the right operated leg. There were no differences between the groups before application of the magnetic stimuli. On day 1, before magnetic treatment, PWFs in the NSC, LMS and HMS groups were 6.0 ± 1.5, 4.9 ± 0.8 and 6.9 ± 2.1 respectively. After 30 min of magnetic stimulation, PWFs in the LMS and HMS groups decreased slightly to 1.8 ± 1.4 and 3.6 ± 1.8, respectively. However, there were no differences between the three groups on day 5. The left, unoperated paws showed no response to cold stimuli at any point during the test.

DISCUSSION

Several previous studies using magnetic applications have suggested that non–invasive magnetic therapy could be effective for the improvement of neuropathic pain and practical for clinical application. However, the experimental conditions in each of these studies were extremely diverse in terms of the type of magnetic fields, field densities and animal models used, so that ideal magnetic stimulation conditions are still unclear. In the current study, we evaluated the effect of alternating magnetic stimulation on an established neuropathic pain model, the rat CCI model, using a unique magnetic stimulation apparatus. This system can produce an alternating magnetic field of an appropriate uniform intensity at a selected region.

We used rats with mechanical allodynia, which had reduced pain thresholds of 10 g or less, and hyperalgesia to heat and cold stimuli in their nerve–injured paws at 2 weeks after the surgery. The levels of hyperalgesia in the three groups of rats in this study on day 1 before the magnetic stimulation were almost the same as those in animals in other studies that satisfied the criteria for the development of neuropathic pain using von Frey filaments. The level of hyperalgesia in the NSC group remained unchanged throughout the rest of the experimental period. After magnetic stimulation, the threshold of mechanical allodynia increased in the treated rats. The strength of the magnetic stimulation used in the HMS group was more effective and succeeded in significantly increasing the PWTs as compared to the NSC group on day 5. This might indicate a cumulative effect of magnetic stimulation. Although magnetism was applied to the lateral sides of both femurs, no effects appeared in the left, unoperated paws. Our results are the first to demonstrate that magnetic stimulation alleviates mechanical allodynia in neuropathic pain rats. Magnetic stimulation also improved the ∆PWLs to thermal stimuli on day 1 in the experiment on thermal hyperalgesia, although the improvement was not statistically significant. On the other hand, there were no significant differences in cold hypersensitivity between the three groups. Although we counted the number of paw lifts in response to spread of acetone as the criterion for measuring cold hypersensitivity, other criteria such as response grades or cumulative times might be more suitable for precise evaluation.

It has been reported that mechanical allodynia is caused by the stimulation of peripheral terminals of Aβ–fibers of sensitized neurons in the spinal dorsal horn. On the other hand, noxious heat stimuli are transmitted to the spinal cord through C– and some Aδ–fibers. Although different processes may exist in the transmission of mechanical allodynia and heat hyperalgesia, magnetic stimulation probably attenuates these processes in the nerve injured sciatic nerve with-
Several previous studies using specific magnetic stimulation have reported that neuropathic pain in rat models was improved by repeated treatments. Nishi et al. showed that paw withdrawal latency against thermal stimulation improved with time in response to a weak electromagnetic field from day 3 to day 14 in CCI rats\textsuperscript{13}. However, they did not evaluate mechanical allodynia. Further, they suggested that insufficient nerve growth factor (NGF) might possibly be involved in the development of neuropathic pain and that magnetic field stimulation exerted beneficial effects via acceleration of neuronal regeneration induced by NGF. On the other hand, Mert et al. reported that, specifically, a pulsed magnetic field improved the high blood glucose concentrations, mechanical allodynia and thermal hyperalgesia in streptozotocin-induced acute and chronic diabetic rats\textsuperscript{12}. These findings were obtained by exposing the entire body of rats to very weak magnetic fields, below 2 mT. However, the precise mechanism of the effects of magnetic stimulation on neuropathic pain in diabetic rats was not known, whether through direct magnetic effects or due to improvement in the diabetic status of rats.

Regarding neuromodulation therapies, SCS was reported to be effective for mechanical allodynia and hypersensitivity to heat and cold in neuropathic rat models\textsuperscript{15,16,17}. In these experiments, exposure of rats to a single 30-min treatment was used, although Maeda et al.\textsuperscript{11} showed the 4-day cumulative efficacy of use of only low frequencies (4 and 60 Hz) of electrical stimulation in improving hypersensitivity in spared nerve injury (SNI) rats. These results suggested that the cumulative effects of magnetic stimulation could also potentially ameliorate the symptoms of neuropathic pain, similar to that seen with electrical stimulation. We applied the magnetic forces locally to the injured sciatic nerve. Magnetic forces were generated horizontally from one pole of the apparatus to the other. Therefore, the rat’s spinal cord would also have been exposed to a weak magnetic force, as shown in Fig.2. However, unlike magnetic stimulation, which is a non-invasive procedure, SCS is an invasive procedure, requiring implantation of leads in the spinal cord. Hence, although the mechanism of the efficacy of magnetic therapy and its precise sites of neural action are not known, magnetic therapy might prove to be effective in the treatment of intractable pain.

In conclusion, using alternating magnetic stimulation of 25 mT and 70 mT intensities, we demonstrated that high-strength magnetic stimulation more effectively improved neuropathic pain in CCI rats. Magnetic stimulation, being a non-invasive treatment, could be an effective non-pharmacological treatment alternative in patients with chronic pain.

ACKNOWLEDGEMENTS
The authors wish to thank Mr. Daichi Kurihara for his excellent technical support during our study, and Mr. Masahiro Onoda for designing the magnetic apparatus.

REFERENCES


Address for correspondence: Kazuo Okamoto
Research and Development Center,
Terumo Corporation
1500 Inokuchi, Nakai-machi, Ashigarakami-gun,
Kanagawa 258-0151, Japan
Tel.: +81-465-81-4129 / Fax: +81-465-81-4154
E-mail: Kazuo_Okamoto@terumo.co.jp

47
PAIN RESEARCH Vol.26 2011