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Research Article

Effects of Intraarticular Injection of Anti-Nerve Growth Factor Neutralizing Antibody on Pain in Osteoarthritis Rat

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ABSTRACT

Background: Nerve Growth Factor (NGF) has been recognized as an important mediator of chronic knee pain caused by Osteoarthritis (OA). We investigated the effect of intraarticular injection of anti-NGF neutralizing antibody on pain-related behavior and histological changes in Mono-Iodoacetate (MIA)-induced OA rats.

Methods: All rats had OA induced by intraarticular injection of 1 mg MIA. Anti-NGF neutralizing antibody (0.1 or 0.5 mg/kg) was intraarticularly or intraperitoneally administered at 14, 21, 28, 35 days after MIA injection. In the control group, saline was intraperitoneally administered in a same timing. Pain-related behavior was assessed by paw withdrawal threshold to punctate stimulation of the hind-paw. OA progression was assessed using Osteoarthritis Research Society International histological grading. The effect of combination of anti-NGF therapy and forced treadmill running (30 cm/s for 60 min: an intense amount for rats) on OA progression was also evaluated.

Results: Intraarticular injection of anti-NGF neutralizing antibody (0.5 and 1 mg/kg) and intraperitoneal injection (1 mg/kg) significantly reversed the OA-induced pain behavior. Analgesic effects of intraarticular injection on OA-induced pain behavior were significantly greater than intraperitoneal injection at a same dose. There were no significant differences in OA progression between control (MIA) and MIA plus anti-NGF antibody (intraarticular and intraperitoneal injection). Forced treadmill running did not affect the OA progression in all groups.

Conclusion: Analgesic effects of intraarticular injection of anti-NGF neutralizing antibody were greater than intraperitoneal injection, which means that intraarticular injection is an effective route for anti-NGF antibodies. The MIA-induced OA model failed to clarify histological differences caused by anti-NGF therapy or combination of the therapy with forced treadmill running. Future research should seek to confirm the influence of anti-NGF antibody on OA progression.

Keywords: Knee joint; Osteoarthritis; Pain; Nerve growth factor; Anti-nerve growth factor antibody; Intra-articular injection; Osteoarthritis rat model

INTRODUCTION

Knee pain is a major source of disability and hospital visit in patients with knee Osteoarthritis (OA). There is a variety of pharmacological interventions available for knee OA pain, including acetaminophen Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) and cyclooxygenase-2 inhibitors, opioid, hyaluronic acid injections and corticosteroid injections. However, with disease progression, pain management using existing medication frequently becomes more challenging. Therefore, development of new therapies is strongly required.

Nerve Growth Factor (NGF) has been recognized as an important mediator of chronic knee pain caused by OA [1,2]. The effects of NGF are mediated by at least two receptors with different binding affinity: the high affinity receptor TrkA [3] and the low affinity receptor p75 neurotrophin receptor [4]. NGF blockade can be achieved using antibodies or TrkA-IgG fusion protein that bind NGF and prevent its interaction with TrkA and p75 receptors. Recent clinical trials in patients with knee OA showed that therapies blocking NGF remarkably reduced joint pain [5,6]. In human OA, the levels of NGF are elevated in synovium [7]. The increased NGF expression in synovium was associated with symptomatic knee OA [7]. In our previous animal study, half of dorsal root ganglion neurons innervating the synovium in rat knee joints were TrkA immunoreactive [8]. The TrkA expression in synovium afferents gradually increased, with disease progression in Mono-sodium Iodoacetate (MIA)-induced OA in rats [9]. These results support that increased NGF immunoreactive cells in synovium might contribute to OA pain. Here, we hypothesized that effects of local administration of anti-NGF antibody on OA pain and NGF expression in synovium were greater than systemic administration.

Regarding adverse effects related anti-NGF antibody, a higher incidence of rapidly progressive osteoarthritis (RPOA) were observed in previous clinical trial of patients with knee OA [10,11]. However, the relevance between anti-NGF antibody and RPOA remains uncertain. Therefore, it is also important to evaluate the influence of anti-NGF antibody on OA progression in animal study.

It has been reported that MIA injection into the rat knee joint disrupts chondrocyte metabolism, leading to cell death and subsequent loss of articular cartilage with synovitis and subchondral bone changes [12,13]. The joint damage observed in the MIA model is similar to the joint damage observed in OA [14,15]. This model is an established and well-characterized preclinical model of OA and can be used to study the effects of drugs on pain.

The purpose of this study was to clarify effects of Intraarticular (IA) injection of anti-NGF neutralizing antibody on pain and OA progression in MIA-induced OA in rats. Specifically, we investigated pain-related behavior and histological changes in different administration route (IA versus Intraperitoneal (IP)). We also evaluated the effect of combination of anti-NGF therapy and forced treadmill running on histological changes.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (6 weeks old, weight 250-300 g) were used in this study. All experiments were approved by the Animal Care and Use Committee of Kochi University. All outcome measurements were made by an observer blinded to treatment.

Induction of OA

After being anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneal), all rats were injected with 1 mg of MIA in 25µl of saline (Sigma-Aldrich, St. Louis, MO) using a 27 G needle with a Hamilton syringe inserted through the patellar ligament into the intra-articular space of the left knee.

Drugs

Anti-NGF neutralizing antibody (0.1 or 0.5 mg/kg in 50µl of saline) (anti-NGF-2.5S, Sigma-Aldrich, St. Louis, MO) was intraarticularly or intraperitoneally administered at 14, 21, 28, 35 days after MIA injection. In the control group, 50 µl saline was intraarticularly administered in a same timing.

Pain-related behavior tests

Pain-related behavior was assessed by paw withdrawal threshold to punctate stimulation of the hind-paw using von Frey filament. Rats were put inside a plexiglass cage placed on an elevated mesh steel platform. Von Frey filaments of varying bending forces (0.4, 0.6, 1, 1.4, 2, 4, 6, 8, 10, 15, 26 g) were applied to the plantar surface of the bilateral paw in ascending order of bending force. Each filament was applied three times for approximately 2-3 s periods or until a withdrawal response was evoked. After a response, the paw was retested with the filaments in descending order until no response occurred at which point the filaments were again applied in ascending order until the response could once again be evoked. The final bending force to induce leg withdrawal was recorded three times. The median value was recorded as mechanical threshold of the paw [9].

Treadmill running protocol

The forced treadmill running was started after the first anti-NGF neutralizing antibody IP or IA injection, or saline injection in the running groups, (n = 6 for each group). The rats were subjected to exercise 5 days a week for 4 weeks using a motor-driven treadmill designed for rodents at a constant speed of 30 cm/s for 60 min. The exercise is an intense amount for rats [16].

Histological evaluation of knee joint

Left knee joints were obtained after all pain-related behavior tests at 6 weeks after MIA injection. The knee joints were placed in 10% formalin for 3 days, decalcified by formic acid for 12 days, and embedded in paraffin. Five-micrometer sections were cut and stained with Safranin O and fast green. Histological changes of knee joints were scored according to the Osteoarthritis Research Society International (OARSI) grading system criteria (from 0 (best) to 5 (worst)) [17]. Data were presented as the median [interquartile range]. The most severe lesion of the femoral condyle on each frontal section was scored. The histological scoring is performed on the three most severely affected consecutive sections (at 200 μm intervals). The values for each parameter are then averaged across the three scored sections per knee joint. We compared the OARSI histological score between control (MIA), MIA plus anti-NGF (IP) and MIA plus anti-NGF (IA) (n = 6 rats for each group) in no running and running group.

Statistical analysis

Statistical analyses were performed with JMP, Version 10 (SAS Ins. Cary, NC). Two-way analysis of variance with Tukey's test was used to compare pain-related behavior tests. Kruskal-Wallis test with Steel-Dwass test was used to compare the OARSI histological scores. P<0.05 was regarded as statistically significant.

RESULTS

Pain-related behavior tests

MIA injection decreased mechanical threshold in the hind paw and the knee joint. IA injection of anti-NGF neutralizing antibody at a dose of 0.1 and 0.5 mg/kg and IP injection at a dose of 0.5 mg/kg significantly reversed mechanical hyperalgesia of the hind paw and the knee joint compared to control (MIA). Analgesic effects of IA injection on mechanical threshold in the knee joint and the hind paw were significantly greater than IP injection at a same dose (Figure 1).

Histological analysis of the knee joint

Histology of knee joints showed intra-articular injection of MIA into the knee progressed articular cartilage and subchondral bone damage. Figure 1 & 2 shows representative photographs of knee joints. There were no significant differences in the OARSI score between all groups (Figure 3). The anti-NGF neutralizing antibody (IP and IA injection) and forced treadmill running did not affect the OA progression (Figure 3).

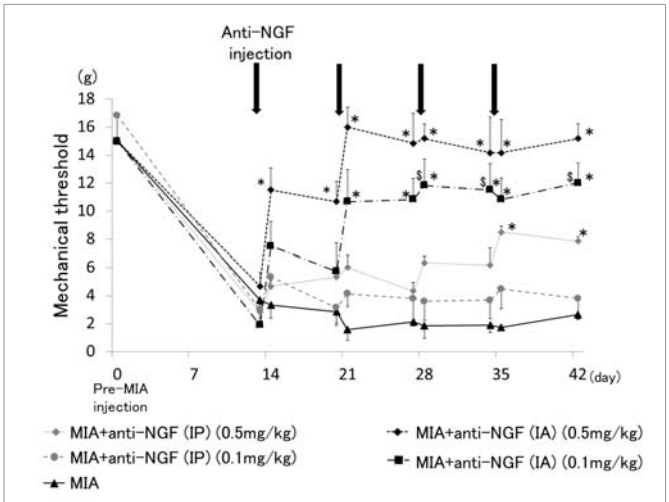

Figure 1: Effect of anti-NGF neutralizing antibody on mechanical threshold in the hind paw.
*p < 0.05 versus MIA, $p < 0.05 versus MIA + anti-NGF (IP) (0.1 mg/kg). Data displayed as mean ± SE.
MIA: Mono-Iodoacetate; NGF: Nerve Growth Factor; IP: Intraperitoneal; IA: Intraarticular.

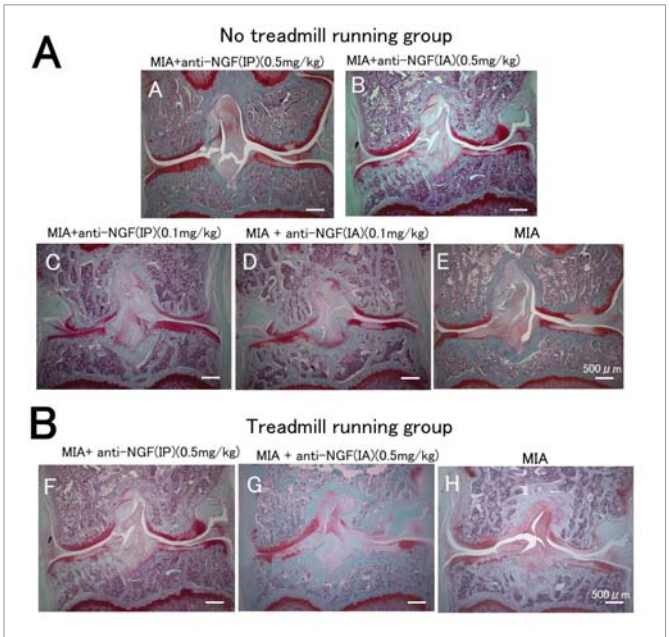

Figure 2: Figure A shows that representative photographs of knee joints of anti-NGF (IP) (A and C), anti-NGF group (IA) (B and D) and MIA (E) in no treadmill running group. Figure B shows that representative photographs of knee joints of anti-NGF (IP) (F), anti-NGF group (IA) (G) and MIA (H) in treadmill running group.
MIA: Mono-Iodoacetate; NGF: Nerve Growth Factor; IP: Intraperitoneal; IA: Intraarticular.

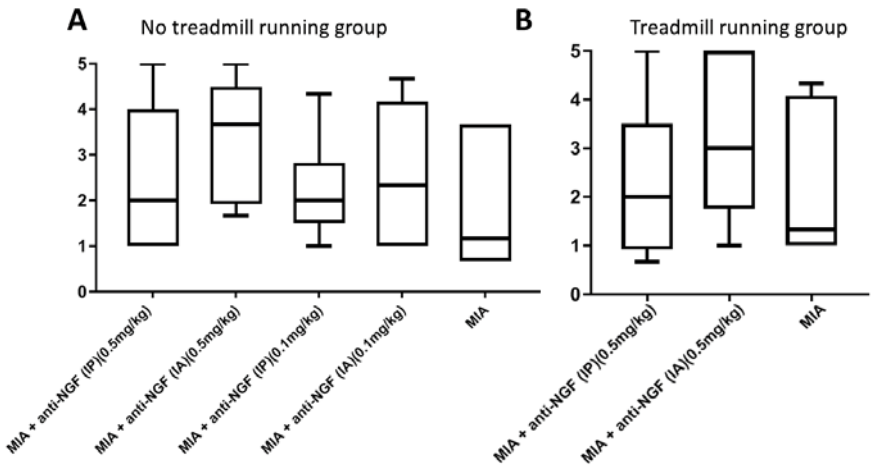

Figure 3: The OARSI histological scores in no treadmill running group (A) and treadmill running group (B).Data were presented as the median [interquartile range]. There were no significant differences in all groups.
MIA: Mono-Iodoacetate; NGF: Nerve Growth Factor; IP: Intraperitoneal; IA: Intraarticular.

DISCUSSION

This is the first study to evaluate effects of IA injection of anti-NGF neutralizing antibody on pain in MIA-induced OA in rats. Our results showed anti-NGF neutralizing antibody inhibited OA-induced pain behavior by MIA injection. The analgesic effects of anti-NGF neutralizing antibody IA injection were greater than IP injection. IA injection was effective even if the drug concentrations were ineffective by systemic administration. In rat MIA-induced OA, NGF and the receptor TrkA are upregulated in the synovium in the knee [9,18], and the upregulations are involved in the maintenance and development of OA pain [1]. Previous study showed that systemic administration of anti-NGF antibody attenuates OA pain behavior in MIA-induced knee OA [19-21]. Our results suggest that inhibition of local NGF is important in order to reduce the knee OA pain. IA injection of hyaluronic acid or corticosteroid is widely used as a pain treatment for knee OA. IA injection may be also an effective route for anti-NGF antibodies. Indeed, recent double-blind and placebo-controlled study showed that IA injection of TrkA inhibitor in knee OA subjects reduced pain with a numerically functional gain and an acceptable safety profile [22].

Our histological analysis showed that administration of anti-NGF neutralizing antibody, forced treadmill running and the combination did not make significant differences in OA progression. Previous clinical trial showed that the event rate of RPOA increased as a function of the tanezumab dose, and administration of tanezumab combined with NSAID further increased the rate [10,11]. The higher incidence of RPOA in the clinical trial suggests that the combination therapy of tanezumab and NSAID is unfavourable. In this study, we tried to clarify the effect of combination of anti-NGF therapy and forced treadmill running on OA progression for the first time. Because there is concern that OA will be progressed by administration of anti-NGF antibody to highly active patients. However, our results showed that the combination did not have significantly greater OARSI score than the others. Consequently, the 1mg MIA-induced OA model failed to clarify histological differences caused by anti-NGF therapy or combination of the therapy with forced treadmill running. The reasons are that 1mg MIA-induced joint degeneration may be too severe and our study was not powered to detect rare adverse effects on joint structure. Previous study reported that treatment with tanezumab prevented gait deficiency and resulted in more severe cartilage damage in the rat medial meniscal tear model [23]. Meanwhile, no deleterious effects were observed in joints or bones in normal monkeys, rats, or mice administered high doses of anti-NGF monoclonal antibody [24]. Further investigation is needed to clarify RPOA in anti-NGF therapy. This study has several potential limitations. Adverse effects that included RPOA and joint replacement were more common in patients treated with anti-NGF antibody and NSAIDs than either treatment alone [2,10,11]. This study did not demonstrate combination effects of anti-NGF antibody with NSAIDs. Because previous studies already showed that the combination therapy is unfavourable, and our primary objectives were effects of IA injection of anti-NGF antibody on pain, therefore, we focused effects of the antibody alone. Second, we used anti-NGF neutralizing antibody for this study. We also did not evaluate differences in efficacy between anti-NGF neutralizing antibody and anti-NGF monoclonal antibody.

In conclusion, analgesic effects of IA injection of anti-NGF neutralizing antibody were greater than IP injection, which means that IA injection is an effective route for anti-NGF antibodies. The MIA-induced OA model failed to clarify histological differences caused by anti-NGF therapy or combination of the therapy with forced treadmill running. Future research should seek to confirm the rare adverse effects on joint structure in anti-NGF therapy.

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AUTHOR CONTRIBUTIONS

All authors approved the final version to be published. K.A. had full access to all of the data in the study and takes responsibility

for the integrity of the data and the accuracy of the data analysis. K.A. designed the experiments, analyzed and interpreted results, and wrote the manuscript. K.A. did histological analysis and Reika Shiraishi did pain-related behavior tests. M.I., Y.O. and M.I. analyzed and interpreted the results.

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