学位論文（要約）

Study on the local administration of the nerve growth factor antibody on knee joints' pain in a rat osteoarthritis model

(ラット変形性関節症モデルの膝関節痛に対する神経成長因子抗体局所投与に関する研究)

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【Background and Objectives】

Osteoarthritis (OA) is the most common type of arthritis and contributes to an economic burden on both patients and the society. Clinically, the symptoms of OA include pain, joint stiffness, and disability. Pain is particularly important in all clinical problems as it is not only the cause of hospital visits for treatment, but also the main cause of poor quality of life and social labor loss. Current pharmacological treatment for OA pain using traditional analgesics is partly effective and accompanied by serious side effects. Recently, a humanized immunoglobulin G2 monoclonal nerve growth factor (NGF) antibody, which has been used as an analgesic agent for OA, has gained a significant relevance in relieving OA-associated pain in a clinical trial. Single intravenous injections can effectively relieve chronic pain in patients with OA of the knee joint and last for several weeks. However, the clinical phase 3 experiment of anti-NGF antibody has been on hold by the Food and Drug Administration in 2015 because of its adverse effect, with all of the patients presenting with progressively worsening OA and subsequently requiring total joint replacement in 1 of 13 phase 3 studies. Moreover, other adverse effects, such as paresthesia, arthralgia, pain in the extremity, and headache, were also observed after the systemic administration of tanezumab, and these effects remain a safety concern for patients.

Even so, because of the high effectiveness of anti-NGF antibody treatment for relieving chronic pain such as OA pain, researchers keep focusing on developing the anti-NGF antibody treatment. As OA only affects a limited number of joints, intra-articular injection therapy appears to be a more attractive alternative for patients compared with other alternative treatments. Local injection can largely decrease the risk of systemic exposure and the incidence of adverse effects. Moreover, local injection can reduce the effective dosage, possibly preventing the aggravation of adverse effects and reducing the economic burden on patients. Therefore, the local administration of anti-NGF antibody, such as its intra-articular injection into the OA joints, might be a preferable way to maintain its effectiveness for the treatment of chronic pain and to reduce the incidence of adverse effects.

However, the hypothesis regarding the local treatment of anti-NGF antibody has not been verified yet. Hence, this study aimed to investigate whether the low-dose intra-articular injection of anti-NGF antibody can reduce OA pain and avoid the adverse systemic effects using a rat OA model.

【Methods】

8-week male SD rats were used in this experiment. Rats were randomly divided into 6 groups. Each group contained a sample size of 6 rats. Osteoarthritis-like pain was

induced by injecting with mono-iodoacetate (MIA, 0.5mg/ 25μl saline solution) into rats' right knee joint capsules. 4 groups of rats (MIA groups) were injected with MIA and the other 2 groups of rats (sham groups) were injected with saline solution (25μl). After two weeks, different doses (1 μg, 10 μg, and 100 μg) of anti-NGF antibodies and saline solution were intra-articularly injected into their right knees for MIA group. Highest dose of anti-NGF antibody (100 μg) and saline solution were injected respectively in sham groups to observe the effect of anti-NGF antibody on joint tissues. Injection were performed one time a week from the end of second week. Pain behavior performance were confirmed by behavior test including weight bearing and von frey filament test. Behavior tests were performed two times a week from week 0 (before injection of MIA). All rats were sacrificed at the end of week 6 and rats' knee joints were collected and submitted for macroscopic evaluation. Histological evaluation was also performed after H&E and safranin o staining of collected rats' knee joints.

【Results】

The results of the behavioral tests showed that MIA injection (MIA groups) can cause impaired weight bearing ($p < 0.005$) and decreasing threshold regarded as allodynia ($p < 0.01$) as signs of pain from the first week, and rats receiving saline injection (sham groups) did not present any pain behavioral changes. After the intra-articular injection of anti-NGF antibody, 100 μg anti-NGF antibody treatment effectively relieved the pain of the OA model rat, as evidenced by improved weight-bearing performance (saline, 1 μg, 10 μg vs 100 μg from week 3, $p < 0.001$), whereas 1 μg and 10 μg showed no effect on relieving pain. However, allodynia, which was also induced by MIA injection, did not improve with anti-NGF antibody injection of all doses.

The results of macroscopic evaluations showed that MIA injection can induce the damage in the cartilage, imitating OA characterized by cartilage erosions. No erosions were showed in sham groups rats' knee joints cartilage that received saline injection. The injection of anti-NGF antibody of all doses and saline had no evident adverse effects on the joints in both MIA and sham groups. Consistent with these results, the histological evaluations based on H&E and safranin O staining showed that disorganized cartilage structure, reduction of safranin-O staining and destroyed tidemark integrity can be observed in MIA groups but knee joints cartilage remains normal in sham group. No significant difference in each MIA and between two sham group indicated that the injection of anti-NGF antibody had no negative effects on cartilage histology. These results showed that during the progression of MIA induced OA, anti-NGF antibody injection did not obviously interrupt the pathological progression of OA.

【Discussion】

Anti-NGF antibody has recently been used as an analgesic drug for chronic pain, but adverse effects, including progressively worsening cartilage degeneration, are considered particularly problematic in OA. The evaluation of safety data of systemic treatment of anti-NGF antibody showed that issues associated with safety were detected in both clinical and nonclinical cases. In this study, we found that low dose of 100 μg anti-NGF antibody could alleviate the MIA-induced pain without deterioration of OA. For systemic injection, it usually requires 10 mg/kg, approaching to 5 mg/injection. In fact, intra-articular injection has been considered a more cost-effective treatment for OA compared with systemic injection. Direct delivery of drugs requires low effective dose, which can decrease the risk of side effects and damages to other unimpaired tissues. However, systemic injection is known to improve both weight-bearing asymmetry and mechanical allodynia. The intra-articular injection of NGF antibody only showed an effect on weight bearing. Mechanical allodynia is a painful sensation stimulated by light touch. Although the mechanism of allodynia is incompletely understood, the involvement of alterations in mechano-transduction and sensory neurons in the central nervous system has been described by several studies. The increasing expression of NGF under OA condition, contributes to the change in receptor sensitivity, or the changes in the dorsal root ganglion may result in central sensitization. Our current results suggest that the intra-articular injection of anti-NGF antibody may provide insufficient analgesic effect on allodynia-related pathologies such as complex regional pain syndrome.

Furthermore, although OA pain has been relieved by anti-NGF antibody injection, complications of OA, such as cartilage degeneration, still require treatment. The mechanism by which topical administration of anti-NGF antibody does not suppress allodynia, but does not exacerbate OA, is still unknown. Further study is required to clarify the association between these two phenomena.

【Conclusion】

In conclusion, based on the results of this study, the effective dose of anti-NGF antibody was significantly lower in intra-articular injection than that in systemic injection without accelerating the OA progression. Moreover, intra-articular injection might be an alternative approach to systemic injection for the treatment of patients with OA. Further experiments are required to improve the intra-articular injection treatment of anti-NGF antibody for protecting cartilage degeneration and elucidate the mechanism of allodynia in OA.