学位論文内容の要旨
（Summary of Dissertation）

博士の専攻分野の名称　博士（医　学）　氏 名　田　園
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（Dissertation Title）

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)

【Background and Objectives】Osteoarthritis (OA) is the most common arthritis which contributes to an economic burden on patients and society. Pain is particularly important in all clinical problems as it's not only the cause of patients going to the hospital for treatment, but also the main cause of declining quality of life and loss of social labor. Current pharmacological treatment for OA pain are partly effective and accompanied by serious side effects. Recently, a humanized IgG2 monoclonal nerve growth factor (NGF) antibody has gained a great achievement in relieving OA pain of patients in clinical trial. Single intravenous injections can effectively relieve joint pain of in patients with OA of knee and last for several weeks. However, the clinical experiment of NGF antibody has been held by Food and Drug Administration, because of its adverse effect in which most patients presented progressively worsening OA resulting in requirement of total joint replacement. Also, the high frequency of adverse effects occurred after systemic administration of tanezumab such as paresthesia, arthralgia, pain in extremity, and headache also remains safety concern. Because the high effectiveness of anti-NGF treatment for reliving OA pain, researchers keep focusing on developing the anti-NGF treatment. Since OA only affects limited number of joints, intra-articular injection therapy appears to be more attractive alternative for patients. Local injection can largely decrease risk of systemic exposure and the incidence of adverse effects and release an economic burden on patients. In fact, local injection can minimize adverse effects generated by given in lower concentrations, which may lead to reduce expenses and economic burden on patients Therefore, we hypothesized that intra-articular injection of NGF antibody with a lower dose may reduce OA pain and avoid the adverse systemic effects. The purpose of our study is to evaluate the efficacy of intra-articular injection of NGF antibodies using a rat osteoarthritic model.

【Materials and Methods】OA-like pain model was established by single injection of 0.5 mg mono-iodoacetate (MIA) into right knee joint of 8-week-old male Sprague Dawley rats. Saline and different dose of NGF antibody were injected into right knee once a week from week 2 after injection of MIA. Evaluation included behavior test, macroscopic scoring and histological evaluations. Behavior test including weight-bearing test and von Frey filament test were performed before and after MIA injection and continue for 6 weeks. Rats were sacrificed at the end of week 6 after MIA injection and the right knee joints were collected. Macroscopic score was performed right after collection and evaluated by Likert scale. The degenerations of cartilage were microscopically examined and scored by Mankin scoring. 5 μm sections of knee joints were prepared and performed for fluorescent staining.

【Results】Florescent staining indicated that MIA injection can increase the concentration of NGF in the synovium tissue surrounding affected joints. Injection of NGF antibody neutralized the NGF in tissue and therefore reduced the signal of NGF staining. Results of behaviors test showed that MIA injection can cause impaired weight bearing ($P<0.005$) and allodynia ($P<0.01$) as signs of pain from 1st week. 100 μg NGF antibody treatment relieved the pain of rat OA model as evidenced by weight bearing performance but not allodynia. Results of macroscopic evaluations showed that MIA injection can induced cartilage damage and the injection of NGF antibody has no obvious adverse effects on joint. Consistent with these results, histological evaluations of H&E and safranin O showed that injection of NGF antibody has no negative effects on cartilage pathology. Results showed that MIA injection impaired weight bearing and allodynia as signs of pain. Of note, NGF antibody treatment relieved the pain of rat OA model as evidenced by improved weight bearing performance but not allodynia. In contrast, no significant differences were observed in macroscopic and histological scores of knees in NGF antibody-treated rats and controls.

【Discussion】NGF antibody has recently been used as an analgesic drug for chronic pain, but the adverse effects of the progressively worsening cartilage degeneration are particularly considered to be problematic for OA. According to the evaluation of safety data of systemic treatment of NGF antibody, the issues associated with safety were detected in both clinical and nonclinical. In this study, we found that low dose of 0.1 mg NGF antibody could alleviate the MIA-induced pain without deterioration of OA, suggesting that intra-articular administration of NGF antibody may be an effective treatment for OA, especially in mono- or oligo-articular OA. Moreover, intra-articular injection can reduce the effective dose of NGF antibody (0.1 mg / knee) compared to the dose used for systemic injection. Systemic treatment of NGF antibody for chronic pain using animal OA model usually requires 10 mg/kg, approaching to 5mg/ injection. Intra-articular injection has been widely used as cost effective treatment for OA comparing with systemic treatment. This directly delivery of drugs requires low effective dose which can decrease the risk of side effects and damage to other unimpaired tissue. Therefore, we can assume that local treatment of NGF antibody may be an appropriate strategy to reduce the dosage and thereby reduce the incidence of side effects and the exposure to the whole body. This is the first study to elaborate this hypothesis. Although systemic injection is known to be able to improve both weight-bearing asymmetry and mechanical allodynia, the intra-articular injection of NGF antibody only showed effect on weight bearing while didn't present any effect on improving allodynia of rat hind paw which induced by MIA injection. Mechanical allodynia is associated with the involvement of alterations in mechano-transduction and sensory neurons in the central nervous system. Our current results suggest that intra-articular injection of NGF antibody may provide insufficient analgesic effect on allodynia-related pathologies such as complex regional pain syndrome. Whether local injection of NGF antibody can effectively reverse the complex regional pain syndrome changes established during the NGF rising phase needs further exploration and experiments. An intra-articular injection treatment may have some disadvantages such as infection, rejection reaction, damage of tissue. Also, the progression of OA pathological such as cartilage degeneration still needs to be treated. More experiments are required to clarify the mechanism of NGF antibody does not suppress allodynia but does not exacerbate OA.

【Conclusion】Conclusion of this research is that local injection largely reduced effective dose of NGF antibody as compare to systemic injection without accelerating the OA progression. This might be alternative approach to overcome the drawback of systemic injection for treatment of OA patients. Further experiments are needed to improve intra-articular injection treatment of NGF antibody and elucidate the mechanism of allodynia in OA.