INJECTION OF NUCLEAR FACTOR-KAPPA B DECOY INTO SCIATIC NERVE SUPPRESSES MECHANICAL ALLODYニア AND THERMAL HYPERALGESIA IN RAT INFLAMMATORY AND NEUROPATHIC PAIN MODELS

Introduction
Proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β) or IL-6 are well-known mediators of inflammatory or neuropathic pain such as sciatica. Recently, it was reported that the transcription factor, nuclear factor-kappa B (NF-kB) plays a crucial role in regulating proinflammatory cytokine gene expression and is regarded to be one of the most important targets for therapeutic intervention against inflammatory diseases such as rheumatoid arthritis, asthma, or inflammatory bowel disease. Other recent experiments found that NF-kB decoy oligodeoxynucleotides (ODNs) which contained synthesized cis elements to block the activation of promoters of proinflammatory cytokine genes effectively suppressed cytokine expression. We hypothesized that inhibiting NF-kB gene expression with NF-kB decoy may suppress mechanical alldynia or thermal hyperalgesia in rat inflammatory and neuropathic pain models.

Materials and Methods
NF-kB decoy labeled with FITC (decoy-FITC) was induced into explant culture (in vitro study) and endoneurally injected into the sciatic nerve (in vivo study), and the transduction efficiency of NF-kB decoy into DRG neurons were investigated. Cell size distributions of FITC-positive neurons were also measured. For behavioral testing, 9 rats received plantar injections of 50µl of complete Freund’s adjuvant (CFA) and caused CFA-induced inflammation (inflammatory pain model), and 9 rats were performed surgery in which their sciatic nerve was constricted partially as Seltzer model (neuropathic pain model). Rats in both models were divided into three groups: Decoy group - single endoneural injection of 10µl of NF-kB decoy (n=6); Saline group - single endoneural injection of 10µl of saline (n=6); or Naïve group - untreated (n=6). The day before surgery and on days 1 to 14 after surgery, behavioral testing was performed using a Dynamic Planter Aesthesiometer and a Hargreaves device with a heat source respectively.

Results
The total transduction efficiency of decoy-FITC was 54% in vitro and 21% in vivo. In cell size distributions, of FITC-positive neurons counted, 39% were small, 33% were medium, and 30% were large-sized in vitro. On the other hand, 48% were small, 35% were medium and 18% were large-sized in vivo (Fig. 1). In behavioral testing, mechanical allodynia was suppressed significantly from 2 to 14 days (Fig. 2), and thermal hyperalgesia was suppressed significantly from 2 to 3 days in decoy group in inflammatory pain model. On the other hand, in neuropathic pain model, mechanical allodynia was suppressed significantly from 3 to 14 days, and thermal hyperalgesia was suppressed significantly from 2 to 14 days in decoy group in decoy group (Fig. 3).

Discussion
In the current study, we first evaluated whether NF-kB decoy ODNs were transduced into DRG neurons in vitro. Results in explant cultures indicated that NF-kB decoy ODNs were transduced into small to large DRG neuron cells with a transduction efficiency exceeding 50%. On the other hand, from the clinical aspect, selective nerve root block is one of the most popular treatment for radiculopathy of patients with lumbar disc herniation. Thus, following the in vitro study, we performed an in vivo study to evaluate whether NF-kB decoy injected into the sciatic nerve from a location just proximal to the sciatic notch was conveyed to the DRG. Results from the current study demonstrated that NF-kB decoy was conveyed to the DRG with a total transduction efficiency of 20.5%. Additionally, NF-kB decoy reduced mechanical allodynia and thermal hyperalgesia after CFA injection into rat hindpaws or partial sciatic nerve ligation. These results suggest that NF-kB decoy injected as a selective nerve root block might be effective in reducing inflammation and facilitating clinical improvement in patients with inflammatory pain or neuropathic pain. While NF-kB decoy was observed to be transduced into all sizes of neurons, it preferentially accumulated in the unmyelinated small-sized neurons associated with pain perception, compared with myelinated large-sized neurons associated with proprioception, inducing the discrepancy in suppression between mechanical allodynia and thermal hyperalgesia. These results suggest that it may be possible that NF-kB decoy has varying effects on different types of neurons and/or that the duration of its effectiveness varies with neuron type.