Transcription Factor Decoy NFкB Inhibits the Expression of Cytokines and Pain Markers in Rat Dorsal Root Ganglion Organ Cultures

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Introduction: Dorsal root ganglion (DRG)-derived sensory neurons are targets in studies related to neuropathic pain. It has been reported that the pro-inflammatory cytokines [tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1)] and pain markers [activating transcription factor-3 (ATF-3) and calcitonin gene-related peptide (CGRP)] are induced in DRGs of neuropathic pain models.¹,² DRG organ culture systems have been widely used as models of peripheral neuropathies because effects on the DRGs can be isolated, unlike the in vivo situation.² Moreover the advantage of this organ culture system is that the original architecture of the DRG and its association with neurons is preserved. In dissociated cultures, Schwann cells and resident macrophages are lost.¹

The transcription factor, nuclear factor kappaB (NFκB), is a heterodimeric DNA binding protein that plays a critical role in the regulation of many pro-inflammatory mediators. The transfection of decoy oligodeoxynucleotide (ODN) containing NFκB cis element has been reported to be a feasible therapeutic approach for disc degeneration and osteoarthritis.⁴,⁵ Therefore, we hypothesized that treatment of organ-cultured DRGs with NFκB decoy ODN is effective in suppressing the gene expression of pro-inflammatory cytokines and pain-related molecules.

The specific aim of this study is to determine if NFκB decoy ODN suppresses the gene expression of pro-inflammatory cytokines and pain markers in a DRG organ culture system as a model of peripheral neuropathies.

Methods: All experiments were performed upon approval by the Animal Care and Use Committee at our institution. Thirteen Sprague-Dawley rats (200-250g) were used. Bilateral lumbar DRGs from L3 to L5 levels were removed by cutting the proximal and distal nerve root of the DRGs without contacting the DRGs. DRGs for the non-cultured group were immediately preserved in RNAlater (non-cultured group, n=9). Other DRGs were free-floating cultured in 0.5 ml of DMEM only (control group), or with 1% insulin-transferrin-selenium, 10 μM scrambled ODN (SCR group) or 10 μM NFκB decoy ODN (Decoy ODN group) for 48 hours at 37°C 5% CO₂ in air (for measurement of gene expression; n=9 per group, for cell viability study; n=6 per group).

Cell viability: For visualization of cell viability following the free-floating culture of DRGs for 48 hours, DRGs were treated with LIVE/DEAD (Life Technologies) and subjected to semi-quantification analysis using a confocal microscope. For quantification of cell viability at 48 hours, the culture medium was
collected and assayed for LDH activity, which is an indicator of cell death, using the Cytotoxicity Detection kit (Roche). The values of percent LDH activity were calculated using the LDH absorbance of lysed DRGs cultured with 0.1% Tween 20 (lysis control) and intact cultured DRGs.

**Measurement of gene expression in DRGs:** Total RNA was isolated from DRGs using the RNeasy kit (Qiagen) after pulverization. RNA was reverse-transcribed to cDNA and amplified using specific primers (Qiagen). q-PCR was performed using gene-specific primers for cytokines [interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α)] and pain molecules [nerve growth factor (NGF) and cyclooxygenase-2 (COX-2)]. 18s rRNA was used as the internal control.

**Statistical Analyses:** Each group were analyzed by two-way ANOVA with Fisher PLSD post-hoc test, which accounts for multiple comparisons.

**Results:** Cell viability: Live cell number by Live/Dead assay in DRGs cultured for 48 hours, was similar to those found in non-cultured DRGs. There was a tendency for decreased LDH activity between the Control and 10 μM NFκB decoy group (p=0.06), while no difference between the Control and 10 μM Scrambled ODN group was found.

Gene expression changes due to organ culture (Fig. 1A and B): The expression of IL-1β, COX-2 and NGF in DRGs cultured for 48 hours was significantly increased compared to those in non-cultured DRGs (p<0.01). However the expression of TNF-α was not significantly induced by 48 hours culture of DRGs.

Effect of NFκB on cytokines in cultured DRGs (Fig. 1A): NFκB decoy significantly suppressed the expression of IL-1β compared to Control (p<0.01) and Scrambled ODN (p<0.05). NFκB decoy significantly suppressed the expression of TNF-α compared to Scrambled ODN (p<0.01).

Effect of NFκB on pain molecules in cultured DRGs (Fig. 1B): NFκB decoy significantly suppressed the expression of COX-2 and NGF compared to Control (p<0.01) and Scrambled ODN (p<0.01).

**Discussion:** In this study, which used an in vitro model of peripheral neuropathy, the results showed that NFκB decoy suppressed the gene expression of pro-inflammatory cytokines and pain molecules after 48 hours in a rat DRG organ culture system. This inflammatory change may be induced by axonal damage due to a dissection procedure, and may simulate nerve injury in vivo.

The possible mechanism of NFκB decoy ODN-induced inhibition is not revealed in this experiment. Recent studies revealed that nerve injury induces the translocation of c-Jun N-terminal kinase (JNK) into the nucleus; JNK then activates IκB kinase; this will induce phosphorylation of IκB, and subsequent activation of NFκB. Therefore, we can speculate that the NFκB decoy ODN interrupts the NFκB signaling pathway, leading to the suppression of pro-inflammatory cytokines and pain molecules. In vivo, the intrathecal injection of NFκB decoy suppressed allodynia as well as induction of ATF-3 and CGRP in a rat neuropathic pain model. Both in vivo and in vitro data support our contention that treatment with NFκB decoy may suppress not only the induction of ATF-3 and CGRP, but also those of pro-inflammatory cytokines and pain molecules in our model of neuropathic pain.
Significance: NFkB Decoy ODN treatment resulted in a significant inhibition of inflammatory responses after axon damage in an in vitro organ culture system. In vivo studies will provide us further mechanistic understanding by confirming that blocking of pro-inflammatory cytokines by decoy NFkB ODN reduces inflammatory changes after nerve damage.

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