Pain-related Differential Expression of NGF, GDNF, IL-6, and Their Receptors in Human Vasculitic Neuropathies

Masahiko YAMAMOTO, Yasuhiro ITO, Norimasa MITSUMA, Naoki HATTORI and Gen SOBUE

Abstract

**Objective** Pain-related differential expressions of nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF) and interleukin-6 (IL-6), and their receptors were investigated in human vasculitic neuropathies.

**Materials and Methods** The mRNA levels of pain-related neurotrophic factors, NGF, GDNF and IL-6, were examined in the sural nerves of 22 painful and non-painful patients with acute necrotizing vasculitic neuropathies, together with their concomitant soluble receptors (p75, GFRα-1 and IL-6Ra).

**Results** The mRNAs for these factors and receptors in the lesioned nerves were up-regulated to a variable extent in both groups. NGF mRNA expression was more closely correlated with that of p75 in painful neuropathy with relatively preserved large fiber density, compared with non-painful neuropathy, though the NGF mRNA level in painful neuropathy was lower than that in non-painful neuropathy. GDNF was closely associated with GFRα-1 in mRNA levels regardless of the pain state, but IL-6 was not associated with IL-6Ra.

**Conclusion** The differential expression of neurotrophic factors and their cognate soluble receptors in human vasculitic neuropathy suggests that NGF, which was effectively transferred to sensory axons with p75, may induce pain.

(Key words: glial cell line-derived neurotrophic factor, nerve growth factor, pain, receptors, vasculitic neuropathy)

Introduction

Nociception is regulated by a large number of endogenous molecules of neurotrophic growth factors and neuropoietic cytokines (1). Nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) have been found to promote the survival of sensory neurons with small myelinated or unmyelinated fibers. Cytokines, such as interleukin-6 (IL-6), are known to lower the threshold of nociception, especially in inflammatory conditions. These pain-related factors act via binding to their cognate receptors in axons and the cell bodies of neurons. It has been reported that axotomy by itself, resulting in Wallerian degeneration, is sufficient to elicit pain (2). However, the mechanism of pain in humans induced by nerve lesions is not fully understood; the possible pain generators include the nerve injury site, dorsal root ganglia (DRG), the dorsal horn of the spinal cord, and nociceptors.

We have now examined the mRNA expression profiles of NGF, GDNF, and IL-6, and their concomitant receptors in the acute phase of human vasculitic neuropathies in terms of pain. In acute necrotizing vasculitic neuropathies, the nerve pathology is almost exclusively acute axonal degeneration analogous to Wallerian degeneration due to ischemic damage (3, 4). By correlating the expression levels of these neurotrophic factors with those of their receptors in painful and non-painful states, we have tried to obtain pain-provocative profiles of the neurotrophic factors and their receptors in human axonal neuropathic lesions of vasculitic neuropathies, indicating that a neurotrophic signal acts as a mediator of neuropathic pain.

Materials and Methods

Part of the sural nerve specimen was obtained from 22 diagnostic sural nerve biopsies in patients with acute necrotizing vasculitic neuropathies. The patients consisted of 12
patients with Churg-Strauss syndrome (8 women, 4 men; mean age, 56.2 years; age range, 43–80 years) and 10 patients with polyarteritis nodosa (3 women, 7 men; mean age, 61.3 years; age range, 43–79 years). Sural nerve biopsies were performed within 2 weeks after the onset of neuropathy, and the samples were examined as described previously (3, 5–9). Informed consent was obtained from each subject. Patients were asked just before the biopsies by the same observer whether they had actual neuropathic pain in the legs (10). The patients were deemed to have painful neuropathy if they had spontaneous pain regardless of the presence or absence of dysesthesia (11). The difference between pain and only unpleasant, but not painful, paresthesia was explained to every patient. Seven age-matched sural nerve specimens with a normal morphology were obtained from autopsied patients after acute death from myocardial infarct or cerebrovascular disease, and served as controls. The autopsy was performed within 3 hours postmortem, after informed consent was granted.

One portion of the sural nerve specimens was immediately frozen in liquid nitrogen for assessment of mRNA levels. The mRNA levels of NGF, GDNF, IL-6 and their receptors were determined by semiquantitative radioisotopic reverse transcription-polymerase chain reaction (RT-PCR) as described previously (5, 7, 12). The appropriate number of PCR cycles for each specific cDNA was determined in the linear range of cycles prior to onset of the plateau. The linear relationship between the amount of the template and the PCR product was also ascertained with respect to cDNA input. The radioactive signals of the PCR product were analyzed quantitatively using a phosphorimage analyzer (BAS-2000II; Fujix, Tokyo, Japan) and standardized against that of cyclophilin as an internal control. Other portions of the nerve segments were processed for morphological assessment of the nerve fibers (3, 6, 10). The density of large and small myelinated fibers was calculated in toluidine blue-stained semithin sections. The density of unmyelinated fibers was determined from electron micrograph as described previously (3, 10).

For statistical analysis, Mann-Whitney U-test was used for comparison of the mRNA levels between the groups. Spearman's correlation test was performed for correlation analysis.

**Results**

Morphometric analysis showed a decrease of total myelinated and unmyelinated fiber densities in painful and non-painful neuropathy (Table 1). Large and small myelinated fiber densities were reduced in both groups, while the loss of large myelinated fibers occurred to a lesser degree, resulting in a smaller ratio of small to large fibers in painful neuropathy compared to non-painful neuropathy and controls.

### Table 1. Fiber Densities in Painful and Non-painful Neuropathies

<table>
<thead>
<tr>
<th>Pain</th>
<th>Myelinated fiber density (no./mm²)</th>
<th>Small/Large</th>
<th>Unmyelinated fiber density (no./mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Large</td>
<td>Small</td>
<td>Total</td>
</tr>
<tr>
<td>+ (P)</td>
<td>411±550***;:;*</td>
<td>1,275±835**c</td>
<td>1,686±1,291**c</td>
</tr>
<tr>
<td>- (N)</td>
<td>127±181***c</td>
<td>755±722***c</td>
<td>882±838***c</td>
</tr>
<tr>
<td>Controls (C)</td>
<td>3,068±294</td>
<td>5,122±438</td>
<td>8,190±511</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SD. *p<0.05; **p<0.01; ***p<0.001 as compared with each other among painful (P; n=11) and non-painful (N; n=11) neuropathy and controls (C; n=7). Control values are based on previously published reports (3, 10).

### Table 2. mRNA Levels and Their Correlation in Painful and Non-painful Neuropathies

<table>
<thead>
<tr>
<th>Pain</th>
<th>mRNA levels</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGF</td>
<td>GDNF</td>
</tr>
<tr>
<td>+ (P)</td>
<td>0.23±0.02</td>
<td>0.62±0.13**c</td>
</tr>
<tr>
<td>- (N)</td>
<td>0.44±0.12**c</td>
<td>0.48±0.11**c</td>
</tr>
<tr>
<td>Controls (C)</td>
<td>0.13±0.03</td>
<td>0.12±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SE. *p<0.05; **p<0.01; ***p<0.001 as compared with each other among painful (P; n=11) and non-painful (N; n=11) neuropathy and controls (C; n=7). Control values are based on previously published reports (3, 10).
painful neuropathy compared with non-painful neuropathy. The densities of small myelinated and unmyelinated fibers were not significantly different between the two groups, but they tended to decrease more in painless neuropathy, reflecting the greater severity of neuropathy.

Furthermore, we assessed whether the mRNA expression of NGF, GDNF, IL-6 and their cognate receptors influenced the pain state in human vasculitic neuropathy. These neurotrophic factors and their receptors were up-regulated to a variable extent irrespective of pain (Table 2). The expression of NGF, IL-6 and IL-6Rα elevated significantly in non-painful, but not painful neuropathy, while GDNF, p75 and GFRα-1 expression increased in both groups. Correlation analysis revealed a very close association between NGF and p75 in painful neuropathy, and also demonstrated a good correlation of GDNF with GFRα-1 in painful and non-painful neuropathy.

**Discussion**

Painful neuropathies, including diabetic, alcoholic and amyloidotic neuropathies, all involve small fibers (10). It has been suggested that small fiber lesions cause pain only when there is also a large fiber lesion. Recently, Wu et al reported that the spontaneous activity of uninjured unmyelinated fibers with neighboring degenerating nerve fibers in Wallerian degeneration produces neuropathic pain (2). The present morphometric findings that unmyelinated fiber density was similar in painful and non-painful vasculitic neuropathy supports the view that intact unmyelinated fibers play an important role in neuropathic pain.

Both NGF and GDNF have been shown to regulate the sensitivity of nociception in adults (1). The peptidergic DRG neurons with small myelinated or unmyelinated fibers respond to NGF, while the non-peptidergic unmyelinated neurons labeled with the isolection IB4 are reactive to GDNF (13). NGF mRNA expression was increased to a higher extent in non-painful neuropathy, in which myelinated and unmyelinated fibers were extensively involved, being supported by the evidence that NGF is expressed in unmyelinated Schwann cells as well as in injured myelinated Schwann cells (14). Efficient retrograde transport of NGF associated with p75, which was suggested from the close correlation of NGF and p75 in this study, could lead to increased excitability of small DRG neurons with the TrkA receptor to pain. NGF can directly sensitize the pain response of these neurons (15). p75 expressed on Schwann cells, which do not bear TrkA, might act to enhance the presentation of NGF to axons, thereby promoting its binding to TrkA and retrograde transport of the NGF-TrkA complex (16). Actually, animals lacking p75 have an increased thermal threshold with impaired retrograde transport of NGF (17). Moreover, NGF transported from the sites of Wallerian degeneration also induces sympathetic sprouting in the DRG, which is involved in pain provocation.

In addition, a previous report showing that GDNF is expressed in unmyelinated Schwann cells, which also synthesize NGF, is consistent with the present findings showing that the levels of GDNF expression did not differ between painful and non-painful neuropathy with a similar density of unmyelinated fibers (1, 13). Nerve lesions are known to lead to a significant up-regulation of GDNF and GFRα1 in Schwann cells and stimulate the release of GFRα1 (7). Soluble GFRα1 can activate the Ret receptor on regenerating axons in the same presenting mechanism as seen in p75 (18). Although a good correlation between GDNF and GFRα1 implies enhanced transport of GDNF to the cell bodies of DRG neurons with Ret, the lack of difference in correlation in painful and non-painful neuropathy indicates that GDNF seems unlikely to affect the sensitization of DRG neurons to pain.

The expression of IL-6 and IL-6Rα mRNAs was independently up-regulated in proportion to the insult strength in vasculitic neuropathies, as shown in the morphometry and expression study, which is in agreement with the animal model experiment for Wallerian degeneration (9). Although a previous report showing that IL-6 deficit mice have a reduced sensitivity for heat pain suggests a relationship between IL-6 and pain sensation (19), no observation of pain elicitation by IL-6 was seen in human vasculitic neuropathy.

The association of NGF and pain is complex, and NGF has contradictory effects on pain in nerve injury in experimental and clinical usage: NGF relieves pain, but NGF can also exacerbate pain. As demonstrated in this study, in situ levels of NGF and p75 but not only NGF in nerve lesions, which is thought to regulate the retrograde NGF-TrkA signaling in sensory neurons, may determine the threshold of pain sensation. Furthermore, since the increased GDNF and GDNF-GFRα1 association did not relate to the painful state in vasculitic neuropathy, the exogenous administration of GDNF to treat painful neuropathy might be more valuable than that of NGF, which possibly induces pain as an adverse effect (20, 21).

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**References**

5) Sobue G, Yasuda T, Mitsuma T, Ross AH, Pleasure D. Expression of...


