Pretreatment with Interleukin-1 Enhances Survival of Sympathetic Ganglionic Neuron Grafts

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Abstract
The effects of pretreatment with interleukin-1 (IL-1) on neurite growth and cell survival of rat superior cervical ganglion (SCG) explants were examined. Neonate rat SCG explants were cultured with serum-containing growth media. Pretreatment with IL-1β (30 U/ml) for 3 hours stimulated neurite outgrowth of the SCG explant, which was inhibited by the addition of anti-nerve growth factor (NGF) antibody to the growth medium. Adult rat autologous SCG with the same pretreatment and non-treated ganglia were transplanted into the lateral ventricle. Histological evaluation of the grafts 2 weeks after transplantation revealed that the pretreated SCG cells survived better. Pretreatment with IL-1 may achieve the NGF-mediated neurotrophic effect in sympathetic ganglionic neurons resulting in enhanced graft survival.

Key words: interleukin-1, sympathetic neuron, neural transplantation, nerve growth factor, tissue culture, Parkinson’s disease

Introduction
Implantation of catecholaminergic neuron grafts from fetal mesencephalic tissue, adrenal medulla, and sympathetic ganglionic neurons is a possible treatment for Parkinson’s disease, a progressive disorder which presents with many problems in long-term medical therapy and severity of symptoms. We previously investigated the potential of autologous sympathetic ganglia as a graft tissue and proved this approach is effective in rodent and primate models of Parkinson’s disease.7,12,13 Our studies strongly suggested that grafted neurons restore impaired functions although the mechanism was not clearly determined. Thus, better graft survival is essential for a favorable outcome in transplantation therapy.

Nerve growth factor (NGF) is an essential protein for supporting the growth and maintenance of sympathetic neurons.6b Sympathetic ganglia contain an abundance of non-neuronal cells such as Schwann cells and fibroblasts which can synthesize NGF.2l Interleukin-1 (IL-1), a macrophage-derived cytokine, apparently regulates NGF synthesis in non-neuronal cells, as the NGF-messenger ribonucleic acid (mRNA) content of rat sciatic nerve segments cultured for 3 days was markedly (14-fold) enhanced by the presence of IL-1.6b These findings suggest that treatment of sympathetic ganglia with IL-1 may induce adequate NGF synthesis in non-neuronal cells within the ganglia to achieve a neurotrophic effect on the ganglionic neurons. The present study investigated whether pretreatment with IL-1 enhances neurite growth of cultured sympathetic neurons and neuronal survival in a rat transplant model.

Materials and Methods
1. Tissue culture study
Superior cervical ganglia (SCG) were removed from neonatal (postnatal day 1-3) Sprague-Dawley rats under ether anesthesia, decapsulated, and divided into two explants. Some explants were incubated with Dulbecco’s modified Eagle’s medium (DMEM) containing 30 U/ml human recombinant IL-1β.
(specific activity $5 \times 10^7$/µg; Genzyme, Cambridge, Mass., U.S.A.) at 37°C for 3 hours, rinsed with Hanks' balanced solution four times, then grown in DMEM supplemented with 10% fetal calf serum, penicillin (50 U/ml), and streptomycin sulfate (10 µg/ml) at 37°C in a humidified 5% CO$_2$ atmosphere. The other explants were cultured in the same growth medium without the IL-1 treatment. Some IL-1-pretreated explants were cultured in the presence of anti-mouse NGF antibody (5 ng/ml; Boehringer-Mannheim, Mannheim, Germany).

The NGF content in the ganglia was measured after 5- and 10-hour culture. The tissue samples were homogenized in 0.1 M Tris-HCl buffer (pH 7.6) containing 1 M NaCl, 2% bovine serum albumin, 2 mM ethylenediaminetetra-acetic acid, and 80 trypsin-inhibitory units of aprotinin per liter. The homogenates were centrifuged at 20,000 g for 30 minutes, and the supernatants were used for NGF assay with a two-site highly sensitive enzyme immunoassay for the mouse β-NGF. The NGF content of freshly prepared ganglia was also determined as a control. NGF levels in the ganglia with or without the IL-1 treatment were expressed as percentages of the mean control value.

After 2-day culture, neurite length and area of outgrowth were quantitatively estimated with a computed image-analyzing system. The neurite length was determined by measuring the distance between the end of the longest neurite and the explant border. The area of neurite outgrowth was determined by subtracting the area of the explant from that of the envelope containing all the neurite fronts.

II. Transplantation study

The transplantation study used 13 male Sprague-Dawley rats weighing 200–250 g. The SCG were removed from the rats and decapsulated in Hanks' solution. The SCG were treated as in the in vitro study for the IL-1-treated group (n = 7). The treated SCG were autologously transplanted into the right lateral ventricle of rats under sodium pentobarbital anesthesia according to the stereotactic coordinates of Pelligrino et al. SCG without IL-1 treatment were transplanted in the control group (n = 6). Two weeks after transplantation, the animals were deeply anesthetized with sodium pentobarbital and perfused transcardially with 300 ml of heparinized saline, followed by 500 ml of 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 2–4°C. The brains were removed and postfixed for 8 hours in the same fixative. Following dehydration in 20% sucrose, coronal sections 35-µm thick were cut using a cryostat. Free-floating brain sections were examined for tyrosine hydroxylase (TH) using an avidin-biotinperoxidase complex method and anti-TH antibody at a dilution of 1:600 (Chemicon, Temecula, Cal., U.S.A.). TH-positive grafted neurons were counted in the 35-µm thick sections at intervals of 70 µm throughout the graft. Counting at intervals

Fig. 1 Micrographs, showing sympathetic ganglion explants cultured in serum-containing growth medium for 2 days. ×25. upper: IL-1-treated ganglion explant in culture. Many fine neurites originate from the explant. middle: Non-treated ganglion explant in culture. Neurites are fine and short. lower: IL-1-treated ganglion explant in culture containing anti-NGF antibody (5 ng/ml). The neurite outgrowth stimulated by the IL-1 pretreatment is inhibited.
ensures that the same neuron is not counted twice, and provides general information about the number of grafted sympathetic ganglionic neurons, but is likely to underestimate the total TH-positive cell numbers. Cell body areas were measured with the image-analyzing system. The areas were obtained from five regions of interest in each graft, and the means used as data.

III. Statistical study

The values of data are indicated as mean ± SE. The data was statistically analyzed with the Mann-Whitney U-test.

Results

I. Tissue culture study

The NGF content in freshly prepared SCG was 1443.0 ± 428.0 pg/g tissue. The NGF contents of the IL-1-pretreated SCG after 5- and 10-hour culture were 236.6 ± 34.1% and 764.2 ± 109.5% of the control, respectively. In contrast, the NGF content of non-treated SCG after 10-hour culture was 31.9 ± 9.2% of the control (NGF content after 5 hours was not determined). The in vitro culture experiment showed that IL-1 pretreatment stimulated neurite outgrowth from the SCG explant, while the addition of antibody to mouse NGF inhibited the IL-1-induced growth to the level of neurite outgrowth from the non-treated SCG explant (Fig. 1). Table 1 shows the neurite length and neurite outgrowth area of the SCG explant in all groups after 2-day culture.

II. Transplantation study

Both the IL-1-treated and control groups demonstrated TH-positive cells surviving in the periphery of the grafted ganglia, forming a shell composed of a cluster of cells. The ganglionic neurons in the IL-1-treated grafts survived around the entire periphery of the ganglia, but the neurons in the non-treated grafts did not (Fig. 2). Moreover, the peripheral shell of surviving neurons in the IL-1-treated group was much thicker. The IL-1-treated group demonstrated a significantly greater number of TH-positive cells as well as a greater mean area of TH-positive cell bodies (Table 2). The non-treated grafts showed some indications of neuronal shrinkage.

Table 1 Neurite outgrowth from sympathetic ganglia after 2-day culture

<table>
<thead>
<tr>
<th></th>
<th>Neurite length (µm)</th>
<th>Neurite growth area (mm²)</th>
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<tbody>
<tr>
<td>No treatment</td>
<td>(n = 9) 321 ± 160</td>
<td>0.537 ± 0.369</td>
</tr>
<tr>
<td>IL-1 treatment</td>
<td>(n = 12) 1096 ± 133*</td>
<td>1.380 ± 0.313</td>
</tr>
<tr>
<td>IL-1 treatment + anti-NGF antibody</td>
<td>(n = 10) 375 ± 194</td>
<td>0.519 ± 0.352</td>
</tr>
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Data are mean ± SE. *p < 0.05, significantly different from no treatment group. anti-NGF antibody; addition of anti-NGF antibody (5 ng/ml) to growth media, n: number of analyzed ganglia.

Table 2 TH-positive cells in grafted sympathetic ganglia

<table>
<thead>
<tr>
<th></th>
<th>Cell number</th>
<th>Cell body area (µm²)</th>
</tr>
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<tbody>
<tr>
<td>No treatment</td>
<td>1425 ± 899 (N = 6)</td>
<td>322 ± 63 (n = 238)</td>
</tr>
<tr>
<td>IL-1 treatment</td>
<td>3082 ± 1020* (N = 7)</td>
<td>388 ± 60* (n = 375)</td>
</tr>
</tbody>
</table>

Data are mean ± SE. *p < 0.05, significantly different from no treatment group. N: number of rats, n: number of analyzed cells.

Fig. 2 Micrographs of TH-immunohistochemical staining of sympathetic ganglion grafts 2 weeks after intraventricular autografting. TH-positive cells survive exclusively in the peripheral portion of the graft. ×25. left: Graft with IL-1 treatment, right: graft without treatment.

Discussion

The present results clearly demonstrate that neurite outgrowth in neonatal SCG cultures and cell survival of grafted adult SCG are enhanced by pretreatment with IL-1. Furthermore, the in vitro study strongly suggests that this effect is mediated by the neurotrophic action of NGF. However, how long the neurotrophic action of induced NGF can be main-

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tained remains unclear.

The temporal relationship between the level of NGF-mRNA and NGF in IL-1-treated non-neuronal cells of the peripheral nervous system has not been investigated. The NGF-mRNA content of cultured astroglial cells treated with IL-1 (10 U/ml) reaches the maximum after incubation for several hours and then gradually decreases but remains elevated for up to 72 hours. In contrast, NGF secretion significantly increases above the control level after 14 hours and reaches a maximum after 38 hours, and remains elevated for an additional 34 hours. The difference in the time courses may involve both the time lag between the transcriptional and the translational processes and the difference in the stability of these substances. IL-1 induction of NGF-mRNA in peripheral non-neuronal cells is discernible at a concentration of 1 U/ml and is maximal at about 10 U/ml, with cellular NGF-mRNA reaching the maximal level after 3 hours of treatment, followed by a gradual decay. However, if the IL-1 treatment (30 U/ml) is interrupted after 3 hours as in our experiment, the decay of cellular NGF-mRNA will be relatively fast because the estimated half-life of NGF-mRNA is 90 minutes. Our study showed that IL-1 treatment for 3 hours caused the NGF content of the SCG to increase after 5 hours and further increase after 10 hours of incubation.

The present transplantation study provides preliminary evidence that pretreatment with IL-1 to induce a temporary increase in NGF content in the SCG will inhibit the loss and shrinkage of adult ganglionic neuron grafts. The significance of our results might be supported by the following findings. Previous studies have shown that 70-90% of grafted SCG neurons die, probably due to ischemia, within the first 24 hours of grafting, followed by a slow progressive loss. Interruption of the NGF supply from peripheral target tissues has shown that the half-life of NGF in sympathetic ganglia is 4-5 hours, and NGF is apparently essential for the maintenance of mature sympathetic neurons because in vivo administration of anti-NGF antibodies inhibits axon regeneration and often kills neurons in sympathetic ganglia. Therefore, ischemia and deficiency of NGF in the SCG may contribute to the initial cell loss. An increased NGF level in the SCG just prior to grafting will protect grafted neurons from death in the initial step of transplantation. Further study is required to determine the optimum IL-1 treatment conditions to achieve the best NGF level and outcome for the graft.

References

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