focal metabolism and hemodynamics in mesiolimbic temporal lobe epilepsy. Epilepsia 1993;34(suppl 2):186 (abstract)
15. Herscovitch P, Markham J, Raichle ME. Brain blood flow measured with intravenous $H_2^{15}O$. I. Theory and error analysis. J Nucl Med 1983;24:782–789

The Effect of Systemically Administered Recombinant Human Nerve Growth Factor in Healthy Human Subjects

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This phase I double-masked, randomized, placebo-controlled study evaluated the safety of single intravenous or subcutaneous doses of recombinant human nerve growth factor (rhNGF) in healthy human volunteers at doses ranging from 0.03 to 1 μg/kg. No life-threatening adverse events were seen at any dose. At doses above 0.1 μg/kg, subjects reported mild to moderate muscle pain, primarily in the bulbar and truncal musculature. The duration and severity of these myalgias varied in a dose-dependent manner, and women appeared to be more susceptible than men. Intravenous rhNGF produced earlier and more pronounced systemic effects than did identical subcutaneous doses. Subjects receiving subcutaneous rhNGF noted hyperalgesia at the injection site, a local effect persisting up to 7 weeks, that also varied in a dose-dependent manner. Antibodies to NGF were not detected in any subject. These results indicate that systemically administered rhNGF exerts a characteristic and reproducible biological effect in healthy subjects at very low doses and in a dose-dependent manner.

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Nerve growth factor (NGF) has an important physiological role in the development and maintenance of both sympathetic and sensory neurons and their neuronal extensions [1], and in the regeneration of lesioned peripheral nerves [2]. Administration of NGF can prevent cell death that would otherwise occur following injury [3]. Neurotrophic factors such as NGF have been proposed as possible candidates to treat or prevent central and peripheral nervous system diseases [4–6]. With the availability of large quantities of NGF, this therapeutic approach is logistically feasible. This double-masked, placebo-controlled, single-dose, phase I study of escalating doses of recombinant human NGF (rhNGH) was performed in healthy human volunteers (1) to assess safety and tolerance, (2) to determine pharmacokinetics, and (3) to determine if single doses would cause antibody formation or changes in nerve conduction parameters.

Materials and Methods

Subjects

Thirty-three men and 12 nonpregnant women between ages 18 and 35 years of age (median, 27 yr) and weighing between 50 and 98 kg (median, 69 kg) participated. Their generally good health was determined by standardized assessments, including nerve conduction studies and quantitative sensory testing (Vibratron II, Clifton Heights, NJ). All gave written informed consent, and the study was approved by local institutional review boards.

After admission, the subjects fasted overnight and then were given rhNGF or placebo, as determined by random allocation, either intravenously (iv) or subcutaneously (sc). Blood samples were collected both before and after dose for measurement of plasma NGF levels by a two-site immunosorbent assay that had a lower limit of detection of 1.56 ng/ml. Subjects were monitored in the hospital for at least 24 hours after administration of the test compound; they then returned 2, 4, and 8 weeks later. At 2 weeks, the initial screening assessment was repeated. At each outpatient visit symptoms were assessed, and blood was drawn for detection of serum antibodies to rhNGF by a radioimmunoprecipitation assay.

The subjects' characteristics were tabulated by using summary statistics. Adverse experiences and laboratory abnormalities were recorded by dose and for each individual subject. The safety of rhNGF was evaluated by comparing dosage groups to placebo groups, using χ^2 analysis of groups.

Results

There were no statistically significant differences in any of the baseline characteristics among the groups studied (data not shown).

In the 1.0 μg/kg iv dose group, each of 3 subjects who received rhNGF developed diffuse myalgias beginning about 60 to 90 minutes after administration, worsening over the next 4 to 6 hours, and then slowly resolving over 2 to 8 days. The subjects reported mild to moderate pain with swallowing, pain in the masseter muscles increased by chewing, sore throat, and pain with eye movements. Sometimes abdominal and limb muscles were involved. These myalgias were described

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Table 1. Neuromyalgia Syndrome

	Route				
	iv[a]	sc	iv	sc	placebo
Doses (μg/kg)	1 + 0.3	1 + 0.3	0.1 + 0.03	0.1 + 0.03	—
No. with neuromyalgias/ no. in dose group	5/5	7/11	0/8	0/8	0/13

[a] $p < 0.001$, by χ^2 analysis of the 1 + 0.3 dose tiers compared with the 0.1 + 0.03 dose tiers.

iv = intravenous; sc = subcutaneous.

Table 2. Injection-Site Hyperalgesia (Subcutaneous Dosing)

	Dose (μg/kg)			
	1	0.3	0.1	0.03
No. with hyperalgesia/ no. in dose group	3/4	6/7	3/4	4/4
Duration of hyperalgesia in those who developed it (days)				
Mean	27	22	22	12
Range	14–41	1–49	16–28	3–27

as though one had run a marathon without preconditioning, or as though one had "done a thousand sit-ups or push-ups." All 4 subjects receiving 1.0 μg/kg sc experienced mild, diffuse myalgias, which were milder in the 2 males, began 5 to 7 hours after the dose was given, and resolved over the next 2 to 5 days. During the course of the myalgias, no laboratory (including creatine kinase [CK] measurements) abnormalities were noted. In the 0.3 μg/kg IV dose tier, the 2 subjects (both male) developed similar symptoms. These symptoms began about 90 to 150 minutes after rhNGF administration, worsened over the next 2 to 3 hours, and then slowly resolved over 1 to 2 days. In the 0.3 μg/kg sc rhNGF dose group, none of the 4 male subjects experienced anything other than trivial effects after rhNGF injection. By contrast, each of 3 female subjects experienced mild myalgias that began about 8 to 9 hours after rhNGF injection and resolved over 1 to 3 days. These myalgias were similar to those reported by the men receiving intravenous doses but were predominantly located in the epigastrium and lower thorax. For the 0.03 and 0.01 μg/kg dose tiers, no serious adverse symptomatic events or laboratory abnormalities were detected (Table 1).

Most subjects receiving rhNGF subcutaneously experienced injection-site hyperalgesia, which was described as increasing tenderness to touch or heat in an area approximately 2.5 cm in circumference (Table 2). The skin in this area was described as "extra-sensitive"; for example, subjects would avoid leaning against the region because it was uncomfortable. Several subjects mentioned that the region felt "different" when they were taking a shower, but none reported spontaneous pain, nor did any feel the need to take analgesics for the hyperalgesia. The duration of this effect was dependent on the dose received (see Table 2). This effect resolved completely in all subjects, and no redness, warmth, or swelling was present at the injection site.

Plasma levels of NGF were detectable only in those individuals receiving rhNGF 1.0 μg/kg iv. The maximal measured concentration was in the 5-minute sample, ranging from 3.6 to 7.38 ng/ml. The concentrations fell from that point and were below the lower level of detection in the 2 male subjects by 60 minutes after the dose but were still detectable in the female subject for up to 3 hours after the dose. The data were insufficient to allow formal pharmacokinetic modeling or parameter determinations.

No significant changes in blood pressure or pulse were observed after dosing. Antibodies to NGF were not found in any subject. Subjects who received placebo did not experience myalgias or injection-site hyperalgesia. In all subjects, nerve conduction studies and neurological examinations were normal at the 2-week follow-up.

Discussion

This study establishes that single doses of systemically administered rhNGF cause potent and reversible biological effects in healthy human volunteers. At doses above 0.1 μg/kg, these effects were similar in all subjects studied but were more severe and occurred at a lower threshold in women than in men. These biologic effects were transient, and no measurable clinical or electrophysiologic effects on nerve function could be detected. In particular, in this closely monitored situa-

tion, no subject experienced a significant change in blood pressure or pulse, as has been seen in animals given large doses of rhNGF.

The biologic effects of rhNGF in healthy human subjects were two. First, a systemic effect, diffuse myalgias, occurred after both intravenous and subcutaneous dosing in those receiving doses above 0.1 μg/kg (see Table 1). This phenomenon was less potent and more delayed after the subcutaneous dose. Usually, the myalgias were most severe in neck and throat muscles, with centripetal spread to the abdominal area and the limb muscles. One interpretation of this topography is that the shorter sensory fibers are affected first, reflecting the different time for retrograde transport of NGF along axons of short versus long nerve fibers. Alternatively, the topography could reflect the shorter distances from the affected regions' dorsal root ganglion cells to their central connections. Myalgias were not associated with weakness or an elevation in serum CK and varied in intensity and duration depending on the dose of rhNGF administered. It is interesting that in men and women given the same dose, this effect was more pronounced in women, possibly reflecting the longer times NGF was detectable in plasma.

The second biologic effect of rhNGF was an area of hyperalgesia at the site of subcutaneous injection. This hyperalgesia was also correlated in time course with the dose administered. This effect may reflect either up-modulation of central connections of nociceptive afferents or increased sensitivity of local sensory fibers, as has been reported in the rat [7, 8]. In that model, NGF treatment of adult rats caused both heat and mechanical hyperalgesia effects, presumably similar to those described by our subjects. The mechanisms underlying these experimental observations are unknown [7, 9].

In our subjects, both biological effects observed may be due to rhNGF interacting with its high-affinity receptor *trk* [10], which is localized to the small-diameter, substance P–expressing, dorsal root ganglion cells [11, 12] that project to laminae I and II of the dorsal horn [13] and mediate nociception. The differences in time of onset of the myalgic complaints between intravenous and subcutaneous administration suggest the more rapid presentation of NGF to its receptor after intravenous administration compared with subcutaneous administration.

This study has defined a range of tolerated doses for a single injection of rhNGF in humans. At some of these doses, biologic effects occur that clearly reflect drug actions on the peripheral nervous system. The effect of rhNGF on damaged peripheral sensory fibers is currently unknown but is being investigated.

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References

1. Levi-Montalcini R. The nerve growth factor 35 years later. Science 1987;237:1154–1162
2. Raivich G, Kreutzberg GW. Nerve growth factor and regeneration of peripheral nervous system. Clin Neurol Neurosurg 1993;95(suppl):S84–S88
3. Kromer LF. Nerve growth factor treatment after brain injury prevents neuronal death. Science 1987;235:214–216
4. Hefti F, Hartikka J, Knusel B. Function of neurotrophic factors in the adult and aging brain and their possible uses in the treatment of neurodegenerative diseases. Neurobiol Aging 1989;10: 515–533
5. Apfel SC, Lipton RB, Arezzo JC, Kessler JA. Nerve growth factor prevents toxic neuropathy in mice. Ann Neurol 1991;29: 87–90
6. Apfel SC, Arezzo JC, Lipson LA, Kessler JA. Nerve growth factor prevents experimental cisplatin neuropathy. Ann Neurol 1992;31:76–80
7. Lewin GR, Ritter AM, Mendell LM. Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. J Neurosci 1993;13:2136–2148
8. Ritter AM, Lewin GR, Mendell LM. Regulation of myelinated nociceptor function by nerve factor in neonatal and adult rats. Brain Res Bull 1993;30:245–249
9. Lewin GR, Mendell LM. Nerve growth factor and nociception. Trends Neurosci 1993;16:353–359
10. Bothwell M. Keeping track of neurotrophin receptors. Cell 1991;65:915–918
11. Davies AM, Bandtlow C, Heumann R, et al. Timing and site of nerve growth factor synthesis in developing skin in relation to innervation and expression of the receptor. Nature 1987;326: 353–358
12. Ritter AM, Lewin GR, Kremer NE, Mendell LM. Requirement for nerve growth factor in the development of myelinated nociceptors *in vivo*. Nature 1992;350:500–502
13. Ruit KG, Elliott JL, Osborne PA, et al. Selective dependence of mammalian dorsal root ganglion neurons on nerve growth factor during embryonic development. Neuron 1992;8:573–587