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Pharmacokinetics and biodistribution of a small radioiodine labeled nerve growth factor fragment

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Nerve growth factor (NGF) exerts various actions on neuronal and non-neuronal tissues and has potential therapeutic utility, but difficulties in using the whole protein have stimulated interest in small NGF fragments. We radioiodinated a small cyclic peptide derived from NGF using the Bolton-Hunter method [^{125}I-C(92-96)], and confirmed binding to high affinity NGF receptors by cross-linkage analysis. Pharmacokinetic characteristics in intravenously injected mice were T $^1/_2\alpha$ 5.2 min, T $^1/_2\beta$ 121.3 min, clearance 11.8 ± 0.5 m*l*/min, and volume of distribution 69.7 ± 4.6 m*l*. Dose-proportionate increases in areas-under-curve and peak-concentrations indicated linear pharmacokinetics. Biodistribution data revealed that clinically relevant doses allowed C(92-96) accumulation sufficient to elicit biological responses in receptor expressing organs including the lungs, liver, spleen, and pancreas.

Key words: nerve growth factor, peptide, radioiodine, pharmacokinetics, biodistribution

INTRODUCTION

NERVE GROWTH FACTOR (NGF) mediates the differentiation and survival of peripheral sympathetic and sensory neurons (Pierchala et al., 2004),[1] and also exerts a variety of biological effects on NGF responsive non-neuronal tissues, including promotion of pancreatic islet cell survival and function,[2] muscle cell regeneration,[3] angiogenesis,[4] and immune modulation.[5]

Although NGF has been proposed as a therapeutic agent for neuropathies as well as for various non-neurological disorders,[2,4,6,7] multiple challenges accompany the clinical application of whole NGF including the high cost of large-scale production, difficulty of working with large polypeptides, and poor *in vivo* stability. Small molecule NGF fragments or analogues could be a feasible strategy to overcome some of these obstacles,[8] and may also help unveil the physiologic and pharmacologic role of NGF-receptor interaction on target tissues. Whole NGF is composed of a tightly associated dimer made up of parallel protomers, each of which has seven β-strands linked by three β-turns and a series of reverse turns.[9] The C-D loop of the molecule mediates binding of NGF to the high-affinity tyrosine kinase receptor TrkA.[10] Hence, small synthetic peptides derived from this loop have been synthesized as NGF analogues that are proteolytic stable and bind to TrkA receptors with high affinity.[11–14] These molecules can act as antagonists[11,13] or agonists[12] for the receptor, and therefore have potential utility as therapeutic agents or as tools to help uncover the physiology of NGF action.

To fully exploit biologic ligands as investigative tools or as therapeutic agents in living subjects, it is essential to recognize how they are handled by the body and delivered to target tissues. Whereas the pharmacokinetic and biodistribution properties of whole NGF have been well characterized,[15–17] they have not been thoroughly investigated for small NGF peptides, except for a previous study that evaluated the tumor targeting properties of a ^{99m}Tc labeled NGF fragment.[14] In this study, we therefore investigated the *in vivo* pharmacokinetics and biodistribution in mice of a small cyclic peptide derived from the C-D β-turn region of NGF [C(92-96)] that is known to bind to TrkA receptors with a K_d value of 100 nM.[9]

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MATERIALS AND METHODS

Radioiodination of the small NGF peptide

In these experiments, we used a small peptide composed of a NGF fragment containing the sequence CTDEKQC (C(92-96); m.w. 826), which was conformationally constrained in a cyclic form by a disulfide bond between the two cysteine residues. The peptide was radioiodine labeled using the Bolton-Hunter method. Briefly, 1 μg Bolton-Hunter reagent (Pierce Chemical Co., Rockford, IL) was radiolabeled with 37 MBq $Na^{125}I$ by incubating it with 400 μg chloramine-T for 15 sec at room temperature. Immediately, the reaction extracted the ^{125}I-labeled Bolton-Hunter reagent with 5 μl DMF and 100 μl benzene. The aqueous phase was removed from the mixture and the organic phase was transferred into a new tube. The organic phase was evaporated with a gentle stream of N_2. Subsequently, 8 μg of the C(92-96) peptide in 2.5 μl 1 M bicarbonate buffer (pH 8.5) was added the tube and incubated for 1 hour on ice. To the reaction tube was added 0.5 m*l* of dilution buffer (0.05 M sodium acetate buffer, pH 5.2 containing 0.4% acetic acid). The product was then purified by HPLC at a flow rate of 2 m*l*/min (0–50% CH_3CN/0.1% TFA in H_2O, 50 min), and the desired fraction was eluted at 22 min.

Cross-linking assay between radioiodine small NGF peptide and TrkA receptor

To confirm binding to the TrkA receptor, 37 kBq of ^{125}I-C(92-96) was incubated with 0.5 μg of a recombinant human TrkA/Fc chimera (R&D System, Inc.; MW 67.4 kDa) at 37°C for 2 hr. The mixture was then incubated with 2 mM of 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride (EDAC) as a cross-linker at room temperature for 30 min. After incubation, the mixture was separated on a 10% non-reducing polyacrylamide gel along with molecular size markers. ^{125}I-C(92-96) that did not undergo incubation with TrkA/Fc was loaded as control. The gel was dried and a film was exposed to obtain an autoradiograph, which was visually evaluated for radioactive bands.

Pharmacokinetics of radioiodine small NGF peptide in normal mice

All animal experiments were performed under protocols approved by institutional guidelines on the use and care of animals. Normal ICR mice (CrjBgi of Charles River Technology, Orient, Korea; AAALAC number 001003) were intravenously injected as a bolus via the tail vein with 0.5 MBq of ^{125}I-C(92-96) with varying amounts of added non-radiolabeled C(92-96) to final doses of 0.6, 6, or 60 μg (n = 3 for each group). Following injection, 5 μl of blood was serially collected from the tail vein at predetermined intervals ranging from 4 min to 6 hr after injection. The blood samples were measured for radioactivity on a gamma counter along with standards, and C(92-96) concentrations in each sample were calculated in ng/m*l* using the ratio of sample counts compared to the standard solution counts.

The half-life of blood activity was calculated using one-phase and two-phase exponential decay equations, the latter of which produced early distribution ($T^1/_2\alpha$) and late elimination ($T^1/_2\beta$) half-lives. The critical pharmacokinetic parameters of half-life ($T^1/_2$), clearance (CL), and volume of distribution (Vd) were calculated with GraphPad Prism® V. 3.02 software (GraphPad Software Inc.) using previously described methods.[18] $T^1/_2$ was calculated as 0.693 divided by k, where k is the elimination rate constant derived from the linear regression between time and log blood-concentration. CL was calculated as the administered dose divided by the total area under curve (AUC) for the time-concentration relation. The AUC from zero time to the last sample was determined by the trapezoid rule, and the area from the last data point to infinite time was calculated as the last measured concentration divided by k. Vd was calculated as the product of $T^1/_2$ and CL divided by 0.693. Linearity of C(92-96) pharmacokinetics was assessed by evaluating the correlation between AUC and peak concentration with the administered C(92-96) dose.

Biodistribution of radioiodine small NGF peptide in mice

The biodistribution of ^{125}I-C(92-96) was evaluated in ICR mice following the last blood sampling for pharmacokinetic analysis, which was 6 h post-injection. Blood and major organs were promptly excised, weighed, and measured for radioactivity on a gamma counter. ^{125}I-C(92-96) uptake in each organ was expressed as mean ± SD of % injected dose per gram-tissue (%ID/g). Peptide accumulation levels were calculated in ng per gram-tissue by multiplying the injected C(92-96) dose to %ID/g values for each organ. The significance of difference in uptake levels between the three different injected peptide doses was analyzed by one-way analysis of variance, with p values < 0.05 considered significant. Benferroni's multiple comparison tests were performed as post hoc tests to determine the significance of the difference between any two groups.

RESULTS

Synthesis of radioiodine small NGF peptide and cross-linkage with TrkA receptor

^{125}I-C(92-96) was radiolabeled with an average labeling efficiency of 22%, and the specific activity of the purified fraction used for experiments was 851 GBq/mM. Polyacrylamide gel analysis demonstrated a clear radioactive band between the 90 and 100 kDa region in the lane for ^{125}I-C(92-96) incubated and cross-linked with chimeric TrkA receptors but not in the control lane (Fig. 1). As a result of glycosylation, the recombinant TrkA chimera appears to migrate as a protein of this size under non-reducing conditions. This finding confirms that ^{125}I-C(92-

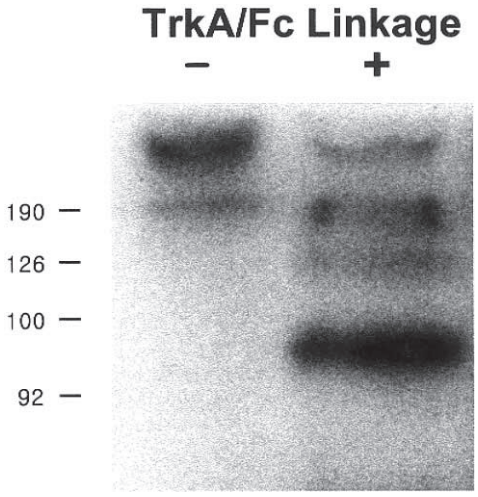

Fig. 1 Polyacrylamide gel analysis of ^{125}I-C(92-96) cross-linked to the TrkA receptor. ^{125}I-C(92-96) cross-linked with a TrkA/Fc chimera (+ lane) clearly demonstrates a radioactive band, which does not appear in the control lane loaded without incubation with TrkA (– lane).

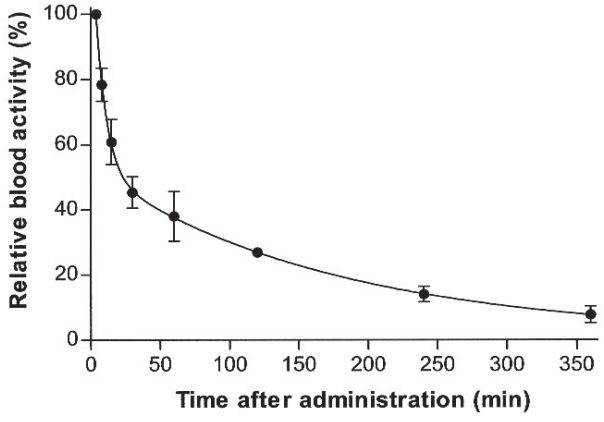

Fig. 2 Blood clearance profile of intravenously injected ^{125}I-C(92-96) reveals a biexponential pattern of elimination. Data are mean ± SD of blood radioactivity relative to that at 4 min postinjection, obtained from 3 normal ICR mice.

96) retains binding characteristics to the high affinity NGF receptor.

Pharmacokinetics of radioiodine small NGF peptide

On the time-activity curve obtained from serial blood sampling in ICR mice intravenously injected with ^{125}I-C(92-96) with tracer doses of the peptide (600 ng), half initial activity was reached approximately 30 min post-injection. Nonlinear curve fitting with a one-phase exponential decay equation revealed a half-life of 20.6 min (95% confidence range between 15.1 to 32.3 min). Curve fitting with a two-phase exponential decay equation demonstrated an early distribution $T^1/_2\alpha$ and late elimination $T^1/_2\beta$ of 5.2 min and 121.3 min, respectively (Fig. 2). The

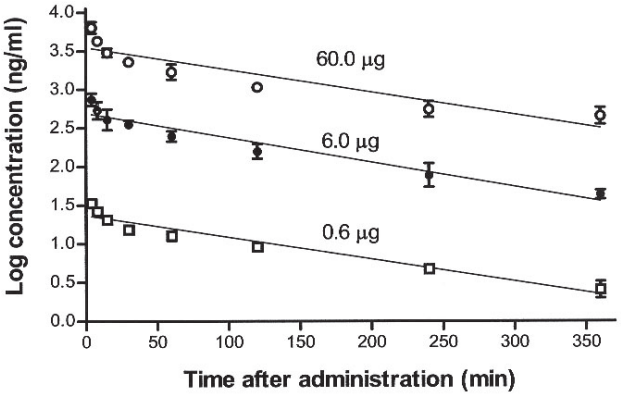

Fig. 3 Semilog plot of time—blood concentration profiles of intravenously injected C(92-96) according to injected dose. Normal ICR mice were tail vein injected with ^{125}I-C(92-96) mixed with non-radiolabeled C(92-96) to final doses of 0.6, 6, or 60 μg. Data are mean ± SD of blood C(92-96) concentration in ng/m*l*, obtained from 3 animals for each group.

log blood concentration of C(92-96) plotted against time demonstrated a *k*-slope of 0.0028, and of the critical pharmacokinetic parameters, CL was 11.8 ± 0.5 m*l*/min, $T^1/_2$ was 4.1 ± 0.4 hr, and Vd was 67.7 ± 4.6 m*l*. In animals injected with 6 or 60 μg of C(92-96), the pattern of blood clearance remained essentially unchanged (Fig. 3), and there were proportionate increases in AUCs ($r^2 = 0.992$; Fig. 4A) and peak blood concentrations ($r^2 = 0.967$; Fig. 4B) according to the injected C(92-96) dose. These findings indicate that intravenously injected C(92-96) follows linear pharmacokinetics when administered with doses between 0.6 and 60 μg in mice.

Biodistribution of radioiodine small NGF peptide

The tissue biodistribution of ^{125}I-C(92-96) at 6 hr according to injection dose expressed as %ID/g, and the level of C(92-96) accumulation in each organ expressed as ng per gram-tissue are shown in Table 1. Excluding the thyroids (9.1 ± 4.3%ID/g for 0.6 μg peptide), activity was highest in the kidneys suggesting renal excretion as a route of elimination for the small peptide NGF analogue. Significant thyroid activity suggested the presence of some free iodide released from the radiolabel. There was relatively high activity in the spleen, liver, lungs, and pancreas, and with 60 μg C(92-96) peptide administered, calculated accumulation levels were above 70 ng per gram-tissue for these organs. The myocardium and skeletal muscle had moderate uptake levels, and as expected, accumulation of the peptide in brain tissue was low (< 0.2%ID/g tissue). There were no significant differences in ^{125}I-C(92-96) uptake levels in any of the organs tested between animals injected with different doses of C(92-96).

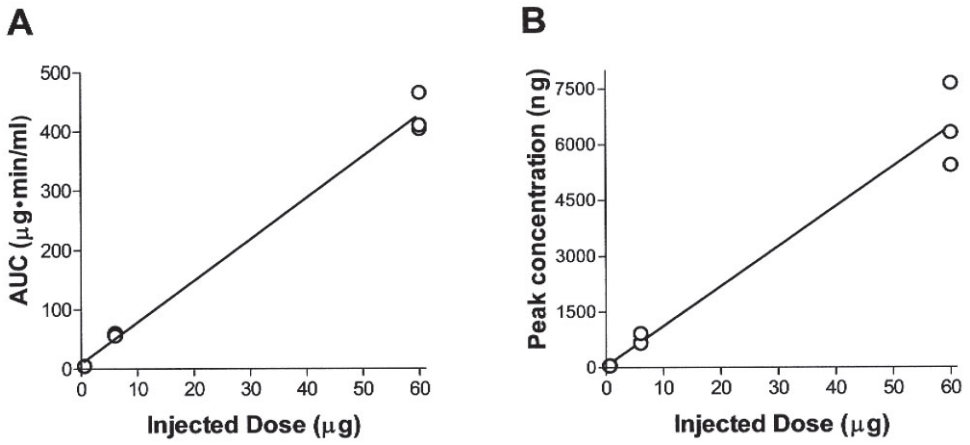

Fig. 4 Correlation between injected C(92-96) dose and (A) areas under curve and (B) peak values of blood C(92-96) concentration. Data are obtained from normal ICR mice intravenously injected with 0.6, 6, or 60 μg of C(92-96) (n = 3 for each group).

Table 1 ^{125}I-C(92-96) biodistribution and C(92-96) accumulation levels at 6 hr after intravenous injection in normal ICR mice

	Biodistribution (%ID/g-tissue)			Accumulation (ng/g-tissue)		
	0.6 μg Group (n = 3)	6 μg Group (n = 3)	60 μg Group (n = 3)	0.6 μg Group (n = 3)	6 μg Group (n = 3)	60 μg Group (n = 3)
Blood	0.77 ± 0.04	0.69 ± 0.09	0.62 ± 0.15	4.6 ± 0.2	41.6 ± 5.6	374.4 ± 89.8
Heart	0.09 ± 0.03	0.07 ± 0.05	0.08 ± 0.04	0.6 ± 0.2	4.2 ± 3.1	49.1 ± 22.6
Lung	0.42 ± 0.15	0.25 ± 0.13	0.25 ± 0.02	2.5 ± 0.9	14.8 ± 7.9	151.8 ± 13.0
Liver	0.34 ± 0.15	0.38 ± 0.19	0.37 ± 0.12	2.1 ± 0.4	22.9 ± 11.3	218.8 ± 70.5
Kidney	1.21 ± 0.17	1.36 ± 0.81	1.78 ± 1.10	7.2 ± 1.0	81.4 ± 48.5	1065.9 ± 662.2
Spleen	0.70 ± 0.31	0.48 ± 0.11	0.71 ± 0.11	4.2 ± 1.8	28.8 ± 6.6	425.8 ± 67.9
Pancreas	0.08 ± 0.03	0.07 ± 0.04	0.12 ± 0.03	0.5 ± 0.2	4.2 ± 2.6	71.9 ± 17.4
Muscle	0.06 ± 0.02	0.05 ± 0.01	0.04 ± 0.00	0.4 ± 0.2	2.7 ± 0.9	25.7 ± 2.8
Brain	0.02 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.1 ± 0.0	0.7 ± 0.3	6.1 ± 2.8

Values are given as mean ± SD of ^{125}I-C(92-96) activity expressed as % injected-dose per gram-tissue, or C(92-96) concentration in nanogram per gram-tissue calculated from total injected doses.

DISCUSSION

In this study, we investigated the *in vivo* kinetics and distribution of radioiodine labeled C(92-96), a small NGF fragment peptide that binds TrkA receptors with high affinity. C(92-96) radioiodine labeled via the Bolton-Hunter reagent was confirmed to bind to a synthetic chimeric TrkA/Fc protein that is known to sequester endogenous NGF and block the survival effects of NGF in cultured neurons.[19] Pharmacokinetic studies after intravenous injection in mice revealed this small peptide to clear from the blood at a rate comparable to that reported for whole β-NGF. By injecting 3 different doses of the peptide we found that peak blood concentrations and AUCs were directly proportional to the administered dose. This is consistent with linear pharmacokinetics and implies that clearance of C(92-96) is not affected by the administered dose due to saturation of transport or metabolic systems. Biodistribution results demonstrated relatively high accumulation of C(92-96) in the spleen, lungs, liver, and pancreas, and high kidney activity suggests renal excretion as a route for its elimination.

In our pharmacokinetic study, half of the initial ^{125}I-C(92-96) activity was reached at approximately 30 min after intravenous injection. $T^1/_2\alpha$ and $T^1/_2\beta$, values were 5.2 min and 2 hr, respectively, and the terminal half-life calculated from the log-concentration plot was 4.1 hr. These results are comparable with the 36.3 min plasma half-life of intravenous ^{125}I labeled β-NGF in rats observed by Pradier et al.,[15] and the early distribution $T^1/_2\alpha$ of 5.4 min and late elimination $T^1/_2\beta$ of 2.3 hr for intravenous 2.5S NGF in rats observed by Tria et al.[16] Similarly, a terminal plasma half-life of 4.1 hr has been reported for ^{125}I labeled recombinant human NGF subcutaneously administered in cynomolgus monkeys.[17] Thus, our findings indicate that despite their substantially smaller molecular size, blood clearance of this small NGF peptide is not faster but comparable to that of whole NGF, which

is likely to be an advantage for *in vivo* delivery to target tissue. Although the reason for the longer than expected circulation time is not clear, it may be partly related to a difference in binding to proteins such as serum α_2-macroglobulin, which has been proposed to bind whole NGF and result in its rapid clearance through the liver.[16]

In the present study, there was high renal activity, suggesting urinary excretion as a route of ^{125}I-C(92-96) elimination. There was high thyroid activity, which indicates radioiodine dehalogenation that may have partly affected our measurements of ^{125}I-C(92-96) kinetics. However, since thyroid uptake accounted for less than 10% of injected activity, we do not believe that dehalogenation was substantial enough to diminish the validity of our findings. Nevertheless, it should be mentioned that our study would have been significantly strengthened by *in vivo* stability and metabolic studies. Accumulation was relatively high in the spleen, liver, lungs, and pancreas, and modest in the myocardium and skeletal muscle. These results are similar to the study of Nguyen et al. where subcutaneously administrated radioiodine labeled recombinant human NGF in monkeys showed the highest 8 hr radioactivity, excluding the thyroid, lymph nodes and adrenals, to be in the kidneys, followed by the liver and spleen.[17] Also, LeSauteur and coworker observed that ^{99m}Tc labeled C(92-96) intravenously injected in tumor bearing mice had the highest 28 hr uptake in the kidneys, followed by the liver, lung and spleen, heart, and muscles in that order.[14] Although we did not attempt to directly confirm receptor specific accumulation in the tissues, the known presence of significant amounts of NGF receptors in the spleen,[20] pancreas,[21] lung[22] and liver,[23] together with a similar accumulation pattern with different NGF derived molecules suggest that the observed distribution of C(92-96) reflects the presence of specific NGF receptors in at least some of these organs.

Administration of 60 μg of the peptide in mice correlates to 2 mg/kg, which is within the 1 to 10 mg/kg dose range of NGF used in the treatment of peripheral neuropathy in animal models. In mice injected with 60 μg of C(92-96), most of the organs tested, excluding the brain and skeletal muscle, accumulated the peptide in levels above or close to the 100 nM K_d value for TrkA binding, which roughly translates into 80 ng per gram of tissue. This suggests that small peptide NGF analogues administered at this dose can reach concentrations in the majority of target tissues necessary to elicit biological responses. Low C(92-96) accumulation in the brain is consistent with the inability of most hydrophilic peptides to cross the blood-brain barrier, but this does not preclude the potential utility of such NGF peptides for investigations of their action on peripheral nerves or non-neuronal target tissue.

CONCLUSION

In conclusion, we have investigated the *in vivo* pharmacokinetic properties and biodistribution in mice of C(92-96), a small peptide NGF analogue that binds to TrkA receptors. Intravenously administered ^{125}I-C(92-96) cleared from the blood at a rate comparable to that known for whole NGF protein, and followed linear pharmacokinetics between doses of 0.6 and 60 μg. In addition, intravenous administration of clinically relevant doses of C(92-96) allows major organ accumulation in concentrations sufficient to induce biologic effects through its target receptor.

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REFERENCES

1. Pierchala BA, Ahrens RC, Paden AJ, Johnson EM Jr. Nerve growth factor promotes the survival of sympathetic neurons through the cooperative function of the protein kinase C and phosphatidylinositol 3-kinase pathways. *J Biol Chem* 2004; 279: 27986–27993.
2. Miao G, Mace J, Kirby M, et al. *In vitro* and *in vivo* improvement of islet survival following treatment with nerve growth factor. *Transplantation* 2006; 81: 519–524.
3. Rende M, Brizi E, Conner J, Treves S, Censier K, Provenzano C, et al. Nerve growth factor (NGF) influences differentiation and proliferation of myogenic cells *in vitro* via TrKA. *Int J Dev Neurosci* 2000; 18: 869–885.
4. Emanueli C, Salis MB, Pinna A, Graiani G, Manni L, Madeddu P. Nerve growth factor promotes angiogenesis and arteriogenesis in ischemic hindlimbs. *Circulation* 2002; 106: 2257–2262.
5. Bonini S, Rasi G, Bracci-Laudiero ML, Procoli A. Nerve growth factor: neurotrophin or cytokine? *Int Arch Allergy Immunol* 2003; 131: 80–84.
6. Apfel SC, Schwartz S, Adornato BT, et al. Efficacy and safety of recombinant human nerve growth factor in patients with diabetic polyneuropathy: A randomized controlled trial. rhNGF Clinical Investigator Group. *JAMA* 2000; 284: 2215–2221.
7. Apfel SC. Neurotrophic factors in the therapy of diabetic neuropathy. *Am J Med* 1999; 107: 34S–42S.
8. Pollack SJ, Harper SJ. Small molecule Trk receptor agonists and other neurotrophic factor mimetics. *Curr Drug Targets CNS Neurol Disord* 2002; 1: 59–80.
9. Nanduri J, Vroegop SM, Buxser SE, Neet KE. Immunological determinants of nerve growth factor involved in p140trk (Trk) receptor binding. *J Neurosci Res* 1994; 37: 433–444.
10. Kaplan DR, Hempstead BL, Martin-Zanca D, Chao MV, Parada LF. The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. *Science* 1991; 252: 554–558.
11. LeSauteur L, Wei L, Gibbs BF, Saragovi HU. Small peptide mimics of nerve growth factor bind TrkA receptors and affect biological responses. *J Biol Chem* 1995; 270: 6564–6569.

12. Maliartchouk S, Feng Y, Ivanisevic L, et al. A designed peptidomimetic agonistic ligand of TrkA nerve growth factor receptors. *Mol Pharmacol* 2000; 57: 385–391.
13. Debeir T, Saragovi HU, Cuello AC. A nerve growth factor mimetic TrkA antagonist causes withdrawal of cortical cholinergic boutons in the adult rat. *Proc Natl Acad Sci USA* 1999; 96: 4067–4072.
14. LeSauteur L, Cheung NK, Lisbona R, Saragovi HU. Small molecule nerve growth factor analogs image receptors *in vivo*. *Nat Biotechnol* 1996; 14: 1120–1122.
15. Pradier P, Jalenques I, Dalle M, Reuling R, Despres G, Romand R. Distribution and metabolism patterns of plasma 7S- and beta-NGF in the adult male rat. *J Physiol Paris* 1994; 88: 273–277.
16. Tria MA, Fusco M, Vantini G, Mariot R. Pharmacokinetics of nerve growth factor (NGF) following different routes of administration to adult rats. *Exp Neurol* 1994; 127: 178–183.
17. Nguyen CB, Harris L, Szonyi E, et al. Tissue Disposition and Pharmacokinetics of Recombinant human nerve growth factor after acute and chronic subcutaneous administration in monkeys. *Drug Metabolism and Disposition* 2000; 28: 598–607.
18. Schoenwald RD. *Pharmacokinetics in drug discovery and development*. Florida; CRC Press, 2002.
19. McMahon SB, Bennett DL, Priestley JV, Shelton DL. The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule. *Nat Med* 1995; 1: 774–780.
20. Lomen-Hoerth C, Shooter EM. Widespread neurotrophin receptor expression in the immune system and other nonneuronal rat tissues. *J Neurochem* 1995; 64: 1780–1789.
21. Kanaka-Gantenbein C, Tazi A, Czernichow P, Scharfmann R. *In vivo* presence of the high affinity nerve growth factor receptor Trk-A in the rat pancreas: differential localization during pancreatic development. *Endocrinology* 1995; 136: 761–769.
22. Ricci A, Felici L, Mariotta S, et al. Neurotrophin and neurotrophin receptor protein expression in the human lung. *Am J Respir Cell Mol Biol* 2004; 30: 12–19.
23. Bailie MB, Roth RA, Contreras ML. Nerve growth factor binding sites on hepatic parenchymal cells. *In Vitro Cell Dev Biol* 1992; 28A: 621–624.