Published in final edited form as:

J Pharm Sci. 2009 October ; 98(10): 3640–3646. doi:10.1002/jps.21674.

Delivery of Nerve Growth Factor to brain via intranasal administration and enhancement of brain uptake

Siva Ram Kiran Vaka¹, S M Sammeta¹, Lainy B Day², and S. Narasimha Murthy^{1,*}

¹Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, University, Mississippi 38677

²Department of Biology, The University of Mississippi, University, Mississippi 38677

Abstract

The main objective of the study was to investigate the efficacy of chitosan to facilitate brain bioavailability of intranasally administered nerve growth factor (NGF). *In vitro* permeability studies and electrical resistance studies were carried out across the bovine olfactory epithelium using Franz diffusion cells. The bioavailability of intranasally administered NGF in rat hippocampus was determined by carrying out brain microdialysis in Sprague Dawley rats. The *in vitro* permeation flux across the olfactory epithelium of NGF solution without chitosan (control) was found to be 0.37 ± 0.06 ng/cm²/h. In presence of increasing concentration of chitosan (0.1%, 0.25% and 0.5% w/v) the permeation flux of NGF was found to be 2.01 ± 0.12 , 3.88 ± 0.19 and 4.12 ± 0.21 ng/cm²/h respectively. Trans-olfactory epithelial electrical resistance decreased $\sim 34.50 \pm 4.06$ % in presence of 0.25 % w/v chitosan. The $C_{\max}$ in rats administered with 0.25 % w/v chitosan and NGF was 1008.62 ± 130.02 pg/ml which was significantly higher than that for rats administered with NGF only 97.38 ± 10.66 pg/ml. There was ~ 14 fold increase in the bioavailability of intranasally administered NGF with chitosan than without chitosan. Chitosan can enhance the brain bioavailability of intranasally administered NGF.

Keywords

Nerve growth factor; chitosan; nose to brain; bioavailability; targeting; microdialysis

INTRODUCTION

Delivery of therapeutic quantities of neurotrophins to the brain interstitial fluid is believed to be the key for successful treatment of neurodegenerative disorders. However, delivering neurotrophins to the brain is a significant challenge due to the presence of the blood-brain and the blood-cerebrospinal fluid barriers.¹ Currently, invasive methods involving injection or implantation of neurotrophins directly into brain tissue (intraparenchymal or intra cerebroventricular or intrathecal administration) and cerebrospinal fluid are the only methods in clinical practice.² Therefore, there is an urgent need to develop a simple, patient compliant and effective method of delivering neurotrophins to the brain for the treatment of neurodegenerative disorders.

Intranasal administration is a potential route for drug delivery to the brain that bypasses the blood brain barrier (BBB).³ This route of administration is relatively more patient compliant than injections and also allows more frequent administration due to its noninvasiveness.

*Corresponding Author: Tel: 662-915-5164, Fax: 662-915-1177, murthy@olemiss.edu.

Moreover, the plausibility of a nose to brain pathway for delivery of macromolecules, like NGF, has been demonstrated previously.^{4–6}

NGF is a hydrophilic, dimeric protein of ~30 kDa which is known to facilitate neurogeneration and survival of neurons in *in vitro* and preclinical experiments.⁷ Prior studies on nose to brain delivery of NGF have used the total amount of NGF in the brain tissue homogenate as a measure of successful delivery.^{8–9} However, the NGF concentration in brain interstitium (perineural fluid that bathes the neuronal cell surfaces) as opposed to total concentration in the brain tissue is more relevant in predicting the therapeutic effectiveness, adverse effects, and toxicity of neurotrophins.

Therefore, in the present study the time course of concentration of NGF in the brain interstitial fluid following intranasal delivery of NGF was studied by microdialysis technique.

Although the existence of nose to brain pathway is well known, the potential for drug delivery to the brain via this pathway has been limited by barrier properties of olfactory epithelium. The barrier at the olfactory epithelium is attributed to the presence of tight junctions and efflux transporters. To deliver therapeutically relevant amounts of drug to the brain, the factors limiting the drug uptake across the olfactory epithelium need to be counteracted with the use of permeation enhancers. The ability of chitosan to enhance the permeation of NGF across the olfactory epithelium was discovered during preliminary *in vitro* screening of enhancers. Chitosan is a polycationic linear polysaccharide (biodegradable) that is bioadhesive and able to interact strongly with the mucus layer.¹⁰ Some reports exist on the ability of chitosan and its derivatives to improve the bioavailability of macromolecules across the nasal epithelium.^{11–13} There is also evidence that chitosan may improve the bioavailability of macromolecules in the brain when injected i.v. or parenterally. For instance, chitosan based microemulsion significantly increased the brain uptake of i.v. injected nobiletin in mice.¹⁴ When doxorubicin loaded chitosan-poly acrylic acid hollow nanospheres are administered parenterally to different organs of mice, a significant amount of drug reached the brain. Confocal laser scanning microscopy confirmed that the hollow chitosan-poly acrylic acid nanospheres are potential carriers for drugs.¹⁵ Chitosan may assist paracellular transport of drugs by transiently opening the tight junctions existing between the epithelial cells and suppressing the efflux transporters as has been shown in Caco-2 cell culture studies and in animal models.^{16–18} Continued investigation of chitosan assisted uptake of various drugs via distinct pathways is necessary to determine the full potential of this carrier. In the present study, it was found that chitosan can enhance the trans-olfactory epithelial transport of NGF in turn leading to relatively higher bioavailability in the brain.

MATERIALS AND METHODS

Chemicals

Krebs-Ringer bicarbonate buffer (KRB, premixed powder), crystal violet, gelatin (300 bloom), chitosan (MW ~250 kDa, 75–80% deacetylation) were procured from Sigma chemicals (St. Louis, MO). NGF, 2.5 S, murine and NGF E_{max} ImmunoAssay system were purchased from Promega Corporation, Madison, WI.

Olfactory epithelium

The bovine olfactory epithelium was purchased from Pel-Freez Biologicals, Arkansas. Bovine olfactory epithelium is a good model for human olfactory epithelium with respect to drug transport and metabolic pathways in nasal epithelium.¹⁹ The tissue was excised freshly

and supplied frozen. The tissue was used within 24 hours of excision. The tissue was thawed for 30 minutes before mounting on the diffusion cell.

***In vitro* Experimental set up**

A vertical Franz diffusion cell (Logan instruments, Somerset, NJ) was used for permeation experiments across the bovine olfactory epithelium. The temperature of the chamber was regulated at $37 \pm 1^\circ\text{C}$ by water circulation. Bovine olfactory epithelium was sandwiched between two compartments of the diffusion apparatus, one serving as the donor and other as the receiver compartment. The olfactory epithelium side was in contact with the upper donor compartment and the ventral side with the receiver compartment. The active diffusion area was 0.64 cm^2 . Ag/AgCl electrode wires of 2 mm in diameter (obtained from *In vivo* Metric, Heraldsburg, CA) made in the form of circular ring were placed 2 mm away from the olfactory epithelium in both donor and the receiver compartments. The donor and the receiver compartments were filled with 100 μl and 5 ml KRB, respectively for electrical resistance measurements. The AC electrical resistance of the epithelium was measured by placing a load resistor R_L (100 k Ω) in series with the epithelium. Voltage drop across the whole circuit (V_O) and across the epithelium (V_E) was measured using an electrical set up consisting of a wave form generator and multimeter (Agilent Technologies, Santa Clara, CA). For measuring the resistance, a small voltage of 100mv was applied at 10 Hz and the olfactory epithelial resistance in k Ω was approximated from the following formula.

$$R_E = \frac{V_E R_L}{V_O - V_E}$$

Where, R_E is the olfactory epithelium resistance and R_L is the load resistor in k Ω .

Preparation of chitosan and NGF mixture solution

The chitosan solution was prepared by dissolving required quantity of chitosan in a fraction of 1% glacial acetic acid solution prepared in Kreb's ringer buffer (pH 5.5). The second fraction of the buffer system was used to dissolve the NGF. The NGF and chitosan solutions were mixed by vortexing. The control solution was prepared for *in vitro* studies in the similar way but without incorporation of chitosan.

***In Vitro* Permeation studies**

The donor and the receiver compartments were filled with 100 μl of NGF solution (100 $\mu\text{g}/\text{ml}$ prepared in KRB containing 1% acetic acid, pH 5.5) and 5 ml KRB respectively. The receiver solution was stirred with a magnetic bead (600 rpm) for uniform mixing. To study the effect of chitosan, different concentrations of chitosan 0.1%, 0.25% and 0.5% w/v were incorporated into the NGF solution as described in the previous section. The amount of NGF permeated across the bovine olfactory epithelium was determined by measuring aliquots obtained from the receiver compartment at one hour intervals for 6 hours. Samples were assayed for NGF by ELISA as described below.

Calibration of microdialysis probe

The *in vitro* calibration of microdialysis probes (CMA 12) was carried out by successively immersing the probes in dialysis media containing 50, 75 and 100 $\mu\text{g}/\text{ml}$ of NGF and continuously perfusing with KRB solution at a flow rate of 2.0 $\mu\text{l}/\text{min}$ using a syringe pump controller (BASi, West Lafayette, IN). Probes were equilibrated for a period of 30 minutes and then the samples were collected at regular intervals of 1h for a period of 5h. The

percentage recovery was calculated as the ratio of dialysate concentration to its concentration in the dialysis medium surrounding the probe.

***In vivo* Microdialysis**

In vivo experiments were carried out in male, Sprague-Dawley rats (250–300 g, Harlan Company, Indianapolis, IN, U.S.A.) under ketamine (80 mg/kg) and xylazine (10 mg/kg) anesthesia (i.p injection). The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Mississippi (Protocol # 07–025). The rats were divided into 3 groups (n=3 for each).

Intranasal vehicle control group (0.25% chitosan in KRB)

Intranasal NGF group (NGF solution prepared in KRB)

Intranasal NGF + Chitosan group (NGF solution prepared in 0.25% chitosan in KRB)

A microdialysis probe (CMA 12) was inserted into the hippocampal region (anterior-posterior = 5.6 mm, medio-lateral = 5 mm, dorso-ventral = 7mm, from bregma) after securing the rat on a stereotaxic frame (Harvard instruments, Holliston, MA). Using a microinjection pump, the microdialysis probes were equilibrated by perfusing KRB at a rate of 2 µl/min for a period of one hour. NGF solution (100 µg/ml in 100µl) was administered directly into the posterior segment of the nose using a microsyringe connected with a soft polymer capillary. The animals were given maintenance doses of anesthesia (ketamine 40mg/kg + xylazine 10mg/kg) intravenously as needed. The microdialysis fractions were collected at hourly intervals for 6 hours following intranasal administration and the dialysate was assayed by ELISA (see below). The change in the concentration of NGF over time was used to calculate the pharmacokinetic parameters using a noncompartmental pharmacokinetic model. The area under the curve was calculated by trapezoidal rule. The maximum concentration (C_{max}) was directly read from the plot.

Assay of NGF

The NGF in the receiver compartments from *in vitro* experiments and the microdialysates from *in vivo* studies were assayed by ELISA using “NGF E_{max} ImmunoAssay system” following the protocol supplied by the manufacturer. ELISA is capable of discriminating the intact peptide from its metabolites and reflects only the actual content of NGF, unlike in the case of radiolabeled substrates where the label could be associated either with intact neurotrophins or its metabolite such that measuring the total label content does not reflect the actual concentration of neurotrophins.^{20–21} The sensitivity of the NGF assay used was about 7.8 pg/ml.

Microscopic Studies

The probe implantation site was examined by microscopic examination of coronal brain sections. The rats were euthanized via carbon dioxide inhalation. Brains were placed in formalin for days and then embedded in 8% gelatin. The gel block was hardened and cryoprotected in 20% sucrose formalin for 2 days before sectioning at 50 µm on a Leica 1800 cryostat. Sections were mounted, stained with cresyl violet, and cover slipped with DPX.

Data Analysis

Mann-Whitney U test was used for statistical analysis of *in vitro* and *in vivo* data. p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The *in vitro* permeation flux of NGF (control) across the olfactory epithelium was found to be 0.37 ± 0.06 ng/cm²/h. In presence of increasing concentration of chitosan (0.1%, 0.25% and 0.5% w/v) the permeation flux of NGF was found to be 2.01 ± 0.12 , 3.88 ± 0.19 and 4.12 ± 0.21 ng/cm²/h respectively. However, there was no statistically significant ($p = 0.06$) difference in the permeation of NGF when 0.25% and 0.5% chitosan was used. Therefore, 0.25% chitosan was used for all our future studies (Fig. 1a). In order to determine the effect of chitosan on permeability status of the olfactory epithelium, the electrical resistance was monitored before and after incorporation of chitosan.

Trans-olfactory epithelial electrical resistance has been reported to reflect the permeability status of the epithelial membranes.²² Therefore, the electrical resistance was measured in the current studies and the membranes with resistance > 2 kohms only were used for the *in vitro* permeation studies. In presence of the chitosan the trans-olfactory epithelial electrical resistance dropped significantly ($p = 0.003$) by $\sim 34.50 \pm 4.06$ % after 30 minutes indicating the ability of the chitosan to permeabilize the olfactory epithelial membrane. Electrical resistance is considered to be a rather qualitative parameter and did not show any quantitative dependence on the concentration of the enhancer. The most intriguing observation of the study was the recovery of trans-olfactory epithelial electrical resistance following replacement of chitosan with the KBR in the donor compartment after the permeation studies. The electrical resistance of the membrane was found to recover almost completely within 1–2 hours (Fig.1b). This suggests that chitosan acts as an enhancer by a mechanism which permeabilizes the membrane reversibly and not by mechanical disruption of the membrane. Similar observations found with chitosan in other biological membranes have been attributed to the opening and closing of tight junctions during permeabilization and recovery phases respectively.¹⁶⁻²³ Based on these observations, it can be speculated that chitosan could be acting as a drug permeation enhancer across the olfactory epithelium by opening the tight junctions.

The drug level in the extracellular fluid is known to determine the drug activity and toxicity in any tissue. Microdialysis is a technique which is most commonly used to investigate the release of neurotransmitters in the brain.^{24–28} The technique is capable of sampling the drug from the perineural fluid of the brain. Therefore, microdialysis was carried out to investigate the time course of free NGF in the perineural fluid. Moreover, CNS disorders such as Alzheimer's and Parkinson's are caused by degeneration of neurons specifically in the hippocampus region. The nose to brain pathway for drugs is well established and has been reported to deliver the NGF directly to the hippocampus region of the brain where most of the NGF receptors are situated.⁹ Therefore, in the present study, the nose to brain delivery of NGF was investigated by carrying out brain microdialysis in the hippocampal region.

A representative photomicrograph showing the probe implantation site which included the rat hippocampal region is shown in figure 2a. The recovery of the microdialysis probe was 4.2 ± 0.3 %. The basal value of unbound NGF in rats was 163.31 ± 3.36 pg/ml. The basal NGF levels observed in our studies are lower than that reported in prior studies. Hoener et al., determined the endogenous level of NGF in rat hippocampus as 738 pg/ml.²⁹ The same was reported to be 1336 pg/ml by Fawcett et al.³⁰ This could be due to the fact that the previous reports on nose to brain delivery of NGF were based on the measurements of NGF in whole tissue homogenates.⁹⁻²¹ Whereas, in this project the reported NGF value represents the free NGF present in the extracellular fluid.

The C_{max} in rats treated with chitosan and NGF was 1008.62 ± 130.02 pg/ml which was significantly higher than that for rats given NGF only 97.38 ± 10.66 pg/ml ($p = 0.014$). The brain bioavailability of intranasally administered NGF was enhanced by ~14 fold as calculated by ratio of area under the curve up to 6 hours in chitosan vs non-chitosan groups (Fig. 2b). However, there was no significant difference in the elimination half life of NGF between these two groups (Table I). These results support our hypothesis that the bioavailability of NGF in the brain is enhanced by the use of chitosan acting at the olfactory epithelium.

Considerable anatomical differences between human and rats with respect to olfactory epithelium and the brain may influence how the current results translate into human applications. In rats, the nasal olfactory mucus covers approximately 50 % of the total nasal epithelium as opposed to 5% in humans. The cerebrospinal fluid volume (~160 ml in humans vs 0.25 ml in rats) is replaced every 1.5 h in rats compared to every 5 h in humans. The brain interstitial fluid volume is ~260 ml in humans versus 0.3 ml in rats. Therefore, the nerve growth factor levels achieved in rats by intranasal administration may be an overestimation of what could actually be achieved in humans. However, these facts reinforce the necessity of drug bioavailability enhancers like chitosan for achieving clinically efficacious levels of drug in the human brain via the nasal pathway given the small epithelium area.

The present study is the first report on the pharmacokinetics of unbound NGF in the brain interstitial fluid which is the most critical measure for determining the therapeutic effect, side effects and toxicity of NGF. The present report is the first to demonstrate the potentiation of brain uptake of intranasally administered NGF via the olfactory pathway. At this stage it is not known how safe chitosan is for frequent administration into the nasal cavity. However, the present study offers an excellent model for further investigations and demonstrates that the olfactory epithelium could be rendered more permeable, thus enhancing the brain uptake of intranasally administered NGF.

Acknowledgments

The project was funded by Grant # P20RR021929 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. We thank Dr. Karen E. Sabol and Dr. Premalatha Balachandran for technical help.

REFERENCES

1. Thorne RG, Frey WH. Delivery of neurotrophic factors to the central nervous system. pharmacokinetic considerations. *Drug Disp.* 2001; 140:907–946.
2. Kordower JH, Palfi S, Chen EY. Clinicopathological findings following intraventricular glial-derived neurotrophic factor treatment in a patient with parkinson's disease. *Ann Neurol.* 1999; 46:419–424. [PubMed: 10482276]
3. Hanson LR, Frey WH II. Strategies for Intranasal Delivery of Therapeutics for the Prevention and Treatment of NeuroAIDS. *J Neuroimmune Pharmacol.* 2007; 2:81–86. [PubMed: 18040829]
4. Shilpley MT. Transport of molecules from nose to brain: transneuronal anterograde and retrograde labeling in the rat olfactory system by wheat germ agglutinin-horseradish peroxidase applied to the nasal epithelium. *Brain Res Bullet.* 1985; 15:129–142.
5. Barnett EM, Perlman S. The olfactory nerve and not the trigeminal nerve is the major site of CNS entry for mouse hepatitis virus, strain JHM. *Virology.* 1993; 94:185–191. [PubMed: 8386871]
6. Baker H, Spencer RF. Transneuronal transport of peroxidase-conjugated wheat germ agglutinin (WGA-HRP) from the olfactory epithelium to the brain of the adult rat. *Expt Brain Res.* 1986; 63:461–473.

7. Martinez HJ, Dreyfus CF, Jonakait GM, Black IB. Nerve growth factor promotes cholinergic development in brain striatal cultures. *Neurobiology*. 1985; 82:7777–7781.
8. Frey WH, Liu L, Chen XQ, Thorne RG, Fawcett JR, Ala TA, Rahman YE. Delivery of ¹²⁵I-NGF to the brain via the olfactory route. *Drug Delivery*. 1997; 4:87–92.
9. Chen X, Fawcett JR, Rahman Y, Ala TA, Frey WH. Delivery of nerve growth factor to the brain via the olfactory pathway. *J Alzheimer's Disease*. 1998; 1:35–44.
10. Illium L, Farraj NF, Davis SS. Chitosan as a novel nasal delivery system for peptide drugs. *Pharm Res*. 1994; 11:1186–1189. [PubMed: 7971722]
11. Illium L. Nasal drug delivery: possibilities, problems and solutions. *J Control Release*. 2003; 87:187–198. [PubMed: 12618035]
12. Tengamnuay P, Sahamethapat A, Sailasuta A, Mitra AK. Chitosan as nasal absorption enhancers of peptides: comparison between free amine chitosans and soluble salts. *Int J Pharm*. 2000; 197:53–67. [PubMed: 10704793]
13. Lubben I, Verhoef JC, Borchard G, Junginger HE. Chitosan and its derivatives in mucosal drug and vaccine delivery. *Eur J Pharm Sci*. 2001; 14:201–207. [PubMed: 11576824]
14. Yao J, Zhou JP, Ping QN, Lu Y, Chen L. Distribution of nobiletin chitosan-based microemulsions in brain following i.v injection in mice. *Int J Pharm*. 2008; 352:256–262. [PubMed: 18053660]
15. Hu Y, Ding Y, Ding D, Sun Mingjie, Zhang L, Jiang X, Yang C. Hollow chitosan/poly(acrylic acid) nanospheres as drug carriers. *Biomacromolecules*. 2007; 8:1069–1076. [PubMed: 17326676]
16. Dodane V, Khan MA, Merwin J. Effect of chitosan on Caco-2 on epithelial permeability and structure. *Int J Pharm*. 1999; 182:21–32. [PubMed: 10332071]
17. Sosnik A, Carcaboso AM, Chiappetta DA. Polymeric Nanocarriers: New endeavors for the optimization of the technological aspects of drugs. *Recent patents on biomed Engineering*. 2008; 1:43–59.
18. Werle M, Hoffer M. Glutathione and thiolated chitosan inhibit multidrug resistance P-glycoprotein activity in excised small intestine. *J Control Release*. 2006; 111:41–46. [PubMed: 16377016]
19. Schmidt MC, Simmen D, Hilbe M, Boderke P, Ditzinger G, Sandow J, Lang S, Rubas W, Merkle HP. Validation of excised bovine nasal mucosa as in vitro model to study drug transport and metabolic pathways in nasal epithelium. *J Pharm Sci*. 2000; 89:396–407. [PubMed: 10707019]
20. Krewson CE, Klarman ML, Saltzman WM. Distribution of nerve growth factor following direct delivery to brain interstitium. *Brain Res*. 1995; 680:196–206. [PubMed: 7663977]
21. Lapchak PA, Hefti. Emerging pharmacology of nerve growth factor. *Prog. Neuro-Psychopharmacol. Bio Psychiat*. 1991; 15:851–860.
22. Narai A, Arai S, Shimizu M. Rapid decrease in transepithelial electrical resistance of human intestinal Caco-2 cell monolayers by cytotoxic membrane perturbants. *Toxicol In Vitro*. 1997; 11:347–354. [PubMed: 20654321]
23. Smith J, Wood E, Dornish M. Effect of Chitosan on Epithelial Cell Tight Junctions. *Pharm Res*. 2004; 21:43–49. [PubMed: 14984256]
24. Young AM. Intracerebral microdialysis in the study of physiology and behavior. *Rev Neurosci*. 1993; 4:373–395. [PubMed: 7903896]
25. Westerink BH. Brain microdialysis and its application for the study of animal behaviour. *Behav. Brain Res*. 1995; 70:103–124. [PubMed: 8561902]
26. Mas M, Gonzalez-mora JL, Hernandez L. In vivo monitoring of brain neurotransmitter release for the assessment of neuroendocrine interactions. *Cell Mol Neurobiol*. 1996; 16:383–396. [PubMed: 8818403]
27. Bradberry CW. Applications of microdialysis methodology in nonhuman primates: practice and rationale. *Crit Rev Neurobiol*. 2000; 14:143–163. [PubMed: 11513243]
28. Chang Q, Savage LM, Gold PE. Microdialysis measures of functional increases in Ach release in the hippocampus with and without inclusion of acetylcholinesterase inhibitors in the perfusate. *J Neurochem*. 2006; 97:697–706. [PubMed: 16579834]
29. Honer MC, Hewitt E, Conner JM, Costello JW, Varon S. Nerve growth factor content in adult rat brain tissue is several-fold higher than generally reported and is largely associated with sedimentable fractions. *Brain Res*. 1996; 728:47–56. [PubMed: 8864296]

30. Fawcett JR, Chen X, Rahaman Y, Frey WH. Previously reported nerve growth factor levels are underestimated due to an incomplete release from receptors and interaction with standard curve media. *Brain Res.* 1999; 842:206–210. [PubMed: 10526111]

Figure 1.

a) *In vitro* permeability of NGF across the bovine olfactory epithelium from control (NGF solution only) and NGF + 0.25% w/v chitosan solution. **b)** Relative electrically resistivity of the bovine olfactory epithelium during permeation studies. After 6 hours of permeation study, the NGF solution in the donor compartment was replaced with KRB buffer and the electrical resistivity was monitored (*Recovery phase*). The data points provided are an average of six trials and error bars represent the standard error of mean (SEM).

Figure 2.

a) A representative picture of rat brain showing the location of microdialysis probe (arrowheads) in the hippocampus region. **b)** Concentration time profile of NGF in rat hippocampus following intranasal administration. The data points represent baseline adjusted values and are averages of three animals with SEM as error bars.

Table I

Pharmacokinetic parameters of intranasally (IN) administered NGF group and NGF + 0.25% w/v chitosan group.

Parameters	IN NGF group	IN (NGF + 0.25% w/v Chitosan) group	p value
C _{max} (pg/ml)	97.38 ± 10.66	1008.62 ± 130.02	0.014
AUC (pg h/ml)	209.79 ± 14.06	2692.46 ± 94.74	0.002
Elimination half life (1/h)	0.45 ± 0.08	0.52 ± 0.05	0.06