# Intranasal Nerve Growth Factor administration improves neurological sequelae after Acquired Brain Injury

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**Summary of purpose**

Nerve Growth Factor (NGF) is a neurotrophin that promotes neural recovery and plasticity after experimental brain injury, supporting neuronal growth, differentiation and survival of brain cells. Only a few studies by our group reported NGF administration in pediatric patients with impaired neurological functions after acquired brain injuries (Table 1). Currently, no effective therapies can restore neuronal loss or produce substantial clinical improvement in this kind of patients. Therefore, it is of primary importance to investigate a novel pharmacological approach for these children.

The aim of our studies was to investigate the pharmacological effects of NGF administration on brain functions in two children, respectively affected by a traumatic brain injury-dependent serious motor and cognitive impairment and by a non-traumatic severe neurological impairment due to a Streptococcus agalactiae devastating meningitis.

**Methodology**

Case A: Four-year-old boy with tetraplegia, neurological bladder and unresponsive wakefulness syndrome (UWS), incapable of swallowing, inable to speak and without reflex movements in response to command.

Case B: Seven-week old female infant suffering from severe post-infectious complications: Multi-Cystic Encephalomalacia (MCE) complicated by tetraventricular hydrocephalus, tetraparesis, severe neuropsychological impairment, dysphagia and absent brainstem reflexes.

Six months after brain injury, after standardized medical, neuro-intensive and rehabilitative care, treatment with intranasal NGF was taken into consideration. This treatment was approved by our University's Ethical Board and by the children's parents, who provided written informed consent.

Our first patient received four cycles of intranasal murine NGF (0.1 mg/kg, twice a day for 10 consecutive days), while the second one was treated, for the first time ever, with intranasal human recombinant NGF (Cenegermin, Dompè SpA.). She received five monthly cycles of intranasal hr-NGF (0.1 mg/kg, three times daily for 7 consecutive days). NGF was delivered through a mucosal atomizer device (MAD) in both patients and each cycle was repeated at one month-intervals.

**Results**

NGF administration improved PET/CT (Figure 1A, 1B), SPECT/CT (Figure 2A, 2B), and EEG assessments (Figure 3), as well as main cognitive processes and clinical and neurological functions. The first patient acquired voluntary movements of his legs, arms and fingers, improved facial mimicry and phonation, attention and verbal comprehension. He recovered the ability to cry, cough reflex, control of oral motility and feeding capacity, improved his bowel and urinary functions and, finally, made some spontaneous respiratory efforts. After hr-NGF treatment, the second patient showed significant improvements in facial mimicry, attention, motor reactions, oral motility and feeding capacity. She also recovered some hypothalamic functions and her cough reflex was restored.

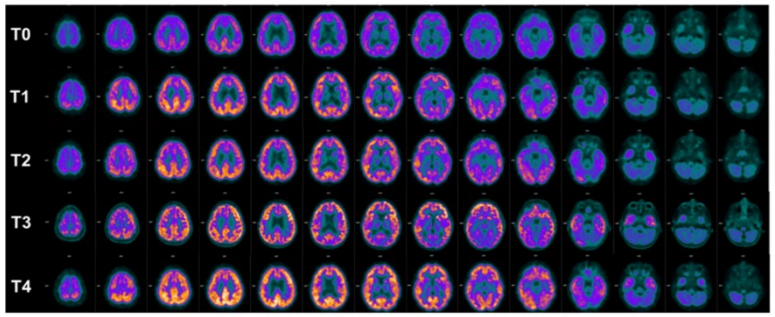


**Figure 1 A. PET/CT.**

18F-FDG images of brain axial slices performed 6 months after head injury, before starting NGF treatment in patient A (T0). The others intranasal NGF administrations were repeated at one-month intervals (T1, T2, T3, T4). A severe global reduction of 18F-FDG uptake in all cortical and subcortical regions in both brain hemispheres (T0) was observed. When comparing the last PET/CT (T4) with the basal PET/CT (T0), an increase in glucose metabolic activity of at least 50% was found in all cerebral regions; only in right and left superior frontal lobe and in right and left cerebellum, the glucose metabolic activity increased of 41%, 41%, 30%, and 32%, respectively.

**Table 1. Summary of clinical trials with NGF in children**

| Disease | Study type | NGF type and dosage | Delivery route | Outcome | Side effects |
|---|---|---|---|---|---|
| Lower limb crush syndrome | Single case patient study [a] | Mouse NGF. 10 µg every eight hours for seven days | Subcutaneous | Reduction of overall ischemic area. Reduction of the area undergoing calcaneal escharatomy | Not reported |
| Optic glioma and advanced optic nerve atrophy | Five patients case study [b] | Mouse NGF. 1 mg total over 10 days in daily applications | Topical (eye) | Improvement in visual evoked potentials (VEP) | Not reported |
| | Single patient case study [c] | Mouse NGF. 1mg total over 10 days in daily applications | Topical (eye) | Reversible improvements of visual function and electro-physiological measurements | Not reported |
| | Prospective randomized double-blind phase II clinical trial (18 patients) [d] | Mouse NGF. 0.5 mg total over 10 days in daily applications | Topical (eye) | Improvements of electro-physiological parameters. Improvement of visual field | Not reported |
| Hypoxic-ischemic perinatal brain injury | Two patients case study [e] | Mouse NGF. 0.1 mg/day for 10 days | ICV | Improvement in the comatose status, increase alpha/theta ratio in the EEG, reduction of malacic areas and improvement in right temporal/occipital cortices perfusion | Not reported |
| | Two patients case study [f] | Mouse NGF. 0.1 mg/day for 10 days | ICV | Improvement in EEG and SPECT parameters. An increase of doublecortin in CSF | Not reported |
| Acquired traumatic brain injury, TBI | Single patient case report [g] | Mouse NGF. 0.1 mg/kg over 10 days, twice-daily administration. Four cycles repeated at monthly intervals | Intranasal | Improved functional assessment PET/CT, SPECT/CT, MRI, EEG, VEP. Improved clinical parameters | Not reported |
| Acquired non-traumatic brain injury, nTBI (GBS meningitis) | Single patient case report [h] | Human recombinant NGF. 0.1 mg/kg over 7 days, thrice-daily administration. Five cycles repeated at monthly intervals | Intranasal | Improved functional assessment PET/CT, SPECT/CT. Improved clinical parameters | Not reported |
| Acquired traumatic brain injury, TBI | Phase II study, single arm clinical trial, exploratory [i] | Human recombinant NGF. 0.1 mg/kg over 7 days, thrice-daily administration. Four cycles repeated at monthly intervals | Intranasal | Ongoing | Ongoing |

[a] Chiaretti A *et al.* (2002), [b] Falsini B *et al.* (2011), [c] Chiaretti A *et al.* (2011), [d] Falsini B *et al.* (2016), [e] Chiaretti A *et al.* (2008), [f] Chiaretti A *et al.* (2005), [g] Chiaretti *et al.* (2017), [h] Eftimiadi G, *Thesis* (2019).

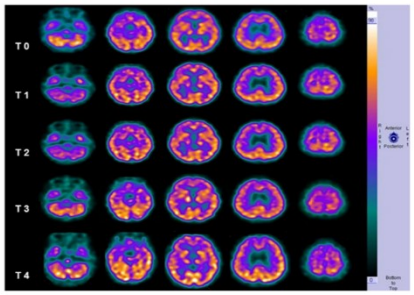


**Figure 2A. SPECT/CT**

$^{99m}$Tc-HMPAO SPECT/CT images before (T0) and after intranasal NGF administration (T1, T2, T3, and T4) to patient A. The baseline SPECT/CT scan revealed moderate hypoperfusion in the right frontal cortex and mild hypoperfusion in the right anterior temporal, left frontal cortices, and cerebellum. At the end of NGF treatment an increase in the radiotracer uptake was found in the right frontal (+19%, p = 0.01 ), right anterior temporal (+35%, p = 0.007), left frontal (+8% , p = 0.30) and left occipital (+13% , p = 0.01) cortices, as well as in the right and left cerebellar hemispheres (+18% , p = 0.03 and +12% , p = 0.07, respectively), by calculating percentage differences in the mean activity concentration of selected VOIs between T0 and T4 SPECT/CT scans.

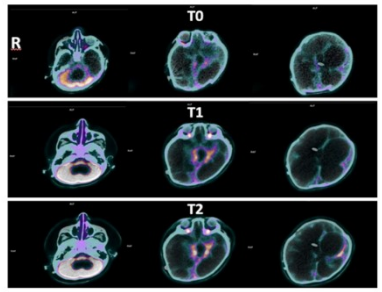


**Figure 1B. PET/CT.**

$^{18}$F-FDG images of brain axial slices performed before NGF treatment (T0) and after the second (T1) and fifth (T2) cycle of intranasal NGF administration to patient B. The first PET/CT study showed a severe global reduction of $^{18}$FDG uptake in all cortical and subcortical regions, suggesting a severe and diffuse hypometabolism. The other assessments were repeated at the end of each cycle, at one-month intervals. When comparing the last PET/CT (T2) with the basal PET/CT (T0), glucose metabolic activity increased by 76% in the deep frontal area, 64% in the thalami and 52% in the cerebellum.

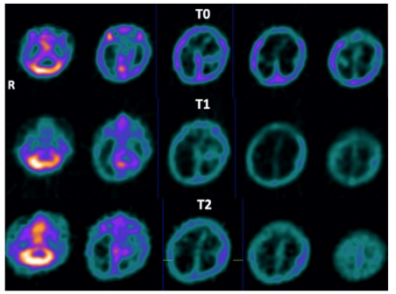


**Figure 2B. SPECT/CT.**

$^{99m}$Tc-HMPAO SPECT/CT axial images before NGF treatment (T0) and after the second (T1) and fifth (T2) cycle of intranasal NGF administration to patient B. The baseline SPECT/CT scan revealed marked bilateral hypoperfusion in the cortical hemispheres. At the end of NGF treatment, 36% increase in the radiotracer uptake was found in the cerebellum by calculating the percentage differences in the mean activity concentration of the selected VOIs between T0 and T2 SPECT/CT scans.

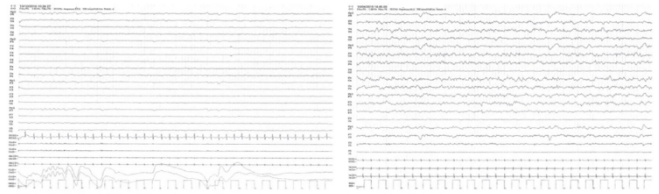

**Figure 3. EEG before and after NGF treatment in patient B.**

EEG recording performed 6 months after brain injury and before the beginning of NGF treatment showed severe low-voltage background activity, sometimes with a tendency to the isoelectric line. Sporadic theta-delta activity in the right fronto-temporal regions could be appreciated. Sleep was scarcely distinguished from wakefulness and the activity was markedly and diffusely depressed. The EEG examination performed after the end of NGF treatment showed an improvement in the electrical cerebral activity: a quantifiable 4-5 Hz background theta activity is evident bilaterally on the anterior regions (right>left), intermixed with abundant diffuse rapid rhythms.

**Conclusion**

Two children with severe acquired brain injuries were successfully treated for the first time ever with the non-invasive intranasal administration of NGF. The olfactory nerve permits a direct, effective route to the CNS, capable of penetrating the BBB with no safety issues and no risk of adverse events.

**Next steps/practical applications**

Further controlled, randomised, double blind studies are needed for a better understanding of the neuroprotective mechanisms of the intranasal NGF administration. The current preliminary findings and the ease of administration of the drug appear to be a promising and safe rescuing strategy for the treatment of children with neurological sequelae due to severe acquired brain injuries.