Title: Inhalation of growth factors and apo-transferrin to protect and repair the hypoxic-ischemic brain

Author: M. Guardia Clausi P.M. Paez L.A. Pasquini J.M. Pasquini

PII: S1043-6618(16)00016-5
DOI: http://dx.doi.org/doi:10.1016/j.phrs.2016.01.010
Reference: YPHRS 3036

To appear in: *Pharmacological Research*

Received date: 30-11-2015
Revised date: 12-1-2016
Accepted date: 12-1-2016

Please cite this article as: Guardia Clausi M, Paez PM, Pasquini LA, Pasquini J.M.Inhalation of growth factors and apo-transferrin to protect and repair the hypoxic-ischemic brain.*Pharmacological Research* http://dx.doi.org/10.1016/j.phrs.2016.01.010

Inhalation of growth factors and apo-transferrin to protect and repair the hypoxic-ischemic brain

Running title: Inhalation of drugs and brain injury.

Guardia Clausi M[1,2]., Paez PM[3]., Pasquini LA[1], Pasquini JM[1*]. jpasquin@qb.ffyb.uba.ar

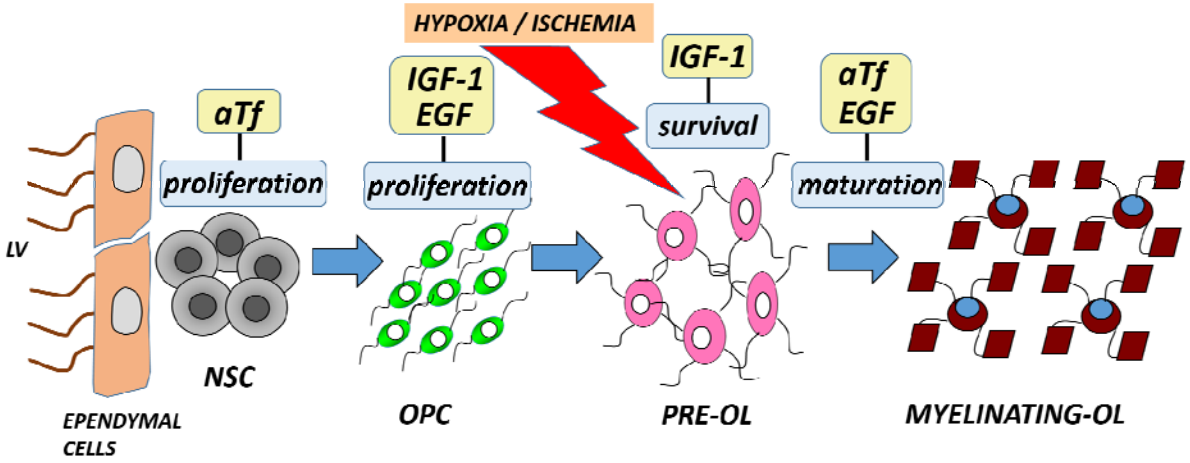
[1]Department of Biological Chemistry, School of Pharmacy and Biochemistry and IQUIFIB-CONICET, Universidad de Buenos Aires, Argentina.

[2]Department of Pharmacology, Physiology and Neuroscience, Rutgers-New Jersey Medical School, Newark, NJ, USA.

[3]Hunter James Kelly Research Institute, Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, SUNY, University at Buffalo. NYS Center of Excellence, 701 Ellicott St., Buffalo, New York 14203, United States.

[*]Corresponding author at: Department of Biological Chemistry, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956, C1113, Buenos Aires, Argentina. Tel.: 54-11-4964-8287/88, Fax: 54-11-4962-5457.

Graphical abstract



ABSTRACT

Hypoxic-ischemic brain damage is a major contributor to chronic neurological dysfunction and acute mortality in infants as well as in adults. In this review, we summarize recent publications demonstrating that the intranasal administration (INA) of apo-transferrin (aTf) and different growth factors provides neuroprotection to the mouse and rat brain after a hypoxic-ischemic event. The intranasal delivery of growth factors such as insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) has been found to improve neurological function and reduce infarct size in adult rats after a hypoxic-ischemic event. On the other hand, INA of aTf and epidermal growth factor (EGF) were effective in reducing white matter damage and inflammation and in promoting the proliferation and survival of oligodendroglial progenitor cells (OPCs) in a model of hypoxic-ischemic encephalopathy. Therefore, data summarized in this review suggest that INA of growth factors and aTf can be used in combination in clinical treatment in order to protect and repair the hypoxic-ischemic brain.

Keywords: Hypoxia-ischemia; inhalation; growth factors; apo-transferin; oligodendrocytes; demyelination; remyelination.

Drug instillation

A promising way to deliver drugs to the brain is the intranasal route. The olfactory region is the only site where the central nervous system (CNS) is in contact with the external environment due to the presence of the olfactory receptor neurons, whose axons end in the olfactory bulb. Illum and coworkers (2000; 2004) [1,2] determined the existence of a direct pathway connecting nose to brain and provided details of the possible routes of entry of substances introduced into the nose of different animals and humans. The authors postulated that, depending on the size, charge and hidro or lipophilicity of the molecule, different substances could be transported into the brain. Essentially, two routes have been proposed for the direct passage of peptides and proteins from the nose to the brain: an intraneuronal and an extra-neuronal pathway [2]. Intraneuronal transport includes the internalization of the peptide into olfactory neurons, followed by axonal transport. However, this route poses a great risk of proteolysis, resulting from lysosomal degradation, and requires several hours for substances to reach the olfactory bulb [2]. It therefore seems more probable that peptide molecules travel by the extracellular route, passing through patent intercellular gaps in the olfactory epithelium to diffuse into the subarachnoid space [2].

Intranasal administration (INA) has many advantages from a clinical point of view: it is noninvasive and easily carried out given the capacity of bypassing the blood-brain barrier [3,4]. The potential utility of INA derives from the fact that biologically effective concentrations of neuropeptides and proteins can reach the human brain without serious systemic side effects. Such effects limit the systemic administration of peptides to quantities too small to exert significant effects in the brain. A wide range of studies has explored the transport of various drugs from the nasal cavity to the brain and, although most of them have been conducted in rat models, studies in mice, rabbits and monkeys have also been reported. INA has not only been used in the basic research field [5-8], but has also found applications in human health [9,10]. Several reports confirm the positive outcome of nose-to-brain delivery not only for drug

molecules with various molecular weights [11,12] but also for living cells [13,14]. Hanson et al (2009) [11,12] reported that INA targets deferoxamine to the brain and reduces systemic exposure, and that intranasal deferoxamine prevents and treats stroke damage after middle cerebral artery occlusion in rats. On the other hand, Danielyan and collaborators (2001) [13,14] have revealed noninvasive intranasal delivery of stem cells to the rat brain for the first time, showing that the intranasal application of mesenchymal stem cells resulted in the appearance of cells in the olfactory bulb, cortex, hippocampus, striatum, cerebellum, brainstem and spinal cord. Therefore, INA represents a highly promising alternative to target and deliver stem cells or neurotrophic factors to the brain with the option of chronic application.

Intranasal administration of growth factors

The INA of neurotrophic factors and other substances, including certain hormones, has received increasing attention in recent years [15]. Different reports on successful INA of insulin-like growth factor-1 (IGF-1) in the treatment of various brain injuries have been recently published. Thorne and colleagues (2004) [8] demonstrated that IGF-1 administered intranasally in rats can reach distant areas such as the cerebral cortex, the hypothalamus, the cerebellum, the brain stem and the medulla in concentrations considered to be of therapeutic value **(Figure 1)**. The intranasal delivery of nerve growth factor (NGF) has been reported to ameliorate or prevent neurodegeneration and memory deficits in the AD11 mouse model of Alzheimer's disease [16,17]. In addition, NAP (an 8-amino acid peptide derived from activity-dependent neuroprotective protein ADNP) has been observed to improve the performance of normal and cognitively impaired rats in the Morris water maze test. Moreover, NAP has been shown to alleviate anxiety and enhance cognition after chronic intranasal treatment [18]. Additionally, the intranasal delivery of activity-dependent neurotrophic factor (ADNF) to the brain has been reported to play a neuroprotective role [19]. Most importantly, intranasal neurotrophins such as

fibroblast growth factor-2 and heparin-binding epidermal growth factor-like growth factor have been shown to enhance neurogenesis in the subventricular zone of the adult mouse brain [20].

Growth factor inhalation and hypoxic-ischemic brain injury

There are no clinically relevant treatments for people suffering hypoxic-ischemic brain injury and the accessibility of different therapeutic molecules to the specific brain damage areas remains problematic. In recent years, growth factor inhalation has been studied as a therapy for hypoxic-ischemic brain injury or brain stroke. IGF-I has been shown to exert protection against stroke when administered intracerebro-ventricularly in rats, although this invasive method of administration is not practical for the large number of individuals who require treatment. However, intranasal delivery of IGF-1 has been found to improve neurological function in adult rats after hypoxic-ischemic brain damage [21,22]. In a work by Liu et al. (2001), INA of IGF-1 was shown to significantly reduce infarct volume and improve neurological function following focal cerebral ischemia in rats, solving deficit in motor, sensory, reflex and vestibulomotor functions [21]. Similarly, intranasal delivery of IGF-1 has been found to recuperate neurological function in adult rats after middle cerebral artery occlusion [21,22]. Intranasal IGF-1 significantly reduced infarct volumes and hemispheric swelling and improved neurologic function, assessed by the postural reflex, flexor response and adhesive tape tests. In the same line, Lin et al. (2009) confirmed that INA of IGF-1 is an effective way to target this growth factor to the neonatal rat brain following cerebral hypoxia-ischemia [23]. Intranasal delivery of IGF-1 not only attenuated pathological changes induced by hypoxia-ischemia in the neonatal brain, but also enhanced neurological functions [23] **(Figure1)**. It has been also demonstrated that IGF-1 treatment activates the pAkt pathway and inhibits the activation of caspase-3 after cerebral hypoxia ischemia [23]. Moreover, it has been shown to promote the proliferation of neural progenitor cells during the tissue repair stage in a neonatal hypoxic-ischemic model [23]. Similarly, Yang and colleges (2009) [12] have evaluated dose effectiveness in the intranasal

delivery of vascular endothelial growth factor (VEGF) in the treatment of experimental stroke, reporting that INA of VEGF was effective in reducing infarct volume, improving behavioral recovery and enhancing angiogenesis in the stroke brain [12].

The epidermal growth factor (EGF) is an important player in the development of oligodendrocytes [24]. Using an established model of preterm brain injury, Scafidi et al. (2014) have demonstrated that INA of heparin-binding EGF immediately after hypoxic injury decreases oligodendroglia cell death, increases the production of new oligodendroglial cells and promotes brain recovery [25]. Furthermore, these interventions diminish ultrastructural abnormalities and alleviate behavioral deficits in white-matter-specific paradigms [25]. Thus, these results provide direct evidence that INA of EGF at a specific time after the hypoxic damage is clinically feasible and potentially applicable to the treatment of premature children with white matter injury.

In summary, these studies indicate that INA of growth factors holds significant promise as a noninvasive and efficacious method for the treatment of hypoxic-ischemic brain damage **(Figure 1).**

Intranasal administration of apo-transferrin

Previous studies have shown that the intracerebral injection of apo-transferrin (**aTf**) alleviates white matter damage and accelerates remyelination in neonatal rat models of neurodegeneration [26-28]. Nevertheless, the intracerebral injection of aTf might not be adequate for clinical treatments. Therefore, the development of less invasive techniques for the delivery of aTf to the CNS has been investigated in order to use this protein in clinical studies and, in particular, our group has explored the possibility of delivering aTf into the brain using INA of radioactive iodine-labeled aTf. ^{125}I-aTf of high specific activity was prepared and delivered through the nostrils of anesthetized young rats. Two hours later, the animals were perfused and the brains excised. The cerebral hemispheres were divided into three areas (anterior, middle and posterior, including the brain stem and cerebellum) and the distribution of

radioactivity present in the tissue was analyzed by autoradiography of coronal brain slices. Our results show that, although in small amounts, the radiolabeled aTf introduced through the nostrils reached distant areas of the brain (**Figure2**), which suggests that INA is a feasible procedure to deliver aTf into the brain.

Similar experiments were performed in a model of hypoxic-ischemic encephalopathy [29]. We have found that aTf reaches the brain parenchyma and increases its presence in the different areas of the CNS. We have also shown that aTf was present in the right olfactory bulb and in the frontal and posterior brain in both the control and hypoxic-ischemic animals after INA. The mechanisms of protein transport from the nasal cavity to the brain are not entirely known, although several possible pathways have been proposed [2]. Our results indicate that anterograde axonal transport is the pathway for aTf delivery into the perinatal mouse brain. In support of this conclusion, a very low concentration of aTf was detected in the olfactory bulb when cytochalasine B or colchicine was administered before the INA of aTf. Colchicine inhibits microtubule assembly and reduces axonal transport [30], while, in the optic nerve, cytochalasin B is an inhibitor of neurofilament axonal transport [31]. However, further studies are necessary to describe the mechanism of transport of aTf from the olfactory area to the brain.

Apo-transferrin inhalation and hypoxic-ischemic brain damage

In the CNS, transferrin (Tf) is produced by oligodendrocytes and is vital for normal brain development. We have found that aTf is essential for oligodendrocyte maturation and myelination in vitro as well as in vivo. Since 1994, our laboratory has published a number of paper describing the effects of aTf on oligodendroglial cell differentiation and myelination. We have reported that a single intracranial injection of aTf upregulates the expression of diverse myelin constituents and significantly increases myelin deposition, especially in areas close to the lateral ventricles in rats [32,33]. This promyelinating effect was also seen in primary cultures of oligodendrocytes [34], as well as in oligodendroglial cell lines treated with aTf [35,36]. We

have demonstrated that aTf modulates the expression of myelin basic protein (MBP) through different signaling pathways and, furthermore, we have shown that aTf overexpression promotes oligodendrocyte differentiation and myelination of cortical neurons [36,37].These data have been confirmed by other authors who showed that aTf regulates MBP expression [38] and that transgenic mice overexpressing the human Tf gene in the CNS evidence increased myelination [39].

In a rat model of neonatal hypoxia-ischemia encephalopathy (**HIE**) [29], we have demonstrated that the INA of aTf can remyelinate areas of demyelination. Intranasal delivery of aTf decreased astrogliosis and neuronal loss in HIE animals and increased oligodendrocyte survival in different areas of the brain [29]. We also found that the INA of aTf enhanced the proliferation of OPCs in the corpus callosum and the subventricular zone and protected these cells against apoptotic death after the hypoxic-ischemic incident. For instance, the number of PDGFRα-positive OPCs was higher in mice treated with aTf than in untreated brains two days after the hypoxic-ischemic event [29]. Additionally, the number of OPCs positive for caspase-3 in the corpus callosum, cortex and striatum was lower in aTf-treated hypoxic-ischemic mice. A summary of aTf effects on the neonatal hypoxic-ischemic brain is shown in **Figure 1**. These results seem to indicate that aTf is an inducer of myelinating oligodendrocytes in the neonatal mouse brain in acute demyelination caused by HIE. Additionally, this study shows that the intranasal delivery of aTf promotes the survival and maturation of OPCs after demyelination and suggests that the INA of aTf can be used for clinical treatment to induce remyelination in demyelinating hypoxic-ischemic events.

ACKNOWLEDGMENTS The authors thank Agencia Nacional de Ciencia y Tecnología and Universidad de Buenos Aires for supporting our research.

The authors declare no competing interests.

REFERENCES

1 Illum L. Transport of drugs from the nasal cavity to the central nervous system. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 2000;11:1-18.

2 Illum L. Is nose-to-brain transport of drugs in man a reality? The Journal of pharmacy and pharmacology 2004;56:3-17.

3 Thorne RG, Frey WH, 2nd. Delivery of neurotrophic factors to the central nervous system: Pharmacokinetic considerations. Clinical pharmacokinetics 2001;40:907-946.

4 Merkus FW, van den Berg MP. Can nasal drug delivery bypass the blood-brain barrier?: Questioning the direct transport theory. Drugs in R&D 2007;8:133-144.

5 Alcala-Barraza SR, Lee MS, Hanson LR, McDonald AA, Frey WH, 2nd, McLoon LK. Intranasal delivery of neurotrophic factors bdnf, cntf, epo, and nt-4 to the cns. Journal of drug targeting 2010;18:179-190.

6 Cai Z, Fan LW, Lin S, Pang Y, Rhodes PG. Intranasal administration of insulin-like growth factor-1 protects against lipopolysaccharide-induced injury in the developing rat brain. Neuroscience 2011;194:195-207.

7 Scranton RA, Fletcher L, Sprague S, Jimenez DF, Digicaylioglu M. The rostral migratory stream plays a key role in intranasal delivery of drugs into the cns. PloS one 2011;6:e18711.

8 Thorne RG, Pronk GJ, Padmanabhan V, Frey WH, 2nd. Delivery of insulin-like growth factor-i to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 2004;127:481-496.

9 Jogani VV, Shah PJ, Mishra P, Mishra AK, Misra AR. Intranasal mucoadhesive microemulsion of tacrine to improve brain targeting. Alzheimer disease and associated disorders 2008;22:116-124.

10 Pathan SA, Iqbal Z, Zaidi SM, Talegaonkar S, Vohra D, Jain GK, Azeem A, Jain N, Lalani JR, Khar RK, Ahmad FJ. Cns drug delivery systems: Novel approaches. Recent patents on drug delivery & formulation 2009;3:71-89.

11 Hanson LR, Roeytenberg A, Martinez PM, Coppes VG, Sweet DC, Rao RJ, Marti DL, Hoekman JD, Matthews RB, Frey WH, 2nd, Panter SS. Intranasal deferoxamine provides increased brain exposure and significant protection in rat ischemic stroke. The Journal of pharmacology and experimental therapeutics 2009;330:679-686.

12 Yang JP, Liu HJ, Wang ZL, Cheng SM, Cheng X, Xu GL, Liu XF. The dose-effectiveness of intranasal vegf in treatment of experimental stroke. Neuroscience letters 2009;461:212-216.

13 Danielyan L, Schafer R, von Ameln-Mayerhofer A, Bernhard F, Verleysdonk S, Buadze M, Lourhmati A, Klopfer T, Schaumann F, Schmid B, Koehle C, Proksch B, Weissert R, Reichardt HM, van den Brandt J, Buniatian GH, Schwab M, Gleiter CH, Frey WH, 2nd. Therapeutic efficacy of intranasally delivered mesenchymal stem cells in a rat model of parkinson disease. Rejuvenation research 2011;14:3-16.

14 Danielyan L, Schafer R, von Ameln-Mayerhofer A, Buadze M, Geisler J, Klopfer T, Burkhardt U, Proksch B, Verleysdonk S, Ayturan M, Buniatian GH, Gleiter CH, Frey WH, 2nd. Intranasal delivery of cells to the brain. European journal of cell biology 2009;88:315-324.

15 Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL. Sniffing neuropeptides: A transnasal approach to the human brain. Nature neuroscience 2002;5:514-516.

16 Capsoni S, Giannotta S, Cattaneo A. Nerve growth factor and galantamine ameliorate early signs of neurodegeneration in anti-nerve growth factor mice. Proceedings of the National Academy of Sciences of the United States of America 2002;99:12432-12437.

17 De Rosa R, Garcia AA, Braschi C, Capsoni S, Maffei L, Berardi N, Cattaneo A. Intranasal administration of nerve growth factor (ngf) rescues recognition memory deficits in ad11 anti-ngf

transgenic mice. Proceedings of the National Academy of Sciences of the United States of America 2005;102:3811-3816.
18 Alcalay RN, Giladi E, Pick CG, Gozes I. Intranasal administration of nap, a neuroprotective peptide, decreases anxiety-like behavior in aging mice in the elevated plus maze. Neuroscience letters 2004;361:128-131.
19 Gozes I, Giladi E, Pinhasov A, Bardea A, Brenneman DE. Activity-dependent neurotrophic factor: Intranasal administration of femtomolar-acting peptides improve performance in a water maze. The Journal of pharmacology and experimental therapeutics 2000;293:1091-1098.
20 Jin K, Xie L, Childs J, Sun Y, Mao XO, Logvinova A, Greenberg DA. Cerebral neurogenesis is induced by intranasal administration of growth factors. Annals of neurology 2003;53:405-409.
21 Liu XF, Fawcett JR, Thorne RG, DeFor TA, Frey WH, 2nd. Intranasal administration of insulin-like growth factor-i bypasses the blood-brain barrier and protects against focal cerebral ischemic damage. Journal of the neurological sciences 2001;187:91-97.
22 Liu XF, Fawcett JR, Thorne RG, Frey WH, 2nd. Non-invasive intranasal insulin-like growth factor-i reduces infarct volume and improves neurologic function in rats following middle cerebral artery occlusion. Neuroscience letters 2001;308:91-94.
23 Lin S, Fan LW, Rhodes PG, Cai Z. Intranasal administration of igf-1 attenuates hypoxic-ischemic brain injury in neonatal rats. Experimental neurology 2009;217:361-370.
24 Aguirre A, Dupree JL, Mangin JM, Gallo V. A functional role for egfr signaling in myelination and remyelination. Nature neuroscience 2007;10:990-1002.
25 Scafidi J, Hammond TR, Scafidi S, Ritter J, Jablonska B, Roncal M, Szigeti-Buck K, Coman D, Huang Y, McCarter RJ, Jr., Hyder F, Horvath TL, Gallo V. Intranasal epidermal growth factor treatment rescues neonatal brain injury. Nature 2014;506:230-234.
26 Adamo AM, Paez PM, Escobar Cabrera OE, Wolfson M, Franco PG, Pasquini JM, Soto EF. Remyelination after cuprizone-induced demyelination in the rat is stimulated by apotransferrin. Experimental neurology 2006;198:519-529.
27 Badaracco ME, Ortiz EH, Soto EF, Connor J, Pasquini JM. Effect of transferrin on hypomyelination induced by iron deficiency. Journal of neuroscience research 2008;86:2663-2673.
28 Guardia Clausi M, Pasquini LA, Soto EF, Pasquini JM. Apotransferrin-induced recovery after hypoxic/ischaemic injury on myelination. ASN neuro 2010;2:e00048.
29 Guardia Clausi M, Paez PM, Campagnoni AT, Pasquini LA, Pasquini JM. Intranasal administration of atf protects and repairs the neonatal white matter after a cerebral hypoxic-ischemic event. Glia 2012;60:1540-1554.
30 Han Y, Malak H, Chaudhary AG, Chordia MD, Kingston DG, Bane S. Distances between the paclitaxel, colchicine, and exchangeable gtp binding sites on tubulin. Biochemistry 1998;37:6636-6644.
31 Jung C, Chylinski TM, Pimenta A, Ortiz D, Shea TB. Neurofilament transport is dependent on actin and myosin. The Journal of neuroscience : the official journal of the Society for Neuroscience 2004;24:9486-9496.
32 Cabrera OE, Bongiovanni G, Hallak M, Soto EF, Pasquini JM. The cytoskeletal components of the myelin fraction are affected by a single intracranial injection of apotransferrin in young rats. Neurochemical research 2000;25:669-676.
33 Marta CB, Paez P, Lopez M, Pellegrino de Iraldi A, Soto EF, Pasquini JM. Morphological changes of myelin sheaths in rats intracranially injected with apotransferrin. Neurochemical research 2003;28:101-110.
34 Paez PM, Marta CB, Moreno MB, Soto EF, Pasquini JM. Apotransferrin decreases migration and enhances differentiation of oligodendroglial progenitor cells in an in vitro system. Developmental neuroscience 2002;24:47-58.

35 Paez PM, Garcia CI, Davio C, Campagnoni AT, Soto EF, Pasquini JM. Apotransferrin promotes the differentiation of two oligodendroglial cell lines. Glia 2004;46:207-217.

36 Paez PM, Garcia CI, Campagnoni AT, Soto EF, Pasquini JM. Overexpression of human transferrin in two oligodendroglial cell lines enhances their differentiation. Glia 2005;52:1-15.

37 Paez PM, Garcia CI, Soto EF, Pasquini JM. Apotransferrin decreases the response of oligodendrocyte progenitors to pdgf and inhibits the progression of the cell cycle. Neurochemistry international 2006;49:359-371.

38 Espinosa-Jeffrey A, Kumar S, Zhao PM, Awosika O, Agbo C, Huang A, Chang R, De Vellis J. Transferrin regulates transcription of the mbp gene and its action synergizes with igf-1 to enhance myelinogenesis in the md rat. Developmental neuroscience 2002;24:227-241.

39 Saleh MC, Espinosa de los Monteros A, de Arriba Zerpa GA, Fontaine I, Piaud O, Djordjijevic D, Baroukh N, Garcia Otin AL, Ortiz E, Lewis S, Fiette L, Santambrogio P, Belzung C, Connor JR, de Vellis J, Pasquini JM, Zakin MM, Baron B, Guillou F. Myelination and motor coordination are increased in transferrin transgenic mice. Journal of neuroscience research 2003;72:587-594.

40 Hill JM, Ruff MR, Weber RJ, Pert CB. Transferrin receptors in rat brain: neuropeptide-like pattern and relationship to iron distribution. Proc. Natl. Acad. Sci. USA 1985; 82: 4553-7

Figure Captions

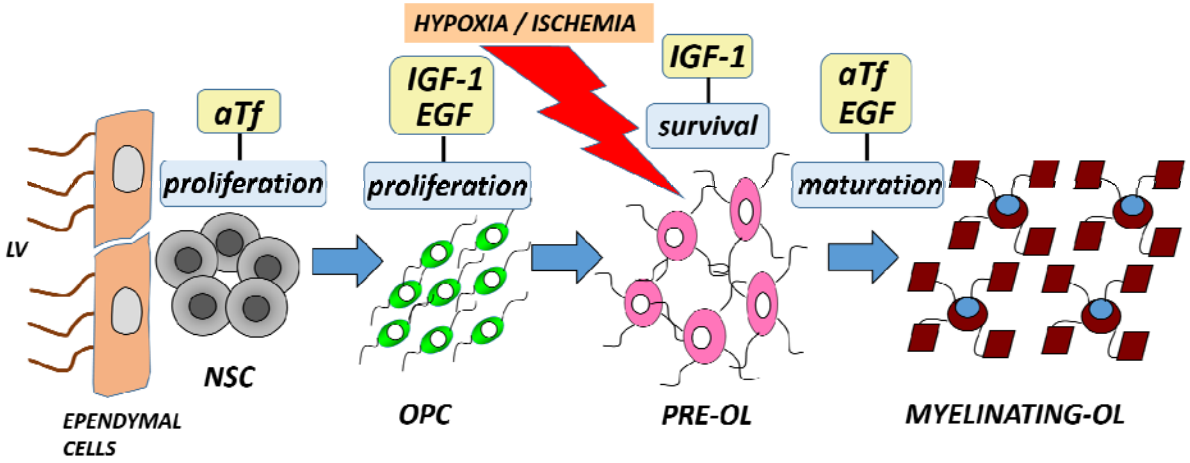


Figure 1: Promyelinating effects of intranasal apotransferrin (aTf), EGF and IGF-1 after neonatal hypoxia ischemia. Intranasal aTf, EGF and IGF-1 restore white matter development disrupted by a hypoxic ischemic insult (H/I). aTf induces proliferation of neural stem cells (NSC) located in the wall of the lateral ventricle (LV). IGF-1 and EGF promote the proliferation of oligodendrocyte precursor cells (OPCs). IGF-1 prevents apoptosis of pre-oligodendrocytes (pre-OL), which are particularly vulnerable to H-I. aTf and EGF accelerate the maturation of pre-oligodendrocytes to become myelinating oligodendrocytes.

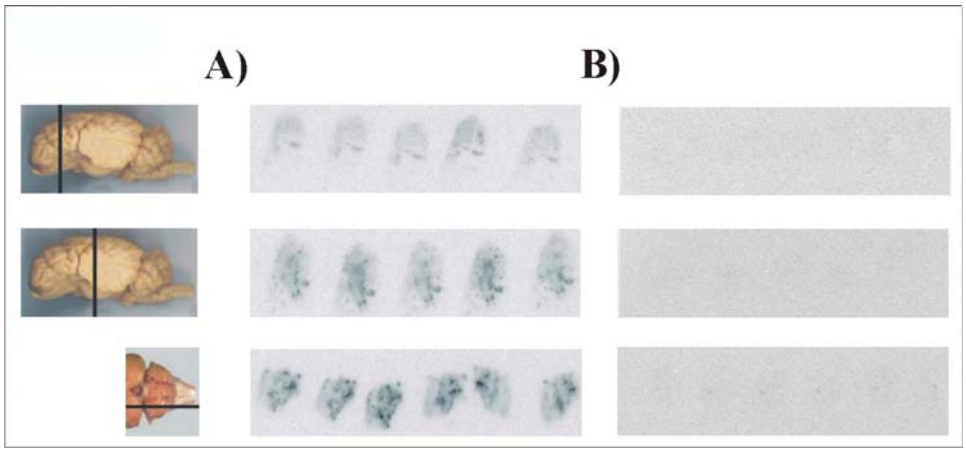


Figure 2: No radioactivity was found in the controls treated with unlabeled aTf. Tissue radioactivity in brains treated with iodine-labeled aTf (^{125}I-aTf) was evidenced by the presence of the intact molecule of aTf, which was checked by gel electrophoresis followed by radioautography, and the identification of the radioactive band by its molecular weight. No radioactive byproducts were detected. This indicates quite clearly that aTf can be safely delivered into the brain by intravascular infusion. Experiments were done following the method described in Hill et al.,1985 with slight modifications