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Review

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

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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.

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1. 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 et al. [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].

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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. [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 et al. [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.

2. 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 et al. [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 (Fig. 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].

3. 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., 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. 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] (Fig. 1). 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 et al. [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. 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 (Fig. 1).

4. 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