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Intranasal Administration of a Naked Plasmid Reached Brain Cells and Expressed Green Fluorescent Protein, a Candidate for Future Gene Therapy Studies

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Background. Intranasal administration (Int adm) has been well-studied and offers the possibility to deliver larger molecular weight biologics, such as proteins, viral vectors, nanoparticles, and naked plasmids to the brain and treat a variety of diseases in the central nervous system. The predominant challenge in this field is finding efficient vectors that are capable of crossing the blood-brain barrier (BBB).

Objectives. Here, we investigated whether a naked plasmid (pIRES-hrGFP-1a), could cross the BBB, reach brain cells and express green fluorescent protein (GFP) after int-adm and propose it as candidate for future gene therapy studies.

Material and Methods. Thirty-six mice were divided into 2 groups. Eighteen animals were assigned to each cluster. Mice from experimental groups received 25 μg of pIRES-hrGFP-1a. The control groups received 25 μl of PBS. Plasmids were given intranasally by applying little drops in both nostrils. Twenty-four hours later, the mice were sacrificed, and their brains were removed. Later, PCR, RT-PCR, and immunohistochemical techniques were performed.

Results. pIRES-hrGFP-1a crossed the BBB and was mainly detected in the olfactory nerves (20%) and hypothalamus (16%). In contrast, GFP/18S-expressing mRNAs were detected mostly in the olfactory bulbs (95%), frontal cortex (71%) and amygdala (60%). GFP was detected in the olfactory bulb, hippocampus, frontal cortex and brainstem at 24 h.

Conclusions. pIRES-hrGFP-1a could be considered a good candidate for gene therapy studies. In the future could be cloned some therapeutic genes in the pIRES-hrGFP-1a and could transcribe and translates deficient proteins that are required to restore a function.

Key Words: Gene therapy, Intranasal administration, pIRES-hrGFP-1a, Green fluorescent protein.

Conflict of Interest: We all authors declare that there is no actual or potential conflict of interest.

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Introduction

Since early last century, intranasal administration (Int-Adm) has been used as an access point to the central nervous system (CNS) for some viruses, such as poliovirus, and for certain substances, such as potassium ferricyanide and ammonium citrate ferric (1−3) But, until the early 1970s, this approach began to draw the attention of scientists as a potential route of administration for various substances, such as albumin, gold colloid, and horseradish peroxidase conjugated-wheat germ agglutinin (WGA-HRP), for their transport from the epithelium of the nasal cavity to the brain (4). The blood-brain barrier (BBB) is associated with low permeability because it possesses tight junctions, which limit the passage of molecules through the space between endothelial cells. Moreover, there are many examples of low molecular weight drugs and polymeric nanoparticles capable of crossing the BBB to reach the brain, but most high-molecular-weight substances are incapable of crossing the BBB under normal conditions (5). Int-Adm provides a non-invasive method to deliver high molecular weight substances, such as proteins, viral vectors, naked plasmids, nanoparticles, stem cells, and recombinant particles, to the brain (5−10). Int-Adm is also a potentially useful strategy for treating a variety of neurological diseases, including Parkinson's disease, Multiple Sclerosis, stroke, Alzheimer's disease, psychiatric disorders, and epilepsy (11−17). During last decade, several examples in which therapeutic substances delivered by Int-Adm reach the brain have been observed. For example, in 2008, Nonaka et al. studied the therapeutic potential of galanin-like peptide (GALP) for treating obesity and related conditions. They demonstrated that GALP reaches the hypothalamus and olfactory bulb (18). Another study used recombinant human nerve growth factor (rhNGF) for the treatment of Alzheimer's disease. It was administered to rats as nose drops and the results showed that rhNGF reached the olfactory bulb within an hour (19). Moreover, Ross et al. in 2004 administered IFN-β1b to rats and found that it reached the brain and phosphorylated an IFN receptor tyrosine in the CNS (20). The following are other examples where plasmids were delivered to the CNS by Int-Adm: a) the plasmid pCMVβ (7.2 kb) encoding the *β-galactosidase* gene was detected in the brain 15 min after its administration (21) and b) the plasmids pCMVβ (7.2 kb) and pN2/pCMVβ (14.2 kb), which both encode the *β-galactosidase* gene, were detected in the olfactory bulb, septal area, striatum, hypothalamus, thalamus, midbrain, hippocampus and cerebellum 15 min after their administration (22). We have previously reported pCI-neo (5.4 kb) as a DNA vaccine (pGQH) against rabies. Seventy-two hours after its intranasal administration to mice, pGQH and glycoprotein mRNA were detected in the medium and posterior brain areas (23). Here, we investigated whether a naked plasmid, the plasmid (pIRES-hrGFP-1a), could cross the blood-brain barrier, reach brain cells and express green fluorescent protein after intranasal administration at 24 h. It would be interesting to determine whether this vector could be applied for future gene therapy studies.

Materials and Methods

pIRES-hrGFP-1a and Plasmid Purification

The plasmid pIRES-hrGFP-1a contains a multiple cloning site (MCS) that is followed by the internal ribosome entry site (IRES) and linked to the sequence encoding humanized recombinant (hrGFP). Expression of the bicistronic transcript is induced by the cytomegalovirus (CMV) promoter, which is located upstream of the MCS. The SV40 polyadenylation signals downstream of hrGFP direct proper processing of the 3′-end of the mRNA (Vitality hrGFP Mammalian Expression Vectors, Agilent Technologies, Inc. La Jolla, California, USA). The design allows the expression of the gene of interest to be monitored at the cellular level due to the expression of hrGFP. Moreover, it allows the expression and independent translation of two genes as the same transcript. Plasmids were grown in *Escherichia coli* (DH5α). Bacteria were cultivated in Luria Bertani (LB) medium (1% tryptone, 0.5% yeast extract, and 1% NaCl), and 100 μg/mL of ampicillin was added to 2.5 l of LB medium. After 24 h of growth, the plasmid was purified using the commercial System Endo-free Plasmid Giga Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Animals

Thirty-six mice (female, Balb/c, 8−10 weeks old) were randomly divided in 2 groups. Group A, which included a total of 18 mice and was the experimental group, was distributed into three subgroups with six mice in each cluster (E1, E2 and E3). Group B, which included 18 mice and was the control group, was distributed into three subgroups with six mice in each group (C1, C2 and C3). Mice were housed in filter-top cages and provided with sterile food and water *ad libitum* in the animal facility at the Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social (IMSS). This work was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (Mexican Official Law, NOM-062-ZOO-1999). The scientific research was approved by the Committee on Ethics of Animal Experiments at IMSS (Authorization number R-2011-785-041).

The experimental mice groups received 25 μg of the plasmid pIRES-hrGFP-1a diluted in 25 μl of phosphate buffer solution (PBS). The control groups received 25 μl of PBS. Plasmids were given intranasally by applying little drops in both nostrils. Twenty-four hours later, the mice were sacrificed, and their brains were removed. Mice from