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Ophthalmic administration of a DNA plasmid harboring the murine Tph2 gene: evidence of recombinant Tph2-FLAG and serotonin levels increased in brain structures

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Abstract

Tryptophan hydroxylase-type 2 (Tph2) is the first, rate-limiting step in the biosynthesis of serotonin (5-HT) in the brain. The ophthalmic-administration (Op-Ad) is a non-invasive method that allows delivering genetic vehicles through the eye and reach the brain. In this work, the murine Tph-2 gene was cloned in a non-viral vector (pIRES-hrGFP- 1a), generating pIRES-hrGFP-1a-Tph2, plus the Tag- FLAG. Recombinant Tph2-FLAG was detected and tested in vitro and in vivo, where 25µg of pIRES-hrGFP-1a-Tph2- FLAG was Op-Ad to mice. The construct was capable of expressing and producing the recombinant Tph2-FLAG. Assays in vivo showed that the construct efficiently crossed the Hemato-Ocular-Barrier and the Blood-Brain-Barrier, reaching brain cells, passed the optical nerves, and transcribed mRNA-Tph2-FLAG in different brain areas. The recombinant Tph2-FLAG was observed in amygdala and brainstem, mainly in raphe dorsal and medial. Relative Tph2 expression of three-fold over basal level was recorded three days after Op-Ad. Besides, we have evidence that recombinant Tph2-FLAG was functional during the 5-HT biosynthesis; this was observed in vitro and in vivo. The 5HT levels were increased in HEK293 cells transfected (6.02ng/mL) vs control (2.87ng/mL) at 48 h after transfection. Moreover, in mice showed an increase with respect to the control: in the amygdala (325ng/mL//125ng/mL) and in the hypothalamus (104ng/mL//68ng/mL), respectively. These results demonstrated that IRES-hrGFP-Tph2-FLAG, administrated through the eyes was capable of reaching the brain, transcribing, and translating an exogenous Tph2 gene. In consequence the 5HT was increased in amygdala and hypothalamus at seven days after treatment. In conclusion, this study showed the feasibility of delivering therapeutic genes, such as the Tph2, the first enzyme, rate- limiting step in the 5-HT biosynthesis. Besides, these results support the possibility to use this innovative idea with therapeutic purposes and could be a strategy for those patients that possess risk TPH2 SNPs associated with depression disorder, and suicidality.

Biography:

Emiliano Tesoro-Cruz works for Immunology and Infectology research unit at the National Medical Center "La Raza", IMSS, Mexico. He has experience in the field of virology and participated in the development of a DNA vaccine against rabies by intranasal administration using animal models such as mice, rabbits, dogs and cats. Currently, his research aim is in the field of depression from a basic point of view, as well as clinic research. Specifically, in basic research, he proposes a gene therapy for depression, using non-viral vectors administered ocular and intranasally in animal models. In the field of clinical research, he is studying different TPH2 gene SNPs, which some of them have been reported with association for depression and suicide attempt. The TPH2 gene codes for the tryptophan hydroxylase type 2, the key enzyme for the brain serotonin biosynthesis.

Speaker Publications:

1. Oviedo N, Ortiz-Borrayo L, Hernández-Sánchez J, Jiménez-Badillo S E, Tesoro-Cruz E, Moreno-Navor E and Aguirre-Alvarado C (2018) Human CATSPER1 promoter is regulated by CREB1 and CREMτ transcriptional factors in vitro. Archives of Medical Research 49(3):135-46.

2. Oviedo N, Manuel-Apolinar L, Orozco-Suárez S, Juárez-Cedillo T, Bekker Méndez V C and Tesoro-Cruz E (2017) Intranasal Administration of a Naked Plasmid Reached Brain Cells and Expressed Green Fluorescent Protein, a Candidate for Future Gene Therapy Studies. Archives of Medical Research 48(7):616- 622.

3. Minerva Mata-Rocha, Edith Alvarado-Cuevas, Javier Hernandez-Sanchez, Doris Cerecedo and Ricardo Felix, Adriana Hernandez-Reyes, Emiliano Tesoro-Cruz and Norma Oviedo (2013) Molecular cloning and analysis of the catsper1 gene promoter. Molecular Human Reproduction. Molecular Human Reproduction 19(5):336-347.

4.I Feria-Romero, K Chávez-Rueda, S Orozco-Suárez, F Blanco-Favela, F Calzada-Bermejo, L Chávez-Sánchez, L Manuel-Apolinar, R Hernández- González, Á Aguilar-Setién and E Tesoro-Cruz (2011) Intranasal anti-rabies DNA immunization promotes a Th1- related cytokine stimulation associated with plasmid survival time. Archives of Medical Research 42(7):563-571.

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