SAFE AND LONG-LASTING TRANSGENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM USING HERPES SIMPLEX VIRUS-1AMPLICON VECTORS

Silvia Zucchini[1], Marie Soukupova[1], Pascal Trempat[2], Coline Perrier-Biollay[2], Selene Ingusci[1], Hervè Berthommé[2], Michele Simonato[1]

[1]Department of Medical Science, Section of Pharmacology, Neuroscience Center, University of Ferrara, National Institute of Neuroscience and Laboratory of Technologies for Advanced Therapy (LTTA), Technopole of Ferrara, Ferrara - Italy, [2]Bioviron, Université Claude Bernard Lyon 1, Villeurbanne - France

Introduction: Disorders affecting the central nervous system (CNS) entail important unmet medical needs. Their pathogenic mechanisms are not yet completely understood, and therapeutic approaches, when available, are not satisfactorily effective and safe. A promising strategy may be gene therapy, that allows the delivery of therapeutic genes into defined target cells. In this direction, one major hurdle to overcame is to find an efficient tool for gene transfer. Among different types of viral and non-viral vectors that can be used to vehicle therapeutic genes into specific brain areas or cells, HSV-1based amplicon vectors (viral particles containing concatameric repetitions of an expression cassette) may represent an interesting option. The aim of this study was to characterize different amplicon vectors designed with a set of diverse promoters driving two reporter genes. These vectors have been tested in a longitudinal study *in vivo* to evaluate target cells, duration of transgene expression and safety.

Materials and methods: Amplicon vectors have been generated with expression cassettes driven by different promoters: the ubiquitous chicken beta-actin enhanced promoter (ECBA), the neuronal tissue-specific synapsin enhanced promoter (ESYN) and the viral promoter IE4/5. The expression cassette contained two reporter genes: luciferase and GFP. All amplicon vectors have been stereotaxically injected into the right dorsal hippocampus of mice at two viral titers. Animals were monitored at different time points, from 1day to 6months after injection, evaluating the bioluminescence level (BL) induced by luciferase expression, acquired using the IVIS Lumina *in vivo* imaging system. Moreover, immunohistochemistry analysis has been performed on brain sections prepared from injected mice sacrificed at different time points.

Results: All tested amplicon vectors were able to efficiently infect neuronal cells and transgene expression lasted differently depending on the promoter. When injected at the same (low) titer, amplicons containing the ESYN or ECBA promoters expressed the luciferase transgene for 6months with similar kinetics: the BL peaked at the 1day and decreased dramatically over 4weeks, subsequently maintaining low but stable levels up to 6months. On the other hand, the BL in mice injected with IE4/5amplicon reached high levels at 1dpi to rapidly diminish and totally disappear at 7days. These effects were dose-dependent, increasing with an increase in vector titer. Using immunohistochemistry, we were able to detect many GFP-positive cells at the level of principal cells in the injected hippocampus, while no infected cell was observed in the contralateral hippocampus, indicating that the vectors lose their ability to be retrogradely transported. Double immunofluorescence demonstrated that transgene expressing cells were almost exclusively neurons. Moreover, by using hematoxylin-eosin and Fluoro Jade C staining we observed lack of immune cell infiltration and no ongoing neurodegeneration.

Discussion and conclusions: The amplicon vectors employed in this study may be useful and safe for gene therapy. The time-course of transgene expression depends on the promoter; IE4/5may be used for short-term gene therapy, whereas ESYN or ECBA may be used for long-term treatments. In conclusion, these tools represent a promising system for the delivery of therapeutic genes for the treatment of neurological diseases such as epilepsy, Parkinson's disease or Alzheimer's disease.