Fundação Bial Grant 15/04

"Pain control from the brain: gene therapy in the treatment of chronic pain control"

FINAL REPORT

The main goal of the project was to develop strategies that will induce intense and sustained analgesia for chronic pain control through the manipulation of pain control centres in the brain using gene therapy strategies. Our gene therapy assays focused in a main component of the endogenous supraspinal pain modulatory system: the dorsal reticular nucleus (DRt). It is an unique area since it is pain facilitatory region easily accessed under stereotaxic control which made us reason that it is possible to inhibit this region through local injections of suitable viral vectors, which were based on replication-defective Herpes Simplex type 1 (HSV-1). Several studies were performed in order to define the transgenes and promoters more suitable in the vector construction: ***i)*** identification of neurotransmitters produced by brain neurons transduced upon HSV-1 injections in the DRt, ***ii)*** behavioural evaluation of opioidergic manipulation of the DRt during acute and chronic pain conditions; ***iii)*** analysis of the neurochemical changes induced by chronic pain at the DRt and ***iv)*** evaluation of the effects of chronic pain installation in the pronociceptive effects of the DRt.

I n summary, the studies showed that ***i)*** the neurons transduced upon the injection of a HSV-1 reporter virus in the DRt were opioidergic (enkephalin-immunoreactive) and noradrenergic (tyrosine hydroxylase (TH)-immunoreactive); ***ii)*** the effects of local delivery at the DRt of opioid agonists were opposite in acute and chronic pain; ***iii)*** chronic pain induced a depression in the expression of opioid peptides and their receptors at the DRt and ***iv)*** the DRt maintain its pronociceptive effects during chronic pain.

Based on the main results referred above we constructed viral vectors based on HSV-1 and which overexpressed pre-proenkephalin (PPE) at the DRt or were directed to transducer only the noradrenergic afferents of the DRt. In the first case, we used a vector in which the overexpression of PPE is controlled by the human cytomegalovirus promoter. The results showed that the viral vector induced inhibition of acute pain that lasted for 4 days whereas in chronic pain the vector induced hyperalgesia. This was probably associated to the changes of the opioidergic system during chronic pain which were referred above and suggest that gene therapy strategies should overexpress the missing receptors. The HSV-1 vector directed to transducer only the noradrenergic afferents of the DRt contained a TH transgene under the control of the TH promoter. The transgene was inserted in antisense sequence to decrease the release of noradrenaline at the DRt, which was based on previous studies with pharmacological manipulation of the nucleus. The results show that the vector induced analgesia in a chronic pain model during 21 days. This was associated to a decrease of noradrenaline release at the DRt and was reversed by local administration of a $\alpha 1$-adrenoreceptor agonist (phenylephrine).

One important achievement of the project was also to be have a PhD student fully trained in the construction of viral vectors that will allow the studies to continue in the future without depending on the collaboration of foreign labs. The perspectives deriving from the present project are to use viral vectors that may correct the neurochemical changes induced by chronic pain at the supraspinal pain modulatory system

INDICATORS OF SCIENTIFIC ACHIEVEMENT

1) Publications

1. Pinto M, Lima D & Tavares I. (2007). Neuronal activation at the spinal cord and medullary pain control centres after brief stimulation of an inflamed joint: a c-*fos* study in the monoarthritic rat. *Neuroscience*, **147**:1076-1089.
2. Tavares I & Lima D (2007) From neuroanatomy to gene therapy: searching for new ways to manipulate the supraspinal endogenous pain modulatory system. *J. Anatomy*, **211**: 261-268.
3. Martins I, Araújo S, Wilson SP, Lima D & Tavares I. (2007). Neuropathic pain is attenuated by a viral vector targeting noradrenergic input to the dorsal reticular nucleus. *Eur. J. Pain*, **11**: S125.
4. Pinto M, Lima D.& Tavares I (2006). Correlation of noxious evoked c-fos expression in areas of the somatosensory system during chronic pain: involvement of spino-medullary and intra-medullary connections. *Neurosci. Lett.*, 409: 100-105.
5. Lima D, Castro AR & Tavares I. (2006). Descending modulation of secondary hyperalgesia in the monoarthritic rat: role of antinociceptive and pronociceptive medullary centres in the differential nociceptive activation of NK1- and $GABA_B$-expressing spinal cord neurons. *Program N° 248.13/R11. 2006 Neuroscience Meeting Planner, Atlanta, GA: Society for Neuroscience, 2006.*
6. Pinto M, Tschudy F, Sousa M, Wilson SP, Lima D & Tavares I . (2006). Opioid-mediated control of pain modulation from the medullary dorsal reticular nucleus: a gene therapy and pharmacological study in the monoarthritic rat. *Program N° 249.21/S24. 2006 Neuroscience Meeting Planner, Atlanta, GA: Society for Neuroscience, 2006.*
7. Martins I, Pinto M, Pereira S, Wilson SP, Lima D & Tavares I. (2005). Gene therapy for chronic pain control: perspectives of HSV-1 mediated gene transfer to supraspinal centres. *Molecular Therapy*, vol.11, Suppl. 1, S63.
8. Martins I, Pinto M, Tavares-Marques A, Pereira S, Wilson SP, Lima D & Tavares I. (2005). Gene therapy for pain control from supraspinal areas: perspectives of directing transgene expression to noradrenergic pathways. *Abstracts of the 11th World Congress on Pain*, IASP Press, Seattle, pp. 306.

9. Martins I, Pinto M, Wilson SP, Lima D & Tavares I. (2005). Gene therapy for pain control by manipulation of supraspinal areas: what did we learn from the studies with replication defective forms of HSV-1? *Sinapse*, 5 (1): 105.
10. Pinto M, Lima D & Tavares I. (2005). C-*fos* expression in the spinal cord of monoarthritic rats induced by brief mobilization of the inflamed paw. *Sinapse*, 5 (1): 109.
11. Pinto M, Lima D & Tavares I. (2005). Nociceptive activation of neurons of in lamina I and supraspinal modulatory nuclei in response to brief stimulation of a chronically inflamed joint. *Program Nº 623.10. 2005 Abstract Viewer/Itenerary Planner. Washington, DC: Society for Neuroscience, 2005. Online.*

2) Comunications

1. Castro AR, Lima D & Tavares I. (2007). Role of antinociceptive and pronociceptive supraspinal centres in the differential nociceptive activation of NK1 and $GABA_B$-expressing spinal neurons during secondary hyperalgesia. *10th Meeting of the Portuguese Society for Neurosciences*, Ofir.
2. Martins I, Araújo S, Wilson SP, Lima D & Tavares I. (2007). Attenuation of neuropathic pain by a viral vector targeting noaradrenergic input to the dorsal reticular nucleus. *10th Meeting of the Portuguese Society for Neurosciences*, Ofir.
3. Pinto M, Tschudy F, Sousa M, Wilson S. Lima D & Tavares I. (2007). Gene and pharmacological manipulation of opioid medullary dorsal reticular neurons in the monoarthritic rat. *10th Meeting of the Portuguese Society for Neurosciences*, Ofir.
4. Pinto M., Tschudy F, Sousa M, Lima D & Tavares I. Opioidergic control of pain modulation from the medullary dorsal reticular nucleus during the course of monoarthritis: a pharmacological and gene therapy study. *PENS Summer School 'The brain as a target for inflammatory processes*", Berlim, 2006.
5. Tavares I , Castro AR & Lima D. Descending modulation of nociceptive activation of spinal cord neurons expressing NK1 or $GABA_B$ receptors during chronic pain. *Joint Meeting of the Anatomical Society of Great Britain and Ireland and the Spanish Anatomical Society*, Madrid, 2006.
6. Pinto M., Tschudy F, Sousa M, Lima D & Tavares I. Manipulation of opioidergic systems in the brain with gene therapy and pharmacological approaches. *XLI Congresso da Sociedade Portuguesa de Microscopia*, Braga, 2006.

7. Martins I, Pinto M, Wilson SP, Lima D & Tavares I. (2005). Migration of HSV-1 from pain control areas of the caudal medulla oblongata and neurochemical characterization of transfected neurons. *25th European Winter Conference on Brain Research*, Les Arcs.
8. Martins I, Pinto M, Wilson SP, Lima D & Tavares I. (2005). Gene therapy for chronic pain control by targeting brainstem regions devoted to pain modulation. *Gene and Cell Therapy, Institut Pasteur EurΩConferences & Inserm*, Paris.

C) **Lectures**

Tavares I. (2005). *The « whys » and « hows » of gene therapy at the nervous system. 25th European Winter Conference on Brain Research*, Les Arcs.

D) **Organization of scientific sessions**

Tavares I. & Lima D. (2005). "*Gene therapy at the nervous system: the present and the future*". *25th European Winter Conference on Brain Research*, Les Arcs.