

DEVELOPMENT OF A PROTOCOL FOR A RAT SPINAL CORD PROTEOME

**Felix Gil¹, Gerardo Avila², Veronica M. Darde³, Tatiana Martin-Rojas¹,
F. Vivanco^{4,5}, Julian S. Taylor², Maria G. Barderas¹**

¹Laboratorio de Fisiopatología Vascular. Hospital Nal. Parapléjicos. SESCAM. Toledo. Spain;

²Laboratorio de Función Sensitivo Motora;

³Unidad de Proteómica. Hospital Nacional de Parapléjicos. Toledo;

⁵Servicio de Inmunología. Fundación Jiménez Díaz. Madrid;

⁶Departamento de Bioquímica y Biología Molecular I. Universidad Complutense de Madrid.

Introduction: Several proteomic techniques have been developed for neurodegenerative and psychiatric disorder research, and in particular in the detection of differences between healthy individuals and patients suffering from such diseases¹. Although brain and cerebrospinal fluid samples from patients with different central nervous system (CNS) disorders have been extensively studied, less research has been focused on spinal cord protein content and the changes induced after injury and the presence of associated symptoms such as neuropathic pain. Indeed spinal cord injury is characterized by a complex relationship between several molecular cascades and the development of a progressive pathophysiology².

In the present study we aimed to describe total protein content in the spinal cord of healthy rats, employing different proteomics tools. With this objective we have developed a new sequential protocol for protein extraction from rat spinal cords.

Methods: Spinal cords were extracted from 10 week old male rats injected with an overdose of Pentobarbital. The tissue was homogenized in two consecutive protein extraction buffers; the first that most of the soluble proteins and the second the more insoluble proteins. Finally the samples were analyzed using 2DE and LC-MS/MS.

Results: This work presents a very simple, fast and efficient method for spinal cord protein extraction. Approximately 400 to 600 spots were resolved in the 2DE experiments and the LC-MS/MS analysis detected hundred of proteins that complement the 2DE results.

The application of new proteomics protocols to the spinal cord may be a useful tool for the study and identification of differential molecular changes associated with CNS pathologies such as spinal injury and/or neuropathic pain. Altogether, these data could contribute to detect specific markers or potential drug targets for spinal cord injury and neuropathic pain management.