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Diabetes and Obesity

COMBINATORIAL GENE THERAPY STRATEGY TO TARGET OXIDATIVE AND ENDOPLASMIC RETICULUM STRESSES IN TYPE 2 DIABETIC NEUROPATHY

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1. Project summary

Objectives

The main objective of the project is to advance in the knowledge of the molecular mechanisms that lead to the development of type 2 diabetic neuropathy and with special emphasis on the participation of endoplasmic reticulum stress and oxidative stress. The ultimate goal is to design a combined therapeutic strategy with gene therapy tools that could lead to a specific and efficient treatment for this complication of diabetes.

This general objective is divided into the following specific objectives:

1- Functional characterization of diabetic neuropathy in animals with type 2 diabetes.

2- Investigate the molecular mechanisms that lead to the development of diabetic neuropathy by using a proteomic strategy in the peripheral sciatic nerve and in the dorsal root ganglia of type 2 diabetes mice.

3- Validate the changes in biomarkers related to endoplasmic reticulum and oxidative stress in vitro and in vivo in short- and long-term diabetes.

4- Promote antioxidant, anti-inflammatory and neuroprotective treatment in diabetic mice through gene therapy using key proteins related to oxidative and / or reticulum stress such as Klotho or BiP. To study the therapeutic potential of the specific neuronal overexpression of these factors through the intrathecal administration of AAV vectors.

Design and Methodology

We proposed to use the DbDb mouse model, which bears a mutation in the leptin receptor and presents the typical characteristics of type 2 diabetes. Diabetic neuropathy in these animals will be monitored through functional studies such as motor and sensory nerve conduction tests and through studies of pain and temperature sensitivity as well as sweating function. In addition, histological analysis will allow the quantification of intraepidermal innervation and sweat glands. Molecular analyses will be performed to study the cellular signaling pathways affected, such as reticulum or oxidative stress. Morphometric quantification of the tibial nerves of diabetic animals

and controls will allow us to determine the level of myelination and / or axonal degeneration of these mice.

To further investigate the molecular mechanisms that lead to the development of diabetic neuropathy, a proteomic study is proposed to characterize in more detail the UPR response and oxidative stress in sciatic nerve and dorsal root ganglia.
These results will be validated by quantitative PCR and Western blot analysis in cell lines of Schwann cells or sensory neurons and in tissues of the peripheral nervous system of diabetic mice to identify the best therapeutic target.

Finally, we propose a gene therapy strategy for diabetic neuropathy using AAV vectors combining action against oxidative and endoplasmic reticulum stresses.

Work plan

- Functional characterization of diabetic neuropathy in animals with type 2 diabetes to determine the appropriate age for proteomics studies.
- Study of proteomics in the sciatic nerve and in the dorsal root ganglia of mice with type 2 diabetes.
- Validation of proteomic results in vitro, in Schwann and dorsal root ganglia cell lines, and in vivo in sciatic nerve and DRG at different times of diabetes.
- Gene therapy through the intrathecal administration of AAV vectors to allow neuronal overexpression of key proteins with antioxidant, anti-inflammatory and neuroprotective effects.

2. Results obtained

Functional characterization of diabetic neuropathy in animals with type 2 diabetes to determine the appropriate age for proteomics studies.
First of all, we set out to determine the onset of functional changes due to diabetic neuropathy in the peripheral nervous system of the mouse model with type 2 diabetes, to establish the best age to detect changes in proteomic studies, and also to set up the basis for the most relevant functional, biochemical and molecular functions to be studied after the treatment with the therapeutic vectors.

Functional studies such as algesimetry to detect pain sensitivity, electrophysiological studies to assess nerve conduction capacity, and sweating tests which indicate the degree of innervation of the sweat glands, were performed. Immunohistochemical analyzes allow us to quantify the number of intraepidermal nerve fibers and finally, the morphometric study of the tibial nerves to determine the level of myelination and / or axonal degeneration of these mice.

Altogether, the exhaustive study of the different factors involved in the characterization of diabetic neuropathy indicates that at 13-15 weeks of diabetes the DbDb mice already present the pathology, so we decided to choose this age to begin the proteomic studies. On the other hand, the high variability between groups of animals showed that we would always need a control group from the untreated animal model to compare the gene therapy data.

Study of proteomics in the sciatic nerve and in the dorsal root ganglia of mice with type 2 diabetes.

We performed independent proteomic studies of two tissues directly involved in diabetic neuropathy, the dorsal root ganglia (DRG) and the sciatic nerve, using tandem mass tag (TMT) and liquid chromatography with mass spectrometry analysis (LC / MS / MS) to better understand the mode of action of the disease and elucidate new targets for the development of advanced therapeutic strategies. The study was carried out comparing diabetic animals at 13-15 weeks of diabetes with their controls, an age that corresponds to the beginning of the functional changes indicative of diabetic neuropathy in the murine model. Proteomic analysis has allowed us to obtain functional clusters using different algorithms concluding that the peripheral nervous system of mice with type 2 diabetes shows a dysregulation of glucose and lipid metabolism; structural protein abnormalities, including cytoskeletal-related proteins; a dysregulation of molecular chaperones, related to protein folding which could cause an increase in reticulum stress; protein alterations related to oxidative stress such as glutathione or peroxyredoxins; and an increase in inflammatory and acute-phase response proteins, which could provide protection against inflammation and oxidative stress. Some pathways have been found differentially modified in DRG and sciatic nerve, such as the cellular translation machinery, directly related to the energy depletion observed in DRG. In DRG we have also seen a greater increase in antioxidant enzymes, which suggests that sensory neurons would experience a more important

response to stress than the sciatic nerve. One of the most altered proteins we have found is MUP, which is extremely diminished in these tissues and this reduction correlates with the progression of diabetes. MUP is located in the extracellular matrix and could be related to its degradation, present in other inflammatory and neurological diseases, so it would most likely point to a new function of this protein. We are currently continuing our studies in this regard.

Validation of proteomic results in vitro, in Schwann and dorsal root ganglia cell lines, and in vivo in sciatic nerve and DRG at different times of diabetes.
We conducted studies on Schwann cell and dorsal root ganglia cell lines to confirm that proteins altered in proteomic studies were also modified at the cellular level. We also thoroughly studied ER and oxidative stress in these lines under hyperglycemic and hyperlipidemic conditions, confirming our hypothesis. In vivo, in sciatic nerves and DRG of mice with type 2 diabetes, the results are less clear, but they also tend to suggest an increase in the alteration of these two pathways as the time of diabetes lengthens and neuropathy appears. In in vivo studies, we have been able to confirm the proteomics data for several altered proteins, validating the information obtained and the molecular mechanisms of development of the neuropathy, as explained in the previous section.

Gene therapy through the intrathecal administration of AAV vectors to allow neuronal overexpression of key proteins with antioxidant, anti-inflammatory and neuroprotective effects.
First, we selected the AAVrh10 serotype from among several AAV serotypes tested intrathecally and intravenously as the most efficient to transduce the peripheral nervous system. We administered AAV vectors overexpressing the protein BiP, a reticulum stress-related chaperone, and Klotho, a protective protein against oxidative stress, intrathecally in the diabetes model, but unfortunately we saw no changes in the development and progression of diabetic neuropathy.

For this reason, we did not perform the double therapy with BiP and Klotho, as we had proposed, but we replaced it with another molecule, neuregulin 1 (Nrg1) that we had previously used successfully in a model of amyotrophic lateral sclerosis, the SOD1G93A transgenic mouse. Nrg1 is a protein involved in protection against oxidative and ER stress, apart from being neuroprotective and stimulating myelination, both in the

central and peripheral nervous systems, through the induction of transcription factors involved in myelination. The overexpression of Nrg1 type III by AAVrh10 vectors in diabetic mice made it possible to ameliorate diabetic neuropathy. Electrophysiological studies showed an improvement in motor and sensory conduction tests compared to untreated diabetic animals, and correlated with a decrease in the number of degenerated axons and myelinating fibers of greater diameter. Furthermore, recovery of the expression of Nrg1-III and its ErbB receptor led to normalized cell signaling and restored levels of myelin proteins and myelinating transcription factors.

3. Relevance for possible future implications

Diabetes mellitus has become one of the most serious health problems of our time. Estimates indicate that by 2030 its prevalence will reach epidemic proportions and will affect 439 million people worldwide. Currently it already affects more than 6.5% of the world population. This will be an unprecedented challenge for the next generations, with profound social, economic and health impacts. Diabetes complications account for the greatest volume of disability, decreased life expectancy, and economic costs related to diabetes. The most common and debilitating complication of diabetes is diabetic neuropathy, which affects both sensorimotor and autonomic components of the peripheral nervous system and is developed by between 60 and 70% of patients, being the complication of diabetes that causes the majority of patients. hospitalizations. Sensorimotor polyneuropathy that develops in diabetic patients is the most common peripheral neuropathy and can affect all types of nerve fibers. Abnormal sensory perception in diabetic patients includes loss of pain and temperature sensation, as well as burning sensation and skin hyperesthesia, initially affecting the feet and lower legs and later the hands and arms. In more advanced stages, foot ulcers and neuropathic deformity appear, which ultimately cause 40% of non-traumatic limb amputations.

The results of our project helped us to gain a better understanding of the disease to elucidate new targets and design advanced therapeutic strategies, based on the mechanism of action of the disease. We are still studying the role of MUP in the central nervous system, but the results of gene therapy with Nrg1-III in the mouse model with diabetic neuropathy are encouraging, although further studies are still needed before reaching the clinic.

4. Scientific bibliography generated

Publications

M Leal-Julià, J Vilches, A Onieva, S Verdés, A Sánchez, M Chillon, X Navarro, A Bosch.
Proteomic quantitative study of dorsal root ganglia and sciatic nerve in type 2 diabetic mice.
Under review in *Diabetes*.

Kagiava A, Richter J, Leal-Julià M, Tryfonos C, Sargiannidou I, Christodoulou C, Bosch A, Kleopa KA.
Efficacy of AAV Serotypes to target Schwann cells after intrathecal and intravenous delivery.
Submitted to *Molecular Therapy Methods and Clinical Development*.

Ariza L, Leal-Julià M, García-Lareu B, Sanchez A, Pagès G, Homs J, Carretero A, Ruberte J, Chillón M, Corfas G, Navarro X, Bosch A.
Downregulation of Nrg1 type III signaling is an early event in murine diabetic neuropathy.
Submitted to *Neurobiology of Disease*.

Leiva-Rodriguez T, Romeo-Guitart D, Herrando-Grebulosa M, Muñoz-Guardiola P, Polo M, Bañuls C, Petegnief V, Bosch A, Lizcano JM, Apostolova N, Forés J, Casas C.
GRP78/BiP overexpression triggers PINKK1/IP3R-mediated neuroprotective mitophagy.
Experimental Neurology, in press.

Congress communications

Leal-Julia M, Pagès G, Casas C, Chillón M, Bosch A
Characterizing in vitro models of type 2 diabetic peripheral neuropathy
Poster
2017 Peripheral Nerve Society Annual Meeting
Publication: Journal of Peripheral Nervous System
Place: Sitges, Barcelona Date: July 2017

Leal-Julià M, Vilches J, Navarro X, Chillon M, Bosch A

Proteomic quantitative study of dorsal root ganglia and sciatic nerve in type 2 diabetic mice

Poster

XI Simposio de Neurobiología, Societat Catalana de Biologia

Place: Barcelona Date: November 2018

PhD Thesis

Marc Leal Julià: *Proteomic study of dorsal root ganglia and sciatic nerve in type 2 diabetic mice.*

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