CHARACTERIZATION OF NOVEL SUPPRESSORS OF NEURONAL TOXICITY IN MYOTONIC DYSTROPHY TYPE 1

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1. Summary of the project

Although the majority of myotonic dystrophy (DM1) research to date has focused on the muscular aspects of the disease, DM1 patients and families report that central nervous system (CNS) related problems associated with this disease are usually the most worrisome and difficult to deal with. In this project the neurobiological substrates of DM1 were better characterized using new animal models to identify therapeutic targets developed with innovative methodologies for drug discovery. For this purpose we have evaluated candidate compounds, identified after detection in Drosophila, in a recently described mouse model for DM1: a knockout of the Mbnl2 gene. These animals showed central brain disorders of DM1 suggesting that the main characteristics of the brain DM1 are attributable to the expression of toxic RNA and the sequestration of MBNL2. Behavioural, neurochemical and pharmacological studies were used to evaluate the therapeutic potential of known compounds in Mbnl2 knockout mice of medium and advanced age. Parallel to the evaluation of compounds, we advanced in the establishment of correlations between the different animal models of DM1 (mice and flies) to determine useful therapeutic objectives for the different DM1 brain disorders. Finally, the demonstrated utility of the DM1 fly model based on the CNS for successful chemical molecule screening was used to increase the panel of candidates to be tested in the mouse model, focusing on a specific evaluation of compounds with the ability to cross the blood-brain barrier. The results obtained in this project allowed the identification of 2 compounds: methylphenidate and mirtazapine to alleviate the progressive neuropsychological deficits and the neurodegeneration observed in animal models of DM1.

2. Results

1. The mouse model deficient in the Mbnl2 gene (Mbnl2 KO) is relevant for the CNS alterations associated with DM1. Specifically, young mice (males and females) that lack this gene show dysregulation of circadian activity, depressive symptoms and cognitive deficits. However, in male mice, these alterations appear to be more specific, since they are not related to changes in locomotor activity. In addition, we found that the lack of function of the Mbnl2 gene induced progressive cognitive and affective alterations in mice that were associated with hyperactivation of the dopaminergic

system in the medial prefrontal cortex, including an increase in extracellular dopamine levels, increased expression of the genes of *Dat, Drd1 and Drd2*, and deep disturbances in neural activity and microgliosis in the medial prefrontal cortex and the hippocampus.

2. Different Drosophila lines were successfully generated, where they sought to mimic a central aspect of DM1: the expression of toxic repeats (CTG expansion) and recreating the lack of endogenous muscleblind function (silencing of Mbl). These aspects were selectively induced in different types of neurons to observe their effect both at the cellular and functional levels. The different Drosophila generated lines are relevant for alterations in CNS in patients with DM1. In particular, different functional phenotypes (flight, survival, climbing) showed in most cases a worsening confirming a strong implication of the toxicity of the expression of a CTG expansion or the interference of Mbl in functional aspects controlled by the brain. Interestingly, studies at the cellular, histochemical and metabolomic level of the expression of CTG expansions in Drosophila dopaminergic neurons reproduced to a large extent the process of neurodegeneration and cerebral atrophy presented by patients.

3. Various biomarkers related to neurotoxicity and neurodegeneration in Drosophila were successfully determined. Specifically, the expression of CTG expansions or silencing of Mbl in dopaminergic neurons causes a significant deregulation of energy metabolism: the levels of ATP and glucose are significantly reduced, as has been detected by positron emission tomography in the temporal and frontal lobes of patients with DM1. Interesting additional markers have been identified that are involved in processes of inflammation and oxidative stress, as well as specific degenerative processes and alterations in neurotransmission (low levels) that not only affect the dopaminergic system.

4. In order to find new therapeutic targets a high throughput screening in vivo of drugs contained 3805 Pfizer, Prestwick, GreenPharma Otava and SPO Life Chemicals libraries using a phenotype in Drosophila caused pupal death was performed by the expression of CTG expansions. There were 61 hits (1.6% of compounds) that offered a significant increase in the birth of adult flies. A global analysis of the results obtained after screening in flies showed that an important percentage of the hits (18%) have to do with modulating the activity of the main groups of neurotransmitters: glutamate,

GABA, dopamine and serotonin. This allowed the identification and validation of 2 compounds: methylphenidate and mirtazapine as potentially useful to alleviate the progressive neuropsychological deficits and neurodegeneration observed in animal models of DM1.

5. Behavioural, biochemical and molecular studies in mice showed that Mbnl2 KO chronic treatment with methylphenidate reverses behavioural deficits, changes in the expression of genes Dat and Drd2 specifically, and increased expression of proinflammatory microglia in the prefrontal cortex present in this model of CNS alterations in DM1.

6. In order to create an animal model of neurodegeneration in specific areas related to behavioural disturbances (frontal cortex and hippocampus), a new line of conditional knockout mouse lacking the gene Mbnl2 selectively in glutamatergic forebrain neurons was developed (NEX-cKO). This new mouse model recapitulates the cognitive and affective alterations observed in the pathology of DM1. In addition, these mice show neurodegeneration and proliferation of the microglia in the sub-granular area of the dentate gyrus, and a deregulation of the mRNA of the H1-type histaminergic and serotonergic type 1A receptors in the hippocampus.

7. The low dose of an atypical antipsychotic, mirtazapine could reverse the cognitive and affective alterations observed in NEX-CKO mice to prevent neurodegeneration and proliferation of microglia in the granular subzone of the dentate gyrus, where neurogenesis takes place. Mirtazapine also reversed the deregulation of the mRNA of histaminergic type H1 and serotonergic type 1A receptors in the hippocampus.

3. Relevance with possible future implications

At present, there is no effective treatment for the neuropsychological disorders associated with DM1. The process of searching for new therapeutic candidates has a very powerful tool in the use of models for DM1 in Drosophila melanogaster and in mice deficient in the Mbnl2 gene that specifically recapitulate the CNS alterations associated with DM1. Specifically, we have identified that an important target is

dopaminergic neurons and known drugs for repositioning in DM1 have been proposed, which will be explored in collaboration with the industry in this sector, for example the company SOM Biotech. In addition, the project has provided two new pharmacological objectives: methylphenidate and mirtazapine as possible treatments for the cognitive and affective alterations experienced by patients with DM1. These results provide a new pharmacological objective for the treatment of DM1 and reveal new mechanisms of action for these effects, opening new ways for a more complete therapeutic orientation of the pathology of DM1.

The detection of a significant reduction of ATP production in neurons expressing toxic RNAs or having silenced muscleblind expression has also been significant. It is very probable that the insufficient generation of energy contributes to the reduction in the number of dopaminergic neurons in the brain of these models so that to investigate the origin of this reduction, and to supply the energy deficit, can constitute important therapeutic targets in the treatment of neurodegeneration. In this sense we will continue with the research on nutritional supplements that could rescue these deficits, for example ATP supplements, in a similar way to our collaboration with the company MyoGem Health in the development of the Myo-DM supplement for patients with DM1.

4. Literature generated

1. Carla Ramon-Duaso, Thomas Gener, Marta Consegal, Cristina Fernández-Avilés, Juan José Gallego, Laura Castarlenas, Maurice S. Swanson, Rafael de la Torre, Rafael Maldonado, M. Victoria Puig and Patricia Robledo. Methylphenidate Attenuates the Cognitive and Mood Alterations Observed in Mbnl2 Knockout Mice and Reduces Microglia Overexpression. Cerebral Cortex, 2018; 1–20. doi: 10.1093/cercor/bhy164.

2. Carla Ramon-Duaso, Cristina Fernández-Avilés, Estela Selma Soriano, Beatriz Llamusí, Rubén Artero, Manuel Pérez-Alonso, Rafael de la Torre and Patricia Robledo. Conditional mutagenesis of the Mbnl2 gene in the neocortex and hippocampus recapitulates DM1 neuropsychopathology and the atypical antidepressant mirtazapine reverses these alterations. Sent to “Brain, Behavior, and Immunity”.

3. Estela Selma Soriano, Beatriz Llamusi, Ariadna Bargiela, Rubén Artero Manuel Pérez Alonso. Dopamine system dysfunction contributes to myotonic dystrophy neurological phenotypes. (In preparation)

4. Estela Selma Soriano, Beatriz Llamusi, Ariadna Bargiela, Rubén Artero Manuel Pérez Alonso. Identificación de compuestos con actividad terapéutica en modelos neuronales de distrofia miotónica en Drosophila. (In preparation)