Metabolic and morphological characterization of the Mecp2-308 truncated mouse model of Rett syndrome: effects of a treatment activating Rho GTPases

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Introduction -
Rett syndrome (RTT) is a pervasive developmental disorder, primarily affecting girls. RTT causes a wide variety of debilitating symptoms and no cure currently exists. Mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2) have been found to be responsible for about 90% of classical RTT cases. After the discovery of a monogenic origin for RTT, several lines of mice carrying endogenous MeCP2 mutations have been developed, recapitulating several RTT symptoms. Cytotoxic Necrotizing Factor 1 (CNF1) is a bacterial toxin that selectively activates Rho GTPases, thus reshaping actin cytoskeleton, enhancing neurotransmission and synaptic plasticity. Here we investigated the effects of CNF1 administration to contrast the RTT phenotype in MeCP2-308 truncated mice by in vivo ¹H MRI and MRS.

Methods -
Fully symptomatic adult MeCP2-308 male mice were intracerebroventricular (icv) treated with CNF1 and first evaluated in a battery of behavioral tests focused on RTT symptomatology. At the end of behavioral testing, animals undergo MRI and MRS scanning to evaluate genotype- and treatment-induced differences in volume and metabolism.

MR examinations were performed on a VARIAN Inova MRI/MRS system operating at 4.7 T, by using a volume coil as transmitter and a surface coil constructed for mouse head as receiver (RAPID Biomedical). Multislice fast spin echo (TR/TEeff = 3000/70 ms, ns = 2, slice thickness 1 mm, matrix 128 x 256) sagittal images were acquired to localise the regions of interest. Single voxel localised spectroscopy (PRESS, TR/TE = 4000/23 ms, ns = 256) were collected from: prefrontal cortex (PFC), 6.8 μl and hippocampus (Hip), 11.7 μl. Spectra were analysed by LCModel fitting program. The unsuppressed water signal was used for metabolite quantification. Multislice fast spin echo axial images (TR/TEeff = 3200/60 ms, ns = 2, slice thickness 1 mm, 24 slices, matrix 256 x 256, FOV =25 x 25 mm², which correspond to voxel resolution of 0.1x0.1x0.6 mm³) were acquired for corpus callosum volumetric analyses. Statistical analysis was performed by using ANOVA (2 x 2 genotype and treatment).

Results and Discussion -
CNF1 treatment resulted in a global increase of nocturnal locomotor cyclicity, amelioration of both motor coordination capabilities and memory of fear-conditioned response. MRS revealed a significant increase in the amount of taurine after CNF1 in hippocampus and striatum whereas the same effects were not evident in prefrontal cortex. Interestingly these effects were more evident in the MeCP2 308 genotype [F(1, 13) = 3.33, p = 0.05]. MRS results from hippocampal region are shown in Fig.1. Inositol (Ins, a marker for glial functionality) is significantly increased by CNF1 treatment in hippocampal region [F (1, 11) = 7.28, p = 0.02] whereas it appears to be reduced in prefrontal cortex of MeCP2-308 mice [F (1, 11) = 4.28, p = 0.06]. Significant reduction in the thickness of the genu of the corpus callosum has been detected by MRI in MeCP2-308 mutant mice in comparison with wild type values [F (1,6) = 9.17 p = 0.02] (Fig. 2).

Conclusions -
Our data indicate that CNF1 icv treatment (and thus consequent activation of RhoGTPases) affects metabolism of taurine and inositol. In the MeCP2-308 phenotype these effects result in a normalization towards wt-like values. CNF1-induced alterations of these two markers indicate that CNF1 impacts oxidative stress pathways (taurine) and glial functionality (inositol) both recently identified as direct targets of MeCP2 dysfunction in RTT mouse models and RTT patients. In addition, MRI analysis revealed a significant reduction of the corpus callosum, a result in substantial agreement with MRI results in RTT patients.

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