IN-VIVO EFFECTS OF KNOCKING-DOWN METABOTROPIC GLUTAMATE RECEPTOR 5 IN THE SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder due to loss of upper and lower motor neurons (MNs). The etiology of ALS is multifactorial and several in-vitro and in-vivo studies demonstrate that MN damage and death is sustained by both neuronal and non-neuronal degeneration. One major cause for MN degeneration in ALS is represented by Glu-mediated excitotoxicity. In this scenario, Group I metabotropic glutamate (Glu) receptors (mGluR1, mGluR5), the only excitatory mGluRs, are involved in the regulation of important cellular processes altered in ALS and are over-expressed in different experimental model of the pathology. Activation of Group I metabotropic Glu receptors (mGluR1 and mGluR5) at glutamatergic spinal cord nerve terminals has been reported to produce abnormal Glu release in the widely studied SOD1G93A mouse model of ALS. We also reported that halving mGluR1 expression in the SOD1G93A mouse had a positive impact on survival, disease onset, disease progression and on a number of cellular and biochemical readouts of ALS.

Following the same path, here we investigated the role of mGluR5 in ALS. We generated a SOD1G93A mouse strain with partial receptor reduction (SOD1G93AmGluR5+/-) by crossing the SOD1G93A mutant with the mGluR5 heterozigous Grm5-/+ mouse.

SOD1G93AmGluR5+/- mice showed prolonged survival probability. The survival age mean of SOD1G93A mice was 134 ± 1.50 days and it was shifted to 153 ± 1.54 days in the case of SOD1G93AGrm5-/+ mice ($p<0.001$); the Kaplan-Meier graph showed a significant ($p<0.001$) survival probability amelioration. Body weight as a measure of the pathology onset, decreased significantly starting at day 114 or 120 in male and female SOD1G93A mice, respectively, and it was shifted to 132 days in both sex SOD1G93AGrm5-/+ mice ($p<0.05$).

These clinical scores were paralleled by a significant preservation of MNs in the ventrolateral horn of 120-130 days old mouse spinal cord (24.9±0.7 in WT, 8.0±0.4 in SOD1G93A, 24.4±0.7 in Grm5-/+, and 14.6±0.5 in SOD1G93AGrm5-/+ mice; $p< 0.01$ vs. SOD1G93A mice); a significant reduction of astrogliosis, measured by the dampening of GFAP over-expression, ($p<0.001$; SOD1G93AGrm5-/+ vs. SOD1G93A mice) and microgliosis, measured by the dampening of CD-11β over-expression, ($p<0.001$; SOD1G93AGrm5-/+ respect to SOD1G93A mice).

Halving mGluR5 in SOD1G93A also normalized the abnormal cytosolic Ca2+ concentration measured under basal conditions (256.70 ± 2.65 nM in SOD1G93A mice and 211.25 ± 9.45 nM in SOD1G93AGrm5-/+ mice; $p<0.05$), and after stimulation with 15 mM KCl (471.70 ± 28 nM in SOD1G93A mice and 325 ± 15.90 nM in SOD1G93AGrm5-/+ mice; $p<0.05$) or by group I mGluR activation with 0.3 μM 3,5-DHPG (526.50 ± 7.50 nM in SOD1G93A mice and 451.20 ± 20.68 nM in SOD1G93AGrm5-/+ mice; $p<0.05$). Release of Glu paralleled Ca2+ concentration changes in

SOD1G93AGrm5-/+ vs. SOD1G93A mice. Either the basal release (about 76% increase; $p<0.001$) and those evoked by 15 mM KCl (74% increase; $p<0.05$) or 0.3 µM 3,5-DHPG (135% increase; $p<0.05$) were significantly elevated in SOD1G93A vs. WT mice and significantly reduced in SOD1G93AGrm5-/+ mice .

Finally, we tested motor abilities in SOD1G93A and SOD1G93AGrm5-/+ in a number of tests (Rotarod, posterior limb extension reflex and gait), in which motor skills were monitored three time a week from day 90 of life. Unexpectedly, only male SOD1G93AmGluR5+/- mice showed improved motor performances vs. SOD1G93A mice, while SOD1G93AmGluR5+/- females did not.

These results demonstrate that the halving of mGluR5 in SOD1G93A mice has a significant positive impact on the disease progression. These evidences support the idea that blocking Group I may represent a potential effective pharmacological approach to ALS.