Results: Immobilisation time (IT) in FST after the administration of imipramine was shorter than the control, same as for subgroups treated with AE I, II and VS. In the subgroup treated with fluoxetine, IT in TST was shorter than the control time, and the same was observed in subgroups treated with AE I, II and VS.

Significant binding energies were found for Serotonin Reuptake Transporter (SERT) and venlafaxine (−7.20 kcal/mol) and verbacoside (−6.61 kcal/mol), and for the Leucine Transporter (LeuT), the homologue of the noradrenaline reuptake transporter, and verbenalin (−6.27 kcal/mol) and caffeic acid (−5.85 kcal/mol).

Conclusion: In both pharmacodynamic tests the antidepressive effect of AE and VS has been confirmed. Venlafaxine and verbacoside binding energies and poses in interaction with SERT were similar to those of paroxetine. For LeuT, venlafaxine showed both a similar binding energy and pose to that of imipramine, whereas caffeic acid showed only a similar binding energy.1-4

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PS231

Effects of Vitamin D on the expression of markers of principal neurons, interneurons and astrocytes in cerebral cortex and hippocampus in gerbils exposed to transient global cerebral ischemia

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Aim: Examination of the effects of vitamin D pretreatment on the expression of markers of principal neurons (NeuN), inhibitory interneurons (PV) and astrocytes (GFAP) in cerebral cortex and hippocampus in gerbils who were exposed to transient global cerebral ischemia.

Introduction: Brain ischemia may cause serious damage to the cells in the central nervous system. Vitamin D has an important role in brain injury treatment due to its neuroprotective effects.

Methods: Gerbils were divided in 5 groups: control group; two groups that underwent ischemia and then reperfusion for three (I/R3d) and seven days (I/R7d) and two groups that were treated with vitamin D before I/R (vitD + I/R3d and vitD + I/R7d). Complete blood supply to the brain was cut off for 10 minutes and reperfusion lasted 3 and 7 days. They were daily treated with vitamin D for 7 days prior ischemia. Expression of proteins was detected using Western blot.

Results: No changes were detected in expression of NeuN markers in cortex of experimental groups, while there was increase in expression in hippocampus in groups I/R7d and vitD + I/R7d in comparison to the control group and group vitD + I/R3d. Expression of PV in cortex was significantly reduced in group I/R7d in comparison to group I/R3d, whereas in hippocampus the expression was significantly higher in group vitD + I/R3d than in group I/R3d. Expression of GFAP has significantly risen in all groups in comparison to the control group whereas in hippocampus there was a rise in groups vitD + I/R3d, I/R7d and vitD + I/R7d in comparison to the control group. There was also a rise of GFAP expression in groups treated with vitamin D (vitD + I/R3d and vitD + I/R7d) in comparison to those that have not been treated (I/R3d, I/R7d).

Conclusion: Vitamin D has positive effect on astrocytes in both structures of gerbils that underwent global cerebral ischemia, especially in hippocampal region.

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PS238

Identification of genetic modifiers of somatic CAG instability in Huntington's Disease by in vivo CRISPR – Cas9 genome editing

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Aim: To develop an experimental platform for in vivo investigation of candidate genetic modifiers of somatic CAG instability in Huntington’s disease.

Introduction: Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion within the huntingtin gene (HTT). Despite being a monogenic disorder, for which the mutation has been known for some time now, no cure or disease-modifying therapy is available, indicating that novel approaches are critical.

Somatic CAG repeat instability, characteristic of mutant HTT alleles, is inversely correlated with patient age of onset and may contribute to HD pathogenesis. This phenotype, common to other trinucleotide repeat disorders, was previously shown to be DNA mismatch repair (MMR) dependent. The DNA repair machinery was further implicated as a modifier of HD age of motor onset in a recent genome-wide association study, underlining its promise as a relevant disease mechanism that could potentially be therapeutically targeted.

In this study, we are developing a CRISPR/Cas9-based approach that will enable the investigation of candidate genetic modifiers of HD age of onset as potential modifiers of somatic CAG repeat instability in a HD mouse model.

Methods: We have developed CRISPR reagents against known and candidate genetic modifiers of somatic CAG instability in Huntington’s disease. In preliminary experiments, we treated HD mice with CRISPR reagents against Mlh1 and investigated the level of gene editing achieved as well as the impact on liver CAG instability.

Results: We were able to significantly suppress the CAG expansion process in the liver of HD mice by knocking out the Mlh1 gene in our in vivo CRISPR platform. The efficiency achieved in modifying the instability phenotype makes us very confident that we will be able to test and validate additional candidate modifiers. To that end, we have already validated reagents for efficient knockout of a subset of known and candidate modifier genes and we have developed assays that will allow detailed characterization of gene editing at these sites.

Conclusion: We have successfully developed an in vivo CRISPR-Cas9-based platform that allows for knocking out genes of interest in the liver of adult mice, and subsequently perturb the somatic CAG expansion process. We will next use this tool to test the role that candidate genes might play in that disease-relevant process. While the scope of this project was liver oriented, future work will also be aimed at targeting the striatum which is the main site of HD-related pathology.

References

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