In vitro and *in vivo* studies on novel pcsk9 inhibitors as pharmacological approach for the treatment of alzheimer's disease

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Aim: Impairment of cholesterol homeostasis is one of the multiple etiopathological mechanisms at the origin of both cardiovascular and neurodegenerative diseases. The PCSK9 protein, known for its role in the degradation of hepatic LDLR and plasma cholesterol regulation, is expressed also in the CNS, where it exacerbates β-amyloid neurotoxicity and reduces neuronal cholesterol uptake, suggesting an involvement in AD. This study proposes an *in vitro* screening of molecules (MR) with inhibitory activity on PCSK9, selecting the best compounds to test their activity on cerebral cell models and their *in vivo* tolerability.

Methods: 30 newly synthesized compounds were tested at increasing concentrations on human hepatoma cells (HepG2) to evaluate their cytotoxicity and efficacy in inhibiting PCSK9. MR-3 was tested on human neuroblastoma cells (SH-SY5Y) overexpressing PCSK9 to assess neurotoxicity and cholesterol uptake. Cytotoxicity was determined through MTT assay; PCSK9 secretion was quantified with an ELISA kit; and radioisotopic techniques measured cholesterol uptake . Three compounds were selected to be tested *in vivo* on C57BL/6 mice at a dose of 40 mg/Kg for 7 days to evaluate: tolerability with SHIRPA test; plasma lipid profile by ELISA assay; biodistribution in plasma and brain through LC-MS/MS.

Results: Among the tested compounds, MR-3, MR-532, MR-533 demonstrated no sign of cytotoxicity and the greatest efficacy on HepG2 cells (IC_{50}=1.7μM; 5.7μM; 6.1μM). Neuronal cholesterol uptake was restored after treatment with MR-3 at 10μM (p<0,05). MR-3, MR-532, and MR-533 exhibited good in vivo tolerability; MR-3 and MR-532 were detected in plasma and brain tissue.

Conclusions: Preliminary *in vitro* screening allowed the identification of MR-3, MR-532, MR-533 as promising PCSK9 inhibitors. The outcome of MR-3 on neuronal cholesterol uptake may suggest a neuroprotective effect to be further investigated. *In vivo* treatment with selected inhibitors shown absence of toxicity, however, it is necessary to bring proof of efficacy.

Effect of lipid-lowering therapies on lipoprotein(a) levels: a meta-analysis of randomized controlled trials

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Aim: Epidemiological studies, Mendelian randomized studies, and genome-wide association studies confirmed that elevated lipoprotein(a) [Lp(a)] concentration is an independent risk factor for cardiovascular diseases. However, no approved therapy for patients with elevated Lp(a) levels is available. Our aim is to investigate to what extent PCSK9 inhibitors (PCSK9i), statins, and ezetimibe affect Lp(a) level.

Methods: This meta-analysis was conducted according to the PRISMA guidelines. Databases were searched from inception to February 2023. Inclusion criteria were: (1) randomized controlled trials (RCTs) in adults (≥18 years), phase II, III or IV; (2) English language; (3) reporting the effects on Lp(a) levels; (4) with intervention duration more than 3 weeks. Pooled estimates were assessed by a random-effects model. Between-study heterogeneity was tested and measured by Cochrane's Q test and I^2 statistics.

Results: Overall, 51 RCTs were included for PCSK9i (39,271 participants), 35 RCTs for statins (15,425 participants), and 14 RCTs for ezetimibe (5,607 participants). Starting from a baseline Lp(a) level of 33.12 mg/dL, participants treated with PCSK9i compared to placebo experienced an additional reduction in Lp(a) levels of -26.34% (95%CI -28.83 to -23.85). Lp(a) levels were marginally reduced by statins by -3.43% (95%CI -9.09 to 2.23) from a baseline Lp(a) level of 15.87 mg/dL, although this reduction was not statistically significant. Finally, ezetimibe had a negligible and still not statistically significant effect on Lp(a) levels (0.51% [95%CI -1.67 to 2.70]), from a baseline Lp(a) level of 20.80 mg/dL.

Conclusions: Among the lipid-lowering approaches evaluated, only PCSK9i seemed to lower Lp(a) levels. Further research is requested to understand whether it translates into a clinically relevant cardiovascular benefit.