EFFECT OF PCSK9 INHIBITION ON ALZHEIMER'S DISEASE-RELATED PARAMETERS IN HUMAN CEREBRAL CELL MODELS

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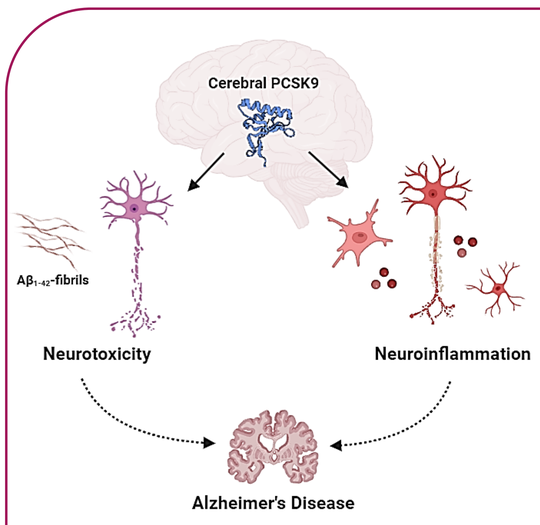
M. Ugolotti[1], B. Papotti[1], M.P. Adorni[2], L. Giannessi[1], I. Rossi[3], M. G. Lupo[3], N. Ferri[3], A. Vilella[4], W. Kukula-Koch[5], M. Radi[1], F. Bernini[1], F. Zimetti[1]

[1]Department of Food and Drug, University of Parma, Italy; [2]Department of Medicine and Surgery, University of Parma, Italy; [3]Department of Medicine, University of Padua, Italy; [4]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Italy; [5]Department of Pharmacognosy with Medicinal Plants Garden, Medical University of Lublin, Poland

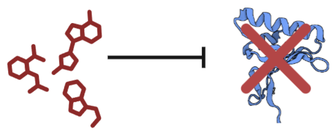
BACKGROUND AND RATIONALE



Brief representation of cerebral PCSK9 functions related to AD

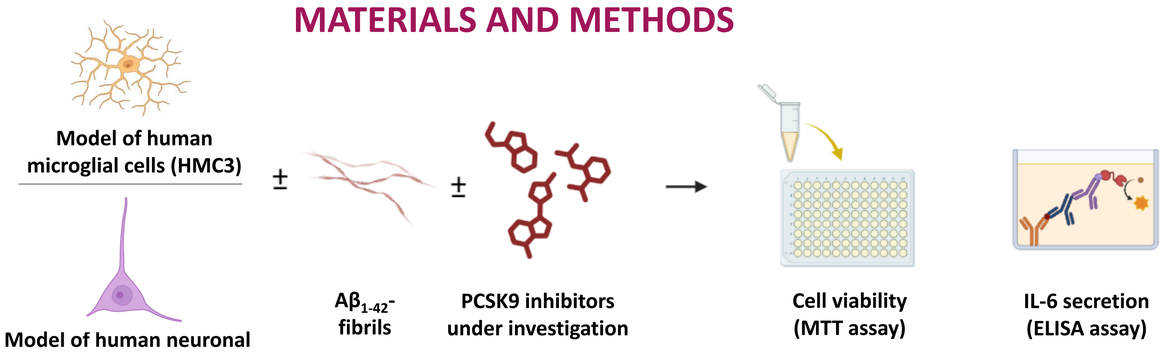
The proprotein convertase subtilisin/kexin 9 (**PCSK9**), beyond regulating plasma cholesterol, is **expressed** also in the **central nervous system** (CNS), where a pathogenetic role in AD has been postulated. **Elevated levels** of this protein have been found **in cerebrospinal fluid of AD patients**[1]; *in vitro* **PCSK9 exacerbates β-amyloid (Aβ) neurotoxicity** and **neuroinflammation**.[2] Moreover, **PCSK9 genetic deletion ameliorates cognitive performance** and **protects against Aβ deposition** and **neuroinflammation in 5XFAD mice**.[3]

AIM OF THE STUDY

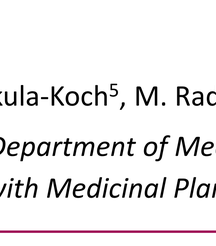


This research aims to investigate the **potential protective effect** of **PCSK9 pharmacological inhibition** with **natural** and **newly-synthesized molecules**.

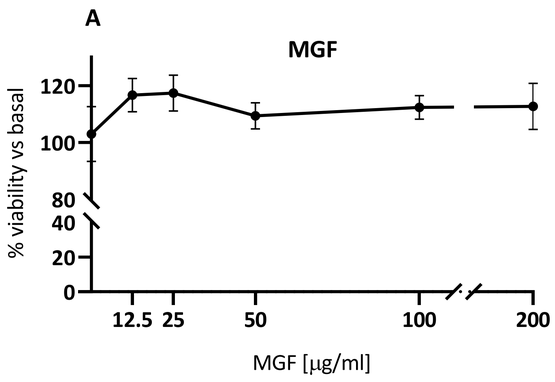
MATERIALS AND METHODS

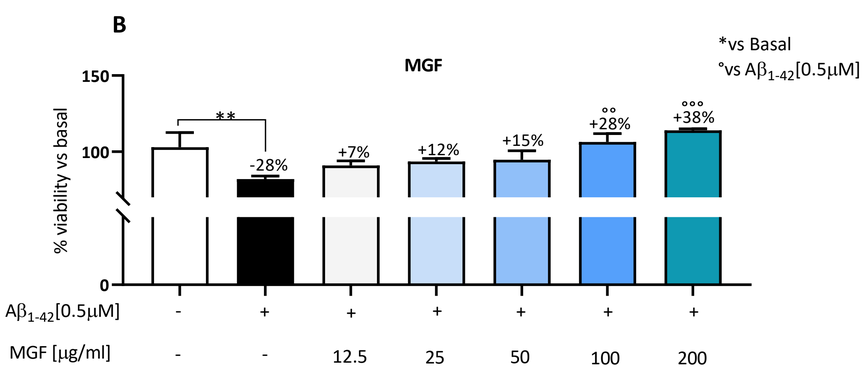


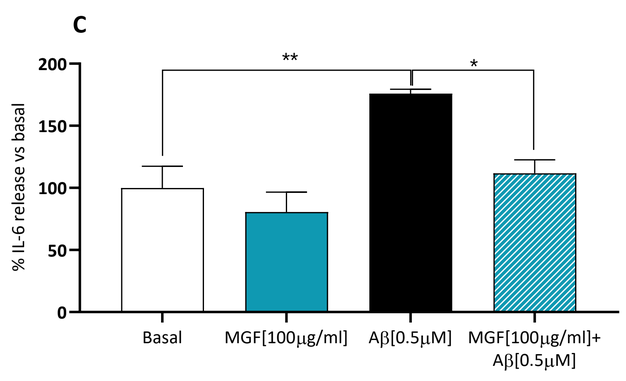
1) STUDY ON A NATURAL PCSK9 INHIBITOR: MAGNOFLORINE



In vitro experiments on HMC3 cells



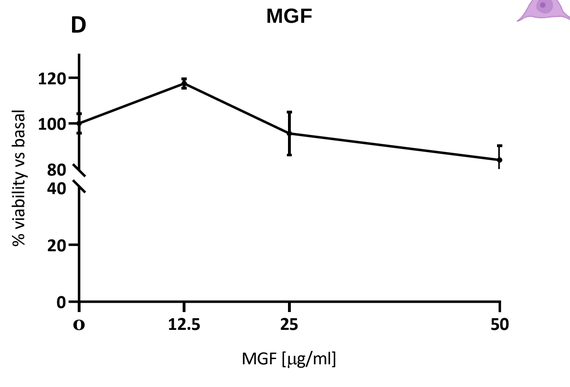


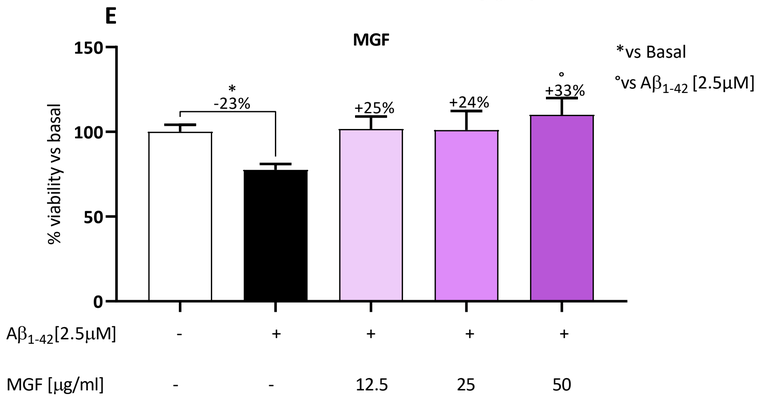


Treatment with Magnoflorine (MGF), a plant-derived molecule and Berberine analogue, **did not affect microglial viability** at the concentrations tested (***Figure 1A***). **Aβ-fibrils reduced viability** (-28%; p<0.01) was **dose-dependently restored by Magnoflorine, with a complete rescue at the concentration of 100μg/ml** (p>0.05 vs basal condition, ***Figure 1B***). Furthermore, **Magnoflorine at 100μg/ml significantly reduced Aβ-triggered IL-6 release** (p>0.05 vs basal condition, ***Figure 1C***).

A neuroprotective effect was observed also in IMR-32 cells, where a concentration of 50μg/ml was well-tolerated and allowed the complete recovery of neuronal viability (p>0.05 vs basal condition, ***Figure 1D, 1E***).

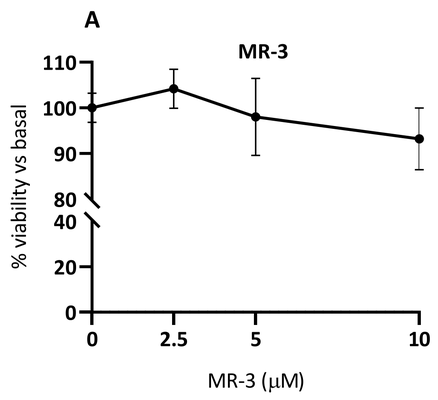
In vitro experiments on IMR-32 cells

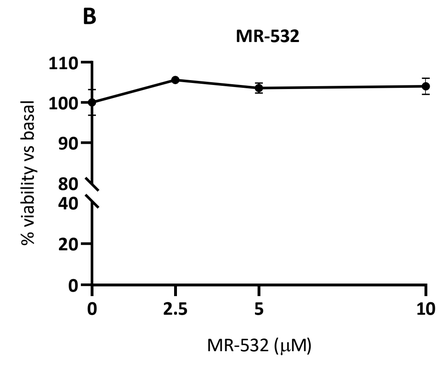


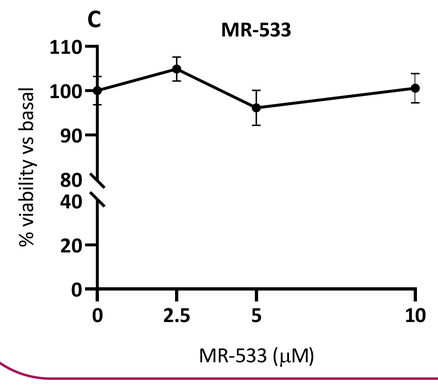


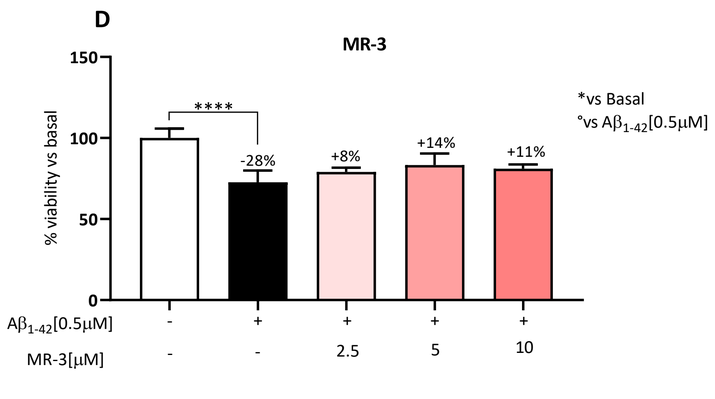
2) STUDY ON *EX NOVO* SYNTHESIZED PCSK9 INHIBITORS: MR COMPOUNDS

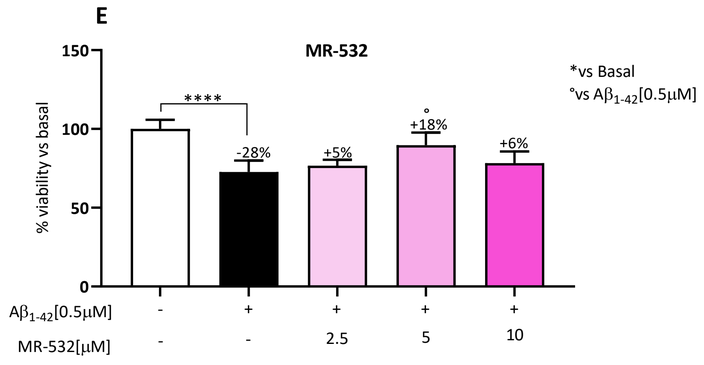
In vitro experiments on HMC3 cells

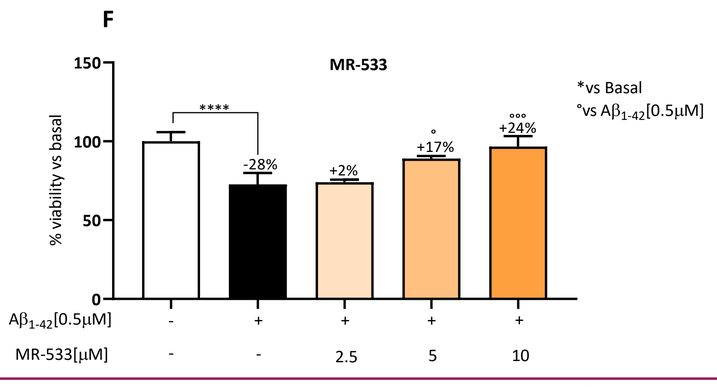


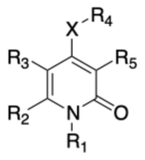




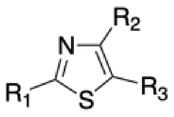








2-pyridone derivatives

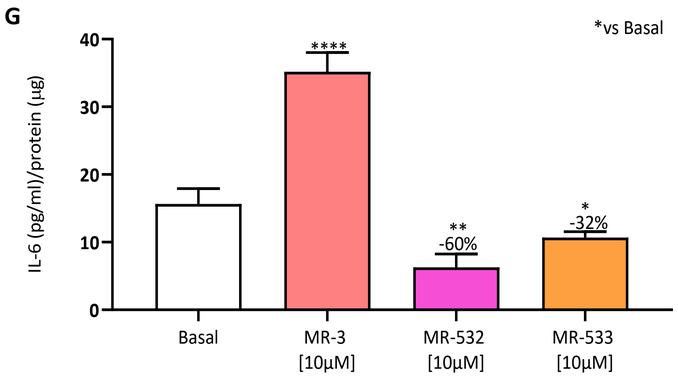


Amino-thiazole derivatives

Previous *in vitro* test on HepG2 cells

Compound ID	Cell viability inhibition IC_{50}(μM)	PCSK9 inhibition IC_{50} (μM)
MR-3	32.4	1.7
MR-532	35.7	5.7
MR-533	>50	6.1

MR-3, MR-532 and **MR-533** – with proven PCSK9 inhibition activity on hepatoma cells (HepG2) - **did not show sign of cytotoxicity** at all concentrations tested in HMC3 cells (***Figure 2A, 2B, 2C***). **Microglial viability**, significantly **reduced after incubation with Aβ-fibrils** (-28%; p<0.0001), **was dose-dependently restored by all three synthetic PCSK9 inhibitors** (***Figure 2D, 2E, 2F***), **with the most evident effect for MR-533 at 10μM** (p>0.05 vs basal condition, ***Figure 2F***).



MR-532 and **MR-533 at 10μM significantly reduced IL-6 microglial release** under basal conditions (-60%, p<0.01; -32%, p<0.05, respectively), while MR-3 increased its secretion (p<0.0001, ***Figure G***).

CONCLUSIONS

PCSK9 pharmacological inhibition acts positively on Aβ-induced neurotoxicity suggesting a **neuroprotective effect**. In addition, **PCSK9 inhibitors carry out a pivotal function in the modulation of neuroinflammation**, potentially opening the way for the development of new approaches in the treatment of AD.

REFERENCES

1. Zimetti, Francesca et al. *Journal of Alzheimer's disease*(2017); 2. Papotti, Bianca et al. *International journal of molecular sciences* (2022); 3. Vilella, Antonietta et al., *Brain Behav Immun*., 2024

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