Final Scientific Report and summary for the research project –PCE-56-2022

For the period June 2022- December 2024

Title: STUDY OF MECHANISMS UNDERLYING THE PROTECTIVE EFFECTS OF ARTEMISININS IN ALZHEIMER`S DISEASE WITH SPECIAL REFERENCE TO ADULT HIPPOCAMPAL NEUROGENESIS

Cod project: PN-III-P4-PCE-2021-1089

Alzheimer's disease (AD) is the most prevalent form of dementia contributing to 60–80% of all cases. No single therapeutic strategy is yet established which might be able to prevent, stop the progression or even cure AD. Actually artemisinin, derived from the plant Artemisia annua and its derivatives such as artesunate represent the most effective and safe therapies for malaria. In addition to their anti-malarial potencies these drugs show a broad spectrum of systemic and cellular effects involving anti-inflammatory and anti-carcinogenic properties. Recently, several publications have shown that artemisinins have also neuroprotective and anti-amyloidogenic effects in vitro and in animal models. In our previous studies in plaque stage AD-mice (12 months) artemisinin or its semisynthetic derivative artesunate modulated synapse components, amyloid plaque load and APP-processing in the cortex and hippocampus.

The hippocampus is one of the most and primarily affected regions of the brain in AD. The hippocampus includes the dentate gyrus, where in the subgranular zone neurogenesis generating new neurons and glia cells persists through adulthood in rodents and humans.

The adult hippocampal neurogenesis (AHN) is a complex multistep process comprising four phases: proliferation, migration, differentiation, and maturation. Transcription factor expression and a set of marker proteins have been established to distinguish the different stages occurring during the transition from stem cell to mature neuron. Inhibitory, particularly GABAergic signaling has been shown to intervene in all stages of adult neurogenesis in humans and rodents.

Within this project we proposed to study the therapeutic potential of artemisinins as probable modulators of adult hippocampal neurogenesis in the APP/PS1 mice as model for AD.

For this purpose, WT and APP/PS1 mice were recruited in experimental groups without and with treatment (artemisinin or artesunate) and divided in two subgroups: 1: end of the experiment at 3 months of age, and 2: end of the experiments at 12 months of age, corresponding early and late stages of the disease, respectively. Brain cryosections from each group were analyzed by extensive immunostaining studies and/or biochemical analyses for expression, localization and co/localization of proteins of interest including markers of proliferation, AHN stages and/or marker proteins of inhibitory synapses/neurotransmission.

Data evaluated to date support, that treatment with artesunate can modulate the process of AHN if administered early in the disease progression (6-12 weeks). The results indicate as a level

for intervention by artesunate in the complex process of AHN the progression from nestin to DCX-positive progenitors and immature granule cells. In parallel, the findings of these experiments further evidence changes in the protein expression level of specific inhibitory receptor subtypes in the granular and/ or subgranular layer of the dentate gyrus, with known regulatory roles in different stages of AHN indicating as possible molecular targets of artesunate Important, these effects can be observed at a similar level in both male and female mice and also by a low dose 1mg/kg artesunat, essential to avoid or minimalize unwanted side effects.

The results of this project deliver new information that should provide new insights into the mechanisms underlying ARMs effects in AD, contributing to elucidate their therapeutic potential in AD, essential for eventual consideration in clinical trials.

Results of this study were presented at national and international neuroscience meetings (e.g. FENS 2024, Vienna) and published in original research articles in well-respected neuroscience journals:

1. Kuhse J, Groeneweg F, Kins S, Gorgas K, Nawrotzki R, Kirsch J, Kiss E. Loss of Extrasynaptic Inhibitory Glycine Receptors in the Hippocampus of an AD Mouse Model Is Restored by Treatment with Artesunate. **Int J Mol Sci.** 2023 Feb 27;24(5):4623. doi: 10.3390/ijms24054623.
2. Kiss E, Kins S, Gorgas K, Venczel Szakács KH, Kirsch J, Kuhse J. Another Use for a Proven Drug: Experimental Evidence for the Potential of Artemisinin and Its Derivatives to Treat Alzheimer's Disease. **Int J Mol Sci**. 2024 Apr 9;25(8):4165. doi: 10.3390/ijms25084165.

A third publication is in preparation:

Rajender R, Eggert S, Schilling S, Banicevic M, Kiss E, Maritzen T, Mueller U, Kins S. Trans-dimerization of the Amyloid Precursor Protein family induces pre- and postsynaptic differentiation through distinct signaling pathways

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