Neuroprotection and cognitive improvement using VACNO and SanFlow in the scopolamine model for Alzheimer's Disease

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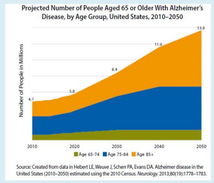
INTRODUCTION

Normal aging is associated with the development of low-grade chronic neuroinflammation that contributes to cognitive decline of age-related pathologies. There is an urgent need to develop novel anti-neuro inflammatory chronic therapeutic agents to prevent dementia of Alzheimer's disease (AD).



The sixth leading cause of death among all US adults, AD is an irreversible progressive neurodegenerative disorder that currently afflicts nearly 6 million Americans. The rates of AD deaths increased more than 50 % between 1999 and 2014. Current estimations predict that by 2050, nearly 14 million people in US will be afflicted with AD.

We have used scopolamine-induced amnesia as a preclinical pharmacological model of AD, and have investigated the efficacy of extracellular superoxide dismutase (SOD3) mimetics to prevent the scopolamine-induced amnesia following single or repeated doses of scopolamine in a rat model. Here, we investigate two novel anti-inflammatory drugs in the prevention of memory and cognitive impairment in an established rat model of AD.

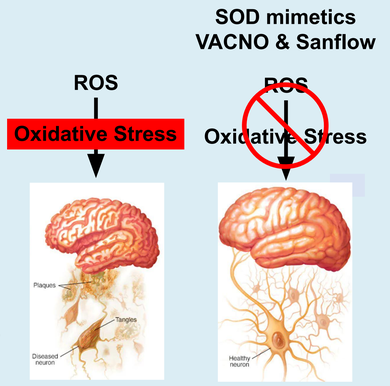


Fig.1. Oxidative Stress is involved in various network pathways of AD hypotheses

SUPEROXIDE DISMUTASE MIMETICS BEING EVALUATED

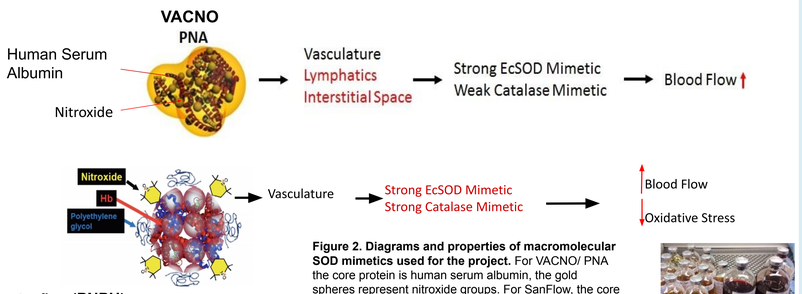


Figure 2. Diagrams and properties of macromolecular SOD mimetics used for the project. For VACNO/ PNA the core protein is human serum albumin, the gold spheres represent nitroxide groups. For SanFlow, the core protein is bovine hemoglobin, the blue shell represents PEGylation and yellow spheres represent caged nitroxides. Unique aspects of each drug is highlighted in red text.

PURPOSE

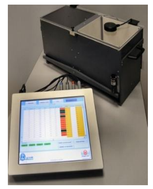
- Oxidative stress in the brain vasculature and tissue induces vascular dysfunction & neural inflammation.
- SOD mimetics VACNO and Sanflow are macromolecular antioxidants known to protect the brain from stroke damage by reducing oxidative stress.
- Since oxidative damage leading to vasculature defects is associated with AD, we predict that these drugs may protect against neuronal damage seen in progression of AD and prevent or reverse memory loss associated with the progression of the disease.
- The aim of this study is to compare VACNO to Sanflow in potential to protect the brain from cognitive impairment and neuronal damage in scopolamine AD rat model.

STUDY DESIGN AND METHODS

Hypothesis: use of macromolecular SOD-mimetics Vacno and / or Sanflow will reduce scopolamine induced cognitive dysfunction and neurological damage

Rat line:	Wistar Han
Breeder:	Envigo
Age at start:	~10-11 weeks
Sex:	males
Number of animals:	42

Wistar Han® Rat



Ugo Basile Passive Avoidance

Treat scopolamine/ vehicle
↓
Treat +/- Vacno/ Sanflow / Donepezil
↓
Day 7-8: Examine effects on cognitive function using step-through passive avoidance test
↓
Tissue histopathology

Table 1: Group allocation

	Scopolamine		Compound		
Group	Scopolamine	Treatment route	Test item	Treatment route	n
A	Vehicle (scop.)	i.p.	VACNO	i.p.	7
B	Vehicle (scop.)	i.p.	SanFlow	i.v.	7
C	1.0mg/kg	i.p.	vehicle	i.p.	7
D	1.0mg/kg	i.p.	VACNO	i.p.	7
E	1.0mg/kg	i.p.	SanFlow	i.v.	7
F	1.0mg/kg	i.p.	Donepezil	p.o.	7

SANFLOW TREATED RATS SHOW HIGHER MEMORY RETENTION

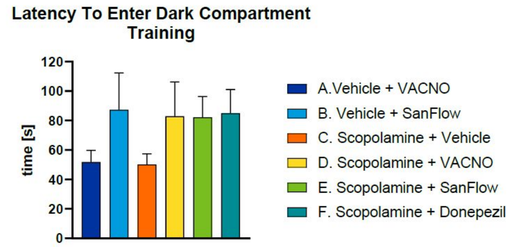


Figure 3. Passive Avoidance training during the acquisition /training phase indicated no significant difference in latency between all treatment groups. Graphs represent latency to enter dark compartment during the training phase (Data are presented as group mean + SEM. Statistics: Kruskal- Wallis test followed by Dunn`s multiple Comparison test group C vs. all.

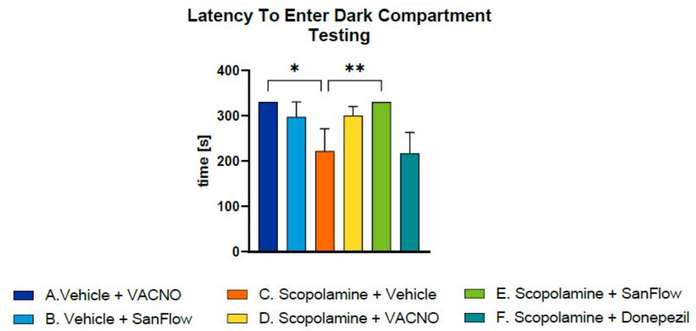


Figure 4. Passive avoidance measurements conducted during the testing phase indicated scopolamine + Sanflow treated rats showed significantly increased latency to enter dark compartment compared to scopolamine /vehicle treated rats. Graph represent latency to enter dark compartment during the testing phase. Data are presented as group mean + SEM. Statistics: Kruskal-Wallis test followed by Dunn`s multiple Comparison test group C vs. all. * $p < 0.05$, ** $p < 0.01$.

VACNO AND SANFLOW REDUCE ASTROGLIOSIS

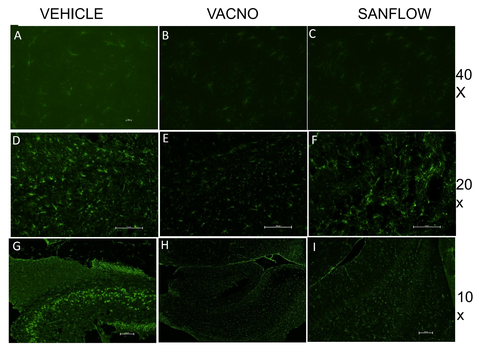


Figure 5. VACNO and Sanflow reduce scopolamine induced astrocyte activation. In scopolamine treated rats, GFAP staining indicates VACNO (B-H) and Sanflow (C-I) reduces staining and astrocyte activation as compared to Vehicle treatment (A-G). Magnification 40x (A-C); 100x (D-F); 400x (G-I). Eyepiece-10x.

CONCLUSIONS

· All animals which were enrolled in the study were in a fair health condition and could perform all tasks throughout the entire project. Treatment was well tolerated, and no animal died during the treatment period.

·Passive Avoidance testing assessing amygdala and hippocampal dependent memory revealed no statistically significant difference in latency between all treatment groups during the training phase.

· During the testing phase, animals treated with Scopolamine + Sanflow showed statistically significant increased latency to enter the dark compartment compared to animals treated with only Scopolamine, suggesting increased memory retention in Sanflow treated rats.

· Reduced Glial fibrillary acidic protein (GFAP) immunoreactivity in VACNO + scopolamine and Sanflow + scopolamine treated rats compared to animals treated with scopolamine alone suggests that both VACNO and Sanflow reduce scopolamine induced astrocyte activation, suggesting decreased astrogliosis and a protective effect on neurons.

FUTURE DIRECTIONS

1. To understand the mechanism of action of SanFlow and VACNO and the associated effects on the brain we will utilize a more advanced AD model with amyloid β oligomers (AβOs) which trigger an inflammatory response and play an important role in the pathogenesis of AD.
2. To examine the ability of SanFlow and VACNO to provide protection to human neurons and astrocytes in the AβOs induced model of AD.
3. To determine the ability of SanFlow and VACNO to reverse the cognitive effects in the AβOs induced model of AD

ACKNOWLEDGMENTS

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