SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: BM1402
STSM title: The protective effects of 18α-glycyrrhetinic acid and omega-3 fatty acids in 5xFAD mouse model of Alzheimer's disease
STSM start and end date: 12/01/2018 to 05/03/2018
Grantee name: Aleksandra Mladenovic Djordjevic

PURPOSE OF THE STSM

AD is one of the most common age-related neurodegenerative disorders worldwide, characterized by the accumulation of amyloid plaques, composed of aggregated amyloid-β peptides (Aβ), mainly Aβ42. The peptides are formed by sequential proteolytic processing of the amyloid-precursor protein (APP) that can be cleaved by two distinct pathways, the amyloidogenic and nonamyloidogenic pathway. Both of these pathways can be manipulated by genetic, environmental and pharmacological approaches. However, so far, there is effective treatment for AD. .

The purpose of this STSM was to examine the effect of omega-3 fatty acids and 18-alpha GA supplementation on APP processing and generation of Aβ42 using a transgenic mouse model of Alzheimer's disease (AD). Two important omega-3 fatty acids, DHA and EPA, are shown to influence both amyloidogenic and nonamyloidogenic processing of APP. On the other hand, 18α-glycyrrhetinic acid (18-alpha GA), a proteasome activator, has been shown to decrease Aβ deposits in model organisms by enhancing levels of proteasome activities.

Previously in a pilot experiment has been conducted in the host lab with a small number of animals and only one concentration of 18-alpha-GA. The results indicated that it is possible to increase the activity of the proteasome in the 18-alpha-GA- and omega-3-treated groups. This activation most probably influenced APP processing and decreased the number of amyloid beta plaques in the cortex. However, additional experiments and additional number of animals were needed to confirm this link. There were indications that gender- and region-specific response is present. Due to the small number of experimental animals in the pilot experiment, only one concentration of 18-alpha-GA was tested (in the literature there was no data about in vivo treatment, thus the experimental concentration was chosen by extrapolation of previously published data for in vitro experiments). One of the aims of this STSM was to treat an additional group of animals with higher (twice as much as the first one) concentration of 18-alpha–GA (20ug/g animal's weight), in order to exam is there a dose-dependent response. We also aimed to confirm and expand previously observed results by testing additional 6 animals per group (3 males and 3 females). In addition, our goal was to determine the frailty index (FI) for all the treated animals, as increased frailty is observed in neurodegenerative disorders and has never been tested in 5xFAD mice before.

As the identification and testing of potential molecules for ageing interventions e.g. geroprotectors is one of the main priorities of the MouseAge Action, the main purpose of this STSM was to provide preliminary data

about potential therapeutic use of omega-3 fatty acids and 18α-glycyrrhetinic acid in AD.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Animal model used in this project was 5xFAD transgenic mouse model that represents an early-onset model of AD. Both female and male 5xFAD transgenic mice were exposed to the omega-3 fatty acids supplementation and 18-alpha GA treatment. As planned, treatments started at 2-months of age and lasted for 4 weeks in the home laboratory. This time window has been chosen as it is considered as an early phase of AD pathology, known to be suitable for therapeutics application. The total amount of 34 animals was used. Animals were divided into several groups, depending of the treatment performed:

1. 5xFAD transgenic animals without any treatment (3 males+ 3 females); marked as controls (ctrl)

2. 5xFAD transgenic animals treated with 18α-glycyrrhetinic acid (10ug/g animal's weight) dissolved in 8%DMSO (3 males+ 3 females); marked as "activator x1"

3. 5xFAD transgenic animals treated with 18α-glycyrrhetinic acid (20ug/g animal's weight) dissolved in 8%DMSO (5 males+ 5 females); marked as "activator x2"

4. 5xFAD transgenic animals treated with omega-3 fatty acids (100ul per animal of commercially available omega-3 fatty acids in a final concentration of: DHA 12mg and EPA 180mg) (3 males+ 3 females)

5. 5xFAD transgenic animals treated with 8% DMSO (3 males+ 3 females); marked as "DMSO"

In the host laboratory STSM applicant determined proteasome activity in the cortex and hippocampus of all samples. These two brain structures were chosen as they play the key role in AD pathology. During the first week of the STSM visit applicant was learning how to prepare samples and perform the analysis. Then the samples were analysed. 64 mice tissue samples were used (34 cortex and 34 hippocampus). Tissues were adequately prepared (proteins were extracted in a manner that preserves proteasome activity), protein concentration was measured and 3 different key proteasome activities were determined (CT-L, T-L, and PGPH activities) by hydrolysis of Suc-LLVY-AMC, Boc-LRR-AMC, and Z-LLE-AMC fluorogenic peptides. Data were statistically analysed.

In addition, the presence and amount of amyloid plaques was determined in both structures by immunohistochemical labelling of tissue sections for amyloid beta 42 specific antibody and Thioflavin S.

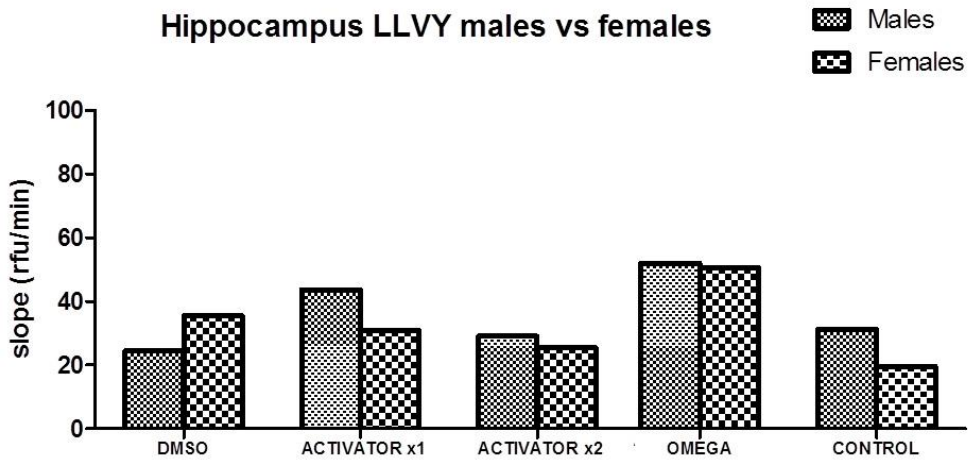
The results were further analysed for statistical significance.

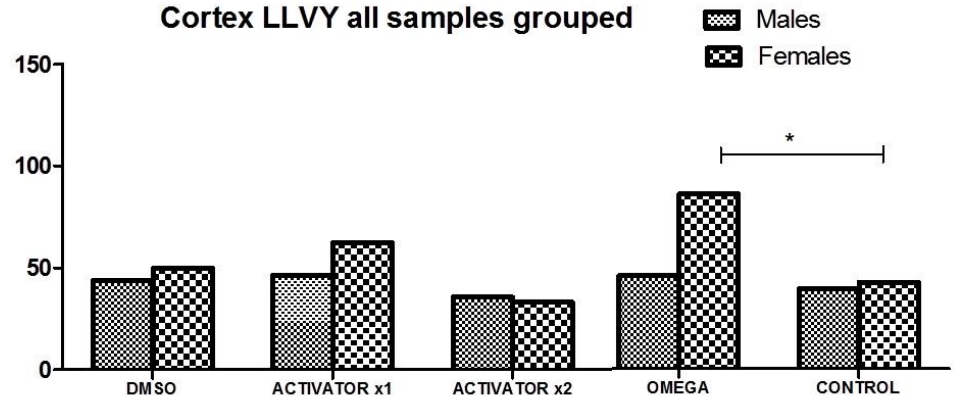
DESCRIPTION OF THE MAIN RESULTS OBTAINED

A detailed analysis of frailty in 5xFAD mice under the different treatments showed that there are no differences among animals. Detailed examination of both clinical signs of frailty (clinical evaluation of the integument, the musculoskeletal system, the auditory system, the ocular system, the neurological system, the digestive system, the urogenital system, the respiratory system and the body weight) and phenotype frailty assessment (we used five frailty criteria, with four of them adapted for animal research and unintentional weight loss as the fifth one; criteria were: grip strength determined by grip test, walking speed determined by velocity (cm/s), physical activity level tested in the open field test (cm/10 min) and rope test) showed no signs of significant frailty in experimental groups.

The analysis of proteasome activities showed the following results:

There were no difference between samples regarding Boc-LRR-AMC, and Z-LLE-AMC proteasome activity. Suc-LLVY-AMC proteasome activity, that is considered to be the most specific activity of the proteasome and the most significant one, showed great variations between samples (please see the graphs and tables below the text). Analysis showed that there is an increase in the LLVY activity of the proteasome in the 18-alpha-GA- and omega-3-treated groups in comparison to the control group of mice.

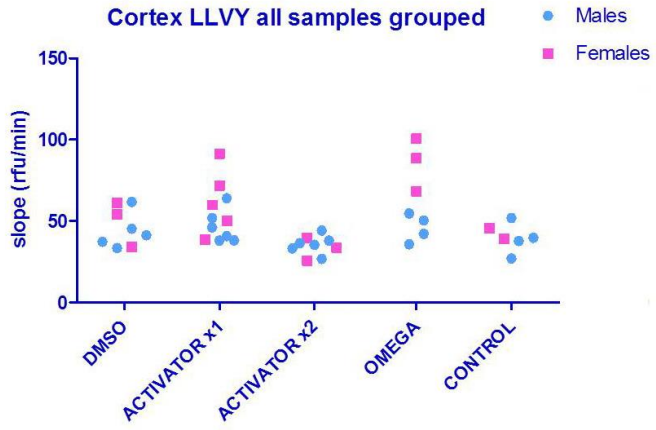


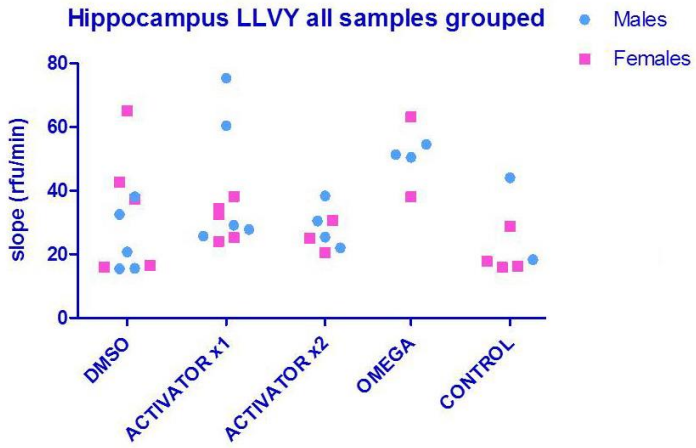


	CORTEX GROUPED STATS 2017+2018				
	DMSO	ACTIVATOR	ACTIVATORx2	OMG3	CONTROL
MEAN	46.1	53.75	34.33	62.95	40.2
Diff. to CONTROL	5.9	13.55	-5.87	22.75	
%Diff. to CONTROL	14.7%	33.7%	-14.6%	56.59%	

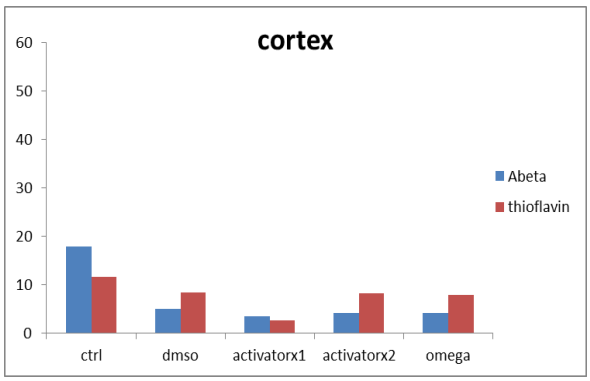
	HIPPOCAMPUS GROUPED STATS 2017+2018				
	DMSO	ACTIVATOR	ACTIVATORx2	OMG3	CONTROL
MEAN	29.9	37.2	27.4	46.3	17.08
Diff. to CONTROL	12.82	20.12	10.32	29.22	
%Diff. to CONTROL	75.1%	117.8%	60.4%	171.08%	

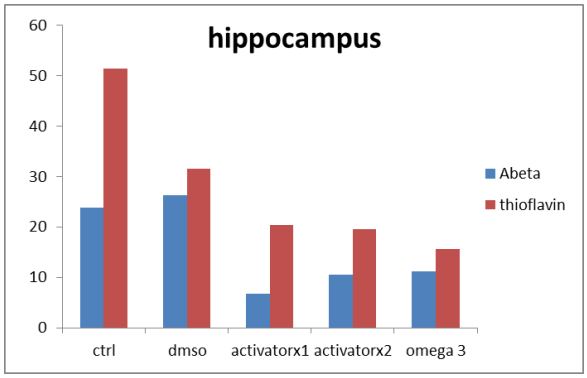
Additionally, when we analyzed individual samples, we detected a significant variation between samples, especially a gender-based variation in the response to treatment in the cortex. We detected a greater response to both 18-alpha GA and omega-3 fatty acid in females compared to males (please see the graphs below).





Analysis of the number of amyloid beta plaques in the cortex and hippocampus showed the following: although the thioflavin staining and immunohistochemical staining with a specific amyloid beta antibody showed some differences in the number of plaques, the number of amyloid beta plaques was decreased in experimental subjects treated both with 18alpha GA (both applied doses) and omega-3 fatty acids in comparison to the control (untreated) and DMSO treated animals. A significant difference in the number of plaques between cortex and hippocampus was observed. Statistical analysis is still in progress.





FUTURE COLLABORATIONS (if applicable)

(max.500 words)

As we have obtained very interesting results about possible protective and therapeutic effects of both 18-alpha-GA and omega-3 fatty acids, we plan to continue with the project. The future collaboration will include long lasting treatment with these two compounds in order to confirm improvement at the behavioural level. As common behavioural deficits can be observed in 5xFAD mice not earlier than 5-6 months of age, we plan to treat animals starting from the 2nd month of age until they are 8-9 months old. During that period, animals will be monitored continuously and tested at appropriate time points in a battery of behavioural tests in order to determine the status of frailty and cognitive outcome. Both research labs will be included in these experiments accordingly.