Effects of antioxidant in mouse model for Alzheimer's: A behavioral phenotyping and microdialysis study

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1 INTRODUCTION

Evidence both supporting and refuting the benefits of antioxidants on Alzheimer's disease and cognitive performance is abundant, leading to uncertainty in the field.

In rodents, treatment with Sulforaphane inhibited Aβ oligomer production, reduced tau hyperphosphorylation, oxidative stress, and neuroinflammation, and prevented decline in spatial learning and memory in a mouse model for AD. It had similar effects in AD-like lesions in a diabetic mouse model (Hou *et al*., 2018, Pu *et al*., 2018). On the other hand, exercise, but not antioxidants reversed impairments in ApoE mice (Chaudhari *et al*., 2016).

Evidence is as inconclusive in humans. Epidemiology studies suggest diets rich in antioxidants reduce risk of AD (Morris, 2009), while clinical studies show AD patients treated with antioxidants have some functional improvement, but no cognitive benefits were observed (Sano *et al*., 1997, Petersen *et al*., 2005).

A randomized, double blind, placebo-controlled clinical trial in mild to moderate AD patients showed that antioxidant treatment did not influence CSF biomarkers related to amyloid or tau pathology, but the treatment did reach its target, as it lowered CSF F2-isoprostane levels in the brain, a biomarker for oxidative stress. However, the treatment may have caused faster cognitive decline (Galasko *et al*., 2012).

In the current study, we attempted to elucidate whether chronic antioxidant treatment could impact AD biomarkers and cognitive performance in the Tg2576 mouse model for Alzheimer's disease. To this aim, we treated 12-week-old female Tg2576 mice with saffron (50 mg/kg daily dose 5-7 days weekly for 17 weeks). Saffron, a potent antioxidant, has been shown to improve learning and memory in an aged mouse model (Papandreou *et al*., 2011), and the neuroprotective and antioxidant effects of saffron have been previously reported (Papandreou et al., 2011, Linardaki *et al*., 2012). After 12 weeks of treatment, behavioral phenotype was evaluated in the Y-maze, open field, EPM, and L/D box. Then, microdialysis was conducted in freely moving animals after 17 weeks of treatment. In dialysates, Aβ 41, 42, and 38, and Tau along with 5-HT, NE, DA, GABA, and Glu were measured.

2 EXPERIMENTAL

Animals

Strain	Sex	Age at 1st dose	Treatment	n
Tg2576	F	12 weeks	Vehicle	10
Tg2576	F	12 weeks	Saffron	10
WT	F	12 weeks	Vehicle	10
WT	F	12 weeks	Saffron	10

Drug administration

Substance	Dose	Volume (ml/kg)	Route	Frequency
Vehicle	--	5 mL/kg	PO	QD (7 days a week, as possible) for 17 weeks
Saffron	50 mg/kg	5 mL/kg	PO	QD (7 days a week, as possible) for 17 weeks

Microdialysis Method Parameters:

Age / Week of dosing:	30 weeks old / 17 weeks of dosing
Prestabilization (h)	1.5
Dialysis flow (µL/min)	0.75
Perfusion fluid (PF)	147 mM NaCl, 3.0 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$
Addition to PF	0.15 % BSA
Sample time (min)	60

2 EXPERIMENTAL

Behavior Method Parameters:

Age / weeks of dosing:	25 weeks old / 12 weeks of dosing
Spontaneous Alternation: (Y-Maze)	Aim: To assess working memory
	Method: Mice were placed in a Y-Maze (arm length: 38 cm and arm width is ~7.5 cm) and left to freely explore for 5 min. Percent alternation (number of alternations * 100 / number of arm visits –2) was used as an index of working memory.
Open Field:	Aim: To assess locomotor activity and anxiety in a novel environment
	Method: Animals were placed in a plastic chamber (16 in X 16 in X 14 in) for 10 min. Time spent in the periphery and center of the arena was recorded.
Light/Dark box:	Aim: To assess anxiety
	Method: Animals were placed in a novel cage, of which 1/3 of the area is covered, creating a dark space and 2/3 is light. Time spent in the light and dark chambers was recorded
Elevated Plus Maze: (EPM)	Aim: To assess anxiety
	Method: Animals were placed in a plastic maze 50cm above the ground, consisting of two closed arms and two open arms (15 x 6 x 30 cm) forming a cross. Time spent in open and closed arms was recorded.

3 RESULTS

Aβ load in hippocampal dialysates:

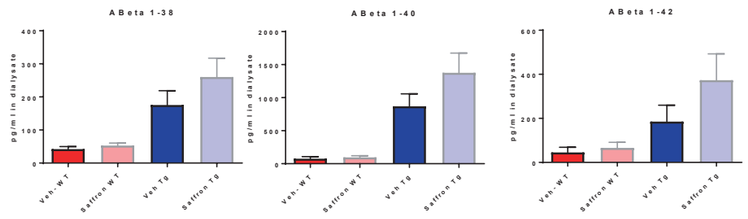

Figure 1: Levels of un-bound Aβ 1-38 and 1-40 (left to right) were significantly higher in transgenic mice than in their wild type littermates at 30 weeks of age. Aβ 1-42 was higher but not significantly in Tg mice compared to WT (one-Way ANOVA).

Behavioral assessment:

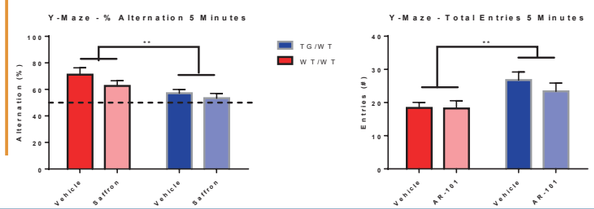

Figure 2: Tg2576 mice had significant reductions in the percent alternation (index of cognitive function) and an increase in total number of entries (index of locomotor activity) in the Y-Maze. (Two-way ANOVA)

3 RESULTS

Behavioral assessment:

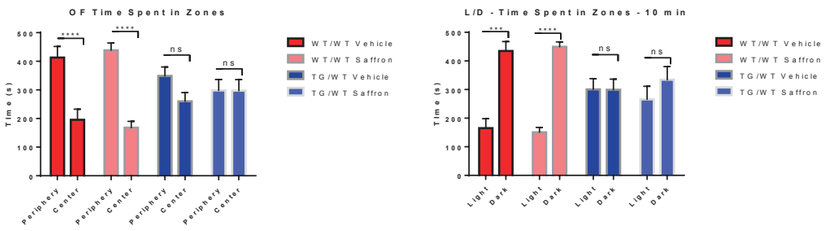

Figure 3: Time spent in periphery vs. center in the open field (OF; left) and in the dark vs. light in the light/dark box (L/D box; right), both indexes for anxiety, were significantly reduced in the Tg2576 mice compared to WT mice (Two-Way ANOVA). Animals that did not pass a blindness test were removed from the L/D box assay.

Neurotransmitter levels in hippocampal dialysates:

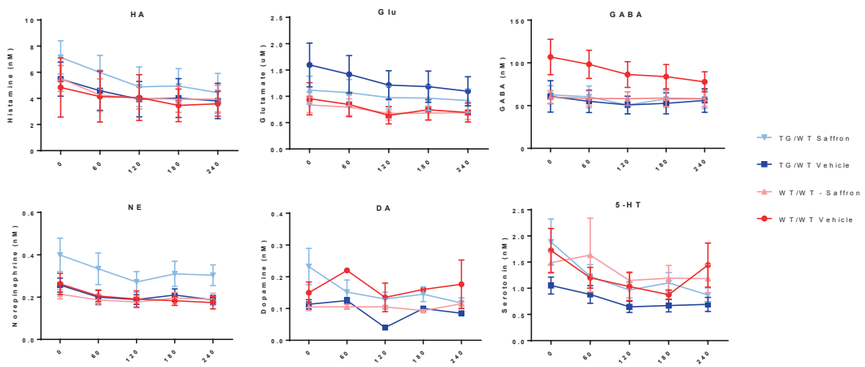

Figure 4 Levels of histamine, glutamate, GABA, norepinephrine, dopamine, and serotonin in the hippocampus of WT and TG2576 mice. Significant differences in GABA and NE were observed between WT and TG animals. Treatment had further effects on GABA and NE, and no other transmitter systems were affected.

4 CONCLUSION

Cognitive and anxiety-related deficits along with neurochemical changes in Aβ and NTS were observed in the Tg2576 mouse model for Alzheimer's Disease. Chronic treatment with the antioxidant saffron at did not affect behavioral deficits or Aβ load, but may have influenced GABA and NE levels.