Evaluating the combination of Lithium and SAM as a Potential Treatment for Alzheimer's disease Pathology in Rat Models

Samuel Rahman
Dr. Claudio Cuello
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Abstract

Alzheimer's disease (AD) is a complex neurodegenerative disorder affecting memory and cognition. There is no available treatment to halt or delay progression of the disease. However, it has been demonstrated in animal models that both s-adenosylmethionine (SAM) and a novel formulation of low dose lithium (NP03) treatments individually do offer some protective effect for AD-related pathologies. No previous study has looked at the combinatorial effects of using both treatments in modifying the progression of AD-like amyloid pathology. In this study, we investigated in the McGill-R-Thy1-APP transgenic rat model of AD-like amyloid pathology whether a combined therapy with SAM and NP03 offered more of protective effect compared to individual treatments. Results showed that a combined treatment was more effective in reducing AD-like pathology than either monotherapy. Not only was the rise of Aβ levels, a pathological hallmark of AD, in transgenic rats reduced more in combined treatment animals than other treatments, but the levels and activity of BACE1, the protein associated with the production Aβ, was also reduced. The knowledge that this combined treatment is more effective in slowing down progression of AD is vital, potentially useful when developing clinical applications and an effective therapy for humans.

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disease that gradually leads to the loss of memory and decline in cognitive ability (Hampel et al. 2012). As the disease progresses, symptoms become more severe and complications ultimately become fatal. The exact cause of AD is unknown and still an active area of research, however it has been characterized by certain factors, such as the accumulation of amyloid beta (Aβ) peptides inside neurons before their aggregation into extracellular plaques, the development of neurofibrillary tangles composed of hyper-phosphorylated tau, increased oxidative stress, inflammation and the loss of neuronal mass (Selkoe, 1991; Yates and Mcloughlin, 2006; Nunomura et al.,2006). There currently is no clinical therapy in preventing or modifying the progression of the disease (Cummings et al., 2018).

Lithium is a drug of particular interest as it has been shown to provide neuroprotective effects that may help preserve cognitive function in patients with bipolar disorder, as well as rescue cognitive function in early stage AD-like pathology animal models with the hopes of translation to AD patients (Wilson et al., 2018). The exact pharmacological mechanism as to how lithium modulates cellular pathways is still being investigated, but it has been linked with the release of second-messengers and downstream signaling molecules which subsequently modulate levels of various transcription factors and gene expression (Oruch et al., 2014).

As a consequence of these changes, lithium has been shown to reduce damage from oxidative stress by reducing free radical formation and lipid peroxidation (Wilson et al., 2018). In addition, lithium has been shown to have anti-inflammatory effects as a result of these downstream effects. (Wilson et al., 2018). The multi-targeted effects of lithium make it an intriguing area for research and a candidate as a potential therapy in AD. Unfortunately, at the

dosage currently prescribed in the clinical setting, lithium does have serious side effects associated with chronic use such as nephrotoxicity, polyuria, nausea, and tremor (Wilson et al., 2018). NP03 is a low-dose lithium formulation where lithium is surrounded in a shell of lipids and surfactants allowing for increased CNS uptake and bioavailability (Wilson et al., 2018; Wilson et al., 2017). Thereby requiring less lithium to be administered and circumvents the negative side effects associated with lithium treatments. The use of NP03 treatments have been shown to reduce the AD amyloid pathology and improve cognitive outcomes in rat models (Wilson et al., 2017). Through modulation of GSK3β, which in turn affect the beta-catenin pathway, Aβ production is reduced (Wilson et al., 2017). As a result of these changes, aspects of cognition such as spatial learning and memory improve (Wilson et al., 2017).

Epigenetic modifications in animal models, specifically DNA methylation, has been shown to be involved in the pathogenesis of several neurodegenerative diseases (Qureshi and Mehler, 2013). What is known so far is that in the brains of AD patients and animal models, global DNA methylation is reduced (Chouliaras et al., 2013). SAM is a universal and ubiquitous methyl donor that participates in various metabolic pathways in the body; administration of SAM can modulate DNA methylation levels (Cavallaro et al., 2016). The use of SAM as a therapy has been considered and it has been shown that chronic administration at early stages can improve cognitive outcomes in animal models (Cavallaro et al., 2016). Restoration of methylation levels affected various genes, which in turn led to less accumulation of Aβ and subsequently there was improvement to cognitive function (Do Carmo et al., 2016). However, these effects only resulted from lower doses of SAM. Higher doses of SAM did affect methylation levels, they did not however provide the reduction in Aβ levels and neuroprotective effects (Carmo et al., 2016).

In this study, McGill-R-Thy1-APP rats, a transgenic animal model that express human

APP, were used. As a result of mutations in these transgenic animal, homozygous animals will, with age, develop AD-like pathology (Leon et al., 2010). They will accumulate elevated levels of intracellular Aβ and develop extracellular plaques Aβ appearing at 6 months along with other hallmarks of the such as decline in cognition (Leon et al., 2010). Of note, treatments were given in early stage of rat development, before development of plaques but where cognitive impairments are already present in order to best model the preventative effects of treatments and their potential in slowing the eventual progression to AD-like pathology.

The aim of this paper is to quantify and assess the efficacy of a preventative combined lithium and SAM treatment in comparison to their monotherapy counterparts in early (pre-plaque) McGill-R-Thy1-APP rats. We hypothesize that preventative combination treatment of lithium and SAM in pre-plaque McGill-APP rats will reduce the progression of Alzheimer's like pathology more efficiently than either NP03 or SAM alone.

Materials and methods

Animals:

McGill-R-Thy1-APP transgenic rats express human amyloid precursor protein (APP) carrying the Swedish and Indiana mutations (Leon et al., 2010). These APP mutations were reported in early-onset (familial) forms of AD in human. These rats thereby mimic the AD-like amyloid pathology, providing a model through which to study progression of AD pathology. Rats were socially housed based on genotype and treatment group. All procedures were carried out in accordance with the guidelines set out by the Canadian Council of Animal Care and were approved by the Animal Care Committee of McGill University.

Study Design:

Prior to my involvement in the project, three month old (pre-plaque) homozygous transgenic (Transgenic) rats and their wild-type (WT) littermates were treated with NP03 (1ml/kg, 40 µg Li/kg body weight) 5 times a week, S-adenosylmethionine (20mg/kg) 3 times a week, a combination of both therapies or vehicle solutions for 12 weeks. Experimental rats were distributed as follow: wild type rats treated with vehicle (n=11), wild type rats treated with NP03 (n=11), wild type rats treated with SAM (n=11), wild type rats treated with both therapies (n=12), transgenic rats treated with vehicle (n=13), transgenic rats treated with NP03 (n=13), transgenic rats treated with SAM (n=12) and transgenic rats treated with both therapies (n=12). The treatments were followed by a 3-week "wash-out" period without the administration of any treatment before carrying behavioral testing and sacrificing the animals.

Immunohistochemistry:

Rats were anesthetized and transcardially perfused with a saline solution. The brains were collected and divided into right and left hemispheres. The right hemisphere was frozen at -80 °C for further analyses. The left hemisphere was fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) for 16 hours, saturated into a 30% sucrose solution in 0.1 M PBS and then sectioned into 40 µm-thick coronal sections using a freezing sledge microtome at -25°C. The sections were stored in a cryoprotectant solution (200mL PBS, 120mL ethylene glycol, 120g sucrose) at -20°C until used for immunohistochemistry.

Rat brain sections were rinsed 3 times for 10 minutes in PBS and were then treated with 3% H_2O_2 + 10% methanol in PBS for 30 minutes. Sections were then rinsed for 10 minutes in PBS and permeabilized by rinsing 2 times for 10 minutes in PBS containing 1% Tween-20 (PBS-T). The sections were blocked for 1 hour in 10% normal goat serum (NGS) in PBS-T for 1

hour at room temperature and subsequently incubated overnight with the primary mouse monoclonal anti-Aβ (McSA1, 1:4000, MediMabs) an antibody recognizing amino acids 1- 12 of human Aβ(Grant et al., 2000). Tissue sections were then washed in PBS-T 3 times for 10 minutes and then incubated in the goat anti-mouse secondary antibody (1:100, MP Biomedical) in 5% NGS in PBS-T for 1 hour at room temperature. This was followed by a 1 hour incubation with mouse anti-peroxidase monoclonal antibody complex (MAP/HRP complex, MediMabs). Sections were then washed and incubated with diaminobenzidine (DAB) (0.6mg/mL PBS) for 10 min (500µL DAB solution/well). The DAB reaction was initiated with 10µL of 1% H_2O_2 in PBS per well and took place for 5 minutes. Sections were washed and left in a shaker overnight. The following day the sections were mounted on slides, dehydrated through a graded alcohol series and with xylenes and then finally cover slipped with Entellan (Sigma Aldrich, USA). Two hippocampal sections per animal were immunolabelled and used for analysis. Imagining was performed using a Zeiss Axiocam microscope running Zen Blue software. To quantify the total amount of McSA1 signal, a region of interest (ROI) was hand-drawn around selected neuron bodies and integrated pixel intensity values were determined for each ROI, using the Image J software (National Institutes of Health, USA).

Electrochemiluminescence-linked immunoassay:

Thirty mg of cortical tissue was homogenized by sonication (3 cycles 2 sec ON, 5 sec OFF) in 10 volumes of TBS buffer (150 mM NaCl, 50 mM Tris HCl, 5 mM EDTA, pH 7.6) complemented with protease inhibitors (1 pellet Complete Mini protease inhibitor (Sigma-Aldrich) per 10ml of buffer). Levels of Aβ38, Aβ40 and Aβ42 were determined by an electrochemiluminescence-linked immunoassay using the Aβ Peptide Panel 1 from Meso Scale Discovery (MSD, Rockville, MD; Cat: N45197A). Plates were processed following

Manufacturer's instructions and were read on a Sector Imager and analyzed using DiscoveryWorkbench software (MSD, Rockville, MD). Aβ peptides concentrations were corrected for protein concentration, as determined by the Lowry assay, and are expressed as pg analyte per mg protein.

Western Blotting:

Twenty mg of Cortical tissue was homogenized in 8 volumes of cell lysis buffer (Cell Lysis Buffer (10X) - Cell Signaling Technology) on ice using a sonicator (3 cycles 2 sec ON, 5 sec OFF) and protein concentrations were assessed using Lowry Protein Assay (BioRad). Normalized loads of each extract (25 ug/lane) were analyzed in triplicate by Western blotting. The primary antibodies applied to the membranes include rabbit anti-pGSK3b (1:1000, Cell signaling, #9323), rabbit anti-GSK3B (1:1000, Cell signaling, #9315), rabbit anti- NF-kB p65 (1:1500, Santa Cruz, #sc-372) and mouse anti-GAPDH (1:1000, Millipore, #MAB374). Primary antibodies were detected using the appropriate HRP-conjugated secondary antibody (1:5000, Jackson labs). Membranes were processed following directions using the Enhanced Chemiluminescent System and were exposed to Hyblot CL films (Denville). Relative integrated optical density was determined using the CLIQS 1D gel analysis software (Total lab Ltd).

ELISA for BACE1 levels and activity:

BACE1 levels in cortical tissue were assessed in duplicate using the Rat BACE1 ELISA kit (Biorbyt cat#orb567219). Cortical extracts were prepared in 8 volumes cell lysis buffer containing protease inhibitors as described above for Western blotting and diluted 1:10 with the dilution buffer provided prior to the assay. BACE1 concentrations were corrected for protein concentration, as determined by the Lowry assay, and are expressed as ng per mg protein.

BACE1 activity was assessed in duplicate using the same extracts than those used for BACE1 levels quantification and the β-secretase (BACE1) Activity Detection Kit (Sigma-Aldrich), an assay involving measuring the fluorescence resonance energy transfer (FRET). Following manufacturer's instruction, enzyme activity was assessed and the fluorescent signal generated was measured using a Synergy fluorimeter at t=0 and t=2hours with excitation set to 320 nm and emission at 405 nm. Fluorescent units (FU) from a blank were assessed and then subtracted from all sample readings generating a standard curve to be used in estimating pmol of substrate cleaved over time. BACE1 activity was corrected for protein concentration, as determined by the Lowry assay, and are expressed as pmol per mg proteins.

Statistics:

Results are expressed as mean ± SEM. Experimental differences were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc comparisons, using GraphPad Prism 5 software (GraphPad Software, USA). Significance was set at $p < 0.05$.

Results:

Intraneuronal Aβ accumulation is reduced by preventive (pre-plaque) treatment with either therapy.

Aβ accumulation is used as an indicator of AD pathology as it has been demonstrated to be elevated in AD patients (Wilson et al., 2016). In order to assess intraneuronal Aβ load in the cortex and hippocampal region, we performed quantitative analyses of McSA1 immunolabeling, an antibody specific to human Aβ from images of the sections. As expected, there was an increase in Aβ levels in McGill-APP transgenic rats as compared to wild type rats, which are devoid of McSA1 immunolabelling (figure 1). The accumulation of Aβ peptides in Transgenic

rats was observed throughout the cortex and in the hippocampal formation and appeared reduced in Transgenic rats treated with either SAM, NP03 or their combination (figure 1 and 2). We then imaged at higher magnification layers V-VI of the cortex, as well as CA1 and subiculum regions of the hippocampus (figure 2) and quantified McSA1 immunoreactivity in CA1 (figure 3). Our results further indicate that Aβ levels significantly decrease in transgenic rats that are treated with NP03 and with a combined SAM-NP03 treatment compared to vehicle treated rats (figure 3). These results suggest that Aβ accumulation can be prevented at pre-plaque stage with a NP03 or combined SAM-NP03 intervention. However, these analyses did not show a difference between combined SAM-NP03 therapy and NP03 treatment alone.

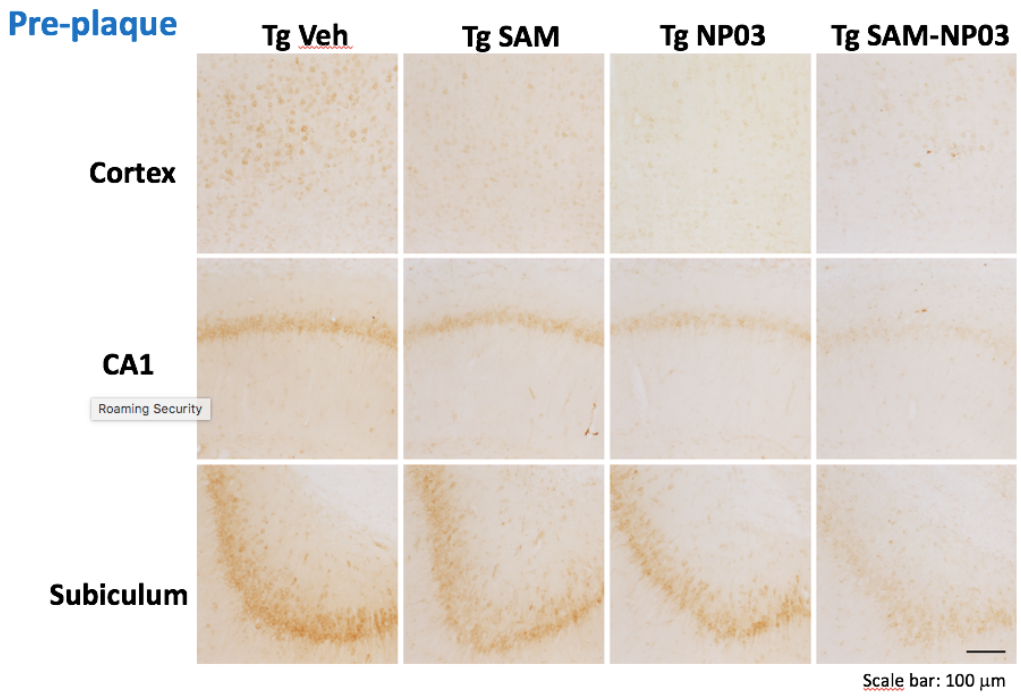

Figure 1. Representative images of McSA1 immunoreactivity (brown) in specific areas of the brain including the cortex and hippocampal regions CA1 and subiculum of wild-type (wt) and pre-plaque McGill-APP transgenic (Tg) rats treated with vehicle, SAM, NP03 or combination of SAM-NP03.

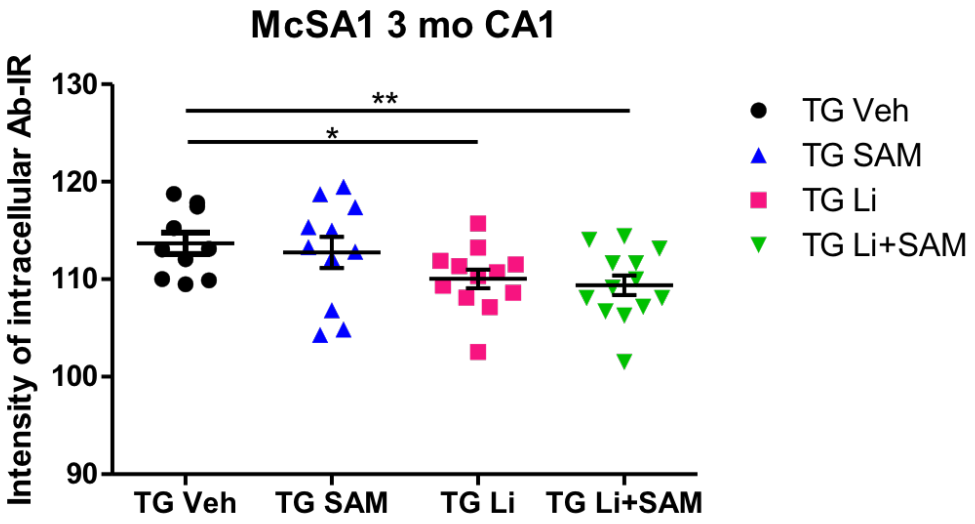

Figure 2: Relative intracellular intensity of McSA1 (Aβ) immunoreactivity in pre-plaque McGill-APP transgenic (Tg) rats treated with vehicle, SAM, NP03 or combination of SAM-NP03. Intensity values recorded as grey level values of the greyscale image.

Decrease in soluble Aβ peptides in cortex

Another method of evaluating progression of AD pathology is measuring levels of soluble Aβ found in brain tissue as at this pre-plaque stage, insoluble Aβ levels are still below detection levels (Do Carmo et al., 2018; Iulita et al., 2014). Based on cleavage of amyloid precursor protein by BACE1, varying fragments of soluble Aβ can be produced. We examined levels of soluble, neurotoxic Aβ 38, Aβ 40 and Aβ 42 peptides using a electrochemiluminescence approach.

As expected, there was a significant increase in the levels of Aβ 38, Aβ 40 and Aβ 42 peptides in vehicle treated transgenic rats compared to vehicle treated wild type rats with Aβ 42 peptides being the most abundant form (figure 4). As well, there was a significant decrease in Aβ38 levels in SAM treated and combined therapy treated rats compared to vehicle treated transgenic rats. However, NP03 treatment alone did not impact Aβ38 levels Transgenic

compared to vehicle-treated transgenic rats. Interestingly, results also indicate that combined therapy with SAM and NP03 was the only treatment regimen capable of significantly reducing Aβ40 levels in transgenic rats (figure 4B).

Finally, Aβ42 levels, the most toxic peptides, were also examined. Both SAM and NP03 monotherapies and combined SAM-NP03 therapies produced a significant reduction in Aβ42 levels with the combined treatment showing a bigger statistical difference compared to the vehicle-treated transgenic group.

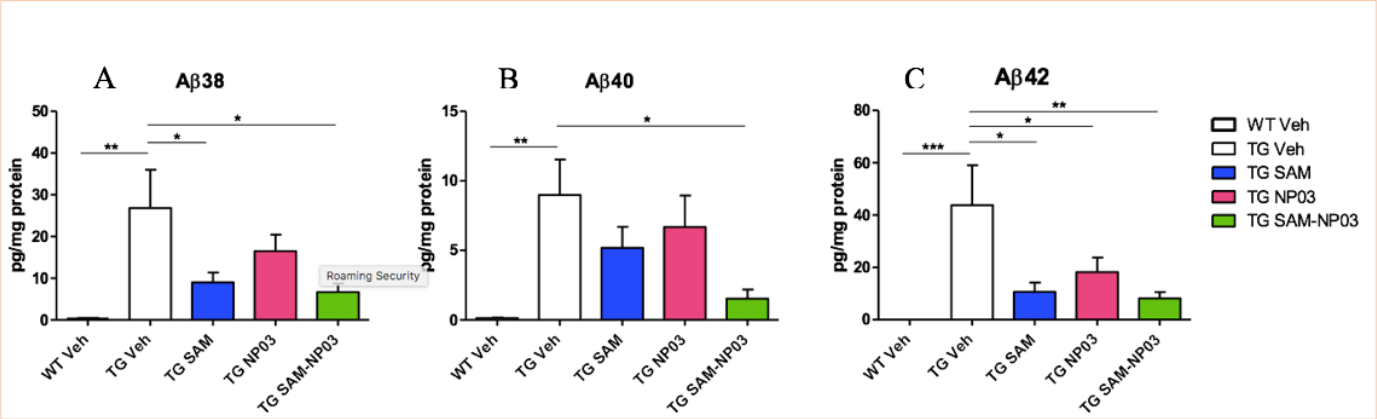

Figure 3: Levels of (A) Aβ38, (B) Aβ40 and (C) Aβ42 peptides in cortical tissue of wild-type (wt) and pre-plaque McGill-APP transgenic (Tg) rats treated with vehicle, SAM, NP03 or combination of SAM-NP03.

Combined SAM-NP03 therapy affect BACE1 levels and activity

Another area under examination was levels of the protein BACE1, involved in the cleaving of amyloid precursor protein into cleaved Aβ fragments. As the precursor to Aβ production, changes in BACE1 expression and activity level are critical to the progression of AD-like pathology. By using an ELISA based assay, we were able to characterize levels of BACE1 protein in brain tissue, which are elevated in vehicle-treated transgenic rats compared to wild-type rats, and witnessed a significant reduction in SAM- and SAM-NP03-treated transgenic rats compared to vehicle treated transgenic rats (figure 5).

In addition, when measuring changes in activity levels of BACE1, we observed a significant increase in the level of BACE1 activity in vehicle-treated transgenic rats compared to wild type rats. This increased activity was then seen to be significantly reduced in NP03- and combined therapy-treated transgenic rats compared to vehicle treated transgenic rats, with the combined therapy producing the most significant difference. However, there was no statistical difference between the combined and the monotherapy regimens (figure 6).

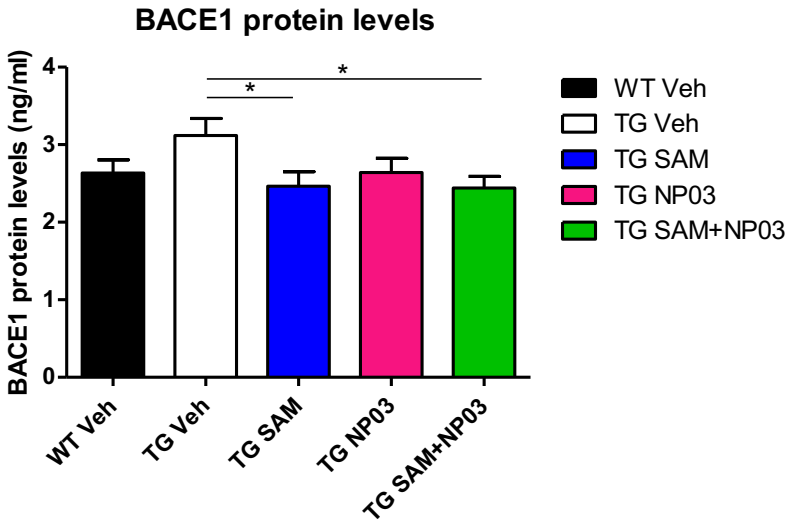

Figure 5: BACE1 protein levels in cortical tissue of wild-type (wt) and pre-plaque McGill-APP transgenic (Tg) rats treated with vehicle, SAM, NP03 or combination of SAM-NP03

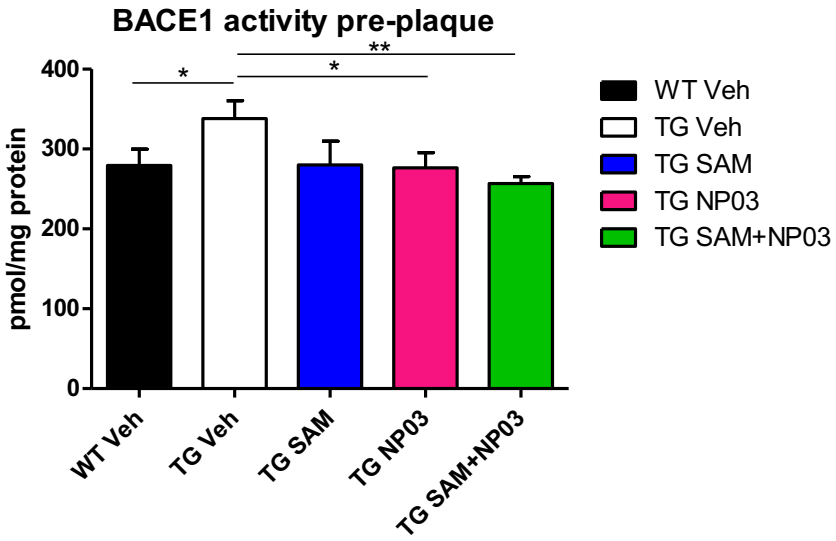

Figure 6: Levels of BACE1 activity in cortical tissue of wild-type (wt) and pre-plaque McGill-APP transgenic (Tg) rats treated with vehicle, SAM, NP03 or combination of SAM-NP03.

Modulation of bioactive molecules

Downstream effects of particular bioactive molecules have been shown to play a vital role in the progression of AD. The two that we have examined so far are GSK3β and NFkB (figure 7). Hyper active GSK3β has been show to play a role in increasing AD amyloid pathology through the beta-catenin pathway (Wilson et al., 2017). Whereas NFkB is a pro-inflammation transcription factor (Liu et al., 2017)

Total GSK3 levels did not vary between treatments or across genotypes. However, the ratio pGSK3β/tGSK3 β levels was significantly reduced in vehicle-treated transgenic rats compared to vehicle-treated wild type rats (figure 8). Further to it, we observed that pGSK3β/tGSK3β levels were elevated in NP03 and SAM-NP03 treated transgenic rats compared to vehicle-treated transgenic rats while SAM treatment did not have a significant effect (figure 8).

NF-kB (p65) levels were also monitored but we could not detect a significant change in expression levels caused by treatments or genotype (figure 9).

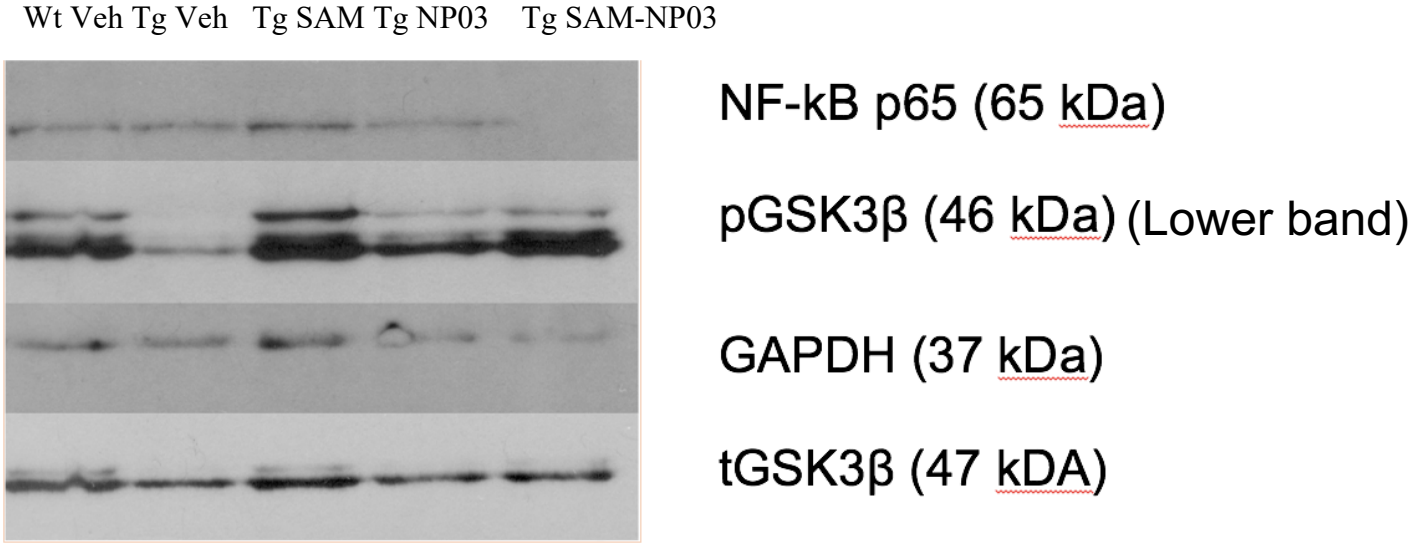

Figure 7: Representative western blot depicting relative expression levels for NFkB subunit p65, phosphorylated GSK3B, GAPDH and total GSK3B in cortical tissue of wild-type (wt) and pre-plaque McGill-APP transgenic (Tg) rats treated with vehicle, SAM, NP03 or combination of SAM-NP03.

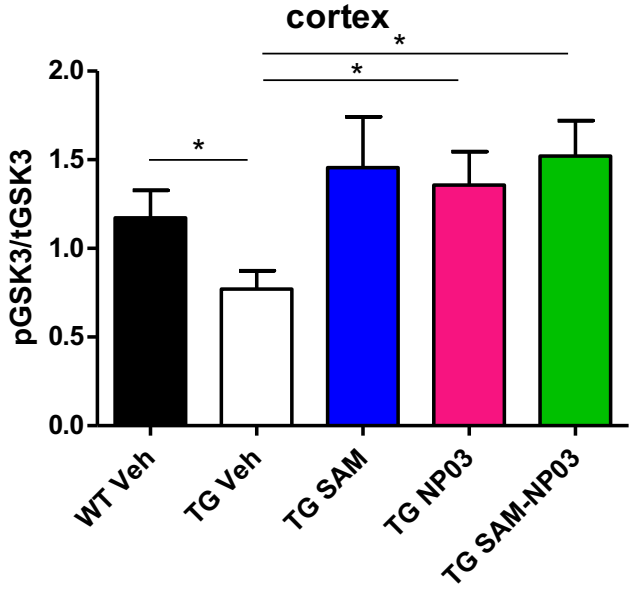

Figure 8: Relative levels of NFkB compared to GAPDH in cortical tissue of wild-type (wt) and pre-plaque McGill-APP transgenic (Tg) rats treated with vehicle, SAM, NP03 or combination of SAM-NP03.

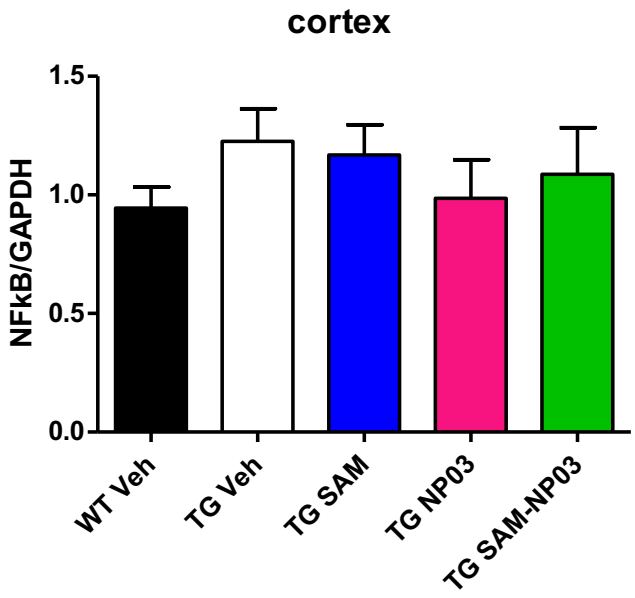

Figure 9: Relative levels of phosphorylatedGSk3β compared to totalGSK3β in cortical tissue of wild-type (wt) and pre-plaque McGill-APP transgenic (Tg) rats treated with vehicle, SAM, NP03 or combination of SAM-NP03.

Discussion

Microdose lithium and SAM treatments are promising candidates for an novel therapy for treating Alzhiemer's disease. Individually, both treatments have shown substantial success in modulating the AD-like pathology progression in animal models (Do Carmo et al., 2016; Wilson et al., 2017). However, as AD is a multi-faceted disease, therapies that interfere with several disease processes, such as multi-targeted combination therapies have a greater potential for intervention and translation to the human AD pathology (Cummings et al., 2016). In addition, application of therapeutics at early disease stages offer the best prognosis (Cummings et al., 2018). Treatment once patients present with symptoms is not favorable as noticible symptoms only occur once there are already significant Aβ plaque formations and severe neuronal loss (Sperling et al., 2014). Any positive effects of therapies will be severely limited because at this point there is significant irreversible damage of the brain, cognition cannot be restored (Sperling

et al., 2014). The goal then is to develop an early treatment that prevents progression of the disease to this point. As with any potential novel therapy, the basis of its efficacy must be first tested in animal models recapitulating aspects of the human AD pathology. In this case we studied the effects of treatments on McGill-R-Thy1-APP transgenic rats. The benefits of using a rat model over the tradition mouse model is that rats are more similar to humans genetically and physiologically. Most importantly, rats have better and more refined motor skills along with substantially more complex behaviors than mice (Do Carmo and Cuello, 2013).

Although there are many theories as to what is the underlying cause of AD, one that hold extreme promise is the amyloid hypothesis. This theory suggests that the resultant AD pathology is due to the increased levels of neurotoxic Aβ (Hardy, 2002). As such, we measured the effects of our treatments in reducing the increase in Aβ load in the brain over time. McGill-APP transgenic rats express mutated human APP. As such, elevated levels of Aβ are produced and deposited in their neurons compared to wild type rats (Leon et al., 2010). Fitting the parameters of the amyloid hypothesis. An ideal treatment, when given early in the pre-plaque stage, should result in Aβ levels that best mimic wild type levels. We observed that although SAM and lithium treatments, when given early, did reduce the Aβ load seen in the neurons after 3 months; individually, the combination of the two treatments reduced it the most. This is in line with our expectations that a combination of these therapies provides a greater protective potential in slowing down the AD-like pathology when given early in animal models compared to SAM and NP03 monotherapy. Of note, no treatment dampened Aβ levels equal to those seen in wild type animals, thus treatments cannot fully stop progression of the disease which is ideally the result of any AD treatment. Albeit this can also perhaps be attributed to the fact that this model carries familial mutations of AD, in which as a result of mutations it is inevitable that transgenic rats

develop AD-like phenotype (Leon et al. 2010). If treatments were used in a model of sporadic AD, cases without a family link, perhaps treatments could affect patient susceptibility and effect factors in the early progression of the disease.

There are many forms of Aβ, resultant of the multiple ways that its precursor APP can be cleaved (Chow et al., 2009). Our study measured levels of Aβ38, Aβ40 and Aβ42, of note is that Aβ42 is the fragment considered most toxic and of interest to our study (Klein et al., 1999). Monotherapy with SAM or NP03 reduced levels of all Aβ fragments. However, it was the combination treatment that reduced it the most. Interestingly, the combination of SAM and NP03 had a greater effect on neurotoxic Aβ42 than on Aβ38 and Aβ40. Again demonstrating that the combined treatment group receives the most protective effects from treatment.

Although simply measuring levels of Aβ can give us some understanding of the efficacy of treatments, it is surface level observation and does not truly explain the underlying reason for the protective effect. Thus, it is critical to understand why or how a treatment works. To produce Aβ, APP must be cleaved through the amyloidogenic pathway first by beta-site APP cleaving enzyme 1 (BACE1) (Cole and Vasser, 2007). As such, we analyzed both levels of BACE1 as well as monitoring its activity. Intervention with treatments lowered both levels of the BACE1 protein and its cleaving activity. Of note is that the combined treatment lowered BACE1 activity more than monotherapy treatments, indicating more favorable outcome by combining treatments. What is important about these findings is that these treatments, when given early reduce the production as well as the activity of BACE1 to levels in line with those of wild type animals. It is not simply a reduction in overall levels of BACE1 which as a result reduces Aβ levels.

Finally, we monitored levels of bioactive molecules such as pGSK3β and NFkB, both involved in the downstream expression of Aβ. pGSK3β has been demonstrated to play a role in

the β-catenin pathway known to be involved in the AD amyloid pathology (Hernández et al., 2009). By inactivating hyperactive pGSK3β, it has been shown to reduce levels of BACE1 and in turn reduce Aβ levels in the brain and animal models. In turn leading to better cognitive outcomes (Hernández et al., 2009). NFkB is a pro-inflammatory mediator that can modulate multiple target related to inflammation through downstream effects and manipulation of second messenger systems (Liu et al., 2017). NFkB did not have a significant change between wild type and transgenic rats. This was also seen from treatment groups, as no treatment significantly modulated expression levels. pGSK3β levels decreased in transgenic rats compared to wild type. This change was then counteracted with treatments that all elevated pGSK3β levels as a factor of total GSK3β. However, the combined treatment did not have a significantly greater increase compared to individual treatments.

Although our experiments garnered favorable results, there are some important limitations. For instance, experiments relied on the use of transgenic animal model. Concerns with the model arise when questioning whether the AD-like phenotype expressed in the transgenic model accurately mimics AD in humans. This transgenic rat model is reliant on the amyloid hypothesis and not evaluating other potential causes such as tau hyper-phosphorylation. In addition, as the data was generated from experiments performed in rats, there are species differences and care must be taken when translating results for humans, even though rats are closer to humans than mice in which most models have been generated.

Conclusion

In summary, combined treatment was more effective in its degree of preventing the expected increase of Aβ levels in the brains of transgenic rats than either SAM and NP03

monotherapy. As well, the combined treatment with SAM and NP03maintained BACE1 levels and activity to be more in line with those seen in wild type animals rather than the elevated levels seen in transgenic animals. pGSK3B levels that had been reduced in transgenic animals were also rescued by combined treatment. NFkB levels were not observed to be changed. In short, combined treatment with SAM and NP03 is a more promising route for preventing AD-like pathology development than individual treatment with lithium or SAM.

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