



**CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY**

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Project Title Alzheimer's and Inflammation: Exploring Enzymatic Pathways Involved in Beta Amyloid Induced TNF alpha Production	
<p style="text-align: center;">Abstract</p> <p>Objectives/Goals A recent theory about the pathology of Alzheimer's disease (AD) is the inflammation hypothesis, which suggests that the inflammatory response in the brain is central to the disease. The objectives of this study were to determine 1) the effect of treatment with fibrillar beta amyloid 1-42 (BA 1-42) on tumor necrosis factor alpha production and proliferation in the J774.2 macrophage cell line and 2) the effect of inhibition of NADPH oxidase, calcineurin, protein kinase C, and NFkB (by treating with the effectors apocynin, fujimycin, tamoxifen, and carnosol respectively) on production of TNF-a and macrophage proliferation. These pathways are responsible for functions such as ROS production, calcium regulation, cell growth, and immune response.</p> <p>Methods/Materials J774.2 macrophages were cultured in DMEM +10% FBS+ 1%Pen-strep , then plated into 24 well plates for treatment with 2.5 uM fibrillar BA alone or with apocynin, carnosol, fujimycin, or tamoxifen in concentrations from 100 nM-300 uM. A 0.03% DMSO vehicle control was also used. After 24 hours, TNF-a concentrations were detected by performing an ELISA assay. At 48 hours cells were stained and counted with Trypan blue, and cell concentration and viability percentages were calculated.</p> <p>Results Using ANOVA, it was determined that apocynin, fujimycin, and tamoxifen significantly decreased TNF-alpha concentration in a dose dependent manner. Carnosol had no significant effect on TNF-a production or cell number until the highest concentrations of 100 and 300 uM, where it appeared to induce significant apoptosis. The data for acetovanillone confirmed observations in existing literature. The IC50 values for apocynin, fujimycin, and tamoxifen were respectively: 9.6 uM, 2.5 uM, and 14.6 uM. Additionally, apocynin, fujimycin, and tamoxifen significantly reduced macrophage proliferation as determined by Trypan blue exclusion.</p> <p>Conclusions/Discussion This study has identified, for the first time, that fujimycin and tamoxifen citrate inhibit BA 1-42 induced TNF-a production and macrophage proliferation. These pathways, calcineurin and protein kinase C, are thus possible therapeutic targets for beta amyloid induced neuroinflammation, specifically Alzheimer's disease. The next step, currently being performed, in this ongoing study is analyzing the effects of beta amyloid treatment on inflammatory gene expression in macrophages and apoptosis in cocultured neurons.</p>	
Summary Statement This study investigated the roles of several different proteins in inhibition of beta amyloid induced tumor necrosis alpha production and macrophage proliferation and identified fujimycin and tamoxifen as novel inhibitors of BA induced TNF.	
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