



CALIFORNIA STATE SCIENCE FAIR 2011 PROJECT SUMMARY

Name(s) Anin Sayana	Project Number 31471
Project Title Discovery of the 2,4-Diaminopyrimidine as a Novel Therapeutic Solution for c-Fms and TNF Induced Rheumatoid Arthritis	
Abstract Objectives/Goals Rheumatoid arthritis has a worldwide estimated prevalence of 2%, according to the Johns Hopkins Arthritis Center. The current methods to treat this disease involve targeting of the pro-inflammatory cytokine Tumor Necrosis Factor (TNF-alpha) and are not entirely effective. Recent research shows that c-Fms plays a major role in joint deterioration associated with rheumatoid arthritis. My project aimed to discover a novel inhibitor of the TNF cytokine and the c-Fms receptor tyrosine kinase. This inhibitor, 2,4-diaminopyrimidine, could potentially lead to an effective treatment for rheumatoid arthritis. Methods/Materials TNF alpha-expressing Rat2 fibroblast cells were cultured in DMEM+10% FBS+1% Pen-Strep. The fibroblasts were then plated into six well plates for treatment with water (control), Imatinib, and 2,4-diaminopyrimidine. 2,4-Diaminopyrimidine was added at concentrations of 5 uM, 15 uM, and 25 uM, and dilutions were determined based on their molecular weight and calculation from the starting stock, which was 75mM. Imatinib was added at the concentrations of 5 mg/ml, 15 mg/ml, and 25 mg/ml. After a 48 hour incubation, an ELISA assay was conducted to detect TNF levels. TNF alpha concentrations were then measured using a plate reader (spectrophotometer) and converted into pg/ml after plotting the standard curve. Results 2,4-Diaminopyrimidine significantly reduced the concentrations of TNF alpha in a dose dependent manner. In comparison to water, 2,4-diaminopyrimidine at 25 uM reduced TNF alpha levels by 22%, 15 uM reduced TNF alpha levels by 19%, and 5 uM reduced TNF alpha levels by 14%. In comparison to Imatinib, 2,4-diaminopyrimidine at 25 uM reduced TNF alpha levels by 19%, 15 uM reduced TNF alpha levels by 15%, and 5 uM reduced TNF alpha levels by 12%. Conclusions/Discussion This research has discovered for the first time that 2,4-diaminopyrimidine inhibits TNF alpha production, supporting my hypothesis. 2,4-Diaminopyrimidine was discovered after investigating into the structures of various inhibitors and analyzing their classifications. In the tests, higher concentrations of 2,4-diaminopyrimidine led to lower levels of TNF alpha. 2,4-Diaminopyrimidine inhibits TNF alpha by binding to the TNF in the cells, preventing its interaction with TNF alpha receptors on the surface of the cells. This discovery could lead to a possible therapeutic solution for c-Fms and TNF alpha induced rheumatoid arthritis.	
Summary Statement In this in vitro study, I investigated and identified 2,4-diaminopyrimidine as a novel inhibitor of Tumor Necrosis Factor Alpha, which could lead to a possible therapeutic solution for c-Fms and TNF induced rheumatoid arthritis.	
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