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THE EFFECTS OF BETA AMYLOID PROTEIN AND METHANESULFONYL FLUORIDE ON COMPLEMENT ACTIVITY

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DEDICATION

To my parents who taught me to enjoy and believe in what I do.

THE EFFECTS OF BETA AMYLOID PROTEIN AND METHANESULFONYL FLUORIDE ON COMPLEMENT ACTIVITY

by

HUGO SANDOVAL, B.S.

THESIS

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ABSTRACT

The beta amyloid protein (Aβ) which forms the core of the extra-cellular plaques found in Alzheimer's Disease has been shown to be involved with complement activation. Activation of the complement system could be a mediator of chronic brain inflammation which is believed to enhance neuronal cell damage and death. Polyacrylamide gel electrophoresis and Western blot techniques were used to study Aβ interaction with the complement proteins. Western Blots were developed with primary antibodies for C2, C3, C4, C5, and Factor B. In these membranes we could detect the cleavage of C2, C3, and C4. Cleavage of Factor B and C5 was not observed. To further the aims of the study a classical pathway hemolysis detection kit was used. Complement activity was reduced by prior incubation of serum with Aβ protein, providing evidence that Aβ activates the classical pathway of complement. Additional evidence of classical pathway activation by Aβ was provided by lysis of human erythrocytes by autologous serum treated with Aβ.

Another part of this study was to evaluate Methanesulfonyl Fluoride (MSF) as a complement inhibitor. MSF has been used as a specific acetylcholinesterase inhibitor. Its mechanism of action is believed to be due to its binding to a serine residue in the active site. Because of this interaction with serine it might also act to inhibit C1r and C1s of the complement system. To study this interaction and to ascertain if some of the benefits observed with MSF are caused by a reduction in brain inflammation, I used the classical pathway hemolysis detection kit. Complement mediated sheep red blood cell lysis was reduced when serum was incubated with MSF prior to incubation with antibody sensitized sheep red blood cells. The concentration of MSF necessary to inhibit

complement mediated lysis was several hundred times higher than the dose being used for treatment of Alzheimer's Disease. Serum from a patient receiving MSF treatment had a normal level of complement activity suggesting that the benefits of MSF are not caused by complement inhibition.

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