Analyzing the Identification of a Novel Biomarker for Parkinson’s Disease
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Introduction

Parkinson’s disease (PD) is a neurodegenerative disease characterized by the death of dopaminergic neurons in the substantia nigra. Uric acid (UA) is an antioxidant produced in response to reactive oxygen species; UA reduces oxidative stress, a mechanism thought to be involved in the pathogenesis of PD. The reaction of UA with the reactive oxygen species (ROS) superoxide, nitric oxide, or peroxynitrite produce identifiable reaction products of allantoin, 6-aminoaracil, and triuret, respectively. Nitric oxide and superoxide can react to form peroxynitrite which is then transformed into triuret by the actions of UA, as seen in Figure 1.

Parkinson’s is not clinically diagnosable until symptoms present; at this point around 50% of dopaminergic neurons have died. This limitation on detection of Parkinson’s disease. In this study, we seek to analyze the methods used to detect biomarkers in the blood of Parkinsonian animals as well as to optimize the methods of sample collection through a study on protein precipitate solvents. We also aimed to identify these products of uric acid reacting with reactive oxygen species in blood of Parkinsonian animals so as to connect the presence of these compounds with the neurodegeneration associated with Parkinson’s. The product of peroxynitrite reacting with uric acid, triuret, was identified at elevated levels in Parkinsonian blood. These results provide preliminary implications for the identification of triuret as a biomarker for early detection of Parkinson’s disease. Furthermore, a study is being conducted to determine the shifts between reactants and products in this reaction.

Study of Protein Precipitate Solvent

Two solvents for precipitation of proteins from blood samples, 0.4M perchloric acid and methanol, were examined through applications of each to a series of blood samples that were spiked (or not spiked) with known concentrations of the biomarker of interest, triuret. A comparison of identical blood samples, shown in Figures 3 and 4, indicates the difference in analysis after treatment with the two different protein precipitate solvents. From this analysis, it can be determined that the use of methanol allows for a clearer analysis of the sample. This process allowed for optimization of the method for preparation of blood samples to be analyzed.

Figure 3: Precipitation of Blood Sample K with Methanol

Figure 4: Precipitation of Blood Sample K with 0.4M Perchloric Acid

Time Study/Future Implications

Currently, a time study is underway to determine the actions of triuret in a blood sample over time. By spiking some blood samples with known concentrations of triuret, some insight may be provided about the actions and reactions of triuret in blood over time. Blood samples were treated with methanol to remove proteins from the blood and were then spiked with known concentrations of triuret. Samples spiked and not spiked were analyzed using liquid chromatography multiple reaction monitoring and results were noted. These samples will now undergo a treatment of 1 month frozen at -80°C and will be analyzed again following this treatment. The purpose of this study is to determine the actions of triuret over time so to gain insight into any potential actions of triuret in blood.

Work Cited

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