CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

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Project Number

S0597

Project Title

The Effect of Carnosine on Glycation-induced Aggregation of a Human Therapeutic Antibody

Abstract

Objectives/Goals

The non-enzymatic reaction of a sugar with a protein is referred to as glycation. The glycation of the therapeutic proteins has the potential to alter the structure, function and stability of the protein. This can lead to a variety of chemical and conformational changes, one of which is the aggregation of the protein therapeutic. Protein aggregation is associated with an increased safety risk if it results in the development of an antibody response to the therapeutic given to the patient. My project goal was to determine if carnosine (CAR), a naturally occurring dipeptide, could inhibit the glycation-induced aggregation of a human antibody.

Methods/Materials

A degradation product of glucose, methylglyoxal (MGO), was used to induce the glycation of a human immunoglobulin gamma (IgG) under various conditions. The treated samples were then buffer-exchanged to stop the glycation reaction by removing the free MGO (and CAR) using Sephadex G-50 spin columns. The aggregated antibody was first separated by size exclusion chromatography (SEC) using two BioSep-SEC-S3000 columns (Phenomenex) in series followed by a standard UV detector (Agilent 1100 Series). Samples were also analyzed using a multi-angle light scatter (MALS) detector using an Agilent 1200 series (Amgen, Inc.). For each sample chromatogram, the peak area percent was integrated using ChemStation software and presented as individual aggregate (High Molecular Weight (HMW), trimer, and dimer) and total aggregate.

Results

The antibody aggregate formation was dependent on the concentration of the antibody and methylglyoxal. Using 5 mg/mL of IgG plus 100 mM of MGO, were measured approximately a 25% increase in IgG aggregation compared to untreated IgG control. Using increasing concentrations of carnosine, a dose-dependent inhibition of IgG aggregation was observed up to a 75% aggregate inhibition compared to IgG control. A decrease in the particle size of the HMW aggregates was confirmed by MALS.

Conclusions/Discussion

The presence of reducing sugars throughout the cell culture production and manufacturing process of a protein therapeutic can result in the glycation-induced aggregation. The addition of carnosine can reduce the glycation-induced aggregation of the protein therapeutic. The reduction in aggregate formation can improve the product quality of the therapeutic, reducing the immunogenicity risk to the patient.

Summary Statement

I have demonstrated that the use of Carnosine can reduce the amount of glycation-induced aggregation, mitigating a leading cause of immunogenicity.

Help Received

Mentored by Dr. Greg Cauchon (Dir, Amethyst), Dr. Dan Mytych (Sci Dir-Amgen) and Dr. Nikki Malhotra (Instructor at TOHS); Experiment#s were performed at the Amgen facilities under supervision by Dr. Dan Mytych. SEC-MALS detection performed at Amgen under supervision of Jill Miller