Withaferin-A in Ameliorating the Effects of High Glucose on Inflammatory and Phagocytic Response of Mouse Macrophages

Trae Whyte
Satyesh K. Sinha, Ph.D., Charles R. Drew University of Medicine and Science

Coordinating Center: Charles R. Drew University of Medicine and Science

ABSTRACT

Rapidly increasing rates of diabetes mellitus (DM) throughout the world represent an emerging epidemic with profound consequences including diabetic nephropathy (DN). Studies indicate that mφ-mediated inflammation correlates with the development of DN. Macrophages exhibit pro- (M1) and anti-inflammatory (M2) phenotypes. Therefore, in the present study, we tested our hypothesis that high glucose suppresses the M2 phenotype and phagocytosis, leading to aberrant cytokine release, and that withaferin-A (an anti-inflammatory molecule) will reduce the pro-inflammatory response of macrophages.

We cultured J-774A.1 macrophage-like cells (ATCC) in RPMI 1640. After reaching 70-80% confluence, the cells were serum starved for 18 hours. Cells were then treated with D-glucose (5, and 25mM) for 24h. We extracted the total protein from the cells to measure the expression level of arginase-1, TGF- beta, LC3 and GPNMB by way of the western blot process. In a second set of experiments, we treated the cells with withaferin-A. In these cells, we will measure the level of secreted pro-inflammatory cytokines in culture media using ELISA.

Preliminary data under our ongoing experiments indicate that high glucose treated macrophages exhibited reduced levels of markers of M2 phenotype (Arginase-1, and TGF- beta) and phagocytosis (GPNMB, and LC3). We are planning to conduct the experiments related to the excretion of pro-inflammatory cytokines in high glucose with and without withaferin-A.

Our results indicate that high glucose modulates the expression of markers of M2 phenotype and phagocytosis in macrophages. We are continuing our experiments to confirm the effects of withaferin-A on the secretion of pro-inflammatory cytokines in high glucose.

Keywords: Withaferin-A, Diabetes Mellitus, High Glucose, Diabetic Nephropathy
ACKNOWLEDGEMENTS

The STEP-UP HS program is supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health, Grant Number: 5R25DK078384-09.