Effects of Fructose-Derived Advanced Glycation End Products on Acetylation of Histones in the Brain

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Effects of Fructose-Derived Advanced Glycation End Products on Acetylation of Histones in the Brain

Abstract
Objective: The objective of this study was to determine the effects of fructose and their advanced glycation end products (fru-AGES) on histone acetylation in microglia, the immune cells of the brain.

Significance: Fru-AGES primarily form as a result of non-enzymatic reactions between fructose and proteins. One result is inflammation in the brain, which can be directly correlated to increased microglia activity. Microglial activity has been shown to be associated with the acetylation of histones, resulting in a change in transcription of inflammatory genes. Elucidation of a direct link between fructose, fru-AGES and histone acetylation would increase understanding the pathophysiology of inflammatory disorders such as Alzheimer’s disease.

Experimental Procedures: An immortalized rat microglial cell line was treated in vitro with control media, fru-AGES or fructose. Histone acetylation was analyzed indirectly through activity of histone deacetylase (HDAC) using the HDAC Glo I/II Assay (Promega). Chemiluminescent product formation was measuring with a spectrophotometer.

Results Obtained: Both treatments with fructose and fru-AGES showed an increase in HDAC activity compared to control by up to 35% and 20%, respectively, correlating to a decrease in global histone acetylation. This is contradictory to initial expectations, as a decrease in acetylation could result in a decrease in transcription of genes. Despite causing an initial inflammatory response, fructose and fru-AGES appear to suppress overall gene transcription.

Conclusion: Previous data show that exposure of microglia to fructose and fru-AGES results in a pro-inflammatory activated state. However, at the level of gene transcription, microglia may be desensitized and less able to respond in the long term.

Disciplines
Pharmacy and Pharmaceutical Sciences

Comments
Introduction
Microglia are considered the immune cells of the central nervous system (CNS). They are found throughout the brain and are in a resting state when their resting state, allowing them to monitor the tissue that surrounds them. Microglia will change into an amoeboid form when they come in contact with a stimulus that is detrimental to the CNS. These triggers can include damaged neuronal cells, foreign matter, plaques, pathogens, or other inflammatory triggers (e.g., advanced glycation end products (AGES)). Fruuctose, or fruit sugar, is a naturally occurring simple sugar that is often found as a backbone with glucose and fructose when they come in contact with the body with glucose and fructose when they come in contact with a protein which provides the AGES used in our experimental procedure. These AGES can then go on to produce inflammation and tissue damage in the body.

Objective
Using prior knowledge of glucose, fructose, and fructose-AGES, the objective of this study was to determine the effects of fructose and their advanced glycation end-products (fru-AGES) on histone acetylation in microglia, the immune cells of the brain. In order to determine this, we studied changes in inflammatory gene expression after microglia were exposed to fru-AGES. In addition, we analyzed histone acetylation in microglia changes in histone deacetylase enzymes activity after being exposed to fructose and fru-AGES.

Methods
Fru-AGES were produced by incubating fructose with bovine serum albumin for 8 weeks at 37°C. An immortalized rat microglial cell line was treated with fructose with a concentration of 0.8, 0.4, 0.2, and 0.1 mM. Samples were collected at 3 hours of treatment with fructose and then the chemiluminescence proportional to the level of HDAC activity. Histone deacetylase (HDAC) activity was measured, showing up to a 37% increase. Increased HDAC activity would correlate to a global decrease in histone acetylation after treatment with fructose or fru-AGES.

Figure 4: A) Cells were treated with Fru-AGES and then the chemiluminescence proportional to the level of HDAC activity was measured, showing up to a 37% increase. Increased HDAC activity would correlate to a global decrease in histone acetylation after treatment with fructose or fru-AGES.

Figure 6: Expression of genes related to microglial activation and inflammation was measured. Three genes, tumor necrosis factor alpha (TNFα), cyclooxygenase-2 (COX2), and toll-like receptor 4 (TLR4) were analyzed for expression after the addition of increasing concentrations of fru-AGES to microglia 24 hours prior. Results determined an increase in both TNFalpha and C5 (0.8 µM) and 0.2 µM treatments resulted in the highest dose of fru-AGES.

References

Conclusions
• Gene expression analyses of TNF alpha and C5 showed up to a 6-fold increase in gene expression with the treatment of fru-AGES 24 hours prior to analysis. The TLR4 gene expression decreased after the treatment with higher doses of fru-AGES. This confirms that fru-AGES elicit an inflammatory response in microglia, producing an activated phenotype.
• Both treatments with fructose and fru-AGES showed an increase in HDAC activity compared to control by up to 80% and 40%, respectively, correlating to a decrease in global histone acetylation. This is contradictory to initial expectations, as a decrease in acetylation could result in a decrease in transcription of genes. Despite increase in inflammatory cytokine gene transcription (TNFalpha and C5), fructose and fru-AGES appear to suppress overall gene transcription, which is reflected in TLR4 expression.
• Previous data show that exposure of microglia to fructose and fru-AGES results in a pro-inflammatory activated state. However, at the level of gene transcription, microglia may be desensitized and less able to respond in the long term.
• It is possible that gene transcription may be altered earlier than 24 hours, and a 3 hour treatment plan for the gene expression assay may be beneficial to obtain in future experiments.