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

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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 deacetlyase (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
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Introduction

Microglia are considered the immune cells of the central nervous system (CNS). They are found throughout the brain and are seen in a ramified, or branched form when in 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)).[1] Fructose, or fruit sugar, is a naturally occurring simple sugar that is often found as a sweetener in many industrial food products such as soft drinks.[2] Fructose is highly prevalent in the average everyday American diet. With high consumption of fructose, its reaction with proteins can cause the formation of fructose advanced glycation end-products(Fru-AGES). One result of the build up of these fru-AGES is inflammation in the brain, which can be directly correlated to increased microglia activity.[3] Microglial activity has been shown to be associated with the acetylation of histones, a chemical modification that increases the accessibility of DNA to transcriptional machinery.[4] Increases in histone acetylation is associated with increase gene transcription, and vice versa. 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.

Objective

Using prior knowledge of glucose, fructose and fru-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 via changes in histone deacetylase enzyme 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.[3] An immortalized rat microglial cell line was treated *in vitro* with control media, fru-AGES or fructose. A quantitative real-time polymerase chain reaction (qRT-PCR) was performed after the treatment of fru-AGES at a 24 hour time point at concentrations of 0.8, 0.4, 0.2, and 0.1 μg/μL. The qRT-PCR measured the gene expression of pro-inflammatory markers such as complement 5 (C5) and tumor necrosis factor α (TNFα) as well as cell-surface receptors such as the toll-like receptor 4 (TLR4). Histone acetylation was analyzed indirectly through activity of histone deacetlyase (HDAC) using the HDAC Glo I/II Assay (Promega Corporation), after 3 hours of treatment with fruAGEs or fructose concentrations of 100, 50 , 25 or 10 mM. Chemiluminescent product formation was measured with a spectrophotometer and directly correlated with HDAC activity.

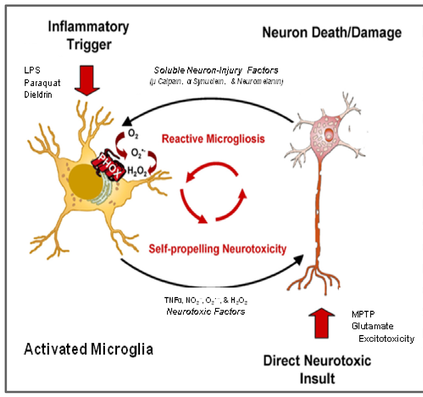

Figure 1: When in contact with inflammatory signals, activated microglia release pro-inflammatory molecules like cytokines and reactive oxygen species. These can be deleterious to neurons in high concentrations suggesting the role in neurodegeneration. This figure is adapted from Lull, 2007.[1]

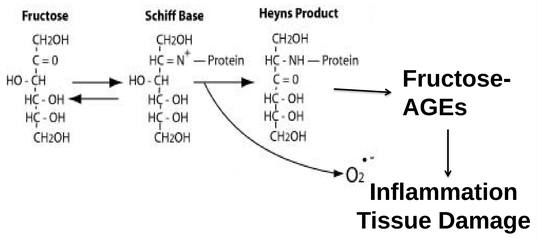

Figure 2: Schematic of the production of Advanced Glycation Endproducts (AGEs). This mechanism can be utilized in the body with glucose and fructose when they come in contact with a protein which produces the AGEs used in our experimental procedure. These AGEs can then go on to produce inflammation and tissue damage in the body.

Histone Deacetylase Activity

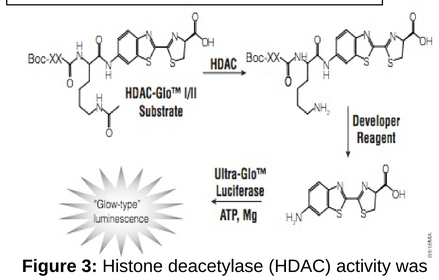

Figure 3: Histone deacetylase (HDAC) activity was measured using the HDAC Glo I/II Assay Kit from Promega Corporation. The assay utilizes the reaction shown above to produce chemiluminescence proportional to the level of HDAC activity. The luminescence glow that can then be measured using a spectrophotometer.[6]

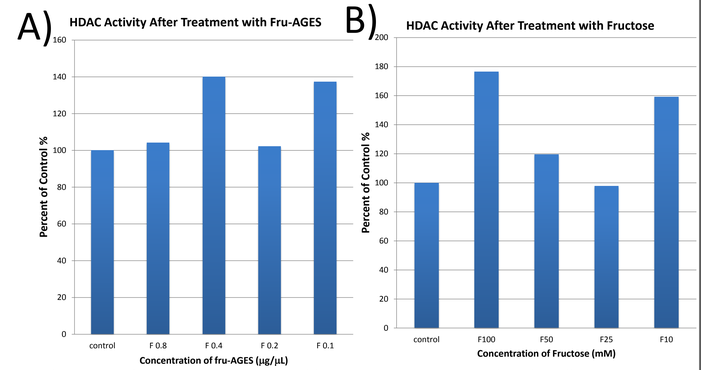

Figure 4: A) Cells were treated with Fru-AGES and then the chemiluminesence from the HDAC activity was measured 3 hours after treatment, showing up to a 20% increase. **B)** Cells were treated with fructose and the HDAC activity was measured, showing up to a 37% increase. Increased HDAC activity would correlate to an global decrease in histone acetylation after treatment with fructose or fru-AGEs.

Taqman Gene Expression

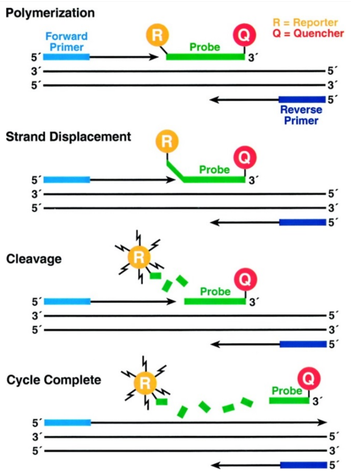

Figure 5: During qRT-PCR, the primers anneal to the DNA, cleaving the probe containing the gene expression markers, causing the probe and quencher to be released from the reporter and the DNA fragment, resulting in a signal from the reporter[5].

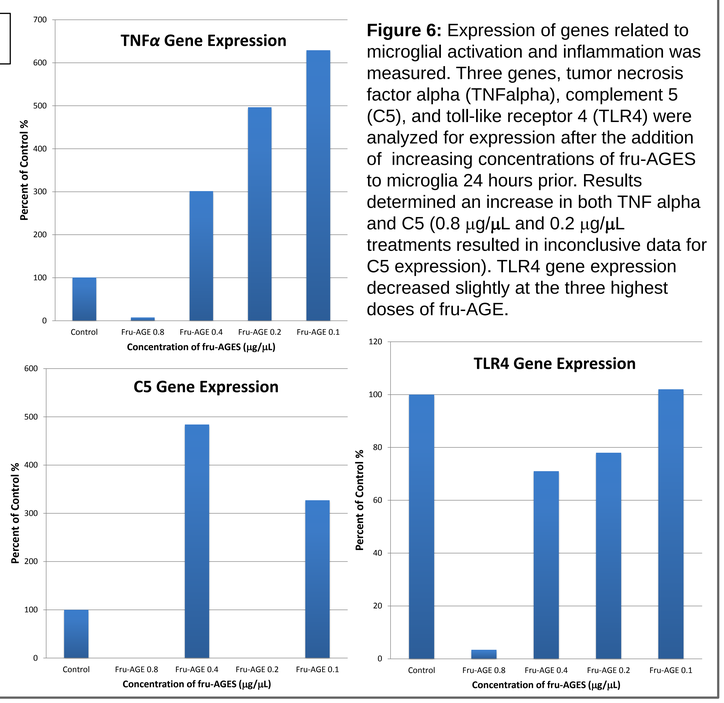

Figure 6: Expression of genes related to microglial activation and inflammation was measured. Three genes, tumor necrosis factor alpha (TNFalpha), complement 5 (C5), and toll-like receptor 4 (TLR4) were analyzed for expression after the addition of increasing concentrations of fru-AGES to microglia 24 hours prior. Results determined an increase in both TNF alpha and C5 (0.8 μg/μL and 0.2 μg/μL treatments resulted in inconclusive data for C5 expression). TLR4 gene expression decreased slightly at the three highest doses of fru-AGE.

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. This 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.

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