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Altered Cell-surface Receptor Levels Result from Fructose Advanced Glycation End Product-Induced Inflammation

Matthew Stryker
St. John Fisher College, mds05927@students.sjfc.edu

Regina Blackley
St. John Fisher College, rcb00745@students.sjfc.edu

Melinda E. Lull
St. John Fisher College, mlull@sjfc.edu

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Abstract

Objective: As a result of the heightened reactivity fructose demonstrates compared to glucose and our current knowledge of glucose advanced glycation end-products, the aim of this research was to further elucidate the proinflammatory pathways involved in the response to fru-AGE exposure, including the effects of fru-AGEs on cell-surface receptor expression. We hypothesized that once microglia were activated in response to fru-AGE exposure, there would be an increase in the expression of RAGE and TLR4 to facilitate the proinflammatory cascade.

Disciplines

Pharmacy and Pharmaceutical Sciences

Comments

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Matthew Stryker[1], Regina Blackley[1], Melinda E. Lull, Ph.D.[2]
[1]Pharm.D. Candidate, Wegmans School of Pharmacy
[2]Department of Pharmaceutical Sciences, Wegmans School of Pharmacy
St. John Fisher College – Wegmans School of Pharmacy, Rochester, New York

Introduction

Fructose, a naturally occurring simple sugar, is also an industrial sweetener that has become a mainstay in the American diet, notably in sugar drinks.[1] A consequence of excess fructose consumption is the non-enzymatic generation of fructose advanced glycation end-products (fru-AGE[s]). The non-enzymatic glycation with fructose is ten times greater compared to glucose.[2] Within the central nervous system, microglia, residential macrophages surveying their microenvironment, become activated when exposed to fru-AGEs. The activation of microglia initiates a cascade of proinflammatory processes. Some of the proinflammatory mediators released include: IL-1β, toll-like receptor 4 (TLR4), tumor necrosis factor α (TNFα) and reactive oxygen species (e.g., H_2O_2). Current evidence suggests that activation of microglia is mediated through the receptor for advanced glycation end-products (RAGE).Although AGE formation is a natural process, the excess exposure to AGEs has been implicated in the pathology of certain diseases, including: diabetic microvascular complications[3] (e.g., retinopathy, nephropathy) and age-related diseases, such as Alzheimer's disease.

Objective

As a result of the heightened reactivity fructose demonstrates compared to glucose and our current knowledge of glucose advanced glycation end-products, the aim of this research was to further elucidate the proinflammatory pathways involved in the response to fru-AGE exposure, including the effects of fru-AGEs on cell-surface receptor expression. We hypothesized that once microglia were activated in response to fru-AGE exposure, there would be an increase in the expression of RAGE and TLR4 to facilitate the proinflammatory cascade.

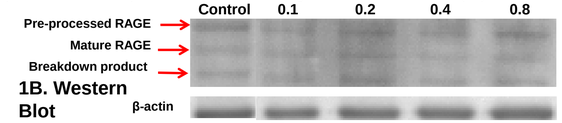
Methods

Fru-AGEs were generated by incubating fructose with bovine serum albumin at 37°C for 8 weeks.[4] An immortalized rat microglial cell line (HAPI) was treated with control media or fru-AGEs *in vitro* for 3 or 24 hours in the following concentrations: 0.1, 0.2, 0.4 or 0.8 mcg/mcL. Gene expression of pro-inflammatory markers and cell-surface receptors was measured using quantitative real-time polymerase chain reaction (qRT-PCR). Genes investigated included the following: complement 5 (C5), interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), receptor for AGE (RAGE) and toll-like receptor 4 (TLR4). Levels of RAGE protein were measured after 24 hours of exposure to fru-AGEs via Western Blot.[5] To further characterize the proinflammatory response, extracellular hydrogen peroxide (H_2O_2) was quantified using the ROS-Glo H_2O_2 chemiluminescent assay (Promega) following a 3 hour incubation with the fru-AGE treatments listed above. Statistically significant changes for each measure were determined using a one-way ANOVA with a Tukey post-hoc test; a p-value < 0.05 was considered significant. All data are expressed as the mean ± SEM.

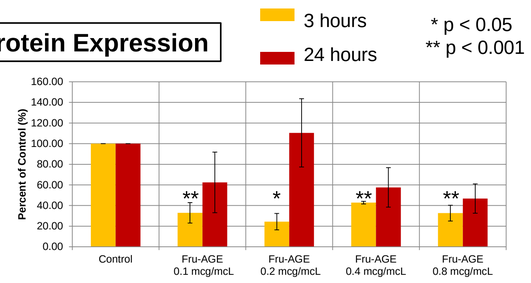
Results

RAGE Gene and Protein Expression

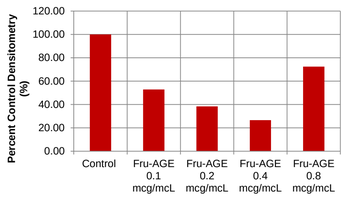
Figure 1: Effects of fru-AGEs on the gene transcription and protein levels of RAGE. RAGE expression decreased significantly across all treatments after 3 hours of exposure to fru-AGEs and there was still a downward trend after 24 hours (ANOVA p = 0.08) (**1A**) . RAGE protein levels also decreased after 24 hours of exposure to fru-AGEs (**1B**, **1C**, **1D, 1E**)**.** This included pre-processed, mature and the breakdown product of RAGE. A representative Western blot image illustrates this same trend (**1B**).



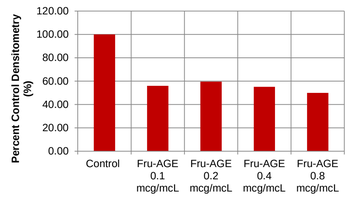
1B. Western Blot



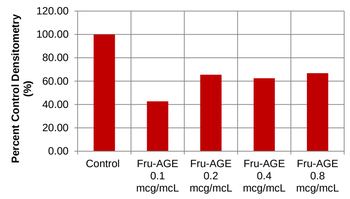
1A. RAGE mRNA



1C. Pre-processed RAGE



1D. Mature RAGE



1E. Breakdown product

Proinflammatory Signals

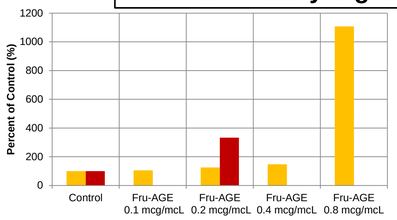


Figure 2: Effects of fru-AGEs on the transcription of C5. After 3 hours of exposure to fru-AGEs, there was no change in the expression of C5, except an increase in expression at 0.8 mcg/mcL. After 24 hours of exposure to fru-AGEs, C5 expression increased.

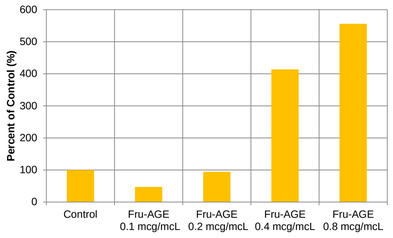


Figure 3: Effects of fru-AGEs on the transcription of IL-1β. After 3 hours of exposure to fru-AGEs, the data suggests that there is a dose-dependent increase in the transcription of IL-1β.

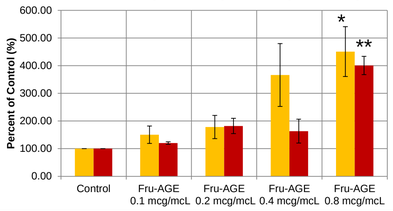


Figure 4: Effects of fru-AGEs on the gene transcription of TNFα. After both 3 and 24 hours of exposure to fru-AGEs, the expression of TNFα increases in a dose dependent manner, which was statistically significant at 0.8 mcg/mcL.

Receptor Expression Changes

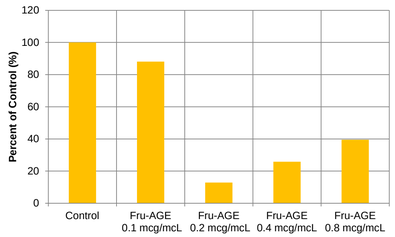


Figure 5: Effects of fru-AGEs on the gene transcription of TLR4. Exposure to fru-AGEs decreased the expression of TLR4 after 3 hours of treatment. The graph above suggests at concentrations ≥ 0.2 mcg/mcL the decrease is more pronounced.

Non-protein Inflammatory Signals

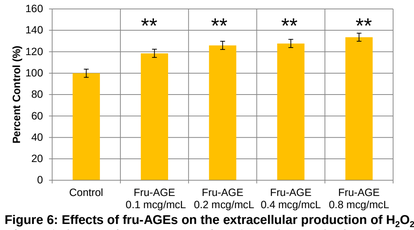


Figure 6: Effects of fru-AGEs on the extracellular production of H_2O_2. After 3 hours of exposure to fru-AGEs, the production of H_2O_2 significantly increased.

Conclusions

- This evidence suggests that exposure to fru-AGEs increases the expression of known mediators of inflammation, including the following: C5, IL-1β, TNFα (statistically significant, dose-dependent acute and chronic effects) and extracellular H_2O_2 (statistically significant dose-dependent effects)
- Cell-surface receptors including RAGE and TLR4 also demonstrate changes in their gene expression when exposed to fru-AGEs. RAGE expression was acute and chronically suppressed after exposure to fru-AGEs. After 3 hours, TLR4 was also suppressed but more replicates are needed.
- Analysis of protein levels support the gene expression trend observed with RAGE. Chronic exposure to fru-AGEs leads to a diminished level of mature RAGE protein.
- The decrease in receptor expression observed with both RAGE and TLR suggests that microglia can become desensitized to noxious stimuli. This observation is concerning because immune cells need to remain sensitized to noxious stimuli to help protect and defend the host.
- This research continues to illustrate and support the hypothesis that excess exposure to fru-AGEs induces an inflammatory state mediated by microglia within the brain and alters cell-surface receptors.
- In addition to their neurodegenerative effects in disease states such as Alzheimer's disease, further investigation about the endogenous effects of fru-AGES should be considered, especially as a result of its accessibility from its use as an industrial sweetener.
- Further areas of research include additional replicates of C5, IL-1β and TLR gene expression and extracellular H_2O_2 production to further validate this hypothesis.

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Disclosure

The authors of this presentation have no personal or financial interest in the subject matter of this presentation.

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