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

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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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Introduction

Fructose, a naturally occurring simple sugar, is also an industrial sweetener that has become a mainstream in the American diet, notably in sugar drinks. A consequence of excess fructose consumption is the non-enzymatic generation of fructose advanced glycation end-products (fru-AGEs). The non-enzymatic glycation with fructose is ten times greater compared to glucose. 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 (TNFα), nitric oxide production induced by advanced glycation endproducts. Molecular Nutritional Food Research. 2010;54:141-50.


Methods

Fru-AGEs were generated by incubating fructose with bovine serum albumin (BSA) at 37°C for 8 weeks. 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 mg/ml. Gene expression of pro-inflammatory markers and cell-surface receptors was measured using quantitative real-time polymerase chain reaction (qRT-PCR). Gene 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. To further characterize the proinflammatory response, extracellular hydrogen peroxide (H2O2) was quantified using the ROS-Glo H2O2 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

Proinflammatory Signals

Figure 1: Effects of fru-AGEs on the gene transcription and protein levels of TNFα. Genes investigated included the following: C5, IL-1β, TLR4. Gene expression and extracellular H2O2 production to further validate this hypothesis.

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 expression increase of C5 following 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, there was no change in the expression of IL-1β, except an expression increase of IL-1β following 24 hours of exposure to fru-AGEs. IL-1β expression increased.

Figure 4: 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 shows an increase at 0.2 mcg/ml, the decrease is more pronounced.

Figure 5: Effects of fru-AGEs on the extracellular production of H2O2. After 3 hours of exposure to fru-AGEs, the production of H2O2 significantly increased.

Figure 6: Effects of fru-AGEs on the gene transcription and protein levels of RAGE. A representative Western blot image illustrates this same trend (β).

Figure 7: Effects of fru-AGEs on the extracellular production of RAGE. After 3 hours of exposure to fru-AGEs, the production of RAGE 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 H2O2 (statistically significant dose-dependent acute and chronic 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 prolonged 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 both RAGE and TLR4 suggesting 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 exposure to fru-AGEs induces an inflammatory response mediated by microglia within the brain and alters cell-surface receptors.

In addition to their neurodegenerative effects in diseases 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 TLR4 gene expression and extracellular H2O2 production to further validate this hypothesis.

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.

Discussion

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

Matthew Stryker: Nothing to disclose

Regina Blackley: Nothing to disclose

Melinda Lull: Nothing to disclose

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