Effect of Grape Pomace Extract on In Vitro CGRP Secretion as a Proxy for Migraine

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Introduction

Migraine
- Painful inflammatory condition, can be traced to the activation of trigeminal nerves, and the subsequent peripheral and central release of neuropeptides.
- Calcitonin gene-related peptide (CGRP) is a widely expressed neuropeptide in central and peripheral neurons, including trigeminal ganglion neurons.
- One of the body’s main pain and inflammatory signaling molecules.
- Elevated levels of CGRP have been correlated with the severity and recurrence of migraine.
- Elevated levels of CGRP have been believed to increase synaptic transmission in the hypersensitive migraine brain (Figure 1). Results in increased perception of sensory inputs as painful stimuli.3

Cell Culture
- The CA77 cell line is known for its propensity to secrete CGRP and is a widely-used cellular model for studying migraine.
- Polyphenols
  - Naturally abundant in grapes, have been shown to have a wide range of beneficial biochemical and pharmacological activities; however, its effect on CGRP regulation has not been extensively investigated.3

Research Question
Can the introduction of non-cytotoxic doses of grape pomace extracts impact secretion of CGRP in stimulated CA77 cells?

Materials
- Grape pomace (Tinta Cao and Cabernet Franc) were obtained from local Virginia wine makers through Dr. John Parry at Virginia State University.
- CA77 cells were obtained from Dr. Andrew Russo, Dept. Molecular Physiology and Biophysics, University of Iowa.
- CGRP rat EIA kit was obtained from Cayman Chemical.

Results and Discussion

Extractions conditions
- 1g of grape pomace powder was extracted in 10 mL of solvent (acetone:water, 50:50, v/v).3
- Solvents were evaporated via nitrogen evaporation followed by lyophilization.
- Dried Extracts were re-dissolved in DMSO for cell treatment.

Cell Culture
- CA77 Cell line: A rat medullary thyroid carcinoma cell line commonly used for CGRP secretion studies.4
- Cultured in a monolayer on laminin-coated plates at 37°C, 5% CO2.

CGRP Secretion Assay
- Cells were incubated with grape pomace extracts in buffer for 1 h, followed by stimulation with 50 mM KCI for 1 h similar to Abbe et al.2
- Incubation solutions were collected and CGRP measured by commercial enzyme immunoassay kit.
- Assays were conducted in triplicate, on two separate conditions.

Data Analysis
- ANOVA was performed with a post-hoc Tukey HSD.

Methodology

Extractions conditions
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Cell Culture
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Cytotoxicity Assay
- Cytotoxicity of grape pomace extracts was assessed via the methylene blue assay after 24 h exposure as reported previously, with modifications.6

CGRP Secretion Assay
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Results and Discussion

Results from the study provide evidence that chemicals in grape pomace extract reduce the levels of CGRP secreted. Other studies using foods with high phenolic concentrations suggest that they have anti-inflammatory properties. In the future, we hope to study the process of how the introduction of polyphenols to the cells result in reducing CGRP secretion levels.

Conclusions
- Treatments, in the doses used, for both grape varieties do not display a toxic effect on CA77 cells when compared to control.
- Both grape varieties decreased CGRP secretion in a dose dependent manner, with more concentrated treatments showing a stronger inhibitory effect.

References


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