The Use of Cannabinoids to Stimulate an Increase of Nitric Oxide Production as a Basis for a Novel Treatment of Chronic Renal Disease

Garrett Hauck
Oak Park River Forest High School
BioGENEius 2017

Introduction

There are 31 million Americans who suffer from chronic renal disease (CRD), and it is agreed upon that a defining characteristic is a lowered amount of nitric oxide (NO) in the kidney.1 This leads to the development of hypertension and atherosclerosis, and contributes to the progression of the disease.2 One of the main factors that causes lowered NO levels is an increased concentration of asymmetric dimethylarginine (ADMA), a nitric oxide synthase (NOS) inhibitor that is elevated in CRD patients due to the lower rate of degradation and excretion in the failing kidney.3 Cannabinoids, a class of molecules derived from cannabis, increase the activity of NOS and stimulate NO production, and (R)-methanandamide (mAEA) is one of the most potent agonists of the cannabinoid receptors.4 In this study, cells from the cell line NRK-52E, established from the kidney of an adult Osborne-Mendel rat, were treated with mAEA after exposure to ADMA in an effort to increase NO production and demonstrate that a cannabinoid pharmaceutical could potentially be used as a treatment for CRD.

Experimental Design

**Purpose:** To establish a basis for a novel pharmaceutical treatment of CRD that works against the cause and fights against progression of the disease rather than decreasing the severity of the symptoms

- **H₂:** If cells are exposed to ADMA with mAEA, then total NO production will increase.

- **H₃:** There will be no statistically significant difference between the individual means of the control and experimental groups.

**Independent Variable:** The chemicals that the cells were exposed to (mAEA, ADMA, both, or neither)

**Dependent Variable:** Total NO production of the cells as measured by nitrite concentration

Procedure

- **Preparation of chemical solutions**

- **Establishment and culture of cells**

- **Cells exposed to chemicals**

- **Griess assay for nitrate determination**

- **Application to Biotechnology**

- **Statistical Analyses**

Results

**Average Nitrite Concentration Produced**

![Figure 2](image)

**Figure 2.** To establish a basis for a novel application to biotechnology.

**TABLE 1.** Single factor ANOVA test

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>14124.77</td>
<td>2</td>
<td>7062.38</td>
<td>3.35</td>
<td>0.05028</td>
<td>3.00</td>
</tr>
<tr>
<td>Within groups</td>
<td>25150.13</td>
<td>33</td>
<td>764.55</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Average Nitrite Concentration (µM)**

![Figure 5](image)

**Figure 5.** Cells exposed to ADMA and fatty acid free bovine serum albumin (faf BSA) solutions were prepared at 120µM and 5mg/mL (Figure 1).

**Cells from cell line NRK-52E were cultured with 12mL DMEM (10% FBS and 1% NEAA) in 75cm² flasks (Figure 2).**

Discussion

There were many obstacles that had to be overcome during the procedure. After two initial rounds of testing, the assay produced no readings. To solve this, the researcher concluded that there were not enough cells and let them grow for an extra day. The third round still did not work. For the next attempt the cells were grown to full confluency, but the assay still produced no readings. The assay was then tried following three separate protocols, each to no avail. Finally, the conclusion was drawn that the phenol red in the DMEM medium was interfering with the acidic conditions necessary for the assay, so cells were resuspended in PBS before the assay, and this procedure gave readings.

Conclusions

Although the calculated p-values (TABLES 1 and 2) were greater than the alpha value of 0.05, the results still show promise for the usage of cannabinoids as a treatment of CRD. The quantitative data show a difference, although insignificant, between groups, supporting the positive effects that come from the interactions between mAEA and ADMA (Figure 5).

Acknowledgments

- Allison Hennings, R.N., B.S.N., M.A.T.
- Mentors: Dr. Eric Kelley, West Virginia University; Professor Allyn Howlett, Ph.D., Wake Forest University

References


*See additional references attached.*