The Effects of Vitamin C, Hydrocortisone, and Thiamine in the Treatment of Sepsis

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ABSTRACT

Bacterial sepsis kills about 250,000 Americans each year. Septic shock, resulting from bacterial sepsis, has a mortality rate of nearly 50 percent. Septic shock is the body’s extreme response to an infection, which is currently untreated throughout the body, resulting in severe tissue damage and organ failure. Tumor necrosis factor (TNF) has been shown to be the primary mediator of the inflammatory response in sepsis. The combination of vitamin C, hydrocortisone, and thiamine given intravenously has been shown to improve the outcome for patients with sepsis and is still being researched. The goal of this research was to test the effects of these drugs on the immune cell production of TNF-alpha in vitro. Minimal research has been done on how the three drugs in combination are treating sepsis.

Human peripheral blood mononuclear cell (PBMC) cultures were isolated from the blood of the student researchers in equivalent concentrations of 5.0x10^5 immune cells per culture. These cultures were exposed to lipopolysaccharide (LPS) of Gram negative bacteria, in a concentration of 1 μg/mL, to stimulate a response similar to in vivo sepsis. Cultures were then incubated for two, four, and six hours with hydrocortisone, vitamin C, and thiamine. The three drugs were added in concentrations scaled to mimic intravenous doses both individually and in combination of all three. After incubation, the supernatant of the cell cultures was extracted, and TNF-alpha was measured via enzyme-linked immunosorbent assay (ELISA) in duplicate to determine each combination effect on immune cell response to LPS. Tukey HSD statistical analysis yielded significant TNF-alpha suppression by hydrocortisone. There was no significant decrease in TNF-alpha levels by vitamin C or thiamine.

INTRODUCTION

Sepsis is caused by an infection and leads to an immune response in the body. Septic shock is the body’s extreme response that causes inflammation throughout the body, resulting in severe tissue damage and organ failure. According to the CDC, more than 1.5 million people in the United States get sepsis each year. Of these, 250,000 die from complications due to sepsis.

Traditional means of treatment have not been ideal with reducing the severity of sepsis in people. In 2016, Dr. Paul Marik, a critical-care physician and head of the general intensive care unit (GICU) at Sentara Norfolk General Hospital in Virginia administered intravenous vitamin C, thiamine, and hydrocortisone as a final life saving attempt for his patients with sepsis. The treatment was effective; the mortality rate of sepsis patients in their ICU dropped from 40.4 percent to 8.5 percent. Those who died did not die directly from sepsis but from comorbidities. The method of action in how the treatment worked was not known. Our research investigated how the combination therapy successfully treated sepsis. In order to ethically study sepsis, cultures of PBMCs were stimulated with LPS, a molecule found in bacteria that the body recognizes as foreign, in order to simulate an in vitro septic system. TNF-alpha has been shown to be the prime mediator in the body’s response to sepsis. It is a cytokine, which is a signaling molecule produced by immune cells, to signal the body’s other cells to respond to the bacteria.

RESULTS

![Figure 1. Tumor Necrosis Factor Alpha (TNF-alpha) Versus Condition Over Time](image)

TABLE 1. Pairwise Comparisons of TNF-alpha vs Condition Over Time Between Donors

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Avg. Effect (pg/mL)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone vs Positive</td>
<td>-2652</td>
<td>0.00002*</td>
</tr>
<tr>
<td>Combination vs Positive</td>
<td>-2668</td>
<td>0.00002*</td>
</tr>
<tr>
<td>Combination vs Hydrocortisone</td>
<td>-16</td>
<td>1.0000</td>
</tr>
<tr>
<td>Donor 1 vs Donor 2</td>
<td>+608</td>
<td>0.03799*</td>
</tr>
</tbody>
</table>

Tukey Honest Significant Difference (HSD) comparisons of tumor necrosis factor alpha (TNF-alpha) production by peripheral blood mononuclear cells (PBMCs) between control and treatment groups, as well as between individual treatments and donors. Average effects of conditions are provided. Significant p-values were determined by exceeding the alpha threshold of 0.05 and indicated by an asterisk (*). All other comparisons had a p-value exceeding 0.05 and were not significant.

DISCUSSION

Hydrocortisone was the only drug that significantly decreased TNF-alpha production.

Although the combination therapy showed a significant decrease in TNF-alpha, this is from the hydrocortisone alone, not a synergistic effect between the drugs as was predicted. This was to be expected, as hydrocortisone is a steroid and is meant to reduce inflammation.

The negative and positive controls seemed to produce the same amount of TNF-alpha.

No extra fluid was added to negative control wells to compensate for the absence of the drugs and LPS solution, which could have resulted in a dilution effect and falsely elevated TNF-alpha concentration.

There could have also been carryover contamination between wells.

The effect of hydrocortisone on donor 1 and donor 2 was similar.

The vitamin C, hydrocortisone, and thiamine combination therapy treats sepsis by some other means than reducing the inflammatory response and TNF-alpha production.