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 that causes inflammation 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* septicemia. 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.

SPECIAL THANKS:

Dr. Matthew J. Nicholaou & Kendal Beazer
For providing guidance and support throughout the project
Kent Criddle MT(ASCP)
For help in ordering supplies
Dr. Marv W. Orrock PharmD
For converting drug concentrations to scale
Weber State Medical Laboratory Sciences Department
For allowing the use of labs, equipment, and supplies
Weber State Office of Undergraduate Research & Ralph Nye Charitable Foundation
For funding the research

RESULTS

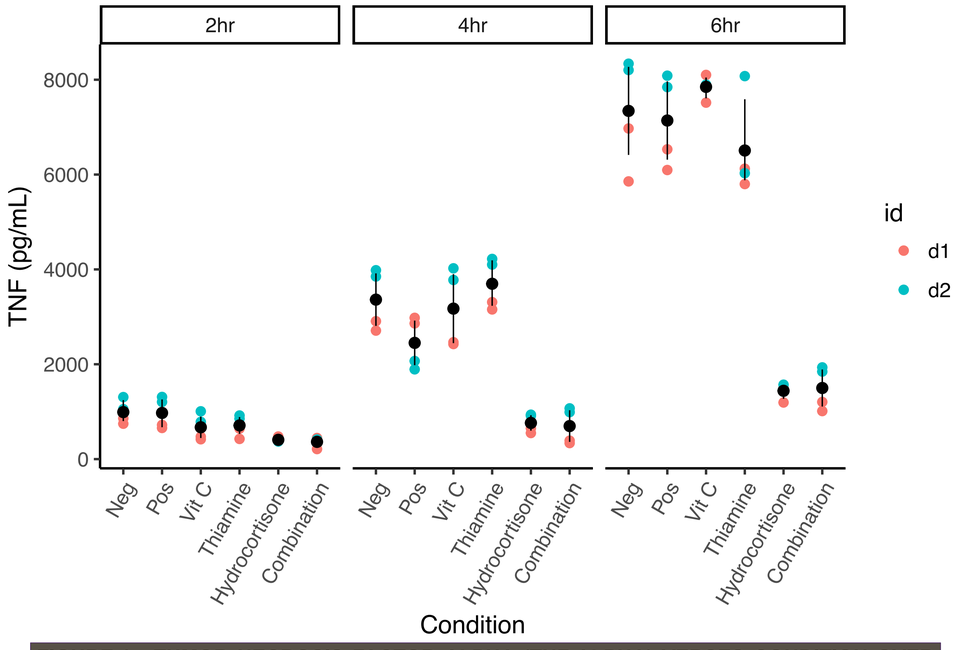

FIGURE 1. TUMOR NECROSIS FACTOR ALPHA (TNF-ALPHA) VERSES CONDITION OVER TIME. Enzyme-linked immunosorbent assay (ELISA) results measuring TNF-alpha in pg/mL versus condition over three time intervals. The red and blue points, d1 and d2, correlate to the donor. The black points indicate the mean TNF-alpha levels between donors and the line corresponds to a 95% confidence interval. Conditions corresponding to Neg and Pos indicate the negative and positive control wells where lipopolysaccharide (LPS) was withheld and added respectively. The other conditions all had LPS added to stimulate TNF-alpha production. The other conditions were the addition of vitamin C , thiamine, hydrocortisone, and a combination of all three drugs.

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

Comparison	Avg. Effect (pg/mL)	p-value
Hydrocortisone vs Positive	-2652	0.00002*
Combination vs Positive	-2668	0.00002*
Combination vs Hydrocortisone	-16	1.00000
Donor 1 vs Donor 2	+608	0.03799*
Donor x Condition	–	0.99790

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 effect 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 0.95 and were not significant.

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METHODS

Human PBMC cultures were isolated from the blood of the student researchers in equivalent concentrations of immune cells. Blood was collected in eight sodium heparin tubes from each of two student researchers using standard venipuncture procedures. Aseptic technique was implemented by use of alcohol pads and povidone iodine swabs at venipuncture site and verified by negative blood cultures at 24, 48, and 72 hours of incubation. Blood was diluted in sterile phosphate buffered saline (PBS) in a 1:2 dilution. In 50 mL conical tubes, 36 mL of diluted blood was added on top of 10 mL of ficoll histopaque (density 1.077 g/mL). PBMCs were isolated by centrifuging the blood with ficoll histopaque at 400xg for 30 minutes with no decelerator brake. The cells were washed three times with sterile PBS and were enumerated with a Beckman-Coulter CBC analyzer. PBMC solutions were diluted and added to 96 well cell culture plates in a concentration of $5.0x10^5$ cells. Cells were suspended in 200 µL of 20 percent fetal bovine serum in sterile PBS. The culture plates were centrifuged at 200xg for 10 minutes. These cultures were then exposed to LPS of *E. coli* (serotype 055:B5) in a concentration of 0.1 µg/mL. Cultures were exposed to hydrocortisone, thiamine, and vitamin C individually and in combination of all three drugs to determine their influence on TNF-alpha production. Drug concentrations added to test wells were 0.1 mg vitamin C, 0.01 mg thiamine, and 0.003 mg hydrocortisone. The cultures were incubated with the drugs for two, four, and six hours with LPS along with two levels of controls. Controls consisted of cultures exposed and unexposed to LPS, both without the addition of drugs. The supernatant was extracted and frozen at -80º C. TNF-alpha was measured in duplicate by an ELISA. The ELISA was run according to the manufacturer's specifications (Thermo-Fisher). The amount of TNF-alpha produced was quantified with the EPOCH 2 microplate reader at a wavelength of 450 nm. Multiple ANOVA models were performed comparing different conditions on TNF-alpha levels, while controlling for time, donor, and effects. A Tukey Honest Significant Difference (HSD) test was used to perform all pairwise comparisons to determine significance.

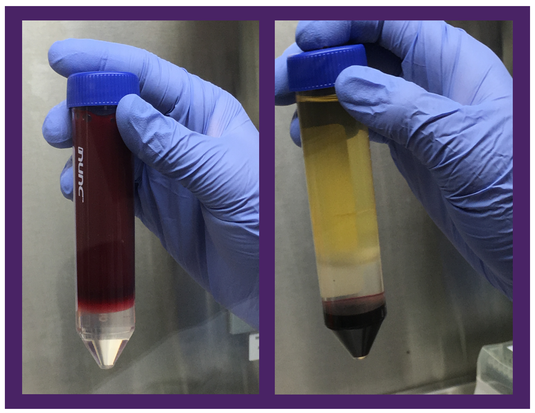

Figure 2. Ficoll separation of heparinized blood. Left image depicts diluted blood poured over ficoll histopaque layer. Right image depicts blood layering after centrifugation. Layers are (from top to bottom): plasma, PBMCs, ficoll, and red blood cells.

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 carry over contamination between wells
- The effect of hydrocortisone on donor 1 and donor 2 cells 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.