Outcomes of Metabolic Resuscitation Using Ascorbic Acid, Thiamine, and Glucocorticoids in the Early Treatment of Sepsis.

ORANGES Trial

NCT#: NCT03422159

Background and Significance:

The global burden of sepsis is substantial with an estimated 15 to 19 million cases per year; the vast majority of these cases occur in low income countries. [1] With more timely diagnosis and improvement in supportive care the 28-day mortality from sepsis in high income countries has declined to about 25%, however, the mortality from septic shock remains as high as 45%. [2,3] Moreover, the mortality from sepsis and septic shock in low income countries is reported to be as high as 60%.[4-6] In addition to short term mortality, septic patients suffer from a numerous short- and long-term complications and are at an increased risk of death for up to five years following the acute event.[7] Over the last 3 decades over 100 phase II and phase III clinical trial have been performed testing various novel pharmacologic agents and therapeutic intervention in an attempt to improve the outcome of patients with sepsis and septic shock; all of these studies have failed to show an improvement in patient outcomes. [8] New therapeutic approaches to sepsis are desperately required; considering the global burden of sepsis these interventions should be effective, cheap, safe and readily available.

A large body of experimental data has demonstrated that both corticosteroids and intravenous vitamin C reduce activation of nuclear factor KB (NFKB) attenuating the release of pro-inflammatory mediators, reduce the endothelial injury characteristic of sepsis thereby reducing endothelial permeability and improving macrocirculatory flow, augment the release of endogenous catecholamines and enhance vasopressor responsiveness.[9-16] In animal models these effects have resulted in reduced organ injury and increased survival. Corticosteroids have been evaluated in several clinical trials, with meta-analysis of these trials demonstrating somewhat conflicting outcomes. [17-20] Low-dose stress corticosteroids have proven to be safe with no increased risk of clinically important complications. [17] While corticosteroids decrease vasopressor dependency the effect on survival is less clear.[19,21]

Several studies have investigated the use of intravenous vitamin C in critically ill patients. Nathens et al randomized 595 surgical ICU patients (91% trauma patients) to receive intravenous vitamin C and vitamin E for up to 28 days. [22] The vitamin combination was associated with a significant reduction in the incidence of multiple system organ failure (p=0.04) with a trend to reduced mortality and length of ICU stay. No adverse effects were noted with the vitamin combination. Fowler et al performed a pilot

study in 24 patients with severe sepsis and septic shock. [23] In this study patients were randomized to placebo (n=8), low dose intravenous vitamin C (50 mg/kg) (n=8) or high dose vitamin C (200mg/kg). Vitamin C attenuated the inflammatory response with both doses of the vitamin being devoid of any side effects. Although the Sequential Sepsis Related Organ Failure Score (SOFA) fell significantly in both treatment arms the study was underpowered to determine any outcome benefit. Zabet and colleagues performed a RCT in which they evaluated the role of intravenous vitamin C in a dose of 100 mg/kg/day (about 7g/day) in 28 surgical ICU patients with septic shock. [24] In this study the mean dose of norepinephrine and duration of norepinephrine administration were significantly lower in the ascorbic acid than the placebo group. The 28-day mortality was significantly lower in the ascorbic acid than the placebo group (14% vs. 64%, p = 0.009). No side effects related to the vitamin C infusion were reported. Tanaka et al randomized 37 patients with severe burn to very high dose vitamin C (about 110g/day) or placebo.[25] Patients who received vitamin C required less fluid resuscitation with a trend towards reduced length of stay and mortality. No adverse effects were noted with the very high dosages of vitamin C. Several studies have administered vitamin C in doses exceeding 100g/day as adjuvant therapy in patients with cancer with no discernable side effects. [26-33] Vitamin C appears to be toxic to normal human cells (not cancer cells) at a concentration on greater than 25 mM. [33] A dose of 6g/day will achieve a steady state serum concentration of about 240uM [34-36] which is about 100 times less than the dose required to cause cellular toxicity. The package insert for vitamin C [37] lists no contraindications or adverse effects of the drug and states that as much as “6 grams has been administered without evidence of toxicity”. The only reported restriction to the use of high dose intravenous vitamin C is in patients with known glucose-6-phospahte deficiency (G6PD) in whom hemolysis has been reported. [38,39] It is important to recognize that patents with sepsis predictably have very low serum vitamin C levels, which can only be corrected with intravenous vitamin C in a dose of more than 3gm per day. [23,34,36] The low or undetectable levels of vitamin C likely result from the metabolic consumption of the molecule as well as increased renal losses. Furthermore, unlike all other mammals, primates and guinea pigs are unable synthesize vitamin C is due to mutations in the L-gulono-_-lactone oxidase (*GLO*) gene which codes for the enzyme responsible for catalyzing the last step of vitamin C biosynthesis. [40] In almost all species, except humans and guinea pigs, vitamin C production increases during stress and is secreted by the adrenal gland; in these species vitamin C is best considered a stress “hormone”. [40] Vitamin C is an essential cofactor for the production of corticosteroids and catecholamines by the adrenal gland. [13,41] Vitamin C has been shown to reverse adrenal suppression caused by induction doses of etomidate during anesthesia.[42,43]

Ascorbate donates a single electron in all its redox reactions, generating the ascorbate radical. This radical is not very reactive with anything but itself. Dismutation of two ascorbate radicals forms a molecule each of ascorbate and dehydroascorbate. Hydrolysis of the lactone ring of dehydroascorbate

irreversibly converts it to 2,3-diketo-1-gulonic acid which is then converted to oxalate. Oxalate is normally excreted by the kidney and serum levels will increase with renal impairment. In patients with renal impairment receiving mega-dose vitamin C, supersaturation of serum with oxalate may result in tissue deposition as well as crystallization in the kidney. [44,45] Glyoxylate, a byproduct of intermediary metabolism, is either reduced to oxalate or oxidized to CO_2 by the enzyme glyoxylate aminotransferase; thiamine pyrophosphate is a co-enzyme required for this reaction. [46] Thiamine deficiency increases the conversion of glyoxylate to oxalate resulting in hyper- oxalosis. [47,48] Donnino and colleagues have demonstrated that thiamine deficiency is common (32%) in patients with sepsis and that treatment with thiamine in these patients reduces mortality. [49] In a post-hoc analysis of this study these authors demonstrated that thiamine decreased the risk of acute kidney injury and the required for renal replacement therapy in all treated patients. [50]

It has previously been suggested that *"...the best hope for therapeutic advances [in sepsis] will depend on broad-base targeting, in which multiple components are targeted at the same time."*[51] Such combination "chemo-therapy" targeting multiple biological pathways is the standard approach in the treatment of malignant disease. While the benefits of vitamin C, hydrocortisone, and thiamine alone are likely limited,[23,49,50,52] we believe that these medications act synergistically to reduce the risk of organ failure and death in patients with sepsis. This hypothesis is supported previous research [53,54] and more recently a set of elegant experiments performed by Barabutis et al. [55] Using a validated pulmonary endothelial monolayer model, these authors demonstrated that hydrocortisone together with vitamin C protected the vascular endothelium from damage by endotoxin while neither agent alone had this effect. Previous research has demonstrated that vitamin C reverses oxidation of the glucocorticoid receptor (GR) a likely manifestation of sepsis.[53] Oxidation of the GR limits binding of the GR to both ligand and DNA responsive units decreasing the activity of glucocorticoids. [53] Furthermore, glucocorticoids increase the expression of the sodium vitamin C transporter-2 (SVCT-2) which is an essential transport protein necessary for vitamin C to be transported intracellularly.[54]

We therefore propose that a "metabolic resuscitation protocol" including vitamin C, corticosteroids and thiamine will limit the development of organ failure and reduce mortality in patients with severe sepsis and septic shock. This postulate is supported by the preliminary findings by Marik et al. [56] In a retrospective before-after clinical study, these authors compared the outcome and clinical course of consecutive septic patients treated with intravenous vitamin C, hydrocortisone and thiamine during a 7-month period (treatment group) compared to a control group treated in during the preceding 7 months.[56] The primary outcome was hospital survival. A propensity score was generated to adjust the primary outcome. There were 47 patients in both treatment and control groups with no significant differences in baseline characteristics between the two groups. The hospital mortality was 8.5% (4 of 47)

in the treatment group compared to 40.4% (19 of 47) in the control group ($p < 0.001$). The propensity adjusted odds of mortality in the patients treated with the vitamin C protocol was 0.13 (95% CI 0.04-0.48, p=0.002). The SOFA score decreased in all patients in the treatment group with none developing progressive organ failure. Vasopressors were weaned off all patients in the treatment group, a mean of 18.3 ± 9.8 hours after starting treatment with vitamin C protocol. The mean duration of vasopressor use was 54.9 ± 28.4 hours in the control group ($p<0.001$). The results of this study provide sufficient information for the design of an adequately powered, pragmatic randomized controlled trial.

Study Design:

Specific Aims of the Study:

The aim of this study is to determine the effect of the combination of intravenous vitamin C, hydrocortisone and thiamine on the clinical course and outcome of patients with sepsis and septic shock.

This study will be performed at Community Medical Center and Monmouth Medical Center Southern Campus. All patients admitted to the ICU with the primary diagnosis of sepsis or septic shock will be screened for inclusion into this study. The diagnosis of sepsis and septic shock will be based on the 2016 Surviving Sepsis Campaign definitions.[63]

Inclusion Criteria:

i. Diagnosis of sepsis or septic shock within 12 hours of admission to the ICU

ii. Informed consent as dictated by IRB and local practice.

iii. Compliance with the 3 hour sepsis bundle

- 30ml/kg of intravenous crystalloid fluid (e.g.: sodium chloride 0.9%) for lactic acid >4 and/or systolic blood pressure <90mmHg / mean arterial pressure <65mmHg
- Lactic acid level drawn
- Broad spectrum antibiotics given after obtaining blood cultures

Exclusion criteria:

i. Age < 18 years

ii. Pregnant

iii. DNR/DNI with limitations of care on admission

iv. Patients with terminal end stage disease (i.e. stage IV cancer, end stage heart failure) that are unlikely to survive to hospital discharge

v. Patients with a primary admitting diagnosis of an acute cerebral vascular event, acute coronary syndrome, active gastrointestinal bleeding, burn or trauma [64-66]

vi. Requirement for immediate surgery [64-66]

vii. Patients with HIV and a CD4 < 50 mm^2 [64-66]

viii. Patients with known glucose-6 phosphate dehydrogenase (G-6PD) deficiency.[39]

ix. Patients with sepsis/septic shock transferred from another hospital

x. Patients with features of sepsis/septic shock > 24 hours after admission

Study end-points:

Primary end-point

i. Time to vasopressor independence. Defined as the time from starting the active treatment/placebo to discontinuation of all pressors

ii. Delta SOFA score, defined as the initial SOFA score minus the day 4 SOFA score

Secondary end-points

iii. 28-day mortality

iv. Hospital mortality

v. PCT clearance (PCT-c) calculated using the following formula: initial PCT minus PCT at 96 hours, divided by the initial PCT multiplied by 100. [67,68]

vi. ICU mortality

vii. ICU length of stay (LOS) and ICU free days. ICU free days is calculated as the number of days alive and out of the ICU to day 28

viii. Hospital LOS

Vitamin C, Hydrocortisone and Thiamine dosing protocol and randomization

Medication dosing.

Based on published clinical data, vitamin C pharmacokinetic modeling, the package insert as well as the preliminary study by Marik et al, Vitamin C will be administered as an intravenous dose of 6gm per day divided in 4 equal doses. [22-24,34-37] This dosage is reported to be devoid of any complications or side effects. Hydrocortisone will be dosed according to the consensus guidelines of the American College of Critical Care Medicine.[52] Thiamine will be administered according to current recommendations in a dose of 200mg q 12 hourly. [69]

This is a double-blind placebo controlled study. Only the dispensing pharmacist will be aware of the treatment allocation. Patients will be randomized to receive either vitamin C/hydrocortisone/thiamine or triple placebo using a random number table provided to the dispensing pharmacists. Each patient will be allocated a unique subject ID which will be linked to the randomization sequence. Only the dispensing pharmacists will have a record of the subject ID and randomization sequence. The vitamin C, vitamin C-placebo, hydrocortisone, hydrocortisone-placebo, thiamine, and thiamine-placebo will be formulated as follows:

Vitamin C: Vitamin C is provided by the manufacturer as a 50 ml vial at a concentration of 500mg/ml. Three (3) ml of vitamin C will be placed in a 50 ml bag of Normal Saline (1500 mg vitamin C in 50ml bag) which will then be infused over 30 minutes (100mL/hr). The bag will be labeled by the pharmacy as "Vitamin C 1.5g or Placebo". The dosing schedule is 1500mg every 6 hours for 4 days (or discontinued earlier if discharged from the ICU).

Once the vial is open, it is only stable for 6 hours. The resulting product is given 12 hour stability. When first ordered, 2 doses are made then and sent to the nurse. For next 12 hours, they will wait until 1 hour prior to make the next batch.

Vitamin C placebo will consist of an identical bag of 50mL normal saline (but with no vitamin C) and will be labeled "Vitamin C or Placebo". Placebo will be infused over 30 minutes as per the infusion instructions of the active vitamin.

Hydrocortisone: Patients will be treated with hydrocortisone 50mg IV push every 6 hours for 4 days (or discontinued earlier if discharged from the ICU). Pharmacists will prepare hydrocortisone 50mg/1mL for the nurse in a 3mL syringe which will be labeled "Hydrocortisone 50mg or Placebo". Hydrocortisone placebo will be provided as an identical 3mL syringe as 1mL of normal saline. Two doses of study medication will be prepared at a time.

Thiamine: Intravenous thiamine will be given in a dose of 200mg q 12 hourly for 4 days (or discontinued earlier if discharged from the ICU). This will be placed in a 50mL bag of Normal Saline labeled "Thiamine 200mg or Placebo" and run over 30 minutes (100mL/hr) Placebo patients will receive a matching 50mL bag of Normal Saline. One dose will be prepared at a time.

Power Calculation:

Based on the results of the preliminary study of Marik et al we project that the combination of hydrocortisone, vitamin C and thiamine could reduce time to vasopressor discontinuation from 54 (+/- 30 hours) versus 30 hours. For the additional primary outcome we projected a greater change of SOFA score of 4 (+/- 3) versus 2. Assuming a type 1 error of 5% (alpha of 0.05) and a power of 80% (the ability to detect a difference between two groups when a difference exists) would require a sample size of 94 patients. To account for dropouts and patients not requiring vasopressor therapy we will therefore aim for a sample size of 140 patients.

Data Collection:

The following de-identified demographic and clinical data will be collected.

i. Age

ii. Sex

iii. Weight

iv. Admitting diagnosis and possible site of infection

v. Blood culture results

vi. Co-morbidities, including diabetes, hypertension, cardiac failure, COPD, malignancy

vii. Requirement for mechanical ventilation (Y/N).

viii. Use of vasopressor agents (Y/N). The hourly dosage of vasopressors will be recorded as the norepinephrine equivalent dosage. [70,71]

ix. Duration of vasopressor use

x. Time to vasopressor independence from start of active drug/placebo

xi. Daily urine output (for first 4 days)

xii. Fluid balance after 24 and 72 hours

xiii. Presence of acute kidney injury (AKI). Presence of AKI: Acute kidney injury (AKI) will be defined using the KDIGO criteria; namely, an increase of the s-creatinine > 0.3 mg/dl or a level > 1.5 times the baseline value. [27] If the baseline s-creatinine is not known a value > 1.5 mg/dl will be regarded as diagnostic of AKI.

xiv. Length of ICU and hospital stay

xv. ICU, hospital, and 28-day mortality

xvi. Routine laboratory data for 4 days including:

a. serum creatinine

b. white cell count (WBC)

c. platelet count

d. total bilirubin

e. PaO_2/FiO_2 ratio

f. Procalcitonin (PCT) – on days 1 and 4

g. lactate level

h. urine oxalate level (day 4)

xvii. HostDx Sepsis genetic testing (day 1) if consent is provided for additional sub-study.

xviii. The patients' admission APACHE II and APACHE IV scores will be recorded. The APACHE IV score allows calculation of the predicted hospital mortality and predicted ICU length of stay (LOS).

ixx. The daily SOFA (Sepsis-related Organ Failure Assessment) score will be recorded for the first 4 treatment days.

The APACHE II score (incrementing score of 0-71) and APACHE IV score (incrementing score 0-286) are standardized measures of disease severity that are used to predict hospital mortality and ICU LOS. [72,73] The SOFA score was designed to sequentially assess the severity of organ dysfunction in patients who are critically ill from sepsis (incrementing score 0-24). [74]. The SOFA scores is calculated 24 hours

after admission to the ICU and daily thereafter. SOFA scores that increase by about 30 percent are associated with a mortality of at least 50 percent.[75]

Data will be collected in the attached Excel spreadsheet (Appendix A – Sepsis Data spreadsheet). No personal identifiers will be recorded on the spreadsheet. We will not be recording names, dates of birth, social security or EMR numbers or any other HPI data in the data collection spreadsheet. Each record will be assigned a unique study ID number (subject ID key). A separate password protected spreadsheet will have a list of subject ID number and the corresponding medical record numbers (Subject ID Key data sheet). This spreadsheet will only be accessible to the principle investor and sub-PI. The pharmacist will have a copy of the "Research Randomization and Dispensing Log" with an additional field which will include the randomization code to active vitamin protocol or placebo. Only the pharmacist will have access to the "Research Randomization and Dispensing Log" until after the study is completed, when it will be made available to the principle investor and sub-PI.

Data analysis:

Summary statistics will be used to describe the clinical data and presented as mean ± SD, median with interquartile range (IQR) or percentages as appropriate. Chi squared analysis with Fisher's exact test (when appropriate) and Student's t test (Mann Whiney U test for non- normal distributions) were used to compare data between the active treatment group and the placebo group with statistical significance declared for probability values of 0.05 or less.

Data Safety & storage:

The main risk to subjects is the accidental release of PHI. Careful record management methods will be in place to ensure this type of privacy breach does not occur.

Subject ID Key: Each subject will be given a unique subject ID number. A subject ID key will be used to match the subjects Financial Identification Number (FIN). The subject ID key, linking the subject ID numbers to the FIN will be kept in a password-protected file on the pharmacy IV room and pharmacy co-investigator computer. Only the research team (PI, sub PI and research pharmacists) will have access to this information, and they will not disclose this information to any other person or entity. The subject ID key will be destroyed as soon as possible after the data set has been completely abstracted and validated for accuracy and completeness.

Data set: Similarly, the data set will be kept in a password-protected file and stored separately from the

subject ID key in the locked office of the study coordinator. Only the research team will have access to this information, and they will not disclose this information to any other person or entity. Three years after the completion of the study, all collected data will be destroyed by permanently deleting electronic copies.

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