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High dose vitamin C in sepsis (HDVCIS): study protocol for a randomized controlled trial

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Abstract

Sepsis is an inflammatory syndrome with life-threatening organ dysfunction resulting from a dysregulated host response to infection. Although the treatment for sepsis has

improved a lot in the last 30 years, sepsis related mortality remains high. In the recent 10 years, high dose intravenous injection of vitamin C, the first line antioxidant of human, has received more and more attention in the field of critical care, its beneficial effect has been demonstrated by several small scale clinical trial and animal studies, but the effect of high dose vitamin C on sepsis in a larger trial seems warranted in further study. Here we will conduct a prospective, multi-center, double-blind, adaptive sample size, randomized, placebo-controlled, clinical trial named as HDVCIS. The trial protocol has been approved by Clinical Trail Ethics Committee of Ruijin Hospital of Shanghai JiaoTong University School of Medicine. This trial has been registered in Chinese Clinical Trial Registry (ChiCTR1800017633) in August 7, 2018. Patients will be recruited from 4 medical centers in China. Its object is to testify the efficacy and safety of high dose vitamin C in the treatment of sepsis.

Keywords

High dose vitamin C, Sepsis, Clinical trial

Background

Sepsis is an inflammatory syndrome with life-threatening organ dysfunction resulting from a dysregulated host response to infection (1). The global annual incidence of sepsis is up to 31 million cases, including 19.4 million cases of severe sepsis, with about 6 million fatalities (2). The incidence in China is 461 per 100,000 population(3) according to latest data.

Although the treatment for sepsis including antibiotics, source control, fluid resuscitation, vasopressors and organ support has improved a lot in the last 30 years,

sepsis related mortality remains high, which was 74 deaths per 100, 000 population in the worldwide (2) and 66.7 deaths per 100, 000 population in China (4). New interventions to improve mortality need to be researched and developed.

In the recent 10 years, high dose intravenous injection of vitamin C, the first line antioxidant of human, has received more and more attention in the field of critical care(5). Its beneficial effect has been demonstrated by several small scale clinical trial and animal studies, such as severe burn(6), severe acute pancreatitis(7), ischemic reperfusion injury (8-10) and sepsis(11). Still, the effect of high dose intravenous vitamin C on sepsis remain controversial according to retrospective work of a cohort study (12) and real-world study (13). Especially, high dose vitamin C was shown to fail to improve mortality, organ function and inflammatory biomarkers by the latest result of CITRIS-ALI study (N=167) (14). Therefore, the effect of high dose vitamin C on sepsis in a larger trial seems warranted in further study.

The purpose of this high dose vitamin C in sepsis (HDVCIS) study is to test if vitamin C reduced 28-day mortality among sepsis patient. We also evaluate effect of vitamin C on recovery of multiple organs function and inflammatory biomarkers level.

Method

We developed HDVCIS study as a prospective, multi-center, double-blind, adaptive sample size, randomized, placebo-controlled, clinical trial. HDVCIS will be conducted in four centers in China. The trial protocol has been approved by Clinical Trial Ethics Committee of Ruijin Hospital of Shanghai JiaoTong University School of Medicine. This trial has been registered in Chinese Clinical Trial Registry (ChiCTR1800017633). A

Standard Protocol Items: Recommendations for Clinical Interventional Trials (SPIRIT) checklist is also available (Additional file 1).

Patients will be recruited from 4 medical centers in China. Any patient admitted to a study site and diagnosed with sepsis according to sepsis 3.0 definition will be considered for enrollment. Specific inclusion and exclusion criteria are as follows:

Inclusion criteria:

Each of the following criteria must be met by a patient before enrollment can be considered:

- Age >18 years
- Met the diagnostic criteria which is suspected infection and organ dysfunction (total SOFA score $\geq$ 2 points).
- Within 72 hours from establishment of sepsis to inclusion
- If it is a woman in childbearing period, she should use contraception for at least one month before screening, and promise to use contraception for the whole study
- Has informed consent form (ICF) signed according to local rules and approved regulations.
- Assign to this consent

Exclusion criteria

- Malignant Tumor

- Patient or their surrogate refuse to receive cardiovascular (vasopressor agent), respiratory (mechanical ventilation) and renal (renal replacement therapy) supports by patient or their surrogate
- With chronic organ failure including:
 - Cardiovascular (need home mechanical or chemical hemodynamic support, such as ventricular assist device milrinone);
 - Chronic kidney disease (eGFR < 60 ml/min or serum creatine > 150 umol/L)
 - Chronic Respiratory failure (requiring supplemental non- invasive oxygen via nasal cannula or continuous positive airway pressure and bi-level positive airway pressure or home mechanical ventilation)
 - Chronic hepatic failure (Child-Pugh score of 10 to 15, Class C).
 - known allergy or contraindication to vitamin C;
 - Has participated in another investigational study
 - Weighs more than 100 kg
 - receiving immunosuppressant treatment or is on chronic high doses (high-dose therapy exceeding 2 weeks of treatment) of steroids equivalent to prednisone/prednisolone 0.5 mg/kg/day, including Autoimmune diseases and solid organ transplant patients
 - Urolithiasis

Screening

Potentially eligible patients will be screened in each participating sites for the timely identification. All patients meeting inclusion criteria will be recorded in the database.

With the exceptions including: not identified in a timely way, decline to participate, presence of any other exclusion criteria.

After screen, the enrolled patients will be informed consent. When patients are not deemed capable of informed (such as intubated state), their legal authorized representatives will be approached and informed consent. In such situation, Attempts to verify and obtain written consent from patient for continued study participation is to be assessed regularly during hospitalization. The enrolled patients will be withdraw from the study if the consent is denied by themselves.

Randomization

Participants will be randomly assigned to the VitC or NS groups in a 1:1 ratio. The randomization sequence will be generated in SAS v. 9.2 (SAS Institute Inc., USA) using stratified block randomization with a block length of 10 and stratified by site. A randomized envelop will be made by a statistician in our clinical research center. The sequence number is marked outside the envelope and the medication puzzle for VitC or NS is sealed inside. The randomization information will be blind to all the participants in the trials except the unblinding drug preparing nurse (UDPN).

After consent is obtained and inclusion and exclusion criteria are verified, PI in each participating site (PS) will hand the unique randomized envelope of this patient to UDPN. The UDPN will unseal the envelope and disposing the VitC or NS into a 50ml syringe according to grouping information inside envelop. UDPN give the opaque syringe to the therapy nurse and independent of the following medical process.

Medication

Patients will receive the first dose of study drugs, or placebos, within 2h of randomization.

The vitamin C (1g/5ml, 200mg/kg/24h) or the controlled 0.9% NS was given by continuous injection through central venous with speed of 5ml/h (1g/h for VitC group).

Vitamin C or NS are to be administered through a separate line.

Other management of randomly assigned patients will be followed by sepsis 3.0 guideline(15). This includes fluid resuscitation, antibiotics, vasopressor titration, mechanical ventilation and ventilator weaning strategies, blood transfusion, nutrition, renal replacement therapy, delirium management and glycemic control.

Data collection

All data were collected using a web-based database in each participating centers (Table 1). Enrolled patient will be evaluated clinically and baseline data including patient demographics, anthropometrics, history, infection source, immune status and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores will be collected at Day 0 of enrollment. Also, the immune function was also evaluated by the number of CD3, CD4, CD8 (+) cell and the level of immunoglobulin A,G and M at Day after enrollment. At Day 1, 3, 7, 14 after enrollment, the organ function indicators and scores (SOFA) will collected. Accordingly, the duration of mechanical ventilation, vasopressor application and renal replacement therapy support. The dose of vasopressor in 28 day were also calculated. The serum lactate will be recorded to

further diagnosis of septic shock. The total volume input and output of the fluid therapy were recorded in the first week after enrollment. The serum level of Vitamin C was tested at Day 0 and Day 3 after enrollment. The level of inflammatory cytokines including IL-2 receptor, IL-1beta, IL-6, IL-10, TNF-a, IL-8 were tested at Day 0 and Day 3.

Outcome

The primary outcome is 28-day mortality. The second outcome is the change of SOFA score in 14 days, the organ support therapies including mechanical ventilation, vasopressor and renal replacement therapy in 28 days, occurrence of adverse events and length of stay (days). The relationship between serum level of vitamin C and primary outcome (28-day mortality) as well as secondary outcomes (change of SOFA score in 14 days, organ support, occurrence of adverse events) were also investigated.

Adverse event reporting

An AE is “any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease” and occurs during a subject’s participation in research (16).

AEs includes:

- 1) crystal in urine
- 2) respiratory failure
- 3) thromboembolic disease
- 4) arrhythmias
- 5) delirium
- 6) anemia

7) coagulopathy

Severe AE (SAE) includes:

- 1) unexplained acute kidney failure;
- 2) death
- 3) life-threatening;
- 4) results in prolongation of the existing hospitalization;
- 5) persistent or significant disability/incapacity

Both AEs and SAEs was evaluated at 8:00 am every day. They will be recorded and reported to PI of each research site and evaluated if they related to this study. Once confirmed, the given medicine should be discontinued immediately. The AE related indicators as well as its development should be monitored closely. Both AEs and SAEs will be summarized.

Data analysis

The primary objective of this trial was to demonstrate the superiority of VitC group, as compared with NS group. The sample size was calculated by PASS 15.0 software (NCSS, Kaysville, UT) with sample allocation ratio of 1:1 between 2 groups. Assuming that there would be the 28-day mortality of 40% in NS group an 30% in VitC group, we would consider VitC injection to be superior if the 28-day mortality was more than 2 percentage points below the 28-day mortality in NS group. We used the superiority test for two proportions, based on a superiority margin of -2%, a one-sided 2.5% of type I error probability, and an 80% of power, evaluation of 552 patients would be

required in each group, and the target sample was increased to 600 patients to allow for an expected rate of loss of 10%.

Continuous variables characterizing each study group will be reported as means with standard deviations or medians with interquartile ranges. Categorical variables will be represented as frequencies and proportions. The primary efficacy analyses will be based on the intention-to-treat principle and per-protocol principle, comparing between-group difference of 28-day mortality according to the randomly assigned treatment. The primary outcome of 28-day mortality will be compared between groups using chi-square test and 2-sided 95% confidence interval will be calculated. Superiority could be concluded if the upper bound of the 95% CI for the between-group difference in 28-day mortality was lower than the –2% superiority margin. The sofa score change from day 0 to day1, day 3, day 7 and day 14 between two groups will be analyzed using generalized linear model. Additional outcomes between groups such as the duration of organ support therapies including MV, vasopressor and CRRT, occurrence of adverse events, together with ICU and hospital length of stay will be compared using chi-square tests or the Wilcoxon rank-sum test, as appropriate. These analyses are 2-sided with no adjustment for multiple comparisons. The correlation between change of VC concentration from day 0 to day 3 and 28 days' mortality as well as changes of sofa will be studied using Pearson correlation analysis. For subjects with missing data on the primary endpoint or secondary endpoints, the approach of last observed status carried forward will be adopted. All analysis in this study was performed two-sided at the 5% significance level. SAS v. 9.2 (SAS Institute Inc., USA) was adopted for all analysis.

Discussion

Here we will conduct a prospective, multi-center, double-blind, adaptive sample size, randomized, placebo-controlled, clinical trial named as HDVCIS. Its object is to testify the efficacy and safety of high dose vitamin C in the treatment of sepsis.

The rationale of supplement of vitamin C in critical illness (such as trauma, sepsis, hemorrhagic shock) has been confirmed for a long time. Severe vitamin C deficiency (plasma concentrations $< 11 \mu\text{mol/L}$) is common (nearly 40%) in patients with sepsis (16). Vitamin C deficiency was reported to be closely related to poor outcome(17), as vitamin C exert important functions: 1) directly erase reactive oxidative species (ROS) and its adverse effect; 2) as a key cofactor for the biosynthesis of endogenous catecholamines, vasopressin, and cortisol to stable the circulatory; 3) anti-inflammatory property; 4) immune enhancing function. There are still several problems need to be resolved, such as the optimal dose and level, and the efficacy and safety need large trial to confirm.

In this trial, we give the vitamin C in a way of sustained injection (200mg/kg/24h, 1g/h) for 4 days. Other published studies (11, 14) or registered clinical trials (NCT03680724) used the same dosage of vitamin C (200mg/kg/24h) for 4 days in total, but they give vitamin C in a bolus injection way. Some registered clinical trials (NCT03509350, NCT03592277) give vitamin C in a dose of 1.5g/q6h combined with other drugs such as Vitamin B1 and hydrocortisone. According to study by De Grooth et al (18), sustained injection 10 gram vitamin C for 48 hours led a sustained level of vitamin C

approximately above 5 times of the normal level, which might be helpful to prevent hypovitaminosis than bolus injection. This might due to lower stable plasma concentration of vitamin C reply with overwhelming oxidative stress better than peak level, still, the optimal drug delivery and plasma level need further investigation.

Protective effect of high dose vitamin C has been shown in several small scale clinical trial, such as Fowler team demonstrated a dose-dependent reduction of organ failure and 28 day mortality(11), but 5 years later Fowler team showed negative result of organ function change in a larger RCT CITRIS-ALI research with sample size of N=167. The difference between CITRIS-ALI and ours are: 1) our sample size will be larger (N=620); 2) severity of disease (sepsis plus ARDS) of the enrolled patients in CITRIS-ALI were worse than ours, so vitamin C administration might be later than ours. Therefore, further evaluation of the effect of vitamin C on sepsis seems warranted.

The most concern of high dose vitamin C safety is its potential harm to renal function and formation of kidney stone(19). A previous case report showed high dose oral intake of vitamin C for 6 years lead high urine oxalate excretion of and formation of ureteral stone in a 9-year-old children. Still, no reported renal stone as adverse event of short term intravenous usage of high dose vitamin C(20). Furthermore, the CITRIS-ALI report no adverse event. In our clinical practice, a few patients was found pink crystal (see supplement material) appear in urine during application of high dose vitamin C without renal function impairment. The urine crystal disappeared after stoppage of vitamin C infusion. This phenomenon was suspected to be related to vitamin C but still under investigation. Anyway, the renal function change of enrolled

patients should arise caution and receive evaluation timely. Patient with history of renal oxalate stones will not enrolled into the study.

Trial status

Currently recruiting. Trial start date was 1st August 2019.

V3.0: 2019.10

List of abbreviations

high dose vitamin C in sepsis (HDVCIS)

vitamin C (VitC)

Standard Protocol Items: Recommendations for Clinical Interventional Trials (SPIRIT)

informed consent form (ICF)

unblinding drug preparing nurse (UDPN)

participating site (PS)

Acute Physiology and Chronic Health Evaluation II (APACHE II)

Adverse event(AE)

Severe AE (SAE)

reactive oxidative species (ROS)

Declarations

- Ethics approval and consent to participate: The trial protocol has been approved by Clinical Trial Ethics Committee of Ruijin Hospital of Shanghai JiaoTong University School of Medicine. And consent has been obtained from all study participants.
- Consent for publication: Not applicable.
- Availability of data and materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.
- Competing interests: The authors declare that they have no competing interests
- Funding: The Clinical Research Project of Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, project number:2018CR004. The funding body plays no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.
- Authors' contributions: Enqiang Mao is the principal of the clinical trial. Bing Zhao to conduct and coordinate. Jian Li to inspect and provide help. Leshan Liu to design the online database. Mengjiao Li and Yihui Wang to assist the conduction and coordination. Silei Sun and Lili Xu to collect the samples. Xing Qi, Mengqi Xie, Yuhua Zhou, Tongtian Ni, Yi Yao, Peili Chen, Meiling Yu, Weisong Jiang to collect the data of cases.
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Trial registration: Chinese Clinical Trial Registry, ChiCTR1800017633. Registered 7 August 2018, <http://www.chictr.org.cn/showprojen.aspx?proj=29851>

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Supplementary Files

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- [Table1FlowChartofHDVCIS.pdf](#)
- [SPIRITChecklistforrandomisedstudies.doc](#)