Thiamine as a Renal Protective Agent in Septic Shock

Statistical Analysis Plan

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Background and Rationale:

Kidney injury during sepsis and septic shock is common and associated with worse overall outcomes.[1] To date, research aimed at attenuating septic kidney injury has largely focused on restoring renal perfusion.[2] Robust pre-clinical and clinical investigation, however, has repeatedly shown that septic renal injury can occur even in the absence of hypoperfusion and that the histopathologic pattern of septic renal injury is predominately one of apoptosis as opposed to ischemia/tubular necrosis.[3-6] Mitochondrial dysfunction leading to cellular apoptosis is an alternative, but largely unexplored, pathway for organ injury in sepsis.[7] Thiamine (vitamin B1) is a critical cofactor for aerobic respiration, and thiamine deficiency has been associated with impaired organ function.[8] The high concentration of mitochondria in the kidney, the importance of thiamine for mitochondrial respiration, and the results of prior studies of thiamine in septic shock[9,10] suggest that thiamine may be an effective agent for the attenuation of septic kidney injury.

Study overview

Study Design and Setting

This was a phase II, multi-center, randomized trial of thiamine (vitamin B1) vs. placebo in patients with septic shock. Enrolling sites were four medical centers in the United States. The trial coordinating center gathered and analyzed the data, and vouches for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. Written informed consent was obtained from the legally authorized representatives of all patients.

Trial Participants

Adult patients ≥18 years of age with septic shock were enrolled in the trial. Septic shock was defined by a suspected or confirmed infection, the receipt of vasopressors for suspected septic shock, and a serum lactate level ≥2mmol/L. To enrich for a population at risk of acute kidney injury (AKI), a serum creatinine level >1.0mg/dL was an inclusion criterion. Exclusion criteria included 1) clinical indication for thiamine administration (alcoholism, known or highly suspected deficiency) or treatment with thiamine beyond the amount found in a standard multivitamin within the last 10 days, 2) Renal replacement therapy within the past 30 days, 3) Comfort measures only or anticipated withdrawal of support within 24 hours, 4) Protected populations (pregnant women, prisoners), and 5) Known thiamine allergy.

Patient flow through the trial will be displayed in a CONSORT diagram (see Appendix Figure 1 for shell figure).

Written informed consent was obtained from all included patients or their legally authorized representatives.

Randomization and Blinding

Randomization: Participants were randomized in a 1:1 ratio to either thiamine or placebo in blocks with random sizes of 2 or 4. The randomization was stratified according to site. An independent statistician created the randomization list using a random number generator.

Blinding: The trial was quadruple-blinded; participants, investigators, the clinical team, and outcomes assessors were blinded to treatment allocation. Only the research pharmacy at each site providing the study drug was aware of the allocation.

Study Registration and Monitoring

The trial was registered at clinicaltrials.gov (NCT03550794).
The trial was conducted under an FDA IND (107, 135).

A Data Safety and Monitoring Board (DSMB) evaluated and monitored the trial for safety. There were no pre-specified stopping rules for futility or efficacy. No statistical interim analyses were performed.

The coordinating center regularly performed trial monitoring as detailed in the Trial Monitoring Plan.

Outcomes and Definitions

Primary Outcome:

Change in serum creatinine level between enrollment and 72-hours after enrollment.

Power Calculation: The planned sample size is based on the primary outcome. In this longitudinal repeated measures study design, we have 4 time points (baseline, 24-hours, 48-hours, and 72-hours), and are interested in measuring the effect of the intervention (thiamine) on the mean level of creatinine at the last time point (72-hours). We hypothesized that at 72-hours, the estimated mean difference (placebo-thiamine) would have similar magnitude as the pilot study's[9] observed mean difference (about 0.51). We used a linear mixed-effects model to estimate the parameters of the variance-covariance structure of the repeated measures data and used these estimates in obtaining the effect estimate of the mean difference at each of the 4 time points, with emphasis on 72-hours. We simulated the data using the estimated variance-covariance components from the pilot data with 0.19 for within-subject variance, and 1.3 for between-subject variance, and the estimated mean differences for the 4 time points from the pilot data. These data went through a linear mixed effects model where linear contrasts were used for estimation at each time point. The non-centrality parameter and the corresponding F-statistic value were then computed. Together with the numerator and denominator degrees of freedom, the estimate of power at each time point was derived. Through different iterations of sample size, at an overall total of 80 patients (40 patients in each arm), we obtained a power of 0.80 at 72-hours with an alpha of 0.05. This method of power analysis for linear mixed-effects model was published in 2000.[11] To account for drop-out and to ensure adequate power at the end of the trial, we planned to enroll a total of 88 patients (44 per arm).

Secondary Outcomes:

Renal replacement therapy
[Time Frame: From date of enrollment until discharge from the intensive care unit (ICU) or date of death, whichever comes first, up to 60 days after enrollment]
Need for renal replacement therapy

Acute renal failure
[Time Frame: From date of enrollment until ICU discharge or death]

ICU-free days
Days alive and free of the ICU between enrollment and day 28 after enrollment.
[Time Frame: From date of enrollment to 28-days after enrollment]

Change in the sequential organ failure assessment (SOFA) score

Change in the SOFA score between enrollment and 72-hours after enrollment
[Time Frame: From date of enrollment 72-hours after enrollment]

Delirium
Incidence of delirium as measured by the Confusion Assessment Method (CAM)-ICU on study day 3
[Time Frame: Day 3 after enrollment]

Change in serum lactate level
Change in serum lactate level between enrollment and 72-hours after enrollment
[Time Frame: From date of enrollment 72-hours after enrollment]

In-hospital mortality
[Time Frame: From date of enrollment until discharge from the hospital or date of death, whichever comes first, up to 60 days after enrollment]

Other biomarkers of renal injury (Serum cystatin C, NGAL, and KIM-1)
[Time Frame: Enrollment to 24-hours]

Peripheral blood mononuclear cell mitochondrial oxygen consumption
[Time Frame: Enrollment to 24-hours]

A table of outcomes by treatment group will be included (see Appendix Table 2)

Statistical Analysis

General Principles

The statistical analyses and reporting will adhere to the CONSORT guidelines.[12,13] All tests will be two-sided, a p-value < 0.05 will be considered significant, and all confidence intervals will have 95% coverage. All analyses will be conducted on a modified intention-to-treat basis only including participants receiving at least the first dose of the study medications. In a double-blind trial, this approach is unbiased while increasing precision.[14]

Baseline Characteristics

A description of the baseline characteristics will be presented by treatment group (see Appendix Table 1). Categorical variables will be summarized by frequencies and percentages. Percentages will be calculated according to the number of patients for whom data are available. Continuous variables will be summarized using means (standard deviations, SD) or medians (interquartile range, IQR) based on the distribution of the data. Statistical tests will not be used to compare baseline characteristics between groups.

Analysis of Primary Outcome

We will compare repeated measures of creatinine levels at each time point (0h, 24h, 48h, 72h) between arms using repeated measures analysis with the dependent variable of creatine and independent variables of treatment group, time, site, and the interaction between time and treatment group. A compound symmetry covariance structure will be used. If a patient receives renal replacement therapy or dies before any time point, creatinine levels will be imputed by carrying forward the last known value before the event with a 20% penalty.

Analysis of Secondary Outcomes

Receipt of Renal Replacement Therapy
Logistic regression controlling for site will be used to compare the incidence renal replacement therapy during the index ICU stay between groups.

Development of acute renal failure
Renal failure during the index ICU stay, which is a composite outcome of all-cause death or Kidney Disease Improving Global Outcomes (KDIGO) stage 3 acute renal failure within the index ICU stay after enrolment. Patients who met KDIGO 3 acute renal failure criteria at the time of initial study drug administration would not be identified as having an adverse kidney event unless they died during the index ICU stay. This outcome will be analyzed using logistic regression controlling for site.

Change in SOFA score
Change in SOFA score will be assessed using the same modeling approach as with the primary outcome. For patients who died prior to 72-hours, a 20% increase in SOFA score will be added to the last observed score prior to death. For patients with missing SOFA values for reasons other than death, the last observed SOFA score for that element will be carried forward without penalty.

Incidence of Delirium
Incidence of delirium on day 3 will be compared using logistic regression controlling for site. Delirium is assessed by the Confusion Assessment Method (CAM). Patients who are sedated on day 3 or otherwise cannot have CAM performed, will not be included in this analysis.

In-hospital mortality
In-hospital mortality will be analyzed using survival analysis. Results will be presented with Kaplan-Meier curves and the groups compared using the log-rank test.[15] Hazard ratios with 95% confidence intervals will be obtained using Cox's proportional hazards models.[16] The proportional hazards assumption will be verified by visual inspection of the Kaplan-Meier curves and testing of scaled Schoenfeld residuals.[17] If the proportional hazards assumption is not met, only the Kaplan-Meier curves and the p-value from the log-rank test will be presented. The patient will be considered to have survived their hospitalization if they remain alive and in the hospital at 60 days.

Change in serum lactate level
Change in serum lactate level will be assessed using the same modeling approach as for the primary outcome. For patients who died prior to 72-hours, a 20% increase in lactate will be added to the last observed score prior to death.

ICU Free Days
ICU-free days will be analyzed using linear regression controlling for site. Patients who are discharged alive from the hospital before 28-days will be assumed to have survived to 28-days.

Other biomarkers of renal injury
Other renal injury biomarkers (cystatin C, NGAL, and KIM-1) will be measured at enrollment and 24-hours and compared using linear regression, controlling for the enrollment level.

Oxygen Consumption
Basal and maximum cellular oxygen consumption will be will be measured at enrollment and 24-hours and compared using repeated measures analysis to account for within subject correlation.

Additional Planned Analyses

A pre-planned sub-analysis of the primary outcome will be performed in the group with baseline thiamine deficiency. Thiamine deficiency will be defined as a plasma vitamin B1 level ≤ 7 nmol/L as has been previously described.[18] Analysis will be performed through inclusion of an interaction term in the primary outcome model.

Analysis of Adverse Events

Rates of serious expected and unexpected adverse events will be reported by group assignment. Proportions of patients with adverse events will be compared between the treatment groups using Fisher's exact test.

Statistical Software

Stata (version 14, StataCorp, College Station, Tx) & R (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria) will be used for all analyses and graphics.

Appendices

Figure 1. Consort Diagram

Figure 2: Trend of Serum Creatinine Over Time by Treatment Group

Figure 3: Trend of Serum Lactate Over Time by Treatment Group

Figure 4: Kaplan-Meier Curve for outcome of In-Hospital Mortality

Figure 5: Biomarkers of Renal Injury over Time

Figure 6: Changes in Cellular Oxygen Consumption over Time

Table 1: Cohort Characteristics

Characteristic	Intervention (N=)	Placebo (N=)
Demographics		
Age (yrs, mean, SD)		
Body mass index (kg/m2, mean, SD, n/N)		
Sex		
Female, n (%)		
Male, n (%)		
Race, n (%)		
White		
Black		
Asian		
More than one race		
Hispanic, n/N (%)		
Past Medical History, n (%)		

Malignancy		
Coronary artery disease		
Congestive heart failure		
Liver Disease		
Chronic Kidney Disease		
Stage 2		
Stage 3		
Stage 4		
Stage unknown		
Clinical Characteristics		
Primary Infectious Source, n (%)		
Pneumonia		
Intra-abdominal		
Urinary tract infection		
Other		
Volume of intravenous fluids prior to study drug (ml, median, IQR)		
Baseline cardiovascular component of the total SOFA score (median, IQR)		
Time from vasopressor initiation to first study drug (hours, median, IQR)		
Time from informed consent to first study drug (hours, median, IQR)		
Mechanically ventilated, n (%)		

Lactate (mmol/L, median, IQR)		
30-day predicted survival, n (%)[f]		
High likelihood		
Uncertain		
Low Likelihood		

Table 2: Primary and Secondary Outcomes

Outcome	Intervention N=	Placebo N=	Effect Estimate (95%CI)	p-Value
Serum Creatinine Over Time (mean, SD, n/N) – Primary Outcome Measure				
Enrollment				
24-hours				
48-hours				
72-hours				
Secondary Outcomes				
In-Hospital Mortality, n (%)				
Receipt of Renal Replacement Therapy, n (%)				
KDIGO Acute Kidney Injury 3, n (%)				
Incidence of Delirium, (n, %)				
Serum Lactate at 24-hours (mmol/L, median, IQR)				
Change in SOFA score over 72 hours (mean difference, SD)				

ICU free days, (days, median, IQR)[g]				

References

1. Schrier RW, Wang W. Acute renal failure and sepsis. *N Engl J Med*. Jul 8 2004;351(2):159-69. doi:10.1056/NEJMra032401
2. Russell JA, Walley KR, Singer J, et al. Vasopressin versus norepinephrine infusion in patients with septic shock. *N Engl J Med*. Feb 28 2008;358(9):877-87. doi:10.1056/NEJMoa067373
3. Kosaka J, Lankadeva YR, May CN, Bellomo R. Histopathology of Septic Acute Kidney Injury: A Systematic Review of Experimental Data. *Crit Care Med*. Apr 7 2016;doi:10.1097/ccm.0000000000001735
4. Maiden MJ, Otto S, Brealey JK, et al. Structure and Function of the Kidney in Septic Shock. A Prospective Controlled Experimental Study. *Am J Respir Crit Care Med*. Sep 15 2016;194(6):692-700. doi:10.1164/rccm.201511-2285OC
5. Legrand M, Dupuis C, Simon C, et al. Association between systemic hemodynamics and septic acute kidney injury in critically ill patients: a retrospective observational study. *Crit Care*. 2013;17(6):R278. doi:10.1186/cc13133
6. Langenberg C, Bagshaw SM, May CN, Bellomo R. The histopathology of septic acute kidney injury: a systematic review. *Crit Care*. 2008;12(2):R38. doi:10.1186/cc6823
7. Fink MP. Cytopathic hypoxia. Mitochondrial dysfunction as mechanism contributing to organ dysfunction in sepsis. *Crit Care Clin*. Jan 2001;17(1):219-37.
8. Donnino MW, Carney E, Cocchi MN, et al. Thiamine deficiency in critically ill patients with sepsis. *J Crit Care*. Dec 2010;25(4):576-81. doi:10.1016/j.jcrc.2010.03.003
9. Moskowitz A, Andersen LW, Cocchi MN, Karlsson M, Patel PV, Donnino MW. Thiamine as a Renal Protective Agent in Septic Shock: A Secondary Analysis of a Randomized, Double-Blind, Placebo-Controlled Trial. *Ann Am Thorac Soc*. Feb 16 2017;doi:10.1513/AnnalsATS.201608-656BC
10. Donnino MW, Andersen LW, Chase M, et al. Randomized, Double-Blind, Placebo-Controlled Trial of Thiamine as a Metabolic Resuscitator in Septic Shock: A Pilot Study. *Crit Care Med*. Feb 2016;44(2):360-7. doi:10.1097/ccm.0000000000001572
11. Verbeke G, Molenberghs G. *Linear mixed models for longitudinal data*. Springer Science & Business Media; 2009.
12. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ*. Mar 23 2010;340:c332. doi:10.1136/bmj.c332
13. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ*. Mar 23 2010;340:c869. doi:10.1136/bmj.c869
14. Fergusson D, Aaron SD, Guyatt G, Hebert P. Post-randomisation exclusions: the intention to treat principle and excluding patients from analysis. Review. *BMJ*. Sep 21 2002;325(7365):652-4.
15. Fleming T, Harrington D. Nonparametric estimation of the survival distribution in censored data. *Communications in Statistics - Theory and Methods*. 1984;13(20):2469–2486.
16. Cox D. Regression models and life tables. *Journal of the Royal Statistical Society*. 1972;Series B(20):187–220.
17. Kleinbaum D, Klein M. *Survival Analysis: A Self-Learning Text*. 3rd ed. Springer; 2013.
18. Donnino MW, Andersen LW, Chase M, et al. Randomized, Double-Blind, Placebo-Controlled Trial of Thiamine as a Metabolic Resuscitator in Septic Shock: A Pilot Study. *Crit Care Med*. Feb 2016;44(2):360-7. doi:10.1097/CCM.0000000000001572