Role of AScorbic acid Infusion in critically ill patients with Transfusion Related Acute Lung Injury (ASTRALI)

Amira kassem[1], Islam Ahmed[1], Gamal Omran[1], Mohamed Megahed[2], and Tamer Habib[2]

[1]Damanhour University Faculty of Pharmacy
[2]Alexandria University Faculty of Medicine

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Abstract

Introduction: In critically ill patients, Transfusion Related Acute Lung Injury (TRALI) remains the leading cause of transfusion-related fatalities in critical care setting and associated with inflammation and oxidative stress state. Recent research raised the potential efficacy of high dose intravenous ascorbic acid in critically ill patients. Objective: The aim of this trial was to investigate the effect of high dose intravenous ascorbic acid (VC) as a targeted therapy for TRALI in terms of serum proinflammatory (interleukin-8, interleukin-1β, C-reactive protein), anti-inflammatory (interleukin-10), oxidative stress (superoxide dismutase, malondialdehyde) markers, and plasma VC levels. Secondary outcomes were oxygenation (PaO2/FiO2 ratio), vasopressor use, duration of mechanical ventilation, ICU length of stay, 7-days mortality and 28-days mortality. Methods: Eighty critically ill patients with TRALI (n=80) were randomized to receive 2.5gm/6hr intravenous vitamin C for 96 hours (ASTRALI group) or placebo. Patients were followed-up to measure the outcomes initially (T0) and at the end of treatment (T96). Results: When compared to control group, ASTRALI group at T96, showed significantly higher median of interleukin-10 (31.6 ± 25.8 Vs. 17.7 ± 12.0 pg/mL, p<0.0001) levels and superoxide dismutase (12876 ± 4627 U/L Vs. 5895 ± 6632 U/L, p<0.0001) activities, lower median C-reactive protein (76 ± 50 Vs. 89 ± 56 mg/L, p=0.033), interleukin-8 (11.8 ± 7.3, 35.5 ± 19.8 pg/mL, p<0.0001), and malondialdehyde (0.197 ± 0.034 Vs. 0.234 ± 0.074 µM/L, p=0.002) levels. Conclusion: High dose ascorbic acid was associated with significantly reduced oxidative stress, reduced pro-inflammatory markers except IL-1β, elevated anti-inflammatory marker, and elevated plasma VC levels

INTRODUCTION

Transfusion-associated lung disorders are fatal respiratory distress after pulmonary edema with direct relationship to transfusion that occurs within 6 hours after blood transfusion. It includes two types: transfusion-related acute lung injury and transfusion-associated circulatory overload. (1-3)

Transfusion Related Acute Lung Injury (TRALI) was defined as "acute non-cardiogenic pulmonary edema typically occurs [?] 6 hours following transfusion of plasma-containing blood products, such as packed red blood cells, fresh frozen plasma, platelets, or cryoprecipitate." (1, 2, 4) In critically ill patients, TRALI remains the leading cause of transfusion-related fatalities and is accompanied by a very significant morbidity and mortality. (5) Survival in such patients is as low as 53% compared with 83% in acute lung injury (ALI) controls. (6)

Three decades TRALI was considered as a rare complication due to its low reported range (0.08% to 15.1% per adult receiving blood transfusion). Subsequently it has been shown to be leading cause of morbidity and mortality after transfusion. (7) Furthermore, the incidence is about to be doubled in critically ill compared to general transfused hospital population. Also, this incidence is likely underreported due to variation in monitoring systems, blood product storage policies, and study design, as well as due to historical lack of

clear definition of TRALI. In densely populated developing countries, incidence has not decreased due to lack of male-only strategy for plasma donation. (1, 3)

The potential recipient risk factors for TRALI (usually called first hit) include older age, hematologic malignancy, trauma, shock, massive transfusion, mechanical ventilation, acute kidney injury, severe liver disease and surgery. (3, 8) Now, TRALI without any risk factor for acute respiratory distress syndrome (ARDS) is called "type I", while in presence of any risk factor or mild ARDS, it is called "type II". (7)

Pathogenesis of TRALI theorized to follow either 'two-hit' or 'threshold' model, with both models hypothesizing that TRALI is mediated by neutrophils. In two-hit model, underlying condition of transfusion recipient, or "first hit," attracts neutrophiles to pulmonary vasculature, while "second hit" from donor product activates neutrophils with resulting pulmonary damage. In threshold model, level of lung neutrophil priming acts as inverse threshold for activation of neutrophils by transfusion product proinflammatory mediators. (3, 9)

TRALI is associated with systemic inflammation characterized by low anti-inflammatory cytokine as interleukin-10 (IL-10), increased pro-inflammatory cytokine as IL-8. (9-11) Regulation of inflammation should include avoidance of overproduction of inflammatory mediators. So, it can be dampened not only by increasing IL-10 but also by decreasing IL-1β release. (12) C-reactive protein (CRP) is an acute phase protein which is up-regulated during infections and inflammation. (13) CRP was recently identified as a novel first hit in TRALI. (14, 15)

Till now, there is no established treatment for TRALI beyond supportive care and monitoring. Recently, potential therapies have been reviewed, and it was concluded that the most promising therapeutic strategies were IL-10 therapy, blocking IL-8 receptors, downregulation of CRP levels, or targeting Reactive Oxygen Species (ROS). (9, 11) Antioxidants, such as high dose vitamins, were recommended for next studies as possibly effective option. (11)

In the lung tissue, activation of neutrophils in response to inflammation leads to pulmonary dysfunction and injury. Activated neutrophils release ROS, proteolytic enzymes, and pro-inflammatory mediators leading to direct tissue damage and prolonged inflammation (16).

Vitamin C hypovitaminosis is observed in 70% of critically ill despite receiving recommended daily doses. This is because decreased intake, limited absorption, altered distribution, and increased metabolism. In response to acute inflammation, plasma levels of vitamin C fall rapidly with unclear mechanisms leading to marked alterations in human tissues including dysregulated inflammation and increased endothelial permeability and edema. (17-19)

Ascorbic acid (VC) is a water-soluble vitamin with pleiotropic functions. It is known to be antioxidant, anti-inflammatory, and immune-supporting molecule. It was found to protect against ROS-induced damage in the epithelial barrier. (20) The excellent ROS-scavenging properties of ascorbic acid are widely acknowledged and correlated with increased superoxide dismutase (SOD) activity and decreased malondialdehyde. (21) Malondialdehyde (MDA) is a well-known final product of polyunsaturated fatty acids peroxidation. MDA is overproduced with increase in free radicals, so it is commonly known as a marker of oxidative stress (22).

Following initial hyper-stimulation of the immune system, levels of anti-inflammatory mediators (IL-10) were found to be enhanced as a compensatory anti-inflammatory response, leading to neutrophil paralysis. Early studies showed that leukocyte chemotaxis could be restored with high doses of ascorbic acid. (20) Also, animal studies showed decreased histological end-organ damage, acute lung injury, and mRNA expression of the pro-inflammatory IL-8. (23)

Neutrophils accumulate ascorbic acid against a concentration gradient resulting in higher levels than plasma (about 100X) (24, 25). Active uptake of ascorbic acid occurs via specialized sodium-dependent vitamin C transporters. Accumulation of dehydroascorbic acid via glucose transporters is also reported in response to inflammation (26). Vitamin C can protect apoptotic cell death pathways and neutrophil clearance via reduction of pro-inflammatory mediators (20, 27).

Over the last 4 years, intravenous ascorbic acid was widely studied in critically ill patients (mostly septic) in different dose regimens. In a recent meta-analysis, intermediate dose (3 - 10 gm per day) was safe, well tolerated and associated with lower mortality. (28) Recent study showed a positive association with antioxidant capacity and plasma VC levels. (29)

The aim of this trial was to investigate the effect of high dose intravenous ascorbic acid as a targeted therapy for TRALI in terms of serum proinflammatory (interleukin-8, interleukin-1β, C-reactive protein), anti-inflammatory (interleukin-10), oxidative stress (superoxide dismutase, malondialdehyde) markers, and plasma VC levels. Secondary outcomes were oxygenation (PaO_2/FiO_2 ratio), vasopressor use, duration of mechanical ventilation, ICU length of stay, 7-days mortality and 28-days mortality.

METHODS

After approval of the Research Ethics Committee of Faculty of Pharmacy, Damanhour University (IRB, 1119PP19), protocol registration (NCT04153487, available from https://clinicaltrials.gov/ct2/show/NCT04153487), full written informed consent was taken from each patient's next of kin to participate and include data in this study. Sample size was calculated using G*Power software (version 3.1.9.4) and setting α error of 0.05, power of 80%, allocation ratio of 1 and effect size of 0.635 for 2-independent means of CRP (two tail t-test). (30)

This double blinded RCT (participant, Care Provider) was carried out on 80 adult (18 – 64 years) critically ill patients diagnosed with TRALI within 6 hours of transfusion at ICU units of two tertiary hospitals between November 2019 and July 2021. Diagnosis of TRALI was defined according to the NHLBI Working Group definitions and or the Canadian Consensus Conference criteria (31, 32) as the following: no evidence of ALI prior to transfusion, onset of ALI [?] 6 hours following cessation of transfusion, hypoxemia (PaO_2/FiO_2 [?] 300 mmHg or SaO2 [?] 90% on room air), radiographic evidence of bilateral infiltrates, and no prior evidence of left atrial hypertension.

Exclusion criteria were pregnancy, childbearing, breastfeeding, hypernatremia, known hypersensitivity to the study drug, parenteral nutrition containing ascorbic acid, active renal stone or history of urolithiasis, acute Kidney Injury, Glucose-6-phosphate dehydrogenase deficiency, iron or copper storage diseases, and immunocompromised patients (cancer or patients on immunosuppression drugs). Moribund patient not expected to survive 24 hours also were excluded. To avoid prior risk of ARDS, home mechanical ventilation (tracheotomy or noninvasive) was also exclusion except CPAP/BIPAP for sleep-disordered breathing.

All patients were subjected directly to data collection, clinical examination, routine laboratory investigations, chest imaging, and transthoracic echocardiography to confirm the inclusion criteria. In addition to their supportive and standard care, patients were randomly allocated with 1:1 ratio, using computer sheet available from randomizer.org, into 2 groups: ASTRALI (AScorbic acid in TRALI) group (n=40) received 2.5 gm IV ascorbic acid every 6 hrs (using black paper-covered syringe pump and infusion set) for 96 hours from diagnosis (10 gm/day for 4 days) (28, 33). Control group (n=40) received placebo in similar regimen. The used normal saline was calculated with daily fluid chart in both groups. Flow chart of the study according to CONSORT 2010 is illustrated in figure 1.

All patients were followed up and treated during the study time. Glucose monitoring using central laboratory measurements were used during 96 hrs of treatment and 36 hrs later. (34) The primary study outcomes were investigated initially at the day of diagnosis (T0) and after 96 hours (T96). Serum samples were assayed for IL-8, IL-10, IL-1β, SOD, and MDA using commercially available research ELISA kits (IL-8 SunRed no.201-12-0090, IL-10 SunRed no. 201-12-0103, IL-1β SunRed no. 201-12-0144, SOD Cayman no. 706002, MDA FineTest (35)). Total VC levels were directly measured at T0, T48, T96, T120, and T148 using plasma samples. The secondary outcomes were calculated starting from the time of enrollment. Mortality rates were calculated as all-cause mortality. All possible adverse events were monitored such as nausea, vomiting, hypersensitivity, acute kidney injury (AKI), and hyperoxaluria. (36, 37).

Statistical Analysis

The collected data were analyzed using software statistical computer package SPSS version 24.0 (IBM Corp., Armonk, NY, USA). Continuous normally distributed variables were expressed as mean ± SD. Continuous non-normally distributed variables were expressed as median ± IQR. Student t-test was used to determine the difference between the two groups of normally distributed variables. Mann-Whitney U test and Wilcoxon signed-rank were used to determine the difference between the two groups of non-normally distributed variables. Categorical variables are presented as number and percentage and were analyzed using the Chi-Square test or Fisher exact test. After Kaplan-Meier analysis, survival curves were compared using Log Rank test. Receiver Operating Characteristics (ROC) was plotted as sensitivity on Y axis and 1-specificity on X axis. The significance level was set at $p< 0.05$.

RESULTS

Regarding demographics, 45 males (56.3%) and 35 females (43.8%) were enrolled. The mean age of all enrolled patients was 49.9 ± 12.884 years. There were no statistically significant differences between the two groups in their sex ($p= 0.652$) or age ($p= 0.062$). The most prevalent causes of admission of all patients were hepatic causes (25%) such as hepatic encephalopathy, ascites, and variceal bleeding. This prevalence of hepatic patients is explained as one of enrollment centers is a hepatology center. All other causes were distributed similarly across the two studied groups without statistically significant differences ($p>0.05$). (Table 1)

The most prevalent medical history was obstructive lung diseases (40%), followed be smoking (35%), atrial fibrillation (33.8%), and hypertension (30%). All medical and drug histories were equally distributed across the 2 groups except diabetes, it was more prevalent in ASTRALI group (45%) than control group (20%) ($p=$ 0.031). The mean APACHE II score was 19.9 ±7.653. The mean SOFA score was 6.3 ± 2.867. Regarding Child Pugh score, most of the enrolled patients were classified as class A (75%). All clinical scores were comparable across the 2 groups (p>0.05). (Table 1)

Regarding the measured primary outcomes of ASTRALI group before (T0) and after treatment (T96), ASTRALI group showed statistically significant reduction of median CRP levels (68 ± 55 Vs. 189.8 ± 96 mg/L,p <0.0001), median IL-8 levels (11.8 ± 7.3 Vs.38.1 ± 17.624 pg/L,p <0.0001), and median MDA levels (0.197 ± 0.034 Vs. 0.280 ± 0.0553 μM/L) ($p< 0.0001$). ASTRALI group showed statistically significant elevations of median IL-10 (31.6 ± 25.8 Vs. 14.1 ± 5.411 pg/mL, $p< 0.0001$), median SOD levels (12876 ± 4627 Vs. 8493 ± 4489 U/L, $p< 0.0001$), and median VC levels (130.5 ± 111.65 Vs. 3.4 ± 2.00 mg/L,$p=$ 0.005*).* ASTRALI group showed comparable median IL-1β levels (11.7 ± 5.338 Vs. 12.4 ± 4.835 mg/L, $p=$ 0.098). (Table 2, Figure 2)

Regarding secondary outcomes, ASTRALI group showed significantly higher median of P/F ratios (342 ± 39) than control group (234 ± 57) at T96 (p <0.0001). (Table 2, Figure 3) ASTRALI group showed significantly lower 7-days mortality rate (15%) than control group (42.5%) ($p= 0.013$). After multivariate logistic regression, ASTRALI group was associated with significant 75.7% reduction of 7-days mortality (OR=0.243 [95% CI: 0.082 – 0.721], $p= 0.011$). (Table 3) Both groups showed comparable 28-days mortality rate without statistically significant differences ($p= 0.173$). (Figure 4)

DISCUSSION

The first trial, invigorated the possible role of high dose IV ascorbic acid in critically ill patients, was published by Marik. (38) Most trials evaluated the role of oral ascorbic acid in critically ill patients failed to show improved outcomes due to limited absorption, gut ischemia, impaired intestinal flora, and different distribution. (39) Animal models showed that the lungs are very susceptible to ascorbic acid deficiency. (40) Also, early reports showed that administration of ascorbic acid was associated with increased alveolar fluid clearance and decreased epithelial permeability. These effects were initiated via increased expression of Na+-K+-ATPase, aquaporin 5, and cystic fibrosis transmembrane conductance regulator. (41)

To our knowledge, this is the first RCT to investigate the role of high dose IV ascorbic acid in patients with TRALI in terms of oxidative stress, pro-inflammatory, and anti-inflammatory markers. Other trials are

still running in patients with close entities for TRALI such as ARDS, sepsis-induced acute lung injury, and COVID-19 pulmonary complications.

Primary outcomes

Regarding the measured primary outcomes after adjustment to its baseline, ASTRALI group showed significant reductions of median adjusted change % of CRP (-54.2% Vs. -48.2%, p= 0.049) and IL-8 (-71.3% Vs -7.2 %, p< 0.0001) levels than control group. ASTRALI group also showed significant elevations of median adjusted change % of IL-10 (170.2% Vs. 34.4%, p= 0.049) and SOD (58.9% Vs -20.8%, p< 0.0001). Both groups showed comparable median adjusted change % after treatment of IL-1β (p= 0.441) and MDA (p= 0.167). These findings were supported by reports that showed that incubation of high concentration ascorbic acid with peripheral blood lymphocytes increased generation of IL-10 and similar IL-1β. (20) Also, the role of high dose ascorbic acid is well identified in sepsis patients to reduce oxidative stress, inflammatory reactions, endovascular, and immunologic dysfunctions. (42)

Zhang et al. (2021) trial, critically ill COVID-19 patients were assigned to receive placebo or 24 gm daily IV infusion of ascorbic acid for 1 week. Although ascorbic acid group showed significantly reduced pro-inflammatory IL-6 levels (19.4 [95%CI: 10.6-29.2]) than control group (158 [95%CI: 15.3-259.6]), results failed to show differences in CRP levels at days 3 or 7. This failure to show improvement in in CRP levels may be due to advanced COVID-19 that were present before ARDS progression.

In CITRIS-ALI trial (2019), patients with septic ARDS were enrolled to receive 50mg/Kg IV ascorbic acid every 6 hrs or placebo for 96 hr. CRP levels showed no statistical difference (54.1 vs 46.1 μg/mL,p =0.33). (43) Although this trial is considered the one of the most promising trials that supposed to show benefits of high dose ascorbic acid for critically ill patients. The primary outcome of the study was the difference in SOFA score at T96. The results of this trial were criticized due to some statistical problems such as Neyman's bias and survivorship bias. These problems lead to underestimation of the true benefits of ascorbic acid. Patients' selection is also questioned because 32% of patients are transferred from another health care facilities. This latency period could alter measuring the outcomes. Finally, plasma VC levels do not fit well with the reported changes of CRP. (44)

Secondary outcomes

Regarding secondary outcomes, ASTRALI group showed significantly higher median of P/F ratios only at T48 (p= 0.003), T72 (p< 0.0001) and T96 (p< 0.0001). ASTRALI group showed significantly lower median of VP days (1 ± 4) than control group (4 ± 5) (p= 0.013). Both groups showed comparable medians of ICU stay (p= 0.649) and MV days (p= 0.611). These findings are supported by the knowledge of that ascorbic acid is a cofactor in the hydroxylase enzymes which are involved in the synthesis of important catecholamines (such as norepinephrine and vasopressin) and downregulation of hypoxia inducible factor 1α. (20) The small sample size used in the current study may affect the significance of outcomes such as MV days, because strong evidence that high dose IV ascorbic acid shortens MV days in critically ill patients was identified in a meta-regression analysis (2020). (45)

Zhang et al. (2021) showed sustained significant improvement of P/F ratio of ascorbic acid group (228.5 ± 72.6) than control (150.7 +- 75.3) (p= 0.01). (35) Saeidreza et al. (2021) studied the effect of IV ascorbic acid (1.5 gm/ 6hrs for 5 days) in COVID-19 patients. Results showed improved peripheral capillary oxygen saturations in ascorbic acid arm over controls. (46)

In Wang et al. (2019) meta-analysis, results showed that ascorbic acid was associated with a decreased MV days, but whether this reflected improved oxygenation was uncertain. (47) Another meta-analysis in 2015 showed significant reduction of VP requirements in critically ill patients. (48) In Zabet et al. (2016) study, IV 25 mg/kg every 6 hrs ascorbic acid in septic shock patients showed lower MV days (standardized mean difference, SMD = -0.73 [95% CI: -1.5-0.04]) and reduced vasopressor requirements than control group (49.64 +- 25.67 Vs. 71.57 +- 1.60 h, p = 0.007). (49)

In contrast, Matthew Li et al. (2021) did not to find any improvement in VP use or ICU days in ascorbic

acid group (1.5 gm/6 hrs) over controls with COVID-19. (50) In CITRIS-ALI trial (2019), ascorbic acid group showed similar oxygenation index and VP use at T96 and T168 of follow up. (43) Also, Flower et al. (2014) showed nearly similar MV days (SMD=0.15 [95% CI: -0.70–1.000]) and VP requirements (SMD= -0.49 [95% CI: -1.35–0.37]) (51)

In terms of mortality, after Kaplan-Meier analysis, survival curves were compared. ASTRALI group showed significantly higher 7-days survival than control group (p= 0.007). Then, Cox regression was used as multiple comparisons (HR= 0.305 [95% CI: 0.120–0.775],p = 0.008). ASTRALI group was associated with 75.7% reduction of 7-days mortality (OR=0.243 [95% CI: 0.082 – 0.721], p= 0.011). This reduction in mortality was partially explained by IL-10 levels after treatment as it was associated with 6.1% reduction (OR=0.939 [95% CI: 0.894 – 0.987],p= 0.013). Receiving ascorbic acid "ASTRALI group" was a fair predictor for 7-days survival (AUROC=0.668, [95% CI: 0.538–0.797],p = 0.019). Also, post treatment IL-10 at a cut-off value of equal or more than 19.55 pg/mL was a good predictor for 7-days survival (AUROC=0.708, [95% CI: 0.584–0.831], p = 0.004).

This finding reflects that ascorbic acid may be associated with better 7-days survival via enhancing IL-10 levels in such patients Although our findings showed short term mortality benefit, results failed to find this mortality benefit after 28-days follow-up. This may be due to the small sample size and long-term ICU complications evolved due to heterogeneity in comorbidities between groups such as diabetes. The prevalence of diabetes was nearly doubled in ASTRALI group than controls (45% Vs. 20%) (p= 0.031).

CITRIS-ALI trial (2019) showed that 28-days mortality was lower in the ascorbic acid group (29.8%) than placebo (46.3%) (p= 0.03). Although this study failed to show different primary outcomes, the Kaplan-Meier survival curves for the two groups were significantly different (p= 0.01) in favor of ascorbic acid group than control. (43) Wang et al. Meta-analysis (2019) showed that IV 3-10 gm/day ascorbic acid for critically ill patients was associated with reduced mortality (OR=0.25 [95% CI: 0.14–0.46)]). (47) In Zabet et al. (2016) study, IV ascorbic acid showed significantly reduced 28-days mortality than control group (14.28% Vs. 64.28%, p = 0.009) (OR=0.09 [95% CI: 0.01 – 0.59]). (49)

In contrast, Zhang et al. pilot trial (2021) showed no difference in 28-days mortality between ascorbic acid group (26 [95% CI: 9.0-28.0]) and control group (22 [95%CI: 8.50-28.0]) (p= 0.57). (35) But this trial showed imbalanced sex distribution across groups. Control group was supported with significantly positive fluid balance than ascorbic acid group on day 3 (463 Vs. -240, p= 0.02). Also, treatment with high dose ascorbic acid was started 10 days after symptoms.

Also, Matthew Li et al. (2021) showed increased in-hospital mortality in ascorbic acid group (88% Vs. 79%, p= 0.049) than control group. (50) But this study had several limitations starting from very small size (n=8 for ascorbic acid group and n=24 for control), retrospective design, and bias liable design. Very late ascorbic acid treatment was initiated, and heterogeneity of baseline characteristics was extended even after propensity score matching to form a control group.

Ferron-Celma et al. (2009), studied the effect of IV 450 mg/d ascorbic acid in septic Patients after abdominal surgery. No difference was detected in 7-days mortality (OR=2.25 [95% CI: 0.38 – 13.47]). (52) In addition to the small dose of ascorbic acid used, this study had very small sample size (n=10) and blinding of outcomes assessment was not clear.

Safety and adverse events

Regarding safety and tolerability of the high dose ascorbic acid, median of VC levels of ASTRALI group were significantly higher than control at T48 (p< 0.0001), T96 (p< 0.0001) and T120 (p< 0.0001). At T144, VC levels returned to normal with was no statistically significant difference between the two groups (p= 0.739). (Figure 3) The two only reported adverse effects were hypernatremia and AKI, with similar distributions across groups (p >0.05).

In a recent pharmacokinetic trial, IV 10 gm/d ascorbic acid bolus or continuous infusion showed varying decrease in VC levels in all groups 48 hrs after the end of infusion, and no significant difference in VC level.

This varying reduction may be due to differences in all pharmacokinetic parameters. In patients without a history of nephrolithiasis, the clinical relevance of hyperoxaluria and stone formation is negligible. (53)

In phase I safety trial, 24 septic ICU patients were randomized to receive IV ascorbic acid infusions every 6 hours for 4 days as 50 mg/kg/d (n = 8), or 200 mg/kg/d (n = 8), or placebo (n = 8). Patients were monitored for hypernatremia, hypotension, tachycardia, and GI adverse effects. Mean baseline plasma VC levels for all patients were 17.9 +- 2.4 μM (normal 50-70 μM). Ascorbic acid infusion rapidly and significantly raised plasma VC levels with no adverse events. (51) In Zhang et al. trial, daily infusion of 24 gm ascorbic acid for 7 days was not associated with significant incidence of AKI, liver injury, cardiac injury, septic shock, or coagulation disorders. (35) In two other trials, no ascorbic acid-related adverse events were identified. (43, 49)

Reports showed that maintaining plasma VC levels in critically ill patients requires increased frequency of dosing (4 times or continuous infusion) due to impaired distribution volume and increased clearance. There is currently no upper limit of IV dose, although nephrolithiasis at doses > 100 gm was reported. (54)

This study had several limitations. Although most of the primary outcomes were significantly different, the small sample size used may affect the significance of secondary outcomes, which may be more clinically important. Some baseline characteristics were not equally distributed across groups such as diabetes. Because one of our enrollment sites was a hepatology center, 25% of patients were hepatic. These deviations may affect the generalizability of our results to general population of critically ill patients with TRALI. It was not easy to enroll patients with TRALI. Although all possible means were used to confirm the diagnosis of TRALI, we cannot guarantee that there were no other forms of lung injury such as sepsis-induced ALI, ARDS or TACO. TRALI is a very difficult diagnosis. About 21.3% of patients were initially admitted with sepsis. Another 13.8% were trauma patients. Although no patients were enrolled with lung contusions and no radiological evidence of lung injury at the time of enrollment, both conditions are risk factors for ARDS. In this study, we focused on levels of pro-inflammatory and anti-inflammatory markers as primary outcomes. We did not add more secondary outcomes to avoid the problems of multiple testing and finding an outcome by chance. Organ failure assessment was not followed-up, it was not the scope of this trial.

CONCLUSION

In the light of the previous results, the use of high dose ascorbic acid (VC) infusion in a mixed population of critically ill patients with Transfusion Related Acute Lung Injury "TRALI" was associated with significantly reduced oxidative stress, reduced pro-inflammatory markers except IL-1β, elevated anti-inflammatory marker, and elevated plasma VC levels. Although it failed to show 28-days mortality benefit, it was associated with better oxygenation, less vasopressor use, and improved 7-days mortality. This short-term mortality benefit is supposed to be via enhanced levels of serum IL-10.

Firstly, we recommend more research about TRALI, especially in developing countries. Further larger studies are recommended to enlighten research community the benefits of high dose ascorbic acid with TRALI, best time to administer, and the exact dose regimen. Failure to show 28-days mortality benefit may reflect the need of longer treatment with ascorbic acid beyond 96 hours. Targeting IL-10 in such patients is also recommended for further research.

Conflicts of interest

Authors declare to have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Sharing

Data are available upon reasonable request. Contact the corresponding author.

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(Table 1): Baseline Characteristics of all the enrolled patients

Overall

(n = 80)

Overall

(n = 80)

ASTRALI group

(n = 40)

ASTRALI group

(n = 40)

Control group

(n = 40)

Control group

(n = 40)

p value

No.

%

No.

%

No.

%

Male

45

56.3

21

52.5

24

60.0

0.652

Female

35

43.8

19

47.5

16

40.0

Age (years)

49.9 ± 12.884

49.9 ± 12.884

52.6 ± 11.582

52.6 ± 11.582

47.2 ± 13.686

47.2 ± 13.686

0.062

Hepatic Sepsis Neurological Trauma Postoperative Drug toxicity AF

20 17 15 11 7 7 3

25.0 21.3 18.8 13.8 8.8 8.8 3.8

10 9 10 4 1 3 3

25.0 22.5 25.0 10.0 2.5 7.5 7.5

10 8 5 7 6 4 0

25.0 20.0 12.5 17.5 2.6 10.0 0

1.000 1.000 0.252 0.518 0.108 1.000 0.241

OLD Smoking AF Hypertension Diabetes HCV Steroids Aspirin Diuretic

32 28 27 24 26 17 32 25 29

40.0 35.0 33.8 30.0 32.5 21.3 40.0 31.3 36.3

17 14 15 11 18 8 15 16 14

42.5 35.0 37.5 27.5 45.0 20.0 37.5 40.0 35.0

15 14 12 13 8 9 17 9 15

37.5 35.0 30.0 32.5 20.0 22.5 42.5 22.5 37.5

0.820 1.000 0.637 0.808 0.031* **1.000 0.820 0.147 1.000**

Child Pugh A B C

60 9 11

75.0 11.3 13.8

30 3 7

75.0 7.5 17.5

30 6 4

75.0 15.0 10.0

0.641

APACHE II

19.9 ±7.653

19.9 ±7.653

18.7 ±8.084

18.7 ±8.084

21.1 ±7.060

21.1 ±7.060

0.166

SOFA score

6.3 ± 2.867

6.3 ± 2.867

6.7 ± 2.928

6.7 ± 2.928

5.9 ± 2.786

5.9 ± 2.786

0.229

SBP mmHg

88.7 ±4.056

88.7 ±4.056

88.5 ±3.658

88.5 ±3.658

88.7 ±4.457

88.7 ±4.457

0.662

DBP mmHg

63.0 ±3.142

63.0 ±3.142

63.2 ±5.331

63.2 ±5.331

101.7 ±6.956

101.7 ±6.956

0.621

HR beats/min

102.2 ±6.185

102.2 ±6.185

102.8 ±64.420

102.8 ±64.420

163 ±88.859

163 ±88.859

0.409

RR cycles/min

28.8 ±2.106

28.8 ±2.106

28.6 ±2.108

28.6 ±2.108

28.9 ±2.122

28.9 ±2.122

0.563

Temp ºC

38.0 ±0.586

38.0 ±0.586

37.9 ±0.616

37.9 ±0.616

38.0 ±0,586

38.0 ±0,586

0.572

Hemoglobin g/dL

9.8 ± 2.296

9.8 ± 2.296

9.8 ± 2.327

9.8 ± 2.327

9.6 ± 2.292

9.6 ± 2.292

0.750

WBCs count x10^9

8412 ± 3444

8412 ± 3444

8675 ± 3630

8675 ± 3630

8151 ± 3273

8151 ± 3273

0.500

PLTs count ξ10^3 /μΛ βλοοδ

164.1 ± 61.195

164.1 ± 61.195

158.1 ± 54.128

158.1 ± 54.128

170.2 ± 67.688

170.2 ± 67.688

0.383

Urea mg/dL

51.1 ± 14.807

51.1 ± 14.807

54.2 ± 16.028

54.2 ± 16.028

48 ± 12.936

48 ± 12.936

0.057

S.Cr mg/dL

1.4 ± 0.561

1.4 ± 0.561

1.5 ± 0.564

1.5 ± 0.564

1.3 ± 0.542

1.3 ± 0.542

0.068

AST U/L

248.0 ± 352.9

248.0 ± 352.9

235.5 ± 288.9

235.5 ± 288.9

260.7 ± 410.4

260.7 ± 410.4

0.752

ALT U/L

242.8 ± 338.9

242.8 ± 338.9

230.7 ± 300.3

230.7 ± 300.3

254.8 ± 377.2

254.8 ± 377.2

0.753

INR

2.7 ± 1.615

2.7 ± 1.615

2.8 ± 1.552

2.8 ± 1.552

2.7 ± 1.695

2.7 ± 1.695

0.839

PCT ng/mL

0.86 ± 0.552

0.86 ± 0.552

0.96 ± 0.499

0.96 ± 0.499

0.75 ± 0.587

0.75 ± 0.587

0.082

Data expressed as mean ±SD

AF: Atrial Fibrillation, OLD: Obstructive lung diseases, HCV: Hepatitis C virus, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HR: Heart Rate, RR: Respiratory rate, Temp: body surface Temperature, WBCs: White Blood Cells, PLTs: Platelets, S.Cr: Serum Creatinine, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase. INR: International Normalized Ratio, PCT: Procalcitonin.

All p values are significant when p[?] 0.05

(Table 2): The measured study outcomes of all the enrolled patients

Outcomes

Overall

(n = 80)

Overall

(n = 80)

ASTRALI group

(n = 40)

ASTRALI group

(n = 40)

ASTRALI group

(n = 40)

Control group

(n = 40)

Control group

(n = 40)

p value

No.

%

No.

No.

%

No.

%

CRP (T0) mg/L

164 +- 67.787

164 +- 67.787

177 +- 79.931

177 +- 79.931

177 +- 79.931

151 +- 50.604

151 +- 50.604

0.082

CRP (T96) mg/L

76 (50)

76 (50)

68 (55)

68 (55)

68 (55)

89 (56)

89 (56)

0.033*

IΛ-1β (T0) mg/L

12.9 ± 5.091

12.9 ± 5.091

12.4 ± 4.835

12.4 ± 4.835

12.4 ± 4.835

13.5 ± 5.346

13.5 ± 5.346

0.364

IΛ-1β (T96) mg/L

11.6 ± 5.408

11.6 ± 5.408

11.7 ± 5.338

11.7 ± 5.338

11.7 ± 5.338

11.4 ± 5.539

11.4 ± 5.539

0.796

IL-8 (T0) pg/mL

40.0 ± 17.001

40.0 ± 17.001

38.1 ± 17.624

38.1 ± 17.624

38.1 ± 17.624

41.9 ± 16.349

41.9 ± 16.349

0.316

IL-8 (T96) pg/mL

18.9 (23.9)

18.9 (23.9)

11.8 (7.3)

11.8 (7.3)

11.8 (7.3)

35.5 (19.8)

35.5 (19.8)

<0.0001*

IL-10 (T0) pg/mL

15.1 ± 5.752

15.1 ± 5.752

14.1 ± 5.411

14.1 ± 5.411

14.1 ± 5.411

16.1 ± 5.967

16.1 ± 5.967

0.114

IL-10 (T96) pg/mL

24.7 (17.3)

24.7 (17.3)

31.6 (25.8)

31.6 (25.8)

31.6 (25.8)

17.7 (12.0)

17.7 (12.0)

<0.0001*

SOD (T0) U/L

8758 ± 4149

8758 ± 4149

8493 ± 4489

8493 ± 4489

8493 ± 4489

9023 ± 3819

9023 ± 3819

0.571

SOD (T96) U/L

10272 (8621)

10272 (8621)

12876 (4627)

12876 (4627)

12876 (4627)

5895 (6632)

5895 (6632)

<0.0001*

MDA (T0) μM/L

0.29 ± 0.055

0.29 ± 0.055

0.28 ± 0.0553

0.28 ± 0.0553

0.28 ± 0.0553

0.30 ± 0.0535

0.30 ± 0.0535

0.302

MDA (T96) μM/L

0.211 (0.063)

0.211 (0.063)

0.197 (0.034)

0.197 (0.034)

0.197 (0.034)

0.234 (0.074)

0.234 (0.074)

0.002*

VC (T0) mg/L

3.6 ± 0.988

3.6 ± 0.988

3.4 ± 1.021

3.4 ± 1.021

3.4 ± 1.021

3.9 ± 0.945

3.9 ± 0.945

0.300

VC (T48) mg/L

22.2 ± 92.48

22.2 ± 92.48

94.9 ± 43.35

94.9 ± 43.35

94.9 ± 43.35

3.0 ± 1.30

3.0 ± 1.30

<0.0001*

VC (T96) mg/L

53.0 (126.40)

53.0 (126.40)

130.5 (111.65)

130.5 (111.65)

130.5 (111.65)

4.2 (2.37)

4.2 (2.37)

<0.0001*

VC (T120) mg/L

19.3 (47.50)

19.3 (47.50)

50.4 (29.98)

50.4 (29.98)

50.4 (29.98)

3.8 (0.62)

3.8 (0.62)

<0.0001*

VC (T144) mg/L

3.4 (0.95)

3.4 (0.95)

3.2 (1.95)

3.2 (1.95)

3.2 (1.95)

3.5 (0.87)

3.5 (0.87)

0.739

P/F ratio (T0)

161 (79)

161 (79)

157 (62)

157 (62)

157 (62)

170 (102)

170 (102)

0.115

P/F ratio (T24)

200 (83)

200 (83)

203 (79)

203 (79)

203 (79)

191 (103)

191 (103)

0.073

P/F ratio (T48)

229 (64)

229 (64)

233 (61)

233 (61)

233 (61)

218 (70)

218 (70)

0.003*

P/F ratio (T72)

255 (84)

255 (84)

298 (67)

298 (67)

298 (67)

233 (36)

233 (36)

<0.0001*

P/F ratio (T96)

289 (125)

289 (125)

342 (39)

342 (39)

342 (39)

234 (57)

234 (57)

<0.0001*

MV days

5 (3)

5 (3)

4 (3)

4 (3)

4 (3)

5 (3)

5 (3)

0.611

ICU days

7 (6)

7 (6)

8 (6)

8 (6)

8 (6)

7 (6)

7 (6)

0.649

VP days

3 (5)

3 (5)

1 (4)

1 (4)

1 (4)

4 (5)

4 (5)

0.019*

7 days-mortality

23

28.8

6

15

15

17

42.5

0.013*

28 days-mortality

33

41.3

13

32.5

32.5

20

50.0

0.173

Hypernatremia

4

5.0

3

7.5

7.5

1

2.5

0.615

AKI

9

11.3

4

10.0

10.0

5

12.5

1.000

Data expressed as mean ±SD or median (IQR).

CRP: C-reactive protein, IL: Interleukin, SOD: Superoxide Dismutase, MDA: Malondialdehyde

P/F ratio: PaO2/FiO2 ratio, AKI: Acute Kidney Injury

All p values are significant when p[?] 0.05

(Table 3):Multivariate logistic regression analysis for 7-days mortality

Variable	**7-days mortality**	**95% C.I.**	**95% C.I.**	p value
	odds ratio (OR)	**LL**	**UL**	
ASTRALI group	0.243*	0.082	0.721	0.011*
Age	0.997	0.959	1.037	0.881
Sex	0.908	0.324	2.541	0.854
Steroids	0.487	0.164	1.446	0.195
Aspirin	0.926	0.293	2.925	0.895
Diuretics	2.053	0.721	5.841	0.178
CRP (T96)	1.000	0.986	1.014	0.982
IΛ-1β (T96)	1.032	0.943	1.129	0.492
IL-8 (T96)	1.032	1.000	1.065	0.050
IL-10 (T96)	0.939*	0.894	0.987	0.013*
SOD (T96)	1.000	1.000	1.000	0.361
MDA (T96)	1.019	0.997	1.041	0.096
VC-T48 change %	0.998	0.992	1.003	0.408
VC-T96 change %	0.999	0.994	1.003	0.513
VC-T120 change %	0.994	0.971	1.018	0.619
VC-T144 change %	0.987	0.955	1.021	0.465
P/F ratio (T24) change %	1.004	0.999	1.010	0.142
P/F ratio (T48) change %	1.004	0.999	1.009	0.099
P/F ratio (T72) change %	1.002	0.998	1.007	0.282
P/F ratio (T96) change %	0.999	0.995	1.004	0.810

CRP: C-reactive protein, IL: Interleukin, SOD: Superoxide dismutase, MDA: Malondialdehyde, VC: Vitamin C, P/F ratio: PaO_2/FiO_2 ratio.

% C.I.: 95% Confidence interval (Lower Limit, Upper Limit)

**p is significant when p[?]0.05*

(Figure 1): Flowchart of the ASTRALI trial

(Figure 2): The measured post-treatment (T96) oxidative stress and inflammatory markers: *oxidative stress (A, B), proinflammatory (C, D, E), anti-inflammatory (F) markers*

(Figure 3): Trend over time oxygenation and VC levels;*(A) Median P/F ratio, (B)* Median VC levels

(Figure 4): Kaplan-Meier survival curves: *7-days survival data on the left (Log Rank p=0.007), 28-days survival data on the right (Log Rank p=0.063)*

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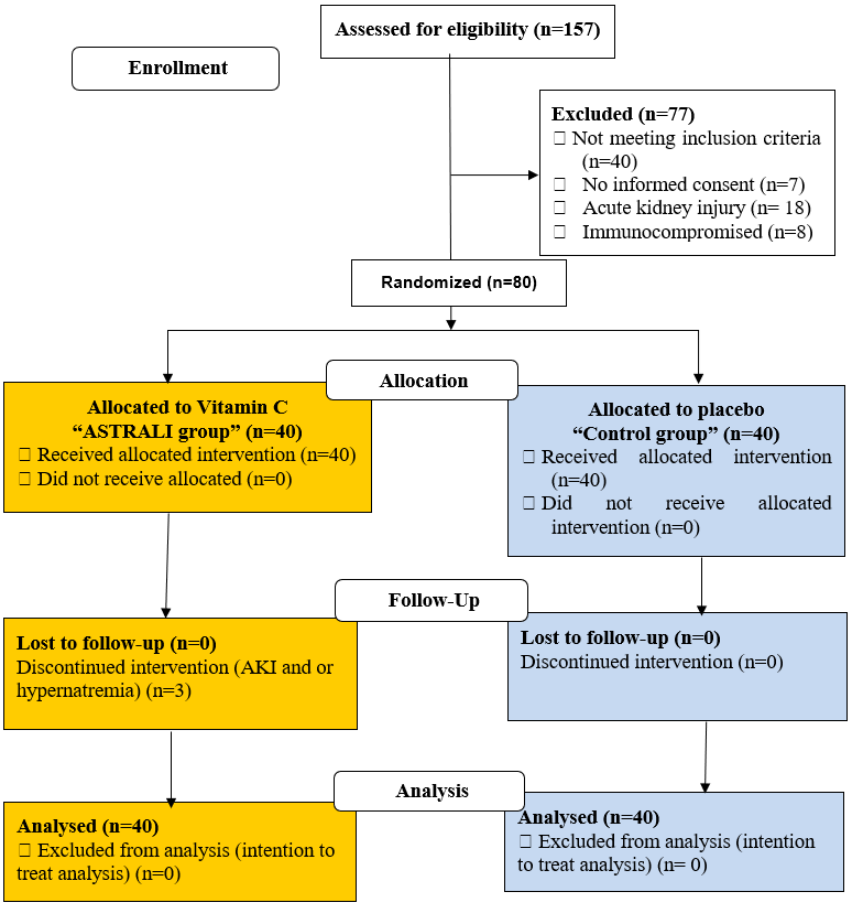
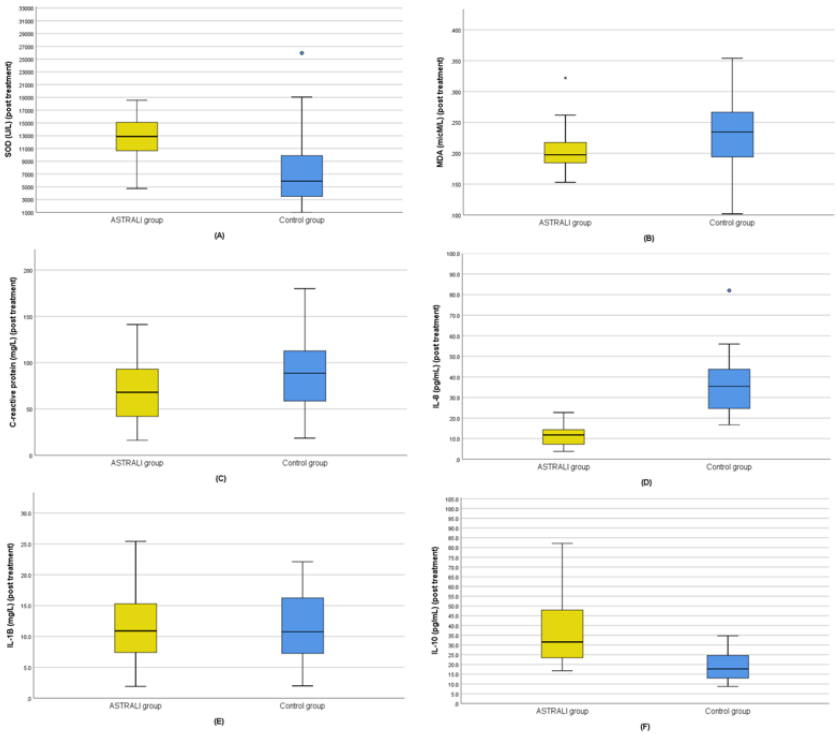
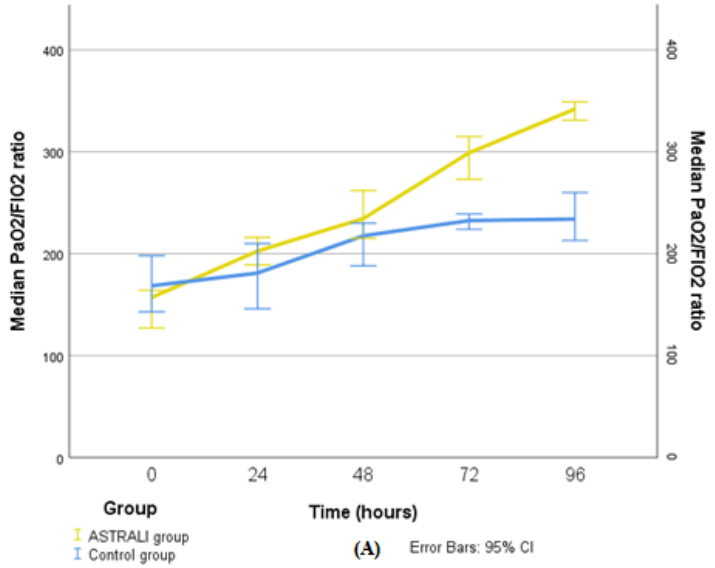
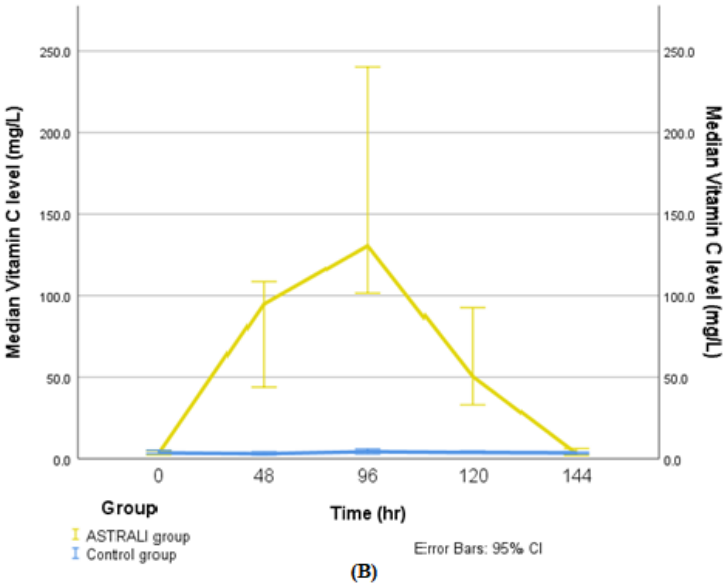
`Tables.docx` available at https://authorea.com/users/351583/articles/542924-role-of-ascorbic-acid-infusion-in-critically-ill-patients-with-transfusion-related-acute-lung-injury-astrali







(A)



(B)