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Inhibiting the Inflammatory Injury After Myocardial Ischemia Reperfusion With Plasma-Derived Alpha-1 Antitrypsin: A Post Hoc Analysis of the VCU- α 1RT Study

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Abstract

Background: Despite the benefits of reperfusion in limiting myocardial injury, the infarct size continues to expand after reperfusion because of secondary inflammatory injury. Plasma-derived alpha-1 antitrypsin (AAT) inhibits the inflammatory injury in myocardial ischemia and reperfusion. To explore the effects of plasma-derived AAT on the inflammatory response to ischemia-reperfusion injury, we analyzed time-to-reperfusion and enzymatic infarct size estimates in a post hoc analysis of the VCU- α 1RT clinical trial ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01936896) NCT01936896).

Methods: Ten patients with ST-segment elevation acute myocardial infarction (STEMI) were enrolled in an open-label, single-arm treatment study of Prolastin C, plasma-derived AAT, at 60 mg/kg infused intravenously within 12 hours of reperfusion. Biomarkers were measured serially over the first 72 hours, and patients were followed clinically for the occurrence of new-onset heart failure, recurrent MI, or death. Twenty patients with STEMI who had been enrolled in previous randomized trials with identical inclusion/ exclusion criteria and had been assigned to placebo served as historical controls.

Results: Time to percutaneous coronary intervention and time to drug did not significantly differ between groups. AAT-treated patients had a significantly shorter time-to-peak creatine kinase myocardial band (CK-MB) values (525 [480–735] vs. 789 [664– 959] minute, $P=0.005$) and CK-MB area under the curve (from 1204 [758–2728] vs. 2418 [1551–4289] U\$day, $P=0.035$), despite no differences in peak CK-MB (123 [30–196] vs. 123 [71–213] U/ mL, $P=0.71$).

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N. A. Abouzaki and S. Christopher equally contributed to the realization of this study.

The authors report no conflicts of interest.

Conclusions: A single administration of Prolastin C given hours after reperfusion in patients with STEMI led to a significant shorter time to peak and area under the curve for CK-MB, despite similar peak CK-MB values. These preliminary data support the hypothesis that Prolastin C shortens the duration of the ischemia-reperfusion injury in patients with STEMI.

Keywords

ischemia; reperfusion; injury; inflammation; biomarkers

INTRODUCTION

Despite the improvements in the therapies in the past 4 decades and the large reduction in early in-hospital mortality, the incidence of heart failure (HF) after acute myocardial infarction (AMI) has not declined.¹ There is therefore an urgent need to find additional treatments to reduce HF after AMI. The initial injury to the heart during AMI is characterized by a direct ischemic injury to the myocardium. This injury is limited by current strategies of prompt reperfusion, and initiatives reducing door-to-drug or door-to-balloon times have shown an improvement in outcomes.² A second phase of injury occurs after reperfusion: only a portion of the potentially salvageable previously ischemic myocardium is effectively saved by reperfusion, whereas a substantial portion continues to die.^{3,4} The intensity of the acute inflammatory response during ST-segment elevation AMI (STEMI) is a predictor of adverse outcome, and anti-inflammatory approaches have been tested to blunt the acute inflammatory response in STEMI.⁵

Administration of plasma-derived alpha-1 antitrypsin (AAT) quenches the inflammatory response in experimental myocardial infarction in the mouse and reduces the amount of injury because of ischemia reperfusion.⁶ AAT was efficacious also when administered with a delay of 30 minutes after reperfusion, therefore providing evidence that the effects of AAT were not only due to reduction of ischemic injury but also due to reduction of the response to the ischemia-reperfusion injury.⁷ To explore the potential translational clinical value of AAT as a mean to inhibit the inflammatory component of the ischemia-reperfusion injury, we performed a post hoc analysis of the time to reperfusion and enzymatic infarct size estimates in 10 patients with STEMI who received AAT as part of a pilot clinical study (VCU- α 1RT), in which the acute inflammatory response was inhibited by approximately 90%.⁸

METHODS

Clinical Trial Design

The details, and main results, of the VCU- α 1RT pilot study (www.clinicaltrials.gov NCT01936896) have already been published.⁸ Briefly, 10 patients received AAT Prolastin C (Grifols Inc, Raleigh, NC), plasma-derived AAT, as an open-label treatment within 12 hours of reperfusion for STE-MI, as a single infusion of 60 mg/kg at a rate of 0.08 mL·kg⁻¹·min⁻¹ (lasting between 12 and 18 minutes), according to the prescribing information approved by the Food and Drug Administration.⁸ The clinical data were compared with data derived from 20 historical control subjects, patients with STEMI who had been enrolled in the VCU-ART

and VCU-ART2 clinical trials^{9–11} that had virtually the same inclusion/exclusion criteria to VCU- α 1RT, and who had been randomly assigned to placebo (NaCl 0.9%), therefore representing a cohort of 20 patients with STEMI previously randomly assigned to placebo.

Calculations of Time-Derived Parameters

We reviewed the emergency department records, cardiac catheterization laboratory reports, and the laboratory data and calculated (Fig. 1):

- Time to percutaneous coronary intervention (PCI): Time between the initial onset of chest pain, per patient report, and the use of an intracoronary device during coronary intervention, not to be confused with door-to-balloon or door-to-device time, which measures time from emergency department arrival to first device use.
- Time from PCI to drug: Time between the initial coronary intervention and the administration of the investigational drug.
- Time-to-peak creatine kinase myocardial band (CK-MB): Time between the initial onset of chest pain, per patient report, and the peak levels of CK-MB, as a measure of duration of myocardial injury,¹² with longer time reflecting prolonged injury.
- Time from drug to peak CK-MB: Time between the administration of the investigational drug and the peak levels of CK-MB—if overall time to drug is not different between groups, this may serve as a surrogate for the progression of the injury after the investigational drug.

Estimation of the Area Under the Curve for CK-MB

Measurement of CK-MB, determined by direct chemiluminescent technology, was obtained at admission and every 6 hours and followed until peak as per clinical practice at our center. The area under the curve for CK-MB could not be measured because there was no consistent CK-MB after peak value. We therefore estimated CK-MB_{AUC} by a formula that reconstructs the typical CK-MB curve.¹³

Measurement of the Inflammatory Biomarkers

Plasma levels of C-reactive protein (CRP—high-sensitivity nephelometry; LabCorp, Burlington, NC) were measured at admission, 72 hours, and 14 days. The area under the curve for CRP was measured using the assumption of a linear change between each point by which $CRP_{AUC} = \frac{1}{2} \cdot ([CRP_{admission} + CRP_{day3}] \cdot 3 \text{ days} + [CRP_{day3} + CRP_{day14}] \cdot 11 \text{ days})$ and measured in $\text{mg} \cdot \text{L}^{-1} \cdot \text{day}$. CRP_{AUC} served as a surrogate for the inflammatory response. Plasma AAT levels were measured at admission and at 72 hours using an immunoturbidimetric assay and used as a surrogate for dose response.

Echocardiography

Doppler echocardiography was performed within 48 hours of admission and at 3-month follow-up weeks later or according to standard of care, as previously described.⁸ Left ventricular end-diastolic and end-systolic volumes were measured using Simpson's formula

from the apical 4 and 2 chamber views, and the left ventricular ejection fraction (LVEF) was calculated.

Clinical Assessment

We assessed the occurrence of death, death due to cardiac causes, acute myocardial infarction (defined as type I, according to the universal classification of AMI¹⁴), and new onset HF (defined as new onset dyspnea associated with clinical, imaging, or laboratory evidence of congestion and requiring the use of loop diuretics).¹⁵

Statistical Analysis

The values are reported as median and interquartile range for potential deviations from Gaussian distribution. Differences between groups were computed using the Mann–Whitney *U* test for continuous variables or Fisher’s exact test for discrete variables. Paired data within the AAT group (before/after) were analyzed using the Wilcoxon test. The Spearman correlation test was used to evaluate the correlation between continuous variables. Unadjusted *P* values are reported, with significance set at 2-tailed 0.05 level. Computations were performed with SPSS 22.0 (IBM, Armonk, NY).

RESULTS

Effects of AAT on Time-To-Peak CK-MB

Figure 1 shows a calculation of the various time intervals in the AAT and placebo groups. There were no significant differences in the time-to-PCI or PCI-to-drug time between the 2 groups. AAT-treated patients had significantly shorter time-to-peak-CK-MB times as measured from time from initial assessment to peak CK-MB value (from 789 [664–959] to 525 [480–735] minute, –33%, *P* = 0.007), mostly reflecting a much shorter drug-to-peak CK-MB time (from 591 [305–818] to 217 [121–344] minute, *P* = 0.005) (Figs. 1, 2).

Effects of AAT on Estimated CK-MB_{AUC}

Despite similar peak CK-MB values in the AAT and placebo groups, the difference in time-to-peak CK-MB value translated into a significantly smaller CK-MB_{AUC}, a surrogate for infarct size (1204 [758–2728] vs. 2418 [1551–4289] U·day, *P* = 0.035 for AAT and placebo), despite no differences in peak CK-MB (123 [30–196] vs. 123 [71–213] U/mL, *P* = 0.71) (Fig. 1).

Dose-Dependent Response of AAT Supplementation

Figure 3 shows the correlation between the interval increase in plasma AAT levels at 72 hours in patients receiving AAT and CK-MB_{AUC}, a surrogate of infarct size. LVEF was available in 5 patients at follow-up and the interval change in plasma AAT correlated with higher final LVEF (Fig. 3).

Clinical Events in AAT-Treated Patients

Follow-up to a median of 13 months showed that 1 patient died suddenly of presumed stent thrombosis early after stenting,⁸ and none of the patients treated with AAT developed HF

after STEMI, as compared with 1 patient who died and 9 patients who experienced HF (50%) in the historical placebo cohort ($P = 0.07$) (Fig. 4).

DISCUSSION

Preclinical research in myocardial ischemia reperfusion has shown that administration of plasma-derived AAT during reperfusion or shortly thereafter significantly limits infarct size by blunting the secondary inflammatory injury after reperfusion.⁶ Within the limitations of this post hoc analysis of a small pilot study, our results extend those preclinical findings to the clinic and suggest that AAT's anti-inflammatory effects (reflected in a reduction in the increase CRP values within the first 72 hours⁸) reduce the duration of the injury (reflected in a reduction in the time-to-peak CK-MB) and resulting in an overall smaller injury (reflected in a reduction in the area under the curve for CK-MB). The proposed benefit seemed to be proportional to the anti-inflammatory effect of AAT and to the dose response to the supplementation (as shown with the correlation with changes in CRP and AAT plasma levels). We also show an extended follow-up of the subjects showing a reassuring lack of new cases of HF.

We need to acknowledge, however, many limitations of this post hoc analysis. This is indeed an unplanned analysis of a small pilot study, without a randomized controlled arm, using historical placebo controlled from other comparable randomized studies performed at the same site at approximately the same time,^{9–11} and as such, imbalances between groups cannot be excluded. We used a formula to calculate CK-MB_{AUC} that, although previously validated, it remains based on several assumptions of linearity of changes in biomarkers over time.¹³ We did not systematically assess for microvascular obstruction after reperfusion and as such cannot exclude that, despite successful PCI, there could have been differences in tissue-level reperfusion between the groups, of note, all 10 of 10 patients (100%) receiving and 18 of 20 (90%) had TIMI 3 flow reported after PCI in the Prolostin C and placebo groups, respectively.

Notwithstanding these limitations, we believe that the clinical data showing protective effects of AAT should be considered in the light of the strong preclinical research in ischemia reperfusion in animals.^{6,7} Moreover, given the safe therapeutic profile of plasma-derived formulations, AAT is very well suited to be further tested in phase II clinical trials in patients with reperfused AMI as an adjunct to reperfusion with the goal of reducing the inflammatory response, infarct size, and improve cardiac remodeling. The VCU- α 1RT pilot safety feasibility study showed no adverse events in 10 patients with STEMI.⁸ Future studies will need to explore treatment with AAT earlier in the Cardiac Catheterization Laboratory, to determine whether administration at time of reperfusion confers a greater benefit in terms of reduction in infarct size. The data presented herein provides further rationale to support the completion of such trial.

CONCLUSIONS

When compared with historical placebo-treated controls, patients with STEMI treated with plasma-derived AAT administered after reperfusion seemed to experience mitigated

ischemia-reperfusion injury because of blunting of the inflammatory response. Although validation in appropriately designed studies is needed, these findings support the hypothesis of protective effects of inhibiting the inflammatory injury after myocardial ischemia reperfusion.

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	Time to PCI	PCI-to- drug time	Time-to- peak CK-MB	Drug-to- peak time	Peak CK-MB	AUC for CK-MB
Placebo	155 min [75-408]	220 min [180-356]	789 min [664-959]	591 min [305-818]	123 U/ml [71-213]	2,418 U*day [1,551-4,289]
Plasma AAT (Prolastin C)	85 min [58-141]	320 min [236-471]	525 min [480-735]	216 min [121-344]	123 u/ml [30-196]	1,204 U*day [758-2,728]
	P=0.10	P=0.67	P=0.007	P=0.005	P=0.71	P=0.035

FIGURE 1.

Time-to-peak CK-MB and estimates of infarct size. We provide a scheme on how the different time-based variables and infarct size estimates are calculated, and a table with the median and interquartile range for each variable divided by group.

FIGURE 2.

Estimated effect of plasma-derived AAT (Prolastin C) on infarct size. Left panel shows estimated infarct size measured as area under the curve (AUC) for CK-MB in the Prolastin C-treated patients and historic placebo-treated patients. Right panel shows differences in time-to-peak to CK-MB between groups.

FIGURE 3.

Interval change in AAT plasma levels and surrogate end points. Correlation between the increase in AAT plasma at 72 hours and infarct size estimate is shown in the Prolastin C–treated patients. Right panel shows correlation between interval change in plasma AAT levels and left ventricular ejection fraction at follow up.

FIGURE 4.

Survival free from HF events. One patient in the Prolastin C group died suddenly, and none experienced HF during midterm follow-up, as compared with 1 patient who died and 9 patients who experienced HF (N = 10, 50%) in the historical placebo cohort ($P = 0.07$).