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Adenosine 2A Receptor Activation Attenuates Ischemia Reperfusion Injury During Extracorporeal Cardiopulmonary Resuscitation

J. Hunter Mehaffey, MD, MSc, **Dustin Money, RRT-ACCS**, **Eric J. Charles, MD**, **Sarah Schubert, MD**, **Angela Fernandez Piñeros, B.Sc.**, **Di Wu, MS**, **Sai Vineela Bontha, PhD**, **Robert Hawkins, MD, MSc**, **Nicholas R. Teman, MD**, **Victor E. Laubach, PhD**, **Valeria R. Mas, PhD**, **Curtis G. Tribble, MD**, **Daniel G. Maluf, MD**, **Ashish K. Sharma, MBBS, PhD**, **Zequan Yang, MD**, **Irving L. Kron, MD***, **Mark E. Roeser, MD***

Department of Surgery, University of Virginia, Charlottesville, Virginia

Abstract

Objective—We tested the hypothesis that systemic administration of an adenosine 2A receptor (A2AR) agonist will reduce multiorgan ischemia-reperfusion injury (IRI) in a porcine model of extracorporeal cardiopulmonary resusitation (ECPR).

Summary Background Data—Advances in ECPR have decreased mortality after cardiac arrest, however subsequent IRI contributes to late multisystem organ failure. Attenuation of IRI has been reported with use of an A2AR agonist.

Methods—Adult swine underwent 20 minutes of circulatory arrest, induced by ventricular fibrillation, followed by six hours of reperfusion with ECPR. Animals were randomized to vehicle control, low-dose A2AR agonist, or high-dose A2AR agonist. A perfusion specialist using a goal-directed resuscitation protocol managed all animals during the reperfusion period. Hourly blood, urine, and tissue samples were collected. Biochemical and microarray analyses were performed to identify differential inflammatory markers and gene expression between groups.

Results—Both treatment groups demonstrated significantly higher percent reduction from peak lactate after reperfusion compared to vehicle controls. Control animals required significantly more fluid, epinephrine, and higher final pump flow while having lower urine output than both treatment groups. The treatment groups had lower urine NGAL, an early marker of kidney injury (p=0.01), lower plasma aspartate aminotransferase, and reduced rate of troponin rise (p=0.01). Pro-inflammatory cytokines were lower while anti-inflammatory cytokines were significantly higher in the treatment groups.

Conclusions—Using a novel and clinically relevant porcine model of circulatory arrest and ECPR, we demonstrated that a selective A2AR agonist significantly attenuated systemic IRI and warrants clinical investigation.

Corresponding Author: Mark E. Roeser MD, University of Virginia, PO Box 800679, Charlottesville, VA 22908, Phone: 434-924-2158, Fax: 434-244-7588, mr8e@virginia.edu.

*Co-Senior Authors

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Mini Abstract

This randomized, double-blinded, placebo controlled large animal study of swine undergoing 20-minutes of circulatory arrest, induced by ventricular fibrillation, followed by 6-hour reperfusion with ECPR. This novel and clinically relevant study demonstrated that addition of a selective A2AR agonist during ECPR significantly attenuated systemic IRI and warrants clinical investigation.

Keywords

Extracorporeal Membrane Oxygenation; ECPR; Ischemia Reperfusion; Adenosine 2a; Cardiac Arrest; Porcine Model; gene expression microarray

Introduction

Cardiac arrest is one of the leading causes of death worldwide, and extracorporeal cardiopulmonary resusitation (ECPR) has emerged as a therapeutic option for refractory cardiac arrest, which was reported in almost 4,000 patients worldwide in 2017[1-3]. Despite advances in resuscitation technology, more than half of patients who experience cardiac arrest die without the return of spontaneous circulation[3, 4]. Even if the restoration of circulation occurs, survivors have a poor prognosis with less than one-third surviving to discharge[3, 4]. Global ischemia during cardiac arrest triggers several pathologic pathways that worsen after reperfusion[5]. Ischemia-reperfusion injury (IRI) after cardiac arrest leads to the development of post-arrest reperfusion syndrome, which is responsible for enhanced tissue damage, often with fatal outcomes[6]. Extensive efforts have been made to prevent the development and progression of post-cardiac arrest syndrome. To date, the only protective intervention proven to reduce this deadly syndrome and improve clinical outcomes is targeted temperature management, which includes induction of mild hypothermia or controlled normothemia[5].

Post-arrest reperfusion injury begins immediately upon reperfusion involving oxidative stress and rapid activation of innate immune cells (lymphocytes, macrophages and neutrophils) with additional contribution from other cells (endothelial and epithelial cells). This phenomenon is well established within the first few hours of reperfusion[7–12]. In the setting of IRI, adenosine is a retaliatory metabolite, which serves largely as a protective agent with anti-inflammatory effects[13–15]. Adenosine mediates its effects through four different G protein-coupled receptors (A1R, A2AR, A2BR and A3R)[16]. Adenosine receptors have been studied extensively, and use of selective adenosine receptor agonists and antagonists have contributed to a better understanding of adenosine signaling in the setting of IRI[17–21]. It is well established that the activity of certain cells during acute inflammation, including lymphocytes, macrophages, monocytes, platelets, and neutrophils, is inhibited by A2AR activation[9, 22, 23]. Resulting in reduction of pro-inflammatory mediators, reduced endothelial adhesion molecule expression, less transmigration of circulating inflammatory cells into tissue, and thus less end-organ damage [13]. The cellular responses are largely mediated through cAMP and result in inhibition of oxidative burst in neutrophils, reduced cytokine release, and inhibition of leukocyte activation[24]. Through the use of selective

A2AR agonists, our lab has demonstrated decreased cellular inflammation with resultant amelioration of IRI in a lung transplant model by LaPar *et al.* and a cardiac model by Yang *et al.* [10, 25, 26]. Additionally, systemically administered, A2AR agonists have been used very successfully in experimental models to reduce tissue injury in the setting of IRI in liver, kidney, bowel, heart, skin, and lung[27–29].

The role of A2AR in post-cardiac arrest reperfusion injury has not been reported. In the current study, we sought to demonstrate the use of a selective A2AR agonist (ATL1223) in a novel, clinically relevant, porcine model of ECPR to attenuate global IRI. We hypothesized that infusion of ATL1223 upon initiation of circulatory support in a porcine model of ECPR would attenuate inflammatory responses and reduce tissue injury in multiple organs (heart, kidney and liver).

Materials and Methods

Animals and Monitoring

The current study was approved by the University of Virginia Animal Care and Use Committee and complied with the 1996 *Guide for the Care and Use of Laboratory Animals* as recommended by the US National Institutes of Health (NIH). Domestic swine of both sexes (35–45 kg) were used for this protocol.

Cardiac arrest

Baseline blood samples, arterial blood gas, hemodynamics, pulmonary mechanics, liver biopsy and urine samples were collected at the start of the experiment. First, a median sternotomy was performed. After administration of 100 U/kg of intravenous heparin (Hospira Inc., Lake Forest, IL), 18F arterial and 32F venous cannulae (Medtronic Inc, Minneapolis, MN) were placed in the ascending aorta and right atrium respectively (Figure 1). Prior to initiating circulatory support, cardiac arrest was induced with direct cardiac fibrillation using a 9V battery (Energizer Brands, LLC, St. Louis MO) and confirmed by direct cardiac visualization and electrocardiography. 20-minutes after cardiac arrest, repeat blood and tissue samples were obtained.

Extracorporeal Cardiopulmonary Resuscitation

After 20 minutes of circulatory arrest, ECMO was initiated at a flow rate of 25 mL/kg/min. Over a five-minute interval, the ECMO flow rate was slowly titrated upward to ≧ 50 mL/kg/min with a goal mean arterial pressure (MAP) of 65 mmHg. Ten minutes after initiating ECMO support and administering electrolytes, defibrillation was performed with 10 J of direct energy using internal cardiac paddles (Figure 2).

Treatment Groups and ECMO Maintenance

This was a randomized, double-blinded placebo controlled large animal study. Low-dose ATL1223 (0.3 ng/kg/min), high-dose ATL1223 (0.6 ng/kg/min) or DMSO vehicle (3 mL/hr, DMSO) was administered via continuous infusion through the arterial limb of the ECMO circuit upon initiation of flow. A clinical ECMO specialist managed the circuit with specific criteria for goal-directed resuscitation. Tissue, blood, and urine samples were collected at

baseline, time of reperfusion (20 minutes) and each subsequent hour for the 6-hour reperfusion period (Figure 2).

Statistical Analysis

All data were reported as mean ± standard error of the mean. Data between all three groups were compared using ANOVA with Tukey's correction for multiple comparisons to determine statistical significance. Each treatment group was compared to the control group using unpaired Student's t-test. Rate of rise of troponin was calculated using linear regression and comparison of best-fit line. Prism 7 (GraphPad Software Inc., La Jolla, CA) was used to perform all statistical calculations. A threshold of $p<0.05$ was used for statistical significance.

Results

Novel Clinically Relevant Large Animal Model of ECPR

Fifteen consecutive animals underwent ventricular fibrillation with 20-minute circulatory arrest and were randomized to initiation of ECPR with DMSO, low-dose ATL1223 or high-dose ATL1223 (n=5/group). All animals were successfully defibrillated to sinus rhythm on first or second attempt after 10 minutes of ECMO and infusion of calcium, magnesium and bicarbonate, with no differences between the groups (Figure 2). All animals were maintained on ECMO support for 6 hours following the previously described protocol to maintain MAP (65–75mmHg), pH (7.2–7.4), and PCO_2 (35–50mmHg), which did not differ between groups. There was no difference in heart rate, pulmonary artery pressure or blood pressure between groups and no adverse drug effects were observed. However, the ATL-treated pigs required significantly less support to maintain the hemodynamic goals.

A2AR Agonism Attenuates Systemic Reperfusion Injury

After 20 minutes of circulatory arrest and initiation of resuscitation with ECPR, all animals demonstrated significant rise in blood lactate with no statistical difference among the groups (DMSO: 6.27 ± 2.4 mg/dL, low-dose ATL1223: 6.74 ± 1.9 mg/dL, high-dose ATL1223: 6.57 ± 1.3 mg/dL, p=0.56). Over the 6-hour reperfusion period, both low and high-dose ATL1223 groups demonstrated significantly lower final blood lactate (Figure 3A). While the DMSO control group had a small decrease in lactate over the 6-hour period (10.7 ± 19.5%), both treatment arms demonstrated better lactate clearance (low-dose ATL1123: 61.4% ± 5.1%, p=0.04; high-dose ATL1223: 63.7% ± 5.9%, p=0.03, Figure 3B).

Plasma concentration of tumor necrosis factor alpha (TNF-α) was significantly different at the end of 6 hours of reperfusion with the highest levels in the control group (DMSO: 0.06 ± 0.005 ng/mL, low-dose ATL1223: 0.04 ± 0.01 ng/mL, high-dose ATL1223: 0.028 ± 0.004 ng/mL, p=0.04, Figure 4A). The final plasma concentration of TNF-α was significantly lower in the high-dose ATL1223 group compared to DMSO control (p=0.001). Final plasma interferon gamma (INF-γ) concentration at 6 hours of reperfusion was not statistically different between groups (p=0.22, Figure 4B). However, the percent increase of INF-γ from baseline was significantly different between groups (DMSO: 225%, low-dose ATL1223: 31.1%, high-dose ATL1223: 9.1%, p=0.01) with DMSO controls being almost ten times

higher. The final plasma concentration of anti-inflammatory cytokine interleukin-4 (IL-4) was significantly different between groups (DMSO: 0.04 ± 0.01 ng/mL, low-dose ATL1223: 0.13 ± 0.04 ng/mL, high-dose ATL1223: 0.26 ± 0.08 ng/mL, p=0.04, Figure 5A) with DMSO controls being the lowest. The high-dose ATL1223 group had significantly higher expression of plasma IL-4 at 6 hours of reperfusion compared to DMSO controls (p=0.03). Finally, interleukin-10 (IL-10), another anti-inflammatory cytokine, was also significantly increased in the ATL1223 treatment groups after 6 hours of reperfusion (DMSO: 0.11 ± 0.01 ng/mL, low-dose ATL1223: 0.202 ± 0.02 ng/mL, high-dose ATL1223: 0.12 ± 0.03 ng/mL, p=0.01, Figure 5B). The low-dose ATL1223 group had significantly higher expression of IL-10 at the end of 6 hours of reperfusion compared to DMSO controls (p=0.01).

A2AR Activation Reduces Fluid Requirements and Improves Hemodynamics

Total fluid requirements trended toward a significant difference between groups (DMSO: 1650 ± 402.8 mL, low-dose ATL1223: 750 ± 115.1 mL, high-dose ATL1223: 850 ± 173.9 mL, p=0.06, Figure 6A) with the control group requiring almost double the volume to maintain hemodynamic parameters. Treated animals (low and high-dose ATL1223 combined) received less total fluid than the DMSO controls (p=0.02). Similarly, total urine output trended toward a dose dependent response to ATL1223 (DMSO: 1022 ± 248.3 mL, low-dose ATL1223: 1495 ± 243.4 mL, high-dose ATL1223: 1821 ± 221.3 mL, p=0.11, Figure 6B) with control animals having the lowest urine output. One DMSO control animal was anuric for the last 4 hours of reperfusion.

The ability to wean off ECMO was significantly different between groups as evidenced by significantly higher final pump flow requirement in the DMSO control group (DMSO: 3.22 ± 0.3 L/min, low-dose ATL1223: 1.76 ± 0.4 L/min, high-dose ATL1223: 1.32 ± 0.3 L/min, p=0.01, Figure 6C). The DMSO control animals required almost twice as much support as the treated animals. Similarly, total vasopressor requirements trended toward a statistical difference between groups (DMSO: 1.5 ± 0.4 mg, low-dose ATL1223: 0.6 ± 0.1 mg, high-dose ATL1223: 0.7 ± 0.3 mg, p=0.17, Figure 6D) with DMSO control animals requiring on average more than twice as much epinephrine over the 6-hour perfusion period. Treated animals (low and high-dose ATL1223 combined) received less total epinephrine than the DMSO controls (p=0.03).

Amelioration of Renal, Hepatic and Cardiac Injury with A2AR Agonism

Urine concentration of NGAL, an early marker of acute kidney injury (AKI), was significantly higher in the DMSO control group at 6 hours of reperfusion versus ATL1223-treated groups (DMSO: 149.6 ± 26.6 mg/dL, low-dose ATL1223: 67.1 ± 10.3 mg/dL, high-dose ATL1223: 68.5 ± 8.3 mg/dL, p=0.01, Figure 7A) with the control groups being more than double compared with treated groups. Both low-dose (p=0.02) and high-dose (p=0.04) ATL1223 groups demonstrated significantly lower urinary expression of NGAL compared to the DMSO controls. This biochemical difference correlates with the finding of reduced urine output in the DMSO controls as mentioned above.

Hepatic injury was assessed with measurement of plasma aspartate aminotransferase (AST), which peaked by 3 hours in the ATL1223 treatment groups but continued to rise in the

DMSO control group (Figure 7B). There was no statistical difference at 6 hours after reperfusion between groups (DMSO: 184.0 ± 16.9 U/L, low-dose ATL1223: 151.8 ± 16.3 U/L, high-dose ATL1223: 146.8 ± 16.6 U/L, p=0.09, Figure 7B). However the difference was significant when the DMSO control group was compared to the combined low and high-dose ATL1223 treatment groups at 6 hours of reperfusion (p=0.048).

Troponin I, a measure of cardiac injury, was elevated and higher in the DMSO control group at 3 and 6 hours of reperfusion, but there was no statistical difference at 6 hours of reperfusion between the groups (DMSO: 26.0 ± 3.8 ng/mL, low-dose ATL1223: 22.7 ± 4.6 ng/mL, high-dose ATL1223: 15.3 ± 4.1 ng/mL, p=0.17, Figure 7C). However, plasma troponin I demonstrated a more rapid rate of rise (m=slope) in the DMSO control animals compared to the ATL groups (DMSO: m= 4.4 ± 0.19, low-dose ATL1223: m=3.8 ± 0.47, high-dose ATL1223: m=2.5 ± 0.24, p=0.01, Figure 7C).

Discussion

Administration of a selective A2AR agonist in a clinically-relevant, large animal model of ECPR resulted in better clinical outcomes compared with ECPR alone as evidenced by lower required pump flows, decreased epinephrine doses, and less fluid administration required to obtain adequate hemodynamics after 6 hours of reperfusion. Reproducible injury was achieved with circulatory arrest and 20 minutes of ischemia, followed by defibrillation and 6 hours of reperfusion with standardized ECMO support. The cardiac arrest and subsequent global reperfusion triggered an extensive inflammatory response and induced multiorgan injury. A2AR agonism attenuated tissue injury as evidenced by reduced plasma lactate, AST, and urine NGAL. The protective effect of A2AR agonist for post-arrest global injury is through inhibition of inflammatory responses and enhancement of organ/tissue survival pathways.

The large animal ECPR model used in the research presented here is clinically relevant and provided reproducible injury. We standardized our approach with pre-arrest central cannulation, which is more durable then strategies used in other porcine models[30, 31]. Different from 30 or 60 minutes of circulatory arrest as used by Rojas and colleagues, we chose a 20-min period due to its clinical relevance and high success rate of electric defibrillation back to sinus rhythm after induction of ECPR[5, 31]. The duration of ECMO support in the present study is longer than that reported by other groups in an effort to capture a more complete assessment of IRI after cardiac arrest and ECPR[5]. The median duration of support for patients undergoing ECPR in clinical practice ranges from 54–130 hours[32]. Furthermore, to fully assess the effects of A2AR agonism in this model we evaluated both standard dosing (0.3 ng/kg/min) and a high-dose of ATL1223 (0.6 ng/kg/min) to determine if there was a dose-dependent effect. The standard dosing was based on prior work in our porcine lung transplant model providing treatment to transplant recipient animals during reperfusion, which was subsequently translated into a clinical trial[19, 25].

Clinically, there is an increasing use of ECPR for post arrest treatment[32]. However, only 35–63% of patients who require ECPR survive to hospital discharge[4, 32]. Several retrospective analyses have demonstrated weaning off ECMO support is the strongest predictor for long-

term survival[4, 33]. Our data established animals treated with a selective A2AR agonist required significantly lower pump flow rates, vasopressors and fluid resuscitation to maintain normal hemodynamic parameters (Figure 6). Seventy percent of animals in the high or low-dose treatment groups were on 2 L/min flow or less by the end of the 6-hour perfusion period and were likely stable enough to tolerate ECMO decannulation. All five animals in the DMSO control group required significantly higher ECMO flow for hemodynamic support with an average final flow over 3.2 L/min. Despite a theoretical risk of bradycardia and hypotension with higher levels of A2AR agonism, these effects were not seen in the present study. There was no difference in heart rate or pulmonary artery pressure between the groups and the DMSO control animals required the highest dosages of vasopressor, fluid and pump support to maintain a MAP 65–85 mmHg. The low and high-dose study group design provides evidence indicating ATL1223 does not induce hemodynamic compromise in this model. Finally, there were no other adverse medication effects identified during this study.

Plasma lactate is a sensitive marker of tissue ischemia and injury that is easily measured and trended in porcine models[34]. Several retrospective studies of clinical ECPR have demonstrated longer time for plasma lactate to return to baseline predicts reduced survival and poor outcomes[2, 35]. In the present study, all animals demonstrated a significant rise in plasma lactate after circulatory arrest with no difference in the peak between groups. Importantly, animals treated with a selective A2AR agonist demonstrated significantly greater reduction in lactate compared to controls, with most treatment animals returning to the normal range by the end of the 6-hour study period (Figure 3). Given the major clinical implications of lactate trends during ECPR following cardiac arrest, these results strongly support the benefit of A2AR agonism in attenuation of post-arrest reperfusion injury.

The improved clinical outcomes observed with A2AR agonist treatment were in part driven by attenuation of the inflammatory response during the post-arrest reperfusion. These data demonstrated a significant reduction in TNF-α expression (Figure 4A), which is a downstream pro-inflammatory cytokine in the nuclear factor kappa beta (NF-κB) pathway induced by IRI[36]. NF-κB has been identified as a critical mediator of cellular inflammation after IRI and correlates with poor outcomes in animal models[37]. Additionally, INF-γ trended towards significantly lower levels in the A2AR agonist treatment groups (Figure 4B). This cytokine is largely produced by T cells and is an important early signal in IRI[38]. Recent work by Borg *et al* demonstrated adenosine pathway dependent inhibition of INF-γ orchestrates cardiac wound healing after myocardial infarction, which likely contributed to the attenuation of cardiac injury observed in our model (Figure 7)[18].

The administration of A2AR agonist increased expression of the anti-inflammatory cytokine IL-4 (Figure 5A). This important signaling molecule has been implicated in the role of polarization of macrophages into an M2 phenotype to stimulate recovery after IRI in kidney and neurologic tissue[39, 40]. IL-10 expression also differed between groups (Figure 5B) and has previously been implicated in hepatic response to IRI through a Kupffer cell dependent mechanism[41]. Our data show that A2AR agonism reduces global IRI and promotes an anti-inflammatory state, as evidenced by increased levels of IL-4 and IL-10. However, future

studies are needed to identify the mechanisms by which A2AR signaling leads to the up regulation of these anti-inflammatory cytokines.

Renal injury after cardiac arrest and ECPR is a common complication with almost 60% of patients experiencing acute renal failure requiring renal replacement therapy[32]. This is a major problem considering the high morbidity, mortality and healthcare costs associated with renal replacement therapy. Clinically, decreased urine output is an early marker of renal injury with rising plasma creatinine lagging 24–36 hours behind[33]. Even with careful protocol driven fluid administration, we demonstrated increased urine output with A2AR agonist treatment (Figure 6B). Furthermore, several studies have correlated higher urine NGAL concentration with increased incidence of renal injury in ICU and cardiac surgery patients[42, 43]. Our data confirm significantly lower urine NGAL in the A2AR agonist treatment groups (Figure 7A). Finally, microarray gene expression analysis identified A2AR-dependent pathways leading to attenuation of renal necrosis (Supplemental Figure 1). In particular, NRF2-mediated oxidative stress response and tight junction signaling represent areas for future research to identify targeted therapies to attenuate renal IRI.

Selective A2AR agonists have been previously demonstrated to attenuate cardiac injury in animal models of ischemia reperfusion[26]. Despite limited translation into human studies, this protective effect may provide significant benefit for patients undergoing ECPR resulting in earlier cardiac recovery and weaning of ECMO support. In the present study, 6 hours did not capture peak troponin levels and the final values were not statistically different between groups. However, we demonstrate that administration of an A2AR agonist reduces the rate of troponin increase in a dose-dependent fashion as measured by linear regression (Figure 7C). Previous studies have suggested peak troponin is a surrogate of total cardiac injury and the rate of rise correlates with the severity of injury[44]. These data are further corroborated in our study by increased pump flow and vasopressor requirements in the control group. Recent work by Woods and colleagues characterized post-cardiac arrest myocardial dysfunction as a calcium/calmodulin dependent phenomenon having profound effects on recovery[45]. These mechanisms likely play an important role in weaning from ECMO support and overall prognosis. Future research will explore the role of these pathways in our ECPR model.

Ischemic hepatitis resulting in acute liver failure in patients undergoing ECPR is one of the most moribund complications following cardiac arrest[46]. Despite recent advances in extracorporeal liver support, limited options exist for treatment of fulminant ischemic hepatitis secondary to IRI after cardiac arrest. Therefore, efforts must focus on prevention and attenuation of primary injury to prevent this deadly complication. Our data support the hypothesis that a selective A2AR agonist reduces hepatic injury after ECPR. AST, one of the earliest clinically relevant markers of liver injury was found to be significantly elevated in the DMSO control animals compared to animals receiving A2AR agonist treatment (Figure 7B). Furthermore, we identified genes in pathways for apoptosis, cell death, and organismal death that showed trends toward down-regulation with A2AR agonist treatment. Similarly, genes affecting cell viability and cell survival showed trends for up regulation in animals receiving A2AR treatment (Supplemental Figure 3). These pathways corroborate our biochemical data demonstrating less liver IRI with A2AR agonist therapy. Future work in liver IRI and ECPR should focus on elucidation of these pathways and identify which genes

are regulated in an A2AR-dependent mechanism and which pathways result from attenuation of secondary injury. These mechanisms may lead to other targeted therapies to prevent this severe complication.

The limitations of this study include the inherent variability of injury and recovery in this large animal model. Furthermore, given the acute 6-hour duration, we were unable to confirm long-term benefit of A2AR agonist therapy. In further studies, an increased sample size, as well as gene expression analysis of samples procured from a longer reperfusion period, may allow for definitive molecular findings. However, given the findings of clinical and biochemical improvement as well as gene expression evidence of molecular injury attenuation, these results support our conclusion. The treatment groups were randomized and researchers were blinded to which animals receive high/low dose ATL1223 or DMSO to prevent bias. Additionally, interventions on the ECMO circuit were standardized with protocol driven management by a clinical perfusion specialist. Finally, neurologic recovery was not evaluated in this study, which is a major predictor of prognosis in patients who experience cardiac arrest and undergo ECPR.

In conclusion, this novel clinically relevant porcine model of ECPR demonstrates that a selective A2AR agonist attenuates systemic IRI. These data establish an improvement in both clinical and biochemical parameters in animals undergoing ECPR with addition of a selective A2AR agonist. Furthermore, we identified specific inflammatory and cell survival pathways in liver and kidney tissues that showed a trend of differential regulation by A2AR agonism in response to systemic IRI. Given the high rate of multi-organ failure and an in-hospital mortality rate approaching 60% in patients undergoing ECPR after cardiac arrest, translation of targeted therapy with a selective A2AR agonist may attenuate ischemia reperfusion injury and improve survival. These data indicate addition of an A2AR agonist to ECPR reduces reperfusion injury following global ischemia and warrants clinical investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclosure

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Abbreviations

ECPR	Extracorporeal Cardiopulmonary Resuscitation
ECMO	Extracorporeal Membrane Oxygenation
IRI	Ischemia-Reperfusion Injury
A2AR	Adenosine 2A Receptor
MAP	Mean Arterial Pressure
PEEP	Positive End-Expiratory Pressure
ACT	Activated Clotting Time
ELISA	Enzyme-Linked Immunosorbent Assay
NGAL	NE Gelatinase136 Associated Lipocalin
AKI	Acute Kidney Injury
TNF-α	Tumor Necrosis Factor Alpha
INF- γ	Interferon Gamma
IL-4	Interleukin-4
IL-10	Interleukin-10
AST	Aspartate aminotransferase
NIH	National Institutes of Health

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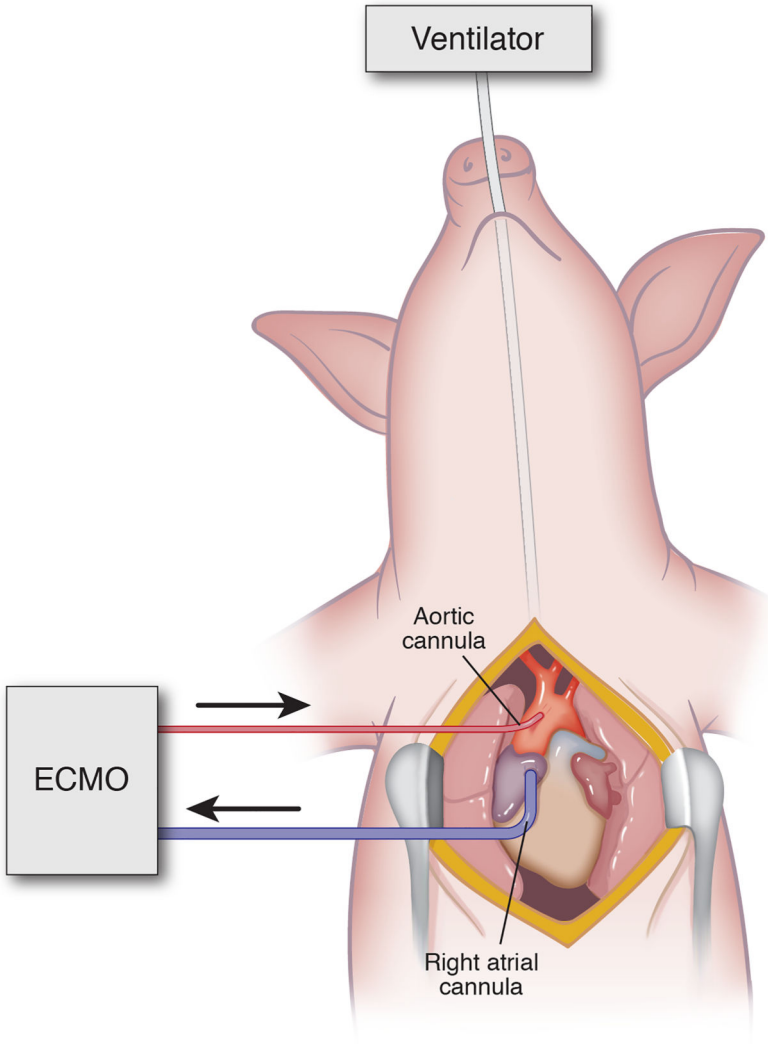

Figure 1.
Porcine Model of ECPR. Median Sternotomy with central ECMO cannulation from right atrium to ascending aorta.

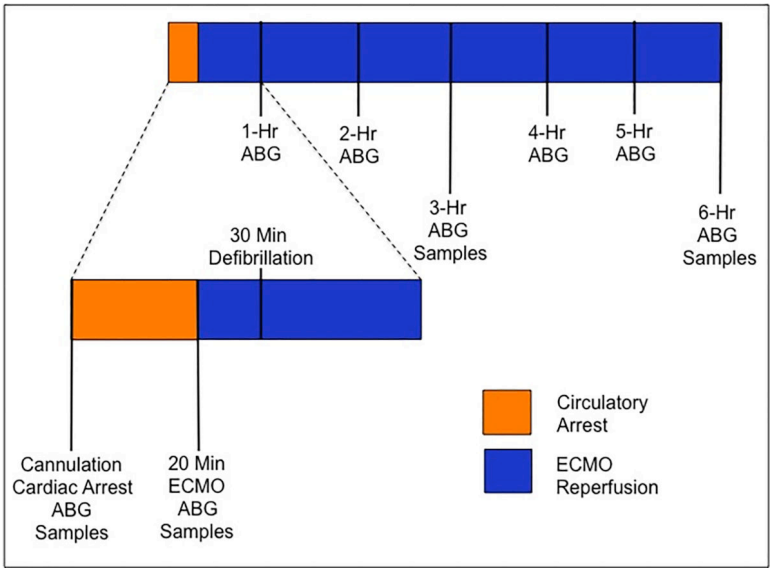

Figure 2.

Experimental methodology describing timing of interventions and data collection. Samples include blood, urine, and liver biopsy. Arterial Blood Gas (ABG) includes lactate, PaO_2, $PaCO_2$, bicarbonate, and pH.

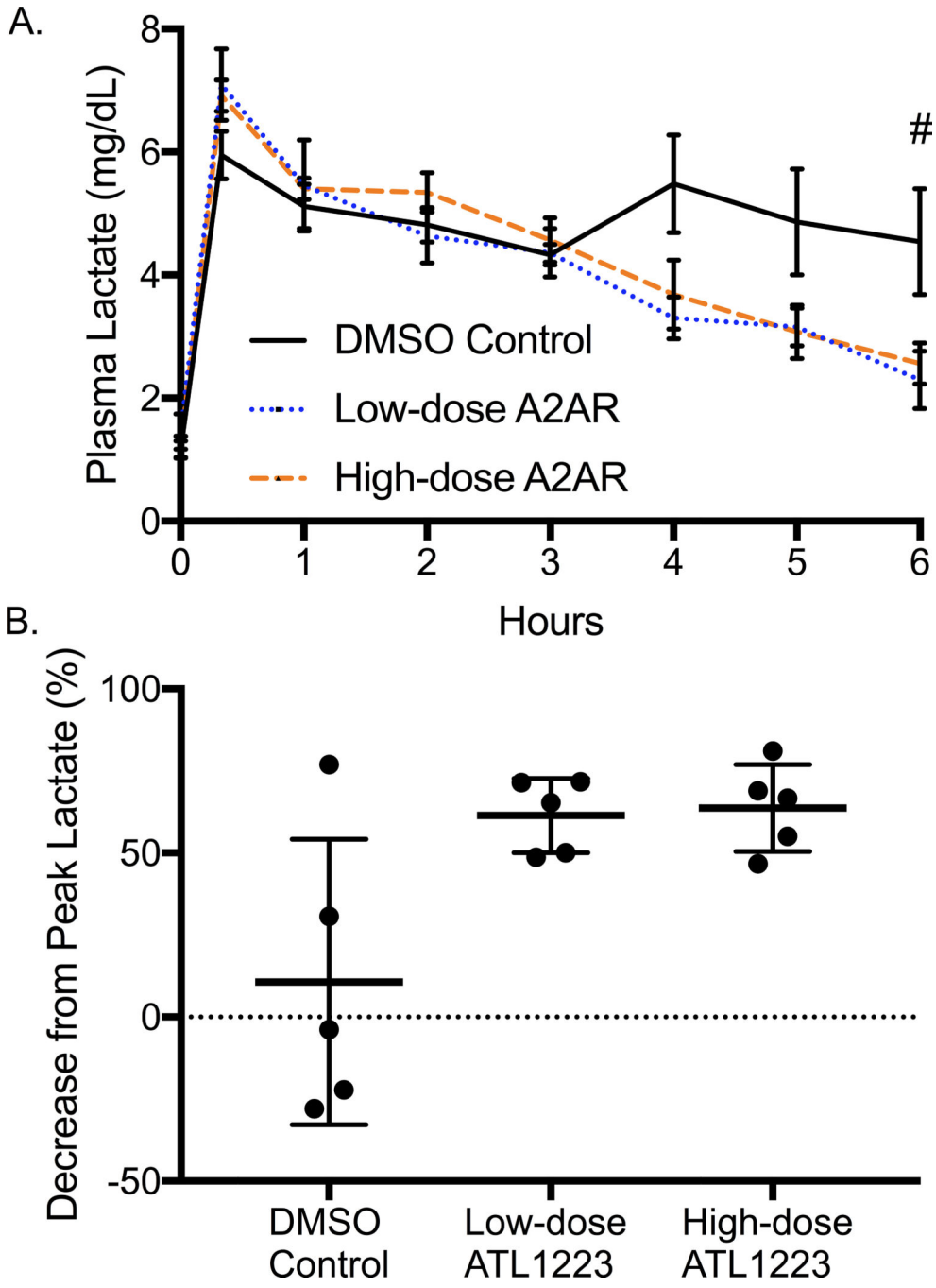

Figure 3.

A: Plasma lactate trends from baseline to 6-hours of ECPR reperfusion (# p=0.04). B: Final percent decrease in plasma lactate (p=0.01) after 6-hours of ECPR reperfusion. Both high dose and low dose A2AR agonism mitigates lactate expression in the plasma.

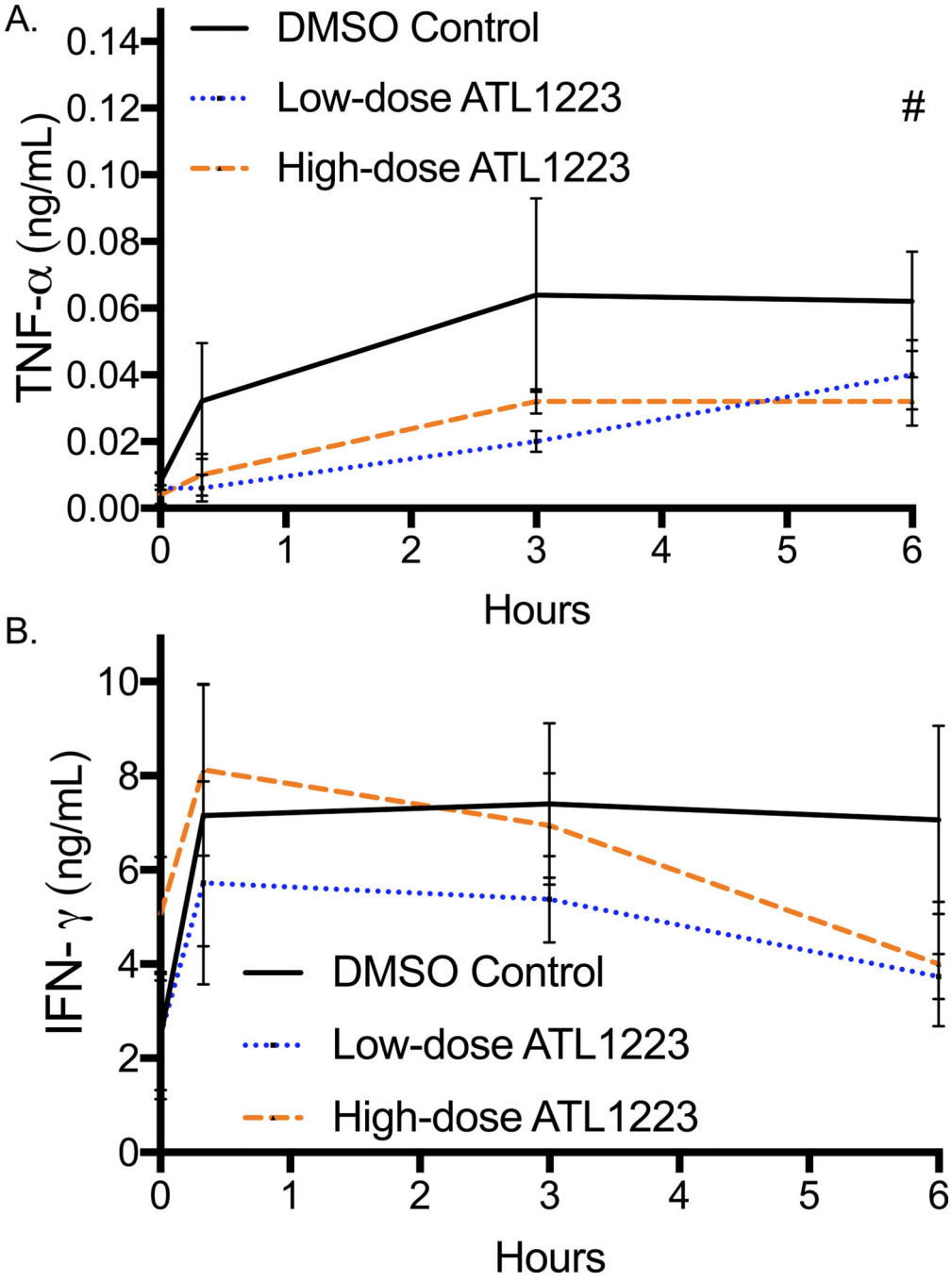

Figure 4.

Plasma pro-inflammatory cytokine expression A: tumor necrosis factor alpha (TNF-α) and B: interferon gamma (INF-γ) decreases from baseline to 6-hours of ECPR reperfusion after A2AAR agonist treatment. # p=0.01.

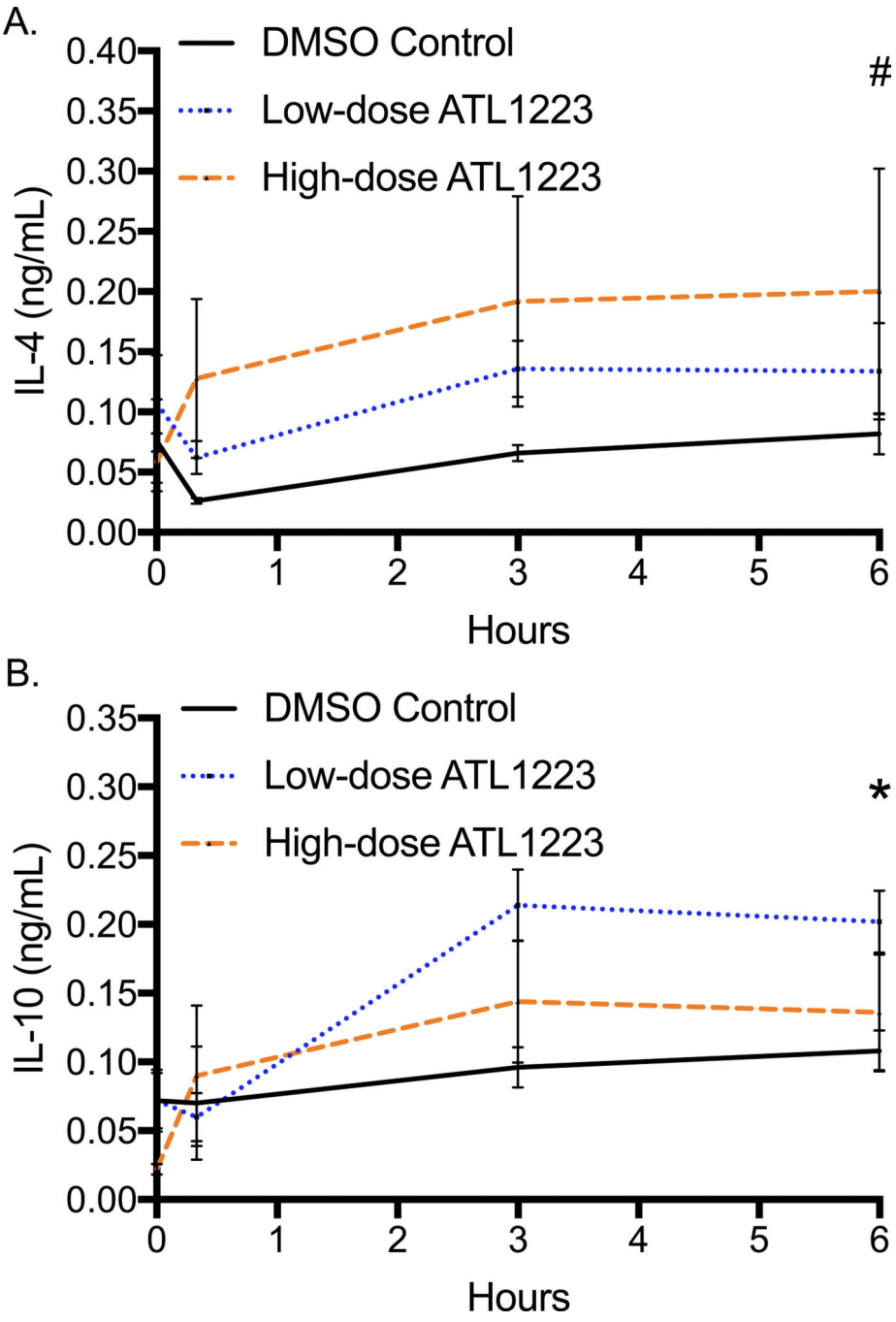

Figure 5.

Plasma anti-inflammatory cytokine expression A: interleukin-4 (IL-4), B: interleukin-10 (IL-10) increases from baseline to 6-hours of ECPR reperfusion. # p=0.04, * p=0.01.

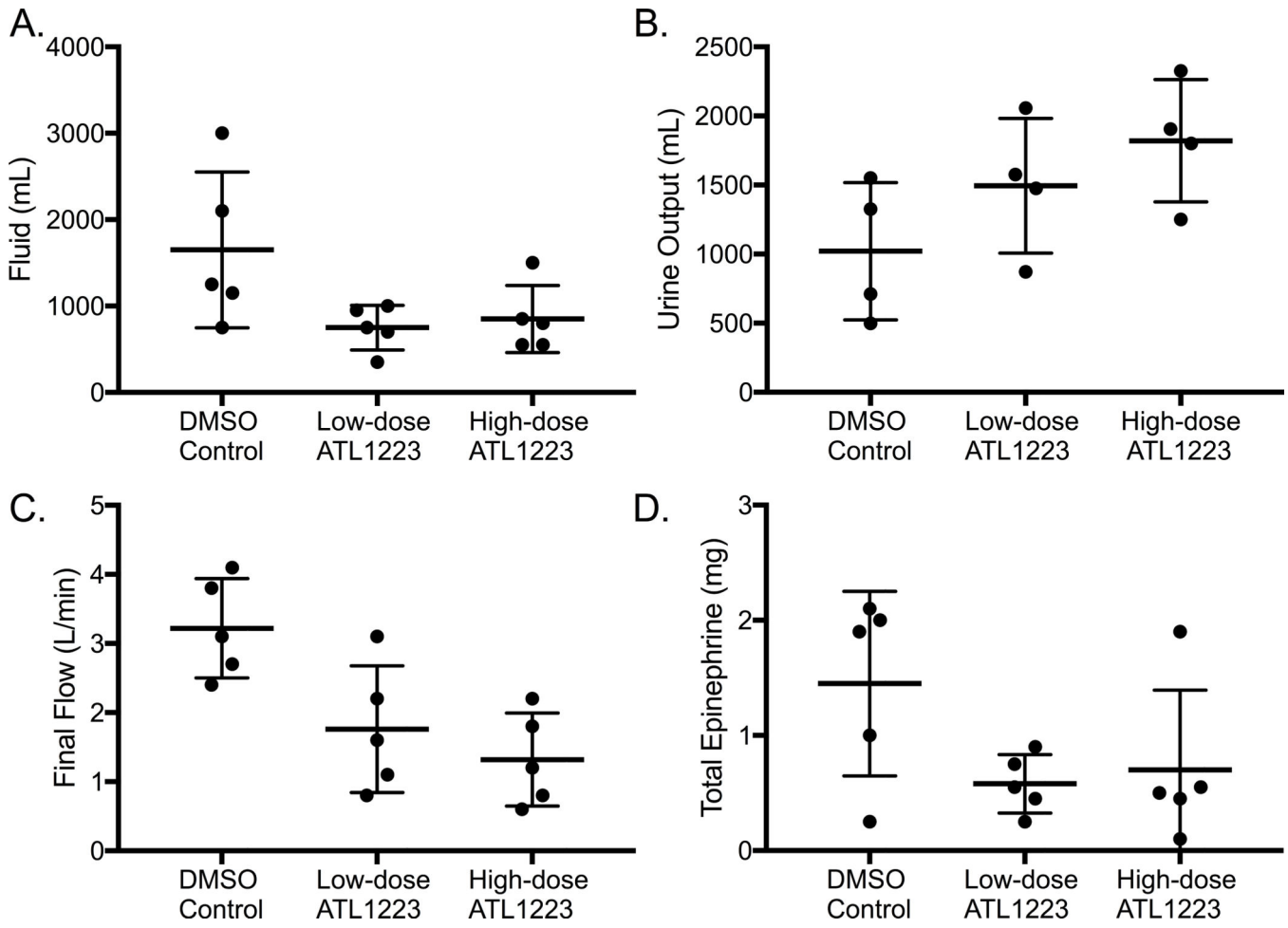

Figure 6.

Fluids and Hemodynamics. A. Intravenous fluid requirement (p=0.06) was lower in the treatment animals B. Urine output (p=0.11) was higher in the treatment animals C. Final flow (p=0.01) was lower in the treatment animals D. Total epinephrine dose (p=0.17) was lower in the treatment groups after 20 minutes of circulatory arrest and 6-hours of ECPR reperfusion.

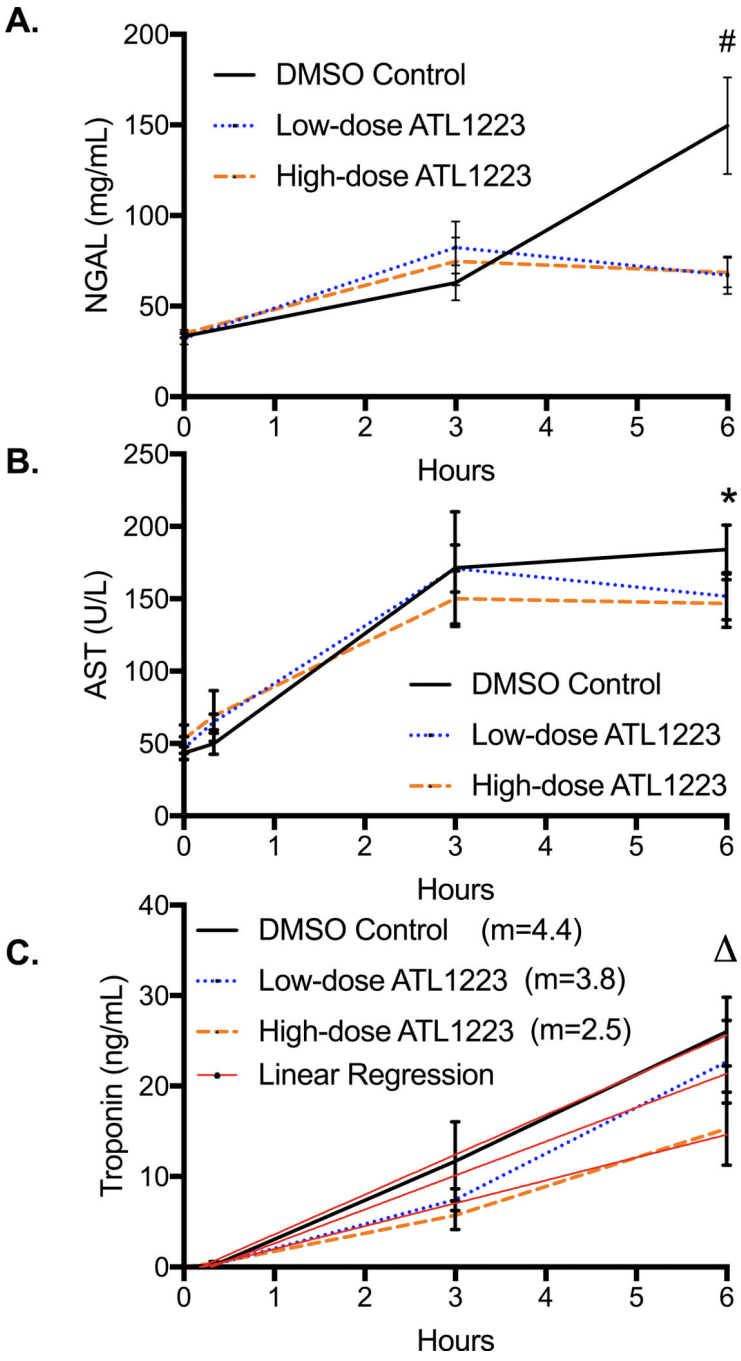

Figure 7.

Plasma biochemical markers of injury in A: Kidney - Urine NE gelatinase136 associated lipocalin (NGAL) B: Liver - Aspartate Aminotransferase (AST) were lower in the treatment groups from baseline to 6-hours of ECPR reperfusion. C: Troponin (m=slope calculated by Linear Regression, p=0.01) had a lower rate of rise from baseline to 6-hours of ECPR reperfusion in the treatment groups. # p=0.001, * p=0.09, Δ p=0.17.