

Adenosine A_{2A} Receptor Agonist Improves Cardiac Dysfunction From Pulmonary Ischemia-Reperfusion Injury

T. Brett Reece, MD, Victor E. Laubach, PhD, Curtis G. Tribble, MD, Thomas S. Maxey, MD, Peter I. Ellman, MD, Patrick S. Warren, BS, Andrew M. Schulman, MD, Joel Linden, PhD, John A. Kern, MD, and Irving L. Kron, MD

Department of Surgery and Cardiovascular Research Center, University of Virginia Health System, Charlottesville, Virginia

Background. Ischemia-reperfusion (IR) injury negatively impacts patient outcome in lung transplantation. Clinically, we observed that lung transplant patients with ischemia-reperfusion injury tend to have cardiac dysfunction. Previous studies have shown that ATL-146e (4-[3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl]-cyclohexanecarboxylic acid methyl ester), a selective adenosine A_{2A} receptor agonist, reduces lung inflammation after ischemia-reperfusion. We hypothesized that pulmonary ischemia-reperfusion causes secondary heart dysfunction and ATL-146e will improve this dysfunction.

Methods. We utilized an in vivo rabbit lung ischemia-reperfusion model. The Sham group underwent 120 minutes single lung ventilation. The IR and ATL groups underwent 90 minutes right lung ischemia with 30 minutes right lung reperfusion. The ATL-146e was given intravenously to the ATL group during reperfusion. Cardiac output and arterial blood gases were monitored, and neutrophil sequestration was measured by myeloperoxidase activity.

Results. Upon reperfusion, cardiac output (mL/min)

significantly dropped in the IR and ATL groups. By 15 minutes reperfusion, cardiac output in the ATL group improved significantly over the IR group and remained significant thereafter. Lung myeloperoxidase activity was significantly reduced by ATL-146e. Although never hypoxic, arterial oxygenation was lower in the IR and ATL groups while central venous pressures and mean arterial pressures were similar among groups. A separate experiment demonstrated that reperfusion with the antioxidant N-(2-mercapto-propionyl)glycine prevented cardiac dysfunction.

Conclusions. Pulmonary ischemia-reperfusion causes cardiac dysfunction independent of preload, afterload, and oxygenation. The ATL-146e improves this dysfunction presumably by the anti-inflammatory effects of adenosine A_{2A} receptor activation on neutrophils. One likely mechanism involves the release of oxidants from the ischemic lung upon reperfusion, which has immediate negative effects on the heart.

(Ann Thorac Surg 2005;79:1189–95)

© 2005 by The Society of Thoracic Surgeons

Lung transplantation has become an important part of the treatment of patients with end-stage pulmonary dysfunction. Approximately 15% of these transplants experience early allograft dysfunction resulting from reperfusion injury of the ischemic donor lung. This injury is difficult to predict or even to define, but the effects are clearly detrimental to patient outcomes. King and colleagues [1] demonstrated that clinically significant reperfusion injury increased in-hospital mortality, mechanical ventilation requirements, intensive care unit (ICU) stay, and hospital stay. Other clinical studies have shown that reperfusion injury leads to poor outcomes after lung transplantation [2–4]. In the study by King and colleagues [1], cardiac dysfunction appeared to play a criti-

cal role in the hospital course of those patients with reperfusion injury that died.

Review of reperfusion injury in other organ systems has demonstrated that reperfusion injury can affect tissue other than the tissue experiencing the ischemic insult. Hind limb reperfusion models have shown increased pulmonary infiltration and hepatic injury. Sorkine and colleagues [5] found that mesenteric ischemia caused increased pulmonary leukocyte sequestration and pulmonary vascular leaks. Palazzo and colleagues [6] studied the effects of unilateral lung ischemia on the opposite lung in a canine model. They demonstrated “similar, but less severe” damage to the nonischemic lung with the

Accepted for publication Sept 21, 2004.

Address reprint requests to Dr Reece, University of Virginia, Department of Surgery, PO Box 801359, Charlottesville, VA 22908; e-mail: tr5q@virginia.edu.

Drs Kron and Linden disclose that they have a financial relationship with Adenosine Therapeutics, LLC.

Table 1. Heart Rates, Mean Arterial Pressures, and Central Venous Pressures After Reperfusion With ATL-146e

	Heart Rate				Mean Arterial Pressure				Central Venous Pressure			
	0 min	15 min	30 min	<i>p</i> Value Within Group	0 min	15 min	30 min	<i>p</i> Value Within Group	0 min	15 min	30 min	<i>p</i> Value Within Group
Sham	154 ± 5	154 ± 5	151 ± 4	0.85	38 ± 2	39 ± 2	37 ± 3	0.86	8.2 ± 0.2	8.2 ± 0.2	8.4 ± 0.3	0.73
IR	175 ± 10 ^a	158 ± 7	157 ± 7	0.01	42 ± 2	38 ± 4	35 ± 4	0.01	8.8 ± 0.4	8.5 ± 0.4	8.6 ± 0.4	0.58
ATL-high	177 ± 7 ^a	160 ± 8	142 ± 12	<0.01	38 ± 4	33 ± 3	32 ± 2	0.02	8.7 ± 0.6	9.0 ± 0.5	9.0 ± 0.6	0.50
ATL-low	141 ± 3	146 ± 6	142 ± 6	0.76	35 ± 1	44 ± 3	33 ± 1	<0.01	8.8 ± 0.4	8.3 ± 0.3	8.2 ± 0.3	0.10
	Between groups: <i>p</i> = 0.10 Time by group interaction: <i>p</i> = 0.004				Between groups: <i>p</i> = 0.61 Time by group interaction: <i>p</i> < 0.001				Between groups: <i>p</i> = 0.66 Time by group interaction: <i>p</i> = 0.31			

^a *p* < 0.001 vs Sham.

Heart rate (beats/min), mean arterial pressure (mm Hg), and central venous pressure (mm Hg) values are expressed as mean ± standard error of the mean. The times shown are minutes after the onset of reperfusion. Statistical results by analysis of variance are shown in bottom row.

ATL-146e = adenosine A_{2A} receptor agonist; IR = ischemia-reperfusion.

reperfusion injury of the injured lung. Weinbroum and colleagues [7] demonstrated that hepatic reperfusion following ischemia can directly induce myocardial and pulmonary dysfunction. These three examples demonstrate that the effects of local ischemia and reperfusion can impair remote organ function presumably through an inflammatory mechanism.

Adenosine and its analogs have been studied extensively over the past thirty years. One such analog, the adenosine A_{2A} receptor agonist ATL-146e (4-{3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydrofuran-2-yl)-9H-purin-2-yl]-prop-2-ynyl}-cyclo-hexanecarboxylic acid methyl ester), has been shown to improve ischemia reperfusion injury in various organs including lungs, kidneys, and spinal cord [8–10]. Adenosine A_{2A} receptors have been found on endothelium and inflammatory cells, such as neutrophils and macrophages. Several models of inflammation have demonstrated that activation of these receptors reduces neutrophil adherence as well as the release of toxic oxidative metabolites [11, 12]. The ATL-146e appears to reduce the inflammatory processes initiated by ischemia and reperfusion [12–14]. It seems reasonable to deduce that by reducing the injury to the reperfused organ, the remote effects of ischemia-reperfusion injury should also be reduced.

We hypothesized that warm pulmonary ischemia-reperfusion (IR) can cause cardiac dysfunction, which can be attenuated by selective adenosine A_{2A} receptor activation by ATL-146e. This study was intended to demonstrate that pulmonary ischemia-reperfusion injury would adversely affect heart function. After establishing the injury and the cardiac dysfunction, we used ATL-146e in an attempt to improve this dysfunction. Further, after demonstrating a cellular explanation for this dysfunction, a separate experiment tested if a possible mechanism of this remote injury involves the rapid release of reactive oxygen species from the ischemic lung, presumably from activated neutrophils, and to test if an antioxidant could produce similar cardioprotection after reperfusion of the ischemic lung.

Material and Methods

Experimental Protocol

We used an in vivo rabbit model of warm pulmonary ischemia-reperfusion injury. The first group of animals (Sham, *n* = 10) underwent 2 hours of single lung ventilation. The second group (IR, *n* = 10) underwent 90 minutes of ischemia followed by 30 minutes of reperfusion on the single ischemic lung. The third group (ATL-high, *n* = 10) was identical to the IR group except that these animals received ATL-146e at 0.04 mg · kg⁻¹ · min⁻¹ for the extent of reperfusion starting 10 minutes before reperfusion. The fourth group (ATL-low, *n* = 10) was the same as ATL-high except that ATL-146e was given at 0.01 mg · kg⁻¹ · min⁻¹. These doses were chosen based on therapeutic effects of these concentrations in rabbit spinal cord ischemia-reperfusion studies by our lab [15]. At 5 minute intervals, hemodynamic variables including central venous pressure, arterial blood gases, and heart rate were monitored in addition to arterial blood gases.

Animals

New Zealand white rabbits (2.75 to 3.6 kg) were used and randomly assigned to the experimental groups. Animal acquisition was under the supervision of the Department of Comparative Medicine and a licensed veterinarian. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and "The Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institutes of Health (National Institutes of Health publication 85 to 23, revised 1985).

In Vivo Pulmonary Ischemia-Reperfusion Model

The animals were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg). An angiocatheter was placed in the lateral vein of both ears for delivery of drug and intravenous fluids (60 mL/h). The neck vessels and trachea were accessed through a midline incision. The

Table 2. Arterial Oxygenation After Reperfusion With ATL-146e

	0 min	5 min	10 min	15 min	20 min	25 min	30 min	p Value Within Group
Sham	294 ± 44	281 ± 46	285 ± 44	293 ± 43	288 ± 44	290 ± 46	308 ± 50	0.99
IR	243 ± 54	189 ± 58	189 ± 58	159 ± 50	156 ± 51	143 ± 51	146 ± 55 ^a	0.02
ATL-high	235 ± 52	192 ± 56	172 ± 47	140 ± 41	160 ± 40	175 ± 42	180 ± 41 ^a	0.11
ATL-low	308 ± 48	197 ± 42	180 ± 41	158 ± 39	145 ± 33	146 ± 34	130 ± 23 ^a	<0.001

^a $p = 0.029$ vs Sham; between groups; $p = 0.15$; time by group interaction: $p = 0.09$ Arterial oxygenation values (PO_2 , mm Hg) are expressed as mean ± standard error of the mean. Statistical results by analysis of variance are shown in bottom row.

ATL-146e = an adenosine A_{2A} receptor agonist; IR = ischemia-reperfusion.

trachea was isolated with sharp dissection, and a tracheotomy was performed. The rabbit was connected to a volume ventilator (Rodent Ventilator Model 683, Harvard Apparatus, Holliston, MA). Tidal volume was set at 15 mL with a rate of 25 breaths per minute and an inspired oxygen concentration of 100%. The ventilator settings during the ischemic period were manipulated according to arterial blood gas measurements to maintain pH between 7.3 and 7.4 (Chiron/243 pH/Blood Gas Analyzer, Chiron Inc, Corning, NY). The carotid artery was cannulated, and the internal jugular vein was accessed. Both these vessels were connected to a pressure monitor (Hewlett-Packard Co, Palo Alto, CA). An esophageal probe was placed to monitor the heart rate. The rabbit was then anticoagulated with heparin (Heparin Sulfate, 500 U/kg) and paralyzed (Vecuronium, 0.2 mg/kg). Body temperature was monitored with a rectal probe and maintained at normothermia using a heating pad.

A sternotomy was preformed after a midline incision was made. Following sternal retraction, the thymus was bluntly dissected away from the pericardium. The pericardium and bilateral pleura were incised. The aortic root was freed circumferentially. An ultrasound flow probe was placed around the ascending aorta (T106 Small Animal Blood Flow Meter, Transonic Systems, Inc, Ithaca, NY).

For lung isolation, a vascular clamp was placed on the right hilum of all animals except the sham. The clamp was in place for 90 minutes, the ischemic period. After the ischemic period, the clamp was removed and transferred to the opposite hilum for the extent of reperfusion. Thus, the left ventricle received blood solely from the ischemic lung during reperfusion. The sham group had the left lung clamped for the entire two hours. Thus, all groups were maintained on single lung ventilation to provide consistency between groups and ensure that blood was not shunted away from the injured lung.

Hemodynamic and Pulmonary Variables

Cardiac output, mean arterial pressure, central venous pressure, and arterial blood gases were monitored and recorded every 15 minutes for the ischemic period and every 5 minutes during the reperfusion period.

Lung Myeloperoxidase Activity

Neutrophil sequestration was assessed by measuring myeloperoxidase (MPO) activity. Lung tissue was flash frozen in liquid nitrogen following the reperfusion pe-

riod. The tissue was resuspended in 50 mmol/L potassium phosphate (pH 7.4), then homogenized for 30 seconds at 4°C. The solution was centrifuged for 15 minutes at 15,000g (Sorvall RC-5b Refrigerated Superspeed Centrifuge, Kendro Laboratory Products, Newton, CT). The supernatant was discarded before the pellet was resuspended in 10 volumes of 0.5% hexadecyltrimethylammonium (HTAB) in 50 mmol/L potassium phosphate (pH 6.4). The solution was then homogenized a second time for 90 seconds at 4°C. The solution underwent sonication and three freeze thaw cycles (alternating liquid nitrogen for 5 minutes and 37°C circulating bath for 5 minutes). The solution was centrifuged for a second time at 15,000g for 15 minutes, and the supernatant was divided into 1 mL aliquots. The assay buffer was made from 10 mg o-diadenosine in 1 mL deionized water, 100 μ L H_2O_2 , and 8.9 mL of 50 mmol/L potassium phosphate (pH 6.0). Aliquots of supernatant (15 μ L) were combined with assay buffer (135 μ L), and incubated at room temperature in a 96 well plate. Absorbance at 460 nm was measured after 2 minutes by spectrophotometry (MRX Revelation Plate Reader, Dynex Technologies, Inc, Chantilly, VA). Protein concentrations were determined for each sample using Coomassie Plus Protein Assay (BioRad, Hercules, CA). The MPO activity was expressed as change in absorbance/mg protein/min (ΔA_{460} /mg/min).

Reperfusion With Antioxidant

To test if secondary cardiac dysfunction could be a result of the release of reactive oxygen species from the ischemic lung, a known antioxidant, N-(2-mercaptopyrionyl)glycine or MPG, was used in an identical setting as with ATL-146e in a separate experiment. The MPG (Sigma, St Louis, MO) was given with reperfusion at a dose of $0.042 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, which this dose has been shown to reduce cardiac dysfunction from heart ischemia-reperfusion in animal studies [16, 17].

Statistical Analysis

Statistics were performed by an independent statistician using repeated measures analysis of variance to make comparisons between groups, within groups over time, and to test for an interaction between group and time. A p value less than 0.05 is considered statistically significant.

Table 3. Cardiac Output After Reperfusion With ATL-146e

	0 min	1 min	5 min	10 min	15 min	20 min	25 min	30 min	<i>p</i> Value Within Group
Sham	126 ± 10	128 ± 10	124 ± 10	122 ± 8.9	120 ± 8.3 ^b	115 ± 6.6 ^b	115 ± 6.6 ^b	112 ± 5.8 ^b	0.11
IR	126 ± 7.1	92 ± 7.5 ^a	88 ± 7.9 ^a	83 ± 7.8 ^a	86 ± 6.7	87 ± 7.0	87 ± 20	86 ± 6.4	<0.001
ATL-high	130 ± 7.3	98 ± 7.2 ^a	105 ± 5.3	102 ± 5.4	107 ± 4.3 ^b	107 ± 4.4 ^b	107 ± 3.8 ^b	112 ± 3.2 ^b	<0.001
ATL-low	121 ± 17	86 ± 7.7 ^a	94 ± 8.0 ^a	96 ± 8.3	97 ± 8.3	94 ± 9.3	95 ± 6.2	93 ± 9.3	<0.001

^a *p* < 0.02 vs Sham; ^b *p* ≤ 0.05 vs IR; between groups: *p* = 0.01; time by group interaction: *p* = 0.004 Cardiac output values (mL/min) are expressed as mean ± standard error of the mean. Statistical results by analysis of variance are shown in bottom row.

ATL-146e = an adenosine A_{2A} receptor agonist; IR = ischemia-reperfusion.

Results

Physiologic Measurements

None of the mean hemodynamic variables or mean arterial blood gas variables differed among groups throughout the ischemic period. Thus, the results shown are limited to the reperfusion period.

The heart rates among groups did not differ throughout reperfusion except for some small acute variations at 0 minutes after reperfusion (Table 1). The mean arterial pressure and the central venous pressure were also not different among groups throughout reperfusion (Table 1).

Arterial Oxygenation

Arterial oxygenation (PO₂) was used as an indicator of lung function and injury. The PO₂ was consistent among groups until reperfusion when some dramatic changes occurred (see Table 2). At the onset of reperfusion, PO₂ was not significantly different among any of the groups. However, PO₂ in IR, ATL-high, and ATL-low groups were all lower than Sham at 5 minutes of reperfusion and at every time point after that (Table 2). The PO₂ in the IR and ATL-low groups was significantly reduced after 30

minutes reperfusion, indicative of declining lung function in this model of ischemia-reperfusion. In addition, the PO₂ was similar between IR, ATL-high, and ATL-low groups, indicating that ATL-146e did not improve lung function during this 30 minute time period. Note that the mean PO₂ of all animals remained supraphysiologic with fraction of inspired oxygen (FIO₂) of 100%.

Cardiac Output

The mean cardiac output (CO) in all groups was similar until reperfusion. One minute after reperfusion, the CO significantly dropped in the IR and ATL groups while CO remained relatively constant in the Sham group (Table 3 and Fig 1). At 15 minutes of reperfusion, CO in the ATL-high group had recovered to a level similar to Sham, of which both were significantly better than IR. By the end of the reperfusion period, CO was nearly identical between ATL-high and Sham, which were both significantly better than IR (Table 3 and Fig 1). The CO in Sham remained significantly better than IR throughout reperfusion. The CO in the ATL-low group dropped signifi-

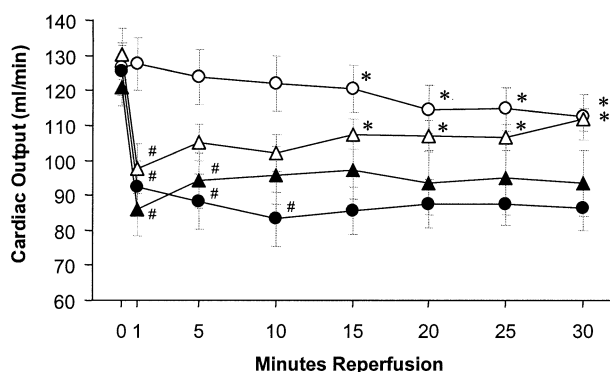


Fig 1. Cardiac output (CO, mL/min) during reperfusion with ATL-146e (an adenosine A_{2A} receptor agonist). The CO in the IR, ATL-low, and ATL-high groups significantly dropped at 1 minute of reperfusion compared to Sham (#*p* = 0.005). By 15 minutes the ATL-high group had a sustained recovery to levels significantly higher than IR (**p* = 0.011), and remained significantly higher than IR thereafter. The CO in ATL-low remained at levels intermediate between IR and ATL-high. See Table 3 for detailed data. ○ = Sham; ● = ischemic-reperfusion (IR); △ = ATL-high; ▲ = ATL-low.

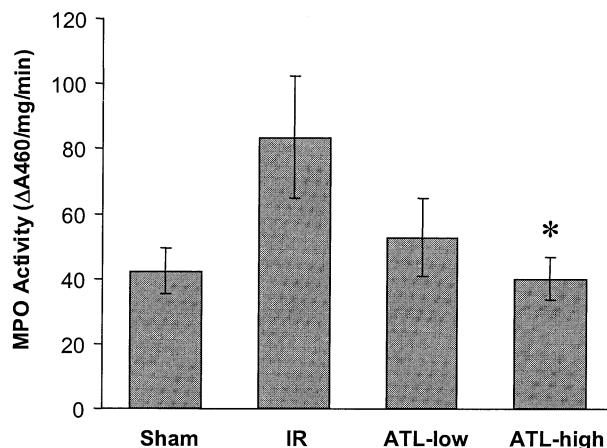


Fig 2. Myeloperoxidase (MPO) activity in lungs after reperfusion with ATL-146e (an adenosine A_{2A} receptor agonist). Pulmonary MPO activity was higher in ischemia-reperfusion (IR) versus Sham (*p* = 0.57), which indicates sequestration of neutrophils in the lung after 30 minutes reperfusion. Importantly, ATL-146e significantly reduced MPO activity in the ATL-high group compared to IR (**p* = 0.049), and reduced MPO activity to an intermediate level in the ATL-low group.

Table 4. Arterial Oxygenation After Reperfusion With MPG

	0 min	5 min	10 min	15 min	20 min	25 min	30 min	p Value Within Group
Sham	457 ± 31 ^a	430 ± 30 ^a	431 ± 27 ^a	430 ± 24 ^a	447 ± 24 ^a	407 ± 21 ^a	441 ± 24 ^a	0.662
IR	308 ± 49	259 ± 50	232 ± 35	207 ± 35	197 ± 25	147 ± 18	197 ± 24	<0.001
MPG	340 ± 60	295 ± 56	263 ± 60	263 ± 61	258 ± 60	248 ± 61	265 ± 68	<0.001

^a $p < 0.001$ vs IR and MPG; between groups: $p < 0.001$; time by group interaction: $p < 0.001$ Arterial oxygenation values (PO₂, mm Hg) are expressed as mean ± standard error of the mean. Statistical results by analysis of variance are shown in bottom row.

IR = ischemia-reperfusion; MPG = N-(2-mercaptopyrionyl) glycine.

cantly compared to Sham with initial reperfusion, then remained at intermediate levels between Sham and ATL-high groups, but without showing any statistically significant difference from either.

Myeloperoxidase Activity

At the end of reperfusion, MPO activity was elevated in IR lungs compared to Sham (Fig 2), indicative of neutrophil sequestration. Importantly, ATL-146e significantly reduced MPO activity at the high dose (Fig 2), and reduced it to an intermediate level at the low dose.

Antioxidant and Cardiac Dysfunction

To test if a possible mechanism of cardiac dysfunction secondary to lung ischemia-reperfusion involved the release of oxidants (reactive oxygen species) from the lung, we utilized a known antioxidant, MPG, in a separate experiment. Here, using the same protocol as with ATL-146e above, MPG was administered during reperfusion of the lung. Similar to that observed with ATL-146e above, MPG did not induce any differences in heart rate, central venous pressure, or mean arterial pressure before or after reperfusion (data not shown). In addition, MPG did not improve lung function during reperfusion as indicated by no significant increases in PO₂ in the MPG group versus the IR (Table 4). Here, both MPG and IR groups displayed significantly reduced PO₂ compared to Sham at all reperfusion times, indicative of lung dysfunction in this in vivo model of ischemia-reperfusion injury. In addition, while MPO activity was significantly increased in the lung after IR, it was not lowered by MPG (data not shown).

In contrast to ATL-146e, which took 15 minutes reperfusion to significantly improve CO, MPG offered significant improvement in CO after only 1 minute reperfusion and throughout the entire 30 minutes reperfusion period (Table 5, Fig 3). In fact, MPG resulted in slightly, but not significantly, higher CO than the Sham group.

Comment

This study demonstrates that reperfusion after warm pulmonary ischemia affects not only the ischemic lung function, but also affects the heart receiving the effluent from the damaged lung. Lung injury was clearly demonstrated by significant reduction in arterial oxygenation with isolated ventilation and perfusion of the ischemic lung. Additionally, MPO activity suggested that neutro-

phils were accumulating in the lungs of the IR group. The reduction MPO activity by the ATL-high group suggests that ATL-146e inhibits the neutrophil sequestration in the lung during ischemia-reperfusion injury. Although heart and lung histology was compared, no differences among groups were demonstrated at this early time point.

The cardiac output was impaired in both the IR and the ATL groups immediately after reperfusion. It did, however, recover in the ATL-high group to levels similar to Sham levels within 15 minutes of reperfusion. No differences were found among any of the groups in other determinants of cardiac performance including preload, afterload, or heart rate. Despite significant differences in the arterial oxygenation between the ATL-high and IR groups, the mean PO₂ was supraphysiologic and, therefore, unlikely to play a role in reducing cardiac function. The cardiac output in the IR animals appears to decline secondary to impaired contractility. This direct cardiac dysfunction thus appeared to be secondary to the effluent from the injured lung and was improved by ATL-146e treatment (ATL-high) after several minutes of continuous therapy.

Other studies have shown encouraging results with ATL-146e at doses that ranged from 0.01 to 0.06 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ [8, 12, 18]. We found that 0.04 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ reduced the resultant pulmonary injury and the secondary cardiac dysfunction. We were not able to demonstrate any appreciable statistical difference compared to IR with the smaller dose of 0.01 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

The cardiac dysfunction immediately followed reperfusion. The literature on reperfusion injury of the heart, liver, and limb has implicated several different substances in affecting the normal function of the heart. The tumor necrosis factor- α (TNF- α), reactive oxygen species, endothelin, and interleukin-1, among others, have all been shown to be produced after reperfusion of an ischemic lung [19-25]. The fact that these substances are released from ischemic lungs does not prove their role in the secondary cardiac dysfunction. However, these same substances have been implicated in the decompensation of cardiac function after reperfusion of the ischemic heart [26-34]. Together or independently, these substances have the potential to lead to the secondary effects we have seen in this study. Other factors yet to be defined could also have a role.

Reactive oxygen species appear particularly suited to cause the immediate remote injury found in this study.

Table 5. Cardiac Output After Reperfusion With MPG

	0 min	1 min	5 min	10 min	15 min	20 min	25 min	30 min	<i>p</i> Value Within Group
Sham	137 ± 6.8	138 ± 9.5	139 ± 7.2	133 ± 7.5	131 ± 7.5	127 ± 7.9	127 ± 7.5	128 ± 7.0	0.10
IR	148 ± 7.7	106 ± 6.8 ^a	107 ± 6.0 ^a	112 ± 6.3 ^a	109 ± 6.2 ^a	103 ± 6.8 ^a	101 ± 7.5 ^a	100 ± 7.7 ^a	<0.001
MPG	149 ± 12	136 ± 14	144 ± 14	143 ± 14	142 ± 12	143 ± 12	143 ± 12	139 ± 11	0.43

^a *p* < 0.05 vs all; between groups: *p* = 0.05; time by group interaction: *p* < 0.001 Cardiac output values (mL/min) are expressed as mean ± standard error of the mean. Statistical results by analysis of variance are shown in bottom row.

IR = ischemia-reperfusion; MPG = N-(2-mercaptopyronyl) glycine.

Reactive oxygen species have both significant production in ischemic lungs and the potential to cause contractile dysfunction in the heart. Weinbroum and colleagues [7] showed that the effluent from reperfusion of the ischemic liver led to impaired cardiac contractility. Specifically, they found that the contractility fell to 70% of baseline. They felt that this dysfunction was secondary to the release of reactive oxygen species in the ischemic effluent since the dysfunction mirrored the increase in the concentrations of lactate dehydrogenase, uric acid, and xanthine oxidase. Leukocytes, especially neutrophils, account for a great majority of the released reactive oxygen species in reperfusion injury. Ischemic parenchyma and endothelium can produce some reactive oxygen species during ischemia, but this production was minimal compared to the production of reactive oxygen species from neutrophils. Importantly, adenosine A_{2A} receptor activation has been shown to decrease neutrophil oxidative activity that may be responsible for the release of reactive oxygen species in ischemia-reperfusion injury [11].

In an effort to further define the mechanism of heart dysfunction in lung ischemia-reperfusion injury, a separate experiment confirmed that a scavenger of reactive oxygen species, MPG, can ameliorate this secondary cardiac dysfunction. The results with MPG did not affect early lung

reperfusion injury evident by PO₂ and MPO levels similar to IR. However, MPG did preserve cardiac output during pulmonary reperfusion, even after only 1 minute reperfusion. Thus, the use of an antioxidant can reduce cardiac dysfunction from lung ischemia-reperfusion. Even though the conclusions are consistent between the MPG and ATL experiments, the PO₂ and CO values are not comparable between the separate studies using different animals at different times. These differences, however, do not impact the conclusions based on these results

Understanding the role of reactive oxygen species in this injury strengthens our theory that inflammatory inhibition by ATL-146e ameliorates cardiac dysfunction over time. In this study, we found that immediately upon reperfusion both the ATL groups and the IR groups experienced impaired cardiac performance. Then, as the drug was able to interact with the previously ischemic compartment and the cells that had been trapped in it, the dysfunction was lessened until it was no longer perceptible. We believe that ATL-146e inactivates the inflammatory cells, notably neutrophils, and thereby lessens the release of reactive oxygen species, or other effectors, that can directly and immediately cause impaired cardiac contractility.

This study demonstrates that secondary or remote cardiac injury does occur with reperfusion of the warm ischemic lung. The secondary dysfunction found in the heart is immediate, implying that the effector is released from the ischemic lung immediately upon reperfusion. This dysfunction can be attenuated with selective adenosine A_{2A} receptor activation by ATL-146e. Although the exact mechanism is not clear, the quick reversal of dysfunction by MPG supports a mechanism involving the release of oxidants from the ischemic lung upon reperfusion, which immediately and negatively impacts heart function. In conclusion, the use of the adenosine A_{2A} receptor agonist, ATL-146e, not only reduces the injury of reperfusion lung, but also reduces remote effects on the heart that may add to the morbidity of pulmonary transplant patients with ischemia-reperfusion injury.

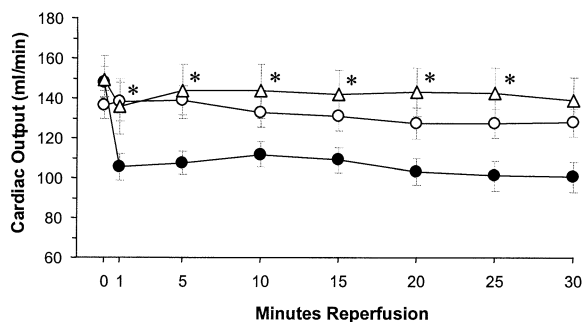


Fig 3. Cardiac output (CO, mL/min) during reperfusion with MPG. The CO was significantly reduced in the IR group beginning 1 minute after reperfusion and thereafter compared to the Sham, demonstrating cardiac dysfunction secondary to lung ischemia-reperfusion. Interestingly, MPG rapidly and significantly improved CO compared to IR starting at 1 minute reperfusion (**p* = 0.012), and this level of protection was maintained throughout reperfusion where CO remained at or above Sham levels with MPG treatment. See Table 5 for detailed data. ○ = Sham; ● = ischemia-reperfusion (IR); △ = N-(2-mercaptopyronyl)glycine (MPG).

We would like to acknowledge the invaluable assistance of Tony Herring, Cindy Dodson, and Sheila Hammond on this project and Mark R. Conaway, PhD, for statistical analysis of this study. This study was funded by the American Heart Association Grant No. 0250222N and National Institutes of Health Grant No. T32HL07849-04.

References

1. King RC, Binns OA, Rodriguez F, et al. Reperfusion injury significantly impacts clinical outcome after pulmonary transplantation. *Ann Thorac Surg* 2000;69:1681-5.
2. Alam S, Chan KM. Noninfectious pulmonary complications after organ transplantation. *Curr Opin Pulm Med* 1996;2:412-8.
3. Fiser SM, Kron IL, McLendon Long S, et al. Early intervention after severe oxygenation index elevation improves survival following lung transplantation. *J Heart Lung Transplant* 2001;20:631-6.
4. McCurry KR, Keenan RJ. Controlling perioperative morbidity and mortality after lung transplantation for pulmonary hypertension. *Semin Thorac Cardiovasc Surg* 1998;10:139-43.
5. Sorkine P, Setton A, Halpern P, et al. Soluble tumor necrosis factor receptors reduce bowel ischemia-induced lung permeability and neutrophil sequestration. *Crit Care Med* 1995;23:1377-81.
6. Palazzo R, Hamvas A, Shuman T, et al. Injury in nonischemic lung after unilateral pulmonary ischemia with reperfusion. *J Appl Physiol* 1992;72:612-20.
7. Weinbroum AA, Hochhauser E, Rudick V, et al. Direct induction of acute lung and myocardial dysfunction by liver ischemia and reperfusion. *J Trauma* 1997;43:627-33.
8. Okusa MD, Linden J, Macdonald T, Huang L. Selective A_{2A} adenosine receptor activation reduces ischemia-reperfusion injury in rat kidney. *Am J Physiol* 1999;277:F404-12.
9. Ross SD, Tribble CG, Linden J, et al. Selective adenosine-A_{2A} activation reduces lung reperfusion injury following transplantation. *J Heart Lung Transplant* 1999;18:994-1002.
10. Cassada DC, Gangemi JJ, Rieger JM, et al. Systemic adenosine A_{2A} agonist ameliorates ischemic reperfusion injury in the rabbit spinal cord. *Ann Thorac Surg* 2001;72:1245-50.
11. Sullivan GW, Rieger JM, Scheld WM, et al. Cyclic AMP-dependent inhibition of human neutrophil oxidative activity by substituted 2-propynylcyclohexyl adenosine A(2A) receptor agonists. *Br J Pharmacol* 2001;132:1017-26.
12. McPherson JA, Barringhaus KG, Bishop GG, et al. Adenosine A(2A) receptor stimulation reduces inflammation and neointimal growth in a murine carotid ligation model. *Arterioscler Thromb Vasc Biol* 2001;21:791-6.
13. Okusa MD, Linden J, Huang L, et al. A(2A) adenosine receptor-mediated inhibition of renal injury and neutrophil adhesion. *Am J Physiol Renal Physiol* 2000;279:F809-18.
14. Sullivan GW, Linden J, Buster BL, Scheld WM. Neutrophil A_{2A} adenosine receptor inhibits inflammation in a rat model of meningitis: synergy with the type IV phosphodiesterase inhibitor, rolipram. *J Infect Dis* 1999;180:1550-60.
15. Cassada DC, Tribble CG, Kaza AK, et al. Adenosine analogue reduces spinal cord reperfusion injury in a time-dependent fashion. *Surgery* 2001;130:230-5.
16. Tanonaka K, Iwai T, Motegi K, Takeo S. Effects of N-(2-mercapto-propionyl)-glycine on mitochondrial function in ischemic-reperfused heart. *Cardiovasc Res* 2003;57:416-25.
17. Xuan YT, Tang XL, Banerjee S, et al. Nuclear factor- κ B plays an essential role in the late phase of ischemic preconditioning in conscious rabbits. *Circulation Res* 1999;84:1095-1109.
18. Cassada DC, Tribble CG, Laubach VE, et al. An adenosine A_{2A} agonist, ATL-146e, reduces paralysis and apoptosis during rabbit spinal cord reperfusion. *J Vasc Surg* 2001;34:482-8.
19. Messent M, Griffiths MJ, Quinlan GJ, et al. Ischaemia-reperfusion injury in the rat is modulated by superoxide generation and leads to an augmentation of the hypoxic pulmonary vascular response. *Clin Sci* 1996;90:47-54.
20. Grisham MB, Granger DN. Metabolic sources of reactive oxygen metabolites during oxidant stress and ischemia with reperfusion. *Clin Chest Med* 1989;10:71-81.
21. Yamada T, Murase N, Maeda T, et al. Protective effect of TNF- α and IL-1 β inhibitor FR167653 on ischemia-reperfusion injury in rat small intestinal transplantation. *Transplant Proc* 1998;30:2638.
22. Shaw MJ, Shennib H, Bousette N, et al. Effect of endothelin receptor antagonist on lung allograft apoptosis and NOSII expression. *Ann Thorac Surg* 2001;72:386-90.
23. Shaw MJ, Shennib H, Tayara L, et al. Endothelin receptor antagonist SB209670 decreases lung allograft apoptosis and improves lung graft function after prolonged ischemia. *J Cardiovasc Pharmacol* 2000;36:S209-11.
24. Chang DM, Hsu K, Ding YA, Chiang CH. Interleukin-1 in ischemia-reperfusion acute lung injury. *Am J Respir Crit Care Med* 1997;156:1230-4.
25. Guidot DM, Kitlowski AD, Hybertson BM, Repine JE. Mitochondrial antioxidant function is a potential mechanism for organ differences in interleukin-1-mediated tolerance to oxidative injury. *Am J Med Sci* 1999;318:308-15.
26. Kadokami T, Frye C, Lemster B, et al. Anti-tumor necrosis factor- α antibody limits heart failure in a transgenic model. *Circulation* 2001;104:1094-7.
27. Blum A, Miller H. Pathophysiological role of cytokines in congestive heart failure. *Annu Rev Med* 2001;52:15-27.
28. Long CS. The role of interleukin-1 in the failing heart. *Heart Fail Rev* 2001;6:81-94.
29. Cain BS, Meldrum DR, Dinarello CA, et al. Tumor necrosis factor-alpha and interleukin-1 beta synergistically depress human myocardial function. *Crit Care Med* 1999;27:1309-18.
30. Di Filippo C, D'Amico M, Marfella R, et al. Endothelin-1 receptor antagonists reduce cardiac electrical instability induced by high glucose in rats. *Naunyn Schmiedebergs Arch Pharmacol* 2002;366:193-7.
31. Marin-Garcia J, Goldenthal MJ, Moe GW. Selective endothelin receptor blockade reverses mitochondrial dysfunction in canine heart failure. *J Card Fail* 2002;8:326-32.
32. Pearl JM, Nelson DP, Wagner CJ, et al. Endothelin receptor blockade reduces ventricular dysfunction and injury after reoxygenation. *Ann Thorac Surg* 2001;72:565-70.
33. Kaminski KA, Bonda TA, Korecki J, Musial WJ. Oxidative stress and neutrophil activation—the two keystones of ischemia/reperfusion injury. *Int J Cardiol* 2002;86:41-59.
34. Suessenbacher A, Lass A, Mayer B, Brunner F. Antioxidative and myocardial protective effects of L-arginine in oxygen radical-induced injury of isolated perfused rat hearts. *Naunyn Schmiedebergs Arch Pharmacol* 2002;365:269-276.