Inflammatory lung injury after cardiopulmonary bypass is attenuated by adenosine A_{2A} receptor activation

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Objective: Cardiopulmonary bypass has been shown to exert an inflammatory response within the lung, often resulting in postoperative pulmonary dysfunction. Several studies have shown that adenosine A_{2A} receptor activation attenuates lung ischemia-reperfusion injury; however, the effect of adenosine A_{2A} receptor activation on cardiopulmonary bypass-induced lung injury has not been studied. We hypothesized that specific adenosine A_{2A} receptor activation by ATL313 would attenuate inflammatory lung injury after cardiopulmonary bypass.

Methods: Adult male Sprague-Dawley rats were randomly divided into 3 groups: 1) SHAM group (underwent cannulation + heparinization only); 2) CONTROL group (underwent 90 minutes of normothermic cardiopulmonary bypass with normal whole-blood priming solution; and 3) ATL group (underwent 90 minutes of normothermic cardiopulmonary bypass with ATL313 added to the normal priming solution).

Results: There was significantly less pulmonary edema and lung injury in the ATL group compared with the CONTROL group. The ATL group had significant reductions in bronchoalveolar lavage interleukin-1, interleukin-6, interferon-γ, and myeloperoxidase levels compared with the CONTROL group. Similarly, lung tissue interleukin-6, tumor necrosis factor-α, and interferon-γ were significantly decreased in the ATL group compared with the CONTROL group. There was no significant difference between the SHAM and ATL groups in the amount of pulmonary edema, lung injury, or levels of proinflammatory cytokines.

Conclusion: The addition of a potent adenosine A_{2A} receptor agonist to the normal priming solution before the initiation of cardiopulmonary bypass significantly protects the lung from the inflammatory effects of cardiopulmonary bypass and reduces the amount of lung injury. Adenosine A_{2A} receptor agonists could represent a new therapeutic strategy for reducing the potentially devastating consequences of the inflammatory response associated with cardiopulmonary bypass.

Since its successful introduction by Dr Gibbon in 1953,[1] cardiopulmonary bypass (CPB) has seen substantial evolution. Despite this, ample evidence exists regarding the massive inflammatory response that is initiated after procedures that use CPB.[2-5] The majority of patients undergoing CPB have sufficient reserve to overcome this inflammatory insult without clinically observable sequelae. In a smaller cohort of patients, however, this inflammatory response can result in system-wide organ dysfunction leading to respiratory failure, renal insufficiency and failure, coagulopathies, neurologic dysfunction, altered hepatic function, and, in an even smaller number of patients, acute respiratory distress syndrome and systemic inflammatory response syndrome.[2,3] The inflammatory response that is triggered is thought to occur from a highly sensitive set of interactions between the normal circulation and the ''foreign'' surface of the CPB circuit, which results in an incremental activation of complement, coagulation, fibrinolytic, and inflammatory responses.[4] In addition, some argue that end-organ ischemia and the subsequent reperfusion injury that results is another powerful player in this inflammatory response.[2] The ability to reconcile this pathologic and potentially lethal process relies on the ability to attenuate the unyielding inflammatory response that is characteristic of CPB.

The role of adenosine in limiting the inflammatory response to CPB is a logical continuation of the success that has been described with adenosine agonists and their widespread effects on ischemia-reperfusion injury in multiple different organ systems.[6-9] The adenosine A_{2A} receptor ($A_{2A}R$) is 1 of 4 G-protein coupled receptors that belong to the adenosine receptor family (A_1, A_{2A}, A_{2B}, A_3). The $A_{2A}R$ is expressed predominantly on inflammatory cells, including neutrophils, macrophages, mast cells, monocytes, and platelets. Once activated, this G-protein coupled receptor leads to an increase in intracellular cyclic adenosine monophosphate, which results in a potent inactivation of inflammatory cells, decreased proinflammatory cytokine production and release, suppressed neutrophil recruitment and activation, decreased

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Funded by a Cardiovascular Surgery Training Grant (NIH 2 T32 HL007849).

Disclosures: Dr Kron is a shareholder in Adenosine Therapeutics, LLC, the corporation that provided the adenosine A_{2A} receptor agonist ATL313.

Received for publication April 28, 2008; revisions received June 6, 2008; accepted for publication July 5, 2008.

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J Thorac Cardiovasc Surg 2008;136:1280-8
0022-5223/$34.00

doi:10.1016/j.jtcvs.2008.07.010

Abbreviations and Acronyms

$A_{2A}R$ = adenosine A_{2A} receptor
BAL = bronchoalveolar lavage
CPB = cardiopulmonary bypass
IFN = interferon
IL = interleukin
MPO = myeloperoxidase
TNF = tumor necrosis factor

oxygen free radical production, and possibly down-regulation of cellular immunity through impaired $CD4^+$ T-cell activity.[10,11]

In this study, we examined the effect of $A_{2A}R$ activation on the inflammatory response within the lung caused by CPB by adding the highly selective $A_{2A}R$ agonist, ATL313 [4-{3-[6-amino-9-(5-cyclopropylcarbamoyl-3,4-dihydroxytetrahydrofuran-2-yl)-9H-purin-2-yl]prop-2-ynyl} piperidine-1-carboxylic acid methyl ester], to the standard bypass priming solution before the initiation of CPB.

Previous research has demonstrated that specific activation of the $A_{2A}R$ subtype has been shown to have significant anti-inflammatory effects.[6,9] Furthermore, our institutional research has shown that the specific activation of the $A_{2A}R$ is mediated by ATL313.[6] We therefore hypothesized that activation of the $A_{2A}R$ in the standard bypass prime before the start of CPB would lead to an attenuation in the pulmonary inflammatory response observed after CPB.

MATERIALS AND METHODS

Animals and Groups

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass) (350–450 g) were used for all studies and received humane care in accordance with the ‘‘Guide for the Care and Use of Laboratory Animals’’ published by the National Institutes of Health (NIH publication No. 85-23, revised 1996). The Animal Care and Use Committee at the University of Virginia reviewed and approved the protocol for this study before experimentation began.

Rats were randomly assigned to 1 of 3 groups (n = 5 for each group): SHAM group, CPB with normal whole blood priming solution (CONTROL group), and CPB with normal whole blood priming solution plus ATL313 (ATL group). The sham operation consisted of all venous and arterial cannulations, as well as full heparinization without CPB.

Surgical Procedure

The rat model of CPB was based on the model for extracorporeal circulation in the rat as developed by Grocott and associates.[12] Rats were anesthetized with 4% isoflurane. After adequate anesthesia was achieved, rats were intubated by direct laryngoscopy with a 14-gauge intravenous catheter (BD Insyte Autoguard, Becton-Dickinson Infusion Therapy Systems Inc, Sandy, Utah) and mechanically ventilated by a small-animal ventilator (TOPO Dual Mode Ventilator, Kent Scientific, Torrington, Conn) with an air-oxygen mixture (F_{IO_2} = 0.5). During the cannulations, ventilation was adjusted to maintain a partial pressure of carbon dioxide (Pa_{CO_2}) between 35 and 42 mm Hg. During the surgical procedure, anesthesia was maintained with 2% to 2.5% isoflurane and adjusted as appropriate to achieve adequate depth of anesthesia. All surgeries were performed with standard sterile techniques. The surgical cannulations and CPB circuit are illustrated in Figure 1. Rectal temperature was monitored and regulated at 37.3°C to 37.6°C using a combination of heat lamps, 2 forced water-heating blankets, and a forced air-warming blanket as needed. Electrocardiography and pulse oximetry were continuously monitored using a small animal portable monitoring system (SurgiVet V3404 Plus, SurgiVet Inc, Waukesha, Wis).

The tail artery was identified through a 1-cm ventral tail incision, encircled with 5-0 silk sutures, and cannulated with a modified 22-gauge intravenous catheter (BD Insyte, Becton-Dickinson Infusion Therapy Systems Inc) to serve as the arterial inflow cannula. After this, the femoral artery was identified through a 1-cm right groin incision and encircled with 5-0 silk sutures. A 24-gauge intravenous catheter (BD Insyte, Becton-Dickinson Infusion Therapy Systems Inc) was then inserted for continuous mean arterial pressure monitoring and intermittent arterial blood gas analysis (Rapidpoint 405 series, Bayer HealthCare LLC, Tarrytown, NY). Next, through a 2-cm midline cervical incision, the right internal jugular vein was identified and encircled with 5-0 silk sutures. A modified 8F multi-orifice pediatric femoral venous cannula (Biomedicus 96830-008, Medtronic Inc, Minneapolis, Minn) was inserted into the vein and gently advanced into the right atrium. The position of the venous cannula was confirmed by previous autopsy studies and allowed for complete drainage of the right atrium, bilateral superior vena cava, and inferior vena cava. On insertion of the venous cannula, 200 IU of unfractionated heparin were given directly via the venous cannula, allowing for an activated clotting time 450 seconds or more.

Cardiopulmonary Bypass Circuit and Procedure

The CPB circuit (Figure 1) consisted of a sterile 5-mL venous reservoir (Bubble Trap Compliance Chamber, Radnoti Glass Technology Inc, Monrovia, Calif). The venous reservoir was connected to a peristaltic

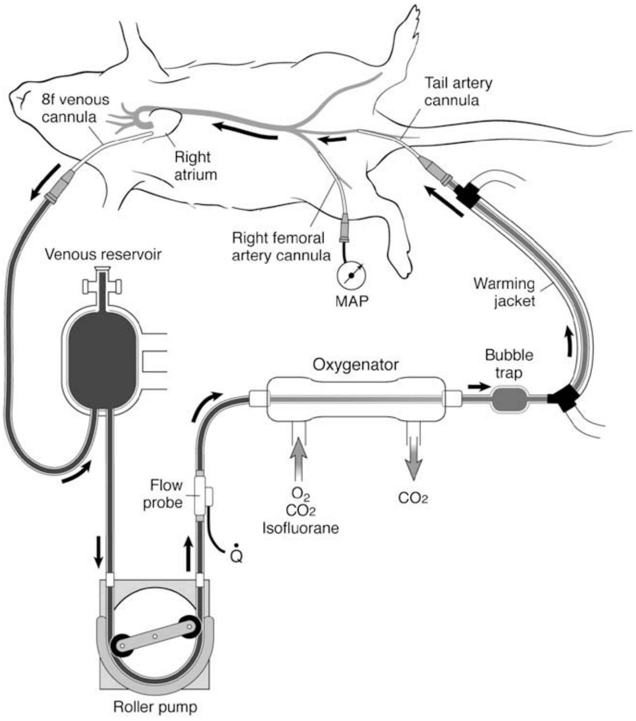

FIGURE 1. Schematic diagram of the rat CPB circuit and surgical cannulations. Ventilator not shown. *MAP,* Mean arterial pressure.

roller-pump (Masterflex, Cole-Parmer Instrument Company, Chicago, Ill) through sterile 1.6-mm internal diameter silicone tubing (Tygon, Cole-Parmer Instrument Company) connected in series to a custom flow probe (Transonic Flowprobe, Transonic Systems Inc, Ithaca NY) used to continuously monitor blood flow rates during CPB. Additional sterile 1.6-mm internal diameter silicone tubing was used to connect the flow probe to an externally warmed sterile hollow-fiber membrane oxygenator (MiniModule, Membrana, Charlotte, NC) with an active surface area of 0.18 m^2. The oxygenator was inverted to serve an additional role as an inline arterial bubble trap and then connected to additional sterile 1.6-mm internal diameter silicone tubing, surrounded by a jacket warmed with circulating water from a separate heat pump, and then to the previously mentioned arterial inflow cannula. Venous drainage was augmented as needed by either adjusting the placement of the venous cannula or changing the height of the venous reservoir relative to the animal to increase or decrease gravity drainage of the right atrium and its associated structures.

The CPB circuit was primed with 45 mL of whole blood obtained from 2 to 3 heparinized (250 IU/kg) donor rats phlebotomized under isoflurane anesthesia via direct cardiac puncture. For the ATL group, ATL313 (Adenosine Therapeutics LLC, Charlottesville, Va) was added directly to the whole blood prime at a dosage based on previous studies from our laboratory.[6] From a 4.6 μmol/L stock solution of ATL313 in normal saline, 1 mL was added to 45 mL of whole blood and gently mixed before pump priming. This resulted in a final dosage concentration of 100 nmol/L. For the CONTROL group, an equivalent volume (1 mL) of vehicle (normal saline) was injected into the 45 mL of whole blood before priming the CPB circuit. After the surgical cannulations, heparinization, and adequate pump priming, the animal was connected to the CPB circuit and extracorporeal circulation was slowly initiated to a final flow rate of 160 to 165 mL/kg/min, which corresponds to 100% of the normal cardiac output in a rat. Once this flow rate was attained, mechanical ventilation was terminated and CPB was carried out for 90 minutes. During CPB, the gas flow to the oxygenator consisted of oxygen, carbon dioxide, and isoflurane. At the conclusion of the 90-minute period, mechanical ventilation was resumed, and all animals were slowly weaned from CPB without the need for inotropes or vasopressors. Once separated from CPB, the rats were decannulated and remained intubated, anesthetized, and mechanically ventilated for an additional 90 minutes.

Physiologic Data and Specimen Collection

Blood pressure, mean arterial pressure, central venous pressure, heart rate, pulse oximetry, temperature, and flow rate were monitored continuously during the bypass period and recorded at baseline and 10, 20, 45, 60, and 90 minutes during the bypass procedure. The same variables (excluding flow rate) were monitored after the cessation of CPB and recorded at 30, 60, and 90-minute intervals. In addition, arterial blood gas analysis was performed at the same prespecified intervals. At the completion of the 90-minute recovery period, the chest was opened and the rat was phlebotomized by direct cardiac puncture. Plasma was collected by centrifugation at 4°C for 20 minutes and stored at –70°C until cytokine analysis was performed. The left lung was then isolated and removed for subsequent tissue cytokine and wet-to-dry weight ratio analysis. Next, a tracheostomy was performed followed by bronchoalveolar lavage (BAL) of the entire right lung. After BAL was performed, the right lung was removed and fixed by intratracheal instillation of 4% paraformaldehyde at 25 cm H_2O pressure.

Lung Wet/Dry Weight Ratio

Lung wet/dry weight ratio was used as a measure of pulmonary edema. Samples of the left lower lobe lung tissue were blotted to remove excess blood and weighed immediately after harvest. These samples were then desiccated under vacuum at 55°C until a stable dry weight was achieved.

Bronchoalveolar Lavage

BAL was performed on all lungs before en bloc removal and permanent fixation. The right lung was isolated and lavaged 3 times with separate 10-mL aliquots of normal saline. The BAL fluid was centrifuged at 1500*g* for 10 minutes at 4°C. The supernatant was then snap-frozen for subsequent analysis.

Myeloperoxidase Content

Myeloperoxidase (MPO) content in BAL fluid was measured using an MPO enzyme-linked immunosorbent assay kit (Cell Sciences, Canton, Mass) and performed according to the manufacturer's instructions. MPO content was used as a broad measure of neutrophil activation and sequestration.

Cytokine Analysis

The protein levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and interferon (IFN)-γ in lung tissue, plasma, and BAL fluid were examined with a Bio-Plex multiplex cytokine enzyme-linked immunosorbent assay system (Bio-Rad Laboratories Inc, Hercules, Calif) and performed according to the manufacturer's instructions. Samples were run in triplicate.

Lung Injury Severity Score

A pathologist, blinded to treatment group, graded each lung sample after appropriate tissue processing and staining (hematoxylin-eosin). Each sample was graded on the presence of the number of macrophages, amount of interstitial infiltrate, and presence of alveolar edema. Each of these 3 categories was given a score of 0 to 3, resulting in a possible score ranging from 0 for uninjured, normal lungs to 9 for the most severely injured lungs.

Statistics

Values are expressed as the mean ± standard error. All statistical analysis was performed by an independent statistician. Analysis of variance and the post hoc Bonferroni test were used to determine whether significant differences existed among the groups.

RESULTS

Physiologic and Arterial Blood Gas Measurements

Physiologic data are detailed in Table 1. The data were similar for all 3 groups at both the baseline and the post-bypass time points. The addition of ATL313 to the bypass prime did not change any of the physiologic parameters measured when comparing the CONTROL group with the ATL group. Among the 3 groups, there were no significant differences noted in temperature, arterial oxygen saturation, or hematocrit at any of the time points captured. In the CONTROL and ATL groups, mean arterial pressure decreased significantly while on CPB compared with SHAM ($P < .05$ for all time points). There were no differences noted between the CONTROL group and the ATL group in terms of CPB flow rates, with each at or slightly above the projected goal flow rate of 160 to 165 mL/kg/min.

In regard to arterial blood gas analysis (Table 2), there were no differences noted between the CONTROL group and the ATL group for pH, Pao_2, $Paco_2$, or HCO_3 at any point during the experiment. When comparing the SHAM group with the CONTROL and ATL groups, the SHAM group was noted to have a higher pH and a slightly lower

TABLE 1. Physiologic data

		CPB					Post-CPB		
	Baseline	**10 min**	**20 min**	**45 min**	**60 min**	**90 min**	**30 min**	**60 min**	**90 min**
MAP (mm Hg)									
SHAM	92 ± 3.6	90 ± 2.0[a]	92 ± 1.6[a]	89 ± 1.6[a]	84 ± 1.5[a]	85 ± 0.9[a]	89 ± 3.5	91 ± 3.0	90 ± 1.1
CONTROL	89 ± 8.9	75 ± 5.6	73 ± 4.5	72 ± 4.4	75 ± 4.1	71 ± 6.3	80 ± 4.5	85 ± 4.1	88 ± 2.8
ATL	88 ± 3.8	76 ± 3.9	72 ± 5.1	76 ± 3.8	75 ± 4.1	72 ± 4.1	82 ± 3.0	86 ± 4.6	86 ± 4.4
CPB flow (mL/kg/min)									
SHAM	-	-	-	-	-	-	-	-	-
CONTROL	-	161 ± 0.3	164 ± 1.1	160 ± 0.7	165 ± 1.2	162 ± 2.3	-	-	-
ATL	-	162 ± 0.1	161 ± 0.9	163 ± 1.2	161 ± 1.7	164 ± 1.7	-	-	-
HCT (%)									
SHAM	37 ± 0.5	-	36 ± 0.7	-	36 ± 1.1	-	-	36 ± 0.4	-
CONTROL	39 ± 1.4	37 ± 0.6	37 ± 0.6	38 ± 0.3	38 ± 0.6	37 ± 1.6	38 ± 0.9	37 ± 1.3	38 ± 1.1
ATL	37 ± 0.6	36 ± 0.5	37 ± 0.9	37 ± 0.6	38 ± 0.7	38 ± 0.2	39 ± 0.7	39 ± 0.6	39 ± 0.5
Temperature (°C)									
SHAM	37.7 ± .04	37.6 ± .07	37.5 ± .11	37.4 ± .05	37.5 ± .03	37.4 ± .06	37.6 ± .06	37.5 ± .07	37.6 ± .06
CONTROL	37.5 ± .12	36.4 ± .09	36.9 ± .07	37.4 ± .15	37.4 ± .07	37.4 ± .04	37.4 ± .09	37.5 ± .08	37.5 ± .11
ATL	37.4 ± .16	36.7 ± .12	37.0 ± .08	37.4 ± .04	37.6 ± .06	37.5 ± .08	37.5 ± .08	37.6 ± .06	37.4 ± .07
Sao_2 (%)									
SHAM	98 ± 0.5	99 ± 1.1	99 ± 1.0	99 ± 0.3	98 ± 0.6	98 ± 0.5	97 ± 0.4	99 ± 0.7	98 ± 0.2
CONTROL	98 ± 0.4	99 ± 0.7	99 ± 0.7	98 ± 0.5	98 ± 0.6	99 ± 0.7	98 ± 0.6	97 ± 0.9	97 ± 0.2
ATL	99 ± 0.2	98 ± 0.6	98 ± 0.9	99 ± 0.7	99 ± 0.7	98 ± 0.7	99 ± 0.3	98 ± 0.3	98 ± 0.5

MAP, Mean arterial pressure; *CPB,* cardiopulmonary bypass; *HCT,* hematocrit; *Sao_2,* arterial oxygen saturation. Values represent mean ± standard error. SHAM (n = 5), CONTROL (n = 5), ATL (n = 5). [a]$P < .05$ versus CONTROL and ATL.

Pao_2 at the 20- and 60-minute time points ($P < .001$). The SHAM group was noted to have a slightly lower baseline Paco_2 and HCO_3 when compared with the CONTROL and ATL groups ($P < .05$).

Cytokine Analysis

Enzyme-linked immunosorbent assay was used to determine whether the addition of ATL313 had any effect on the quantity of proinflammatory cytokines in the lung tissue, BAL fluid, or plasma (Table 3). Within lung tissue, CONTROL animals were found to have marked elevations in IL-1, IL-6, TNF-α, and IFN-γ after the 90-minute recovery period compared with SHAM. In the ATL group, tissue expression of IL-6, TNF-α, and IFN-γ were significantly attenuated. Similar results were observed within the BAL fluid. The CONTROL group was found to have significant

TABLE 2. Arterial blood gas analysis

		CPB					Post-CPB		
	Baseline	**10 min**	**20 min**	**45 min**	**60 min**	**90 min**	**30 min**	**60 min**	**90 min**
pH									
SHAM	7.42 ± .01	-	7.42 ± .01[a]	-	7.45 ± .01[a]	-	-	7.45 ± .01[a]	-
CONTROL	7.42 ± .02	7.39 ± .02	7.37 ± .01	7.39 ± .02	7.39 ± .02	7.38 ± .01	7.41 ± .01	7.38 ± .01	7.39 ± .01
ATL	7.39 ± .02	7.36 ± .03	7.36 ± .04	7.37 ± .03	7.38 ± .03	7.39 ± .03	7.38 ± .02	7.39 ± .01	7.38 ± .01
Pao_2 (mm Hg)									
SHAM	283 ± 11	-	266 ± 30[a]	-	222 ± 13[a]	-	-	298 ± 33	-
CONTROL	289 ± 20	371 ± 53	379 ± 46	392 ± 53	392 ± 49	325 ± 58	297 ± 31	259 ± 24	244 ± 15
ATL	298 ± 34	383 ± 38	400 ± 30	369 ± 41	377 ± 46	381 ± 19	312 ± 30	251 ± 11	293 ± 12
Paco_2 (mm Hg)									
SHAM	36 ± 3[b]	-	37 ± 2	-	38 ± 3	-	-	38 ± 2	-
CONTROL	41 ± 4	39 ± 1	40 ± 1	38 ± 2	37 ± 3	41 ± 2	41 ± 2	39 ± 2	39 ± .4
ATL	42 ± 2	38 ± 2	37 ± 3	40 ± 2	39 ± 1	43 ± 1	42 ± 1	42 ± 1	39 ± 1
HCO_3 (mEq)									
SHAM	24.4 ± 1.5[b]	-	23.9 ± .75	-	23.8 ± .63	-	-	25.1 ± .94	-
CONTROL	26.5 ± 1.4	24.9 ± 1.4	23.1 ± 1.2	23.2 ± 1.2	22.9 ± 1.1	23.1 ± 1.1	23.6 ± 1.7	24.6 ± 2.5	23.9 ± 1.9
ATL	26.1 ± .46	25.1 ± .37	24.2 ± .43	24.1 ± .75	23.5 ± .81	23.7 ± .87	23.8 ± .83	24.3 ± .61	24.1 ± .44

Pao_2, Partial pressure of oxygen; *$Paco_2$,* partial pressure of carbon dioxide; *HCO_3,* bicarbonate. Values represent mean ± standard error. SHAM (n = 5), CONTROL (n = 5), ATL (n = 5). [a]$P < .001$ versus CONTROL and ATL. [b]$P < .05$ versus CONTROL and ATL.

TABLE 3. Cytokine analysis

	Lung tissue (pg/mL)			
	IL-1	**IL-6**	**TNF-α**	**IFN-γ**
SHAM	10,865 ± 1086	403 ± 88	811 ± 90	1870 ± 9
CONTROL	22,839 ± 536[a]	7402 ± 371[b]	2002 ± 148[b]	2530 ± 9[c]
ATL	21,033 ± 694[a]	2136 ± 247	765 ± 129	1770 ± 20
BAL (pg/mL)				
	IL-1	IL-6	TNF-α	IFN-γ
SHAM	382 ± 45	126 ± 41	311 ± 24	75 ± 17
CONTROL	753 ± 45[b]	389 ± 70[b]	330 ± 10	161 ± 17[b]
ATL	434 ± 45	178 ± 12	285 ± 43	52 ± 7
Plasma (pg/mL)				
	IL-1	IL-6	TNF-α	IFN-γ
SHAM	243 ± 13	91 ± 5	193 ± 14	92 ± 10[d]
CONTROL	249 ± 16	102 ± 9	169 ± 4	124 ± 4
ATL	225 ± 6	89 ± 3	165 ± 8	124 ± 8

IL, Interleukin; *TNF,* tumor necrosis factor; *IFN,* interferon; *BAL,* bronchoalveolar lavage. Values represent mean ± standard error. SHAM (n = 5), CONTROL (n = 5), ATL (n = 5). [a]$P < .001$ versus SHAM. [b]$P < .001$ versus SHAM and ATL. [c]$P < .05$ versus SHAM and ATL. [d]$P < .05$ versus CONTROL and ATL.

elevations in IL-1, IL-6, and IFN-γ compared with SHAM. The addition of ATL313 to the bypass prime (ATL group) significantly reduced the expression of IL-1, IL-6, and IFN-γ back to SHAM levels. There were no differences noted in the levels of TNF-α within the BAL fluid for the 3 groups. The levels of IL-1, IL-6, TNF-α, and IFN-γ in the plasma were comparatively low and found to be similar among the 3 groups.

Myeloperoxidase Levels

MPO levels within the BAL fluid were used as an indicator of neutrophil activation and sequestration into alveolar airspace (Figure 2). The CONTROL group was found to have significant elevations in MPO level relative to SHAM. The addition of ATL313 to the standard bypass prime (ATL group) led to a 59% reduction in MPO activity compared with the CONTROL group ($P < .05$). There were no significant differences in MPO activity observed between the ATL group and the SHAM group.

Wet/Dry Weight Ratio Analysis

Wet-to-dry weight ratios were analyzed as an indicator of pulmonary edema (Figure 3). The CONTROL group was found to have a significantly higher wet-to-dry weight ratio compared with SHAM. The addition of ATL313 to the standard bypass prime (ATL group) resulted in a 63% reduction in wet/dry weight ratio compared with the CONTROL group ($P < .001$). There were no significant differences in the wet/dry ratios observed between the ATL group and the SHAM group.

Lung Injury Severity

The CONTROL group had considerably more lung injury relative to SHAM. The addition of ATL313 to the bypass prime resulted in significant improvements in lung histology (Figure 4) and lung injury severity score (Table 4) compared with the CONTROL group ($P < .001$ for overall lung injury severity score). Lung histology and lung injury severity were similar between the ATL group and the SHAM group.

DISCUSSION

This study demonstrates that $A_{2A}R$ activation during CPB results in a significant reduction in the pulmonary inflammatory response observed after CPB in a rat model. The addition of ATL313 to the standard bypass prime, before the initiation of CPB, resulted in significantly decreased levels of the potent proinflammatory cytokines, IL-1, IL-6, TNF-α, and IFN-γ in BAL fluid and lung tissue, and resulted in decreased neutrophil sequestration and activation (decreased BAL MPO level), decreased pulmonary edema, less lung injury, and preserved lung histology. Presumably, these improvements in quantitative inflammatory markers may translate into improved physiology after CPB, although the true effects on lung physiology were not evaluated in this study.

Cardiac surgery using CPB is a well-known trigger of a substantial inflammatory response in both humans after

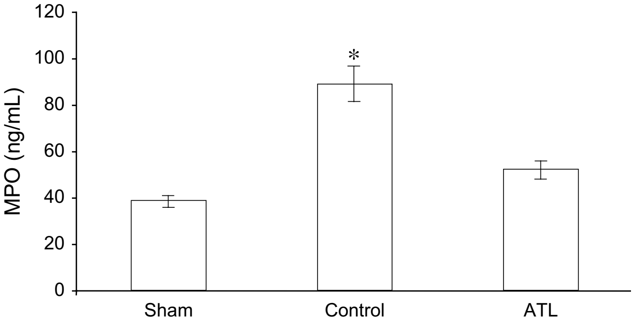

FIGURE 2. BAL MPO. SHAM (n = 5), CONTROL (n = 5), ATL (n = 5). *$P < .05$ versus sham and ATL. *MPO,* Myeloperoxidase.

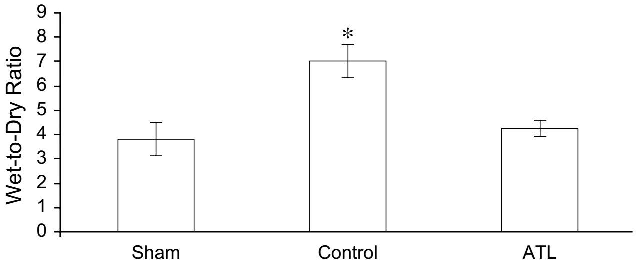

FIGURE 3. Wet/dry analysis. SHAM (n = 5), CONTROL (n = 5), ATL (n = 5). *$P < .001$ versus SHAM and ATL.

routine CPB and in animal models of CPB.[2,4,13-16] This inflammatory response can in turn lead to dysfunction in several organ systems, including the cardiovascular, pulmonary, renal, hepatic, gastrointestinal, hematopoietic, and central nervous systems.[2] This dysfunction can range in severity from mild organ dysfunction to severe life-threatening multiorgan failure. Several mechanisms have been shown to contribute to the inflammatory response during CPB, including direct surgical trauma, ischemia-reperfusion injury, exposure of blood to the foreign surfaces of the CPB circuit, direct release of endotoxin, and changes in body temperature.[2,4] We hypothesize that 2 important mechanisms play a role in the inflammatory response within the lung after CPB in our model: exposure of blood to the foreign CPB circuit and pulmonary ischemia-reperfusion injury.

An important source of inflammatory cell activation in our model is from the interaction of the blood with foreign surfaces of the CPB circuit. Despite improvements in the composition of the internal lining of the circuit, the addition of heparin-coated circuits, leukocyte depletion filters, and ultrafiltration techniques, ample evidence exists describing the persistent activation of complement, coagulation, fibrinolytic, and inflammatory responses during CPB.[2,4] Strong evidence suggests that the end result of these processes is the sequestration and activation of macrophages, eosinophils, neutrophils, mast cells, platelets, endothelial cells, and T lymphocytes.

Another important source of inflammatory activation during CPB may be the result of lung ischemia-reperfusion injury. Because perfusion of the lungs during CPB is limited predominantly to flow received from the bronchial arteries, ischemic injury to the lungs during CPB is a likely consequence, despite seemingly adequate systemic perfusion by the CPB pump. Several studies have demonstrated this in various models of CPB. By using a pig model of CPB, Schlensak and collegues[17] demonstrated a significant decline in bronchial artery blood flow with the onset of CPB, which led to lung inflammatory activation and subsequent injury. They were able to eloquently show that lung injury and inflammation were markedly reduced with controlled pulmonary artery perfusion during CPB. Similar results have been described by others, in both human and animal

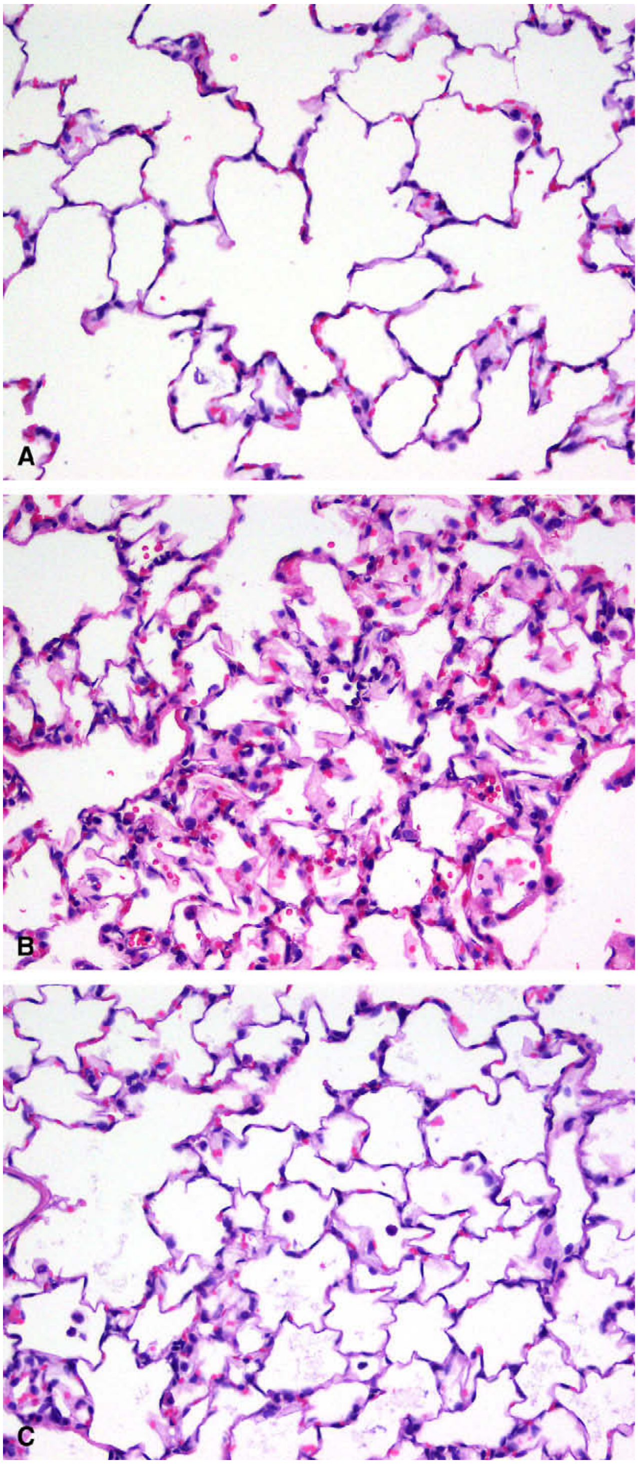

FIGURE 4. Representative hematoxylin-eosin sections of lung tissue. A, SHAM group: Normal-appearing pneumocytes apposed to capillaries are observed, without evidence of inflammatory infiltrate, alveolar macrophages, or edema. B, CONTROL group: Alveolar septa are substantially widened, and there are multiple alveolar macrophages and significant edema. C, ATL group: Alveolar septa are minimally widened by interstitial infiltrate, very few alveolar macrophages are observed, and there is sparse edema.

TABLE 4. Lung injury severity scoring

Group	Interstitial infiltrate	Macrophages	Alveolar edema	Total score
SHAM	0.2 ± 0.17	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.17
CONTROL	0.6 ± 0.21[a]	1.6 ± 0.43[a]	2.1 ± 0.21[a]	4.3 ± 0.41[b]
ATL	0.3 ± 0.21	0.4 ± 0.21	0.1 ± 0.14	0.8 ± 0.17

Values represent mean scores ± standard error. SHAM (n = 5), CONTROL (n = 5), ATL (n = 5). [a]$P < .05$ versus SHAM and ATL. [b]$P < .001$ versus SHAM and ATL.

models of CPB.[18,19] Although we did not directly measure the influence of bronchial artery blood flow in the present study, our results indicate a robust pulmonary inflammatory response after the bypass period with significant elevations in IL-1, IL-6, TNF-α, IFN-γ, MPO, pulmonary edema, and lung parenchymal injury. There was a relative lack of inflammatory activation, as measured by a lack of induction of proinflammatory cytokines, in the plasma, which is in contrast with the results of other investigators. One possible explanation is that our post-bypass recovery time (90 minutes) was not long enough to demonstrate the propagation of systemic inflammation within the plasma.

Adenosine is a primitive signaling molecule that serves to modulate several physiologic responses in the majority of mammalian tissues.[20] More specifically, it has significant anti-inflammatory properties and has been shown to exert a protective role against the development of ischemia-induced cell injury.[6-9,20-22] Specific activation of the $A_{2A}R$ subtype has been shown to be protective against the development of lung ischemia-reperfusion injury.[6,9,23] Previous institutional research has indicated that the activation of the $A_{2A}R$ is mediated specifically by ATL313.[6] Although the global understanding of $A_{2A}R$ agonists and their influences on lung ischemia-reperfusion injury are largely known, the exact mechanisms by which $A_{2A}R$ activation attenuates the inflammatory response induced by CPB have not been well described. Presumably, the action of ATL313 in limiting the inflammatory response after CPB is similar to the mechanism by which $A_{2A}R$ agonism attenuates ischemia-reperfusion injury in other organ systems. There is extensive, well-supported evidence for the role of inflammatory cells in the propagation of the CPB-induced inflammation. $A_{2A}Rs$ have been shown to be present on nearly all inflammatory cells, including macrophages, eosinophils, neutrophils, mast cells, platelets, endothelial cells, and T lymphocytes, and subsequent activation of the $A_{2A}R$ has been shown to be almost uniformly inhibitory in these cell lines.[20,24] Furthermore, $A_{2A}R$ agonists have been shown to decrease the expression of several adhesion molecules, including intercellular adhesion molecule-1, P-selectin, and vascular cell adhesion molecule-1 in myocardial and renal ischemia-reperfusion models.[19,21] Many of these same adhesion molecules have been shown to have important effects in the propagation of CPB-induced inflammation.[25] Therefore, the observed anti-inflammatory activity of ATL313 in our CPB model could involve 2 important mechanisms. First, ATL313 could attenuate the inflammatory response generated by the foreign surfaces of the CPB circuit itself, and second, it could reduce the inflammatory response associated with the relative pulmonary ischemia that occurs during CPB.

CONCLUSIONS

This report demonstrates that the addition of a potent $A_{2A}R$ agonist to the normal priming solution before the initiation of CPB significantly protects the lung from the inflammatory effects of CPB and reduces the amount of lung injury. The ability to adequately protect the lung and other organs from ischemia-reperfusion injury and attenuate the activation of an inflammatory response by the CPB circuit itself suggest that $A_{2A}R$ agonists may represent a new therapeutic strategy for reducing the potentially devastating consequences of the inflammatory response associated with CPB.

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Discussion

Dr Frank Sellke *(Boston, Mass)*. Is there a physiologic correlate to this or is this purely a pharmacologic manifestation?

Dr Lisle. At this point it is purely a pharmacologic manifestation, in that we don't see many physiologic changes when we give the drug. If we give it in a very high dose, it causes substantial vasodilation and hypotension. The dose that we give has been previously tested in our laboratory and found to be the most effective dose without the aforementioned side effects. We were unable to measure pulmonary artery pressure in the rats, which is something that we are currently working on.

Dr Sellke. You found a tremendous benefit to this drug. Is there some cellular mechanism that would explain all these different findings simultaneously?

Dr Lisle. ATL313 is a specific $A_{2A}R$ agonist that belongs to a family of adenosine receptors (A1, A2A, A2B, and A3) that are ubiquitous throughout most tissues and most specific to inflammatory cells, endothelial cells, macrophages, T cells, and neutrophils, and it is shown to be uniformly inhibitory in those cell lines. So it is something that we have been able to show before, but never in this model.

Dr Paul Kurlansky *(Miami, Fla)*. I just wanted to pursue that exact line of thought, because what you have measured is obviously downstream from the impact that this receptor is having. I was wondering if you could give us a little bit of guidance as to exactly where in the inflammatory cascade this receptor is acting. Is this before nuclear factor kappa beta? You have complement release virtually immediately, and yet the downstream effect seems to be ameliorated in several different areas. So I was wondering if you could just mechanistically give us sort of a tour of where you think this is working?

Dr Lisle. I think it is probably working in concert with a few different cell lines at a few different points in the inflammatory pathway. Our laboratory has done some work that you will hear about in a little while, so I don't want to necessarily spoil the punch line, but I think it certainly works on a whole host of those cell lines. I think that there is a substantial inflammatory activation within the bypass pump itself. I also think there is a marked reperfusion injury within the lung after bypass, and my guess is that the drug is helping attenuate both of those inflammatory responses.

Dr Christopher Caldarone *(Toronto, Ontario, Canada)*. The slides went by kind of quick, but I think you said that there weren't any changes in terms of oxygenation and physiologic parameters of the lung, which weakens your argument a little bit that these changes are clinically relevant. Nevertheless, your data are impressive. What plans do you have in terms of what you are going to do with your model to perhaps change it so you could demonstrate clinically relevant changes in the model?

It really is an elegant model, and for those of us who might contemplate setting up a rat bypass model, what was your learning curve like and how technically difficult is it to get one of these studies to go from start to finish?

Dr Lisle. This study is a compilation of about 2 years' worth of work for me in Dr Kron's laboratory. The learning curve was substantially steep, but once you master it, things seem to become a lot easier; at least I'd like to think so.

Dr Caldarone. I think that is the case with all science.

Dr Lisle. To answer your second question, the majority of the animals we bypass go on to survive. Postoperatively, they are extraordinarily hard to keep alive, and they require intense care once they are extubated. We don't see a lot of changes in oxygenation initially, but my guess is that if we took them out further, more than 90 minutes, we would see more significant changes in terms of oxygenation and so forth. The direction we are heading right now is to look at the effects of ventilation during bypass and then see if we can figure out how this inflammatory response fits into the picture of ischemia-reperfusion injury with other organ systems. The hypothesis that we are going on now is that the lung is the centerpiece of this whole inflammatory cascade within the body, resulting in a systemic inflammatory response syndrome because it is relatively ischemic with the cessation of pulmonary artery blood flow relative to the rest of the organs in the body.

Dr William Smythe *(Temple, Tex)*. We have a surgeon in my department that has been working for the last several years on generalized endothelial or microvascular dysfunction in shock states. As you know, that is a whole body ischemia-reperfusion model whereby animals either have septic or hemorrhagic shock, and there is a generalized microvascular permeability issue. This process can involve extrathoracic sites, such as the brain, kidneys, and other tissues or organs, many of which have deleterious effects on patients in the intensive care unit and may lead to patient morbidity and mortality. Do you have any evidence that ATL313 works on microvascular permeability states in other areas of the body besides the lung specifically?

Dr Lisle. There certainly has been a host of literature published on the subject with ATL313 and its effects on adhesion molecules like P-selectin, for example. We haven't been able to evaluate any of that specifically, but it is certainly somewhere we could go in the future.

Dr Yoshiya Toyoda *(Pittsburgh, Pa)*. In your program do you use adenosine during lung transplantation or CPB, and if so, how? Do you put it in the pump prime or give it directly to the lung?

Dr Lisle. We haven't started using adenosine or ATL clinically at the University of Virginia for lung transplantation; however, it is being studied presently in a phase III trial for cardiac imaging. I expect, coming down the pipeline within the next 12 months or so, we will start to see a compound similar to ATL313 in phase II trials for acute inflammation, but nothing at this point.

Dr Toyoda. At the University of Pittsburgh Medical Center, we have been using adenosine clinically since 1997 or so. We administer it directly into the lung through the pulmonary artery with good outcome against ischemia-reperfusion injury.