Activation of A₁, A_{2A}, or A₃ adenosine receptors attenuates lung ischemia-reperfusion injury

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Objective: Adenosine and the activation of specific adenosine receptors are implicated in the attenuation of inflammation and organ ischemia-reperfusion injury. We hypothesized that activation of A₁, A_{2A}, or A₃ adenosine receptors would provide protection against lung ischemia-reperfusion injury.

Methods: With the use of an isolated, ventilated, blood-perfused rabbit lung model, lungs underwent 18 hours of cold ischemia followed by 2 hours of reperfusion. Lungs were administered vehicle, adenosine, or selective A₁, A_{2A}, or A₃ receptor agonists (CCPA, ATL-313, or IB-MECA, respectively) alone or with their respective antagonists (DPCPX, ZM241385, or MRS1191) during reperfusion.

Results: Compared with the vehicle-treated control group, treatment with A₁, A_{2A}, or A₃ agonists significantly improved function (increased lung compliance and oxygenation and decreased pulmonary artery pressure), decreased neutrophil infiltration by myeloperoxidase activity, decreased edema, and reduced tumor necrosis factor- α production. Adenosine treatment was also protective, but not to the level of the agonists. When each agonist was paired with its respective antagonist, all protective effects were blocked. The A_{2A} agonist reduced pulmonary artery pressure and myeloperoxidase activity and increased oxygenation to a greater degree than the A₁ or A₃ agonists.

Conclusion: Selective activation of A₁, A_{2A}, or A₃ adenosine receptors provides significant protection against lung ischemia-reperfusion injury. The decreased elaboration of the potent proinflammatory cytokine tumor necrosis factor- α and decreased neutrophil sequestration likely contribute to the overall improvement in pulmonary function. These results provide evidence for the therapeutic potential of specific adenosine receptor agonists in lung transplant recipients. (*J Thorac Cardiovasc Surg* 2010;140:440-6)

Primary graft dysfunction (PGD), the most severe form of ischemia-reperfusion (IR) injury, continues to be a major cause of morbidity and mortality after lung transplantation. The most recent report from the International Society for Heart and Lung Transplantation reported that PGD was the leading cause of death (28.8% of patient deaths) within the first 30 days after transplant.¹ Although the overall incidence of lung IR injury has remained fairly constant ($\sim$ <25%), the 30-day mortality after transplant has improved over the past 2 decades primarily because of reductions in PGD.² The clinical significance of lung IR injury is further magnified by its association with the development of bronchiolitis obliterans.³ Improving outcomes after lung transplantation and extending the

donor pool and recipient criteria are predicated on the ability to minimize the deleterious inflammatory responses that occur with lung IR injury.

Adenosine is an endogenous mediator that typically serves as a cytoprotective modulator in response to stress. Many studies have demonstrated the protective effects of adenosine in the setting of organ IR injury. Adenosine signals through 4 subtypes of the G protein-coupled receptor, A₁, A_{2A}, A_{2B}, and A₃, all of which are expressed in the lung. Protective effects of adenosine receptor signaling classically occur through second messenger pathways, such as cAMP production or the phospholipase C pathway. Our laboratory has extensively studied selective A_{2A} receptor activation and shown it to provide significant protection against lung IR injury.⁴⁻⁶ However, the effects of specific activation of A₁, A_{2B}, and A₃ adenosine receptors in lung IR injury remain poorly understood. Previous studies have provided evidence that A₁ and A₃ receptors may primarily be involved in anti-inflammatory actions,^{7,8} whereas the A_{2B} receptor may have more proinflammatory actions⁹ in the lung. The objective of this study is to further define the protective effects of adenosine receptor subtypes, using specific agonists and antagonists, on lung IR injury. We hypothesize that specific activation of A₁, A_{2A}, or A₃ receptors provides significant protection from lung injury and dysfunction after IR. The use of specific adenosine receptor agonists and antagonists in an isolated,

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Abbreviations and Acronyms

BAL	= bronchoalveolar lavage
IR	= ischemia-reperfusion
MPO	= myeloperoxidase
PA	= pulmonary artery
PGD	= primary graft dysfunction
TNF- α	= tumor necrosis factor-alpha

blood-perfused rabbit model of lung IR will be used to test this hypothesis.

MATERIALS AND METHODS

Animal Care

New Zealand white rabbits (Burlson Enterprises, Inc, Unionville, Va) of both genders (3.0-3.5 kg) were used in this study and received humane care in accordance with the "Guide for Care and Use of Laboratory Animals," published by the National Institutes of Health (publication no. 85-23, revised 1995). The study protocol was reviewed and approved by the animal care and use committee at the University of Virginia.

Experimental Protocol

Eight experimental groups of lungs ($n = 6/\text{group}$) were compared using an isolated, whole-blood perfused, ventilated rabbit lung model of IR (Kent Scientific, Model TIS3862, Litchfield, CT) as previously described.⁴ All lungs were reperused for 120 minutes after 18 hours of cold ischemic storage, time periods that we previously demonstrated to result in reproducible injury versus sham lungs.⁴ Lungs received vehicle (dimethylsulfoxide), adenosine, or a specific adenosine receptor agonist (with or without its respective antagonist), as summarized in Table 1. Each agonist used in this study is potent and specific for its target: IB-MECA is reported to be approximately 50-fold selective for A_3 versus A_1 or A_{2A} ;¹⁰ ATL-313 is selective for A_{2A} versus A_1 (81-fold) and A_3 (350-fold);¹¹ and CCPA is reported to be approximately 53-fold selective for A_1 versus A_{2A} or A_3 .¹² Each receptor agonist or antagonist was added to the whole-blood perfusate at the beginning of reperfusion (see Table 1 for specific doses). The doses used were based on well-established doses used in previous studies and do not result in significant cardiovascular effects.^{4,13,14} Adenosine was administered by constant infusion (0.75 mg/kg/min) during reperfusion because of its short half-life of several seconds in whole blood.

Harvest Procedure

Animals were randomly assigned to each experimental group. Each animal was anesthetized with intramuscular ketamine (50 mg/kg) and xylazine (5 mg/kg). Tracheal intubation was performed via a tracheostomy, and mechanical ventilation (Kent Scientific, Model RSP1002) was instituted with room air at a respiratory rate of 30 breaths/min. Median sternotomy and thymectomy were performed. The aorta and pulmonary artery (PA) were encircled, and 1000 U/kg heparin was administered intravenously. Prostaglandin E1 (30 mg) was then administered via the PA, and the vena cavae were ligated to initiate ischemia 5 minutes after heparin administration. The PA was then cannulated through a ventriculotomy made within a purse-string placed in the right ventricular outflow tract. After the left ventricle was vented through a left ventriculotomy and the aorta was ligated, 100 mL/kg of buffered Perfadex (Vitrolife, Kungsbacka, Sweden) preservation solution was infused into the PA at a pressure of 30 cm H₂O at 4°C. Topical cooling was achieved with cold saline solution slush. The left atrium was cannulated through the left ventriculotomy with an outflow catheter. The lung-heart block was excised. The lungs were stored inflated at 4°C for 18 hours.

Reperfusion Procedure

A force transducer suspended the heart-lung block, and ventilation was initiated with a 95% oxygen and 5% carbon dioxide gas mixture (Kent Scientific, Model RSP1002). All groups underwent 120 minutes of whole-blood perfusion at 37°C. Atelectasis was grossly eliminated by administering 1 breath of approximately 30 cm H₂O positive end-expiratory pressure once per minute during the first 5 minutes of the stabilization period. Lungs were ventilated at a constant tidal volume of 10 mL/kg with 3 cm H₂O of positive end-expiratory pressure at a rate of 30 breaths/min. The PA and outflow catheters connected the lung-heart block to a venous blood perfusion circuit. New Zealand white rabbits served as fresh venous blood donors. Blood was circulated through a pediatric oxygenator set to deoxygenate the blood and add carbon dioxide to simulate venous blood ($P_{O_2} = 60$ mm Hg/ $P_{CO_2} = 60$ mm Hg). The lungs were subsequently perfused via the PA cannula at 60 mL/min with "venous" blood at 37°C.

Lung Physiology

A dynamic data acquisition program (DASYLab; DASYTEC, USA, Bedford, NH) recorded PA pressure and pulmonary compliance. Pulmonary venous blood samples were collected for blood gas analysis (Bayer 348 pH/Blood Gas Analyzer; Bayer Corp, E Walpole, Mass) at 15, 30, 60, 90, and 120 minutes after initiation of reperfusion.

Lung Wet/Dry Weight

The lung wet/dry weight ratio was used to assess pulmonary edema. After the 120-minute reperfusion period, fresh samples of lung tissue were collected from the right lower lobe of the left lung. Fresh lung samples were blotted to remove excess blood; they were then weighed and desiccated under vacuum at 55°C until a stable dry weight was achieved.

Myeloperoxidase Activity

Neutrophil sequestration was assessed by measuring myeloperoxidase (MPO) activity in left lung tissue, as previously described by our laboratory.⁵

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed on all lungs at the end of the reperfusion period. The right upper and middle lobes were isolated and lavaged with 10 mL normal saline. The BAL fluid was then centrifuged at 1500g for 5 minutes at 4°C. The supernatant was stored at -80°C until analysis.

Measurement of Tumor Necrosis Factor- α

Protein levels of tumor necrosis factor (TNF)- α in BAL fluid were measured using a TNF- α enzyme-linked immunosorbent assay kit (BD Biosciences, San Diego, Calif) as instructed by the manufacturer. Samples were assessed in triplicate.

Statistics

Values are expressed as the mean $\pm$ standard error of the mean. Analysis of variance was used to determine whether significant differences existed among the groups. Tukey's honestly significant difference multiple-comparison test was used to determine which groups were significantly different when the analysis of variance results were significant. Repeated-measures analysis of variance was performed and ultimately allowed us to conclude that PA pressure, lung compliance, and oxygenation changed over time and depended on the group.

RESULTS

Lung Physiology

Selective activation of A_1 and A_{2A} receptors during reperfusion resulted in significant decreases in PA pressure versus control at the end of the 120-minute reperfusion period

TABLE 1. Adenosine receptor agonists and antagonists used

Adenosine receptor	Agonist (dose, source)	Antagonist (dose, source)
A ₁	CCPA (10 nM; Sigma, St Louis, Mo)	DPCPX (100 nM; Sigma)
A _{2A}	ATL-313 (100 nM; Dr J. Linden)	ZM241385 (100 nM; Tocris Bioscience, Ellisville, Mo)
A ₃	IB-MECA (60 nM; Sigma)	MRS1191 (10 nM; Sigma)
All	Adenosine (0.75 mg/kg/min; Sigma)	NA

CCPA, 2-chloro-N(6)-cyclopentyladenosine; ATL-313, 4-[3-[6-amino-9-(5-cyclopropylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl]-piperidine-1-carboxylic acid methyl ester; IB-MECA, 2-chloro-N⁶-(3-iodobenzyl)adenosine-5'-N-methyluronamide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; ZM 241385, 4-(2-[7-Amino-2-[2-furyl][1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]-ethyl); MRS 1191, 3-ethyl-5-benzyl-2-methyl-6-phenyl-4-phenylethynyl-1, 4-(±)-dihydropyridine-3,5-dicarboxylate.

(Figure 1, A). In addition, significant decreases in PA pressure were observed in the A_{2A} agonist-treated group relative to treatment with A₁ or A₃ agonists (Figure 1, A). The A₃ receptor agonist reduced PA pressure somewhat, but not significantly. The A₁, A_{2A}, and A₃ receptor agonists resulted in significant increases in lung compliance (Figure 1, B) and oxygenation (Figure 1, C) compared with control at 120 minutes of reperfusion. Oxygenation was significantly higher in the A_{2A} agonist-treated group versus treatment with A₁ or A₃ agonists (Figure 1, C). The improvements in the aforementioned physiologic measurements by each agonist were all blocked by the concomitant administration of antagonist (Table 2). Although adenosine administration resulted in significant improvement of PA pressure, lung compliance, and oxygenation, these improvements were generally not as great as for the receptor agonists.

Production of Tumor Necrosis Factor- α

Treatment with A₁, A_{2A}, and A₃ receptor agonists, and adenosine resulted in significantly less production of the proinflammatory cytokine TNF- α versus control (Figure 2, A). The administration of each receptor agonist with its respective antagonist blocked the decrease in TNF- α (Table 3). No significant difference in TNF- α production occurred among the agonist-treated groups. Each receptor agonist resulted in significantly lower TNF- α production compared with adenosine alone.

Myeloperoxidase Activity

MPO activity was used as an indicator of neutrophil sequestration into the lung. Treatment with A₁, A_{2A}, and A₃ receptor agonists, and adenosine resulted in significant decreases in MPO activity versus control (Figure 2, B). The administration of each receptor agonist with its respective antagonist blocked the decrease in MPO activity (Table 3). Selective A_{2A} receptor activation resulted in the

FIGURE 1. Lung function. Comparison of PA pressure (A), pulmonary compliance (B), and oxygenation (C) among groups at the end of the 120-minute reperfusion period. * $P < .0002$ versus control. # $P < .02$ versus adenosine. § $P < .02$. PA, Pulmonary artery.

greatest decrease in MPO activity (44% reduction vs control). Each receptor agonist resulted in significantly less MPO activity compared with adenosine alone.

Wet/Dry Weight Ratio

Lung wet/dry weight ratio was measured to assess pulmonary edema. Treatment with A₁, A_{2A}, and A₃ receptor

TABLE 2. Pulmonary function at the end of the 120-minute reperfusion period

Group	PA pressure (mm Hg)	Compliance (cm H ₂ O)	Oxygenation (mm Hg)
Control	32.83 ± 0.48	4.93 ± 0.03	527.7 ± 1.1
A ₁ agonist	24.17 ± 1.19*,§	5.70 ± 0.03*,†	551.7 ± 2.2*,†
A ₁ agonist + antagonist	31.67 ± 0.56†	5.07 ± 0.03†	527.0 ± 2.1
A _{2A} agonist	18.50 ± 0.62*,‡,§	5.87 ± 0.08*,†	569.2 ± 1.1*,‡,§
A _{2A} agonist + antagonist	31.33 ± 0.84†	5.12 ± 0.48†	525.3 ± 2.9†
A ₃ agonist	29.17 ± 1.66†,‡	5.85 ± 0.04*,†	556.2 ± 4.0*,†
A ₃ agonist + antagonist	32.00 ± 1.59†	5.07 ± 0.06†	530.0 ± 3.5
Adenosine	21.67 ± 0.49*	5.33 ± 0.04*	538.3 ± 2.5*

PA, Pulmonary artery. **P* < .0002 vs control. †*P* < .02 vs adenosine. ‡*P* < .01 vs A₁ agonist. §*P* < .02 vs A₃ agonist.

agonists, and adenosine resulted in significantly less wet/dry weight versus control (Figure 2, C). The administration of each receptor agonist with its respective antagonist blocked the decrease in wet/dry weight (Table 3). No significant difference in wet/dry weight occurred between the agonist- and adenosine-treated groups.

DISCUSSION

Pharmacologic modulation of specific adenosine receptors may be a useful therapeutic method for attenuating lung IR injury, thus improving patient outcomes after lung transplantation. Although the anti-inflammatory effects of adenosine are well described, the ability to translate adenosine-related therapeutics into clinical practice requires better understanding of the relationship between adenosine receptor subtypes and end-organ effect. The protective as well as deleterious profile of adenosine receptors with respect to lung IR injury is poorly understood. A modest number of studies have begun to evaluate the role of single adenosine receptors in lung IR injury.^{4,7,15} However, it is difficult to compare and contrast the role of different adenosine receptor subtypes between these various studies and models. The present study better characterizes and compares the effects of specific activation of A₁, A_{2A}, and A₃ receptors in an accepted, reproducible model of lung IR injury.

Of the adenosine receptor subtypes, A_{2A} receptor activation has most consistently demonstrated potent anti-inflammatory properties and has repeatedly attenuated lung IR injury in various studies. For example, A_{2A} agonists have been associated with inhibition of inflammatory cytokine release, reduction of IR-induced apoptotic injury, and diminution of free radical production.^{4,16} In addition, neutrophil-induced IR injury is directly abrogated by selective A_{2A} receptor activation.¹⁷ The attenuation of lung dysfunction and injury after IR by A_{2A} agonists observed in prior studies was confirmed in the present study. Compared with A₁ and A₃ receptor agonists, the A_{2A} agonist provided greater protection (eg, lower PA pressure, improved oxygenation, and reduced MPO activity), possibly because A_{2A} receptors are predominantly expressed on inflammatory cells, including neutrophils, mast cells, macrophages, mono-

cytes, and T cells. It has been shown that A_{2A} receptor activation prevents leukocyte adhesion to endothelial cells and inhibits the release of toxic oxygen products.¹⁸

Studies on the role of A₁ receptor activation in lung IR injury have been somewhat confusing. Although the A₁ receptor has been shown to play a protective role after IR in the heart,¹⁹ intestine,²⁰ liver,⁸ and kidney,²¹ an early study by Neely and Keith¹⁵ concluded that A₁ receptor antagonists block lung IR injury. Paradoxically, A₁ receptor activation with CCPA was shown to exert a cytoprotective role against IR injury in skeletal muscle, an effect that was blocked by the A₁ receptor antagonist DPCPX.²² In the current study, A₁ receptor activation by CCPA improved lung function in association with decreased neutrophil infiltration (MPO activity), inflammatory cytokine production (TNF-α), and edema (wet/dry weight). Notably, these protective effects were blocked by DPCPX. One possible explanation for the differing conclusions between our study and Neely and Keith's study could involve differences in the model. Neely and Keith used an in vivo model in which the left lower lobe in cats with intact chests was rendered ischemic for 2 hours and reperfused for 2 hours. A more likely explanation could involve a difference in DPCPX administration: We used DPCPX at a concentration of 100 nM in the whole-blood perfusate, whereas Neely and Keith used a bolus dose of 6 mg/kg DPCPX to the cat either 30 minutes before ischemia or 1 hour after reperfusion. We estimate that this dose resulted in a blood concentration of DPCPX at least 700-fold greater than our dose of 100 nM, a dose that could lead to unwanted side effects, such as the nonspecific antagonism of other (anti-inflammatory) adenosine receptors. Although 100 nM DPCPX was efficient in blocking the protective effects of the A₁ agonist CCPA in our study, we did not test the effect of DPCPX alone.

Proinflammatory properties of the A₃ receptor have been demonstrated by mast-cell degranulation resulting from administration of IB-MECA.²³ On the other hand, A₃ receptor activation reduces degranulation and free radical formation from eosinophils.²⁴ More anti-inflammatory effects, however, have been associated with A₃ receptor activation. For example, activation of the A₃ receptor was shown to suppress

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FIGURE 2. Lung injury. Comparison of TNF- α levels in BAL fluid (A), MPO activity (B), and lung wet/dry weight ratio (C) among groups at the end of the 120-minute reperfusion period. * $P < .0002$ versus control. # $P < .01$ versus adenosine. § $P < .01$. TNF- α , Tumor necrosis factor; MPO, myeloperoxidase.

superoxide production and chemotaxis in mouse neutrophils.²⁵ Moreover, various studies have demonstrated that A₃ agonists reduce IR-associated myocardial injury.^{26,27} In a study of feline lung IR injury, Rivo and colleagues⁷ showed that A₃ receptor activation with IB-MECA reduced alveolar injury, wet/dry weight, MPO activity, and apoptosis. In support of these data, the current study found that IB-MECA pro-

vided significant protection against lung dysfunction and injury after IR. However, A₃ receptor activation differed from A₁ and A_{2A} receptor activation in that it did not significantly decrease PA pressure versus control.

The administration of adenosine during reperfusion reduced PA pressure and increased lung compliance and oxygenation. The A₁, A_{2A}, and A₃ receptor agonists all improved oxygenation and lung compliance to a greater extent than adenosine alone. Although adenosine infusion did decrease TNF- α production and MPO activity versus control, this was not as great a decrease as observed with the A₁, A_{2A}, and A₃ agonists. This may account for the poorer lung function with adenosine treatment compared with the agonists. Adenosine has also been shown to enhance the release of histamine from mast cells and potentiate the bronchoconstrictor response in patients with chronic obstructive pulmonary disorder and asthma.²⁴ Interactions and cross-talk between the adenosine receptors themselves also remain largely unknown and might account for differences in end-organ effect between adenosine administration and selective adenosine receptor activation. Finally, adenosine could also be activating the A_{2B} receptor, a pathway that may be proinflammatory in this setting.

Although small but significant differences in oxygenation were observed as summarized, all of the Po₂ levels were greater than 500 mm Hg. This high degree of oxygenation would likely not make a large clinical difference. However, we reported Po₂ levels in this study to complete the data and illustrate that the observed significant differences in oxygenation correlated well with other differences in physiologic parameters, such as PA pressure and pulmonary compliance.

As stated in the "Materials and Methods" section, the doses of agonist used in our study were based on well-established doses used in previous studies and do not result in significant cardiovascular effects. Thus, it is unlikely that the protective effects of the agonists were primarily physiologic. The reductions in PA pressure observed with the use of the agonists are likely secondary to the anti-inflammatory effects of the agonists. The mechanism responsible for these anti-inflammatory effects is not completely understood. Adenosine receptors couple, via G proteins, to an intricate network of second messenger signaling pathways, such as modulation of cAMP production or phospholipase C. However, the effects of G protein coupling can differ substantially between receptor subtypes. In addition, they are known to couple to mitogen-activated protein kinases, indicating a possible role in cell growth, differentiation, survival, and death. Current studies in our laboratory are evaluating mechanisms of action and what cell types are most responsible for the protective effects of adenosine receptors in the setting of lung IR injury.

The role of the A_{2B} receptor in lung IR injury remains largely unknown because no selective, potent A_{2B} agonists yet exist. Functional A_{2B} receptors are widely distributed

TABLE 3. Pulmonary inflammation at the end of the 120-minute reperfusion period

Group	TNF- α (pg/mL)	MPO Activity (Δ OD/g/min)	Wet/dry weight
Control	1336 $\pm$ 18.9	0.858 $\pm$ 0.012	3.07 $\pm$ 0.08
A ₁ agonist	759 $\pm$ 14.7*, $\dagger$	0.582 $\pm$ 0.013*, $\dagger$	2.22 $\pm$ 0.05*
A ₁ agonist + antagonist	1308 $\pm$ 18.7 $\dagger$	0.810 $\pm$ 0.015 $\dagger$	2.98 $\pm$ 0.09 $\dagger$
A _{2A} agonist	804 $\pm$ 17.6*, $\dagger$	0.482 $\pm$ 0.017*, $\dagger$, $\ddagger$	2.08 $\pm$ 0.05*
A _{2A} agonist + antagonist	1326 $\pm$ 18.7 $\dagger$	0.847 $\pm$ 0.026 $\dagger$	2.90 $\pm$ 0.18 $\dagger$
A ₃ agonist	778 $\pm$ 10.5*, $\dagger$	0.540 $\pm$ 0.016*, $\dagger$	2.03 $\pm$ 0.08*
A ₃ agonist + antagonist	1287 $\pm$ 13.8 $\dagger$	0.843 $\pm$ 0.150 $\dagger$	3.00 $\pm$ 0.06 $\dagger$
Adenosine	978 $\pm$ 26.0*	0.697 $\pm$ 0.028*	2.41 $\pm$ 0.06*

TNF- α , Tumor necrosis factor; MPO, myeloperoxidase. * $P < .0002$ vs control. $\dagger P < .01$ vs adenosine. $\ddagger P < .01$ vs A₁ agonist.

on cells, including fibroblasts, various vascular beds, hematopoietic cells, mast cells, and endothelium,²⁸ and have recently been shown to be highly expressed on mouse alveolar epithelial cells.²⁹ Activation of A_{2B} receptors has been associated with the release of proinflammatory mediators by activated human mast cells and subsequent release of interleukin-8.³⁰ In addition, results from Sun and colleagues⁹ suggest that A_{2B} receptor signaling influences pathways critical for pulmonary inflammation and injury in vivo. With the recent development of A_{2B} receptor knockout mice, the role of the A_{2B} receptor in lung injury will be better understood. In fact, our laboratory has observed that A_{2B} receptor knockout mice have less lung injury after IR compared with wild-type mice (F. Anvari, MD, unpublished data, May 2010), further supporting a proinflammatory role of the A_{2B} receptor in lung injury.

Study Limitations

The current study has several limitations. One limitation of the isolated rabbit lung model is in the length of study. It is difficult to perfuse the lungs beyond 2 hours and maintain stable lungs, and thus we limited the reperfusion time to 2 hours. However, we think the acute period of reperfusion (ie, the first several hours) is a critical time of injury that determines whether a transplant recipient will experience PGD, which is why we focused on this acute time period. It is possible that longer reperfusion times might lead to different results, and we speculate that some of the parameters used to assess lung protection by adenosine receptor agonists, such as oxygenation, would be even more convincing after longer reperfusion.

Another limitation is that we did not use any mechanical maneuvers, such as controlled low-pressure reperfusion, which is often used in the clinical setting. One reason for this is because we wanted to remain consistent with the model used in our previous studies, which produces significant and reproducible injury. It is possible that such mechanical maneuvers would mask the protection offered by adenosine receptor agonists. However, despite such maneuvers used in transplant recipients, there will always be some level of inflammation induced on reperfusion. It is likely that

a combination of mechanical maneuvers and adenosine receptor agonists would prove most effective at preventing both immediate endothelial injury and inflammation, as well as later injury and infiltration of inflammatory cells. One of the goals of our study was to directly compare the protective effects of the different adenosine agonists. Some of these differences were small but significant, and these differences may not have been detected if we had used mechanical maneuvers to blunt injury.

CONCLUSIONS

The results from the present study suggest that selective activation of A₁, A_{2A}, or A₃ adenosine receptors provides protection after lung IR by significantly attenuating both lung dysfunction and injury. As better adenosine receptor agonists are developed and the interactions between adenosine receptors are further elucidated, the pharmacologic modulation of adenosine receptor signaling will likely play an important role in minimizing the vast deleterious effects of IR injury after lung transplantation. Further characterization of protective as well as potentially detrimental effects of adenosine receptor subtypes is required to maximize the possibilities of adenosine-related therapeutics translating to clinical improvements in patient care.

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