Anesthesiology 2004; 101:1153–9

Activation of A_3 Adenosine Receptors Attenuates Lung Injury after In Vivo *Reperfusion*

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Background: **A_3 adenosine receptor (AR) activation worsens or protects against renal and cardiac ischemia–reperfusion (IR) injury, respectively. The aims of the current study were to examine in an *in vivo* model the effect of A_3AR activation on IR lung injury and investigate the mechanism by which it exerts its effect.**

Methods: **The arterial branch of the left lower lung lobe in intact-chest, spontaneously breathing cats was occluded for 2 h and reperfused for 3 h (IR group). Animals were treated with the selective A_3 receptor agonist IB-MECA (300 μg/kg intravenously) given 15 min before ischemia or with IB-MECA as described, with pretreatment 15 min earlier with the selective A_3AR antagonist MRS-1191, the nonsulfonylurea adenosine triphosphate–sensitive potassium channel–blocking agent U-37883A, or the nitric oxide synthase inhibitor N^w-nitro-L-arginine benzyl ester.**

Results: **IB-MECA markedly ($P < 0.01$) reduced the percentage of injured alveoli (IR, 48 ± 4%; IB-MECA, 18 ± 2%), wet:dry weight ratio (IR, 8.2 ± 0.4; IB-MECA, 4 ± 2), and myeloperoxidase activity (IR, 0.52 ± 0.06 U/g; IB-MECA, 0.17 ± 0.04 U/g). This protective effect was completely blocked by pretreatment with the selective A_3AR antagonist MRS-1191 and the adenosine triphosphate–sensitive potassium channel blocking agent U-37883A but not the nitric oxide synthase inhibitor N^w-nitro-L-arginine benzyl ester.**

Conclusions: **In the feline lung, the A_3AR agonist IB-MECA confers a powerful protection against IR lung injury. This effect is mediated by a nitric oxide synthase–independent pathway and involves opening of adenosine triphosphate–sensitive potassium channels. Therefore, selective activation of A_3AR may be an effective means of protecting the reperfused lung.**

IN the clinical scenarios of lung transplantation, pulmonary thromboembolectomy, thrombolysis, and cardiopulmonary bypass, lung injury secondary to ischemia–reperfusion (IR) is of serious concern. Adenosine has been shown to be a critical modulator of IR injury in several organs. In the kidney, preischemic A_1 or postischemic A_{2a} adenosine receptor (AR) activation protected against IR injury,[1,2] whereas A_3AR activation worsened IR-induced renal failure.[3] Similarly, in the heart, A_1AR agonists administered before ischemia[4] and A_2AR agonists administered before reperfusion[5] attenuated IR injury. In the heart, however, activation of A_3ARs[6–10] exerted early and delayed protective affects, with significant improvement in functional recovery.

In the rabbit and rat lungs,[11–13] the nonselective adenosine analog 2-chloroadenosine and the adenosine A_{2a} receptor agonist significantly decreased the severity of reperfusion injury. Using an *in vivo* feline model, Neely and Keith[14] demonstrated that the A_1AR antagonist attenuated IR lung injury. Although the A_3AR subtype is expressed in the lung,[15,16] its role in IR-induced lung injury has not yet been reported. There fore, in the current study, we evaluated the hypothesis that selective activation of A_3AR would attenuate lung injury in an intact-chest, spontaneously breathing animal model of IR lung injury.[14,17] In addition, as an initial step toward exploring the mechanism of action of the selective A_3AR agonist, we tested whether the effects of this agent were blocked by a nitric oxide synthase (NOS) inhibitor and a nonsulfonylurea adenosine triphosphate–sensitive potassium (K_{ATP}) channel blocker.

Materials and Methods

Adult cats weighing 2.5–3.5 kg were used in this investigation. All experiments were performed in accordance with the guidelines of the Animal Care and Use Committee of the Hebrew University–Hadassah School of Medicine, Jerusalem, Israel, and with the approval of the Institutional Animal Care and Use Committee, Jerusalem, Israel.

Animal Model

A standard reperfusion lung model of injury in intact-chest, spontaneously breathing cats was used, as described previously in detail.[14,17] Briefly, in barbital-anesthetized cats (20 mg/kg intravenous), with the aid of fluoroscopy, a specially designed 6-French triple-lumen catheter was advanced from the left external jugular vein into the lobar artery of the left lower lobe (LLL). After heparinization, the LLL was perfused at 35 ml/min with blood withdrawn from the aorta through a catheter in the femoral artery, using a Harvard peristaltic pump (model 1210; Harvard Apparatus, South Natick, MA). The LLL was isolated by distending a balloon with contrast dye on the LLL arterial catheter. After a 1-h period of stabilization, ischemia of the LLL was induced by discontinuing the Harvard peristaltic pump for 2 h (ischemia period), and the perfusion circuit was then attached to a femoral vein catheter. After 2 h of ischemia,

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Received from the Department of Anesthesiology and Critical Care Medicine, Hadassah University Medical Center, The Hebrew University of Jerusalem, Jerusalem, Israel. Submitted for publication April 21, 2004. Accepted for publication July 13, 2004. Supported by the Chief Scientist's Ministry of Health Grant 4994, Jerusalem, Israel; the Joint Research Fund of the Hebrew University and Hadassah Hospital, Jerusalem, Israel; and the Batsheva de Rothschild Fund, Israel Academy of Science, Jerusalem, Israel.

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the perfusion circuit was reattached to the arterial catheter in the LLL, and the LLL was reperfused (reperfusion period) for 3 h at a rate of 35 ml/min, using a Harvard peristaltic pump, as described above, with blood withdrawn from the aorta. In all of the groups, hemodynamic measurements and arterial blood gases were obtained before ischemia, after 1 and 2 h of ischemia, and after 1 and 3 h of reperfusion.

Experimental Protocol

Cats were randomly assigned to six treatment groups (n = 9).

I: Nonischemic Group. In group I, the LLL was perfused for 4 h (no ischemia).

II: IR Group. In group II, animals were subjected to ischemia and reperfusion of the LLL.

III: A_3AR Agonist Group. In group III, to test the hypothesis that A_3AR activation attenuates IR lung injury, 0.3 mg/kg of the selective A_3AR agonist N^6-(3-iodobenzyl)-*N*-methyl-5'-carbamoyladenosine (IB-MECA; Research Biochemical International, Natick, MA) was administered systemically as an intravenous bolus 15 min before occlusion of the lobar artery.

IV: A_3AR Antagonist plus Agonist Group. In group IV, to ascertain that IB-MECA–induced lung protection is mediated by A_3ARs, in further studies, the ability of an A_3AR antagonist to block the effect of IB-MECA on IR lung injury was evaluated. IB-MECA (0.3 mg/kg intravenous) was given systemically 15 min before occlusion of the lobar artery with pretreatment 15 min earlier with the selective A_3AR antagonist 3-ethyl-5-benzyl-2-methyl-4-phenylethynykyk-6-phenyl-1,4-(±)-dihydropyridine-3,5 dicarboxylate (MRS-1191, 1 mg/kg intravenous; Research Biochemical International).

V: NOS Inhibitor Group. In group V, to determine whether the effect of IB-MECA is mediated by nitric oxide, 100 mg/kg of the NOS inhibitor N^w-nitro-L-arginine benzyl ester (L-NABE; Sigma Chemical, St. Louis, MO) was administered systemically as an intravenous bolus 15 min before IB-MECA, and 15 min later, the lobar artery was occluded.

VI: K_{ATP} Channel–blocking Agent Group. In group VI, to determine whether the effect of IB-MECA is mediated through K_{ATP} channels, 5 mg/kg of the nonsulfonylurea K_{ATP} channel–blocking agent U-37883A (Upjohn, Kalamazoo, MI) was administered systemically as an intravenous bolus 15 min before IB-MECA, and 15 min later, the lobar artery was occluded.

The doses of the A_3AR agonist[18,19] and antagonist[1,3] and their pretreatment times[1,3,7] were selected based on previous *in vivo* studies in mice, rats, and rabbits. Doses and pretreatment times of the NOS inhibitor and K_{ATP} channel–blocking agent have previously been described by Cheng *et al.*[20] using the same feline model.

Injury Assessment

After 3 h of reperfusion, animals received an overdose of pentobarbital sodium (30 mg/kg). For light microscopy, samples of lung tissue were embedded in paraffin, cut into 4-μm slices, and stained with hematoxylin and eosin. The slides were coded and examined in a blinded manner by a single examiner. Fifty microscopic fields at 40× magnification were examined in each section, and the total number of alveoli in the 50 microscopic fields was scored. The severity of alveolar injury was assessed according to the percentage of injured alveoli as defined before.[14,17,21] Briefly, an alveolus was defined as injured if it contained exudate, more than two leukocytes (macrophages or neutrophils), or more than two erythrocytes. The severity of alveolar injury was assessed according to the percentage of injured alveoli (number of injured alveoli divided by the total number of alveoli in the 50 microscopic fields). Excised samples of lung tissue were also snap frozen in liquid nitrogen and stored at −70°C for determination of lung myeloperoxidase.[22–24] Briefly, frozen lung tissue were dissolved in 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide, homogenized, and centrifuged. The homogenate was sonicated on ice for 15 s, frozen at −70°C, thawed three times, and then centrifuged at 40,000*g* for 15 min. Spectrophotometry was used to assay myeloperoxidase in the supernatant. Myeloperoxidase activity was measured by mixing 0.1 ml of the supernatant and 2.9 ml phosphate buffer, 50 mM (pH 6.0), containing 0.167 mg/ml dianisidine hydrochloride (Sigma) and 0.0005% H_2O_2. Absorption at 460 nm was read by a spectrophotometer (Beckman Instruments, Palo Alto, CA). Results were expressed in units of myeloperoxidase per gram of lung weight, each of which was defined as the activity degrading 1 μmol peroxide/min at 25°C. The remainder of the left and right lower lobes was used for determination of lung wet:dry weight ratio after sequential weights demonstrated maximal dehydration at 80°C.

Measurement of Plasma Histamine Concentrations

In preliminary studies, we evaluated the effect of IB-MECA on histamine blood concentrations in nine animals: three received 0.1 mg/kg IB-MECA, and three received 0.3 mg/kg IB-MECA 15 min before ischemia. Baseline blood samples were taken immediately before drug administration and 5 and 15 min after treatment. In addition, in three control animals (nonischemic control group), histamine blood concentrations were measured during the stabilization period. Blood samples were added to an EDTA–saline solution and kept on ice. The blood samples were centrifuged (5,000 rpm for 10 min at 4°C), and the plasma was stored at −20°C until it was analyzed by radioimmunoassay (Immunotech, Marseille, France).

Statistical Analysis

Data were analyzed using SigmaStat (Jandel, San Rafael, CA). Data were analyzed with the Student *t* test when comparing means of two groups or with one-way analysis of variance with the Bonferroni correction for multiple comparisons between groups. Differences were considered significant at $P < 0.05$. Results are presented as mean ± SEM.

Results

The surgical interventions (insertion of the catheter into the lobar artery and inflation of the balloon) and perfusion of the LLL with a peristaltic pump had no effect on lung-injury indices. Both microscopic findings (percent injured alveoli, 2.8 ± 1.0%) in the LLL of group I (nonischemic control group), as well as wet:dry weight ratio (4.8 ± 0.5) and myeloperoxidase activity (1.2 ± 0.2 U/g lung tissue), were not significantly different from those observed in the corresponding right lower lobe (3 ± 1%, 4.1 ± 0.8, and 1.5 ± 0.3 U/g lung tissue, respectively), in which no manipulations were performed.

Effect of IR on Lung Injury

The gross appearance of the lung parenchyma was unremarkable in the nonischemic group. Lungs from the acute IR group showed hemorrhagic lesions extending throughout the entire lobe. Examination of lungs subjected to 2 h of ischemia followed by 3 h of reperfusion revealed marked increase in infiltration of the interalveolar walls by granulocytes, mononuclear cells, and erythrocytes, with thickening of the alveolar septa (fig. 1). The percentage of injured alveoli (group II, 48 ± 2%; fig. 2A) was significantly higher compared with the nonischemic group (group I, 2.8 ± 1%; $P < 0.001$). Also, LLL myeloperoxidase activity (fig. 2B) was significantly higher in the IR group than in the nonischemic group. IR also caused marked lung edema as assessed by wet:dry weight ratios of the LLL (fig. 2C).

Protective Effect of A_3AR Agonist

The effects of the A_3AR agonist and antagonist on indicators of lung injury are summarized in figure 2. Activation of A_3AR with IB-MECA (group III) caused significant attenuation of IR-induced lung injury; the average percentage of injured alveoli was 62% lower (group III, 18 ± 2% *vs.* group II, 48 ± 4%), and myeloperoxidase activity (2.0 ± 0.5 U/g lung tissue) and wet:dry weight ratios (4.8 ± 0.3) were nearly halved compared with values in group II (IR; 4.8 ± 0.6 U/g lung tissue and 8.2 ± 0.4, respectively). The highly selective A_3AR antagonist MRS-1191 given before IB-MECA (group IV) completely abolished the protection provided by pretreatment with IB-MECA; in this group, lung injury parameters were indistinguishable from those measured in the IR group.

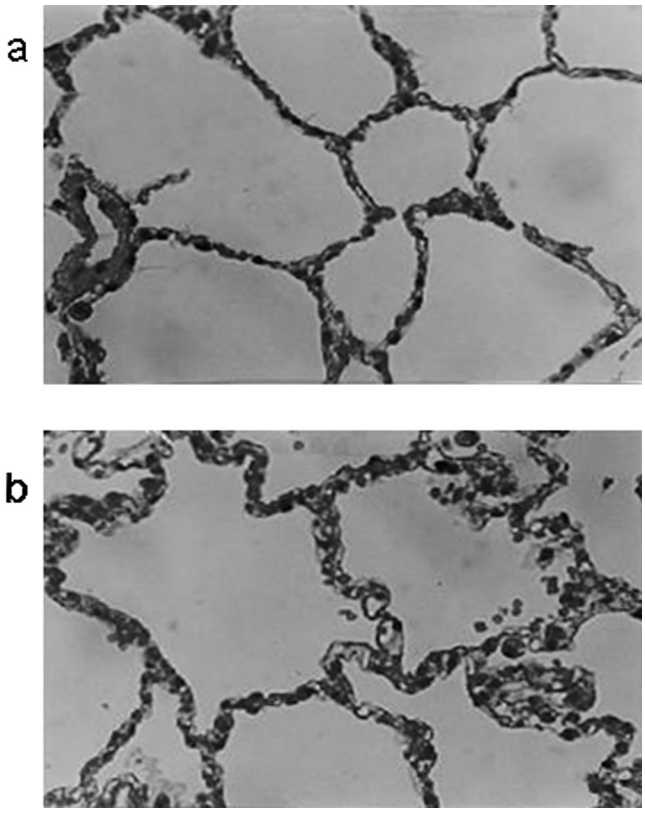

Fig. 1. Representative light micrographs showing structural alteration of alveolar parenchyma from the left lower lobe. (*A*) Nonischemic group (group I); (*B*) ischemia–reperfusion group (group II); magnification ×40. Infiltration with leukocytes and erythrocytes with thickening of alveolar septa is observed in tissue samples from lungs subjected to ischemia and reperfusion.

Effect of L-NABE and U-37883A on IB-MECA–induced Lung Protection

Having found that IB-MECA protected against IR injury in the feline lung, in the next series of experiments, we examined the effect of a NOS inhibitor (L-NABE) and a K_{ATP} channel blocker (U-37883A) on the lung-protective responses caused by IB-MECA. In group V (100 mg/kg L-NABE was administered intravenously before IB-MECA, and 15 min later, IR was induced), percentage of injured alveoli, tissue myeloperoxidase activity, and wet:dry weight ratio in the LLL were significantly lower than in group II and essentially not significantly different from the values in group III (IB-MECA administered before ischemia) (figs. 2A–C), indicating that L-NABE did not block the protective effects of IB-MECA. Pretreatment with U-37883A 15 min before IB-MECA (group VI) blocked the effect of IB-MECA on lung injury parameters, which were not significantly different from those in IR group (group II). The lung-protective effects of IB-MECA cannot be ascribed to the vehicle dimethyl sulfoxide because the same dose of dimethyl sulfoxide had no effect on IR lung damage (data not shown).

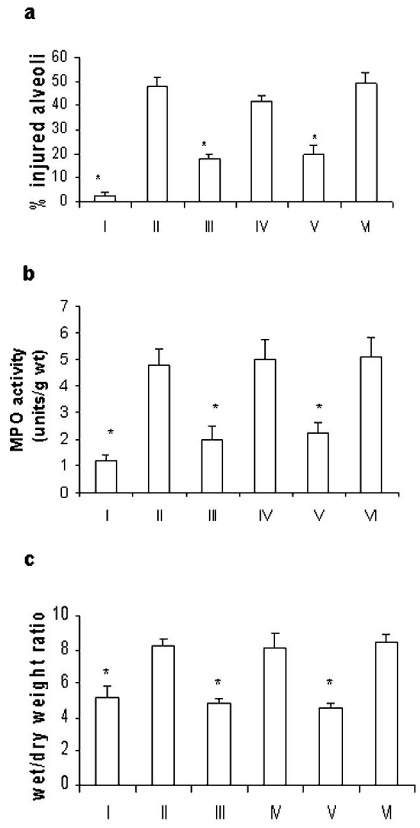

Fig. 2. Percentage of injured alveoli (*A*), tissue myeloperoxidase (MPO) activity (expressed in units of myeloperoxidase per gram of lung weight, each of which was defined as the activity degrading 1 μmol peroxide/min at 25°C) (*B*), and lung tissue wet:dry weight ratio (*C*) in the left lower lobe of the different groups. Values shown are mean ± SEM; n = 9 cats/group. * $P < 0.05$ *versus* groups II, IV, and VI. The groups were as follows: I, nonischemic group; II, ischemia–reperfusion (IR); III, IB-MECA was administered before IR; IV, MRS-1191 pretreatment before IB-MECA and beginning of IR; V, N^{w}-nitro-L-arginine benzyl ester (L-NABE) pretreatment before IB-MECA and beginning of IR; VI, U-37883A pretreatment before IB-MECA and beginning of IR.

Effects on Plasma Histamine and Hemodynamics

In preliminary experiments, plasma histamine concentrations were measure in nine animals: control, 0.1 or 0.3 mg/kg IB-MECA, administered 15 min before ischemia (3 animals/group). Baseline plasma histamine concentrations were similar in all animals (3.8 ± 0.9, 4.7 ± 1.2, and 4.5 ± 1.1 ng/ml, respectively). Plasma histamine concentrations were not altered in the control group or by the administration of 0.1 or 0.3 mg/kg IB-MECA, 5 min (3.7 ± 0.8, 4.9 ± 1.1, and 4.5 ± 0.9 ng/ml, respectively) and 15 min (3.7 ± 0.9, 4.2 ± 0.9, and 4.7 ± 1 ng/ml, respectively) after administration of the drug.

At baseline, heart rate, mean arterial blood pressure, and mean lobar arterial pressures were similar in all groups (table 1). The administration of IB-MECA, MRS-1191, and U-37883A produced no systemic hemodynamic effects. In group V, administration of L- NABE resulted in increases in both mean systemic and mean lobar arterial pressures but did not cause any appreciable

Table 1. Baseline Hemodynamic Variables (Value Recorded 15 min into the Stabilization Period)

Group	Mean Arterial Blood Pressure, mmHg	Heart Rate, beats/min	Mean Lobar Arterial Pressure, mmHg
I	130 ± 10	123 ± 8	4.3 ± 0.5
II	119 ± 8	130 ± 7	3.9 ± 0.7
III	118 ± 8	125 ± 8	4.0 ± 0.4
IV	127 ± 9	136 ± 9	4.4 ± 0.5
V	125 ± 6	131 ± 6	4.3 ± 0.6
VI	127 ± 7	129 ± 7	4.2 ± 0.4

Values shown are mean ± SEM. The groups were as follows: I, nonischemic group; II, ischemia–reperfusion (IR); III, IB-MECA was administered before IR; IV, MRS-1191 pretreatment before IB-MECA and beginning of IR; V, N^{w}-nitro-L-arginine benzyl ester pretreatment before IB-MECA and beginning of IR; VI, U-37883A pretreatment before IB-MECA and beginning of IR.

changes in heart rate (table 2). After reperfusion, the lobar arterial pressure increased significantly in all groups compared with the pressures present in the same lung preischemia, with the maximum lobar arterial pressure reached within approximately 5 min of reperfusion (table 3). The increase in lobar arterial pressure observed during the reperfusion period was significantly smaller in the groups in which IB-MECA was administered before ischemia or before ischemia with NOS inhibitor (groups III and V). This increase in lobar arterial pressure was followed by a gradual decline toward the baseline value with time. At the end of reperfusion, lobar arterial pressure was still significantly increased (compared with baseline lobar arterial pressure) in all groups. The mean lobar arterial pressure, however, was not significantly different among the groups at the end of reperfusion.

Discussion

The role of A_3ARs in IR-induced lung injury remains unclear and is the focus of the current investigation. The current study demonstrates in an *in vivo* IR injury model that pharmacologic activation of A_3 receptors before ischemia confers significant lung protection. Furthermore, administration of the NOS inhibitor L-NABE, in doses previously shown to block NOS activity in this model,[20] did not abolish IB-MECA–induced lung protection. However, administration of the nonsulfonylurea K_{ATP} channel–blocking agent U-37883A, in doses previously shown to block responses to a K_{ATP} channel opener in the same model,[20] caused significant reductions in the lung-protective effect of IB-MECA. Taken together, these results demonstrate that IB-MECA confers powerful protection against IR-induced lung injury by opening K_{ATP} channels and that this lung-protective activity does not require NOS activity.

The A_3AR subtype is the newest characterized member of the adenosine receptor family[14,15] and has been under scrutiny in relation to potential therapeutic approaches for treating inflammatory and neurodegenerative diseases.[25–27] The ability of the A_3AR agonist to affect IR injury

Table 2. Hemodynamic Variables 15 min after Administration of IB-MECA (Group III), MRS-1191 (Group IV), L-NABE (Group V), and U-37883A (Group VI)

Group	Mean Arterial Blood Pressure, mmHg	Heart Rate, beats/min	Mean Lobar Arterial Pressure, mmHg
III before drug administration	125 ± 10	128 ± 8	4.7 ± 0.6
III after drug administration	119 ± 7	132 ± 7	4.7 ± 0.5
IV before drug administration	117 ± 8	137 ± 10	5.1 ± 0.5
IV after drug administration	122 ± 6	142 ± 8	5.3 ± 0.6
V before drug administration	128 ± 8	135 ± 7	4.9 ± 0.6
V after drug administration	159 ± 7*	137 ± 7	14.1 ± 1.0*
VI before drug administration	123 ± 7	133 ± 12	5.2 ± 0.8
VI after drug administration	131 ± 10	129 ± 11	5.3 ± 0.8

Values shown are mean ± SEM. The groups were as follows: III, IB-MECA was administered before IR; IV, MRS-1191 pretreatment before IB-MECA and beginning of IR; V, N^{ω}-nitro-L-arginine benzyl ester (L-NABE) pretreatment before IB-MECA and beginning of IR; VI, U-37883A pretreatment before IB-MECA and beginning of IR.

* $P < 0.05$ *vs.* mean value recorded before drug administration.

in various species, models, and organs has also been previously demonstrated. In the brain, Von Lubitz *et al.*[28] reported that postischemic but not preischemic stimulation of A_3ARs with IB-MECA resulted in cerebroprotection. In the heart, both *in vivo* and *ex vivo* studies demonstrated that the A_3AR subtype elicited protection against infarction,[6–10,18] and that this effect was mediated by K_{ATP} channels.[7,18] In the kidney, however, the highly selective A_3AR agonist IB-MECA worsened renal IR injury, whereas the A_3AR antagonist MRS-1191 protected renal function after ischemia and reperfusion.[1,3] The A_3AR is widely expressed in human tissues, with an abundant expression in the lung,[15,16] but its physiologic function remains unknown. Walker *et al.*[25] postulated a role for A_3 receptors in lung inflammation. These authors demonstrated that A_3ARs were primarily expressed on eosinophils in human lung, where they mediated inhibition of eosinophils chemotaxis, and suggested that A_3AR ligands could be useful agents in the treatment of eosinophil-dependent diseases such as asthma and rhinitis. AR modulation may also significantly affect lung function after IR injury. Previous studies reported that A_2AR agonist and A_1AR antagonist blocked IR-induced lung injury.[11–14] The current results expand these previous observations and elucidate the role of A_3AR. To our knowledge, this is the first study to identify a lung-protective role of A_3 receptors during IR *in vivo* using a selective agonist for these receptors. We predict that pretreatment with IB-MECA provided protection in our animal model by a signaling mechanism similar to that demonstrated in the heart,[7,18] presumably the K_{ATP} channel, because the reduction in lung injury provided by pretreatment with IB-MECA was blocked completely by a K_{ATP} channel–blocking agent.

There are few potential mechanisms for the protecting effects of acute A_3 activation during IR lung injury. The first is through an antiinflammatory mechanism. Reperfusion lung injury has been reported to be mediated by a variety of cellular and humoral factors, including platelets, cytokines, cell adhesion molecules, and neutrophils, which migrate and release proinflammatory mediators and reactive oxygen species.[29] A_3AR has been linked to a variety of antiinflammatory processes, including inhibition of tumor necrosis factor α production from lipopolysaccharide-stimulated murine[30] and human[31] macrophage-like cell lines, inhibition of platelet-activating factor–induced chemotaxis of human eosinophils,[25] inhibition of neutrophil-mediated tissue injury,[32] and inhibition of neutrophil degranulation.[33] A second complementary mechanism by which IB-MECA may have attenuated IR lung injury is by inhibiting apoptosis. In the lung, apoptosis has been shown to be involved in the

Table 3. Mean Lobar Arterial Pressure, mmHg

	Group					
	I	II	III	IV	V	VI
Baseline (before ischemia)	5.1 ± 0.8	5.3 ± 1.3	4.7 ± 0.5	5.3 ± 0.6	14.1 ± 1‡	5.3 ± 0.8
Reperfusion—5 min		26 ± 5*	16 ± 3*†	27 ± 4*	18 ± 2*†	25 ± 3*
Reperfusion—3 h	4.7 ± 1‡§	9 ± 2*	8 ± 2*	10 ± 3*	12 ± 2	10 ± 2*

Values shown are mean ± SEM. The groups were as follows: I, nonischemic group; II, ischemia–reperfusion (IR); III, IB-MECA was administered before IR; IV, MRS-1191 pretreatment before IB-MECA and beginning of IR; V, N^{ω}-nitrol-L-arginine benzyl ester pretreatment before IB-MECA and beginning of IR; VI, U-37883A pretreatment before IB-MECA and beginning of IR.

* $P < 0.05$ *vs.* baseline within a group with use of Student *t* test for paired analysis.

† Different compared with groups II, IV, and VI with use of analysis of variance and Bonferroni correction.

‡ Different compared with the other groups.

§ Value obtained in this group after 4 h of perfusion in nonischemic control group.

process of lung damage after ischemia and reperfusion.[34,35] However, the effect of the A_3 receptor on apoptosis in HL-60 human promyelocytic leukemia cells and rat cardiocytes seems to be dual and opposite. Both agonists and antagonists have been shown to induce apoptosis when administered in high concentration, whereas nanomolar concentrations of selective agonists tend to inhibit apoptosis.[36–38] The effect of A_3AR activation on apoptosis during IR injury of the lung is yet to be explored.

IB-MECA is one of the most potent A_3AR analogs identified, with nearly 50-fold selectivity over A_1 and A_{2A}AR in rats and dogs[10,39] and 13- to 21-fold higher selectivity in the rabbit.[18,40] Nevertheless, it may be argued that IB-MECA may have induced lung protection not only by interacting with A_3ARs, but rather through nonspecific interactions with other ARs. In the current study, intravenous administration of IB-MECA did not produce any effect on heart rate or blood pressure, suggesting that at the dose of 300 μg/kg, this agent does not interact with A_1 or A_{2a} receptors in cats. A recent study, however, showed that A_3AR agonists may bind to A_{2a}ARs.[41] This possibility was addressed using an A_3AR-selective inhibitor, MRS-1191. MRS-1191, which was reported to be useful as an A_3 receptor antagonist across species,[42] blocked the actions of IB-MECA against lung injury, supporting the hypothesis that this agent attenuated IR injury by interacting with the A_3AR. Finally, intravenous administration of IB-MECA did not produce any effect on heart rate or blood pressure, suggesting that at the dose of 300 μg/kg, this agent does not interact with A_1 or A_{2a} receptors in cats. At the doses of 100 and 300 μg/kg, IB-MECA was determined in pilot studies to have no effect on plasma histamine concentration. The lack of hemodynamic effect or increase in plasma histamine concentrations after IB-MECA administration in the current study is in accord with previous studies in rabbits,[7,18] using the same doses of IB-MECA.

In the current study, as well as in previous studies,[14,17,43] exposure of the lung to ischemia and reperfusion caused a prompt increase in pulmonary artery pressure followed by a gradual decline. However, compared with the baseline values, the pressures remained increased during the remainder of lung perfusion. An etiologic role for thromboxane in the generation of pulmonary hypertension after reperfusion was suggested by previous work that reported an increase in the thromboxane A_2 concentrations of lung effluent after IR lung injury,[43,44] which started 5 min after reperfusion and continued for 45 min. The increases in thromboxane concentrations were associated with histopathologic changes, including interstitial edema formation, and were attenuated by a thromboxane receptor antagonist. In our experiments, IB-MECA attenuated the increase in lobar arterial pressure with reperfusion. The effect of the A_3AR agonist on thromboxane release in this model is yet to be defined.

There are several limitations to the current study that prevent immediate extrapolation of the results to the clinical arena. The current study does not provide data on the cell types that are the main target of the A_3AR pathway. Similar to the clinical situation, in the current *in vivo* model, improvement in lung injury with systemic administration of IB-MECA could have been mediated by receptors in lung tissue or blood cells. A_3AR is known to be expressed in resident leukocytes such as macrophages and mast cells and in vascular smooth muscle cells and endothelial cells.[45,46] Also, recent studies demonstrated the inhibitory effect of IB-MECA on neutrophil degranulation and reactive oxygen species production by leukocytes.[47,48] Based on these observations, it remains possible that A_3AR agonists may elicit lung protection through the release of mediators from nonpulmonary cells. In addition, the current study does not evaluate whether the observed protective effects can be obtained when IB-MECA is administered after induction of ischemia, before reperfusion, or during reperfusion. Also, the study does not describe the dose–response characteristics or evaluate the issue of optimal timing of application before ischemia. Finally, caution must be exercised when extrapolating the results to humans because significant species variability exists.

The current data provide comprehensive evidence of *in vivo* lung protection by the A_3AR subtype. The protective actions occurred without hemodynamic effects. If current findings are confirmed in additional models, these results suggest that targeting the A_3AR could be a novel and useful approach to protecting the lungs of patients undergoing lung transplantation, pulmonary thromboembolectomy, thrombolysis, or cardiopulmonary bypass.

The authors thank Nachum Navot (Technician, the Laboratory for Experimental Surgery, Hadassah Hebrew University Medical Center, Jerusalem, Israel) for his outstanding technical assistance.

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