Novel soluble guanylyl cyclase stimulator BAY 41-2272 attenuates ischemia-reperfusion-induced lung injury

Bakytbek Egemnazarov,¹,⁴ Akylbek Sydykov,¹,⁴ Ralph T. Schermuly,¹,⁵ Norbert Weissmann,¹ Johannes-Peter Stasch,² Akpaj S. Sarybaev,⁴ Werner Seeger,¹ Friedrich Grimminger,² and Hossein A. Ghofrani¹

Departments of ¹Internal Medicine II and ²Internal Medicine V, University Hospital Giessen and Marburg, Giessen; ³Pharma Research Center, Bayer HealthCare, Wuppertal, Germany; ⁴National Center of Cardiology and Internal Medicine, Bishkek, Kyrgyz Republic; and ⁵Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany

Submitted 9 July 2008; accepted in final form 8 December 2008

Egemnazarov B, Sydykov A, Schermuly RT, Weissmann N, Stasch JP, Sarybaev AS, Seeger W, Grimminger F, Ghofrani HA. Novel soluble guanylyl cyclase stimulator BAY 41-2272 attenuates ischemia-reperfusion-induced lung injury. Am J Physiol Lung Cell Mol Physiol 296: L462–L469, 2009. First published December 12, 2008; doi:10.1152/ajplung.90377.2008.—The protective effects of nitric oxide (NO), a physiological activator of soluble guanylyl cyclase (sGC), have been reported in ischemia-reperfusion (I/R) syndrome of the lung. Therefore, we studied the effects of BAY 41-2272, a novel sGC stimulator, on I/R injury of the lung in an isolated intact organ model. Lung injury was assessed by measuring weight gain and microvascular permeability (capillary filtration coefficient, K') Release of reactive oxygen species (ROS) into the perfusate was measured during early reperfusion by electron spin resonance (ESR) spectroscopy. Rabbit lungs were treated with BAY 41-2272. N⁵-monomethyl-l-arginine (l-NMMA), or NO to evaluate the effects on I/R-induced lung injury. In untreated lungs, a dramatic rise in K' values and weight gain during reperfusion were observed, and these results were associated with increased ROS production. Both, BAY 41-2272 and l-NMMA significantly attenuated vascular leakage and suppressed ROS release. Additional experiments showed that BAY 41-2272 diminished PMA-induced ROS production by NADPH oxidase. A pharmacological inhibition of the enzyme with consequent reduction in ROS levels decreased I/R injury. NO had only marginal effect on I/R injury. Thus BAY 41-2272 protects against I/R-induced lung injury by interfering with the activation of NADPH oxidases.

Acute lung injury; nitric oxide; oxidative stress; reactive oxygen species; guanosine 3',5'-cyclic monophosphate

ISCHEMIA-REPERFUSION (I/R)-induced injury is defined as cellular damage that occurs after reperfusion of previously viable ischemic tissue. I/R injury may occur during organ transplantation, thrombendarterectomy, myocardial ischemia and revascularization, or stroke (8). The lack of effective therapeutic strategies to treat this disorder remains an issue.

An oxidative stress-induced damage is suggested to serve as a critical mechanism of I/R injury (36). Oxidative stress is characterized by the increased production of reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide, hydroxyl radical, and peroxynitrite. It has been shown that generation of ROS occurs during both ischemia and reperfusion (20, 35). The ability of SOD to attenuate I/R injury suggests that superoxide radical plays the crucial role in pathogenesis of this condition (25). Inhibition of NADPH oxidase, which is the major source of superoxide, has been reported to have a protective effect against vascular leakage in reperfused lungs (7).

The use of nitric oxide (NO) as a therapeutic tool for treating of I/R injury is an issue currently under debate (5). NO is an activator of intracellular soluble guanylyl cyclase (sGC), the activity of which increases the level of the second messenger cGMP. cGMP induces smooth muscle relaxation, reduces microvascular permeability, and inhibits neutrophil-endothelium interactions (18). It has not been elucidated whether NO confers protective or injurious effects. On one hand, exogenous administration of NO demonstrated protective effect in animal models of I/R (3) and in patients after lung transplantation (15), and it has been postulated that NO may protect by inhibition of neutrophil function (11). Other studies, however, have reported that NO inhalation can be detrimental to reperfused lungs (13, 32). Additionally, exogenous NO could bind with ROS to form peroxynitrite, which results in endothelial dysfunction (9).

A novel NO-independent sGC stimulator BAY 41-2272 was recently identified (31). BAY 41-2272 represents a new class of drugs that function by direct stimulation of sGC. This substance has a synergistic effect on stimulation of sGC when combined with NO (30). Stimulation of sGC by BAY 41-2272 may result in decreased I/R injury by restoring the cGMP signaling pathway. Therefore, the aim of our study was to clarify the effect of NO-independent stimulation of sGC on lung I/R injury.

MATERIALS AND METHODS

Chemicals and Reagents

1-Hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine (CPH) was purchased from Alexis (Lausen, Switzerland). Krebs-Henseleit buffer containing 120 mM NaCl, 4.3 mM KCl, 1.1 mM KH₂PO₄, 24 mM NaHCO₃, 2.4 mM CaCl₂, 1.3 mM MgCl₂, and 13.32 mM glucose as well as 5% (wt/vol) hydroxyethylamloylopectin (200,000 Da) was provided from Serag-Wiessner (Naila, Germany). The perfusate for ROS measurements containing the same salts in the same concentration was composed in situ and treated with Chelex (Bio-Rad, Heracles, CA) to remove trace amounts of Fe and Cu.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Animals

All animal experiments were approved by the local authorities. Male New Zealand White rabbits (body wt 2.8–3.5 kg) were used for all experiments. Animals were kept under pathogen-free conditions and handled in accordance with the European Communities recommendations for experimentation.

Isolated Lung Model

The technique of isolated rabbit lung perfusion has been described (26). Briefly, rabbits were anti-coagulated with 1,000 U/kg heparin and deeply anesthetized with ketamine and xylazine. Lungs were removed from the thorax without interruption of perfusion and ventilation and subsequently subjected to a recirculating system. Lungs were perfused with 100 ml/min Krebs-Henseleit buffer and ventilated with a gas mixture comprised of 5.3% CO₂, 21% O₂, 73.7% N₂ (Messner, Siegen, Germany). A tidal volume of 30 ml, 30 breaths/min, and 1 cmH₂O positive end-expiratory pressure (PEEP) using a Harvard respirator were used (cat/rabbit ventilator; Hugo Sachs Elektronik, March-Hugstetten, Germany). Left atrial pressure was set at 2 mmHg (referenced at the hilum), and the whole system was equilibrated at 39°C.

Pressures in the pulmonary artery and the left atrium were registered with pressure transducers, and the data were transferred to a computer. The capillary filtration coefficient (K̅c) was determined gravimetrically from the slope of the lung weight gain curve induced by a 7.5-mmHg step elevation of the venous pressure for 8 min as previously described (27). Lung weight gain was calculated as the difference in organ weight measured directly before and 5 min after each of these pressure elevation maneuvers.

Experimental Protocols

After termination of the initial steady-state period and performance of a control hydrostatic challenge, ischemia was induced in the lungs by stopping the perfusion and ventilation with anoxic gas mixture 5.3% CO₂-94.7% N₂ (Messner). To maintain positive intravascular pressure, the arterial and venous catheters were clamped after adjustment of initial value of arterial pressure to 6 mmHg. During ischemia, lungs were continuously ventilated with the warmed and humidified anoxic gas mixture. At the end of ischemia, ventilation was changed to normoxia, and perfusion was reestablished by increasing the flow from 0 to 100 ml/min stepwise over 3 min. Hydrostatic challenges were performed 30 and 60 min after the onset of reperfusion. Duration of the ischemic period and timings of hydrostatic challenges were designed based on our previous studies (17, 23, 24, 26).

Lungs were treated according to one of the following protocols.

NIC: nonischemic control; lungs were perfused and ventilated throughout 7 h.

I/R: lungs were exposed to anoxic ischemia (applies to all of the following I/R groups) followed by reperfusion; no interventions were performed.

BAY 41-2272: the substance (3 μM) was added to perfusion buffer 5 min before onset of ischemia. This dosage was determined to be the most effective based on previous dose ranging experiments (data not given in detail). NO metabolites (nitrite and nitrate) were determined in perfusate samples by NOA Sievers 280.

Measurement of Exhaled NO

Measurements were performed as described by Spiess et al. (29). Briefly, aliquots of exhaled gas were provided continuously for measurement by chemiluminescence technique to NOA Sievers 280.

Measurement of NO Concentration in Perfusate

NO metabolites (nitrite and nitrate) were determined in perfusate samples by NOA Sievers 280 according to manufacturer’s instructions. Briefly, perfusate probes were sampled from venous effluent before ischemia as well as 3, 30, and 60 min after the onset of reperfusion and frozen immediately and stored under −20°C until measurements were taken. NO reaction products in samples were reduced by vanadium chloride. Resulting gaseous NO was detected by NOA Sievers 280, which was connected to a computer for data transfer and analysis by “NoaWin 32” software (DeMeTec, Langgöns, Germany).
ESR Measurements in Perfusate

The triple-line spectrum of CP• radical, a product of the reaction between spin probe CPH and ROS, was detected by ESR spectroscopy using a MS 100 spectrometer (Magnettech, Berlin, Germany). ESR measurements were performed in field scan with the following settings: microwave frequency 9.78 GHz, modulation frequency 100 kHz, modulation amplitude 2 G, and microwave power 18 mW (34). Spin probe 1 mM CPH was administered 5 min before reperfusion into the perfusate. Samples from the venous outflow of the isolated lung were taken immediately after the start of reperfusion in 50-μl glass capillaries and measured at room temperature. To decrease autoxidation of CPH due to iron, present in perfusate, 50 μM diethylenetriamine pentaacetic acid (DTPA) was applied before CPH.

NADPH Oxidase Activity Assay

Assay was performed as described previously (19). Briefly, 10% (wt/vol) tissue homogenate was prepared in 50 mM phosphate buffer (pH 7.4) containing 1 mM sodium vanadate, Complete protease inhibitor mix (Roche, Mannheim, Germany), and 0.1 mM PMSF. After lysis, homogenates were centrifuged at 20,000 g for 20 min at 4°C to pellet organelles. The supernatant was subjected to 100,000 g for 60 min at 4°C. Pellets, containing plasma membranes and microsomes, were resuspended and used for following analysis. Protein concentration was determined by Bradford assay (Bio-Rad, Munich, Germany). For determination of NADPH oxidase activity, 20 μg of protein were incubated with 1 mM NADPH, 1 mM CPH, and 50 μM DTPA in Chelex-treated PBS (pH 7.4) at 37°C for 30 min. From serial measurements by ESR spectroscopy, rate of ROS production was determined. To calculate rate of superoxide production, parallel reactions in presence of 100 U/ml SOD were performed, for which results are subtracted from values of each sample. Measurements were done using MS 100 spectrometer with settings as described above.

Data Analysis

Data are expressed as means ± SE. Differences between two groups were analyzed by t-test. For multiple comparisons, one-way analysis of variance followed by a post hoc Student-Newman-Keuls test was used. P values <0.05 were considered to represent a significant difference. Statistical analyses were performed using SPSS statistical software.

RESULTS

BAY 41-2272 Attenuates I/R-Induced Lung Injury

Permeability. In the preischemic period, low Kfc values (1.13E-04 ± 0.15E-04 cm3 × s-1 × mmHg × g) indicated intact endothelial barrier properties in all experimental groups. After 4-h ischemic exposure, control lungs displayed dramatically elevated Kfc values (Fig. 1) on reperfusion (Kfc: 13.2e-04 ± 1.80e-04 cm3 × s-1 × mmHg × g) with subsequent severe edema formation. Pretreatment with BAY 41-2272 significantly attenuated vascular leakage and reduced edema (Kfc: 4.39e-04 ± 0.51e-04 cm3 × s-1 × mmHg × g; P < 0.01). Interestingly, complete blockade of NOS activity with l-NMMA also protected against I/R injury (Kfc: 6.00e-04 ± 1.80e-04; P < 0.05). The combination of BAY 41-2272 and l-NMMA had additive protective effect on the microvascular leakage response (Kfc: 3.08E-04 ± 0.71E-04; P < 0.01 vs. I/R). Organ protection with inhaled NO was not as potent as with BAY 41-2272 (Figs. 1 and 2). Coadministration of BAY 41-2272 and inhaled NO resulted in partial loss of organ protection compared with the effect of BAY 41-2272 alone.

Changes in weight gain correlated well with changes in Kfc values (Fig. 2).

Pulmonary arterial pressure. Average pulmonary arterial pressure (Ppa) was determined to be 6.76 ± 0.23 mmHg during the preischemic period with no significant differences observed between groups. BAY 41-2272 application caused a slight decrease in basal vascular tone. Inhibition of NO formation by
1-NMMA did not significantly affect the baseline pulmonary vascular tone. Reperfusion in I/R group caused a transient Ppa increase (16.28 ± 2.14 mmHg) followed by decrease to the baseline level. BAY 41-2272 significantly attenuated Ppa rise after reperfusion (7.63 ± 0.54 mmHg; \( P < 0.01 \) vs. I/R; Fig. 2). 1-NMMA did not significantly affect Ppa increase (15.51 ± 2.46 mmHg). Combined application of BAY 41-2272 and 1-NMMA was less efficient at decreasing transient Ppa rise than BAY 41-2272 alone (12.52 ± 1.21 mmHg). Inhaled NO alone and in combination with BAY 41-2272 did not significantly influence the transient Ppa increase.

Exhaled NO and NO metabolites in the perfusate. NO release detected in the exhaled gas mixture (NOex) was 75.33 ± 5.52 ppb under preischemic control conditions. On initiation of ischemia, NOex decreased immediately and remained low during the entire ischemic period (1.33 ± 1.09 ppb shortly before reperfusion). After the onset of reperfusion, levels of NOex returned to baseline levels (Fig. 3A). NO metabolites in perfusate accumulated continuously in the course of experiment. BAY 41-2272 did not significantly influence NOex and NO metabolites in perfusate. 1-NMMA-mediated inhibition of NOS was confirmed by a rapid drop of NOex and diminished accumulation of NO metabolites after 1-NMMA application. Moreover, 1-NMMA blocked the recovery of NO production after reperfusion (23.8 ± 1.97 ppb; \( P < 0.001 \) vs. I/R). These effects of 1-NMMA were also observed on combined application of BAY 41-2272 and 1-NMMA. As expected, addition of NO to the inspired gas mixture resulted in a significant increase of NOex and NO metabolites, which masked any changes in endogenous NO production.

Concentration of cGMP in perfusate. The concentration of cGMP in I/R group in the perfusate was 0.32 ± 0.07 nM at baseline and was the same for all groups (Fig. 4). The concentration of cGMP slightly increased during the observation period (2.75 ± 0.32 nM). The cGMP concentration significantly increased in the groups treated with BAY 41-2272 and exogenous NO (39.93 ± 0.85 and 29.50 ± 5.39 nM; \( P < 0.001 \) and \( P < 0.01 \) vs. I/R control, respectively), whereas 1-NMMA inhibited increases in cGMP concentrations. Interestingly, combined application of 1-NMMA and BAY 41-2272 resulted in elevated cGMP levels but to a lesser extent than that induced by BAY 41-2272 alone (17.30 ± 0.85 nM; \( P < 0.01 \), BAY 41-2272 + 1-NMMA vs. BAY 41-2272). Most likely, this reveals the NO-independent part of the sGC stimulatory effect of BAY 41-2272. The combination of BAY 41-2272 with inhaled NO induced enormous increase of the cGMP levels (154.13 ± 42.82 nM; \( P < 0.01 \) vs. NO). On the other hand, this dramatic rise was not paralleled by a significant improvement of I/R-induced permeability disturbances (Fig. 1).

**BAY 41-2272 Reduces I/R-Induced ROS Release in Perfusate**

In lungs exposed to ischemia, a significant increase in ROS release in the perfusate on onset of reperfusion was detected, whereas the NIC lungs showed no significant increase (Fig. 5).
Application of SOD reduced significantly CP signal suggesting that the superoxide is the predominant radical produced. BAY 41-2272 as well as L-NMMA diminished ROS release. However, the combination of BAY 41-2272 and L-NMMA did not further decrease the ROS levels in the perfusate. Inhaled NO and the combination of exogenous NO and BAY 41-2272 failed to reduce ROS release in perfusate.

**BAY 41-2272 Inhibits I/R and PMA-Induced NADPH Oxidase Activation**

NADPH oxidase activity was inhibited in the BAY 41-2272-treated lungs as demonstrated in Fig. 6.

![Fig. 6. Influence of BAY 41-2272 on NADPH oxidase activity.](image)

**Fig. 6.** Impact of BAY 41-2272, l-NMMA, and NO on reactive oxygen species (ROS) release into perfusate. Spin probe 1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine (CPH) was added to recirculating perfusate shortly before reperfusion. Five minutes after starting reperfusion, aliquots of the perfusate were taken from lung effluent. Free radicals were measured by electron spin resonance (ESR) spectroscopy immediately after sample acquisition. Average value of the 3 measurements is taken for every experiment. ESR signal intensity reflects the concentration of ROS in the recirculating buffer. All treatment groups except NO and BAY 41-2272 + NO groups demonstrated significant inhibition of ROS release into the perfusate (*P < 0.05 vs. I/R). The data represent the means ± SE. AU, arbitrary units. *P < 0.05 vs. I/R; **P < 0.01 vs. I/R.

During steady-state period, the lungs in PMA group demonstrated stable Ppa (8.63 ± 0.34 mmHg) with no significant differences between groups. PMA stimulation resulted in increased ROS release in the perfusate of isolated lungs, which was accompanied by a pronounced increase in Ppa (Fig. 7). BAY 41-2272 significantly inhibited an increase in both ROS production (Fig. 7A) and Ppa elevation (Fig. 7B). SOD completely suppressed superoxide release accompanied by a reduced pressure response.

**NADPH Oxidase is Involved in Mediation of I/R Injury**

Application of the NADPH oxidase inhibitor apocynin protected against I/R-induced injury (Fig. 8). Apocynin protected against increase in permeability and reduced weight gain (14.4 ± 2.34 g; P < 0.05 vs. I/R; Fig. 8, A and B). Additionally, apocynin reduced reperfusion-induced Ppa increase (Fig. 8C). NADPH oxidase inhibition was confirmed via decreased ROS release in perfusate in apocynin-treated lungs (Fig. 8D).

![Fig. 7. Influence of BAY 41-2272 on PMA-stimulated ROS production and Ppa increase.](image)

**Fig. 7.** Influence of BAY 41-2272 on PMA-stimulated ROS production and Ppa increase. Lungs were stimulated with PMA (1 μM) either in the presence or absence of BAY 41-2272. Control measurements were performed in the presence of SOD (150 U/ml) throughout the experiments. A: changes in the ESR signal intensity (fold increase) by comparison of values before and after 30 min of PMA stimulation. B: influence of BAY 41-2272 on PMA-induced Ppa increase. Ppa values 30 min after PMA stimulation are demonstrated. The data represent the means ± SE. *P < 0.05; **P < 0.01; ***P < 0.001 all vs. PMA.
DISCUSSION

The main findings of this study are: 1) direct stimulation of sGC by BAY 41-2272 protects against I/R lung injury; 2) this protective effect is not modulated by NO; and 3) stimulation of sGC leads to inhibition of NADPH oxidase.

Direct stimulation of sGC by BAY 41-2272 potently prevented an increase in vascular permeability and fluid retention in the BAY 41-2272-treated lungs. Interestingly, BAY 41-2272 inhibited the observed increase in ROS release into perfusate after reperfusion. One of the features of I/R injury is a transient pressure increase after reperfusion. Direct stimulation of sGC by BAY 41-2272 inhibited this increase. Whether the hydrostatic challenge of increased pressure (4, 28) or the permeability disturbance as result of oxidative stress (36) is a driving force of I/R-induced lung injury remains under debate. Hence, BAY 41-2272 seems to influence both possible components that drive lung injury.

BAY 41-2272 has been described as a highly potent and specific NO-independent stimulator of sGC (1), which has an additive effect when combined with NO (30). Therefore, we tested the effect of BAY 41-2272 and inhalative NO coadministration on I/R lung injury. The combination of BAY 41-2272 and NO caused significant increase in cGMP concentration in the perfusate. However, this combination resulted in a loss of protective effect on I/R injury compared with BAY 41-2272 alone. This observation could be partially explained by the adverse effect of peroxynitrite, which is produced from NO in an oxidizing environment.

Although the most effective dose and regimen of NO inhalation were chosen based on comparison of different approaches of NO application (21), inhalation of NO was less efficient to reduce increased permeability compared with BAY 41-2272. Interestingly, increase in cGMP concentration in the perfusate was comparable between groups treated with BAY 41-2272 and NO inhalation, which suggests that both agents had comparable potency in sGC stimulation. In the NO treatment group, ROS release into perfusate was not significantly different from that in the I/R group. Apocynin significantly attenuated the Ppa increase on reperfusion (P < 0.05 vs. I/R). The data represent the means ± SE.

Fig. 8. Impact of the NADPH oxidase inhibitor apocynin on I/R-induced lung injury. NIC lungs were perfused and normoxically ventilated for 6 h. I/R lungs underwent a 4-h period of ischemia and reperfusion without other treatments. Apocynin (1 mM) was applied 5 min before ischemia according to protocol.

A: capillary filtration coefficient (Kfc) was determined gravimetrically from the slope of the LWG curve induced by a 7.5-mmHg step elevation of the venous pressure for 8 min. At 30 min after onset of reperfusion, apocynin treatment group demonstrated a significant reduction of elevated Kfc (*P < 0.05 vs. I/R).

B: LWG was calculated as the difference in organ weight measured directly before and 5 min after each of these pressure elevation maneuvers. Values of LWG at 30 min after reperfusion are shown. Apocynin significantly inhibited LWG increase (P < 0.05 vs. I/R).

C: reperfusion-induced pressure increase demonstrates Ppa on 5th min of reperfusion, when perfusion was completely reestablished. Ppa values are presented in percent relative to I/R group. Apocynin significantly attenuated the Ppa increase on reperfusion (P < 0.05 vs. I/R).

D: free radicals were measured by ESR spectroscopy after reperfusion. Average value of the 3 measurements is taken for every experiment. ESR signal intensity reflects the concentration of ROS in the recirculating buffer. Apocynin-treated group demonstrated significant inhibition of ROS release into the perfusate (**P < 0.01 vs. I/R).
Indeed, in the present study, we observed that L-NMMA has a positive effect on the reduction of I/R-induced lung injury. Complete blockade of NO production by both iNOS and eNOS was achieved as evidenced by very low levels of exhaled NO and cGMP levels in perfusate. This was associated with significant reduction in ROS release in perfusate in the early reperfusion period. Inhibition of the ROS production may be due to prevention of ROS formation by uncoupled NOS. Similar results were observed in another study where treatment with L-NMMA reduced ROS production in the early reperfusion (2).

The combination of BAY 41-2272 and L-NMMA resulted in further reduction of I/R-induced injury. We suggest that L-NMMA and BAY 41-2272, despite having opposite effects on NO-cGMP signaling pathway, have additive effect in this situation because they inhibit two different components involved in the mediation of ROS-induced injury. L-NMMA inhibits uncoupled NO synthases, thereby preventing superoxide production, whereas BAY 41-2272 restores the NO-cGMP signaling pathway, preventing activation of NADPH oxidase. This results in synergistic effect in protection of vascular permeability and weight gain changes.

We suggest that BAY 41-2272 decreases I/R injury by inhibiting NADPH oxidase-derived ROS production, as YC-1, a substance structurally related to BAY 41-2272, has been shown to inhibit formyl-Met-Leu-Phe (fMLP)-induced ROS production (12, 33). NO-cGMP signaling pathway has been shown that L-NMMA exerts its positive effect by preventing free radical formation catalyzed by uncoupled NO synthases and BAY 41-2272 protects against I/R-induced injury by restoring the NO-cGMP signaling pathway and preventing NADPH oxidase activation.

ACKNOWLEDGMENTS

Parts of the doctoral thesis of B. Egemnazarov are included in the manuscript. We thank K. Quanz for assistance in cGMP measurements.

Grants

Funding sources for this study were German Research Foundation [Deutsche Forschungsgesellschaft (DFG), SFB 547] and Excellence Cluster Cardio-Pulmonary System (ECCPS).

References

22. Muzaffar S, Shukla N, Srivastava A, Angelini GD, Jeremy JY. Silde-
nafil citrate and sildenafil nitrate (NCX 911) are potent inhibitors of
superoxide formation and gp91phox expression in porcine pulmonary
and continued ventilation are protective in lung ischemia/reperfusion.
24. Schutte H, Lockinger A, Seeger W, Grimminger F. Aerosolized PGE1,
PGI2 and nitroprusside protect against vascular leakage in lung ischaemia-
Grimminger F. Endogenous nitric oxide synthesis and vascular leakage
in ischemic-reperfused rabbit lungs. Am J Respir Crit Care Med 164:
S, Seeger W, Grimminger F. The PDE inhibitor zaprinast enhances
NO-mediated protection against vascular leakage in reperfused lungs.
27. Seeger W, Walmrath D, Menger M, Neuhof H. Increased lung vascular
permeability after arachidonic acid and hydrostatic challenge. J Appl
28. Singh RR, Laubach VE, Eillman PJ, Reece TB, Unger E, Kron IL,
Tribble CG. Attenuation of lung reperfusion injury by modified ventilation
and reperfusion techniques. J Heart Lung Transplant 25: 1467–1473,
2006.
W. On-line measurement of nitric oxide generation in buffer-perfused
31. Stasch JP, Becker EM, Alonso-Alija C, Apeler H, Dembowsky K,
Feurer A, Gerzer R, Minuth T, Perzborn E, Pleiss U, Schroder H,
Schroeder W, Stahl E, Steinke W, Straub A, Schramm M. NO-
independent regulatory site on soluble guanylate cyclase. Nature 410:
A, Claro MA, Tendillo F, Castillo-Olivares JL. Inhaled nitric oxide
33. Wang JP, Chang I-C, Raung SL, Hsu MF, Huang IJ, Kuo SC.
34. Weissmann N, Kuzkaya N, Fuchs B, Tiyerili V, Schäfer RU, Schütte
H, Ghofrani HA, Schermuly RT, Schudt C, Sydykov A, Egennazarow
B, Seeger W, Grimminger F. Detection of reactive oxygen species in
isolated, perfused lungs by electron spin resonance spectroscopy. Respir
Measurement and characterization of free radical generation in reoxygen-
ated human endothelial cells. Am J Physiol Cell Physiol 266: C700–C708,
1994.
36. Zweier JL, Talukder MA. The role of oxidants and free radicals in