Anesthesiology 2007; 106:124–31

Inhalation of the Phosphodiesterase-3 Inhibitor Milrinone Attenuates Pulmonary Hypertension in a Rat Model of Congestive Heart Failure

Thomas Hentschel, M.D., Ning Yin, M.D.,† Alexander Riad, M.D.,‡ Helmut Habbazettl, M.D.,§ Jörg Weimann, M.D.,‖ Andreas Koster, M.D.,# Carsten Tschope, M.D.,** Hermann Kuppe, M.D.,†† Wolfgang M. Kuebler, M.D.‡‡*

Background: **Most patients with congestive heart failure (CHF) develop pulmonary venous hypertension, but right ventricular afterload is frequently further elevated by increased pulmonary vascular resistance. To investigate whether inhalation of a vasodilatory phosphodiesterase-3 inhibitor may reverse this potentially detrimental process, the authors studied the effects of inhaled or intravenous milrinone on pulmonary and systemic hemodynamics in a rat model of CHF.**

Methods: **In male Sprague-Dawley rats, CHF was induced by supracoronary aortic banding, whereas sham-operated rats served as controls. Milrinone was administered as an intravenous infusion (0.2–1 $\mu g \cdot kg\ body\ weight^{-1} \cdot min^{-1}$) or by inhalation (0.2–5 mg/ml), and effects on pulmonary and systemic hemodynamics and lung water content were measured.**

Results: **In CHF rats, intravenous infusion of milrinone reduced both pulmonary and systemic arterial blood pressure. In contrast, inhalation of milrinone predominantly dilated pulmonary blood vessels, resulting in a reduced pulmonary-to-systemic vascular resistance ratio. Repeated milrinone inhalations in 20-min intervals caused a stable reduction of pulmonary artery pressure. No hemodynamic effects were detected when 0.9% NaCl was administered instead of milrinone or when milrinone was inhaled in sham-operated rats. No indications of potentially adverse effects of milrinone inhalation in CHF, such as left ventricular volume overload, were detected. Moreover, lung edema was significantly reduced by repeated milrinone inhalation.**

Conclusion: **If these results can be confirmed in humans, inhalation of nebulized milrinone may present a novel, effective, safe, and pulmonary selective strategy for the treatment of pulmonary venous hypertension in CHF.**

CONGESTIVE heart failure (CHF) results in a "passive" increase in pulmonary vascular pressure, termed *pulmonary venous hypertension*. Pulmonary venous hypertension causes pulmonary endothelial dysfunction characterized by reduced bioavailability of nitric oxide and increased formation of vasoconstrictors such as endothelin 1[1] and thromboxane A_2.[2] Pulmonary venous hypertension may therefore promote pulmonary vasoconstriction and vascular remodeling and cause a "reactive" increase in pulmonary vascular resistance (PVR).[3,4] Thus, lung vascular responses may further increase pulmonary arterial pressure (PAP) in CHF and augment the risk for right ventricular failure. Clinical studies have identified pulmonary hypertension[5] and reduced right ventricular ejection fraction[6,7] as predictors of increased mortality in CHF. Therefore, patients with CHF may benefit from vasodilatory therapeutic approaches to reduce PVR. However, intravenous or oral administration of vasodilators such as epoprostenol or "inodilators" such as the phosphodiesterase-3 inhibitor milrinone did not reduce, but increased mortality in prospective clinical trials.[8,9] Inhaled vasodilators may circumvent potentially deleterious systemic side effects by acting predominantly on the pulmonary circulation. In a small nonrandomized clinical trial, inhalation of milrinone was shown to reduce PVR in cardiac surgical patients with pulmonary hypertension.[10] However, inhaled vasodilators may also promote hydrostatic lung edema in CHF by opening precapillary sphincters (Kitajew reflex) or cause left ventricular volume overload.[11,12] Here, we determined *in vivo* the effects of both inhaled and intravenous milrinone on pulmonary and systemic hemodynamics and lung water content in an experimental rat model of hypertensive CHF.

Materials and Methods

All experiments were performed in male Sprague-Dawley rats. Animals received care in accordance with the *Guide for the Care and Use of Laboratory Animals*.[13] The study was approved by the local animal care and use committee of the local government authorities (Tierversuchskommission, LAGetSi Berlin, Germany).

Experimental Model of CHF

In juvenile rats of 100 ± 8 g body weight (bw), CHF was induced by supracoronary aortic banding as previously described.[14] In brief, rats were anesthetized by intraperitoneal injection of ketamine (87 mg/kg bw; Pharmacia GmbH, Erlangen, Germany) and xylazine (13 mg/kg bw; Bayer, Leverkusen, Germany). Rats were placed in the supine position, the chest wall was shaved, and a left thoracotomy was performed in the third intercostal space during ventilation with 100% O_2. The ascending aorta was freed from connective

* Senior Resident in Anesthesiology, # Assistant Professor, †† Professor, Department of Anesthesiology, German Heart Institute Berlin, Berlin, Germany. § Associate Professor, Department of Anesthesiology, German Heart Institute Berlin, Berlin, Germany; Institute of Physiology, Charité-Universitaetsmedizin Berlin, Campus Benjamin Franklin. † Senior Resident in Anesthesiology, ‡‡ Associate Professor, Institute of Physiology, ‡ Resident in Cardiology, ** Assistant Professor, Department of Cardiology and Pneumatology, ‖ Associate Professor, Department of Anesthesiology and Intensive Care Medicine, Charité-Universitaetsmedizin Berlin, Campus Benjamin Franklin.

Received from the Institute of Physiology, Charité-Universitätsmedizin Berlin, Berlin, Germany. Submitted for publication January 17, 2006. Accepted for publication September 3, 2006. Supported by the Kaiserin Friedrich Foundation, Berlin, Germany, and the Charité-Universitätsmedizin Berlin, Berlin, Germany. Drs. Hentschel and Yin contributed equally to this study.

Address correspondence to Dr. Kuebler: Institute of Physiology Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Arnimallee 22, 14195 Berlin, Germany. wolfgang.kuebler@charite.de. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

tissue and partially occluded by implantation of a titanium clip (Hemoclip®; Weck Closure System, Research Triangle Park, NC) with a defined internal diameter of 0.8 mm. After surgical closure of the thorax, the rats were allowed to recover from anesthesia. For postoperative analgesia, rats received 250 mg/kg bw of metamizole (Novalgin®; Aventis Pharma, Bad Sodem, Germany) intramuscularly immediately after the operation and on the first postoperative day. Sham-operated rats (*i.e.*, without insertion of a clip) served as controls. After recovery from anesthesia, the animals were placed in cages with free access to water and standard laboratory diet.

Hemodynamic Monitoring

Nine weeks after aortic banding, rats had attained body weights of 360 ± 10 g and were anesthetized by intraperitoneal administration of urethane (0.15 g/100 g bw; Fluka, Buchs, Switzerland), followed by intramuscular injection of ketamine (0.05 mg/100 g bw; Pharmacia GmbH) as previously described.[15] The neck and chest wall were shaved, and rats were placed in the supine position on a thermostatically controlled electric heating blanket (Homeothermic Blanket Control Unit; Harvard Apparatus, March-Hugstetten, Germany) to maintain body temperature at 38°C during the experimental protocol. After tracheostomy, rats were mechanically ventilated (Rodent Respirator 680; Harvard Apparatus) with a tidal volume of 6 ml/kg bw at 110 breaths/min and a peak inspiratory pressure of 10.5 ± 1 cm H_2O. Polyvinyl catheters (internal diameter 0.58 mm; Sims Portex Ltd., Hythe, United Kingdom) were introduced into the aorta and the vena cava *via* the left carotid artery and the right jugular vein, respectively. A median thoracotomy was performed, and catheters were surgically placed into the left atrium and the pulmonary artery *via* the left auricle and the right ventricle, respectively. An ultrasonic flowprobe (Transonic®; Transonic Systems Inc., Ithaca, NY) was positioned around the ascending aorta distal to the branching of the coronary arteries and proximal to the truncus brachiocephalicus. Arterial pressure (AP), central venous pressure (CVP), PAP, left atrial pressure (LAP), and airway pressure as well as aortic flow were continuously monitored and registered on-line with use of the software package DasyLab®32 (DasyLab, Moenchengladbach, Germany). SVR and PVR were calculated using standard equations.[16]

Drug Delivery

For inhalation, milrinone (0.2–5 mg/ml, Corotrop®; Sanofi Winthrop, Paris, France) or NaCl (0.9%) were nebulized using an ultrasonic nebulizer (Optineb®; Nebu-Tec, Elsenfeld, Germany) and inhaled for 3 min at identical peak inspiratory pressures as used throughout the experiment. A 3-min nebulization of 1 mg/ml milrinone resulted in vaporization of 14 μg of the phosphodiesterase-3 inhibitor as determined by microgravimetry. Therefore, the respective dose of 39 μg/kg is analog to inhaled doses in human studies.[10] For intravenous delivery, milrinone (initial bolus of 2–10 μg/kg, followed by 0.2–1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or equivalent volumes of NaCl (0.9%; initial bolus of 1.6 ml/kg, followed by 10 $ml \cdot kg^{-1} \cdot h^{-1}$) were administered by an infusion pump (Perfusor® fm; B. Braun Melsungen AG, Melsungen, Germany) for 10 min.

Fluorescence Microscopy

Alveolar delivery of aerosolized substances was tested by inhalation of nebulized fluorescein isothiocyanate dextran (molecular weight 150 kd; Sigma-Aldrich, Taufkirchen, Germany) instead of milrinone and subsequent fluorescence imaging of subpleural alveoli *in situ* as previously described.[17] In brief, after inhalation of fluorescein isothiocyanate dextran or NaCl (0.9%), lungs were excised, inflated with room air at 5 cm H_2O, and imaged at $\lambda = 480$ nm (Axiotech^{vario} 100HD; Carl Zeiss, Jena, Germany).

Echocardiography

Rats were anesthetized and allowed to breathe spontaneously in the half left-lateral position. The long axis of the left ventricle was imaged by a 15-MHz transducer (SONOS 5500; Philips Medizin Systeme GmbH, Hamburg, Germany) as previously described.[18] From the two-dimensional mode of the long axis, end-diastolic volume (LVEDV) was determined. M-mode recordings from the short axis of the left ventricle were traced to yield ejection fraction, fractional shortening, and the wall thickness of the interventricular septum during diastole. Echocardiographic parameters were measured before and 1 min after milrinone inhalation.

Cyclic Nucleotides

Blood samples (1.5 ml) were obtained before and after inhalation of milrinone in sham-operated and CHF rats. Samples were collected in EDTA tubes, and plasma phosphodiesterases were immediately blocked by addition of IBMX (33 mg/ml; Sigma-Aldrich). After centrifugation at 4°C (5,000*g* for 10 min), plasma aliquots were stored at −80°C and analyzed for cyclic adenosine 3′,5′-monophosphate (cAMP) and cyclic guanosine 3′,5′-monophosphate (cGMP) concentrations by enzyme-linked immunosorbent assay microplate immunoassay kits (R&D Systems, Minneapolis, MN).

Experimental Groups and Protocol

Dose–Response. Congestive heart failure rats were randomized into six groups as specified in table 1, groups i–vi. Hemodynamic measurements were performed before and after drug administration as illus-

Table 1. Experimental Groups

Group	Rats	n	Substance	Route	Dose	Loading Dose	Duration, min	Mode
i	CHF	5	Milrinone	Inhaled	0.2 mg/ml	—	3	—
ii	CHF	5	Milrinone	Inhaled	1 mg/ml	—	3	—
iii	CHF	5	Milrinone	Inhaled	5 mg/ml	—	3	—
iv	CHF	5	Milrinone	Intravenous	0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$	2 μg/kg	10	—
v	CHF	5	Milrinone	Intravenous	0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$	5 μg/kg	10	—
vi	CHF	5	Milrinone	Intravenous	1 $\mu g \cdot kg^{-1} \cdot min^{-1}$	10 μg/kg	10	—
1	CHF	11	Milrinone	Inhaled	1 mg/ml	—	3	—
2	CHF	11	0.9% NaCl	Inhaled	—	—	3	—
3	Control	11	Milrinone	Inhaled	1 mg/ml	—	3	—
4	Control	11	0.9% NaCl	Inhaled	—	—	3	—
5	CHF	11	Milrinone	Intravenous	1 $\mu g \cdot kg^{-1} \cdot min^{-1}$	10 μg/kg	10	—
6	CHF	11	0.9% NaCl	Intravenous	1.6 ml/kg	10 $ml \cdot kg^{-1} \cdot h^{-1}$	10	—
A	CHF	5	Milrinone	Inhaled	1 mg/ml	—	60	Single
B	CHF	5	Milrinone	Inhaled	1 mg/ml	—	60	Repeated
C	CHF	5	—	—	—	—	60	—

Experimental groups for dose–response relations to inhaled and intravenous milrinone in congestive heart failure (CHF) rats (groups i–vi), single drug application of inhaled or intravenous milrinone or NaCl in CHF and control rats (groups 1–6), and repeated milrinone inhalation in CHF rats (groups A–C). Each single milrinone inhalation lasted 3 min, and repeated inhalations were delivered in 20-min intervals (group B).

trated by the flowchart of the experimental protocol (fig. 1A).

Single Drug Application. Animals were randomized into six groups and received inhalation (3 min) or intravenous (10 min) administration of either milrinone or NaCl as specified in table 1, groups 1–6. Blood samples for determination of cyclic nucleotides and mixed venous oxygen saturation (Svo_2) were obtained and replaced by infusion of hydroxyethyl starch (6% hydroxyethyl starch 200/0.5; Fresenius, Bad Homburg, Germany) as illustrated in figure 1A. Hemodynamic parameters were recorded before and after drug administration. At the end of experiments, animals were killed by exsanguination, and the heart and lungs were removed. Hearts were dissected into the free wall of the right ventricle and the left ventricle including the septum and both atria and weighed. Lung wet/dry weight ratio was determined by use of the microwave drying technique.[19]

Repeated Drug Application. Congestive heart failure rats were randomized into three groups (table 1, groups A–C) receiving none, one single, or three repeated milrinone inhalations for 3 min each in 20-min intervals over 1 h as illustrated in figure 1B. Hemodynamic parameters were continuously monitored and recorded every 10 min for 1 h, and lung wet/dry weight ratio was measured at the end of the experiments.

Statistical Analysis

All data are presented as mean ± SEM. Values were compared by Wilcoxon or Friedman tests for intragroup data and by Kruskal–Wallis or Mann–Whitney U tests for intergroup data. Statistical significance was assumed at $P < 0.05$.

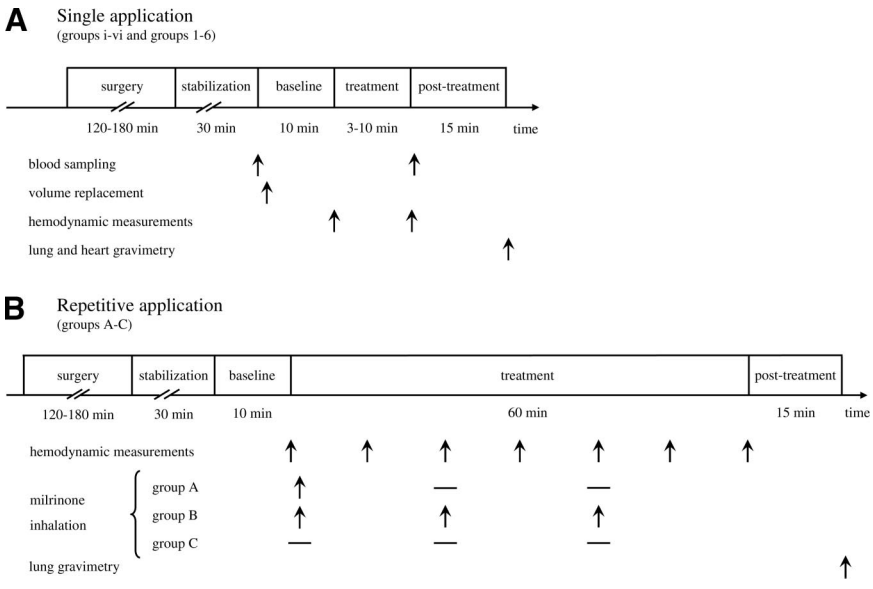

Fig. 1. Experimental flow chart. Experimental protocols used to determine the effects of single (*A*) or repeated (*B*) drug applications. *Arrows* indicate time point of intervention.

Table 2. Cardiovascular and Hemodynamic Characteristics of the CHF Model

	Control	CHF
Heart weight/body weight, %	0.32 ± 0.01	0.49 ± 0.01*
Left ventricular mass, mg	867 ± 30.5	1,274 ± 25.1*
Right ventricular mass, mg	242 ± 15.5	374 ± 10.9*
AP, mmHg	61.8 ± 0.16	62.6 ± 0.14
PAP, mmHg	10.2 ± 0.05	15.1 ± 0.04*
LAP, mmHg	1.90 ± 0.02	4.13 ± 0.03*
CVP, mmHg	2.66 ± 0.02	3.01 ± 0.02
TPG, mmHg	8.33 ± 0.06	11.3 ± 0.04*
Aortic flow, ml/min	48.5 ± 0.24	52.2 ± 0.22
PVR, dyn · s/cm^5	14.7 ± 0.13	20.0 ± 0.10*
SVR, dyn · s/cm^5	109 ± 0.52	107 ± 0.56
PVR/SVR, %	14.2 ± 0.10	19.7 ± 0.08*

Group data are from 22 sham-operated and 44 congestive heart failure (CHF) rats.

* $P < 0.05$ *vs.* control rats.

AP = arterial pressure; CVP = central venous pressure; LAP = left atrial pressure; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; SVR = systemic vascular resistance; TPG = transpulmonary pressure gradient.

Results

Experimental CHF Model in Rats

Within 9 weeks after supracoronary aortic banding, rats developed biventricular cardiac hypertrophy as demonstrated by increased heart/body weight ratio and ventricular masses in comparison with sham-operated animals (table 2). Increased hydrostatic pressures in the left atrium and the pulmonary artery demonstrate the development of CHF. Because the absolute increase in PAP exceeded the increase in LAP, both transpulmonary pressure gradient and PVR were increased, indicating increased pulmonary vascular tone or remodeling. AP, CVP, aortic flow, and systemic vascular resistance (SVR) in CHF rats did not differ from sham-operated animals demonstrating the absence of systemic hemodynamic effects.

Alveolar Delivery

Alveolar delivery of nebulized drugs was tested by inhalation of aerosolized fluorescein isothiocyanate dextran for 3 min. Subsequent fluorescence imaging showed strong and homogeneous fluorescence in all imaged alveoli, whereas no alveolar fluorescence was detected following inhalation of 0.9% NaCl (data not shown).

Dose–Response Relation

To establish dose–response relations for hemodynamic effects of milrinone in CHF, the effects of different drug concentrations delivered either by inhalation or by intravenous infusion on AP and PAP were tested. Inhalation of 1 mg/ml milrinone, a dose previously used in postcardiac surgical patients,[10] resulted in a near-maximal reduction of PAP without significant effects on AP (fig. 2A) and was therefore used subsequently for inhalation treatment. The reduction in PAP was less pronounced ($P < 0.05$) in response to the maximal intravenous dose tested, 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (fig. 2B), which to our knowledge is the highest dose reported for *in vivo* use so far.[20] Lower intravenous doses had a lesser effect on PAP without improving pulmonary selectivity of the vasodilatory response. Therefore, a concentration of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was subsequently used for intravenous delivery.

Milrinone Inhalation in CHF Rats

Inhalation of 1 mg/ml milrinone decreased pulmonary artery pressure similarly in a larger collective of CHF rats (fig. 3A). This effect was not attributable to milrinone effects on aortic flow, *i.e.*, cardiac output, but on a decrease in PVR by 39.9 ± 1.31% ($P < 0.05$) indicating pulmonary vasodilation. In addition, a small but significant decrease in LAP by 30.14 ± 2.29% was registered. AP, CVP, and SVR did not change (data not shown), whereas the PVR/SVR ratio decreased (fig. 3A), demonstrating that inhaled milrinone acted predominantly on the pulmonary circulation. Milrinone inhalation increased Svo_2 from 33 ± 1 to 42 ± 1%, but without reaching significance ($P = 0.09$). These changes were attributable to milrinone and not the inhalation procedure *per se*, because NaCl inhalation had no similar effects.

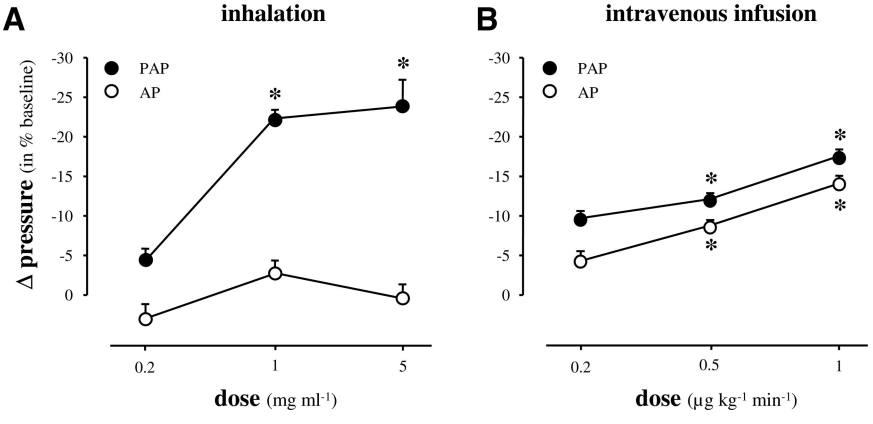

Fig. 2. Dose–response relations. Group data show reduction of pulmonary artery pressure (PAP; *filled circles*) and arterial pressure (AP; *open circles*) relative to baseline in response to different concentrations of inhaled (*A*) or intravenous (*B*) milrinone in congestive heart failure rats. Data are from n = 5 rats in each group. * $P < 0.05$ *versus* baseline.

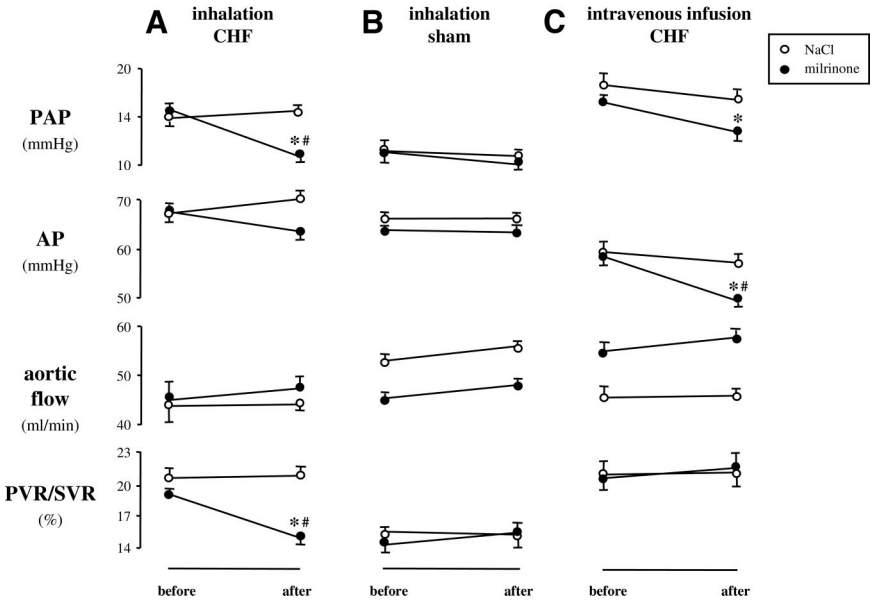

Fig. 3. Hemodynamic effects. Group data show pulmonary artery pressure (PAP), arterial pressure (AP), aortic flow, and the ratio of pulmonary over systemic vascular resistance (PVR/SVR) before and after inhalation (*A* and *B*) or intravenous (*C*) delivery of milrinone (*filled circles*) or NaCl (*open circles*) in congestive heart failure (CHF) rats (*A* and *C*) or sham-operated animals (*B*), respectively. Concentrations for milrinone and NaCl aerosols were 1 mg/ml and 0.9%, respectively. Intravenous doses were 10-μg/kg body weight bolus and 1-μg · kg body weight^{-1} · min^{-1} infusion rate for milrinone and equal volumes for 0.9% NaCl. Data are from n = 11 rats in each group. * *P* < 0.05 *versus* baseline (before). # *P* < 0.05 *versus* NaCl.

Milrinone Inhalation in Sham-operated Rats

In sham-operated rats, inhalation of 1 mg/ml milrinone neither lowered PAP nor changed any of the other hemodynamic parameters, demonstrating the absence of pulmonary or systemic vascular effects in normal rats (fig. 3B).

Intravenous Milrinone in CHF Rats

In CHF rats, PAP (fig. 3C), PVR (−18.96 ± 1.7%), and LAP (−26.03 ± 2.3%) were also significantly reduced by intravenous infusion of 1 μg · kg^{-1} · min^{-1} milrinone ($P < 0.05$). However, intravenous milrinone also caused systemic vasodilation as evident from decreases in AP (fig. 3C) and SVR (−22.23 ± 1.6%). As a result, intravenous milrinone neither reduced the PVR/SVR ratio nor markedly improved Svo_2 (37 ± 2% after milrinone *vs.* 33 ± 2% at baseline; $P = 0.35$).

Repeated Milrinone Inhalations in CHF Rats

After a single milrinone inhalation, PAP reached its minimum after 20 min and then slowly returned to baseline values (fig. 4). To test whether repeated milrinone inhalations may cause sustained pulmonary vasodilation, CHF rats inhaled milrinone in 20-min intervals, which produced a stable reduction of PAP over 60 min (fig. 4).

Lung Edema Formation

Potential propagation of hydrostatic lung edema is a major concern for the use of vasodilators in pulmonary venous hypertension. Analysis of wet/dry weight ratios showed a small but significant increase in CHF rats as compared with sham-operated animals (table 3). However, wet/dry weight ratio was not further increased 15 min after milrinone inhalation or infusion or even 60 min after inhalation of a single milrinone dose. Repeated milrinone inhalations even reduced lung wet/dry weight ratio, indicating that inhaled vasodilators may diminish lung edema in pulmonary venous hypertension.

Echocardiography

In accordance with the characteristics of compensated left heart failure, CHF rats had a higher LVEDV and a thickening of the interventricular septum as compared

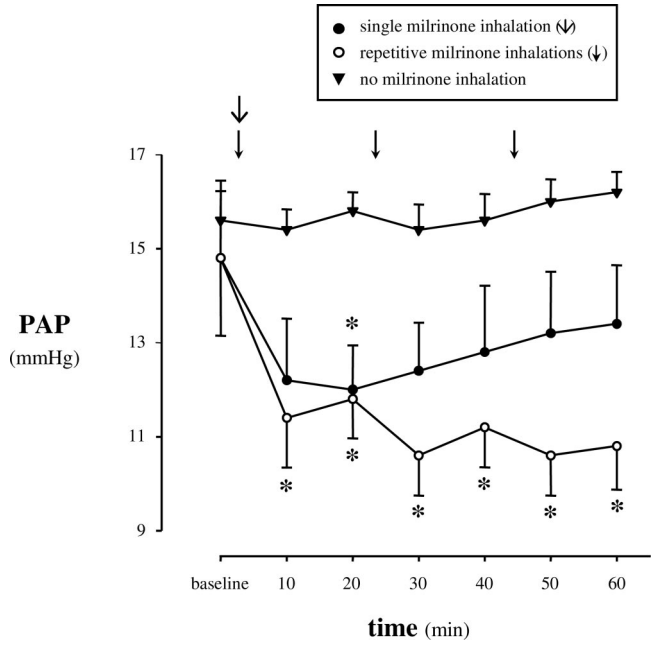

Fig. 4. Repeated milrinone inhalation. Group data show pulmonary artery pressure (PAP) determined in 10-min intervals at baseline and over 60 min in congestive heart failure rats without inhaled drug delivery (*filled triangles*), with a single milrinone inhalation (1 mg/ml; *filled circles*), and with repeated milrinone inhalations in 20-min intervals (*open circles*) as indicated by *arrows*. Data are from n = 5 rats in each group. * *P* < 0.05 *versus* baseline.

Table 3. Lung Edema Formation

Group	Rats	Substance	Route	Mode	Wet/Dry Ratio
1	CHF	Milrinone	Inhaled	—	5.23 ± 0.03*
2	CHF	0.9% NaCl	Inhaled	—	5.17 ± 0.02*
3	Control	Milrinone	Inhaled	—	4.88 ± 0.02
4	Control	0.9% NaCl	Inhaled	—	4.72 ± 0.01
5	CHF	Milrinone	Intravenous	—	5.17 ± 0.02*
6	CHF	0.9% NaCl	Intravenous	—	5.12 ± 0.05*
A	CHF	Milrinone	Inhaled	Single	5.27 ± 0.04
B	CHF	Milrinone	Inhaled	Repeated	4.97 ± 0.01*
C	CHF	—	—	—	5.28 ± 0.04

Group data give wet/dry weight ratio in lungs of congestive heart failure (CHF) and sham-operated (control) rats. *Groups 1–6:* Wet/dry weight ratio determined 15 min after inhalation or intravenous administration of either milrinone or NaCl as indicated. Inhaled concentrations for milrinone and NaCl were 1 mg/ml and 0.9%, respectively. Intravenous doses were 10-μg/kg body weight bolus and 1-$\mu g \cdot kg\ body\ weight^{-1} \cdot min^{-1}$ infusion rate for milrinone and equal volumes for 0.9% NaCl. Data are from n = 11 rats each. * $P < 0.05$ *vs.* NaCl inhalation in control rats (group 4). *Groups A–C:* Wet/dry weight ratio in lungs of CHF rats determined 75 min after a single milrinone inhalation (1 mg/ml, A), repeated milrinone inhalations in 20-min intervals (B), or no milrinone inhalation (C). Data are from n = 5 in each group. * $P < 0.05$ *vs.* CHF without inhalation (group C).

with control animals, but did not differ with respect to ejection fraction or fractional shortening (table 4). Milrinone inhalation did not cause a detectable change in the dynamic geometry of the left ventricle and septum.

Plasma cAMP and cGMP Concentration

Whereas baseline cAMP concentrations were identical in CHF rats and control animals (fig. 5A), baseline cGMP levels were significantly lower in CHF rats (fig. 5B). Milrinone inhalation selectively increased cAMP but not cGMP plasma concentrations in both groups.

Discussion

In a rat model of CHF, the phosphodiesterase-3 inhibitor milrinone reduced pulmonary hypertension and thus right ventricular afterload. Whereas intravenous infusion of milrinone resulted in systemic vasodilation, the action of inhaled milrinone was predominantly confined to the pulmonary vasculature. A sustained reduction of PAP was achieved by repeated milrinone inhalations. No hemodynamic indications of left ventricular volume overload were detected after milrinone inhalation. Edema formation was not amplified, but even reduced by repeated milrinone administrations. Therefore, inhaled milrinone may present an effective and safe new treatment strategy in pulmonary venous hypertension to unload the right ventricle and reduce edema formation.

In the current study, we determined the effects of milrinone in a rat model of CHF induced by supracoronary aortic banding,[14] an experimental model that reflects the pathophysiologic characteristics of compensated CHF due to supracoronary aortic stenosis in humans. Analogous to this clinical picture, increased hydrostatic pressure proximal to the stenosis resulted in left ventricular dilation and hypertrophy, whereas distally measured APs were unchanged. Unaltered left ventricular ejection fraction, fractional shortening, and cardiac output indicate the compensated state of CHF in this model. It should be considered that cardiovascular responses to inhaled or intravenous inodilators may differ in heart failure of alternative etiology such as ischemic heart disease. Because APs were determined distal to the aortic banding site, they do not adequately reflect left ventricular afterload and underestimate SVR by a systematic factor. However, aside from the constant resistance imposed by the aortic band, they yield reliable information on systemic vasomotor responses to intravenous or inhaled vasodilators. Alveolar deposition of the inhaled substances depends on size, charge, and lipophilia. Effective alveolar delivery of nebulized fluorescein isothiocyanate dextran, which exceeds milrinone in molecular weight by a factor of 700, was demonstrated by fluorescence imaging. Physiologic markers such as reduced PVR and increased cAMP plasma levels further confirm the effective delivery of milrinone by inhalation.

Endothelial dysfunction, *i.e.*, an imbalanced release of vasodilatory and vasoconstrictive factors in the lung resulting in pulmonary vasoconstriction and vascular remodeling, has been proposed to aggravate pulmonary hypertension in CHF.[3] Our findings support this concept because reduced cGMP levels measured in plasma of rats with supracoronary aortic banding indicate a deficiency of the endogenous nitric oxide–guanylate cyclase pathway. Consistent with the functional effects of reduced nitric oxide bioavailability, CHF rats had increased transpulmonary pressure gradients and an increased PVR. Inhaled or intravenous vasodilators rapidly reduced PVR to values similar to those measured in sham-operated rats. Because vascular remodeling cannot be reversed within this short time interval, these findings indicate that PVR elevation in CHF resulted predominantly from increased vascular tone. In sham-operated rats, vasodilator therapy had no pulmonary hemody-

Table 4. Echocardiography

	Control		CHF	
	Before	After	Before	After
EF, %	92 ± 2.3	90 ± 3.0	88 ± 4.8	91 ± 2.0
FS, %	55 ± 3.8	56 ± 4.7	55 ± 6.5	57 ± 3.2
LVEDV, μl	142 ± 25.8	140 ± 20.4	192 ± 5.8*	191 ± 6.6*
IVSd, μm	234 ± 23.1	232 ± 25.4	324 ± 27.5*	322 ± 27.8*

Group data give echocardiographic data from control and congestive heart failure (CHF) rats before and 1 min after inhalation of 1 mg/ml milrinone. Data are from n = 4 rats in each group.

* $P < 0.05$ *vs.* control rats.

EF = ejection fraction; FS = fractional shortening; IVSd = thickness of the interventricular septum during diastole; LVEDV = left ventricular end-diastolic volume.

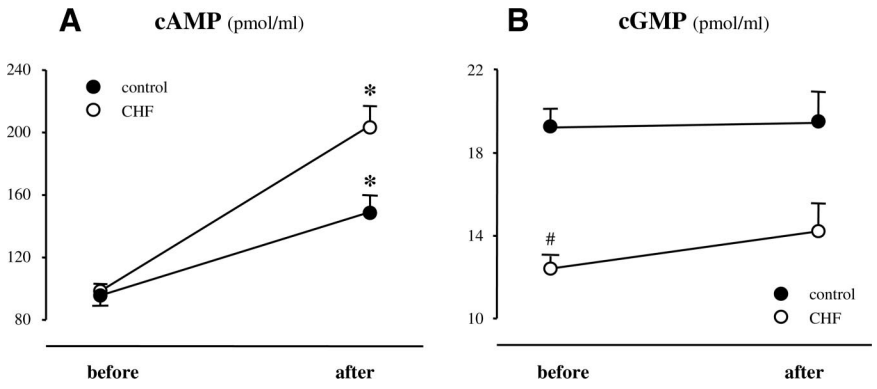

Fig. 5. Cyclic nucleotides. Group data show plasma concentrations of cyclic adenosine 3′,5′-monophosphate (cAMP; *A*) and cyclic guanosine 3′,5′ -monophosphate (cGMP; *B*) before and after inhalation of milrinone (1 mg/ml) in congestive heart failure (CHF; *open circles*) and control rats (*filled circles*). Data are from n = 11 rats in each group. * $P < 0.05$ *versus* baseline (before). # $P < 0.05$ *versus* control.

namic effects, which is in agreement with the notion that muscularized pulmonary vessels have only negligible tone under physiologic conditions.[21]

The vasodilatory phosphodiesterase-3 inhibitor milrinone induces vessel relaxation[22] and increases myocardial contractility[23] by increasing the intracellular concentration of cAMP in smooth muscle cells and cardiomyocytes and has been approved for short-term intravenous treatment of patients with acute decompensated heart failure.[24] Here, milrinone given either by intravenous infusion or by inhalation effectively reduced PVR in CHF rats. In addition, a small decrease in LAP contributed to the pulmonary antihypertensive effect of both treatment strategies. The latter finding suggests additional cardiac effects of milrinone, presumably by improving left ventricular contractility and relaxation.[25] The fact that the positive inotropic effect of milrinone is not reflected by changes in systemic blood flow and AP is likely due to the mechanical outflow obstruction in the aortic banding model. Right-to-left ventricular septal wall interactions may provide an alternative explanation because reduction of right ventricular afterload may decompress the left ventricle. But echocardiographic data do not support this notion, because LVEDV and ejection fraction did not increase with milrinone inhalation in CHF rats.

Pulmonary vasodilation by intravenous infusion of milrinone was dose-dependent and paralleled by a marked hypotensive response in the systemic circulation. Previously, it had been hypothesized that systemic vasodilation may be beneficial in CHF by reducing left ventricular afterload. However, several clinical trials investigating the effects of the calcium channel blocker nifedipine,[26] oral milrinone,[8] the arteriovenous dilator flosequinan,[27] or systemic infusion of the prostacyclin analog epoprostenol (Flolan®; GlaxoSmithKline, Research Triangle Park, NC)[9] revealed that systemic vasodilator therapy in CHF patients is detrimental in terms of clinical outcome. The reasons underlying the negative outcome of these clinical trials are complex and incompletely understood. Besides potential drug interactions and inadequate data on pharmacokinetics, pharmacodynamics, and dose–response relations,[27] adverse effects of systemic vasodilation may aggravate the deterioration of right ventricular contractility, *e.g.*, by reducing coronary perfusion pressure.[28]

Inhaled vasodilators may potentially circumvent these detrimental effects by acting predominantly in the pulmonary circulation with little or no vasodilatory effect in systemic blood vessels. Inhalation of 1 mg/ml milrinone reduced PAP more effectively than the maximal intravenous dose of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$, but without exerting systemic hemodynamic effects. A marked trend toward an increase in Svo_2 in the inhalation group but not in the intravenous group further suggests a beneficial potential for inhaled milrinone. The pulmonary vasodilatory effect of milrinone reached its maximum 20 min after inhalation and returned to a half-maximal effect after approximately 60 min, which is in general agreement with previously reported time profiles.[10,29] Repeated inhalation of milrinone in 20-min intervals warranted a stable reduction of PAP over the observation period of 1 h, demonstrating the potential of this strategy for prolonged treatment in CHF. Further studies in chronically instrumented animals are required to study the effects of long-term treatment by inhaled milrinone and potential rebound phenomena during subsequent weaning.

Major concerns in the treatment of CHF with vasodilators focus on the potential progression of lung edema and left ventricular dysfunction. The so-called Kitajew reflex, a precapillary pulmonary vasoconstrictive response to increased lung capillary pressure, has been postulated to protect the pulmonary capillary bed from excessive pressure increases in pulmonary venous hypertension.[30] Accordingly, vasodilator therapy may cause hydrostatic lung edema in pulmonary venous hypertension. In the current study, we detected a small but significant increase of lung wet/dry weight ratio in CHF rats as compared with sham-operated animals, indicating moderate lung edema, but this was not further aggravated by a single milrinone inhalation. This is in accordance with our findings that inhaled milrinone reduced PAP and vascular resistance in parallel without changing pulmonary flow, so that capillary pressure is likely to remain constant. However, precapillary vasoconstriction may constitute a protective mechanism when cardiac output is increased, and future studies are required to

address the safety of inhaled vasodilators during exercise or β_1-adrenergic stimulation. Repeated milrinone inhalations even reduced lung wet/dry weight ratios, indicating an additional antiedematous effect. This finding may be attributable to decreased capillary filtration due to a reduction in LAP or to a cAMP-mediated enhancement of lung microvascular barrier function.[31,32] Vasodilator therapy has also been proposed to increase left ventricular filling pressure due to an increased venous return to a poorly compliant left ventricle in CHF.[33] However, our data do not support this concept, because milrinone inhalation reduced LAP and lung wet/dry weight ratios without affecting LVEDV or ejection fraction in CHF rats.

Caution must be exercised when transferring findings from animal models to the clinical situation. However, similar pathophysiologic mechanisms and the results of two small nonrandomized trials suggest that inhaled milrinone may also reduce right ventricular afterload in CHF patients. Endothelial dysfunction and increased PVR are characteristics of CHF not only in rats, but also in humans.[3,34] In nine cardiac surgical patients in the immediate postoperative period and in nine heart transplant candidates, inhaled milrinone was shown to induce a vasodilatory effect in the lung in the absence of systemic hemodynamic effects.[10,29] Consistent with the results of our study, inhaled milrinone did not exhibit adverse effects in CHF patients because it reduced pulmonary capillary wedge pressure and increased neither intrathoracic blood volume nor extravascular lung water.[29] Therefore, inhaled milrinone may present an effective and safe clinical strategy for the treatment of pulmonary venous hypertension due to CHF.

The authors thank Sylvia May (Technician, Institute of Physiology, Charité-Universitätsmedizin Berlin, Berlin, Germany) for excellent technical assistance.

References

1. Cody RJ, Haas GJ, Binkley PF, Capers Q, Kelley R: Plasma endothelin correlates with the extent of pulmonary hypertension in patients with chronic congestive heart failure. Circulation 1992; 85:504–9

2. Christman BW, McPherson CD, Newman JH, King GA, Bernard GR, Groves BM, Loyd JE: An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. N Engl J Med 1992; 327:70–5

3. Moraes DL, Colucci WS, Givertz MM: Secondary pulmonary hypertension in chronic heart failure: The role of the endothelium in pathophysiology and management. Circulation 2000; 102:1718–23

4. Delgado JF, Conde E, Sanchez V, Lopez-Rios F, Gomez-Sanchez MA, Escribano P, Sotelo T, Gomez de la Camara A, Cortina J, de la Calzada CS: Pulmonary vascular remodelling in pulmonary hypertension due to chronic heart failure. Eur Heart J 2005; 7:1011–6

5. Abramson SV, Burke JF, Kelly JJ, Kitchen JG III, Dougherty MJ, Yih DF, McGeehin FC III, Shuck JW, Phiambolis TP: Pulmonary hypertension predicts mortality and morbidity in patients with dilated cardiomyopathy. Ann Intern Med 1992; 116:888–95

6. Polak JF, Holman BL, Wynne J, Colucci WS: Right ventricular ejection fraction: An indicator of increased mortality in patients with congestive heart failure associated with coronary artery disease. J Am Coll Cardiol 1983; 2:217–24

7. Di Salvo TG, Mathier M, Semigran MJ, Dec GW: Preserved right ventricular ejection fraction predicts exercise capacity and survival in advanced heart failure. J Am Coll Cardiol 1995; 25:1143–53

8. Packer M, Carver JR, Rodeheffer RJ, Ivanhoe RJ, DiBianco R, Zeldis SM, Hendrix GH, Bommer WJ, Elkayam U, Kukin ML, Mallis GI, Sollano JA, Shannon J, Tandon PK, DeMets DL: Effect of oral milrinone on mortality in severe chronic heart failure. The PROMISE Study Research Group. N Engl J Med 1991; 325:1468–75

9. Califf RM, Adams KF, McKenna WJ, Gheorghiade M, Uretsky BF, McNulty SE, Darius H, Schulman K, Zannad F, Handberg-Thurmond E, Harrell FE Jr, Wheeler W, Soler-Soler J, Swedberg K. A randomized controlled trial of epoprostenol therapy for severe congestive heart failure: The Flolan International Randomized Survival Trial (FIRST). Am Heart J 1997; 134:44–54

10. Haraldsson A, Niels Kieler-Jensen, Ricksten S: The additive pulmonary vasodilatory effects of inhaled prostacyclin and inhaled milrinone in postcardiac surgical patients with pulmonary hypertension. Anesth Analg 2001; 93:1439–45

11. Olschewski H, Olschewski A, Rose F, Schermuly R, Schütte H, Weissmann N, Seeger W, Grimminger F: Physiologic basis for the treatment of pulmonary hypertension. J Lab Clin Med 2001; 138:287–97

12. Natori S, Hasebe N, Jin YT, Matsusaka T, Ido A, Matsuhashi H, Ihara T, Kikuchi K: Inhaled nitric oxide modifies left ventricular diastolic stress in the presence of vasoactive agents in heart failure. Am J Respir Crit Care Med 2003; 167:895–901

13. Institute of Laboratory Animal Resources, Commission on Life Sciences: Guide for the Care and Use of Laboratory Animals, 7th edition. Washington, DC, National Academy Press, 1996

14. Backs J, Haunstetter A, Gerber SH, Metz J, Borst MM, Strasser R, Kübler W, Haass M: The neuronal norepinephrine transporter in experimental heart failure: Evidence for a posttranscriptional downregulation. J Mol Cell Cardiol 2001; 33:461–72

15. Habazettl H, Lindert J, Baeter S, Neumann K, Kuppe H, Kuebler WM, Pries AR, Koster A: Effects of unfractionated heparin, low molecular weight heparin and r-hirudin on leukocyte adhesion in ischemia/reperfusion. Blood Coagul Fibrinolysis 2004; 15:375–81

16. Lahana A, Costantopoulos S, Nakos G: The local component of the acute cardiovascular response to simulated apneas in brain-dead humans. Chest 2005; 128:634–9

17. Brueckl C, Kaestle S, Kerem A, Habazettl H, Krombach F, Kuppe H, Kuebler WM: Hyperoxia-induced reactive oxygen species formation in pulmonary capillary endothelial cells *in situ*. Am J Respir Cell Mol Biol 2005; 34:453–63

18. Walther T, Steendijk P, Westermann D, Hohmann C, Schulze K, Heringer-Walther S, Schultheiss HP, Tschope C: Angiotensin deficiency in mice leads to dilated cardiomyopathy. Eur J Pharmacol 2004; 493:161–5

19. Peterson BT, Brooks JA, Zack AG: Use of microwave oven for determination of postmortem water volume of lung. J Appl Physiol 1982; 52:1661–3

20. Tanaka H, Tajimi K, Kobayashi K: Milrinone improves arterial oxygenation in dogs with acute lung injury induced by oleic acid. J Cardiovasc Pharmacol 1999; 34:806–10

21. Kuebler WM, Uhlig U, Goldmann T, Schael G, Kerem A, Exner K, Martin C, Vollmer E, Uhlig S: Stretch activates nitric oxide production in pulmonary vascular endothelial cells *in situ*. Am J Respir Crit Care Med 2003; 168:1391–8

22. He GW, Yang CQ: Inhibition of vasoconstriction by phosphodiesterase III inhibitor milrinone in human conduit arteries used as coronary bypass grafts. J Cardiovasc Pharmacol 1996; 28:208–14

23. Jaski BE, Fifer MA, Wright RF, Braunwald E, Colucci WS: Positive inotropic and vasodilator actions of milrinone in patients with severe congestive heart failure: Dose-response relationships and comparison to nitroprusside. J Clin Invest 1985; 75:643–9

24. Levy JH, Bailey JM, Deeb GM: Intravenous milrinone in cardiac surgery. Ann Thorac Surg 2002; 73:325–30

25. Tanigawa T, Yano M, Kohno M, Yamamoto T, Hisaoka T, Ono K, Ueyama T, Kobayashi S, Hisamatsu Y, Ohkusa T, Matsuzaki M: Mechanism of preserved lusitropy by cAMP-dependent drugs in heart failure. Am J Physiol Heart Circ Physiol 2000; 278:H313–20

26. Packer M, Lee WH, Medina N, Yushak M, Bernstein JL, Kessler PD: Prognostic importance of the immediate hemodynamic response to nifedipine in patients with severe left ventricular dysfunction. J Am Coll Cardiol 1987; 10:1303–11

27. Van Veldhuisn DJ, Poole-Wilson PA: The underreporting of results and possible mechanisms of "negative" drug trials in patients with chronic heart failure. Int J Cardiol 2001; 80:19–27

28. Langer F, Wendler O, Wilhelm W, Tscholl D, Schafers HJ: Treatment of a case of acute right heart failure by inhalation of iloprost, a long-acting prostacyclin analogue. Eur J Anaesthesiol 2001; 18:770–3

29. Sablotzki A, Starzmann W, Scheubel R, Grond S, Czeslick EG: Selective pulmonary vasodilation with inhaled aerosolized milrinone in heart transplant candidates. Can J Anaesth 2005; 52:1076–82

30. Schamarin PI: About the Kitajew reflex: Mechanism of mitral valve vitium compensation. Terapiszeskij Archiv 1952; 24:79–85

31. Moore TM, Chetham PM, Kelly JJ, Stevens T: Signal transduction and regulation of lung endothelial permeability: Interaction between calcium and cAMP. Am J Physiol 1998; 275:L574–82

32. Tarpey SB, Sawmiller DR, Kelly C, Thompson WJ, Townsley MI: Phosphodiesterase 3 activity is reduced in dog lung following pacing-induced heart failure. Am J Physiol Lung Cell Mol Physiol 2003; 284:L766–73

33. Bocchi EA, Bacal F, Auler JO, Carmone MJ, Bellotti G, Pileggi F: Inhaled nitric oxide leading to pulmonary edema in stable severe heart failure. Am J Cardiol 1994; 74:70–2

34. Loh E, Stamler JS, Hare JM, Loscalzo J, Colucci WS: Cardiovascular effects of inhaled nitric oxide in patients with left ventricular dysfunction. Circulation 1994; 90:2780–5