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Inhibition of Platelet Aggregation by Inhaled Nitric Oxide in Patients with Acute Respiratory Distress Syndrome

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Background: **Nitric oxide inhibits platelet adhesion and aggregation *in vitro*. The aim of this prospective study was to assess the platelet antiaggregating activity of nitric oxide administered to patients with acute respiratory distress syndrome (ARDS) at increasing concentrations.**

Methods: **In six critically ill patients (mean age 37 ± 16 yr) with ARDS (lung injury severity score ≥ 2.2), the lungs were mechanically ventilated with inhaled nitric oxide (1, 3, 10, 30, and 100 ppm) randomly administered. Patients with cardiac dysrhythmias, septic shock, an underlying hemostasis disorder (constitutive or acquired), a platelet count less than 100 Giga/l, or a decreased platelet aggregation and those treated with antiplatelet or anticoagulant agents were excluded. Platelet aggregation was measured without nitric oxide and at each nitric oxide concentration in platelet-rich plasma issued from radial artery. Ivy bleeding time using a horizontal incision was simultaneously performed.**

Results: **After nitric oxide, a non-dose-dependent but statistically significant decrease in *ex vivo* platelet aggregation induced by three aggregating agents was observed: adenosine diphosphate = −56 ± 18%, collagen = −37 ± 18%, and ristocetin = −45 ± 18% ($P < 0.05$). In each individual, Ivy bleeding time remained within normal values measured in healthy volunteers, and variations after nitric oxide did not correlate with changes in platelet aggregation. Simultaneously, arterial oxygenation improved significantly and pulmonary artery pressure decreased significantly.**

Conclusions: **In patients with ARDS and without preexisting coagulation disorders, the beneficial effects of inhaled nitric oxide on arterial oxygenation and pulmonary circulation are associated with a significant inhibition of platelet aggregation. This antithrombotic effect is not associated with a significant prolongation of the bleeding time. (Key words: Anesthetic techniques: mechanical ventilation. Blood: Ivy bleeding time; platelet aggregation. Gases: nitric oxide. Lung: acute respiratory distress syndrome.)**

ACUTE respiratory distress syndrome (ARDS) is characterized by a combination of nonspecific alveolar damage and extensive pulmonary vascular disease.[1] Pulmonary arterial hypertension and increased pulmonary vascular resistance have been identified as markers of the severity of ARDS and are related to vascular thrombosis[2] and pulmonary vasoconstriction. A local and general activation of the coagulation process involving platelets has been described in ARDS.[3] Inhaled nitric oxide is a selective pulmonary vasodilator[4] that decreases pulmonary artery pressure *via* a reduction in pulmonary vascular resistance. When inhaled, nitric oxide acts as a selective pulmonary vasodilator in ventilated lung areas and increases arterial oxygenation by diverting pulmonary blood flow from nonventilated to ventilated lung regions.[5–7]

In vitro studies have shown that nitric oxide causes antiplatelet effects by activating intraplatelet guanylate cyclase and thereby increasing platelet cyclic guanosine monophosphate (cGMP). In turn, cGMP-dependent protein kinase is stimulated, resulting in a reduction in fibrinogen binding to glycoprotein GP IIb/IIIa, inducing partial inhibition of platelet aggregation, inhibition of phosphorylation of myosin light chains and of protein kinase C, stimulation of phosphorylation of the subunit of glycoprotein I, and modulation of phospholipase A2- and C-mediated responses.[8] cGMP-reg-

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Materials and Methods

Patients

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study during an 8-month perio
severe ARDS responding to inhal
of decreased pulmonary artery
arterial oxygenation were enrol
sion criteria were (1) lung injur
or equal to 2.2 and (2) positi
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artery pressure of at least 2 mm
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inhalation at a concentration of
teria were (1) circulatory shoc
arterial pressure < 90 mmHg
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rhythmias; (3) treatment with
agulant agents (aspirin, nonster
agents, heparin, oral anticoagul
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der (constitutive or acquired);
100 Giga/l; and (6) a decrease
defined as a maximal intensity
less than the lowest value obse
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for collagen, and <70% for risto
The trachea of each patient ha
an endotracheal tube that incor

ulated responses decreases intracellular Ca^{2+} by inhibition of agonist-mediated calcium flux.[9] Until the current study, no *ex vivo* study has been performed in patients treated with inhaled nitric oxide regarding its antiplatelet activity. An antithrombotic effect and/or an increase in the bleeding risk might be expected in patients with ARDS receiving inhaled nitric oxide if the antiplatelet effect is of sufficient magnitude. This prospective study was conducted to measure the effect of increasing concentrations of inhaled nitric oxide on platelet aggregation *ex vivo* and Ivy bleeding time *in vivo* in critically ill patients with severe ARDS.

Materials and Methods

Patients

This study was approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale of La Pitié-Salpétrière Hospital and supported by l'Assistance Publique-Hôpitaux de Paris. Written informed consent was obtained from each patient's next of kin. Consecutive patients diagnosed with ARDS on or after admission to the surgical intensive care unit of La Pitié Hospital in Paris (Department of Anesthesiology) were included in this prospective study during an 8-month period. Only patients with severe ARDS responding to inhaled nitric oxide in terms of decreased pulmonary artery pressure and increased arterial oxygenation were enrolled in the study. Inclusion criteria were (1) lung injury severity score greater or equal to 2.2 and (2) positive response to inhaled nitric oxide defined as a decrease in mean pulmonary artery pressure of at least 2 mmHg and an increase in Pa_{O_2} (FI_{O_2} 1) of at least 40 mmHg after nitric oxide inhalation at a concentration of 10 ppm. Exclusion criteria were (1) circulatory shock, defined as a systolic arterial pressure < 90 mmHg or dependence on exogenous catecholamines; (2) documented cardiac dysrhythmias; (3) treatment with antiplatelet or anticoagulant agents (aspirin, nonsteroidal antiinflammatory agents, heparin, oral anticoagulants) or drugs interfering with hemostasis; (4) underlying hemostasis disorder (constitutive or acquired); (5) a platelet count < 100 Giga/l; and (6) a decreased platelet aggregation defined as a maximal intensity of platelet aggregation less than the lowest value observed in healthy volunteers (<20% for adenosine diphosphate (ADP), <75% for collagen, and <70% for ristocetin).

The trachea of each patient had been intubated with an endotracheal tube that incorporates two side ports, one that runs to the distal tip of the endotracheal tube and one more proximal that ends 6 cm from the tip (Hi-Lo Jet Tracheal Tube no. 8, Mallinckrodt, Argyle, NY). These additional channels were used for continuous monitoring of tracheal pressure and tracheal concentrations of inhaled nitric oxide.

Once identified, all patients were sedated and paralyzed with a continuous intravenous infusion of 250 μg/h fentanyl, 1 mg/h flunitrazepam, and 4 mg/h vecuronium, and their lungs were ventilated using continuous positive pressure in a conventional volume-controlled mode (César ventilator, Taema, Antony, France). Minute ventilation was adjusted to maintain Pa_{CO_2} between 35 and 45 mmHg. The fraction of inspired oxygen (FI_{O_2}) was continuously monitored using an oxygen analyzer (Séres 2000, precision = 0.5%) and maintained at 0.85 for the duration of the study. Positive end-expiratory pressure was maintained at 10 cmH_2O and the inspiratory time at 30% to provide optimal alveolar recruitment. In all patients, hemodynamic monitoring included the use of a fiberoptic thermodilution pulmonary artery catheter (Oximetrix Opticath catheter, Abbott Critical Care System-France, Rungis, France) and a radial or femoral arterial catheter.

Nitric Oxide Administration

Nitric oxide was administered to all patients as follows: Nitric oxide was released from three tanks of nitrogen with nitric oxide concentrations of 25, 900, and 2,235 ppm measured using chemiluminescence (Air Liquide, Paris-LaDéfense, France). Nitric oxide was continuously delivered within the inspiratory limb of the ventilator before the Y piece by using a nitrogen flowmeter calibrated in the range 0.250 − 1 l/min. Three intratracheal concentrations of inhaled nitric oxide were administered in a randomized order: 1, 3, 10, 30, and 100 ppm. Nitric oxide concentrations of 1 and 3 ppm were obtained using the 25-ppm tank, nitric oxide concentrations of 10 and 30 ppm were obtained using the 900-ppm tank, and the concentration of 100 ppm was obtained using the 2,235-ppm tank. In each condition, the nitric oxide flow was adjusted to obtain the desired intratracheal nitric oxide concentration, and tidal volume and FI_{O_2} were adjusted to compensate for the added volume of nitric oxide gas. Thus, by keeping respiratory frequency constant, minute ventilation and FI_{O_2} delivered to the patient remained unchanged regardless whether nitric oxide was administered. Control measurements were systematically performed before nitric oxide administration (C_1)

and after the last nitric oxide concentration (C_2). Endotracheal concentrations of nitric oxide and nitrogen dioxide were continuously measured using a chemiluminescence apparatus (NOX 2000 Séres, Aix-en-Provence, France, precision = 0.005 ppm). An operating range of 0–5 ppm was selected for measuring intratracheal nitric oxide concentrations of 1 and 3 ppm and the NOX 2000 was calibrated using a tank of nitrogen containing 0.9 ppm of nitric oxide (Air Liquide). An operating range of 0–100 ppm was selected for measuring intratracheal nitric oxide concentrations of 10, 30, and 100 ppm, and the NOX 2000 was calibrated using a tank of nitrogen containing 25 ppm of nitric oxide (Air Liquide). When a different operating range of measurement was used, systematic recalibration of the NOX 2000 was performed to increase the precision of measurement. Intratracheal gas was continuously sampled using a continuous aspiration of 150 ml/min through the proximal side port of the Mallinckrodt endotracheal tube. At this aspiration flow rate, the time response of the NOX 2000 was about 40 s. Therefore, only mean intratracheal concentrations of nitric oxide were measured, and fluctuations between inspiratory and expiratory concentrations were not evaluated. The NOX 2000 also was used for continuous monitoring of oxygen concentration to ensure that $F_{I_{O_2}}$ was maintained close to 0.85 during nitric oxide administration. This technique of nitric oxide administration (high nitric oxide concentration tank and low flow of nitric oxide) was intended to avoid any significant decrease in $F_{I_{O_2}}$ during nitric oxide inhalation.

Hemodynamic Measurements

In each patient, systolic and diastolic arterial pressures and systolic and diastolic pulmonary arterial pressures were measured simultaneously using the arterial cannula and the fiberoptic pulmonary artery catheter connected to two calibrated pressure transducers (91 DPT-308 Mallinckrodt) positioned at the midaxillary line. Systemic and pulmonary arterial pressures, electrocardiogram, and tracheal pressure (Paw), measured through the distal port of the endotracheal tube, were recorded simultaneously on a Gould ES 1000 recorder (Cleveland, OH) for two different conditions: control (without nitric oxide) and during administration of nitric oxide at a concentration of 3 ppm.

In each phase, when a steady-state was obtained—defined as a relatively constant pulmonary arterial pressure—systolic and diastolic arterial pressures, systolic and diastolic pulmonary arterial pressures, pulmonary capillary wedge pressure, right atrial pressure, and Paw were recorded at a speed of 50 mm/s. Mean arterial pressure was calculated as one-third systolic arterial pressure plus two-thirds diastolic arterial pressure. Mean pulmonary artery pressure was measured by planimetry as the mean of four measurements performed at end-expiration. Systolic and diastolic arterial pressures, systolic and diastolic pulmonary arterial pressures, pulmonary capillary wedge pressure, and right atrial pressure also were measured at end-expiration. Cardiac output was measured using the thermodilution technique and a bedside computer allowing the recording of each thermodilution curve (Oximetrix 3 SO2/CO computer). Four serial injections of 10 ml of saline at room temperature performed at random during the respiratory cycle were used to average the variations in cardiac output related to the inspiratory and expiratory phases of continuous positive-pressure ventilation. Heart rate was measured from the recorded electrocardiogram. Systemic and pulmonary arterial blood samples were simultaneously withdrawn within 1 min after the measurements of cardiac output (after discarding an initial 10-ml heparin-contaminated aliquot). Arterial *p*H, Pa_{O_2}, Pv_{O_2}, and Pa_{CO_2} were measured using a BGE blood gas analyzer (Instrumentation Laboratory-France, Paris, France). Hemoglobin concentration, methemoglobin concentration, and hemoglobin oxygen saturations (Sa_{O_2} and Sv_{O_2}) were measured using a calibrated OSM3 hemoximeter (Radiometer Copenhagen-France, Neuílly-Plaísance, France). We used standard formula to calculate cardiac index, pulmonary vascular resistance index, systemic vascular resistance index, intrapulmonary shunt, and oxygen delivery.

Hemostasis Study

Method of Sampling. Arterial blood samples were collected in 3.8% trisodium citrated tubes (9:1 vol/vol, Becton Dickinson-France, Le Pont de Claix, France) for platelet aggregation measurements and in EDTA tubes (Becton Dickinson) for platelet count and hematocrit. To prevent platelet activation inside the arterial line, 20 ml of arterial blood was first slowly and smoothly sampled and discarded; then, a 20-ml arterial blood sample was collected for platelet aggregation analysis. Blood samples were collected before administration of nitric oxide (control 1), after each concentration of nitric oxide when a 30-min steady-state was obtained, and after nitric oxide cessation (control 2).

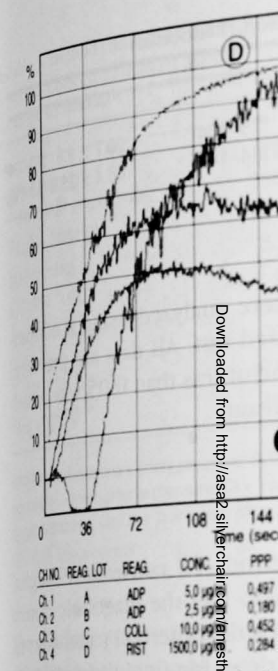

Fig. 1. Maximal intensity and velocit
(line B), and collagen (COLL; line C)
line D) in patient 4 at control (*left*)
to each agonist; CONC = concentrat
MAX TIME = time necessary to reach
of the platelet rich plasma; REAG =

Platelet Aggregation. In a pr
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was prepared from the same blo
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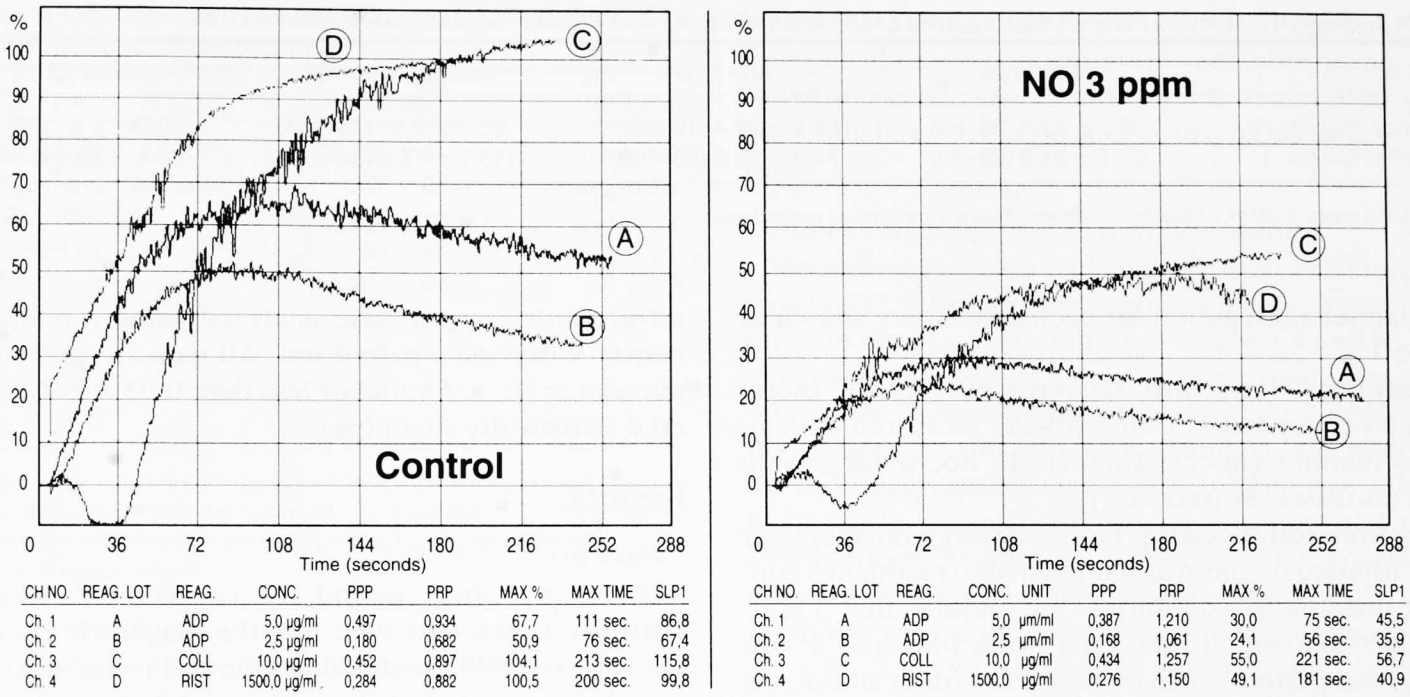

CH NO.	REAG. LOT	REAG.	CONC.	PPP	PRP	MAX %	MAX TIME	SLP1
Ch. 1	A	ADP	5,0 µg/ml	0,497	0,934	67,7	111 sec.	86,8
Ch. 2	B	ADP	2,5 µg/ml	0,180	0,682	50,9	76 sec.	67,4
Ch. 3	C	COLL	10,0 µg/ml	0,452	0,897	104,1	213 sec.	115,8
Ch. 4	D	RIST	1500,0 µg/ml	0,284	0,882	100,5	200 sec.	99,8

CH NO.	REAG. LOT	REAG.	CONC.	UNIT	PPP	PRP	MAX %	MAX TIME	SLP1
Ch. 1	A	ADP	5,0	µm/ml	0,387	1,210	30,0	75 sec.	45,5
Ch. 2	B	ADP	2,5	µm/ml	0,168	1,061	24,1	56 sec.	35,9
Ch. 3	C	COLL	10,0	µg/ml	0,434	1,257	55,0	221 sec.	56,7
Ch. 4	D	RIST	1500,0	µg/ml	0,276	1,150	49,1	181 sec.	40,9

Fig. 1. Maximal intensity and velocity of platelet aggregation induced by 5 µM adenosine diphosphate (ADP; line A), 2.5 µM ADP (line B), and collagen (COLL; line C) and maximal intensity and velocity of platelet agglutination induced by ristocetin (RIST; line D) in patient 4 at control (*left*) and 30 min after 3 ppm of nitric oxide exposure (*right*). CH NO = channel corresponding to each agonist; CONC = concentration of the agonist; MAX % = maximal intensity of platelet aggregation and agglutination; MAX TIME = time necessary to reach maximal intensity; PPP = optical density of the platelet poor plasma; PRP = optical density of the platelet rich plasma; REAG = agonist; SLP1 = velocity of platelet aggregation and agglutination.

Platelet Aggregation. In a previous study, we were unable to detect any nitric oxide-induced inhibition of platelet aggregation and agglutination in patients with acute respiratory failure receiving 2 ppm of inhaled nitric oxide.[10] In fact, this "negative" result was due to a methodologic artifact: any delay between arterial sampling and platelet aggregation analysis may result in a false "normalization" of nitric oxide-induced inhibition of platelet aggregation. This effect is likely related to an *in vitro* loss of nitric oxide. In the current study, the following technique was used for measuring platelet aggregation and agglutination. After arterial sampling, the tubes were centrifuged and platelet aggregation analysis was performed immediately. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 1,500 g for 1 min. Platelet-poor plasma was prepared from the same blood sample by centrifuging blood at 1,500 g for 15 min. The platelet count in PRP was adjusted between 250 and 350 Giga/l by addition of autologous platelet-poor plasma. Aggregation and agglutination tests were performed according to the turbidimetric method of Born.[11] The PRP was incubated under continuous stirring (900 rounds per minute) at 37°C. Platelet aggregation was induced by 2.5 and 5 µM ADP and 10 µg/ml collagen (type I; Helena-France, St. Leu, France). Platelet agglutination was induced by 1.5 mg/ml ristocetin (Helena-France). These automated tests were performed on a Helena Packs 4 aggregometer (Helena-France). The increase in light transmission was recorded for 4 min after addition of the different aggregating agents (agonists). Aggregation and agglutination induced by the different agonists in PRP were evaluated by measuring light transmission in stimulated PRP, assuming that light transmission was 100% in platelet-poor plasma and 0% in nonstimulated PRP. As shown in figure 1, maximal intensity of platelet aggregation was defined as the maximal increase in light transmission and velocity of platelet aggregation as the speed of the increase in light transmission increase, after each aggregating agent, as computed by the software. The software and the aggregometer/computer interface for this operation and the generation of printed platelet aggregations reports were designed and developed by Helena. This technique was first tested in ten healthy volunteers who had not taken any antiplatelet agent for the preceding 10 days. Normal values and ranges for maximal intensity and velocity

Table 1. Normal Values of Platelet Aggregation (ADP and Collagen) and Platelet Agglutination (Ristocetin)

	ADP 2.5 μM	ADP 5 μM	Collagen 10 $\mu g \cdot ml^{-1}$	Ristocetin 1.5 $mg \cdot ml^{-1}$
Maximal intensity (%)	37.2 ± 17.8 (20–65)	55.2 ± 22.4 (19–85)	90.4 ± 8.1 (75–104)	90.7 ± 9.8 (70–104)
Velocity ($\% \cdot min^{-1}$)	54.7 ± 24.9 (29–90)	74.9 ± 25.8 (28–109)	113.7 ± 17.5 (84–147)	88.4 ± 25.9 (58–147)

Values are mean ± SD in 10 healthy volunteers. Ranges are given in parentheses.

of platelet aggregation for each agonist are shown in table 1.

Platelet Count and Hematocrit. Whole blood platelet count and hematocrit were measured with an Argos monitor (Roche, Hoffman La Roche Diagnostic Systems, Basel, Switzerland).

Ivy Incision Bleeding Time. A sphygmomanometer was installed on the arm and inflated to maintain a constant pressure of 40 mmHg. One minute after, a standardized horizontal incision (5 mm long and 1 mm deep) was performed on the forearm with an automated device (Simplate, Organon Teknika, Durham, NC). The incision was blotted every 30 s by a filter paper until the bleeding stopped. Normal values of the bleeding time have been reported to be in the range 4–8 min[12]. Ivy bleeding time was measured immediately after each arterial sampling corresponding to C1, C2, and the different nitric oxide concentrations.

Statistical Analysis

The effects of increasing concentrations of inhaled nitric oxide on platelet aggregation and agglutination were measured using a one-way analysis of variance for repeated measures followed by Fisher's exact test. Nitric oxide 3 ppm-induced inhibition of platelet aggregation was compared for the three agonists (ADP, collagen, and ristocetin) using a one-way analysis of variance for repeated measures followed by a Fisher's exact test. Hemodynamic and respiratory effects induced by nitric oxide 3 ppm were analyzed using a nonparametric Wilcoxon's paired test. All data are presented as mean ± SD. A P value of less than 0.05 was considered statistically significant.

Results

Patients

During the study period, 18 consecutive surgical critically ill patients who met the diagnostic criteria for severe ARDS (inclusion criteria 1) and who responded to inhaled nitric oxide (inclusion criteria 2) were screened for inclusion into the study. Among these patients, one was excluded because he was receiving aspirin, four because they had a baseline platelet count < 100 Giga/l, and seven because they had a baseline decreased platelet aggregating activity, when compared to the healthy volunteers group. Thus, six male patients (age 37 ± 16 yr) were included in the study: Four were admitted after trauma and two after surgical procedures (orthopedic and gastrointestinal surgery). The initial clinical data for the patients, recorded on the day of inclusion into the study, just before initiation of the protocol and during intermittent positive-pressure ventilation (FI_{O_2} 1.0, inspiratory time 30%, and 0 positive end-expiratory pressure) are presented in table 2.

Hemodynamic and Respiratory Changes

Hemodynamic and respiratory data measured with and without nitric oxide are summarized in table 3.

Table 2. Initial Clinical Data of the Six Patients Collected on the Day of Inclusion in the Study

Patient No.	Age (yr)	Sex	Cause of ARDS	Pa_{O_2} (mmHg)	Qs/Qt (%)	MPAP (mmHg)	PVRI ($dyne \cdot s \cdot cm^{-5} \cdot m^2$)	LISS	Hematocrit (%)	Platelet Count ($g \cdot l^{-1}$)	Outcome
1	35	M	MT	256	33.2	31	393	2.25	21.6	206	Survived
2	25	M	MT	154	33.1	19	311	2.25	25.4	270	Survived
3	64	M	SC	126	34.1	18	322	2	27.1	292	Survived
4	26	M	SC	99	35.2	22	255	2.3	24	398	Survived
5	48	M	MT	164	30.2	28	266	2.7	19.5	300	Survived
6	26	M	MT	67	52	39	474	3.7	24.4	232	Survived

MT = multiple trauma; SC = surgical complications following major surgical procedures; ARDS = adult respiratory distress syndrome; PVRI = pulmonary vascular resistance index; LISS = lung injury severity score.

Table 3. Hemodynamic and Respira NO at a Concentration of 3 ppm

	C
MPAP (mmHg)	26
PVRI ($dyne \cdot s \cdot cm^{-5} \cdot m^2$)	337
Pa_{O_2}/FI_{O_2} (mmHg)	151
MAP (mmHg)	83
CI ($l \cdot min^{-1} \cdot m^{-2}$)	4.7
Sv_{O_2} (%)	65
Qs/Qt (%)	36
D_{O_2} ($ml \cdot min^{-1} \cdot m^{-2}$)	477
Pa_{CO_2} (mmHg)	46

MPAP = mean pulmonary artery pressure; PVR
index; MAP = mean arterial pressure; CI = ca
oxygen saturation; Qs/Qt = pulmonary shunt
* $P < 0.05$ *versus* control.

The administration of inhaled
a significant reduction in mean
sure and pulmonary vascular
0.05), whereas heart rate, cardi
pressure, right atrial pressure
wedge pressure, systemic vasc
oxygen consumption, oxygen d
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nificant increase in Pa_{O_2} and Sv
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0.05) after nitric oxide admini
sation of inhaled nitric oxide,
sure and Pa_{O_2} returned to contro
(data not shown).

Blood Methemoglobin Conc
Tracheal Concentrations of

After nitric oxide administrat
of 3 ppm, there was no signific

Table 4. Effects of Increasing Conce Induced by Three Different Agonist

	ADP
Control 1	44
NO 1 ppm	26
NO 3 ppm	21
NO 10 ppm	22
NO 30 ppm	31
NO 100 ppm	29
Control 2	41
Statistical significance (*P*)	

Changes in maximal intensity are expressed in

Table 3. Hemodynamic and Respiratory Effects of Inhaled NO at a Concentration of 3 ppm

	Control	NO 3 ppm
MPAP (mmHg)	26 ± 8	21 ± 8*
PVRI (dyne · s · cm^{-5} · m^2)	337 ± 83	262 ± 75*
Pa_{O_2}/FI_{O_2} (mmHg)	151 ± 70	244 ± 83*
MAP (mmHg)	83 ± 17	81 ± 9
CI (l · min^{-1} · m^{-2})	4.7 ± 0.9	4.4 ± 1.3
Sv_{O_2} (%)	65 ± 8	69 ± 7*
Qs/Qt (%)	36 ± 8	30 ± 4*
D_{O_2} (ml · min^{-1} · m^{-2})	477 ± 82	446 ± 129
Pa_{CO_2} (mmHg)	46 ± 11	45 ± 11

MPAP = mean pulmonary artery pressure; PVRI = pulmonary vascular resistance index; MAP = mean arterial pressure; CI = cardiac index; Sv_{O_2} = mixed venous oxygen saturation; Qs/Qt = pulmonary shunt; D_{O_2} = oxygen delivery.

* $P < 0.05$ *versus* control.

The administration of inhaled nitric oxide resulted in a significant reduction in mean pulmonary artery pressure and pulmonary vascular resistance index ($P < 0.05$), whereas heart rate, cardiac index, mean arterial pressure, right atrial pressure, pulmonary capillary wedge pressure, systemic vascular resistance index, oxygen consumption, oxygen delivery, and oxygen extraction ratio remained unchanged. There was a significant increase in Pa_{O_2} and Sv_{O_2} ($P < 0.05$), with a concomitant reduction in intrapulmonary shunt ($P < 0.05$) after nitric oxide administration. After the cessation of inhaled nitric oxide, pulmonary artery pressure and Pa_{O_2} returned to control values in all patients (data not shown).

Blood Methemoglobin Concentrations and Tracheal Concentrations of Nitrogen Dioxide

After nitric oxide administration at a concentration of 3 ppm, there was no significant increase in methemoglobin plasma concentrations. Mean endotracheal nitrogen dioxide concentration measured using chemiluminescence was found to be 0.05 ± 0.003 ppm after inhalation of 3 ppm of nitric oxide at a FI_{O_2} of 0.85.

Platelet Aggregation and Agglutination

Platelet aggregation and agglutination (maximal intensity and velocity) were significantly decreased after nitric oxide administration (fig. 1, tables 4 and 5). The effect was maximal at 3 ppm of nitric oxide, reaching 50% of the control value but was not dose-dependent in the range 1–100 ppm (figs. 2 and 3). The effects of the three aggregating agents were inhibited by inhaled nitric oxide. At a nitric oxide concentration of 3 ppm, ADP-induced platelet aggregation and ristocetin-induced platelet agglutination were significantly more inhibited than collagen-induced platelet aggregation ($P < 0.05$, maximal intensity only). In four patients, platelet aggregation was measured in duplicate either immediately after blood sampling or after a 20-min delay; although a significant decrease in maximal intensity and velocity was evidenced when platelet aggregation was measured immediately after sampling, no inhibition could be found when platelet aggregation was measured after a 20-min delay.

Bleeding Time

No lengthening of Ivy bleeding time was observed after nitric oxide administration as compared to the control value. Furthermore, bleeding time remained in the normal range in all patients (fig. 4).

Discussion

This study demonstrates that platelet aggregation and agglutination induced *ex vivo* by three agonists—ADP,

Table 4. Effects of Increasing Concentrations of Inhaled NO on Maximal Intensity of Platelet Aggregation and Agglutination Induced by Three Different Agonists: ADP at Two Different Concentrations (2.5 μM and 5 μM), Collagen, and Ristocetin

	ADP 2.5 μM (%)	ADP 5 μM (%)	Collagen 10 μg · ml^{-1} (%)	Ristocetin 1.5 mg · ml^{-1} (%)
Control 1	44.7 ± 18.7	56.7 ± 16.5	81.8 ± 14.5	84.8 ± 12.9
NO 1 ppm	26.3 ± 19.8	33.3 ± 20.3	54.5 ± 16.2	51.5 ± 13.2
NO 3 ppm	21.5 ± 13.9	27.8 ± 11.6	51.0 ± 13.5	46.3 ± 15.2
NO 10 ppm	22.9 ± 10.7	30.8 ± 10.9	58.2 ± 8.8	51.1 ± 12.9
NO 30 ppm	31.3 ± 18.3	38.6 ± 20.7	59.6 ± 13.9	59.8 ± 18.9
NO 100 ppm	29.7 ± 16.1	37.4 ± 14.7	60.1 ± 15.3	52.6 ± 12.2
Control 2	41.1 ± 15.9	52.7 ± 17.4	87.1 ± 7.1	86.4 ± 10.9
Statistical significance (*P*)	0.0001	0.0001	0.002	0.0001

Changes in maximal intensity are expressed in absolute values (%).

Table 5. Effects of Increasing Concentrations of Inhaled NO on Velocity of Platelet Aggregation and Agglutination Induced by Three Different Agonists: ADP at Two Different Concentrations (2.5 μM and 5 μM), Collagen, and Ristocetin

	ADP 2.5 μM (% · min^{-1})	ADP 5 μM (% · min^{-1})	Collagen 10 μg · ml^{-1} (% · min^{-1})	Ristocetin 1.5 mg · ml^{-1} (% · min^{-1})
Control 1	60.3 ± 25.7	69.8 ± 23.0	86.4 ± 16.3	73.8 ± 8.8
NO 1 ppm	35.3 ± 25.7	42.5 ± 22.2	56.6 ± 23.9	42.8 ± 13.4
NO 3 ppm	29.1 ± 20.1	36.0 ± 16.1	50.1 ± 14.3	37.8 ± 15.4
NO 10 ppm	31.5 ± 16.1	38.2 ± 12.6	58.3 ± 10.8	40.0 ± 9.2
NO 30 ppm	36.9 ± 23.9	46.6 ± 21.5	60.1 ± 18.5	48.7 ± 19.7
NO 100 ppm	37.8 ± 20.0	41.3 ± 15.2	62.2 ± 23.7	41.6 ± 14.3
Control 2	54.5 ± 19.5	64.9 ± 18.8	86.0 ± 18.1	74.5 ± 10.9
Statistical significance (*P*)	0.0001	0.0001	0.0125	0.0001

Changes in velocity are expressed in absolute values (% · min^{-1}).

collagen, and ristocetin—is significantly inhibited in patients with ARDS receiving low doses of inhaled nitric oxide. This effect is obtained without any apparent prolongation of the bleeding time, in contrast to what has been reported in healthy volunteers.[13] Platelet aggregation inhibition by nitric oxide was first demonstrated *in vitro* by Mellion *et al.*[14] and then confirmed by Radomski *et al.*[15] The incubation of human PRP with nitric oxide resulted in a concentration-dependent inhibition of platelet aggregation induced by ADP, collagen, and thrombin. Nitric oxide was two- to threefold more potent in human washed platelets than in PRP. However, inhibition of aggregation of human washed platelets decayed (half-life approximately 2 min) and disappeared after 4 min. Furthermore, preincubation of platelets with hemoglobin or Fe^{2+} reduced the antiaggregating activity of nitric oxide. When comparing nitric oxide and prostacyclin-induced inhibition of platelet aggregation, the same authors found that nitric oxide was less potent than prostacyclin. Salvemini *et al.* also demonstrated that *in vitro* nitric oxide completely inhibited thrombin-induced platelet aggregation.[16] However, this inhibition was reversed by oxy-

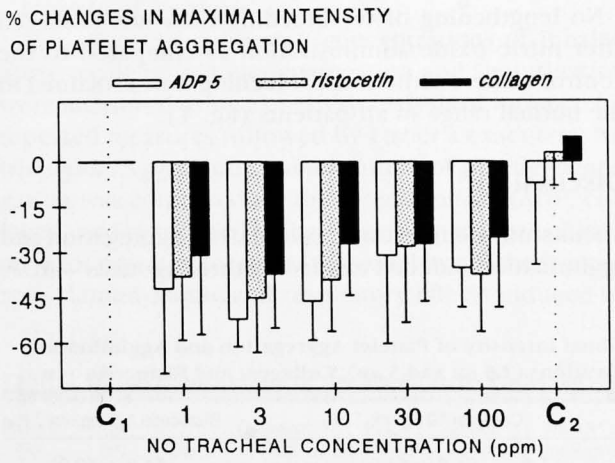

Fig. 2. Changes in maximal intensity of platelet aggregation induced by five randomized incremental concentrations of nitric oxide administered to six patients with acute respiratory distress syndrome. Changes are expressed in percentage of variation from the first control value (C1), which is by definition equal to 0%. Inhaled nitric oxide induced a significant and non-dose-dependent decrease in maximal intensity of platelet aggregation induced by three aggregating agents: 5 μM adenosine diphosphate (ADP), ristocetin, and collagen ($P < 0.01$).

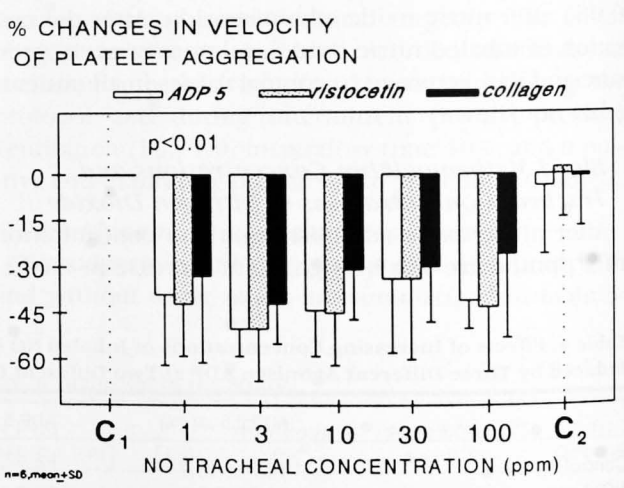

Fig. 3. Changes in velocity of platelet aggregation induced by five randomized incremental concentrations of nitric oxide administered to six patients with acute respiratory distress syndrome. Changes are expressed in percentage of variation from the first control value (C1), which is by definition equal to 0%. Inhaled nitric oxide induced a significant and non-dose-dependent decrease in velocity of platelet aggregation induced by three aggregating agents: 5 μM adenosine diphosphate (ADP), ristocetin, and collagen ($P < 0.01$).

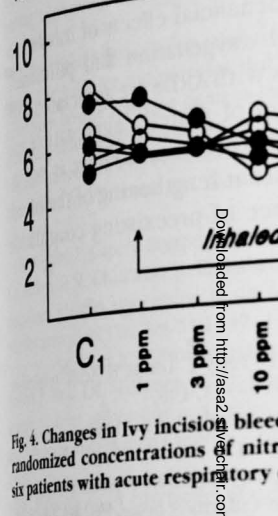

Fig. 4. Changes in Ivy incision bleed
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Despite the fact that, in the
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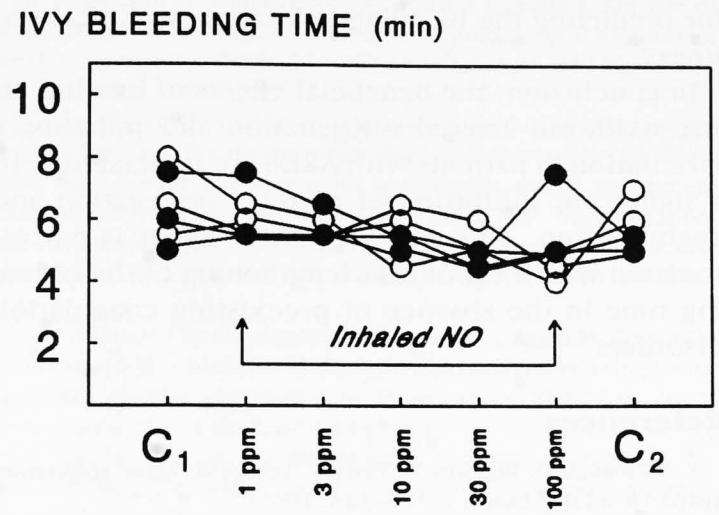

Fig. 4. Changes in Ivy incision bleeding time induced by five randomized concentrations of nitric oxide administered to six patients with acute respiratory distress syndrome.

hemoglobin administered *in vitro* 30 s to 10 min after thrombin-induced platelet aggregation, according to a time-dependent profile.[16] Nitric oxide is unstable, especially when stirred in solution,[17] as in the aggregometer cuvette. The current study confirms the *in vitro* findings that the inhibitory effect of inhaled nitric oxide on platelet aggregation disappears with time. After 20 min of prolonged centrifugation or conservation of the blood sample at room temperature, no platelet aggregation inhibition could be evidenced. Additional studies are required to elucidate the mechanisms by which inhaled nitric oxide-induced inhibition of platelet aggregation vanishes with time.

Despite the fact that, in the current study, inhaled nitric oxide was administered at increasing concentrations ranging between 1 and 100 ppm, nitric oxide-induced inhibition of platelet aggregation did not appear to be dose-dependent, the maximal inhibition being observed at 3 ppm. Further studies are needed to determine whether nitric oxide-induced inhibition of platelet aggregation is, like nitric oxide-induced decrease in pulmonary vascular resistance index,[6] dose-dependent in the range 0.1–3 ppm. The inhibition of platelet aggregation was less pronounced when collagen was used as the agonist as compared to ADP. It may be assumed that this difference was related to the dose of collagen used in the current study. It is likely that, if smaller concentrations of collagen had been chosen, nitric oxide-induced inhibition of platelet aggregation would have been the same with ADP and collagen. Because ristocetin-induced platelet agglutination was inhibited by inhaled nitric oxide to the same extent as was ADP-induced platelet aggregation, it is highly likely that inhaled nitric oxide inhibited both platelet adhesion and aggregation. However, these effects could be measured only *ex vivo*. The effect of inhaled nitric oxide on platelet function could be direct or indirect. Exogenous nitric oxide might increase platelet cGMP or induce the production of a substance that could modulate platelet function. Because nitric oxide-induced effects on platelet functions vanish with time, the true antiplatelet effect of inhaled nitric oxide *in vivo* cannot be assessed with certainty. Golino and Yao have shown that nitric oxide, either endogenous or exogenous, is able to inhibit intravascular platelet aggregation in the Folts model of carotid thrombosis in the rabbit.[18–20] After placing an external constrictor around endothelially injured arteries, cyclic flow reductions due to recurrent platelet aggregation were measured at the site of stenosis using a Doppler probe directly inserted on the artery. Soluble nitric oxide infused in the carotid completely abolished cyclic flow reductions in all animals. These effects were transient, and cyclic flow reductions were restored spontaneously within 10 min after cessation of nitric oxide infusion. Therefore, in the rabbit, nitric oxide was considered as an antithrombotic agent inhibiting platelet aggregation *in vivo* to an extent similar to aspirin and thromboxane synthase inhibitors. Because the antiaggregating potency of nitric oxide on washed platelets was found to be three- to fourfold lower in the rabbit than in humans,[15] it may be assumed that nitric oxide is a potent antithrombotic agent in humans.

Patients with ARDS often experience pulmonary arterial microthrombosis, which may increase pulmonary artery pressure, compromise right ventricular function, promote lung ischemia, and result in the development of severe fibrosis.[3,21] Platelet activation is involved in such a process,[22,23] and antiplatelet agents have been proposed as a prevention of these deleterious effects.[3] However, most of antiplatelet agents, such as prostacyclin and nitroprusside, are associated with important hemodynamic side effects that limit their routine use in patients with ARDS. In addition, most of these agents are nonselective pulmonary vasodilators and worsen gas exchange when administered to patients with acute respiratory failure.[5] In contrast, inhaled nitric oxide, which inhibits platelet adhesion and aggregation, is a selective pulmonary vasodilator and offers the possibility of combining a potent antithrombotic effect with an increase in arterial oxygenation and a reduction of pulmonary hypertension. Platelets are involved in the

early pulmonary hypertensive response observed in ARDS.[3,22] Infusion of ADP into sheep promotes platelet aggregation and generates pulmonary hypertension. The increase in pulmonary artery pressure is not observed if the animals are platelet-depleted.[24] Therefore, it can be hypothesized that an early use of inhaled nitric oxide, by preventing thrombi formation in the pulmonary circulation, could prevent, in part, the increase in pulmonary artery pressure characterizing late stages of ARDS. Inhaled nitric oxide could contribute indirectly to limiting the fixed part of pulmonary hypertension in addition to its well recognized ability to reverse pulmonary artery constriction.

Regarding the bleeding risk, nitric oxide plays a role in primary hemostasis. Remuzzi *et al.* showed that N-monomethyl-L-arginine, a specific inhibitor of nitric oxide formation, normalized bleeding when given to uremic rats.[25] This correction was reversed by giving the animals the nitric oxide precursor L-arginine. The authors have emphasized the role of nitric oxide as a mediator of the bleeding tendency of uremia. However, with respect to the potential effect of nitric oxide on primary hemostasis, in the current study performed in patients with ARDS and without preexisting coagulation disorders, no increase in the bleeding risk could be demonstrated because bleeding time was not prolonged. Several mechanisms might induce a discrepancy between inhaled nitric oxide-induced inhibition of platelet aggregation and its lack of effect on bleeding time. Low hematocrit and platelet count and edema, factors frequently encountered in critically ill patients, may interfere with primary hemostasis and induce a lengthening of Ivy bleeding time. It should be emphasized that, despite the fact that the patients of the current study had a decreased hematocrit (table 2), no prolongation of the bleeding time was observed. Confirming a previous study,[26] we recently found that the level of von Willebrand factor (antigen and activity) frequently is increased in critically ill patients with ARDS.[10] It could interfere with primary hemostasis[27] and contribute to a decrease in the bleeding time. Our results contrast with Högman's showing a 33% prolongation of the bleeding time 15 min after administration of 30 ppm of nitric oxide in the rabbit[28] and in six healthy volunteers.[13] Reasons for these opposite results are not clear, but it may be assumed that factors influencing primary hemostasis might be different in rabbits, in healthy volunteers, and in patients with ARDS. Furthermore, there is a general agreement for considering that bleeding time is neither sensitive nor specific for predicting the bleeding risk in many clinical settings.[29]

In conclusion, the beneficial effects of inhaled nitric oxide on arterial oxygenation and pulmonary circulation in patients with ARDS are associated with a significant inhibition of platelet aggregation and agglutination. This antithrombotic effect is not associated with a significant lengthening of the bleeding time in the absence of preexisting coagulation disorders.

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