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Effects of cardiopulmonary bypass and inhaled nitric oxide on platelets in children with congenital heart defects

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Abstract Nitric oxide (NO) reduces platelet aggregation in vitro. However, repeated measurements of platelet aggregation in infants and small children are impossible due to the large blood samples required. Instead, the expression of different platelet receptors mediating platelet adhesion (CD 36 and CD 42b), activation (CD 42b and CD 61) and aggregation (CD 41a) was measured repeatedly by flow cytometry. First, the expression of platelet receptors was quantified in platelet suspensions of 20 healthy volunteers after incubation with different concentrations of NO (0, 25, 100 and 640 ppm) and compared to changes in platelet aggregation and intrathrombocytic cGMP levels. It was then studied in 21 infants and children before, during and up to 3 days after cardiopulmonary bypass surgery. Seven of these patients required NO inhalation postoperatively. The in vitro experiments showed a reduced expression of the CD 41a, CD 42b and CD 61 receptors with increasing doses of NO, predominantly affecting the CD 41a receptor (−11% at 100 ppm and −20% at 640 ppm). This significant effect is in keeping with the observed NO-induced inhibition of platelet aggregation (−44% at 100 ppm) and the rise in platelet cGMP levels (+69% at 100 ppm). In patients without inhaled NO, the expression of CD 41a was slightly attenuated during cardiopulmonary bypass surgery (−15%) but increased significantly afterwards (2 h: +31%, 1st day: +129%, 2nd day: +120%, 3rd day: +111%). Comparable results were obtained regarding the other adhesion molecules CD 36, CD 42b and CD 61. In patients with inhaled NO the same pattern was observed and analysis of variance did not reveal any significant difference between both groups of patients.

Conclusions NO (≥100 ppm) decreases the expression of different platelet adhesion molecules and platelet aggregation, presumably via an increase in intracellular cGMP. However, due to the low dose range used in the clinical setting (1–40 ppm) this is clinically not relevant. Immediately after cardiopulmonary bypass surgery the expression of these adhesion molecules is reduced, but recovers on the 1st postoperative day.

Key words Inhaled nitric oxide · Platelet aggregation · Platelet surface receptors · Platelet adhesion molecules · Intracellular cyclic guanosine monophosphate

Abbreviations cGMP cyclic guanosine monophosphate · *CPB* cardiopulmonary bypass · *NO* nitric oxide · *PRP* platelet rich plasma

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Introduction

Inhaled nitric oxide (NO) is a promising new therapeutic modality to improve haemodynamics and oxygenation in critically ill patients with a variety of diseases [1, 14, 23, 27]. Possible side-effects of a continuous NO inhalation include an increased concentration of methaemoglobin in blood [15], the formation of NO_2 in the ventilator circuit [6] and the inhibition of platelet aggregation [16, 19]. NO reduces platelet aggregation via activation of intracellular guanylate cyclase and increased cyclic guanosine monophosphate (cGMP) [22]. Whether this effect may cause increased bleeding problems in infants and children with NO inhalation remains unknown. Högman et al. [10] demonstrated a prolonged bleeding time in rabbits after inhalation of 30 ppm NO for 15 min. Repeated measurements of platelet aggregation in infants and small children are impossible due to the large blood samples required. Instead, the expression of different platelet receptors [2] mediating platelet adhesion (CD 36 and CD 42b), platelet activation (CD 42b and CD 61) and platelet aggregation (CD 41a) can be studied repeatedly by flow cytometry (Table 1).

In the present study we investigated whether the expression of these platelet receptors is influenced by cardiopulmonary bypass (CPB) surgery and inhaled NO in children. Moreover, dose-response curves were created in vitro between NO and the expression of these platelet receptors, platelet aggregation and intracellular cGMP. From these data an upper dose limit for inhaled NO was extracted which ensures that platelets are not significantly affected by this new therapy.

Material and methods

Patients and controls

Blood was collected directly into EDTA-tubes only from patients and volunteers who had not taken aspirin or other drugs for the last 10 days. For the in vitro study, venous blood samples were drawn from 20 healthy volunteers (controls) with no evidence of heart disease and a mean age of 28 years. The samples were immediately prepared to yield platelet rich plasma (PRP) and incubated with NO as described below.

The patient study was performed in 46 infants and children with congenital heart disease who were included consecutively at the time of diagnostic cardiac catheterization or CPB surgery. From 25 patients with a mean age of 5.6 years a venous or arterial blood sample was obtained pre-operatively during diagnostic cardiac catheterization (pre-operative study group). The remaining 21 patients with a mean age of 3.1 years were studied in the peri-operative period of corrective CPB surgery (peri-operative study group). Blood samples were obtained via an indwelling catheter placed either into the radial artery or the superior vena cava immediately before and at the end of CPB as well as 2, 24, 48 and 72 h postoperatively. All catheters had been placed for routine haemodynamic monitoring after cardiac surgery but not for study purposes. Cardiac diagnoses were: venticular septal defect ($n = 12$), atrial septal defect ($n = 7$), tetralogy of Fallot ($n = 3$), pulmonary stenosis or atresia ($n = 6$), aortic stenosis or atresia ($n = 3$), transposition of the great arteries ($n = 3$), and others ($n = 12$). Seven of these patients had pulmonary hypertension pre-operatively.

Inhaled NO

Seven of the peri-operative patients received inhaled NO with an initial concentration of 20.4 ± 4.5 ppm after weaning from CPB. The indication for starting NO inhalation was persistent postoperative pulmonary hypertension either due to a previous left-to-right shunt lesion or, in one case, aortic atresia with a previously ductal dependent systemic circulation.

NO (Messer-Griesheim, Duisburg, Germany) was introduced as a NO/N_2 gas mixture into the afferent limb of the ventilator circuit close to the endotracheal tube as previously described [4]. The inspired NO and O_2 concentrations were continuously measured (NO-Gaswarnanlage, Bieler and Lang, Achern, F.R.G; O_2-sensor module, Hewlett-Packard, Böblingen, F.R.G.). When a significant improvement was seen, inhaled NO therapy was continued with 1–20 ppm as required to obtain a stable situation concerning haemodynamics and gas exchange. Along with the clinical improvement the applied NO concentration was reduced daily and finally discontinued. The system for application of inhaled NO was reviewed and approved by the Technical Surveillance Association (TÜV Südwest, Stuttgart, F.R.G.). This study was approved by the ethics committee of the University of Tuebingen. Informed consent was obtained from the parents.

Incubation

For the in vitro study of the effects of exogenous NO on platelets, PRP was incubated with 0, 25, 100 and 640 ppm NO for 45 min with almost no bubbling. The same NO/N_2 gas mixture as in the clinical setting was used. The desired NO concentration was obtained by blending NO gas and pressure air via two separate flowmeters. NO was measured electrochemically (NO-Pac II, Dräger, Lübeck, F.R.G.). Following incubation, the samples were immediately prepared for flow cytometry as well as for measurement of platelet aggregation and platelet cGMP levels.

Flow cytometry

Platelet receptors were labelled by fluorescent antibodies following the manufacturer's instructions. Briefly, 0.5 ml PRP were centrifuged, the pellet resuspended in PBS and washed twice. The final

Table 1 Characterization of selected receptors on the platelet surface [according to [2]]

Receptors	Synonyma	Subunits	Amount of binding sites	Platelet function
CD 36	Collagen receptor	GP IV	12000	Adhesion
CD 41a	Fibrinogen receptor	GP Iib GP IIIa	50000	Aggregation
CD 42b	vWF receptor	GP Ib GP IX	25000	Adhesion, activation
CD 61		GP IIIa		Activation

suspension (1 ml) was prepared in saline with 0.1% NaN_3 and treated with 20 µl of the FITC-labelled antibody for 30 min at 4–8°C. The following antibodies were used: anti CD 36 mouse IgG_1, anti CD 41a mouse IgG_1 kappa, anti CD 42b mouse IgG_1 (Dianova-Immunotech, Marseilla, France) and anti CD 61 mouse IgG_1 (Becton-Dickinson, Mountain View, USA). Flow cytometry was performed by counting 10,000 cells per sample, using a FACScan (Becton-Dickinson, Mountain View, USA) equipped with the Lysis II software. After appropriate gating of the platelet population within the dotplot-diagram (Forward scatter versus side scatter) a histogram was created in which the cells were classified according to their fluorescence. Fluorescence intensity was expressed as arbitrary units (channel number) on a log scale. For further analysis the median channel of the platelet population showing positive fluorescence was estimated.

Platelet aggregation

The final platelet count in the PRP was adjusted to 200–300 $\times 10^3$/µl by adding appropriate amounts of platelet poor plasma of the same patient. Platelet aggregation was monitored according to the turbidimetric method using the Platelet Aggregation Profiler PAP-4 (Mölab, Hilden, F.R.G.). PRP (0.5 ml) was incubated under continuous stirring for 5 min prior to the analysis at a temperature of 37°C. Aggregation was induced with a final concentration of 5 mg/ml collagen. The extent of platelet aggregation was expressed as percentage of the maximal increase in light transmission in stimulated PRP, assuming that light transmission was 100% in platelet post plasma from the same patient and 0% in nonstimulated PRP.

Platelet cGMP assay

After centrifugation of PRP (2000 *g*, 15 min), the supernatant was decanted and the pellet resuspended in 15 mM TRIS buffer (pH 7.0). Then, the platelet count was measured and the sample stored at −20°C until analysis on the following day. Deep freezing provided the necessary rupture of the platelet membranes. The sample was assayed for cGMP without further extraction by enzyme-immunoassay using a commercially available test kit (Amersham, Braunschweig, F.R.G.) and a photometer (Spectra, SLT Labinstruments, Crailsheim, F.R.G.). No extraction procedure was used, since Jakob et al. [11] presented data from the measurement of plasma cGMP, supporting the assumption that this step is not necessary. Because of the low cGMP concentration expected within the sample, the acetylation assay with a range of 0.04–10.24 pmol/ml was used. The final results were expressed as pmol per 10^9 platelets.

Haemodynamic measurements

Routine postoperative haemodynamic monitoring included heart rate, respiratory rate, arterial oxygen saturation obtained by pulsoximetry, systemic arterial pressure and central venous pressure. In some patients indwelling catheters were surgically placed into the pulmonary artery and the left atrium to monitor both pulmonary artery and left atrial pressures. All parameters available were recorded continuously using the Intensive Care Monitoring system (Merlin/Mars, Hewlett Packard, Böblingen F.R.G.). Cardiac output was determined by the Fick principle and indexed to body surface area. Pulmonary vascular resistance index, systemic vascular resistance index, intrapulmonary shunting (Q_S/Q_T) and blood oxygen content were calculated using standard equations.

Statistical analysis

Results are presented as mean ± SEM. Statistical analysis was performed using the PC-based SAS 6.03 software package (SAS Institute, Cary, U.S.A.). The normal distribution of each parameter was verified by univariate analysis of variance. Analysis of variance with adjustments for multiple comparisons (Bonferroni *t*-tests) and analysis of variance for repeated measures were used for statistical comparison. A P value of <0.05 was considered statistically significant.

Results

In vitro study

The platelet count of the control group 218 ± 8.34 $\times 10^3$/µl) was not significantly different from the initial value within the pre-operative (193 ± 14.05 $\times 10^3$/µl) or peri-operative (204 ± 24.67 $\times 10^3$/µl) study group. The fluorescence intensity which reflects the expression of the corresponding adhesion molecule was highest when using the CD 41a antibody. Moreover, the fluorescence intensity of each antibody was increased in the control group after the incubation procedure with pressure air when compared to the pre-operative study group without any incubation (Table 2). However, the effect was well within the range of the in vivo changes induced by CPB surgery (Table 2). The amount of unspecific binding was low in each group.

As shown in Fig. 1, the incubation of PRP from controls with increasing doses of NO led to a significantly reduced expression of CD 41a at 100 ppm (−11%) and 640 ppm (−20%). The platelet receptors CD 42b and CD 61 were significantly reduced only at 640 ppm NO. A concentration of 25 ppm NO did not affect the expression of any platelet adhesion molecule studied, and no changes of the CD 36 receptor were detected. The NO-induced reduction in the expression of the CD

Table 2 Changes in expression of different platelet adhesion molecules [fluorescence intensity in arbitrary units] due to the incubation process itself and CPB surgery

Receptors	Incubation without NO (control group)	Without incubation (pre-operative group)	Effect of CPB (post-operative group)	
			before CPB	max. after CPB
Unspecific Binding	38 ± 4.8	40 ± 3.49	42 ± 5.3	54 ± 12.8
CD 36	576 ± 36.5	552 ± 36.0	560 ± 54.9	687 ± 53.1
CD 41a	1917 ± 84.9	769 ± 60.9	636 ± 81.8	1409 ± 166.2
CD 42b	736 ± 29.6	550 ± 31.2	586 ± 52.0	664 ± 48.0
CD 61	726 ± 22.6	479 ± 25.1	468 ± 29.7	574 ± 49.2

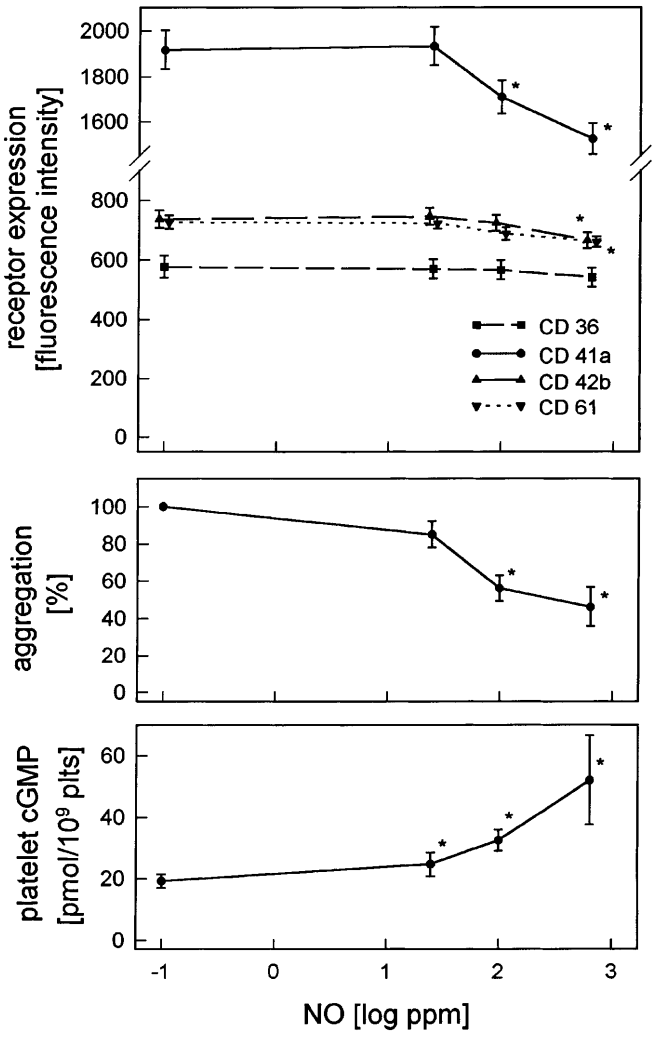

Fig. 1 Effect of increasing doses of NO [log ppm] within the incubation tube on the expression of platelet receptors [fluorescence intensity in arbitrary units], platelet aggregation [% change versus baseline] and platelet cGMP levels [pmol/10^2 platelets], * Statistical significant ($P < 0.05$)

41a receptor was accompanied by a parallel decrease in platelet aggregation and an increase in intraplatelet cGMP levels (Fig. 1).

Patient study

The effect of inhaled NO with an inital concentration of 20.4 ± 4.5 ppm on different haemodynamic and gas exchange parameters is shown in Table 3. Twenty minutes after beginning of NO inhalation, mean aortic pressure, arterial oxygen saturation and partial arterial oxygen pressure were significantly increased, whereas mean pulmonary artery pressure, pulmonary-to-aortic pressure ratio, pulmonary vascular resistance and partial arterial carbon dioxide pressure were significantly decreased. Intrapulmonary right-to-left shunting (Q_S/Q_T) showed a small, but not significant decrease. Methaemoglobin concentration increased significantly from 0.82% to 1.47%. Considering the NO-induced increase in methaemoglobin concentration, oxygen saturation and partial oxygen pressure, the net effect is a significant increase in the blood oxygen content from 16.13 ± 0.51 ml O_2/100 ml blood to 17.4 ± 0.46 ml O_2/100 ml blood. Due to the clinical improvement, the applied NO concentration could be reduced stepwise and discontinued after 2.5–6 days (mean: 4.2 ± 0.6 days).

Table 3 Haemodynamics and gas exchange before and 20 min after beginning of NO inhalation in seven children after CPB surgery for congenital heart disease. The ventilator settings and FiO_2 were kept constant between the two measurements. (*MAP* mean aortic pressure, *MPAP* mean pulmonary artery pressure, *CVP* central venous pressure, *LAP* left atrial pressure, *CI* cardiac index, *SVRI* systemic vascular resistance index, *PVRI* pulmonary vascular resistance index, SaO_2 arterial oxygen saturation, paO_2 partial arterial oxygen pressure, $paCO_2$ partial arterial carbon dioxide pressure, Q_S/Q_T intrapulmonary right-to-left shunt, MetHb methaemoglobin concentrations)

Parameter	Before NO	During NO (20.4 ± 4.5 ppm)
Heart rate [bpm]	134 ± 7.3	135 ± 7.7
MAP [mmHg]	58.1 ± 4.0	63.1 ± 3.2*
MPAP [mmHg]	52.0 ± 10.2	30.4 ± 4.3*
MPAP/MAP	0.85 ± 0.1	0.47 ± 0.05*
CVP [mmHg]	12.2 ± 1.1	11.1 ± 1.1
LAP [mmHg]	11.6 ± 2.3	12.2 ± 2.0
CI [l/min/m^2]	3.41 ± 0.5	3.47 ± 0.5
SVRI [dyn/s/m^2/cm^2]	1038 ± 150	1195 ± 227
PVRI [dyn/s/m^2/cm^2]	665 ± 180	383 ± 148*
SaO_2 [%]	92.9 ± 2.9	98.1 ± 0.7*
paO_2 [mmHg]	71.7 ± 7.8	117.2 ± 17.4*
$paCO_2$ [mmHg]	32.3 ± 1.8	30.3 ± 1.5*
Q_S/Q_T [%]	38.4 ± 10.8	28.7 ± 4.1
MetHb [%]	0.82 ± 0.08	1.47 ± 0.18*

* Statistical significant difference ($P < 0.05$)

To test the influence of age, sex and type of congenital heart defect on platelet count and the initial expression of the four platelet receptors, the data of the pre-operative study group and a subset of data from the peri-operative group, obtained before starting CPB, were pooled. Then the patients were stratified according to age (< 1 year: $n = 9$, 1–3 years: $n = 9$, 3–6 years: $n = 18$ and > 6 years: $n = 10$), sex (male: $n = 25$, female: $n = 21$) and type of defect (left-to-right shunt: $n = 12$, right-to-left shunt: $n = 21$, no shunt: $n = 13$), respectively. One-way analysis of variance revealed no significant effect of any of these parameters on the expression of platelet receptors and the platelet count.

CPB sugery led to a significant decrease in platelet count of approximately 50% (Fig. 2). Patients who later required inhaled NO, had a significantly pronounced decrease of about 70%. The reason for this phenomenon will be shown below. The platelet count remained low for 2 days postoperatively and returned almost to preoperative levels on the 3rd day in patients without inhaled NO or showed only a slight increase in patients with inhaled NO.

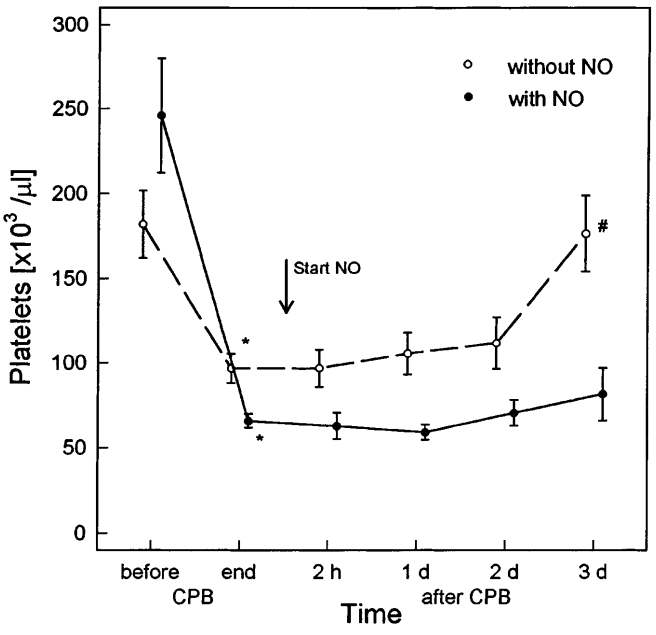

Fig. 2 Changes in platelet count during and after CPB surgery in children. Patients without NO inhalation (○), patients with NO inhalation (●). *Statistical significant ($P < 0.05$) compared to 'before CPB'; **Statistical significant ($P < 0.05$) to 'end of CPB'

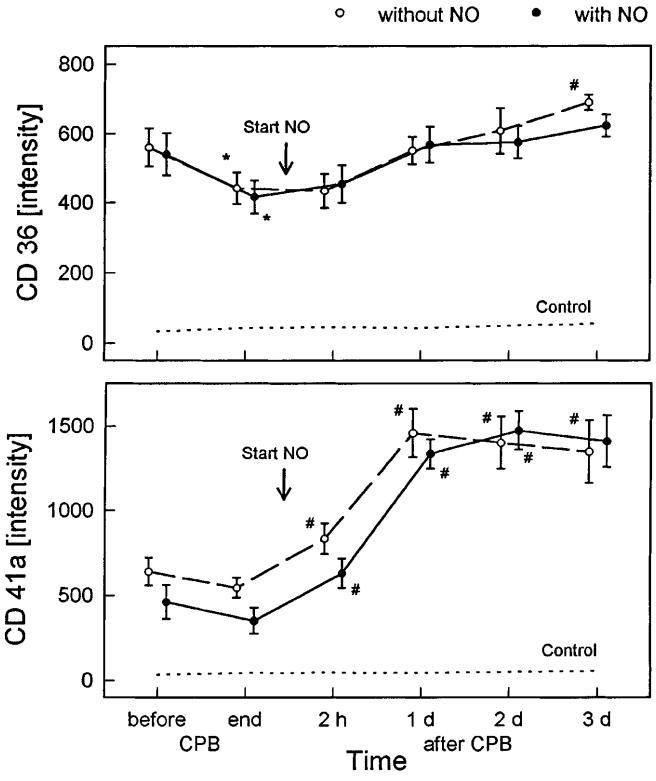

Fig. 3 Expression of the platelet receptors CD36 and CD 41a during and after CPB surgery in children. Patients without NO inhalation (○); patients with NO inhalation (●). *Statistical significant ($P < 0.05$) compared to 'before CPB'; **statistical significant ($P < 0.05$) to 'end of CPB'. *Control*, small amount of fluorescence due to unspecific binding of labelled antibodies

The expression of the platelet receptors CD 36 (−21%), CD 42b (−23%) and CD 61 (−20%) was significantly reduced at the end of CPB in all patients of the peri-operative study group (Figs. 3, 4), whereas the CD 41a receptor (−15%) showed only a slight and not significant attenuation. The extent of unspecific binding was not changed by CPB. Postoperatively, the expression of the receptors CD 36, CD 42b and CD 61 increased again, reaching pre-operative values on the 1st postoperative day and exceeding it slightly on the 2nd and 3rd day. The expression of the CD 41a receptor increased above the pre-operative level shortly after CPB surgery (2 h: +31%) and remained high up to 3 days thereafter (1st day: +129%, 2nd day: +120%, 3rd day: +111%). No significant difference in this pattern between patients with and without NO inhalation was observed. Clinically, no prolonged bleeding after withdrawal of indwelling catheters or drainage tubes was detected in patients with inhaled NO.

The alterations of platelet count and expression of platelet receptors induced by CPB surgery were not significantly related to the parameters sex or type of cardiac defect. However, there was a more pronounced decrease in platelet count induced by CPB in infants (−74.6%) compared to other age groups) (1–4 years: −58.3%, >4 years: −21.9%). The expression of the receptors CD 41a and CD 61 was significantly more decreased in patients with a lower body temperature during CPB (<27°C vs ≥27°C). Likewise, the expression of the CD 61 receptor was significantly attenuated by hypothermic circulatory arrest during cardiac surgery. Of interest, patients who received inhaled NO later on were younger (1.1 ± 0.5 vs 4.0 ± 1.0 years), had a longer CPB time (80 ± 6 vs 72 ± 11 min) and a lower body temperature during CPB (23.8 ± 1.8 vs 27.5 ± 1.7°C) than patients without NO.

Discussion

In the first part of our study it was shown that the expression of the CD 41a receptor on the platelet surface accurately reflects the dose-dependent inhibition of platelet aggregation by exogenous NO. It was confirmed that an increased intraplatelet cGMP level is involved in this process. cGMP decreases intracellular calcium availability, which is the common trigger for the induction of platelet aggregation and the release of granular contents such as serotonin [24] via an inhibition of the influx and the mobilization of calcium from intracellular stores [9]. Calcium availability is further reduced by an elevation of intraplatelet cAMP levels, which enhances calcium uptake into the dense tubular system [13]. Platelets may regulate their aggregation by a negative feedback mechanism via intraplatelet synthesis of NO from L-arginine [21]. Also, the intact endothelium obviously regulates the homeostatic interactions between platelets and the vessel wall by the release of NO and prostacyclin [19].

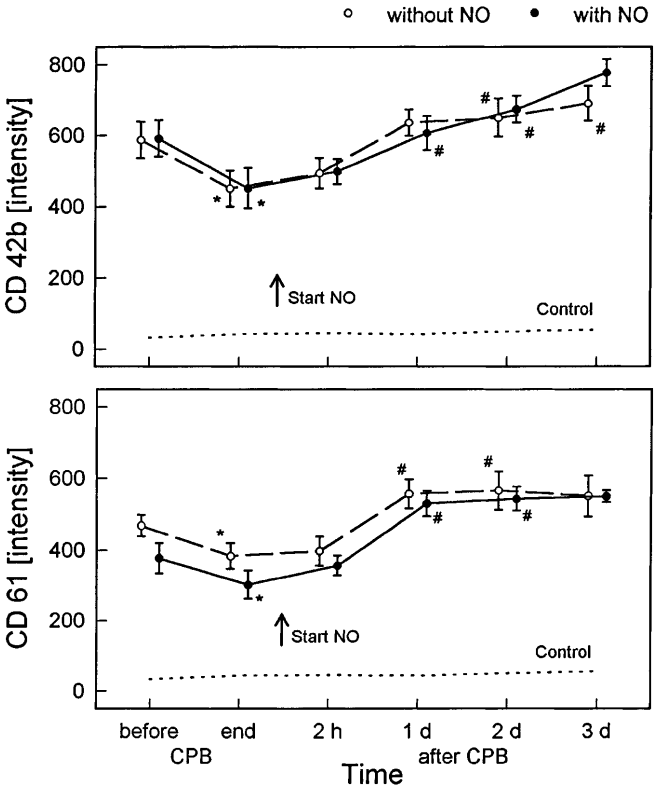

Fig. 4 Expression of the platelet receptors CD42b and CD 61 during and after CPB surgery in children. Patients without NO inhalation (○); patients with NO inhalation (●). *Statistical significant ($P < 0.05$) compared to 'before CPB'; **statistical significant ($P < 0.05$) to 'end of CPB'. *Control* small amount of fluorescence due to unspecific binding of labelled antibodies

The mechanism by which NO alters the expression of platelet receptors is unknown. It is speculated that the cAMP- and cGMP-induced activation of different protein kinases and subsequent phosphorylation of regulator regions of the membrane protein are involved. Evidence for this hypothesis stems from Parise et al. [17], who found a phosphorylation of the cytoplasmatic C-terminal end of GP IIIa (subunit of CD 41a) by protein kinase C in human platelets during activation.

An increase in the expression of platelet receptors in vitro due to the incubation process itself was detected by comparing the fluorescence intensity of the control group at 0 ppm NO with the preoperative study group without incubation. However, because CPB also increased the receptor expression to the same amount, the in vitro situation closely reflected the in vivo situation.

In our in vitro experiments we observed a close correlation of the dose-response to NO between the CD 41a expression and the inhibition of platelet aggregation induced by collagen. In order to compare these results with previously published studies [19], the concentration of dissolved NO in PRP was calculated from the gaseous NO concentration within the incubation chamber. Assuming a solubility coefficient of 4.6 ml NO/100 ml H_2O at 20°C and 1 atm [7], and a molar volume of 22.4 l NO/mol, 25 ppm corresponds to 0.051 μM NO, 100 ppm to 0.205 μM and 650 ppm to 1.332 μM, respectively. A comparable dose range for NO (0.18–1.50 μM) was used by Radomski et al. [19], who found a dose-dependent inhibition of platelet aggregation after incubation of human PRP with a 50% inhibitory concentration IC_{50} of 0.54–0.87 μM NO. This is the same order of magnitude as the concentration of NO which reduces the expression of the CD 41a receptor significantly (0.205 μM NO). In our study, 650 ppm NO (= 1.3 μM) increased the intraplatelet cGMP level three fold, whereas Radomski et al. [20] observed a 16-fold rise when using a considerably higher NO concentration (= 10 μM).

In the clinical part of our study we asked whether inhaled NO even in low concentrations may alter platelet function in children after surgery for congential heart disease. To answer this question we first analysed the alteration of the platelet count and the expression of platelet receptors induced by CPB. The platelet count was markedly decreased (−47%) at the end of CPB and returned slowly to normal on the 3rd postoperative day. Schricker et al. [26] found a similar decrease (−47%) and a slow recovery of the platelet count in adult patients whose CPB time was longer than 60 min. In patients with a shorter bypass time these alterations were considerably less. Therefore, a longer bypass time, the younger age and the deeper hypothermia during CPB are the most likely explanation for the fact that in our study patients who later required inhaled NO had a lower platelet count at the end of CPB.

Possible mechanisms for the decreased platelet count at the end of CPB are haemodilution, an increased filtration of aggregated platelets and an increased adhesion of activated platelets at the subendothelium or the large artificial surface of the perfusion system [26]. For instance, platelets can adhere to surface adsorbed fibrinogen via the CD 41a receptor [8]. A rise in platelet activation with a subsequent release of serotonin from the dense granules can be inferred from the observation that 3 and 24 h after CPB surgery the urinary excretion of the serotonin metabolite 5-hydroxyindoleacetic acid was slightly increased as reported elsewhere [3].

A biphasic change with a reduced expression of platelet receptors at the end of CPB and increase to or above normal values within the first 2 postoperative days was detected. These changes are independent of the absolute platelet count of the sample because by flow cytometry always 10,000 platelets were measured. The reduced expression of all platelet receptors studied might be caused by an active conformational change of the receptor protein, which has been shown for the CD 41a (GP IIb/IIIa) receptor [18]. On the other hand, it could simply reflect an increased filtration during CPB of those platelets which showed an increased expression of receptors mediating aggregation and adhesion. However, an active conformational change or a de-novo synthesis of the receptor protein seems to be the reason for the increased receptor expression on the 1st and 2nd postoperative day, since at that time the platelet count was still low. Besides other factors such as increased fibrin-

ogen levels, the increased expression of platelet receptors obviously compensates for the postoperatively decreased platelet count, because Schricker et al. [26] observed a normal bleeding time already on the 1st day after CPB.

The positive effect of inhaled NO on haemodynamics and gas exchange in the patients of our study is comparable to previously published results from other groups [12, 14, 27]. The same holds true for the dose range which was 1–20 ppm NO. Since inhaled NO did not alter the natural course of changes in platelet receptor expression induced by CPB, we presume that the corresponding platelet functions such as aggregation and adhesion are not disturbed by NO in this dose range. This is in contrast to Samama and co-workers [25], who found a significant decrease of platelet aggregation by about 46% in six patients with adult respiratory distress syndrome after inhaled NO. However, some potential errors are present in this study. First, the decrease was not dose-dependent over a range of 1–100 ppm NO, which is in contrast to the known dose-dependent inhibition of platelet aggregation within this dose range [19]. Second, the Ivy bleeding time remained within the normal range, and variations during NO inhalation did not correlate with measured alterations of platelet aggregation in these patients. In another study, a prolonged bleeding time was detected in rabbits after inhalation of 30 ppm NO for 15 min, and this was thought to be due to a NO-induced inhibition of platelet aggregation [10]. However, neither did Samama and co-workers [25] detect a prolonged bleeding time in their patients, nor was an increased bleeding after withdrawal of drainage tubes or after endotracheal suction observed in our patients postoperatively. Moreover, bleeding time is known as a global test of coagulation and is influenced by many factors such as coagulation proteins, vascular tone and platelet aggregation.

From the in vitro part of our study we concluded that the effects of NO on platelets occurred only at such a high concentration that should not be used in humans anyway, due to other undesired side-effects, e.g. formation of nitric dioxide [6] and methaemoglobin [15]. The in vivo part confirmed this hypothesis. In a dose-response study in a similar group of children after surgery for congenital heart defects, 95% of the maximum effect on pulmonary vascular resistance and oxygen saturation was observed with less than 10 ppm NO [5]. Therefore, it is not necessary to apply high dose inhaled NO (>40 ppm) in the clinical setting.

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ANNOUNCEMENT

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September 11–12, 1998,
Basle, Switzerland
Chairman: Prof. Dr. med. F. Hefti
University Hospital for Pediatric Orthopaedics Basle

Date September 11–12, 1998

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Main Topics "The pediatric foot in practice and clinic"
Clubfoot
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