

Surgery for Congenital Heart Disease

CHD

The endothelin antagonist BQ123 reduces pulmonary vascular resistance after surgical intervention for congenital heart disease

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Objective: Postoperative pulmonary hypertension in children after surgical intervention for congenital heart disease has been attributed to failure of the pulmonary endothelium to provide adequate vasodilation. Although we have shown that the impaired vasodilatory component attributable to the L-arginine–nitric oxide pathway is almost completely reversible, a nonrestorable component persists, implying an additional vasoconstrictive mechanism in postoperative pulmonary endothelial dysfunction. In this study of children after surgical intervention for congenital heart disease, we measured endothelin-1 levels and used BQ123, a selective endothelin-A receptor antagonist, together with inhaled nitric oxide to discriminate dysfunctional pulmonary endothelial vasodilation from endothelin-mediated pulmonary vasoconstriction.

Methods: All children were examined early after surgical intervention in the intensive care unit. Pulmonary vascular resistance (with respiratory mass spectrometry), as well as arterial and venous endothelin-1 levels (measured by means of a quantitative enzyme-linked immunosorbent assay), were determined in 7 children (age range, 3.3–13.7 months; median age, 6.3 months) with intracardiac shunting defects at baseline and during ventilation with a fraction of inspired oxygen of 0.65, with additional BQ123 (0.1 mg/kg infused over 20 minutes), and with inhaled nitric oxide (20 ppm).

Results: Pulmonary vascular resistance decreased from 7.7 ± 3.4 at baseline to 6.1 ± 2.8 Woods units \cdot m⁻² ($P = .022$) at a fraction of inspired oxygen of 0.65 and to 4.7 ± 2.7 Woods units \cdot m⁻² ($P = .013$) during BQ123 infusion. Inhaled nitric oxide had no further effect on pulmonary vascular resistance. Left atrial endothelin-1 levels (1.35–5.12 pg/mL; mean, 2.4 pg/mL) correlated significantly with the decrease in pulmonary vascular resistance in response to BQ123 infusion ($r^2 = 0.89$, $P = .003$).

Conclusion: Postoperative elevation of pulmonary vascular resistance in children after surgical intervention for congenital heart disease is responsive to endothelin-A blockade with BQ123. Increased levels of endothelin-1 predict the response to this therapy, which might become an important addition to the clinical armamentarium in postoperative pulmonary hypertensive disease.

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Increased postoperative pulmonary vascular resistance (PVR) is an important cause of morbidity and mortality in children after surgical intervention for congenital heart disease. It is implicated in prolonged postoperative ventilation and in a considerable number of early postoperative deaths in certain patient groups, such as those with atrioventricular septal defect, truncus arteriosus, and hypoplastic left heart syndrome.¹ It is now recognized that the vascular endothelium plays a pivotal role in the regulation of pulmonary vascular tone by producing a variety of vasodilator and vasoconstrictor messengers. We have demonstrated, in children after cardiac surgery, that despite maximal stimulation of the vasodilatory nitric oxide (NO) pathway with intravenous L-arginine substrate combined with stimulation of the enzyme responsible for synthesis of NO with substance P and the addition of exogenous NO by means of inhalation, PVR still remains substantially increased compared with that in preoperative patients who undergo the same interventions.²

These observations suggest that other endothelial messengers, possibly vasoconstrictive in nature, might play a role in the development of increased PVR in the early postoperative period after cardiac surgery. Cardiopulmonary bypass (CPB) increases the production of the vasoconstrictor endothelin-1 (ET-1) and expression of the endothelin receptor in the lung.³ Furthermore, increased plasma ET-1 levels have been demonstrated in children in the early postoperative period after cardiac surgery, especially those with preoperative left-to-right shunts who are at risk of pulmonary hypertension (PHT).^{4,5} In addition, in an animal model of chronic left-to-right shunt, PHT after CPB was abolished by means of administration of an endothelin-A (ETA) receptor antagonist before CPB.⁶

Recently, an endothelin receptor antagonist has been developed for use in human patients. This agent, BQ123, is a cyclopentapeptide with a more than 800-fold selectivity toward the ETA receptor versus the endothelin-B (ETB) receptor.⁷ BQ123 was shown to reverse the changes in pulmonary arterial pressure after endothelin infusion,⁸ and in adults with congestive heart failure, BQ123 was shown to reduce pulmonary arterial pressure and PVR.⁹

In this study we investigated whether an infusion of BQ123 has beneficial effects on PVR in patients with congenital heart disease after cardiac surgery and whether plasma ET-1 levels predict the magnitude of the response to this endothelin antagonist.

Methods

Patients

The study was approved by our hospital research ethics committee, and written informed consent was obtained from the parents of each child. Children were studied soon after their return from the operating room to the intensive care unit. All had undergone cardiac surgery for intracardiac shunting lesions, including CPB

with modified ultrafiltration.¹⁰ They remained sedated and paralyzed (with vecuronium, midazolam, and morphine) and mechanically ventilated throughout the study. They had been intubated with a cuffed endotracheal tube (Mallinckrodt) to exclude any respiratory gas leaks. Volume-controlled ventilation was delivered by means of a Siemens 900 C ventilator.

A period of 1 to 2 hours between return to the intensive care unit and commencement of the study was allowed for central rewarming, adjustment of sedation and inotropic agents, and tracheal suctioning. Intracardiac shunts were excluded by means of echocardiography. Thereafter, further handling or therapeutic intervention during the study protocol was minimized. For the duration of the study protocol, the cuff of the endotracheal tube was inflated with a pressure below the systemic diastolic blood pressure, and continuous monitoring of hemodynamic pressures, surface electrocardiographic results, pulse oximetry, and end-tidal carbon dioxide concentration was performed.

Hemodynamic and Metabolic Measurements

Systemic and pulmonary arterial pressures, as well as right and left atrial pressures, were measured, and blood samples were taken from the pulmonary artery and the left atrium. The partial pressures for oxygen and carbon dioxide and hemoglobin saturation were measured by means of the spectral absorption method (Chiron 270 CO-oximeter), and the arteriovenous oxygen content difference (in milliliters per liter) was calculated.

Systemic oxygen consumption (in milliliters per kilogram per minute) was continuously determined by using respiratory mass spectrometry with the mixed expirate inert gas dilution method¹¹ and our previously described modification¹² for use in ventilated patients. Special care was taken to detect and exclude any air leaks or carbon dioxide contamination of the monitoring and ventilatory circuits. The mass spectrometer was calibrated directly before the study and then every 30 minutes to exclude any measurement drift.

Cardiac output was derived by using the Fick principle as the systemic oxygen consumption/arteriovenous oxygen content difference. PVR (in millimeters of mercury per liter per minute) and the PVR index (PVRI; ie, PVR per square meter) were derived from the transpulmonary pressure gradient by using a standard formula¹² and reported in Wood Units indexed to body surface area (Wood units per square meter).

Protocol for Evaluation of Pulmonary Endothelial Function

The study protocol was instituted within 1 to 2 hours after discontinuation of CPB and after a cardiorespiratory steady state was confirmed during 5 to 10 minutes of monitoring. All hemodynamic and metabolic measurements were made during the last 2 minutes of each 15-minute condition (Figure 1):

1. ventilation at baseline with a low fraction of inspired oxygen (0.21-0.30);
2. ventilation in increased oxygen (fraction of inspired oxygen of 0.65), which was continued to the end of the protocol to obviate the possible confounding effects of alveolar hypoxia;
3. intravenous infusion of 0.1 mg/kg BQ123 (Clinalfa AG) over 20 minutes to the end of the protocol to block the effects of circulating endothelin; and

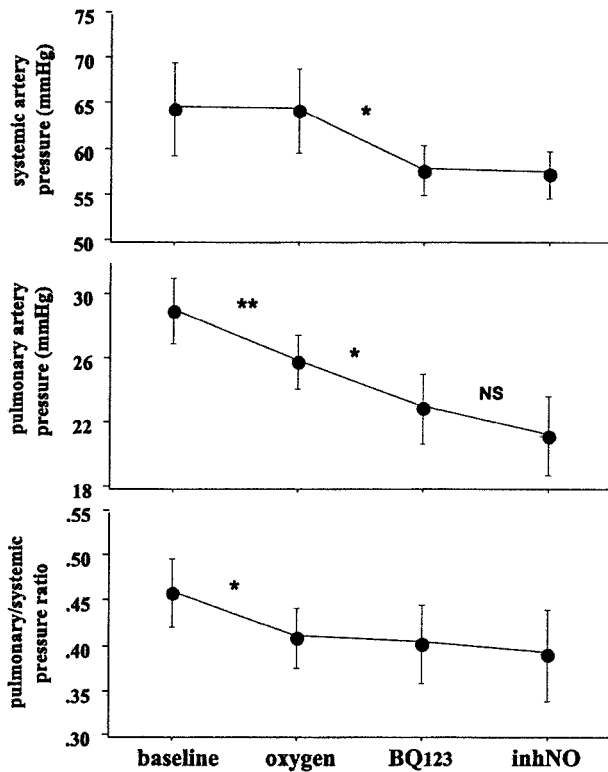


Figure 1. Hemodynamic variables during the study protocol. All values are displayed as means \pm SEM. Statistical significance of the change of a variable in response to the next study protocol condition is indicated as follows: * $P < .05$ and ** $P < .01$. NS, Not significant; inhNO, inhaled NO.

4. inhalation of NO (20 ppm; BOC), which was continued to the end of the protocol to provide direct pulmonary vascular smooth muscle relaxation.

Plasma Endothelin Levels

At each step of the study protocol, 2-mL samples of blood were taken from the pulmonary artery and the left atrium. These blood samples were taken by using ethylenediamine tetraacetic acid as an anticoagulant and were spun down, with the plasma frozen for later analysis. A quantitative sandwich enzyme immunoassay technique in the form of a solid-phase enzyme-linked immunosorbent assay (QuantiGlo for human ET-1, R&D Systems) was used to detect ET-1 (in picograms per milliliter). This assay has a coefficient of variation for repeated measurements of 2.5% within the range of 1 to 20 pg/mL, with a minimum detectable ET-1 concentration of 0.16 pg/mL and a cross-reactivity with big endothelin of 0.01% to 0.02%. All samples were measured in duplicate and diluted both 1:1 and 1:10. They were then pipetted into microplate wells precoated with ET-1-specific monoclonal antibody. After removing the unbound substance, the enzyme-linked immunosorbent assay antibody was added to the wells, and the light produced in response to the addition of luminol-peroxide substrate was measured with a luminometer. The resulting relative light units were

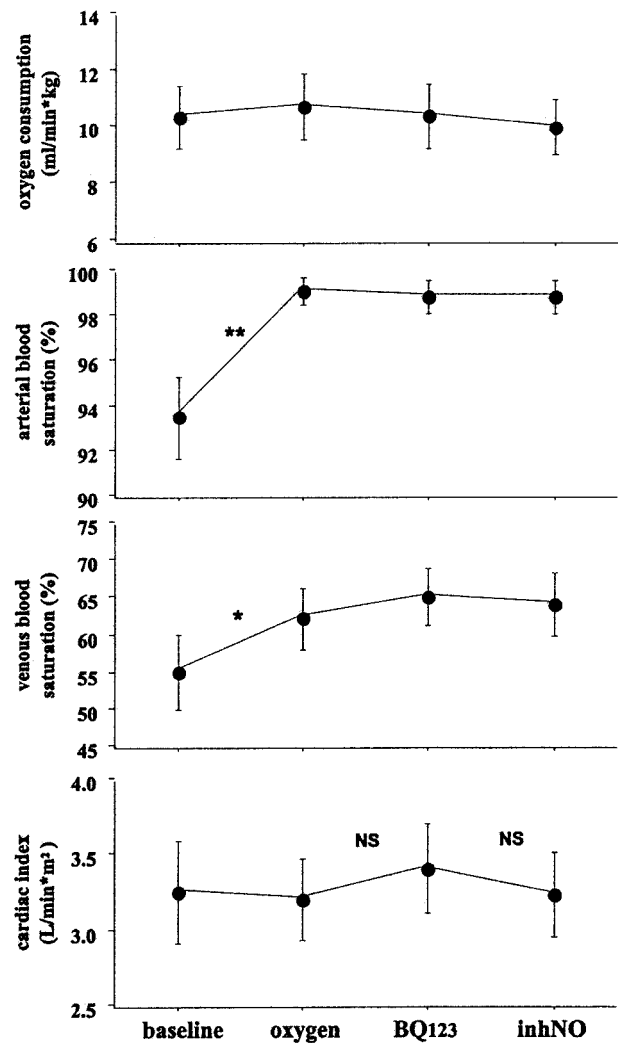


Figure 2. Metabolic variables and derived cardiac output during the study protocol. All values are displayed as means \pm SEM. Statistical significance of the change of a variable in response to the next study protocol condition is indicated as follows: * $P < .05$ and ** $P < .01$. NS, Not significant; inhNO, inhaled NO.

related to a calibration curve obtained from a simultaneously measured standard dilution of known ET-1 concentrations.

Statistical Analysis

All data are expressed as means \pm SD. The effect of each intervention was assessed by using standard statistical methods. The standard Student *t* test for paired data points was used to compare mean values of the same variable within the patient group. Linear regression analysis was used to determine correlations between results.

Results Patients

Seven patients completed the full study protocol (Table 1). All had intracardiac shunting defects and underwent surgi-

TABLE 1. Patient data

Patient No.	Body weight (kg)	BSA	Diagnosis	Age (mo)	CPB (min)	XCT (min)	Temperature (°C)
1	4.9	0.3	VSD	3.27	55	22	32
2	7.5	0.35	VSD	3.30	55	28	22
3	4.4	0.27	VSD	2.83	35	19	32
4	7.5	0.35	VSD	9.27	50	30	32
5	4.7	0.28	cAVSD, T ₂₁	4.53	127	80	25
6	5.8	0.32	cAVSD	0.53	94	61	25
7	10.8	0.5	VSD	13.73	96	54	22
Mean	6.51	0.34		5.35	73	42	27
SD	2.28	0.08		4.55	33	23	5
Median	5.80	0.32		3.30	55	30	25

BSA, Body surface area; XCT, crossclamp time; temperature, lowest temperature during CPB.

cal correction with CPB, according to our standard protocol. Modified ultrafiltration was used immediately after CPB and before decannulation.

Baseline Measurements

Although systemic arterial pressures were in the normal range (65 ± 13 mm Hg), mean pulmonary arterial pressure was increased (29 ± 5 mm Hg) to $46\% \pm 10\%$ of mean systemic arterial pressure. The cardiac index was 3.3 ± 0.9 L \cdot min⁻¹ \cdot m⁻², so that as a result, PVRI (7.7 ± 3.4 Wood units [WU] \cdot m⁻²) and the ratio of pulmonary to systemic resistance ($40\% \pm 9\%$) were increased.

Measured Hemodynamic Variables

Mean pulmonary arterial pressure decreased in response to oxygen supplementation (29 ± 5 to 26 ± 4 mm Hg, $P = .005$) and decreased further during ETA blockade with BQ123 (to 23 ± 6 mm Hg, $P = .039$). Mean systemic arterial pressure decreased during the intravenous administration of BQ123 (64 ± 12 to 58 ± 7 mm Hg, $P = .036$), but cardiac index tended to increase (3.21 ± 0.71 to 3.42 ± 0.77 , $P = .196$, Figures 1 and 2).

Systemic Vascular Resistance and PVR

Mean baseline PVRI decreased from 7.7 ± 3.4 WU \cdot m⁻² to 6.1 ± 2.8 WU \cdot m⁻² (ie, $-14.1\% \pm 25.2\%$; $P = .022$) during supplemental oxygen administration. There was a further decrease of PVRI during BQ123 infusion to 4.7 ± 2.7 WU \cdot m⁻² (ie, $-26.3\% \pm 23.1\%$; $P = .013$). Additional administration of exogenous inhaled NO did not cause a significant further decrease in PVRI ($P = .294$). The systemic vascular pressure/PVR ratio and systemic/pulmonary resistance ratio decreased significantly during oxygen supplementation ($46\% \pm 10\%$ to $41 \pm 8\%$ [$P = .046$] and $40\% \pm 9\%$ to $33\% \pm 9\%$ [$P = .008$], respectively). These ratios then remained unaltered by intravenous infusion of BQ123 (Figure 3).

Endothelin Levels and Pulmonary Endothelin Extraction

At baseline, arterial ET-1 levels were significantly lower than venous ET-1 levels, thus indicating the presence of pulmonary endothelin extraction (Table 2). Neither plasma ET-1 levels nor pulmonary endothelin extraction changed significantly during the protocol (Table 2).

Relationship Between Pulmonary Vasodilator Responses and Endothelin Levels

There was no relationship between baseline PVR and plasma ET-1 levels. However, the percentage reduction in PVR during infusion of BQ123 (ie, $[PVR_{O_2} - PVR_{BQ123}]/PVR_{O_2}$) was significantly related to the venous ($r^2 = 0.85$, $P = .002$) and arterial ($r^2 = 0.89$, $P = .003$, Figure 4) plasma ET-1 levels.

Discussion

This study examined plasma ET-1 levels and assessed the effects of ETA blockade on PVR in children after surgical intervention for significant intracardial left-to-right shunting defects by using CPB. It showed that postoperative baseline PVR, as well as plasma ET-1 levels, were increased when compared with normal values reported by others¹³ and that extraction of endothelin occurred in the pulmonary vascular bed. ETA blockade by means of intravenous infusion of BQ123 decreased PVR, and the magnitude of this effect correlated with the amount of circulating ET-1. After ETA blockade, there was no further additional effect on PVR from inhaled NO.

Biology of Increased PVR After CPB

Increased PVR after CPB is associated with a failure of the pulmonary endothelium to produce the endogenous vasodilator NO, which has been referred to as pulmonary endothelial dysfunction (PED). PED has been demonstrated in primary PHT,¹⁴⁻¹⁷ in secondary PHT caused by congenital

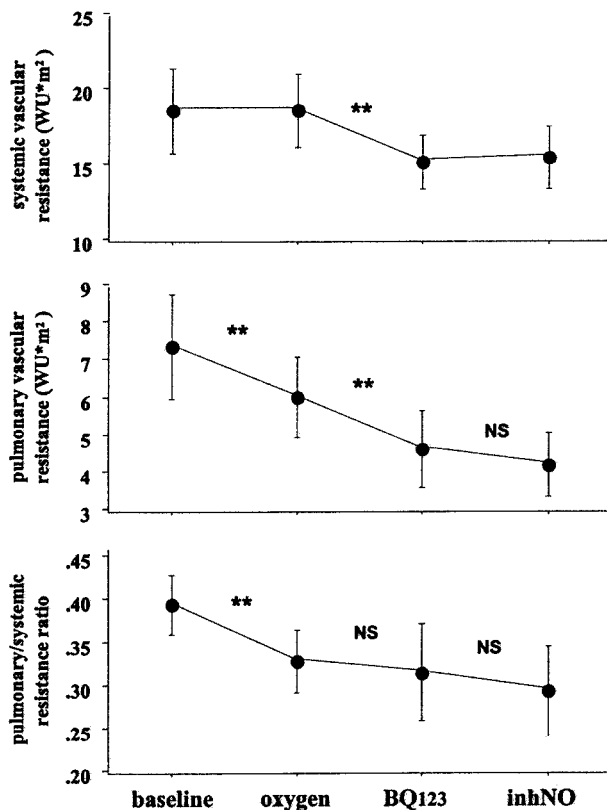


Figure 3. Vascular resistance during the study protocol. All values are displayed as means \pm SEM. Statistical significance of the change of a variable in response to the next study protocol condition is indicated as follows: * $P < .05$ and ** $P < .01$. NS, Not significant; inhNO, inhaled NO.

heart disease,¹⁷ and in the postoperative pulmonary vascular bed after CPB.¹⁸ Although it is logical and clinically useful to support the vasodilating function of the pulmonary endothelium with inhaled NO,¹⁹⁻²¹ some patients experience either no or only partial reversal of increased PVR with this form of therapy. In a previous study we demonstrated that postoperative PED could be reversed by means of maximal enhancement of the NO pathway with L-arginine and substance P. However, PVR remained increased despite additional inhaled NO, suggesting that either smooth muscle relaxation was impaired or that active vasoconstrictive factors might play a role.²

Increased Circulating Endothelin Levels After CPB

We found increased ET-1 levels in our patients.¹³ Endothelins are a class of very potent vasoconstrictors. The most important of these, ET-1, is produced in endothelial cells and secreted locally toward the smooth muscle cell layer in response to shear stress and hypoxia. Its production might also be triggered by activated neutrophil-endothelin interaction²² during CPB, reaching a maximum 6 to 9 hours after

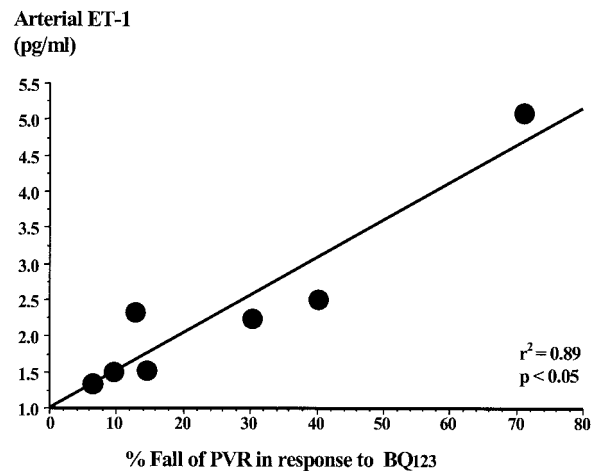


Figure 4. Correlation of plasma endothelin levels and PVR response to ETA blockade. The arterial ET-1 concentration is related to the decrease of PVR in response to BQ123 infusion ($r^2 = 0.89$, $P < .05$).

CPB.²³ However, whether pulmonary vasoconstriction results from endothelin release in the systemic circulation as part of the whole-body inflammatory response to CPB or whether it is due directly to intrapulmonary release of endothelin remains controversial.⁴

Pulmonary Endothelin Metabolism

We found a decrease in circulating ET-1 levels across the pulmonary vascular bed, which cleared 15% to 20% of the venous ET-1, and this remained unchanged during the course of our study protocol. Although in the normal lung there is considerable exchange of endothelin, with both absorption and secretion occurring, it has been observed that these 2 processes are normally at equilibrium, so that there is an overall net zero clearance within the pulmonary vasculature.²⁴⁻²⁶ Although the lung is able to decrease moderately increased endothelin levels by means of passage through an intact pulmonary vascular bed, this extraction function is impaired in severe lung disease.^{27,28} Komai and colleagues⁴ found pulmonary extraction of endothelin only in those postoperative patients who had preoperatively low pulmonary blood flow. In patients with high preoperative pulmonary blood flow and therefore pulmonary endothelial damage, they noted a loss of the pulmonary endothelin gradient. Thus the transpulmonary ET-1 gradient in our study does not suggest severe lung damage or marked PED but rather indicates maintained function of a viable pulmonary endothelium in terms of its capacity to clear ET-1.²⁸

Contribution of Endothelin to Postoperatively Increased PVR

ETA blockade does not decrease PVR in severe lung diseases, such as adult respiratory distress syndrome,²⁹ persis-

TABLE 2. ET-1 data and pulmonary endothelin extraction

	Baseline	O ₂	BQ123	NO
PA (pg/mL)	2.39 ± 0.82	2.62 ± 0.85	2.57 ± 0.99	2.59 ± 1.10
LA (pg/mL)	1.92 ± 0.51	2.14 ± 0.54	2.19 ± 0.72	2.15 ± 0.94
Extraction	17.8% ± 9.1%	16.6% ± 7.4%	12.8% ± 7.6%	17.3% ± 5.5%
P value		.715	.189	.291

PA, Pulmonary artery; LA, left atrium.

tent pulmonary hypertension of the newborn,³⁰ or PHT,³¹ despite demonstrable pulmonary production of endothelin.^{32,33} However, in an experimental model of CPB, ETA blockade before CPB prevented the postoperative increase of both ET-1 and PVR. Furthermore, the decrease in PVR during modified ultrafiltration was attributed to removal of ET-1 in another study.⁴ Going along with these latter studies, we found a decrease of PVR in response to ETA blockade, the degree of which correlated with the circulating ET-1 level. These data suggest a reversible pathophysiologic vasoconstrictor mechanism of ET-1 release in children after surgical intervention for significant intracardial left-to-right shunting defects with CPB.

We did not observe a further significant decrease in PVR with inhaled NO after BQ123. Integrity of the L-arginine–NO pathway is inherent to the effects of endothelin. Impairment of the L-arginine–NO pathway potentiates the effects of endothelin by modulating the vasodilatory effects of ETB receptor stimulation,^{34,35} which relies on intracellular NO as its signal. CPB, by interfering with the L-arginine–NO pathway and increasing endothelin release, might have been expected to interfere with both the ETA and ETB mechanisms. However, in this study we were unable to demonstrate an additional effect of inhaled NO, perhaps providing further evidence that the integrity of the L-arginine–NO pathway is not so severely affected by CPB as previously thought. This concept is in keeping with our previous observations² and underlines that an imbalance of constrictor-dilator mechanisms is produced by CPB. Blockade of the endothelin-constrictor pathway or stimulation-supplementation of the L-arginine–NO dilator pathway seems to be similarly effective.

Limitations

BQ123 was administered intravenously into the systemic circulation and resulted in both a decrease in systemic blood pressure and resistance. However, the decrease in systemic blood pressure was clinically insignificant, and the cardiac index tended to increase. Nonetheless, this might be undesirable in some patients and needs to be borne in mind if used therapeutically. Furthermore, this study was not designed as a dose-ranging study. The effects of higher or lower doses of BQ123 on either the systemic vascular resistance or PVR will need to be assessed in appropriately designed clinical trials.

All our patients were ultrafiltered immediately after CPB. Although modified ultrafiltration results in both reduction of postoperative endothelin levels and PVR, we could demonstrate a significant and clinically relevant further decrease in PVR in our patients. It is interesting to speculate that the effects of ETA blockade might be even more marked in those patients unexposed to modified ultrafiltration.

Finally, our study protocol was designed with the aim of first examining the effects of ETA blockade (to expose the constrictor element to raised postoperative PVR) and then examining for vasodilator failure (by examining the response to inhaled NO). Although theoretically it might have been desirable to examine their effects in a crossover fashion, we believe our study design best addressed the clinical effect of increased circulating endothelins after CPB within the time constraints of a clinical study performed in sedated and paralyzed children after surgical intervention. Indeed, if inhaled NO had been given before ETA blockade, some of the unbalanced constrictor effects might have been obviated, a mechanism that presumably exists during clinical therapy with inhaled NO in these patients. Further studies will be required to examine the effects of ETA blockade in those patients with postoperative PHT resistant or partially responsive to inhaled NO.

Summary

Our data show that ETA blockade with the ETA receptor antagonist BQ123 reduces PVR in children early after cardiac surgery. Furthermore, the reduction in PVR during BQ123 infusion was closely related to the plasma ET-1 levels. The pulmonary extraction of endothelin was preserved, and additional inhaled NO produced no further reduction in PVR, suggesting that endogenous pulmonary bioavailability of NO was not reduced. Thus this study highlights the importance of endothelin in the pathogenesis of PHT after surgical intervention for congenital intracardiac shunting lesions and might open new avenues in its treatment.

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