Endothelin-A Receptor Blockade and Inhaled Nitric Oxide in a Porcine Model of Meconium Aspiration Syndrome

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ABSTRACT

Acute neonatal pulmonary hypertension is associated with increased activation of the endogenous endothelin pathway. We investigated the role of selective endothelin-A receptor blockade using i.v. BQ-123 in a piglet model of meconium aspiration syndrome. Meconium aspiration was induced in 18 anesthetized piglets. Six controls received no further intervention. Six piglets received 1 mg/kg BQ-123 at 120 min, with the addition of 20 ppm inhaled nitric oxide at 240 min. Six commenced nitric oxide therapy at 120 min, and were given i.v. BQ-123 at 240 min. The total study duration was 360 min. Meconium aspiration resulted in acute pulmonary hypertension and elevated endothelin-1 levels in all animals. There were no changes in pulmonary hemodynamics or endothelin-1 levels beyond 120 min in controls. In the group receiving BQ-123 first, this agent alone reduced the pulmonary artery pressure and pulmonary vascular resistance,

and the subsequent addition of inhaled nitric oxide further reduced pulmonary artery pressure. In the group first receiving nitric oxide alone, this reduced the pulmonary artery pressure, and the addition of BQ-123 resulted in a fall in pulmonary vascular resistance. Endothelin-1 levels increased with both agents. BQ-123 was found to be a highly effective pulmonary vasodilator and augmented the effects of nitric oxide in this model of acute pulmonary hypertension. (*Pediatr Res* 56: 353–358, 2004)

Abbreviations

PPHN, persistent pulmonary hypertension of the newborn
PVR, pulmonary vascular resistance
ET, endothelin
iNO, inhaled nitric oxide

PPHN occurs when there is a failure of the normal rapid fall in PVR and increase in pulmonary blood flow with the onset of respiration at birth (1). Meconium aspiration syndrome is the single most common cause of PPHN, and can result in significant morbidity and mortality in term and near-term infants (2).

The normal transition from the high-resistance fetal circulation to the low-resistance postnatal pulmonary circulation is associated with activation of the endogenous vasodilator nitric oxide-cyclic guanosine 5'-monophosphate pathway, and reduced release of the potent pulmonary vasoconstrictor, ET (3). The ET-1 isopeptide is a 21-amino acid polypeptide (4) that is produced within the pulmonary endothelium by the cleavage of its precursor, pro-ET (big ET), by ET converting enzyme. ET-1 is released by the vascular endothelium, and produces its

effects through its interaction with at least two receptor subtypes—the A and B receptors, located on the vascular smooth muscle and endothelium. The pulmonary vasoconstrictor effects of ET-1 result mainly from activation of the G-protein-coupled smooth muscle A receptor, whereas stimulation of the endothelial ET-B receptor has been shown to result in vasodilatation (5). ET-1 is present in high levels at birth, with a gradual fall during the early neonatal period in healthy individuals (6). Elevated ET-1 levels have been demonstrated in animal models of meconium aspiration (7), in neonates with PPHN (3, 8, 9), and in older children (10) and adults (11) with pulmonary hypertension. Furthermore, the plasma ET-1 level appears to positively correlate with disease severity (8, 9, 12).

Therapy for pulmonary hypertension in the neonate is currently targeted at preventing or reversing the sequelae of pulmonary endothelial dysfunction. iNO improves oxygenation and reduces the need for extracorporeal life support in some of the most severe cases (13). However, the individual response to iNO is disease specific (13, 14). In infants with meconium aspiration syndrome, the distribution of iNO, and its

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subsequent efficacy, can be limited by the presence of meconium in the airways, reduced surfactant activity, and widespread ventilation-perfusion mismatch (14). There is a need for continued investigation of adjuncts, or even alternatives to iNO, and in particular exploration of the role of systemic agents for the treatment of acute pulmonary hypertension.

We recently demonstrated an acute fall in PVR, in response to an i.v. bolus of the selective ET-A receptor antagonist BQ-123, in children after surgery for congenital heart disease (15). Acute, selective ET-A receptor blockade has been shown to reduce PVR in piglet and lamb models of hypoxic pulmonary hypertension (16–18) and congenital diaphragmatic hernia (19). Previous investigators have suggested that ET-A receptor blockade may improve smooth muscle responsiveness to iNO in monocrotaline-induced pulmonary hypertension (20), and it has recently been proposed that ET-A receptor antagonists may have a role in the prevention of rebound pulmonary hypertension upon iNO withdrawal (21).

In this study, we have compared for the first time the pulmonary vascular responses to the selective i.v. ET-A receptor antagonist BQ-123 and iNO, and have examined the acute interaction between the two agents in a piglet model of acute pulmonary hypertension secondary to meconium aspiration syndrome.

METHODS

The study conformed to the U.S. *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No 85–23, revised 1996). Eighteen female Danish Landrace piglets weighing 4.9 kg were premedicated with intramuscular ketamine (2 mg/kg) and midazolam (1 mg/kg), followed by an i.v. bolus of ketamine (1 mg/kg) administered through the ear vein. They were anesthetized, ventilated, instrumented for monitoring of systemic blood pressure, pulmonary artery pressure, and cardiac output, and sedated, as previously described (22).

After completing the preparation, hemodynamics were allowed to stabilize for 15 min. Then systemic, central venous, pulmonary arterial, and pulmonary capillary wedge pressures, ventilatory settings, and cardiac output were recorded and an arterial blood gas was taken. Systemic and pulmonary vascular resistances and oxygenation index were calculated using standard equations. Inspired oxygen and tidal volumes were adjusted to maintain an arterial P_{O_2} of 70–100 mm Hg, and carbon dioxide of 35–45 mm Hg. After 30 min, a further set of hemodynamics was recorded, and blood gas analysis was performed; these measurements were taken as baseline (0 min).

Each animal then received a deep intratracheal instillation (3 mL/kg) of a 20% solution of pooled human meconium, prepared as previously described (22). Animals were randomly allocated to one of three study groups. Six animals (controls) did not receive any further therapeutic intervention, and were monitored for 360 min. Six animals received a single dose (1 mg/kg infused over 20 min) of i.v. BQ-123 (Clinalfa AG, Laufelfingen, Switzerland) at 120 min and were given iNO (20 ppm) at 240 min. iNO was continued until the experiment was terminated at 360 min. The six remaining animals received

iNO at 120 min, with a dose of i.v. BQ-123 (1 mg/kg) at 240 min.

An arterial blood gas was taken 30 min after each intervention, and complete sets of measurements (hemodynamics and blood gases) were made hourly. Ventilation was adjusted aiming to achieve the parameters outlined above. Systemic arterial samples were taken for ET-1 assays at 0, 120, 240, and 360 min.

At the end of the study, all animals underwent fluoroscopy with an injection of i.v. contrast to the right atrium to exclude an intracardiac right-to-left shunt, and then were killed with a lethal injection of i.v. pentobarbital.

ET-1 assay. Blood samples were taken using EDTA as an anticoagulant, and were immediately centrifuged at 1000 g at 4°C for 10 min, and the supernatant plasma was stored at –40°C. A quantitative sandwich ELISA technique (QuantiGlo human ET-1 assay, R & D Systems, Minneapolis, MN, U.S.A.) 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 1–20 pg/mL, with a minimum detectable ET-1 concentration of 0.16 pg/mL and a cross-reactivity with big ET of 0.01–0.02%. Samples were measured in duplicate and diluted both 1:1 and 1:10, and were pipetted into microplate wells precoated with ET-1-specific MAb. After removing the unbound substance, the ELISA antibody was added to the wells, and light produced in response to the addition of luminol/peroxide substrate was measured with a microplate luminometer.

Statistics. Measurements in the three groups at each time point were compared with a one-way ANOVA. Within-group responses to interventions (meconium instillation, iNO, and BQ-123) were evaluated with a one-way repeated measures ANOVA, using Bonferroni's correction for multiple comparisons. Results are expressed as mean (SEM); a p value of <0.05 was considered statistically significant.

RESULTS

Meconium Instillation: 0–120 min

Baseline recordings and initial changes in hemodynamic and oxygenation variables after meconium instillation (120 min) were similar for all three study groups; therefore the responses to meconium instillation between 0 and 120 min for the 18 animals will be considered together (Table 1).

Meconium instillation significantly increased the mean pulmonary artery pressure and PVR. Systemic hemodynamics and systemic vascular resistance were unchanged; thus, the ratio of pulmonary-to-systemic vascular resistance increased significantly. Meconium instillation necessitated an increase in inspired oxygen fraction and mean airway pressure to maintain the target level of oxygenation; thus, the oxygenation index more than doubled (mean increase $135 \pm 44\%$). ET-1 levels also increased significantly after meconium instillation.

Changes Occurring Between 120 and 360 min

Changes occurring between 120 and 360 min are described in Table 2 and Figure 1.

Table 1. Hemodynamic parameters for all animals (n = 18) at baseline (0 min) and 120 min after meconium instillation

Parameter	0 min	120 min
Weight (kg)	4.9 (0.11)	—
Cardiac output (mL/kg/min)	169 (10)	176 (10)
Mean PAP (mm Hg)	17.8 (0.7)	24.4 (0.9)*
Mean PCWP (mm Hg)	7.8 (0.6)	7.1 (0.7)
Mean ABP (mm Hg)	59 (1.2)	62 (1.4)
CVP (mm Hg)	7.6 (0.6)	7.3 (0.6)
Mean PVR (units/kg)	0.065 (0.005)	0.106 (0.01)*
Mean SVR (units/kg)	0.33 (0.02)	0.33 (0.02)
PVR/SVR	0.20 (0.01)	0.31 (0.02)*
Paw (cm H ₂ O)	9.7 (0.3)	12.1 (0.5)*
Pao ₂ (mm Hg)	101 (6)	82 (6)*
Oxygenation index	3.0 (0.2)	6.1 (0.7)*
Endothelin-1 (pg/mL)	1.52 (0.21)	2.34 (0.31)*

PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; ABP, arterial blood pressure; CVP, central venous pressure; PVR, pulmonary vascular resistance; SVR, systemic vascular resistance; Paw, mean airway pressure; Pao₂, arterial partial pressure of oxygen.

* $p < 0.05$.

Controls. In the six controls, there were no further significant changes in hemodynamic parameters, or in oxygenation index, for the remainder of the study period. ET-1 levels did not change significantly beyond 120 min.

BQ-123 at 120 min with iNO at 240 min. An infusion of BQ-123 alone significantly reduced pulmonary artery pressure, and tended to increase cardiac output ($p = 0.07$). Pulmonary capillary wedge pressure was unchanged, and PVR fell significantly. Mean arterial blood pressure fell transiently between 120 and 180 min, but increased again to pre-BQ levels at 240 min (Fig. 1); and systemic vascular resistance was unchanged. PVR and the pulmonary-to-systemic vascular resistance ratio were reduced significantly at 240 min.

The addition of iNO to animals after BQ-123 produced significant falls in mean pulmonary artery and pulmonary capillary wedge pressures, but did not change cardiac output. PVR, systemic hemodynamics, ventilatory requirements, and oxygenation were unchanged by iNO.

Infusion of BQ-123 resulted in a significant increase in plasma ET-1 levels between 120 and 180 min, and ET-1 increased further between 240 and 360 min during iNO therapy (Fig. 1).

iNO at 120 min with BQ-123 at 240 min. iNO alone significantly reduced the pulmonary artery pressure. Cardiac output was unchanged, and PVR tended to fall between 120 and 240 min, though this change did not quite reach statistical significance ($p = 0.1$). The addition of BQ-123 at 240 min tended to increase the cardiac output ($p = 0.07$), without producing any change in pulmonary capillary wedge pressure or pulmonary arterial pressure. PVR fell significantly between 240 and 360 min (Fig. 1). Mean systemic blood pressure fell transiently between 240 and 300 min, but this change had reversed by 360 min (Fig. 1).

ET-1 levels were obtained from 16 animals. Samples were unsuitable from one control, and from one animal in the group receiving nitric oxide first. ET-1 increased significantly during iNO alone, and continued to increase after the addition of

BQ-123 (Fig. 1). Ventilatory requirements and oxygenation were unchanged by any of the therapeutic interventions.

At the conclusion of the study, none of the subjects had any evidence of an intracardiac shunt on contrast injection.

DISCUSSION

Our current study has clearly demonstrated that BQ-123 is an effective and relatively selective pulmonary vasodilator in the treatment of pulmonary hypertension in this model of meconium aspiration syndrome. BQ-123 reduced the pulmonary artery pressure without changing wedge pressure, and tended to increase the cardiac output. When given to animals already receiving iNO, BQ-123 further lowered the PVR. There was a minimal (<10%) and transient reduction in systemic blood pressure during the first hour after BQ-123 administration, which self-corrected without intervention. Neither agent produced any detectable effect upon oxygenation, either alone or in combination, in this group of animals without an intracardiac shunt.

Endothelial function is a key determinant of pulmonary vascular tone and development. In recent years, treatments for PPHN have been aimed at controlling or reversing the pulmonary endothelial dysfunction, which otherwise rapidly initiates the unrelenting cascade of pulmonary hypertension, right-to-left shunting, and hypoxemia. iNO has an important role in the acute management of pulmonary hypertension (14). However, the limited response in some patient groups, the potential for rebound upon iNO withdrawal, and more recent financial implications of long-term iNO therapy, coupled with our increasing understanding of other important molecular pathways in the pathophysiology of pulmonary hypertension, remain compelling stimuli for research into other agents (23, 24).

There are few clinical investigations of ET receptor blockade in the treatment of acute pulmonary hypertension in the literature to date. Two previous studies have demonstrated a *preventive* effect of ET-A receptor blockade on the subsequent development of pulmonary hypertension in an acute hypoxic rat model (25) and in a model of meconium aspiration (26). The beneficial effects of other ET-A receptor antagonists in the treatment of acute pulmonary hypertension have recently been demonstrated in lamb (16, 17), and pig (27) models of acute hypoxic pulmonary hypertension, and in a lamb model of congenital diaphragmatic hernia (19). Furthermore, the intravenous combined ET-A and -B receptor antagonist, tezocentan, has been shown to reduce PVR in a model of oleic acid-induced lung injury (28). There are no published studies that compare the clinical effects of ET-A receptor antagonists with iNO in acute pulmonary hypertension, and a single published study has examined the combined influences of ET-A receptor blockade and iNO in an acute hypoxic pulmonary hypertension. In this model, receptor blockade reduced PVR, and this fell further with the addition of iNO (29).

Our current study is therefore unique in a number of ways. First, we have explored for the first time the use of the ET-A receptor antagonist, BQ-123, as a treatment for pulmonary hypertension secondary to meconium aspiration. Second, we have compared the agent with iNO, which is the current

Figure 1. Mean pulmonary artery (PA) pressure, PVR, mean systemic arterial pressure (ABP), and ET-1 levels in controls (closed circle, $n = 6$); in animals receiving BQ-123 as intervention 1 at 120 min followed by iNO as intervention 2 at 240 min (open square, $n = 6$); and in animals receiving iNO as intervention 1 with BQ-123 as intervention 2 (open triangle, $n = 6$). Significant changes after each intervention are shown as follows: for controls (open circle); for group receiving BQ-123 first (closed square); for group receiving iNO first (closed triangle). Meconium instillation increased mean PA pressure, PVR, and ET-1 significantly in all animals. There were no further significant changes in controls. BQ-123 as intervention 1 lowered PA pressure and PVR, and further reduced PVR as intervention 2. iNO reduced PA pressure when given as intervention 1, and as intervention 2, but did not independently lower PVR. ABP fell transiently during the first 60 min after BQ-123, but recovered spontaneously during the next 60 min. ET-1 increased with both interventions.

preferred therapy for neonatal pulmonary hypertension (13). Third, we have examined the interaction between the two therapies in this model.

The pulmonary hypertension that followed meconium aspiration was accompanied by an increase in ET-1 levels. In the normal neonate, ET-1 levels are elevated at birth, and have been shown to fall as the elevated PVR of the transitional circulation naturally falls with adaptation to extrauterine life (6). ET levels are further elevated in neonates with persistent pulmonary hypertension when compared with control (6–9).

ET-1 levels in our control group followed a near-identical pattern to PVR. That is, the acute, and significant, elevation over the first 120 min was followed by a plateau without further significant change. Kuo previously observed a correlation between changes in ET-1 levels and elevation of PVR in a model of meconium aspiration similar to our own (7). The administration of BQ-123 in our study resulted in a further increase in plasma ET-1 despite a fall in PVR. This pattern of response has been demonstrated by others in acute and chronic models of hypoxic pulmonary hypertension (18, 29), the proposed mechanism being an increase in “unbound” ET-1 after receptor blockade.

The effect of iNO on ET-1 is more complex. In this study, we have shown a clear rise in ET-1 levels in all animals when given iNO. The rise seen in those first given BQ-123 and then nitric oxide could potentially be interpreted as a “continued” response to the BQ-123 rather than a response to iNO itself. However, the increase in ET-1 in the group first receiving iNO alone would strongly support an independent effect of iNO on ET-1 release.

Our observation of an increase in ET-1 during iNO treatment would agree with some but not all previous investigations. Truog *et al.* (30) demonstrated a reduction in ET-1 levels during short-term iNO therapy in neonates with PPHN. An insignificant rise in ET-1 was observed by Christou *et al.* (3) during the first 24 h of iNO therapy in infants with PPHN. However, in healthy lambs, acutely increased plasma ET-1 levels were observed in response to iNO, lending weight to the suggestion that ET-1 may play a role in the pathophysiology of rebound pulmonary hypertension upon iNO withdrawal (21, 31). Similar findings have been observed in children after surgery for congenital heart disease and adults with acute respiratory distress syndrome (32); and increased lung tissue expression of ET-1 has been demonstrated in a model of lung injury after iNO exposure (33). The exact explanation for an increase in ET-1 during iNO therapy is unclear: proposed mechanisms include nitric oxide-induced alterations in ET-1 gene expression (34), or displacement of the ET-1 from the A receptor in the presence of iNO (35).

The benefits of long-term ET receptor antagonists in the treatment of chronic pulmonary arterial hypertension are becoming increasingly recognized. Recent clinical literature suggests beneficial effects on functional capacity in adults (36, 37) and children (38) receiving long-term therapy with the combined oral ET-A and -B receptor antagonist bosentan.

Our current study has important new implications for the clinical treatment of acute pulmonary hypertension. Administration of BQ-123 alone produced relatively greater pulmonary

Table 2. Hemodynamics and gas exchange during the study period

	Controls (meconium only)				BQ-123 at 120 min, NO at 240 min			
	0 min	120 min	240 min	360 min	0 min	120 min	240 min	360 min
Mean PAP (mm Hg)	19.0 (1.0)	25.0* (1.6)	26.0 (2.3)	27.8 (2.0)	19.0 (1.7)	25.5* (1.9)	23.2 (2.0)†	21.2‡ (1.6)
CO (mL/kg/min)	150 (20)	154 (12)	160 (14)	167 (11)	178 (21)	165 (12)	195 (24)	186 (24)
PVR (units/kg)	0.075 (0.01)	0.124* (0.02)	0.129 (0.023)	0.127 (0.022)	0.060 (0.01)	0.100* (0.01)	0.067† (0.01)	0.071 (0.01)
AoP (mm Hg)	56 (2)	61 (2)	66 (3)	69 (2)	59 (4)	60 (4)	60 (3)	60 (4)
SVR (units/kg)	0.35 (0.05)	0.35 (0.03)	0.38 (0.04)	0.37 (0.03)	0.30 (0.03)	0.32 (0.03)	0.29 (0.05)	0.31 (0.05)
PVR/SVR	0.22 (0.02)	0.35* (0.04)	0.34 (0.04)	0.34 (0.04)	0.20 (0.01)	0.31 (0.03)*	0.27† (0.06)	0.26 (0.04)
Pao ₂ (mm Hg)	100 (5)	75 (5)	77 (9)	71 (6)	96 (7)	76 (9)	80 (5)	75 (4)
OI (units)	3.7 (0.4)	6.6 (0.9)*	7.2 (1.0)	7.7 (1.0)	3.0 (0.5)	6.0 (1.8)*	6.0 (1.0)	5.3 (1.2)
Paco ₂ (mm Hg)	38 (1.5)	38 (1.5)	41 (2.3)	39 (2.3)	41 (2.3)	42 (3.0)	44 (0.1)	42 (3.0)
ET-1 (pg/mL)	1.26 (0.16)	2.04* (0.35)	2.13 (0.36)	2.24 (0.40)	1.35 (0.32)	1.68* (0.35)	2.25† (0.36)	2.83‡ (0.37)

CO, cardiac output; OI, oxygenation index. Within-group statistically significant changes: * $p < 0.05$, 120 min vs 0 min; † $p < 0.05$, 240 min vs 120 min; ‡ $p < 0.05$, 360 min vs 240 min.

Table 2. (Continued)

	iNO at 120 min, BQ-123 at 240 min			
	0 min	120 min	240 min	360 min
Mean PAP (mm Hg)	17.0 (1.0)	23.0* (1.1)	19.3† (1.2)	19.0 (1.5)
CO (mL/kg/min)	176 (21)	200 (22)	192 (17)	203 (29)
PVR (units/kg)	0.060 (0.00)	0.101* (0.02)	0.084 (0.01)	0.071‡ (0.01)
AoP (mm Hg)	61 (3)	64 (3)	64 (3)	60 (3)
SVR (units/kg)	0.34 (0.04)	0.31 (0.03)	0.32 (0.03)	0.30 (0.06)
PVR/SVR	0.18 (0.02)	0.31 (0.05)*	0.26 (0.02)	0.30 (0.03)
Pao ₂ (mm Hg)	97 (11)	76 (8)	84 (6)	80 (4.5)
OI (units)	2.8 (0.4)	5.7 (1.4)*	7.1 (2.3)	8.4 (2.0)
Paco ₂ (mm Hg)	38 (1.5)	39 (2.3)	43 (1.0)	41 (1.5)
ET-1 (pg/mL)	1.05 (0.25)	1.78* (0.32)	2.56† (0.25)	3.13‡ (0.35)

CO, cardiac output; OI, oxygenation index. Within-group statistically significant changes: * $p < 0.05$, 120 min vs 0 min; † $p < 0.05$, 240 min vs 120 min; ‡ $p < 0.05$, 360 min vs 240 min.

vasodilatation than did iNO alone, with minimal systemic side effects, and without any deterioration in oxygenation. BQ-123 tended to increase cardiac output, and, when given in addition to iNO, resulted in a slight but further reduction in PVR. ET-A receptor blockade may well have a role in the treatment of PPHN secondary to meconium aspiration syndrome, and potentially from other aetiologies.

Limitations. There are a few potential limitations within our study. First, our choice of the dose of BQ-123 (1 mg/kg) was based upon the doses used in earlier animal studies (39, 40). Second, the animals studied were about 2 wk old, and thus may have different, or less exaggerated, hemodynamic responses than piglets in the first hours of life. No animal had an intracardiac shunt, which could potentially influence (or limit) any acute effect of an intravenous pulmonary vasodilator upon oxygenation. However, a number of previous investigations of pulmonary vasodilators in models of acute pulmonary hypertension (27, 29, 41) and, specifically, therapies for meconium aspiration syndrome (42, 43) have been explored in more mature animals. Indeed, having demonstrated an acute rise in ET-1 levels coincident with an increase in PVR after meconium aspiration, it would seem appropriate to investigate the subsequent actions of an ET-A receptor antagonist in our model.

CONCLUSION

The selective ET-A receptor antagonist, BQ-123, is a highly effective pulmonary vasodilator in this model of acute pulmo-

nary hypertension secondary to meconium aspiration syndrome. BQ-123 did not produce any sustained systemic side effects and did not impair oxygenation. When given to subjects already receiving nitric oxide, BQ-123 resulted in a subtle additional reduction in PVR. Targeting the pulmonary endothelium using ET-A receptor antagonists may represent a safe alternative or adjunctive therapeutic approach in the acute management of persistent pulmonary hypertension of the newborn.

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