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**Key Words**
Indomethacin infusion
Cerebral blood flow/oxygenation
Preterm babies

**Abstract**
Although an indomethacin-induced decrease of brain perfusion in preterm infants has been well established, the acute effects of this vasoactive drug on cerebral hemodynamics and oxygenation are not well documented. Using near infrared spectroscopy we monitored in 6 very preterm infants changes in cerebral blood volume (ΔCBV) and cytochrome oxidase concentration (ΔCytaa₃), used as relative measures of changes in brain perfusion and as an indicator for cellular oxygenation of brain tissue, during and up to 1 h after indomethacin infusion. ΔCBV showed a quick blood-pressure-related increase as compared to baseline (preindomethacin values) during indomethacin infusion (averaged maximal increase 13%), followed by a sharp decrease below baseline values (averaged maximal decrease 24%). There was a sustained recovery to baseline during the registration period. ΔCytaa₃ showed a small, early increase in 4 of 6 babies, followed by a substantial decrease below baseline in 5 babies. ΔCytaa₃ showed only a partial recovery in those 5 babies during the study period. We conclude that a therapeutic dose of indomethacin may cause substantial swings in brain perfusion and a marked and rather longstanding decrease in Cytaa₃, suggesting a decrease in cellular oxygenation of brain tissue. Awareness of these effects may be important in sick preterm babies during periods of pulmonary and cardiac instability.
Introduction

Indomethacin, a prostaglandin inhibitor, has been used extensively for noninvasive closure of a hemodynamically important patent ductus arteriosus (PDA) in the preterm infant. Indomethacin has a constrictive action on the vascular beds of important organ systems in the newborn animal and human neonate [1-4]. Several studies showed an increase of the resistance of the cerebral vascular bed with a subsequent decrease in brain blood flow up to 40% for at least 1 h after administration of a therapeutic dose of indomethacin to preterm babies [2, 5].

Although immediate significant increases in arterial blood pressure have been reported during indomethacin infusion in experimental and clinical studies [6, 7], the impact of these acute hemodynamic perturbations on the preterm cerebral circulation, which has a vulnerable autoregulation [8], and on metabolism, is not well documented.

We therefore monitored cerebral perfusion and cellular oxygenation of brain tissue in 6 preterm babies, using near infrared spectroscopy (NIRS), during and up to 1 h after intravenous infusion of 0.1 mg/kg indomethacin.

Patients and Methods

The study was performed on preterm infants, who received an initial intravenous dose of 0.1 mg/kg of indomethacin for noninvasive closure of PDA. The indomethacin was infused over a 5-min period. The diagnosis of PDA was made on clinical symptoms and radiographic features, and confirmed by Doppler echocardiographic investigation. Informed parental consent was obtained in all infants. The study was approved by the scientific board of the Department of Pediatrics.

Assessment of Cerebral Hemodynamics and Oxygenation by NIRS

The head of the neonate is relatively transparent to near infrared light. Hemoglobin (Hb) and cytochrome oxidase (the terminal member of the mitochondrial respiratory chain) are natural chromophores and both have an oxygenation-dependent absorption in this wavelength region. By selection of the appropriate wavelength, algorithms have been developed to convert absorption changes into changes in concentration of oxygenated Hb ($\Delta$HbO$_2$), deoxygenated Hb ($\Delta$HbR), total Hb ($\Delta$Hb = $\Delta$HbO$_2$ + $\Delta$HbR) and cytochrome oxidase ($\Delta$Cytaa$_3$) in mmol/l [9, 10]. Quantification of the NIRS variables into absolute concentration changes presupposes that the pathlength is known. The interoptode distances in the present study ranged from 5 to 6 cm. The optical pathlength is supposed to be considerably greater than the interoptode distance, due to significant scattering of light in brain tissue. Similar to earlier studies by others we assumed an optical pathlength of 4.4 times the interoptode distance [9]. The NIRS instrument used (Radiometer, Copenhagen, Denmark), consisted of four semiconductor laser diodes with wavelengths of 904, 845, 805 and 775 nm. The lasers were operated sequentially, and pulsed with a repetition rate of 500 Hz for 200 ns. We placed the transmitting and receiving optodes on the parietal-frontal regions on both sides of the head above the ear. Changes in optode position will cause changes in pathlength which results in absorption changes not related to changes in cerebral blood or tissue oxygenation. We therefore used a proper fixation method of the optodes as described earlier [11]. The energy emitted by each diode was well within the orders of the British Standards Institute safety limits (BS 4803).

Relative changes in cerebral blood volume ($\Delta$CBV) were calculated using the following equation: $\Delta$CBV = $\Delta$Hbtotal $\times$ 0.89/ [Hb], where [Hb] is the large vessel hemoglobin concentration in g/dl [12]. Changes in $\Delta$CBV are thus relative changes from the baseline value and expressed in ml/100 g brain tissue. Earlier studies showed a good relationship with changes in actual brain blood flow, determined with the $^{133}$Xe clearance method [10, 13]. We therefore considered that $\Delta$CBV indicated changes in brain perfusion, if changes in $\Delta$CBV were caused predominantly by changes in $\Delta$HbO$_2$. $\Delta$Cytaa$_3$ is supposed to indicate changes in the oxidation level of the intracerebral mitochondrial enzyme cytochrome oxidase and has been used as a relative measure of brain cell oxygenation [12, 14]. Changes in $\Delta$Cytaa$_3$ are thus relative changes from the baseline value and are expressed in mmol/l.

Study Design, Data Collection and Analysis

NIRS registrations started at least 30–60 min before the start of the study to exclude the possibility of system drift. Indomethacin infusion started at the mo-
ment a stable baseline was reached for at least 5–10 min. The recordings ended 1 h after the completion of the indomethacin infusion.

Changes relative to baseline in HbO₂ (ΔHbO₂), HbR (ΔHbR), Hb₉tot and Cytaa₃ (ΔCytaa₃) were determined every 4 s and stored in a personal computer for off-line analysis and calculation of ΔCBV. Transcutaneous-derived arterial PO₂ and PCO₂ (tcPO₂ and tcPCO₂), and arterial blood pressure from an indwelling catheter (2 patients) were simultaneously determined every 4 s and stored in the computer. In 4 patients blood pressure was measured using an oscillometric method (Dynamap, Criterion, Tampa, Fla., USA) just before the start of indomethacin infusion and then every 2 min up to 6 min, until completion of the infusion. Further on, blood pressures in these 4 infants were measured at 10, 20, 30, 40, 50, and at 60 min after the start of indomethacin infusion. Hematocrit was determined at regular intervals.

Statistical Analysis

For statistical purposes the 4-second interval values of ΔCBV, ΔCytaa₃, mean arterial blood pressure (MABP), tcPO₂, and tcPCO₂ were averaged per 2-min time interval. Because it appeared from our data (see ‘results’) that changes in arterial blood pressure during indomethacin infusion might play an important role in the simultaneously detected changes in ΔCBV and ΔCytaa₃, we evaluated the relation between the various variables during indomethacin infusion (0–6 min) separately from those obtained after completion of the indomethacin infusion (7–60 min).

To investigate which factor(s) was (were) responsible for the changes in ΔCBV or ΔCytaa₃ during indomethacin infusion, we selected those variables which are known to be involved in brain perfusion: MABP, tcPO₂ and tcPCO₂. We used the (averaged) values of these variables collected at baseline, and at 1–2, 3–4 and 5–6 min after the start of indomethacin infusion. A multiple linear regression model was used. The regression equation was:

\[ Y = b_0 + b_{\text{MABP}} \cdot \text{MABP} + b_{\text{tcPO}_2} \cdot \text{tcPO}_2 + b_{\text{tcPCO}_2} \cdot \text{tcPCO}_2 + \Sigma a_{iv} v_{in} \]

where ΔCBV or ΔCytaa₃ are the dependent variables and b₀ its mean over all the runs. The MABP, tcPO₂ and tcPCO₂ were taken as independent variables. These independent variables were either introduced or removed from the equation, based on their significance level (p < 0.05). To assess the interpatient variability (IV) five patient variables for the 6 patients were also introduced as independent variables. The patients were coded using the effect coding technique [15]. This coding for interindividual (patient) variability was primarily done to account for potential confounding variables that might obscure the effect of the chosen independent variables. The overall model was tested on significance by an F test and related p value. The R² of the regression equation gives the correlation between the predicted ΔCBV/ΔCytaa₃, based on the independent variables of this regression model and the actual ΔCBV/ΔCytaa₃ values. In other words: how well the model fits the data. Furthermore, each independent variables was tested whether it had a significant effect on ΔCBV/ΔCytaa₃ by a partial F test and related p value. A more detailed explanation has been given by Glantz and Slinker [15].

To investigate a possible relation between brain perfusion (ΔCBV) or oxygenation (ΔCytaa₃), MABP and tcPO₂ and tcPCO₂ after the completion of the indomethacin infusion, we repeated the multiple linear regression model as described above. We used the (averaged) values of these values collected at 10, 20, 30, 40, 50 and 60 min after the start of the indomethacin infusion.

To further elucidate the (individual) relationship between MABP and ΔCBV (see ‘results’), we used simple linear regression analysis. To test whether or not a curvilinear fit showed a better correlation as compared to linear regression, we used a polynomial regression analysis.

Differences between hemodynamic variables and extra oxygen need before indomethacin and at 5, 30 and 60 min were investigated by analysis of variance for repeated measurements followed by Scheffe’s F test if statistically significant differences were obtained. To investigate whether or not intraindividual differences between pre- and 12 h postindomethacin hematocrit existed, the Student’s t test for paired observations was used. p values of less than 0.05 were considered statistically significant.

Results

Nine patients were initially included in the study. In 3 of these 9 patients it was not possible to obtain reliable NIRS recordings for at least 1 h (2 patients) or reliable blood pressure measurements (1 patient). The remaining 6 patients all met the clinical signs for PDA (characteristic murmur, bounding pulses, hyperdynamic precordium) and Doppler echo-
cardiographic investigation confirmed PDA: a diastolic reverse flow in the main pulmonary artery with a predominantly left-to-right flow through the ductus arteriosus. Moreover, all infants were mechanically ventilated (Infant star, Infrasonics Inc., San Diego, Calif., USA) and none of them could be weaned from the ventilator. The patient characteristics of these 6 infants are shown in table 1. During the study period, the infants were stable and no major changes in ventilator settings were necessary. Table 2 gives PDA-related data (mean values ± SD of MABP, pulse pressure, heart rate and extra oxygen need) as a function of time. Only MABP upon completion of the indomethacin infusion was significantly higher as compared to the MABP before indomethacin administration. The other variables did not differ during the study period. tcPO2 values were stable in each infant and within the normal range (mean ± SD: 61 ± 11 mm Hg), tcPCO2 values were slightly elevated in 2 infants but stable in each infant (mean ± SD: 52 ± 6 mm Hg). Individual hematocrit values did not differ before indomethacin infusion as compared to values 12 h after its administration.

### Individual Patterns of ΔCBV and ΔCytaa3

Individual patterns of ΔCBV and ΔCytaa3 are shown in figure 1. There was a biphasic response of ΔCBV after indomethacin administration: A variable but quick increase in ΔCBV in 5 of the 6 patients (patients 1–5) as compared to baseline during indomethacin-infusion with an average maximal increase of 0.4 ml/100 g brain tissue, followed by a decrease below baseline in all patients after completion of the infusion (average maximum decrease: 0.6 ml/100 g brain tissue). These changes were almost exclusively caused by

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>GA, weeks</th>
<th>BW, g</th>
<th>Age, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.3</td>
<td>835</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>30.6</td>
<td>1,835</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>25.0</td>
<td>672</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>28.6</td>
<td>900</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>30.9</td>
<td>1,730</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>27.0</td>
<td>1,000</td>
<td>7</td>
</tr>
<tr>
<td>Mean ± 1 SD</td>
<td>27.9±2.3</td>
<td>1,162±450</td>
<td>14.5±7.4</td>
</tr>
</tbody>
</table>

### Table 2. Mean values ± SD of MABP, pulse pressure, heart rate and extra oxygen need (FiO2) as a function of time

<table>
<thead>
<tr>
<th></th>
<th>Before indomethacin</th>
<th>After indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>30 min</td>
</tr>
<tr>
<td>MABP, mm Hg</td>
<td>35±3</td>
<td>46±6*</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>26±5</td>
<td>23±8</td>
</tr>
<tr>
<td>Heart rate</td>
<td>154±13</td>
<td>145±15</td>
</tr>
<tr>
<td>FiO2</td>
<td>0.43±0.18</td>
<td>0.45±0.21</td>
</tr>
</tbody>
</table>

* p < 0.01 vs. before indomethacin.
Fig. 1. Individual patterns of ACBV and ACV(3) as a function of time.
changes in ΔHbO₂. Four patients showed a (mostly) sustained recovery to baseline during the recording time, but in the 2 other patients ΔCBV was well below baseline at the end of the NIRS registrations. ΔCytaa₃ showed a small early increase in 4 of the 6 babies (average maximum increase: 0.9 mmol/l) and a decrease well below baseline in 5 babies with no or an incomplete recovery to baseline at the end of the registration period (average maxi-
mum decrease: 3 mmol/l). One patient showed a second persistent increase in ΔCytaa₃ as compared to baseline value from 10 min post-indomethacin.

**Relationship between ΔCBV or ΔCytaa₃, and MABP, tcPO₂, and tcPCO₂ during Indomethacin Infusion**

We were able to construct significant regression models for both ΔCBV and ΔCytaa₃ (p values equations; <0.05 and <0.05). Only MABP appeared to be a significant predictor of ΔCBV (partial F value: 8.12, p < 0.05), whereas tcPO₂ and tcPCO₂ did not. There was no relation between ΔCytaa₃, and MABP, tcPO₂, or tcPCO₂. In both equations (ΔCBV and ΔCytaa₃) the interpatient variability was not significant.

Figure 2a shows the individual relationships between MABP and ΔCBV. The correlation coefficients (r) ranged from 0.56 to 0.98. The overall correlation coefficient appeared to be 0.74 (p < 0.0001), but increased to 0.86 (p < 0.0001) when a curvilinear fit was used (3rd order polynomial regression analysis). This curvilinear relationship is shown in figure 2b.

**Relationship between ΔCBV or ΔCytaa₃, and MABP, tcPO₂, and tcPCO₂ after Completion of Indomethacin Infusion Onward**

Also here we were able to construct significant regression models for both ΔCBV and ΔCytaa₃ (p values equations; <0.01 and <0.01). MABP, tcPO₂ or tcPCO₂ had no influence on ΔCBV or ΔCytaa₃ during the remainder of the study period. It must be mentioned, however, that MABP, tcPO₂ and tcPCO₂ were rather stable during this part of the study. The interpatient variability was significant in both equations (ΔCBV: F set 40.18, p < 0.0001; ΔCytaa₃: F set 10.51, p < 0.001).

**Discussion**

Although the magnitude of the individual changes were quite variable, the present study showed that intravenous infusion of indomethacin induced an arterial blood pressure-related increase of ΔCBV, whereas after the completion of the infusion ΔCBV decreased well below baseline in almost all patients. Assuming that normal CBV in these preterm babies is in the order of 2.5–3.5 ml/100 g brain tissue [12, 16], this means an approximately 13% increase and 24% decrease of CBV, respectively. As far as changes in CBV reflect changes in actual brain blood flow, the present study indicates substantial indomethacin-induced swings of brain perfusion in at least some of these preterm babies.

Although indomethacin has been shown to enhance the autoregulatory ability of the neonatal cerebral vascular bed [17], an initial, arterial blood pressure related increase in brain perfusion during indomethacin infusion, suggesting lack of cerebral autoregulation, has not yet been reported. Experimental studies report that the autoregulatory ability of the cerebral vascular bed is already operative in early fetal life, but that the autoregulatory range is narrow and resting cerebral perfusion pressure is very near its lower limit [18, 19]. Also in very preterm infants less than 32 weeks of gestation with a postnatal age between 12 and 72 h, cerebral autoregulation has been reported, again only over a narrow range (from 32 to 41 mm Hg) of mean arterial pressures [8]. Another study in preterm babies reported similar findings [20]. Although regulatory ability improves with advancing gestational and postnatal age and the range of blood pressures at which brain perfusion is constant is higher in more mature newborns [19, 21], Lou et al. [22] reported that especially in distressed (preterm) babies cerebral autoregulatory ability was often impaired. Fig-
Figure 2a shows the individual best fits between ΔCBV and MABP of our study group during indomethacin infusion by linear regression analysis. It can be questioned, however, whether or not this is the appropriate manner to analyse these data for all patients: For instance, in patients 1, 3 and 4 the highest MABP values may have exceeded the upper limit at which autoregulation of the cerebral circulation was still operative, whereas in patient 5, the patient with the most advanced gestational age, the pattern of MABP-related changes suggests that the pre-indomethacin MABP was below the lower limit of autoregulatory ability of the cerebral vascular bed. In all instances a curvilinear fit rather than a linear fit might be more appropriate. This is supported by the curvilinear fit of the pooled results shown in figure 2b, which in fact show a blood-pressure-independent brain perfusion of between 35 and 42 mm Hg, surprisingly similar to earlier studies in preterm newborns with comparable gestational age [8]. However, our data must be interpreted with caution because of the small study population and heterogeneity in gestational and postnatal age.

The subsequent decrease in brain perfusion confirms earlier studies in newborn animals and preterm babies. In all these studies a decrease in brain blood flow (velocity) was reported up to 40% of pre-indomethacin values and lasting up to 2 h after indomethacin administration [2, 5, 23, 24]. Although recent studies showed a beneficial effect of early indomethacin treatment in very preterm babies with regard to periventricular-intraventricular hemorrhages [25], Leffler et al. [1] showed that cerebral oxygen consumption was decreased in hypotensive piglets that were treated with therapeutic dosages of indomethacin. As far as ΔCytaa₃ indicates changes in cellular oxygenation of brain tissue, our study provides further evidence that the indomethacin-induced drop in brain perfusion decreases brain tissue oxygenation in pulmonary and hemodynamically stable preterm newborns [26]. However, there is some concern that ΔCytaa₃ does not properly reflect changes in brain cell oxygenation. The use of wrong algorithms for calculation of changes in ΔCytaa₃ [10] and the low energy requirement of the brain cell in the preterm neonate may mask fluctuations in the oxidation-reduction level of cytochrome oxidase [27] and affect the reliability of ΔCytaa₃ as a marker of actual changes in oxidation of the enzyme cytochrome oxidase.

It is less likely that the hemodynamic changes, including the changes in ΔCBV, were related to an indomethacin-induced (transient) ductus arteriosus closure. In earlier studies in preterm infants, we found that the clinical signs of PDA subsided only 4 h after indomethacin administration [2, 3]. Moreover, in a very recent study in 20 preterm infants, in which we assessed the influence of indomethacin on cardiac and pulmonary hemodynamics, it appeared that ductal patency and pulmonary hemodynamics were not effected after indomethacin treatment until 4 h after its administration [28]. The (hemodynamic) data shown in table 2 also suggest that no important alterations occurred with respect to ductal patency during the study period.

Despite the results of the present study, indomethacin will remain an important drug for noninvasive closure of PDA. Moreover, its use for prevention of severe intraventricular hemorrhages [20] seems very promising. We propose, however, very slow infusion rates of indomethacin to avoid as far as possible blood-pressure-related acute increases in brain blood flow [26]. Furthermore, one should be aware that an indomethacin-induced decrease in brain perfusion and oxygenation in the sick preterm babies may compromise cerebral metabolism during periods of pulmonary and cardiac instability.
References