Original Articles

Prostaglandin Levels: Predictors of Indomethacin Responsiveness

Cathy Hammerman, William Zaia,* Stuart Berger, Elene Strates, and Abdul Aldousany**

Department of Pediatrics, University of Chicago, Chicago, Illinois, USA

SUMMARY. Pretreatment plasma dilator prostaglandin levels were measured in 16 premature infants with patent ductus arteriosus in an attempt to correlate abnormally elevated levels with clinical responsiveness to indomethacin therapy. Nine of the 16 infants responded well to indomethacin, with complete disappearance of their murmurs by 48 h. Eight of these nine infants had elevated baseline 6 keto $PGF_{1\alpha}$ levels (>500 pg/ml).

In contrast, seven of the 16 infants did not respond to indomethacin, and six of these had 6 keto $PGF_{1\alpha}$ within the normal range (<500 pg/ml). PGE_2 levels varied in the same general direction, but lacked the specificity and sensitivity of the 6 keto $PGF_{1\alpha}$ levels. Thus, 6 keto $PGF_{1\alpha}$ levels seem to correlate with, and may eventually be helpful in predicting, clinical indomethacin responsiveness in the premature neonate with patency of the ductus arteriosus.

KEY WORDS. Patent ductus arteriosus — 6 keto prostaglandin $F_{1\alpha}$ — Prostaglandin E_2 — Indomethacin

Patency of the ductus arteriosus remains a significant problem in premature neonates. Current medical therapy usually involves the use of indomethacin, a prostaglandin synthetase inhibitor. However, it is difficult to predict which babies will respond to indomethacin and which will not. Attempts have been made in the past, with variable success, to correlate clinical responsiveness with other clinical factors including the baby's age [17] at the time of therapy, and plasma indomethacin levels [1]. Therapeutic responsiveness does not appear to be related to peak plasma indomethacin levels [2, 12, 23]. However, some correlation between PDA outcome and the area under the plasma concentration time curve of indomethacin has been detected [3, 22, 23]. Still, predictability remains uncertain in the majority of cases. In this study, we have attempted to utilize pretreatment plasma dilator prostaglandin levels to predict clinical responsiveness of the ductus arteriosus to indomethacin therapy.

Ductal patency in the premature is known to be associated with increased concentrations of dilator prostaglandins [10, 16]. Indomethacin, in fact, is effective in closing the ductus precisely because it inhibits dilator prostaglandin synthesis. Thus, it is expected that when the concentration of these prostaglandins is significantly elevated, indomethacin is more likely to be therapeutically successful than when it is not elevated. In this context, pretreatment levels of two dilator prostaglandins have been examined—6 keto prostaglandin $F_{1\alpha}$, a stable metabolite of prostacyclin, and PGE_2—in an attempt to correlate abnormally elevated circulating levels of these prostaglandins with a good therapeutic response to indomethacin therapy.

Materials and Methods

Subjects

Any premature infant admitted to the intensive care nursery of Wyler Children's Hospital from September 1983 to September 1985 was a candidate for the study if he/she was evaluated clinically to have a patent ductus arteriosus (PDA), and was being considered for indomethacin therapy. The diagnosis of PDA was made by clinicians (neonatologists and/or pediatric cardiologists) not involved in the study. This diagnosis was based on the presence of an intraclavicular and precordial systolic murmur consistent with PDA, plus any two of the following: bounding pulses, diastolic pressure of ≤25 mmHg, pulmonary plethora on chest

---

* Currently of Cook County Hospital, Chicago, Illinois, USA.
** Currently of University of Miami, Miami, Florida, USA.
Address reprint requests to: Dr. Cathy Hammerman, Department of Pediatrics, Box 325, University of Chicago, 5825 Maryland Avenue, Chicago, IL 60637, USA.

Table 1. Patient characteristics

	Nonresponders (n = 6)	Partial responders (n = 2)	Responders (n = 8)
Day of life treated	10 ± 4	10 ± 0	8 ± 4
Fluids prior to treatment (cc/kg/day)	117 ± 15	124 ± 43	115 ± 18
CVD score	6 ± 2	9 ± 1[a]	5 ± 2
Birth weight (g)	990 ± 245[a]	995 ± 205	1298 ± 302
Gest. age (wk)	29 ± 1	29 ± 1	30 ± 2
Peak FiO$_2$	32 ± 11	45 ± 0	47 ± 20
PIP (cm H$_2$O)	19 ± 5	18 ± 3	21 ± 5

[a] $p < 0.05$ when compared to responders. CVD score: modified cardiovascular distress score; PIP, peak inspiratory pressure; and fluids, peak FiO$_2$, and PIP all refer to the 24-h period prior to treatment.

x-ray, cardiomegaly on chest x-ray, or LVPEP/LVET ≤ 0.30 on M-mode echocardiogram.

Babies were excluded only if there was evidence of other congenital heart disease or persistent fetal circulation upon examination by a neonatologist and/or pediatric cardiologist, or if baseline prostaglandin levels could not be obtained prior to initiation of indomethacin therapy.

This protocol was reviewed and approved by the Clinical Investigation Committee of the University of Chicago.

Procedure

Once the diagnosis was confirmed, parental consent was obtained. Next, a clinical evaluation of the severity of the ductus was performed. Baseline arterial blood samples were drawn through indwelling lines for measurement of plasma 6 keto prostaglandin F$_{1\alpha}$ and PGE$_2$ levels.

The baby was then treated clinically with intravenous indomethacin according to the usual clinical protocol in our nursery, which is three doses of 0.2 mg/kg given at 12-h intervals. All babies were restudied at 48–72 h following the last dose of indomethacin with a repeat prostaglandin profile and clinical evaluation in order to determine response. Response at 48 h was determined by either the continued presence or the disappearance of the PDA as defined by the above criteria.

Laboratory Methods

Prostaglandin Radioimmunoassay (RIA). 1.5-cc blood samples were drawn through indwelling arterial lines for consistency and to avoid hemolysis. Blood was collected into chilled heparinized (100 U/ml) syringes which contain a final concentration of indomethacin of 2×10^{-5} M. They were centrifuged immediately at 2000 g at 4°C for 10 min, and the plasma was harvested and frozen at −60°C pending analysis by RIA, based on the methods of Coker [6] and Fitzpatrick [8] as modified by us [13].

The accuracy of this method for detection of a known amount of prostaglandin ranges from 92% to 114%. Our intraassay and interassay coefficients of variation are 5%–15% and

11%–15%, respectively. Cross-reactivity of the 6 keto PGF$_{1\alpha}$ antisera with PGE$_2$ is 0.64%; cross-reactivity of the PGE$_2$ antisera with 6 keto PGF$_{1\alpha}$ is 0.01%. Sensitivity varies somewhat from assay to assay, but generally the smallest amount of 6 keto PGF$_{1\alpha}$ measured is 100 pg/ml and the smallest amount of PGE$_2$ is 50 pg/ml.

Discrete values which are reported are the means of duplicate determinations performed on each sample. In our laboratory control levels of PGE$_2$ in healthy newborns are < 100 pg/ml, and normal 6 keto PGF$_{1\alpha}$ levels are < 500 pg/ml based on samples taken from 20 neonates without evidence of patent ductus arteriosus during the first week of life. (These are composed of ten premature infants with a mean birth weight of 983 g and ten term infants with a mean birth weight of 3105 g. Prostaglandin levels in both groups of healthy controls were similar.)

Analysis of Data. Means and standard deviations were computed for each of the descriptive characteristics of the nonresponders, the partial responders, and the responders. Means could not be calculated for the prostaglandin levels of the groups because several of the samples did not have discrete values. Categorical variables were compared using chi-square analysis. Statistical significance was accepted at p values of <0.05.

Results

A total of 16 babies were evaluated as described above. (Several babies were lost to study because of failure of notification prior to therapy or inability of one of the investigators to obtain a sample prior to treatment.) Eight (50%) of the 16 responded to the indomethacin with a complete disappearance of their ductal murmur by 48 h following therapy. Two infants (13%) had a partial response with a decrease in the intensity of their ductal murmur, but not complete disappearance. Six babies (37%) did not respond at all to their indomethacin therapy. Five of these six required surgical ligation soon thereafter and the other one expired. Our indications for surgical intervention include the presence of a persistent murmur consistent with the diagnosis of PDA, plus either (a) an inability to wean from the ventilator (in the absence of any intercurrent infection), or (b) evidence of congestive heart failure (tachycardia, pulmonary edema, and cardiomegaly) not adequately controlled with fluid restriction and diuretics.

There were no differences between the responders and the nonresponders in terms of mean postnatal age at the time of therapy or in the mean fluid administration during the 24 h prior to indomethacin treatment. In addition, all infants had RDS, all were receiving lasix, and all were being mechanically ventilated. There were no differences in the severity of attendant respiratory distress as assessed by their assisted ventilation requirements within the 24 h prior to therapy, or in the incidence of hypoxic or acidotic episodes within the 24 h prior to therapy. Although the nonresponders did tend to

Table 2. Prostaglandin data

Pretreatment 6 keto $PGF_{1\alpha}$ (pg/ml)	Posttreatment 6 keto $PGF_{1\alpha}$ (pg/ml)	Pretreatment PGE_2 (pg/ml)	48-h response	Long-term response
153	NA	82	P	D
160	NA	<50	P	S
207	256	<50	P	S
294	182	<50	P	S
<500	NA	<100	P	S
<500	NA	<100	P	S
250	NA	<50	1	C
1320	32	644	1	S
662	174	<50	A	C
861	260	<50	A	C
938	495	<50	A	C
1095	NA	208	A	C
1177	NA	<100	A	C
1368	NA	207	A	C
>5000	1030	354	A	S
>5000	NA	NA	A	C

Normal 6 keto $PGF_{1\alpha}$: <500 pg/ml; and normal PGE_2: <100 pg/ml.

48-h response: P, PDA present; 1, PDA improved; and A, PDA absent.

Long-term response: D, died, PDA present; S, surgical ligation; and C, PDA closed.

NA, not available.

be smaller, there was no difference between the gestational ages of the two groups (Table 1).

Three of the eight responders did have abnormally elevated plasma levels of PGE_2, as compared to none of the nonresponders. This difference in the incidence of abnormally elevated PGE_2 levels is not significant ($p > 0.05$; chi-square analysis).

There were significant differences, however, in the pretreatment levels of the prostacyclin metabolite, 6 keto $PGF_{1\alpha}$. All eight of the responders had abnormally elevated levels, as predicted (Table 2). In contrast, none of the non-responders had elevated 6 keto $PGF_{1\alpha}$ levels. This difference between the incidence of elevated 6 keto $PGF_{1\alpha}$ levels in the responders vs. the nonresponders is significant ($p < 0.01$ by chi-square analysis).

There were, as noted, two partial responders. One infant had normal baseline levels of both PGE_2 and 6 keto $PGF_{1\alpha}$. He had a decrease in intensity of his ductal murmur at 48 hours, with subsequent spontaneous complete closure. The second infant had elevated levels of both PGE_2 and 6 keto $PGF_{1\alpha}$ prior to treatment. This infant's murmur was, in fact noted to be decreased at 48 h when it was decided to ligate him rather than wait for closure. At surgery his ductus was observed to be very small.

There was some additional variance in long-range outcome. In two of the eight infants there was an initial response—their ductal murmur disappeared—but subsequently their PDA reopened, and one of these did eventually require surgical ligation.

Interestingly, these were the two infants with the highest pretreatment dilator prostaglandin levels.

Posttreatment 6 keto $PGF_{1\alpha}$ levels were measured in seven of the 16 infants studied—in two of the nonresponders, one of the partial responders, and four of the responders. All of these posttreatment levels measured were within the normal range except for one—in the one infant who subsequently developed a recurrence.

Thus, pretreatment 6 keto $PGF_{1\alpha}$ levels were predictive of short-term clinical responsiveness to indomethacin therapy in 88% of the cases. They were not as helpful in predicting recurrences. PGE_2 levels were less useful, yielding 63% false negative predictions, even in short-range evaluations.

Discussion

Although indomethacin is currently the best pharmacologic therapy available for treatment of patent ductus arteriosus, there continues to be a significant number of cases in which it remains unsuccessful. The efficacy of indomethacin in the literature varies from 18% [19] to 91% [15]. In our group, 63% responded to indomethacin, which is similar to the 79% ductal closure rate reported by the National Collaborative Indomethacin Study [12]. In the collaborative study, 26% subsequently reopened, yielding a permanent closure rate of 53%. Among our infants, the permanent closure rate was 50%.

Furthermore, there are certain toxicities attendant to the use of indomethacin; some real and some as yet theoretical. These include transient renal failure, as well as the as yet more hypothetical concerns of vasoconstriction in the gastrointestinal tract [9] or in the eye [7], possibly leading to necrotizing enterocolitis and/or retrolental fibroplasia, respectively, and decreased platelet aggregation via inhibition of the thromboxane synthesis possibly leading to bleeding tendencies [11]. It would therefore be beneficial to be able to predict which babies will respond to the drug and thereby hopefully preclude the necessity of giving a potentially toxic drug to babies who are not likely to respond to it. Furthermore, giving unnecessary medication to these patients is likely to delay more effective therapy, i.e., surgical ligation.

Ductal patency in the premature baby is associated with increased concentrations of dilator prostaglandins [10, 14, 16]. Indomethacin functions as a general inhibitor of prostaglandin synthesis. Therefore it is expected that when the concentration of dilating prostaglandins is significantly elevated, the indomethacin is more likely to be therapeutically successful.

Elevations of both PGE_2 and 6 keto $PGF_{1\alpha}$ have been demonstrated in conjunction with patent ductus arteriosus. Which of these dilators is the most significant clinically remains controversial. It has been shown that more prostacyclin (PGI_2) than PGE_2 is produced by ductal tissue [20]; however, it has also been demonstrated that PGE_2 is more potent in mediating ductal vasodilation [5]. 6 keto $PGF_{1\alpha}$ is a stable metabolite of prostacyclin. Its levels reflect prostacyclin synthesis, while being easier and more reliable to measure. We have previously demonstrated that elevated 6 keto $PGF_{1\alpha}$ levels are earlier, more sensitive indicators of clinically significant ductal dilation than are PGE_2 levels [14]. It is therefore not surprising that in this study, although PGE_2 levels in general seemed to vary in the same direction as 6 keto $PGF_{1\alpha}$ levels, they did not predict indomethacin response with the same sensitivity or specificity. Elevations of 6 keto $PGF_{1\alpha}$ levels were better correlated with indomethacin responsiveness.

One possibility as to the mechanism of ductal response to indomethacin can be proposed from the consistent decline observed in 6 keto $PGF_{1\alpha}$ following treatment. Indomethacin seems to produce decreases in circulating prostaglandin levels in both the responders and the nonresponders. Thus, what differentiates the two groups does not appear to be whether or not the drug is successful in inhibiting prostaglandin synthesis, nor whether or not there are adequate plasma levels of indomethacin. What seems to be important is what the baseline level of

dilator prostaglandin production was before treatment, and thereby, indirectly, whether the ductus arteriosus was highly dependent on prostaglandins to sustain its patency.

Of those who respond initially to indomethacin therapy, 20%–35% are likely to suffer a recurrence at some point after therapy is completed [18]. In our group, ductuses were subsequently reopened in 25% of the responders. Seyberth et al. [21] found that in many of the infants treated with indomethacin for patency of the ductus arteriosus there is a resurgence of prostaglandin production within 5½ days after completion of indomethacin treatment. This occurs as indomethacin levels fall and suppression of prostaglandin synthesis is thereby removed. In this light, it is interesting to note that our two recurrences occurred in the infants with the highest basal 6 keto $PGF_{1\alpha}$ levels. It seems reasonable to speculate that when indomethacin inhibition is removed in these infants with excessively elevated ongoing prostaglandin production, this prostaglandin production may return to sufficiently high levels to mediate a reopening of the ductus. It is also possible that these infants with extremely elevated pretreatment dilator prostaglandin levels would benefit from a more prolonged course of indomethacin therapy in order to inhibit prostaglandin synthesis long enough for anatomic closure of the ductus to occur.

The significance of the birth weight discrepancy between the two groups is questionable. It is theoretically possible that we are selecting out a group of larger infants who by virtue of their size, have both higher levels of circulating prostaglandins and a better therapeutic response to indomethacin. This argument is troublesome, however, since the literature seems to implicate precisely the opposite pattern in prostaglandin development [4], i.e., smaller babies are expected to have the higher prostaglandin levels. Another possible factor to be considered when evaluating this data is that we were concurrently conducting a study of prophylactic indomethacin treatment in the very low birth weight infant, which may have decreased the proportion of smaller babies in the group of responsive PDAs. Thus, we do not feel that these differences in birth weight, although statistically significant, are clinically significant in determining the association noted here.

In conclusion, although indomethacin is currently the best pharmacologic therapy available for the treatment of patent ductus arteriosus, there continues to be a significant number of cases in which it remains unsuccessful. Since there are potential toxicities attendant to the use of indomethacin, it would be beneficial to be able to predict clinical responsiveness to the drug. Our data show that ele-

vated pretreatment plasma 6 keto $PGF_{1\alpha}$ levels are correlated with and thus, potentially predictive of a good therapeutic response to indomethacin. Pretherapeutic levels within the normal range, on the other hand, are associated with treatment failures. Elevated plasma PGE_2 levels are also generally associated with good therapeutic response, but are neither as sensitive nor as specific.

References

1. Alpert B, Lewins M, Rowland D, Grand M, Olley P, Soldin S, Swyer P, Coceani F, Rowe R (1979) Plasma indomethacin levels in preterm newborn infants with symptomatic patent ductus arteriosus: clinical and echocardiographic assessments of response. *J Pediatr* 95:578–582

2. Bhat R, Vidyassagar D, Fisher E, Hastreiter A, Ramirez J, Burns L, Evans M (1980) Pharmacokinetics of oral and intravenous indomethacin in preterm infants. *Dev Pharmacol Ther* 1:101–110

3. Brash A, Hickey D, Graham T, Stahlman M, Oates J, Cotton R (1981) Pharmacokinetics of indomethacin in the neonate. *N Engl J Med* 305:67–72

4. Clyman R, Mauray F, Koerper M, Wiemer F, Heymann M, Rudolph A (1978) Formation of prostacyclin (PGI₂) by the ductus arteriosus of fetal lambs at different stages of gestation. *Prostaglandins* 16:633–641

5. Clyman R, Mauray F, Roman C, Rudolph A (1978) PGE₂ is a more potent vasodilator of the lamb ductus arteriosus than is either PGI or 6 keto $PGF_{1\alpha}$. *Prostaglandins* 16:259–264

6. Coker S, Clarke B, Zeitlin I (1982) Radioimmunoassay techniques for the determination of the local release of prostaglandins and thromboxanes. *J Pharmacol Methods* 7:207–217

7. Cotton RB, Stahlman M, Bender H, Graham T, Catterton W, Kovar I (1978) Randomized trial of early closure of symptomatic patent ductus arteriosus in small premature infants. *J Pediatr* 93:647–651

8. Fitzpatrick F, Gorman R, McGuire J, Kelly R, Wynalda M, Sun F (1977) A radioimmunoassay for thromboxane B₂. *Anal Biochem* 82:1–7

9. Friedman W, Fitzpatrick K (1980) Effects of prostaglandins, thromboxanes and inhibitors of their synthesis on renal and gastrointestinal function in the newborn period. *Semin Perinatol* 4:143–156

10. Friedman Z, Demers L (1978) Essential fatty acids, prostaglandins and respiratory distress syndrome of the newborn. *Pediatrics* 61:341–346

11. Friedman Z, Whitman V, Maisels J, Berman W, Marks K, Vesell E (1978) Indomethacin disposition and indomethacin-induced platelet dysfunction in premature infants. *J Clin Pharmacol* 18:272–279

12. Gersony W, Peckham G, Ellison R, Miettinen O, Nadas A (1983) Effects of indomethacin in premature infants with patent ductus arteriosus: results of a national collaborative study. *J Pediatr* 102:895–906

13. Hammerman C, Strates E, Berger S, Zaia W, and Aldousany A (1986) Prostaglandins and systolic time intervals in the assessment of patent ductus arteriosus. *Crit Care Med* 14:462–465

14. Hammerman C, Zaia W, Lee K (1983) Do plasma prostaglandin levels predict patent ductus arteriosus development? [abstr]. *Soc Pediatr Res* 17:315A

15. Harris J, Merritt T, Alexson C, Longfield L, Manning J (1982) Parenteral indomethacin for closure of the patent ductus arteriosus. *Am J Dis Child* 136:1005–1008

16. Lucas M, Mitchell A (1978) Plasma prostaglandins in preterm neonates before and after treatment for patent ductus arteriosus. *Lancet* 2:130–132

17. McCarthy J, Zies L, Gelband H (1976) Age-dependent closure of the patent ductus arteriosus by indomethacin. *Pediatrics* 62:706–711

18. Mellander M, Jeheup B, Lindsterom D, Palme C, Graham T, Stahlman M, Cotton R (1984) Recurrence of symptomatic patent ductus arteriosus in extremely premature infants treated with indomethacin. *J Pediatr* 105:138–143

19. Neal W, Kyle J, Mullett M (1977) Failure of indomethacin therapy to induce closure of patent ductus arteriosus in premature infants with respiratory distress syndrome. *J Pediatr* 91:621–623

20. Pace-Asciak C, Rangaraj G (1977) The 6 keto $PGF_{1\alpha}$ pathway in the lamb ductus arteriosus. *Biochem Biophys Acta* 486:583–585

21. Seyberth H, Muller H, Wille L, Pluckthun H, Wolf D, Ulmer H (1982) Recovery of prostaglandin production associated with reopening of the ductus arteriosus after indomethacin treatment in preterm infants with respiratory distress syndrome. *Pediatr Pharmacol* 2:127–141

22. Vert P, Bianchetti G, Marchal F, Monin P, Morselli P (1980) Effectiveness and pharmacokinetics of indomethacin in premature infants with PDA. *Eur J Clin Pharmacol* 18:83

23. Yeh T, Luken J, Raval D, Thalji A, Carr I, Pildes R (1983) Indomethacin treatment in small versus large premature infants with ductus arteriosus: comparison of plasma indomethacin concentration and clinical response. *Br Heart J* 50:27–30