Published in final edited form as:

J Perinat Med. 2011 January ; 39(1): 55–58. doi:10.1515/JPM.2010.113.

Effect of ibuprofen on bilirubin-albumin binding affinity in premature infants

Sanjiv B. Amin* and **Nicholas Miravalle**

Division of Neonatology, Department of Pediatrics, University of Rochester Medical Center, Rochester, NY, USA

Abstract

Aim—To evaluate the effect of ibuprofen on bilirubin-albumin binding affinity and unbound bilirubin in premature infants.

Study design—A prospective study with subjects serving as their own controls was performed on <30 weeks' gestational age infants with unconjugated hyperbilirubinemia and who received ibuprofen for patent ductus arteriosus. Infants with congenital malformation, TORCH infections, and conjugated hyperbilirubinemia were excluded. Total serum bilirubin (TSB) and unbound bilirubin (modified peroxidase test) were measured prior to (baseline) and after (follow-up) initiation of ibuprofen. The bilirubin/albumin equilibrium association binding constant was calculated using albumin, TSB, and unbound bilirubin.

Results—Ten infants were studied. The mean TSB between baseline (5.9 ± 1.7 mg/dL) was higher than that at follow-up [4.9 ± 1.7 mg/dL]. Mean unbound bilirubin at baseline (0.75 ± 0.65 μ g/dL) was similar to that at follow-up (0.63 ± 0.46 μ g/dL). No difference existed between mean baseline binding constant (49 ± 50 L/ μ mol) and that at follow-up (44 ± 36 L/ μ mol). The ratio of unbound bilirubin with and without ibuprofen, index of displacing effect, was 0.88 (95% CI 0.63–1.14).

Conclusions—Ibuprofen may not be associated with bilirubin displacing effect in relatively stable premature infants with mild to moderate unconjugated hyperbilirubinemia.

Keywords

Bilirubin:albumin ratio; free bilirubin; hyperbilirubinemia; patent ductus arteriosus

Introduction

Patent ductus arteriosus (PDA) is extremely common in very premature infants and untreated symptomatic PDA may be associated with chronic lung disease [15]. Pharmacological closure of PDA with indomethacin, a prostaglandin inhibitor, has remained the mainstay of treatment in premature infants over the last three decades. Recently, ibuprofen lysine was approved by the US Food and Drug Administration (FDA) for use in treatment of PDA for premature infants. Recent meta-analyses and systematic reviews, evaluating the efficacy and toxicity of ibuprofen lysine compared with indomethacin, concluded that the efficacy on PDA closure was similar but ibuprofen lysine was associated

with lower serum creatinine values and lower risk of oliguria compared to indomethacin [6, 13, 16].

Although the findings of meta-analyses suggest a small therapeutic superiority in favor of ibuprofen, there is a theoretical concern from *in vitro* studies for a potential bilirubin displacing effect and secondary increase in free or unbound bilirubin (bilirubin not bound to protein, B_f) concentration because ibuprofen is highly (approx. 95%) bound to protein [1, 6]. B_f crosses the blood-brain barrier [8] and recent studies have demonstrated strong association of B_f as measured by the peroxidase method using the FDA approved UB analyzer with bilirubin-induced neurotoxicity in premature infants [2, 4, 9]. Therefore, potential risk of bilirubin-induced neurotoxicity exists with ibuprofen administration in premature infants. However, the results of *in vitro* studies that evaluated the bilirubin displacing effect of ibuprofen are inconclusive [1, 3, 10]. In addition, only a single secondary report has been previously published that explored the bilirubin displacing effect of ibuprofen in premature infants [6]. We have been measuring B_f and bilirubin-albumin binding affinity in premature infants for a National Institute of Health funded bilirubin study using the modified peroxidase test with two peroxidase concentrations. We, therefore, undertook this prospective nested study to evaluate the effect of ibuprofen lysine on bilirubin-albumin binding affinity and B_f using the modified peroxidase test. The study was approved by the Institutional Research Review Board, and informed consent was obtained from the parents.

Methods

The study was designed as a prospective observational study with subjects serving as their own control. Premature infants born at <30 weeks gestational age (GA) at the University Hospital between November 2007 and July 2009, were enrolled prospectively in the National Institute of Health funded bilirubin study, and who received ibuprofen lysine for the management of PDA during the first two weeks after birth were eligible for the study. GA was assessed by obstetrical dating criteria or, when obstetrical data was inadequate, by Ballard examination. Infants with major congenital malformations, toxoplasmosis, rubella, cytomegalovirus infection and herpes simplex (TORCH) infections, conjugated hyperbilirubinemia [elevated direct or conjugated hyperbilirubinemia > 1.0 mg/dL if the total serum bilirubin (TSB) is <5.0 mg/dL or > 20% of the TSB if the TSB is ≥ 5.0 mg/dL], and resolved unconjugated hyperbilirubinemia (TSB ≤ 2 mg/dL) were excluded.

Indomethacin is the preferred drug for the management of PDA at the study site. In the presence of renal dysfunction, ibuprofen is preferred for the management of PDA and used at the discretion of attending neonatologist. The unit policy is to administer ibuprofen lysine as an initial dose of 10 mg/kg intravenously followed by 5 mg/kg/dose every 24 h for two additional doses.

Blood samples were collected from each enrolled subject before the administration of ibuprofen (baseline samples), and between the first (10 mg/kg) and second dose (follow-up samples), in amber colored serum separator tubes to protect from light. Each blood sample was immediately centrifuged in the dark and the separated serum was analyzed in limited light to measure TSB and B_f concentration ($\mu\text{g/L}$) by a validated modified peroxidase method [14] using a FDA approved UB UA-1 analyzer (Arrows Co., Ltd., Osaka, Japan). The samples were analyzed for B_f within two weeks after collection and storage in -20°C or -80°C freezer by a single investigator. The peroxidase test was modified to also include B_f measurement at half the standard peroxidase concentration to avoid underestimating the equilibrium B_f ($B_{f\text{eq}}$). In addition, the albumin concentration was measured using the bromocresol green method [11] to allow calculation of the bilirubin/albumin equilibrium

association binding constant (K , L/ μ mol) from the equation below. The binding constant quantifies the relative strength of bilirubin-albumin binding (the greater the K the stronger the binding). The coefficients for converting TSB (mg/dL), B_f (μ g/dL), and albumin (g/dL) to μ mol/L are 17.1, 0.0171, and 151, respectively.

$$\frac{\text{TSB} - B_f}{B_f([\text{Albumin}] - \text{TSB} + B_f)} = K$$

Infants received intravenous lipid emulsion at the discretion of the attending neonatologist. Phototherapy was used according to the institutional guidelines for the management of hyperbilirubinemia in premature infants that is currently based on TSB concentrations, birth weight, and presence of clinical factors that increases the risk for bilirubin encephalopathy. Acidosis and hypoxia were evaluated as clinically indicated at the discretion of the attending neonatologist.

The sample size calculation was based on findings of published *in-vitro* studies that suggest that ibuprofen may increase B_f by a factor of more than 2 [1, 10]. Our preliminary findings (unpublished) suggest the mean B_f to be approximately 0.65 ± 0.45 μ g/dL for 24–30 weeks' GA infants during the first two weeks after birth. We used a difference of > 1.3 standard deviation (SD) for sample size calculation to approximate the magnitude of ibuprofen-induced bilirubin displacing effect reported in *in-vitro* studies. Assuming the correlation between the baseline and follow-up B_f to be more than 0.2, 10 subjects would allow detection of an actual difference of more than 1.3 SD in the mean difference in B_f between baseline (before ibuprofen) and follow-up (after ibuprofen) with an α level of 0.05 and power of 0.80.

Statistical analyses and sample size calculation were performed using Stata 10 (Stata Corporation, College Station, TX, USA). The B_f and K as baseline (before ibuprofen) were compared with respective follow-up (post ibuprofen) B_f and K using the paired *t*-test or Wilcoxon signed-ranks test (if not normally distributed) with the hypothesis that the mean or median difference between the paired measurement is zero. All tests were two sided and a $P < 0.05$ was considered statistically significant.

Results

A total of 13 subjects who were enrolled in the primary bilirubin study between November 2007 and July 2009 at the university hospital received ibuprofen for PDA management. Three infants who were started on ibuprofen after unconjugated hyperbilirubinemia was resolved were not considered for this secondary study. The remaining 10 premature infants met the study criteria and were evaluated. The mean GA and birth weight were 25.9 (range 24.1–27.4) weeks and 928 g (range 600–1300 g), respectively. Seven (70%) infants were Caucasians and five (50%) were male. The mean postnatal age when baseline blood samples were collected was 117.5 h (range 48–323 h) whereas the mean postnatal age when follow-up blood samples were collected was 131.1 h (range 60–335 h). The mean duration after ibuprofen administration when the follow-up bilirubin albumin binding assays were performed was 9 h (range 2–15 h). The clinical characteristics of subjects at baseline and at follow-up are shown in Table 1. There was no significant difference in the amount of intravenous lipid intake at baseline and follow-up in study subjects (Table 1). There was significant difference in glucose intake between baseline and follow-up. However, there was no association between glucose intake and B_f concentration at baseline and follow-up. Similarly, there was no association between glucose intake and K . Eight infants had blood gases performed at baseline and follow-up. The mean pH at baseline was not significantly

different from follow-up. There were no subjects with hypoxia ($\text{PaO}_2 < 40$ mm Hg) at baseline and follow-up. There was no significant difference in the number of subjects receiving phototherapy between baseline and at follow-up.

The mean B_f and K at baseline and follow-up are shown in Table 2. There was significant difference in mean TSB concentration and TSB/albumin molar ratio between baseline and follow-up with the TSB concentrations and TSB/albumin molar ratios significantly lower in the follow-up samples. There was no difference in B_f concentrations and K between baseline and follow-up. The mean ratio of B_f with (follow-up) and without ibuprofen present (baseline), a measure of how strongly the drug interferes with bilirubin albumin binding, was 0.89 (95% CI 0.63–1.14).

Discussion

Current literature suggests that B_f is capable of crossing the blood-brain barrier and causing bilirubin-induced neurotoxicity in neonates [2, 4, 8, 9]. Therefore, a drug that displaces bilirubin from albumin, resulting in increased B_f concentration, should be avoided in neonates with unconjugated hyperbilirubinemia. Ibuprofen, a prostaglandin inhibitor, is nearly 95% bound to proteins and therefore has a potential to displace bilirubin from albumin binding sites. The bilirubin-displacing effect has not been well studied *in vivo*. Therefore, although ibuprofen has been approved by the FDA for the management of PDA, the use of the drug has been cautioned against in the presence of hyperbilirubinemia. Our findings in premature infants suggest that ibuprofen for PDA management at the recommended initial dosage of 10 mg/kg administered intravenously may not be associated with a significant bilirubin displacing effect in the presence of unconjugated hyperbilirubinemia in very premature infants. Our findings imply that the risk of bilirubin-induced neurotoxicity may not be increased by ibuprofen administration after the first two postnatal days in doses recommended for PDA treatment in extremely premature infants.

Several *in-vitro* studies used bilirubin-albumin solution or pooled newborn sera [1, 3, 10]. It appears from *in-vitro* studies that the risk of ibuprofen-induced bilirubin displacing effect depends on plasma concentration of ibuprofen, bilirubin:albumin ratio, and intrinsic ability of albumin to bind ibuprofen and bilirubin. Ambat et al. [3] reported from an *in-vitro* study that significant bilirubin displacement was seen only with a high bilirubin:albumin ratio of 2.0 and ibuprofen concentration of 285 $\mu\text{g/mL}$ (1387 $\mu\text{mol/L}$) when using saturation index test on bilirubin-albumin solution. The same group of investigators using a peroxidase method reported that B_f was significantly higher when ibuprofen (142 $\mu\text{g/mL}$ concentration) was added to newborn serum or bilirubin-albumin solution containing 10 mg/dL bilirubin compared to 5 mg/dL bilirubin suggesting that the displacing effect varies as a function of bilirubin:albumin ratio. Ahlfors et al. [1] demonstrated using the peroxidase method in an *in-vitro* study that the bilirubin displacing effect depended on ibuprofen plasma concentration. Ibuprofen significantly increased B_f concentration and a ratio of B_f with and without ibuprofen only at ibuprofen concentration > 50 $\mu\text{g/mL}$ in pooled newborn sera with bilirubin albumin ratios of 0.3 and 0.36 [1]. Similar association between bilirubin-displacing effect with ibuprofen concentration was demonstrated by Cooper-Peel et al. [10] with a reverse displacement method using cord sera with a bilirubin:albumin ratio of 0.5. Cooper-Peel et al. [10] reported none to minimal bilirubin displacing effect with ibuprofen concentration < 50 $\mu\text{g/mL}$, however, there was an increase in B_f by a factor of four with ibuprofen concentration of 154 $\mu\text{g/mL}$ (750 $\mu\text{mol/L}$).

The finding of *in-vitro* studies may not be applicable in sick and very premature infants with PDA. Sick and very premature infants are more likely to have reduced albumin binding capacity and binding affinity, endogenous bilirubin displacers, such as free fatty acids, and

conditions, such as acidosis and hypoxia that can magnify the displacing effect [4, 5]. Thus, the potential for displacement of bilirubin may be greater with competing drugs, such as ibuprofen at these or lower plasma concentrations of ibuprofen and lower bilirubin:albumin ratio [12]. We found no evidence of displacing effect as measured by bilirubin-albumin binding affinity and ratio of unbound bilirubin with and without ibuprofen in relatively stable and very premature infants. Although none of the premature infants were hypoxic, most premature infants had acidosis, presumably from symptomatic PDA. All premature infants were also receiving intravenous lipids that may be associated with altered bilirubin-albumin binding affinity [5]. Our finding of absence of increase in unbound bilirubin with ibuprofen therapy is consistent with a previous secondary report in premature infants [6]. The observed decline in TSB and bilirubin:albumin ratio at the follow-up period is probably a therapeutic effect of ongoing photo-therapy which most subjects were receiving during the study period.

The major strength of the study is the prospective evaluation with patients serving as their own control. Secondly, we evaluated displacing effect using bilirubin-albumin binding affinity in addition to the ratio of B_f with (follow-up) and without ibuprofen. The major limitation of our study is that the findings may not be applicable during the first two days after birth as all the subjects received ibuprofen after the first two days. Plasma concentrations of ibuprofen are higher during the first two days after birth [7]. Second, our findings are based on random samples drawn between drug dosages. The findings may not be applicable to the time period corresponding to the peak ibuprofen concentrations. We did not measure ibuprofen concentrations, but the peak ibuprofen concentrations after intravenous administrations are usually seen soon after IV administration (1–2 h) of the initial dose and may exceed 50 $\mu\text{g/mL}$ [17]. Therefore, it is possible that a significant transient bilirubin displacing effect may only be seen soon after IV administration. Third, our findings may also not be applicable at bilirubin:albumin molar ratio of ≥ 0.5 as most subjects in our study had bilirubin:albumin molar ratio < 0.5 .

In summary, our findings suggest that ibuprofen lysine when used at recommended dosage for the management of PDA after the first two postnatal days may not be associated with significant bilirubin displacing effect in relatively stable premature infants with mild to moderate hyperbilirubinemia. Since the bilirubin displacing effect appears to depend on plasma concentration of drug and bilirubin:albumin molar ratio, future larger prospective study including measurement of peak ibuprofen concentrations is warranted in sicker premature infants with jaundice to exclude the possibility of transient increase in unbound bilirubin concomitant with the peak plasma concentration of ibuprofen and or in the presence of severe jaundice with bilirubin:albumin molar ratio > 0.5 .

Acknowledgments

We are grateful to our research coordinator for data collection. We are also thankful to Dr. Wang H, biostatistician, for performing sample size calculation for the study. The study was funded by NIH DC 6229-04 and UL1 RR 024160 from the NCRR.

References

1. Ahlfors CE. Effect of ibuprofen on bilirubin-albumin binding. *J Pediatr.* 2004; 144:386–388. [PubMed: 15001951]
2. Ahlfors CE, Amin SB, Parker AE. Unbound bilirubin predicts abnormal automated auditory brainstem response in a diverse newborn population. *J Perinatol.* 2009; 29:305–309. [PubMed: 19242487]

3. Ambat MT, Ostrea EM Jr, Aranda JV. Effect of ibuprofen L-lysinate on bilirubin binding to albumin as measured by saturation index and horseradish peroxidase assays. *J Perinatol*. 2008; 28:287–290. [PubMed: 18200021]
4. Amin SB. Clinical assessment of bilirubin-induced neurotoxicity in premature infants. *Semin Perinatol*. 2004; 28:340–347. [PubMed: 15686265]
5. Amin SB, Harte T, Scholer L, Wang H. Intravenous lipid and bilirubin-albumin binding variables in premature infants. *Pediatrics*. 2009; 124:211–217. [PubMed: 19564302]
6. Aranda JV, Thomas R. Systematic review: intravenous ibuprofen in preterm newborns. *Semin Perinatol*. 2006; 30:114–120. [PubMed: 16813969]
7. Aranda JV, Varvarigou A, Beharry K, Bansal R, Bardin C, Modanlou H, et al. Pharmacokinetics and protein binding of intravenous ibuprofen in the premature newborn infant. *Acta Paediatr*. 1997; 86:289–293. [PubMed: 9099319]
8. Bratlid D. How bilirubin gets into the brain. *Clin Perinatol*. 1990; 17:449–465. [PubMed: 2196140]
9. Calligaris SD, Bellarosa C, Giraudi P, Wennberg RP, Ostrow JD, Tiribelli C. Cytotoxicity is predicted by unbound and not total bilirubin concentration. *Pediatr Res*. 2007; 62:576–580. [PubMed: 18049372]
10. Cooper-Peel C, Brodersen R, Robertson A. Does ibuprofen affect bilirubin-albumin binding in newborn infant serum? *Pharmacol Toxicol*. 1996; 79:297–299. [PubMed: 9000255]
11. Dumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta*. 1971; 31:87–96. [PubMed: 5544065]
12. Hansen TW. Ibuprofen for closure of patent ductus arteriosus: is it really safe in hyperbilirubinaemic infants? *Eur J Pediatr*. 2003; 162:356. author reply 7. [PubMed: 12692722]
13. Ohlsson A, Walia R, Shah S. Ibuprofen for the treatment of patent ductus arteriosus in preterm and/or low birth weight infants. *Cochrane Database Syst Rev*. 2005; 4:CD003481. [PubMed: 16235321]
14. Roca L, Calligaris S, Wennberg RP, Ahlfors CE, Malik SG, Ostrow JD, et al. Factors affecting the binding of bilirubin to serum albumins: validation and application of the peroxidase method. *Pediatr Res*. 2006; 60:724–728. [PubMed: 17065581]
15. Rojas MA, Gonzalez A, Bancalari E, Claure N, Poole C, Silva-Neto G. Changing trends in the epidemiology and pathogenesis of neonatal chronic lung disease. *J Pediatr*. 1995; 126:605–610. [PubMed: 7699543]
16. Thomas RL, Parker GC, Van Overmeire B, Aranda JV. A meta-analysis of ibuprofen versus indomethacin for closure of patent ductus arteriosus. *Eur J Pediatr*. 2005; 164:135–140. [PubMed: 15717178]
17. Van Overmeire B, Touw D, Schepens PJ, Kearns GL, van den Anker JN. Ibuprofen pharmacokinetics in preterm infants with patent ductus arteriosus. *Clin Pharmacol Ther*. 2001; 70:336–343. [PubMed: 11673749]

Table 1

Clinical characteristics before (baseline) and after (follow-up) ibuprofen.

	Baseline	Follow-up	P-value
Intravenous lipid intake (g/kg/day) ^a	1.5±0.8	1.6±0.8	0.3 ^b
Intravenous glucose intake (mg/kg/min) ^a	7.1±2.2	7.9±2.1	0.04 ^b
Concurrent blood pH ^a	7.24±0.03	7.23±0.04	0.67 ^b
Subjects (%) with hypoxia (PaO ₂ ≤40 mm Hg)	0	0	NS
Subjects (%) on phototherapy	8/10	7/10	0.60 ^c

^aMean±SD.^bpaired *t*-test.^cFisher exact test.

SD=standard deviation.

Table 2

Effect of intravenous ibuprofen on bilirubin-albumin binding and unbound bilirubin in premature infants.

	Baseline (before ibuprofen)	Follow-up (after ibuprofen)	P-value
Total serum bilirubin (mg/dL)			
Mean±SD (range)	5.9±1.7 (4–8.9)	4.9±1.7 (3.1–8.7)	0.01 ^a
Unbound bilirubin equilibrium (µg/dL)			
Mean±SD (median)	0.75±0.65 (0.70)	0.63±0.46 (0.61)	0.2 ^b
Binding constant (L/µmol)			
Mean±SD (median)	49±50 (30)	44±36 (29)	0.87 ^b
Bilirubin:albumin molar ratio			
Mean±SD (median)	0.24±0.06 (0.22)	0.20±0.15 (0.20)	0.015 ^b

^a Paired *t*-test,^b Wilcoxon signed-ranks test.

SD=standard deviation.