Published in final edited form as:

J Neonatal Perinatal Med. 2014 January 1; 7(3): 223–228. doi:10.3233/NPM-1477013.

Intestinal Fatty-Acid Binding Protein and Metronidazole Response in Premature Infants

Mario R. Sampson, PharmD^{1,2}, Barry T. Bloom, MD³, Antonio Arrieta, MD⁴, Edmund Capparelli, PharmD⁵, Daniel K. Benjamin Jr., MD, PhD, MPH^{1,6}, P. Brian Smith, MD, MPH, MHS^{1,6}, Gregory L. Kearns, PharmD, PhD^{7,8}, John van den Anker, MD, PhD⁹, and Michael Cohen-Wolkowicz, MD, PhD^{1,6,a} on behalf of the Best Pharmaceuticals for Children Act – Pediatric Trials Network Administrative Core Committee*

¹Duke Clinical Research Institute, Durham, NC, USA

²UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, USA

³Wichita Medical Research and Education Foundation, Wichita, KS, USA

⁴Children's Hospital–Orange County, Orange, CA, USA

⁵Schools of Medicine and Pharmacy, University of California–San Diego, La Jolla, CA, USA

⁶Department of Pediatrics, Duke University, Durham, NC, USA

⁷Division of Pediatric Pharmacology and Therapeutic Innovation, Children's Mercy Hospital, Kansas City, MO, USA

⁸Department of Pediatrics, University of Missouri–Kansas City School of Medicine, Kansas City, MO, USA

⁹Division of Pediatric Clinical Pharmacology, Children's National Medical Center, Washington, DC, USA

Abstract

Objectives—In premature infants with suspected intra-abdominal infection, biomarkers for treatment response to antimicrobial therapy are lacking. Intestinal fatty acid-binding protein (I-FABP) is specific to the enterocyte and is released in response to intestinal mucosal injury. I-FABP has not been evaluated as a surrogate marker of disease response to antimicrobial therapy. We examined the relationship between metronidazole exposure and urinary I-FABP concentrations in premature infants with suspected intra-abdominal infection.

Study design—We conducted an intravenous metronidazole pharmacokinetic study, collecting 3 urine samples per infant for I-FABP concentration measurements. We analyzed the relationship

^aAddress for correspondence: Michael Cohen-Wolkowicz, Duke Clinical Research Institute, 2400 Pratt Street, Durham, NC, 27705, USA. Phone: 919-668-8812. Fax: 919-668-7058. michael.cohenwolkowicz@duke.edu.

*See Acknowledgments for listing of committee members.

Conflicts of interest: Drs. Sampson, Bloom, Arrieta, Kearns, and van den Anker have no conflicts to report.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

between I-FABP concentrations and measures of metronidazole exposure and pharmacokinetics, maturational factors, and other covariates.

Results—Twenty-six samples from 19 premature infants were obtained during metronidazole treatment. When analyzed without regard to presence of necrotic gastrointestinal disease, there were no significant associations between predictor variables and I-FABP concentrations. However, when the sample was limited to premature infants with necrotic gastrointestinal disease, an association was found between average predicted metronidazole concentration and I-FABP concentration ($p=0.006$).

Conclusion—While a predictive association between urinary I-FABP and metronidazole systemic exposure was not observed, the data suggest the potential of this endogenous biomarker to serve as a pharmacodynamic surrogate for antimicrobial treatment of serious abdominal infections in neonates and infants.

Keywords

necrotizing enterocolitis; biomarkers; pharmacokinetics; premature infants; antimicrobial agents

Fatty acid-binding proteins are small (14-15 kD) proteins that transport fatty acids in enterocytes. Human intestinal fatty acid-binding protein (I-FABP) is found in mature enterocytes of the small intestine [1]. This protein is rapidly released in blood in response to intestinal mucosal injury [2]. Studies in mature enterocytes vs. fetal intestinal cell lines, and in human organ cultures from older children vs. fetuses, suggest that immature enterocytes respond to inflammatory stimulation with excessive pro-inflammatory cytokine production [3]. This inflammatory response may be associated with the development of necrotizing enterocolitis, a disease that results in intestinal necrosis in premature infants. In this population, urinary I-FABP (I-FABP_u) has been investigated as a biomarker for early diagnosis and disease severity [4–7]. As I-FABP can be easily measured in urine, it is a suitable biomarker for evaluation in premature infants because blood volume is a limiting factor for laboratory monitoring in this population.

There are very limited data on the abundance of I-FABP as a function of early development. One study used human gut specimens from aborted fetuses ranging from 13–20 weeks gestation, jejunal biopsies from a 2-year-old child, and jejunal and colonic samples from adults. I-FABP expression was detected throughout the intestine in the fetal samples. Also, I-FABP expression was similar in the 15-week fetus, 2-year-old, and adults, but lower in the 20-week fetus [8].

Treatment of complicated intra-abdominal infections (e.g., necrotizing enterocolitis [NEC]) in premature infants is empirical and based on clinical signs/symptoms and radiographic findings. Metronidazole, an antibiotic with a broad anaerobic spectrum and excellent tissue penetration, is often included in treatment regimens for these infections. While empiric use of metronidazole in NEC is common, its efficacy is not adequately supported [9].

As I-FABP has not been evaluated as a surrogate marker of disease response to antimicrobial therapy, we explored the association between metronidazole exposure and I-FABP_u concentrations in critically ill premature infants.

Methods

Clinical study

We enrolled premature infants with gestational ages (GA) ≥ 32 weeks and postnatal ages (PNA) < 91 days with suspected serious infection in a prospective, open-label, metronidazole pharmacokinetic (PK) and safety study [10]. Urine samples were collected (3 per infant, before, during, and after treatment) for I-FABP concentration measurements; all samples were included in the analysis regardless of collection time relative to metronidazole treatment. We defined during-treatment as the time between first dose of metronidazole and one dosing interval following the last dose. Necrotic gastrointestinal diseases were defined as NEC or intestinal perforation including spontaneous intestinal perforation. Urine was collected by placing a cotton ball in the diaper and squeezing the soaked cotton ball to collect 200 μ L of urine in a cryovial.

Bioanalytical methods

We determined I-FABP and creatinine concentrations from each urine sample. I-FABP_u concentrations were measured using a commercially available enzyme-linked immunosorbent assay (Hycult Biotech, PA, USA). Urine creatinine concentrations were measured by spectrophotometry using a method validated according to Food and Drug Administration guidance. Briefly, we centrifuged urine for 5 minutes at 2200 g. Next, we combined 5 μ L urine, 100 μ L sodium hydroxide, and 100 μ L picric acid, covered the mixture, and shook it for 30 seconds. The samples were incubated at 37°C for 15 minutes, and a spectrophotometer reading was obtained at 490 nm. Intra-run and inter-run coefficients of variation were 12.8% and 7.8%, respectively.

We measured metronidazole concentrations in plasma using a liquid chromatography-mass spectrometry method [10].

Metronidazole PK analysis

Metronidazole concentration-time data were analyzed as previously described [10]. We used intermittent infusion equations to predict steady-state metronidazole concentrations at the time of I-FABP collection and calculated average predicted metronidazole concentration ($C_{avg,met}$) using the following equation and each infant's PK parameters and dosing: median metronidazole maintenance dose [mg/kg]/metronidazole clearance [L/h/kg]/median dosing interval [h].

Statistical analysis

We normalized I-FABP concentrations by urine creatinine concentrations (referred to as I-FABP_u:Cr) to normalize for renal function and compare our results to prior evaluations of I-FABP in premature infants [4–7]. Box plots were employed to examine the association between during-treatment I-FABP_u:Cr concentration and categorical predictors (such as race, ethnicity, sex, and underlying diagnosis). Linear regression with clustering by patient was used to examine the association between during-treatment I-FABP_u:Cr concentration and continuous predictors, including demographic variables (GA, PNA, postmenstrual age, body weight, current weight), laboratory values (serum creatinine, albumin, hematocrit),

days of concomitant antimicrobial exposure, and metronidazole exposure/PK (cumulative dose, clearance, volume of distribution, $C_{avg,met}$, maximum and minimum concentration at steady state, area under the curve at steady state, elimination half-life, and cumulative area under the curve). We transformed (natural-log) non-normally distributed variables and, to avoid negative values, added a constant to all values of transformed variables such that the minimum value of the variable was one [11]. We considered linear regression associations to be significant if the p-value for the slope coefficient was <0.05 . We conducted the analysis using Stata 12 (StataCorp, College Station, TX).

Results

Patients and samples

Twenty-two premature infants contributed 33 I-FABP_u samples at the following times with respect to timing of metronidazole treatment: two were obtained before, 26 during, and five post treatment. Because a small number of before- and post-treatment samples were collected, only during-treatment I-FABP_u:Cr samples were included in the statistical analysis. The during-treatment analysis contained 26 samples from 19 premature infants with median (range) GA of 25.3 weeks (24–31) and PNA of 31 days (2–83). Thirteen premature infants contributed one during-treatment sample, five contributed two, and one contributed three samples. Patients and samples were also analyzed with regard to presence or absence of necrotic gastrointestinal disease; ten samples were contributed from seven premature infants with necrotic gastrointestinal disease, and 16 samples from 12 infants without. All premature infants were treated with other antimicrobials, including gentamicin (79%), piperacillin-tazobactam (53%), and vancomycin (47%) (Table 1).

During-treatment I-FABP associations

During-treatment I-FABP_u samples were obtained at a median (range) of 1.9 days (0.1–8.1) after the start of metronidazole treatment and 5.8 hours (0.7–22.9) post metronidazole dose. I-FABP_u concentrations were 5244 pg/mL (815–54,407), and I-FABP_u:Cr concentrations were 5.4 pg/nmol (1.1–49.6). When analyzed without regard to presence of necrotic gastrointestinal disease, there were no significant associations between I-FABP concentrations and measures of metronidazole exposure and pharmacokinetics, maturational factors, days of concomitant antimicrobial exposure, or other covariates. Among premature infants with and without necrotic gastrointestinal disease, median I-FABP_u:Cr concentrations were not different ($p=0.45$). However, among premature infants with necrotic gastrointestinal disease, increasing I-FABP_u:Cr was associated with increasing $C_{avg,met}$ ($p=0.006$) (Figure 1).

Discussion

Our data indicate that concentrations of I-FABP_u:Cr appear to be associated with metronidazole exposure in premature infants with clinically suspected intra-abdominal infection. It is unclear if this association is due to the natural course of the disease during treatment or a potential effect of metronidazole on the assay used to measure I-FABP concentrations, as has been observed with other drugs and biomarkers [6]. It is possible that

metronidazole concentrations were higher in sicker premature infants who had potentially worse gastrointestinal necrosis. Also, the relationship between $C_{avg,met}$ and response to therapy/clinical outcome has not been established. The small sample size, variability in I-FABP_u:Cr concentrations, and variability in metronidazole plasma concentrations likely precluded finding statistically significant relationships between I-FABP_u and most of the covariates examined.

Previous studies of I-FABP_u:Cr in premature infants with suspected or proven NEC reported limited information on associations between I-FABP_u:Cr and infant maturation. One study reported an association of lower I-FABP_u with increased GA, uncorrected for urinary creatinine (mean GA of 28.8 weeks for premature infants with I-FABP <800 pg/mL and mean GA of 26.4 weeks for premature infants with I-FABP ≥ 800 pg/mL, $p < 0.01$) [4]. Similarly, another study of 22 premature infants (16 with NEC, median [range] 34 weeks [28–41] GA) showed that decreasing I-FABP_u:Cr was associated with increasing GA ($p = 0.005$) and admission weight ($p = 0.011$) [1]. This more mature cohort of premature infants with mostly NEC diagnoses, compared with our cohort with mostly non-NEC diagnoses, is in contrast with the lack of association observed between GA and I-FABP_u:Cr in our sample. The difference may be related to an imprecise estimate in our cohort, different pathophysiology of NEC in preterm vs. term infants [12], or changes in I-FABP abundance with maturation. Thus, GA may be a potential confounder when I-FABP is used as a biomarker for antimicrobial treatment response.

A specific biomarker for response to metronidazole in premature infants with serious intra-abdominal infection is lacking. Our data suggest I-FABP_u:Cr merits further study as a potentially valuable biomarker for response to metronidazole, and potentially other antimicrobial agents, in the treatment of intra-abdominal infections in premature infants. This protein deserves further study to validate its clinical utility as a functional biomarker. A larger study in premature infants with NEC is necessary to clarify the relationship between I-FABP_u:Cr and antimicrobial exposure.

Acknowledgments

Mario Sampson was supported by training grant T32GM086330 from the National Institute of General Medical Sciences. Research reported in this publication was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under award number UL1TR001117.

The Pediatric Trials Network Administrative Core Committee

Katherine Y. Berezny, Duke Clinical Research Institute, Durham, NC; Jeffrey Barrett, Children's Hospital of Philadelphia, Philadelphia, PA; Matthew Laughon, University of North Carolina at Chapel Hill, Chapel Hill, NC; Andre Muelenaer, Virginia Tech Carilion School of Medicine, Roanoke, VA; T. Michael O'Shea, Wake Forest Baptist Medical Center, Winston Salem, NC; Ian M. Paul, Penn State College of Medicine, Hershey, PA; Kelly Wade, Children's Hospital of Philadelphia, Philadelphia, PA; Thomas J. Walsh, Weill Cornell Medical College of Cornell University, New York, NY.

The Eunice Kennedy Shriver National Institute of Child Health and Human Development: David Siegel, Perdita Taylor-Zapata, Anne Zajicek, Alice Pagan.

The EMMES Corporation (Data Coordinating Center): Ravinder Anand, Traci Clemons, Gina Simone.

The study was approved by the institutional review board at each enrolling site: Wesley Medical Center, Duke University Medical Center, and Children's Hospital of Orange County. Informed consent was obtained from parents/legal guardians.

Dr. Capparelli receives salary support from the United States government (U54 HD071600-01) and research support from Trius, Cerexa Pharmaceuticals, Abbott, and Theravance. Dr. Benjamin receives support from the United States government for his work in pediatric and neonatal clinical pharmacology (1R01HD057956-05, 1K24HD058735-05, UL1TR001117, and NICHD contract HHSN275201000003I) and the nonprofit organization Thrasher Research Fund for his work in neonatal candidiasis (www.thrasherresearch.org); he also receives research support from industry for neonatal and pediatric drug development (www.dcri.duke.edu/research/coi.jsp). Dr. Smith receives salary support for research from the National Institutes of Health (NIH) and the National Center for Advancing Translational Sciences of the NIH (HHSN267200700051C, HHSN275201000003I, and UL1TR001117); he also receives research support from industry for neonatal and pediatric drug development (www.dcri.duke.edu/research/coi.jsp). Dr. Cohen-Wolkowicz receives support for research from the National Center for Advancing Translational Sciences of the NIH (UL1TR001117), the Food and Drug Administration (1U01FD004858-01), the Biomedical Advanced Research and Development Authority (BARDA) (HHSO100201300009C), the nonprofit organization Thrasher Research Fund (www.thrasherresearch.org), and from industry for drug development in adults and children (www.dcri.duke.edu/research/coi.jsp).

References

1. Pelsers MM, Namiot Z, Kisielewski W, et al. Intestinal-type and liver-type fatty acid-binding protein in the intestine. Tissue distribution and clinical utility. *Clin Biochem.* 2003; 36:529–35. [PubMed: 14563446]
2. Kanda T, Fujii H, Tani T, et al. Intestinal fatty acid-binding protein is a useful diagnostic marker for mesenteric infarction in humans. *Gastroenterology.* 1996; 110:339–43. [PubMed: 8566578]
3. Nanthakumar NN, Fusunyan RD, Sanderson I, et al. Inflammation in the developing human intestine: A possible pathophysiologic contribution to necrotizing enterocolitis. *Proc Natl Acad Sci USA.* 2000; 97:6043–8. [PubMed: 10823949]
4. Derikx JP, Evennett NJ, Degraeuwe PL, et al. Urine based detection of intestinal mucosal cell damage in neonates with suspected necrotising enterocolitis. *Gut.* 2007; 56:1473–5. [PubMed: 17872576]
5. Evennett NJ, Hall NJ, Pierro A, Eaton S. Urinary intestinal fatty acid-binding protein concentration predicts extent of disease in necrotizing enterocolitis. *J Pediatr Surg.* 2010; 45:735–40. [PubMed: 20385280]
6. Thuijls G, Derikx JP, van Wijck K, et al. Non-invasive markers for early diagnosis and determination of the severity of necrotizing enterocolitis. *Ann Surg.* 2010; 251:1174–80. [PubMed: 20485148]
7. Mannoia K, Boskovic DS, Slater L, Plank MS, Angeles DM, Gollin G. Necrotizing enterocolitis is associated with neonatal intestinal injury. *J Pediatr Surg.* 2011; 46:81–5. [PubMed: 21238645]
8. Levy E, Menard D, Delvin E, et al. Localization, function and regulation of the two intestinal fatty acid-binding protein types. *Histochem Cell Biol.* 2009; 132:351–67. [PubMed: 19499240]
9. Shah D, Sinn JK. Antibiotic regimens for the empirical treatment of newborn infants with necrotising enterocolitis. *Cochrane Database Syst Rev.* 2012; 8:CD007448. [PubMed: 22895960]
10. Cohen-Wolkowicz M, Sampson M, Bloom BT, et al. on behalf of the Best Pharmaceuticals for Children Act – Pediatric Trials Network. Determining population and developmental pharmacokinetics of metronidazole using plasma and dried blood spot samples from premature infants. *Pediatr Infect Dis J.* 2013; 32:956–61. [PubMed: 23587979]
11. Osborne, JW. The effects of minimum values on data transformations. Paper presented at the Annual Meeting of the American Educational Research Association; New Orleans, LA. April 1-5; 2002.
12. Lin PW, Nasr TR, Stoll BJ. Necrotizing enterocolitis: recent scientific advances in pathophysiology and prevention. *Semin Perinatol.* 2008; 32:70–82. [PubMed: 18346530]

Figure 1. During-treatment average predicted metronidazole concentration vs. urinary I-FABP:Cr in premature infants with necrotic gastrointestinal disease. $P=0.006$; $R^2 = 0.65$; slope (95% CI) = 0.10 (0.04, 0.16).

Table 1
During-treatment Premature Infant Demographics

	Necrotic GI disease (n=7)	Non-necrotic GI disease (n=12)	Overall (n=19)
I-FABP _u :Cr (pg/nmol)	6.53 (2.02–49.6)	4.05 (1.14–46.0)	5.44 (1.14–49.6)
GA (weeks)	25.7 (24–31)	25.1 (24–31)	25.3 (24–31)
PMA (weeks)	33.0 (27.7–35.6)	28.4 (25.1–38.9)	33.1 (25.1–38.9)
PNA (days)	32.5 (13–68)	26.0 (2–83)	31 (2–83)
Serum creatinine (mg/dL)	0.4 (0.3–1.9)	0.9 (0.1–1.4)	0.7 (0.1–1.9)
Birth weight (g)	806 (488–1466)	740 (431–1252)	750 (431–1466)
White	5 (26%)	7 (37%)	12 (63%)
Male	3 (16%)	2 (11%)	5 (26%)
Hispanic	2 (11%)	3 (16%)	5 (26%)
NEC	4 (21%)	0 (0%)	4 (21%)
Intestinal perforation	3 (16%)	0 (0%)	3 (16%)
Abdominal distention	0 (0%)	1 (5%)	1 (5%)
Hematochezia & GERD	0 (0%)	1 (5%)	1 (5%)
Inguinal hernia	0 (0%)	1 (5%)	1 (5%)
No GI diagnosis	0 (0%)	9 (47%)	9 (47%)
Gentamicin use	4 (21%)	11 (58%)	15 (79%)
Piperacillin-tazobactam use	2 (11%)	8 (42%)	10 (53%)
Vancomycin use	6 (32%)	3 (16%)	9 (47%)
Ampicillin use	0 (0%)	6 (32%)	6 (32%)
Cefuroxime use	1 (5%)	0 (0%)	1 (5%)

GI=gastrointestinal; PMA=postmenstrual age; GERD=gastroesophageal reflux disease. Necrotic disease defined as NEC or intestinal perforation. N=number of premature infants; values are median (range) or number (%).