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Developmental Regulation of the Gut-Liver (FGF19-CYP7A1) Axis in Neonates

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Abstract

Introduction: Fibroblast growth factor 19 (FGF19) is a gut-derived hormone that regulates the expression of CYP7A1, the rate-limiting enzyme in bile acid (BA) synthesis pathway. Dysregulation of the FGF19-CYP7A1 (gut-liver) axis is associated with cholestatic liver disease. Infants, especially preterm infants and those with intestinal failure are at high risk for developing cholestatic liver disease. The activity of the gut-liver axis has not been characterized in this population. Our objective was to assess relationships between circulating FGF19 concentrations and CYP7A1 activity in neonates.

Materials and Methods: Plasma samples were obtained longitudinally from term and preterm infants (22 – 41 weeks gestation) hospitalized in a neonatal intensive care unit. Infants with congenital and acquired gastrointestinal disorders were excluded. Plasma levels of 7α-hydroxy-4-cholesten-3-one (C4), a marker of CYP7A1 activity, were quantified using HPLC-MS/MS. Plasma FGF19 concentrations were quantified by ELISA. Data were analyzed using linear regression models and structural equation modeling.

Results: 181 plasma samples were analyzed from 62 infants. C4 concentrations were undetectable prior to 30 weeks' gestation and, thereafter, increased with advancing gestational age and with volume of enteral feeds. They did not correlate with serum FGF19 concentrations, which decreased with advancing gestational age and volume of enteral feeds.

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Discussion: The activity of CYP7A1, the rate-limiting BA synthetic enzyme in adults, is developmentally regulated and undetectable in newborns less than 30 weeks' gestation. FGF19 concentrations do not correlate with CYP7A1 activity, suggesting that the gut-liver axis is not functional in infants. High FGF19 concentrations at birth in infants less than 37 weeks' gestation is a novel finding, and its source and role in preterm infants warrants further investigation.

Rationale—The intestinal hormone, fibroblast growth factor 19 (FGF19), is a major regulator of CYP7A1, the rate limiting enzyme in bile acid (BA) synthesis. Recently, dysregulation of the gut-liver (FGF19-CYP7A1) axis has been implicated in adult cholestatic liver disease, and animal studies have shown that exogenous FGF19 protects against liver injury. Given the therapeutic potential related to this signaling pathway, we sought to characterize the association between CYP7A1 and FGF19 in term and preterm infants. We conducted a prospective, observational study that measured *in vivo* CYP7A1 activity and FGF19 concentrations in 62 term and preterm infants (n = 181 samples). We found that CYP7A1 activity is developmentally regulated; its activity is undetectable prior to 30 weeks' gestation and increases with advancing gestational age and volume of enteral feeds. Contrary to expectation, we demonstrated that FGF19 is expressed at birth in preterm infants and decreases over time, even as enteral feeds increase. Using structural equation modeling, we were able to show that CYP7A1 activity does not correlate with FGF19 concentrations. Our results suggest that the gut-liver axis is not upregulated in preterm and term infants and that neonates with cholestatic liver disease will unlikely benefit from supplemental FGF19. We also report novel findings of elevated FGF19 concentrations in preterm infants at birth and speculate that there may be an extra-intestinal source of FGF19 that is developmentally expressed in these infants.

Keywords

Fibroblast growth factor 19; cholesterol 7α-hydroxylase; cholestasis; liver disease

Introduction

Fibroblast growth factor 19 (FGF19) is a gut-derived hormone that plays a critical role in the regulation of bile acid (BA) metabolism. Under normal feeding conditions, the liver secretes BA into the proximal small intestine, and after aiding in fat digestion, these BA are reabsorbed in the distal small intestine. Reabsorption of BA by ileal enterocytes activates nuclear receptor farnesoid X receptor (FXR), which upregulates the expression of FGF19. FGF19 is then secreted into the portal circulation and binds to its cell surface receptor, fibroblast growth factor receptor 4 (FGFR4) on the plasma membrane of hepatocytes. Binding of FGF19 to FGFR4 and activation of the co-receptor β-klotho triggers a number of downstream signaling pathways, which collectively suppress the expression of the rate-limiting enzyme of the classical BA pathway, cholesterol 7α-hydroxylase (CYP7A1) (1, 2). Enterohepatic circulation of BA also activates hepatic FXR, which plays a minor role in suppressing CYP7A1 expression (3). Thus, in healthy individuals, the intestinal FXR-FGF19-CYP7A1 axis (i.e. gut liver axis), is the predominant regulator of BA synthesis. Activation of this axis suppresses BA synthesis and limits BA accumulation in hepatocytes by a negative feedback mechanism (Supplemental Figure).

Dysregulation of the gut-liver axis due to ileal disease, ileal resection, or lack of enteral feedings has been associated with increased BA synthesis and/or liver disease (4–8). Consistent with these findings, studies in mice have revealed that exogenous FGF19 protein or constitutive ileal FXR activation protects against cholestatic liver injury (9–11). Neonates, particularly preterm infants and those with intestinal failure are often dependent on prolonged parenteral nutrition (PN) and are at high risk for cholestatic liver disease (12, 13); which suggests that FGF19 activity may be deficient in neonates and that therapeutic manipulation of FGF19 signaling may be a promising therapeutic strategy. However, the gut-liver axis has not been characterized in this population. Moreover, animal studies have described that various components of the gut-liver axis, including expression of intestinal FXR/FGF19, hepatic FGFR4/β-klotho, and CYP7A1 are developmentally regulated in pups compared to adult animals (14). A study of term infants has shown that induction of FGF19 does not happen in the first few days after birth but does occur by four months of age (15). The objective of this study was to describe the developmental regulation of FGF19 and CYP7A1 activity in term and preterm infants and to determine whether FGF19 concentrations are directly associated with CYP7A1 activity in this population. In order to describe a “normal” neonatal gut-liver axis, infants with congenital or acquired gastrointestinal diseases were excluded from this study.

Materials and Methods

This was a prospective observational study using samples from term and preterm infants admitted to the neonatal intensive care unit (NICU) at Bristol-Myers Squibb Children’s Hospital, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ. The study was approved by the Rutgers Institutional Review Board, and written informed consent was obtained from all parents/legal guardians. Selection criteria included viable infants born at 22 – 42 weeks’ gestation between June 2015 and May 2016. Infants with congenital gastrointestinal disease, acquired gastrointestinal disease (including necrotizing enterocolitis, infectious hepatitis, and direct hyperbilirubinemia > 2 mg/dL), and whose mothers had limited proficiency in English were excluded.

Infant Clinical Data

Clinical data were collected from the electronic medical record. Baseline data included sex, gestational age, birth weight, route of delivery, and maternal race. Gestational age in completed weeks was determined using first trimester ultrasound information and/or date of last menstrual period. Corrected gestational age was defined as gestational age at birth plus postnatal age. Infant nutritional data, including the volume of enteral feeds (ml/kg/d), were collected on the date of blood sampling.

Sample Collection

Residual blood samples that remained after completion of clinical laboratory testing were collected, when available, throughout the infants’ stay in the NICU. For this study, samples were obtained on admission (before the first feed) and then at approximately 6AM on subsequent days, when infants had been *nil per os* for 3 hours based on the routine feeding

schedule. Blood samples were collected in heparin-containing tubes and centrifuged at 3,000*g* at 4°C for 10 minutes. Plasma was separated and stored at −80°C until analysis.

Sample Analyses

CYP7A1 activity was quantified by measuring plasma concentrations of 7α-hydroxy-4-cholestene-3-one (C4), a validated marker of CYP7A1 activity (16) using liquid chromatography/tandem mass spectrometry (LC-MS/MS) as described by Camilleri et al. (17) (Mayo Laboratories, Rochester, MN). Plasma FGF19 concentrations were measured by quantitative sandwich enzyme linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) according to manufacturer's instructions.

Statistical Analyses

Descriptive statistics are given as mean (standard deviation) or median (interquartile range) for continuous data, and as number (%) for nominal data. Data were analyzed using simple and multiple linear regression using JMP 13 (SAS Institute, Raleigh NC) and by structural equation modeling using Stata 14 (StataCorp, College Station, TX). Structural equation modeling allowed for simultaneous analysis of the inter-related variables (i.e. corrected gestational age, volume of enteral feeds, FGF19 concentrations, and C4 concentrations) and quantification of total, direct, and indirect effects of these variables with each other. For example, multiple regression may show associations between Variables A and B, and Variable C. Structural equation modeling may demonstrate that Variable A is directly associated with changes in Variable C (a direct effect), and that Variable A is also associated with changes in Variable B that in turn affects C4 concentrations (an indirect effect). 'Patient' was used as a clustering variable. Statistical significance was set at $p < 0.05$.

Results

Infant Characteristics

A total of 62 infants (55% male) were enrolled in the study (Supplemental Table 1); 45% Caucasian, 18% African American, 15% Asian, 6% Hispanic, and 16% of other or undefined ethnicity. The mean gestational age was 32.7 ± 4.4 weeks, with 31% of the infants > 34 weeks' gestation, 58% between 28 – 34 weeks' gestation, and 11% < 28 weeks' gestation. The mean birth weight was 1883.0 ± 964.0 g, with 23% weighing > 2500 g, 35% between 1500 – 2500 g, and 42% < 1500 g at birth. Eighty-five percent of the infants were appropriate for gestational age at birth. Fifty-eight percent of the infants were delivered by cesarean section. All preterm infants < 35 weeks' gestation were started on total PN on the day of birth. Enteral feeds were introduced in the first 2–3 days of life and advanced by 20–30 mL/kg/day according to physician discretion. Full-volume feeding (a minimum of 120 mL/kg/day) was attained at 20.8 ± 5.3 days for infants less than 28 weeks' gestation, and 11.8 ± 7.8 days for infants between 28 – 34 weeks' gestation. Infants greater than 34 weeks' gestation were feeding *ad libitum* by 2.7 ± 1.3 days.

Developmental Regulation of C4 and FGF19

C4 concentrations were measured in 168 samples from 57 infants. Simple linear regression analysis was used to examine the association between C4 concentrations and corrected

gestational age (Figure 1A). C4 was not detectable before 30 weeks' gestation and increased with advancing gestational age (r^2 adjusted 0.13, $p < 0.0001$).

FGF19 concentrations were measured in 123 samples from 47 infants, and simple linear regression analysis was used to examine the association between FGF19 concentration and corrected gestational age (Figure 1B). FGF19 decreased with increasing corrected gestational age (r^2 adjusted 0.14, $p < 0.0001$).

Relationship between C4 and FGF19 using Linear Regression

Simple linear regression analysis showed a significant negative association between FGF19 and C4 (r^2 adjusted 0.13, $p = 0.0001$). Multiple regression analysis was then performed to adjust for the effects of corrected gestational age ($p = 0.01$) and volume of enteral feeds ($p = 0.0007$) on C4. Once these confounding variables were accounted for, the analysis revealed no association between FGF19 and C4 concentrations ($p = 0.48$). FGF19 concentration was negatively associated with corrected gestational age and volume of enteral feeds ($p = 0.0003$ and $p < 0.0001$, respectively).

Regulation of C4 and FGF19 using Structural Equation Modeling

The effects of corrected gestational age, volume of enteral feeds, and FGF19 concentrations on C4 are shown in Figure 2 and Supplemental Table 2. Corrected gestational age and enteral feeds have a direct positive association with C4 concentration ($p = 0.007$ and $p < 0.001$, respectively) and corrected gestational age also has an indirect positive association with C4 ($p = 0.007$). However, FGF19 was not independently associated with C4 concentration ($p = 0.391$).

The effects of corrected gestational age and volume of enteral feeds on FGF19 are also shown in Figure 2 and Supplemental Table 2. Corrected gestational age ($p = 0.016$) and enteral feeds ($p < 0.001$) are directly and negatively associated with FGF19. In addition, corrected gestational age has an indirect negative effect on FGF19 via enteral feeds ($p < 0.006$).

Discussion

Our study is the first to characterize *in vivo* CYP7A1 activity longitudinally in term and preterm infants using plasma C4 concentrations. We found that CYP7A1 activity is undetectable in infants < 30 weeks' corrected gestational age and that its activity is upregulated by both advancing gestational age and volume of feeds. The developmental regulation of BA synthetic enzymes has been well documented in animal models (14, 18–22) and inferred from analyses of BA composition in human fetal and neonatal serum, urine/amniotic fluid, and stool (23–26). These findings are consistent with studies suggesting that alternative BA synthetic pathways are active early in fetal/neonatal life and that the classic pathway (in which CYP7A1 is the rate-limiting enzyme) may become the predominant pathway some time later in postnatal life.

Contrary to expectation, we demonstrated that FGF19 is expressed at birth in preterm infants and decreases over time, even as enteral feeds increase. These findings differ from those in

adults, where FGF19 is physiologically induced in enterocytes by exposure to BAs after eating, providing negative feedback to decrease further hepatic BA synthesis (27). Elevated expression of FGF19 in preterm infants is a novel finding, and its function is unclear. Studies of human and animal fetuses have shown that FGF19 is expressed in various tissues and may play a role in early organogensis (28–30). We reason that the high FGF19 concentrations observed before 37 weeks' gestation are likely extra-intestinal in origin. Maternal and placental sources of FGF19 cannot be excluded. Further studies are needed to identify the source and role of FGF19 in fetal and preterm infant development.

Finally, by using generalized linear modeling and structural equation modeling, we have shown that FGF19 is not associated with CYP7A1 activity, suggesting that the gut-liver axis is not developed in neonates less than 42 weeks' corrected gestation. Given that CYP7A1 activity is upregulated by 30 weeks' gestation, we speculate that this lack of intestinal regulation of BA synthesis is due to delayed expression of intestinal FGF19 and/or hepatic FGF4R/β-klotho. Studies of older infants and children are needed to identify the postnatal induction of this axis.

Our study also highlights the limitations of using simple linear regression models when analyzing complex relationships, such as the regulation of BA synthesis. Data analysis using simple linear regression showed a significant negative association between FGF19 and C4. However, this model failed to take into account the major confounding variables of corrected gestational age and volume of enteral feeds, which act both directly and indirectly on C4 and are unique considerations for preterm infants. When appropriate models were used to analyze the data, no significant association between FGF19 and C4 was found.

There were a number of limitations to our study. First, the vast majority of infants enrolled in our study were preterm infants between 28 and 34 weeks gestational age. Therefore, we cannot directly assess the effect of gestational age on the expression of FGF19 or its effect on C4, or comment on the strength of our observations at the extremes of gestational age. Secondly, we were not able to analyze samples from infants greater than 42 weeks' corrected gestational age, as all infants were discharged home before this age. This limited the length of study for the larger and more mature infants. Finally, we did not take into account other variables that may affect FGF19 and CYP7A1 activity. We controlled for the known diurnal effects and timing of enteral feeds by analyzing samples taken at a specific time of day, prior to the infant's enteral feed. However, we could not accout for factors such as type of enteral feed (formula vs. breastmilk), characterization of PN (i.e. amount of intravenous lipid emulsion), triglyceride levels, and bile acid concentrations as this clinical information was unavailable for analysis.

In conclusion, the gut-liver axis is not developed in infants corrected to less than 42 weeks' corrected gestation. The activity of CYP7A1, the predominant BA synthetic pathway in adults, is undetectable in newborns less than 30 weeks' gestation, and is not associated with FGF19 concentrations. Therefore, it is unlikely that neonates with cholestatic liver disease will benefit from supplemental FGF19. We also report novel findings of elevated FGF19 concentrations in preterm infants at birth and speculate that there may be an extra-intestinal

source of FGF19 that is developmentally expressed in these infants. Further studies are needed to determine the source and role of FGF19 in fetuses and preterm infants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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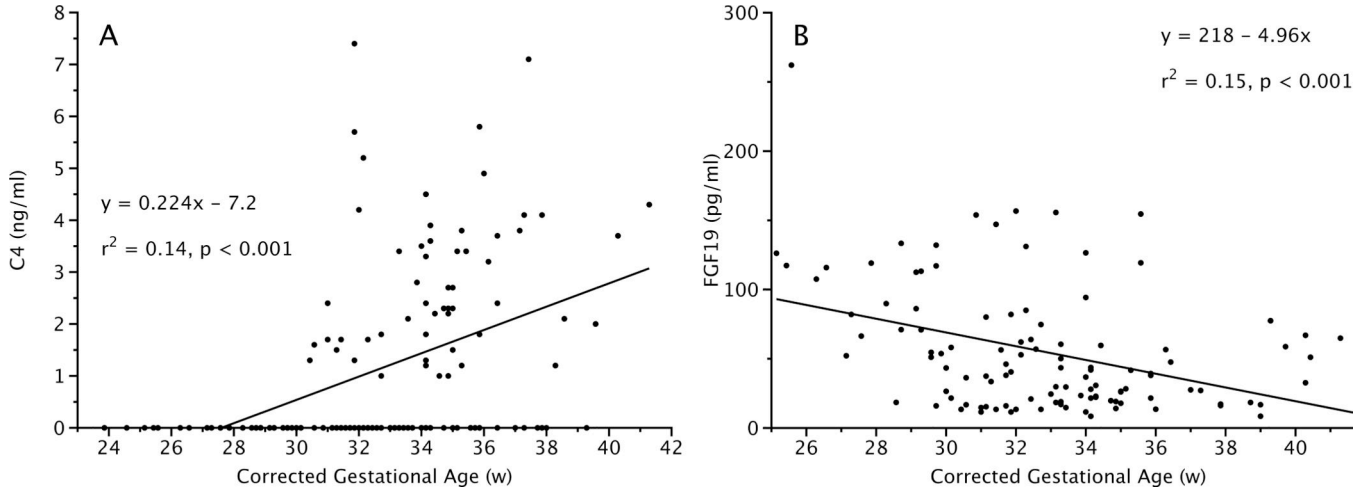

Figure 1.

Correlation of C4 (A) and FGF19 (B) with corrected gestational age using simple linear regression analysis.

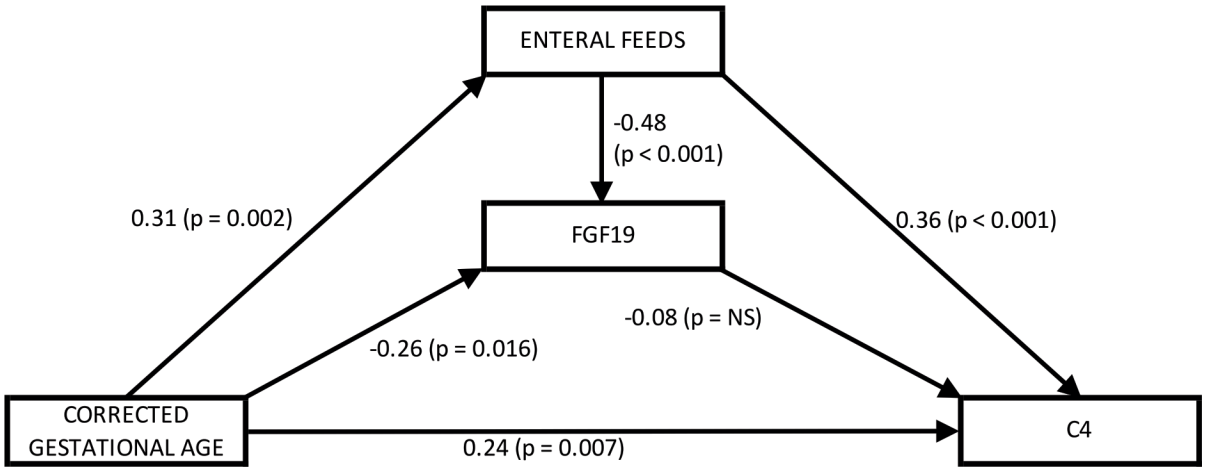

Figure 2.

Structural equation modeling with standardized coefficient weights (p-values) on the associations between corrected gestational age (weeks), volume of enteral feeds (ml/kg/d), FGF19 (pg/mL), and C4 (ng/mL).