Randomized Controlled Trial of Talactoferrin Oral Solution in Preterm Infants

Michael P. Sherman, MD, PhD[1], David H. Adamkin, MD[2], Victoria Niklas, MD[3,*], Paula Radmacher, PhD[2], Jan Sherman, PhD[1,4], Fiona Wertheimer, DO[3], and Karel Petrak, PhD[5,†]

Objective To evaluate the safety and explore the efficacy of recombinant human lactoferrin (talactoferrin [TLf]) to reduce infection.

Study design We conducted a randomized, double blind, placebo-controlled trial in infants with birth weight of 750-1500 g. Infants received enteral TLf (n = 60) or placebo (n = 60) on days 1 through 28 of life; the TLf dose was 150 mg/kg every 12 hours. Primary outcomes were bacteremia, pneumonia, urinary tract infection, meningitis, and necrotizing enterocolitis (NEC). Secondary outcomes were sepsis syndrome and suspected NEC. We recorded clinical, laboratory, and radiologic findings, along with diseases and adverse events, in a database used for statistical analyses.

Results Demographic data were similar in the 2 groups of infants. We attributed no enteral or organ-specific adverse events to TLf. There were 2 deaths in the TLf group (1 each due to posterior fossa hemorrhage and post-discharge sudden infant death), and 1 death in the placebo group, due to NEC. The rate of hospital-acquired infections was 50% lower in the TLf group compared with the placebo group ($P < .04$), including fewer blood or line infections, urinary tract infections, and pneumonia. Fourteen infants in the TLf group weighing <1 kg at birth had no gram-negative infections, compared with only 3 of 14 such infants in the placebo group. Noninfectious outcomes were not statistically significantly different between the 2 groups, and there were no between-group differences in growth or neurodevelopment over a 1-year posthospitalization period.

Conclusion We found no clinical or laboratory toxicity and a trend toward less infectious morbidity in the infants treated with TLf. *(J Pediatr 2016;175:68-73)*.

Trial registration ClinicalTrials.gov: NCT00854633.

Hospital-acquired infections represent the majority of diseases affecting preterm infants in neonatal intensive care units (NICUs).[1] Because hospital-acquired infections are associated with increased length of hospital stay and significant increases in the cost of care, the American Academy of Pediatrics has called for strategies to reduce hospital-acquired infections in NICUs.[2,3] Among hospital-acquired infections, bacteria resistant to broad-spectrum antibiotics cause >50% of patient-associated diseases,[4] which has led to an emphasis on antibiotic stewardship.

Modified health care practices have reduced hospital-acquired infections in extremely preterm infants,[2,3] but do not address the underlying immaturity of the mucosal and systemic immune systems.[5] Maternal milk is known to reduce the occurrence of bacteremia and necrotizing enterocolitis (NEC).[6,7] Biomolecules in human milk are proposed to synchronously modify the intestinal microbiome and nascent gut and boost systemic immunity, thereby reducing susceptibility to infection.[5,8] Extremely preterm infants (birth weight <1 kg) are the most vulnerable to infection because maternal colostrum is limited immediately after birth, or because intestinal dysmotility hinders full enteral feedings for days to weeks.

Human milk proteins enhance the development of intestinal epithelia, facilitate a healthy intestinal microflora, establish host defenses, and heighten mucosal defenses. We propose that the human milk protein lactoferrin partly explains these beneficial effects.[9,10] Commercial recombinant human lactoferrin (talactoferrin [TLf]) TLf became available 20 years ago.[11] We found that feeding

AE	Adverse event
bLF	bovine lactoferrin
CoNS	Coagulase-negative staphylococci
FDA	Food and Drug Administration
IND	Investigational New Drug
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
RCT	Randomized controlled trial
SAE	Serious adverse event
TLf	Talactoferrin
VLBW	Very low birth weight

From the [1]Division of Neonatology, Department of Child Health, University of Missouri, Columbia, MO; [2]Division of Neonatal Medicine, Department of Pediatrics, University of Louisville, Louisville, KY; [3]Division of Neonatal Medicine, Children's Hospital Los Angeles, Keck School of Medicine at the University of Southern California, Los Angeles, CA; [4]Sinclair School of Nursing, University of Missouri, Columbia, MO; and [5]Agennix Inc, Houston, TX

*Current address: Prolacta, City of Industry, CA.

†Current address: F.J.S. de Oro, 2835 Piso 5-2, 1425 Capital Federal Buenos Aires, Argentina.

Funded by the National Institutes of Health (HD057744 to Agennix, Inc [PI: K.P. and M.S.]). K.P. served as project coordinator and Vice-President for Research at Agennix, Inc until 2012. The other authors declare no conflicts of interest.

Portions of the study were presented at a Mead Johnson Pediatric Nutrition Institute, Boston, MA, April 23-24, 2015.

http://dx.doi.org/10.1016/j.jpeds.2016.04.084

TLf prophylactically to neonatal rats prevented morbidity and mortality caused by enteroinvasive *Escherichia coli*.[12] Our research then became focused on enteral lactoferrin deficiency that occurs in the early life of immature infants. We hypothesized that administering TLf would be safe, and conducted a randomized controlled trial (RCT) to assess its safety and efficacy in very low birth weight (VLBW) infants.

Methods

In this double-blinded RTC, TLf or placebo was administered to infants with birth weight between 750 and 1500 g starting within 24 hours of birth and continuing through 28 days of life (ClinicalTrials.gov: NCT00854633). We excluded infants who had a major congenital malformation, chromosomal abnormality, documented prenatal or intrapartum neonatal infection, or absence of parental consent, or were moribund at birth. We enrolled and randomized subjects between July 1, 2009, to March 17, 2012. Hospital discharge was followed by a 1-year outpatient follow-up period. Agennix Inc (Houston, Texas), the trial sponsor, ended the final data analyses in December 2013.

Agennix provided TLf to 3 academic health care systems in the US. Agennix used good manufacturing practice and suspended TLf in sterile, endotoxin-free, phosphate-buffered saline. The excipient served as the placebo with excellent color matching. The Institutional Review Board for each site approved the study. We obtained written consent from the parents or legal guardians before 24 hours of age. Thereafter, each institutional pharmacy randomized a subject via a central computer system (inVentiv Clinical Solutions, Houston, Texas). The inVentiv Web Response system also recorded data about participants in this RCT. Agennix sponsored the research under Food and Drug Administration (FDA) Investigational New Drug (IND) policies and procedures. The study design included a Data Safety Monitoring Board, a centralized serious adverse event reporting system, and periodic onsite monitoring visits that verified clinical, health care, laboratory, and radiologic data and pharmacy record keeping.

We randomized infants to receive either TLf solution (150 mg/mL) at a dose of 300 mg/kg/day or an identical volume of the excipient. Doses were administered every 12 hours via nasogastric tube from days of life 1 through 28 or until discharge, whichever occurred first. We extrapolated the dose of TLf from lactoferrin consumed during enteral breast milk feeding (150 mL/kg/day) with the content of lactoferrin in human milk estimated at 2 mg/mL. In all subjects, we administered the first dose before 24 hours of age. We adjusted the dose at weekly intervals if the weight increased by ≥10% from a previous weight.

Primary and Secondary Outcomes

The primary outcome was a significant reduction in hospital-acquired infections, including bacteremia, pneumonia, urinary tract infection, meningitis, and NEC. Our criteria were based on Centers for Disease Control and Prevention definitions for hospital-acquired infections.[13] The sponsor established strict criteria for infection, including bloodstream infections, pneumonia, urinary tract infections, meningitis and NEC.[14] A diagnosed infection required antibiotics for ≥72 hours.

Secondary outcomes were mortality, duration of hospitalization, time to regain birth weight, and the time to reach full enteral feeds. Disease-related morbidities included medically or surgically treated patent ductus arteriosus, intracerebral hemorrhage, periventricular leukomalacia, retinopathy of prematurity, chronic lung disease (defined as O_2 therapy at 36 weeks postconceptual age), suspected NEC, clinical sepsis syndrome, and neonatal inflammatory response syndrome. We defined a clinical sepsis syndrome as a negative blood culture, but with clinical and laboratory findings necessitating empiric antibiotic therapy. These criteria included elevated inflammatory markers, namely serial C-reactive protein level ≥1.5 mg/dL, abnormal serial white blood cell count or an elevated immature/total neutrophil ratio (≥0.3), central thermal instability, apnea and bradycardia, or respiratory distress. We established suspected NEC as a clinical scenario that involved a cessation of enteral feedings and initiation of antibiotics based on gastric residuals, occult or gross blood in the stool, abdominal distention, and radiographs showing dilated bowel loops and an abnormal bowel gas pattern, but without a sentinel loop or pneumatosis intestinalis.

Safety Assessment

We used the MedDRA system to report safety outcomes to the FDA.[15] All investigators underwent training in the use of this grading and severity scoring system. This system reports adverse events (AEs) and severe adverse events (SAEs) on a daily basis using an FDA-accepted measurement scale.[15] The FDA mandates daily recording of clinical information during the 28-day prophylactic period, with weekly recording until discharge. At the 6- and 12-month postdischarge visits, we collected growth measurements, including head circumference, health outcomes, and developmental progress using the Bayley screener III.[16] If the subject did not return, we contacted the primary care physician or parents by telephone, or parents by US mail, to ascertain the infant's clinical status.

Agennix did not require any specific collection of clinical findings, laboratory tests, or radiologic studies. The FDA IND program requires information on daily weight and abdominal circumference, vital signs, physical examination findings, type and duration of respiratory support, O_2 saturation, volume and type of enteral feeding, intravenous fluids and total parenteral nutrition volume and composition, urinary output, gastric residual volumes, number and description of feces passed, and concomitant medications. The study protocol collected laboratory and radiographic test data as ordered by the supervising neonatal attending. Test data included complete blood count, C-reactive protein, complete metabolic and electrolyte panels, blood gas analyses, gross or occult blood in feces, and results of all