Journal of Pediatric Surgery 49 (2014) 961–965

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

Oral relaxin maintains intestinal blood flow in a rat model of NEC☆,☆☆,★

Paul J. Matheson [a,b,1], Sarah K. Walker [b,1], Alexandra C. Maki [b], Saad P. Shaheen [a], R. Neal Garrison [a,b], Cynthia D. Downard [c,*]

[a] Robley Rex Veterans Affairs Medical Center at Louisville, KY
[b] Hiram C. Polk, Jr., M.D. Department of Surgery, University of Louisville, Louisville, KY
[c] Division of Pediatric Surgery, Hiram C. Polk, Jr. M.D. Department of Surgery, University of Louisville, Louisville, KY

ARTICLE INFO

Article history:
Received 21 January 2014
Accepted 27 January 2014

Key words:
Necrotizing enterocolitis
Intestinal vasoconstriction
Relaxin
Intestinal blood flow

ABSTRACT

Purpose: Intestinal vasoconstriction is a critical step in development of necrotizing enterocolitis (NEC). Relaxin (RLXN), a hormone found in breast milk but absent from formula, is a potent vasodilator. We hypothesized that relaxin-supplemented feeds with an NEC protocol would decrease NEC severity and increase intestinal blood flow.

Methods: Timed-pregnant Sprague–Dawley rats were randomly assigned to CONTROL, NEC, NEC + 1xRLXN, or NEC + All Feeds RLXN, and all but CONTROL underwent NEC protocol. NEC + 1xRLXN and NEC + All Feeds RLXN groups were fed relaxin-supplemented formula with the last feed or every feed. At 48 h of life, intestinal blood flow was measured at baseline and after application of 2.5% Delflex® solution.

Results: The addition of relaxin to NEC group feeds (1x or All Feeds) improved the degree of ileal injury. Ileal blood flow was decreased in the NEC pups compared to the CONTROLS, but the addition of relaxin to one feed increased baseline ileal blood flow in the NEC group compared to NEC alone. Furthermore, the addition of relaxin to ALL feeds significantly increased baseline ileal blood flow.

Conclusion: Pups who received relaxin with all feeds had substantially increased ileal perfusion compared to control pups. Our data suggest that relaxin supplementation maintains intestinal blood flow and results in less histologic NEC.

Necrotizing enterocolitis (NEC) predominantly affects premature infants, and those who are formula fed are 6 to 10 times more likely to develop NEC compared to similar infants who receive breast milk [1]. While the benefits of breast feeding are widely touted, the components specific to breast milk and absent from formula, especially those with effects on intestinal development and immune protection, are subject to investigation as potential therapeutic targets in NEC.

Relaxin (RLXN) is a 6 kDa hormone of pregnancy that leads to relaxation of the pubic symphisis [2]. Relaxin is also a potent vasodilator which is responsible for the relative volume expanded state of pregnancy and is associated with decreased renal vascular resistance during pregnancy [3]. The cardiovascular effects of relaxin have made it a novel treatment in heart failure, and current phase three clinical trials of recombinant relaxin have shown improved outcomes, decreased mortality, and decreased end organ damage in this clinical scenario [4–6].

We have previously shown that enteral relaxin supplementation given for 24 h increases intestinal blood flow in an animal model of NEC [7]. The purpose of this project is to 1) evaluate relaxin as a preventative agent for NEC, 2) to analyze its utility as a rescue therapy when NEC is suspected, and 3) to evaluate its effect on intestinal perfusion when used in conjunction with direct peritoneal resuscitation.

1. Materials and methods

The research protocol was approved by the Institutional Animal Care and Use Committee, Biohazard Safety Committee, and Research and Development Committee prior to studies. Four timed pregnant Sprague–Dawley dams (Harlan, Indianapolis, IN) were maintained in an AAALAC-approved Veterinary Medical Unit at the Robley Rex VA Medical Center in Louisville, KY for at least one week prior to delivery of pups. Dams were acclimated on a 12 h light–dark cycle and were allowed rat chow and water *ad libitum*. The rat pups were randomized to group by litter for inclusion in the CONTROL group (n = 12) which were vaginally delivered and dam fed, or experimentally-induced NEC groups (3 groups, n = 11-13 per group) which were delivered by Caesarian section 12 h prematurely under carbon dioxide anesthesia.

☆ Dr. Matheson contributed to the project design, execution (animal care and study), data collection, data analysis, and performed final critical revision of the manuscript.
☆☆ Dr. Walker contributed to the project design, execution (animal care and study), data collection, and provided the initial draft of the manuscript.
★ Supported by James R. Petersdorf Fund of Norton Healthcare and Kosair Charities.
* Corresponding author at: Division of Pediatric Surgery, Hiram C. Polk, Jr., M.D. Department of Surgery, University of Louisville, 315 E. Broadway, Ste 565, Louisville, KY 40202.
E-mail address: c0down01@louisville.edu (C.D. Downard).
[1] Drs. Matheson and Walker have contributed equally to this study.

http://dx.doi.org/10.1016/j.jpedsurg.2014.01.032
0022-3468/

The CONTROL group was time-matched at post-delivery hour of life to the NEC groups.

As previously reported, experimental NEC was induced by formula feeds via gastric gavage every 4–5 h, intermittent hypoxia (100% nitrogen gas for 60 s) and hypothermia (4 °C for 10 min) every 12 h, and a single gastric dose of lipopolysaccharide at 12 h of life (Sigma-Aldrich, St. Louis, MO) [8–10]. The formula feeds consisted of 20 g Similac 60/40 (Ross Pediatrics, Columbus, OH) dissolved in 100 mL Esbilac (Pet-Ag, New Hampshire, IL) puppy formula. Feeds were started at 0.1 mL/feed and were advanced to 0.15 mL/feed at 24 h of life and 0.2 mL/feed at 48 h of life. These feeds were calculated to supply approximately 200 kcal/kg/day caloric intake.

The experimental protocol is outlined in Fig. 1. At birth, NEC pups were randomized to the following groups: 1) NEC alone (n = 11), 2) NEC with a single oral supplemental dose of rat relaxin (0.25 ng/feed, Sigma-Aldrich, St. Louis, MO) in the final feed prior to blood flow experiments (n = 12, NEC + 1xRLXN), or 3) NEC with oral supplemental doses of relaxin (0.25 ng/0.1 mL) in all formula feeds (n = 13, NEC + All Feeds RLXN). CONTROL animals were dam fed and received no relaxin supplementation. At 48 h of life, the pups were weighed and anesthetized with isoflurane with induction of 3.5% and maintenance of 1.0% on 1 L oxygen per minute. All animals were separated from their littermates and underwent laparotomy for the study of ileal blood flow by laser Doppler flowmetry (Periflux system, Perimed AB, Järfälla, Sweden). Gross appearance of the intestines was recorded for signs of necrosis, hemorrhage, gaseous distension, or perforation. Any blood or stool that was present was flushed from the peritoneum with prewarmed saline (37.0 °C). Body temperature was maintained at 37 °C by feedback controller. A 7-site integrating flow probe was placed over the terminal ileum and organ blood flow was recorded. Correct positioning of the flow probe was verified visually at each time point for the duration of the experiment. Warmed saline was dripped on the peritoneum and the animals were allowed to equilibrate for 20 min prior to initiation of the flow protocol. In each animal, laser Doppler perfusion was recorded in the ileum at two baseline time points 10 min apart. If flow was stable (less than 10% difference) during that baseline period the study was continued. No pups were excluded for unstable blood flow at baseline. After the completion of the baseline period, a 2.5% peritoneal dialysis solution prewarmed to 37.0 °C was dripped in the peritoneum and flow was recorded for an additional 10 min to evaluate if the alteration in intestinal blood flow seen with relaxin supplementation works against or in concert with our previously demonstrated changes in intestinal blood flow seen with the addition of peritoneal dialysis solution [11].

At the completion of the laser Doppler flowmetry studies, the degree of bladder distension in the pups was observed and recorded. The grading scale used was: 0 when the bladder appeared completely empty, 1 when the bladder diameter was > 0 and < 1 mm, 2 when bladder distension was > 1 and < 2, and 3 when bladder distension was > 2 mm. In addition, ileum samples were obtained, and placed in 10% neutral buffered formalin for 16 h and then placed in 70% ethanol for hematoxylin and eosin (H&E) staining. Histopathology scores for signs of NEC were obtained from a pathologist masked to group and experimental protocols. The samples were graded as follows: Grade 0, normal or no damage; Grade 1, epithelial cell lifting or separation; Grade 2, sloughing of epithelial cells to mid villus level; Grade 3, necrosis of entire villus; or Grade 4, transmural necrosis [12].

All data are expressed as mean ± standard error of the mean (SEM). Differences between groups (CONTROL, NEC, NEC + 1xRLXN, and NEC + All Feeds RLXN) and time points (baseline1, baseline2, DPR1 min, DPR5 min, and DPR10 min) were determined by two-way analysis of variance (ANOVA) using SigmaPlot for Windows 11.1.0.102 (Systat Software, Inc., San Jose, CA). Differences between baseline intestinal perfusion, bladder distension, body weights, or histopathologic scores were determined by one-way ANOVA. The null hypothesis was rejected *a priori* at $P < 0.05$. When differences were found using ANOVA, the *post hoc* Tukey–Kramer honestly significant difference test was applied.

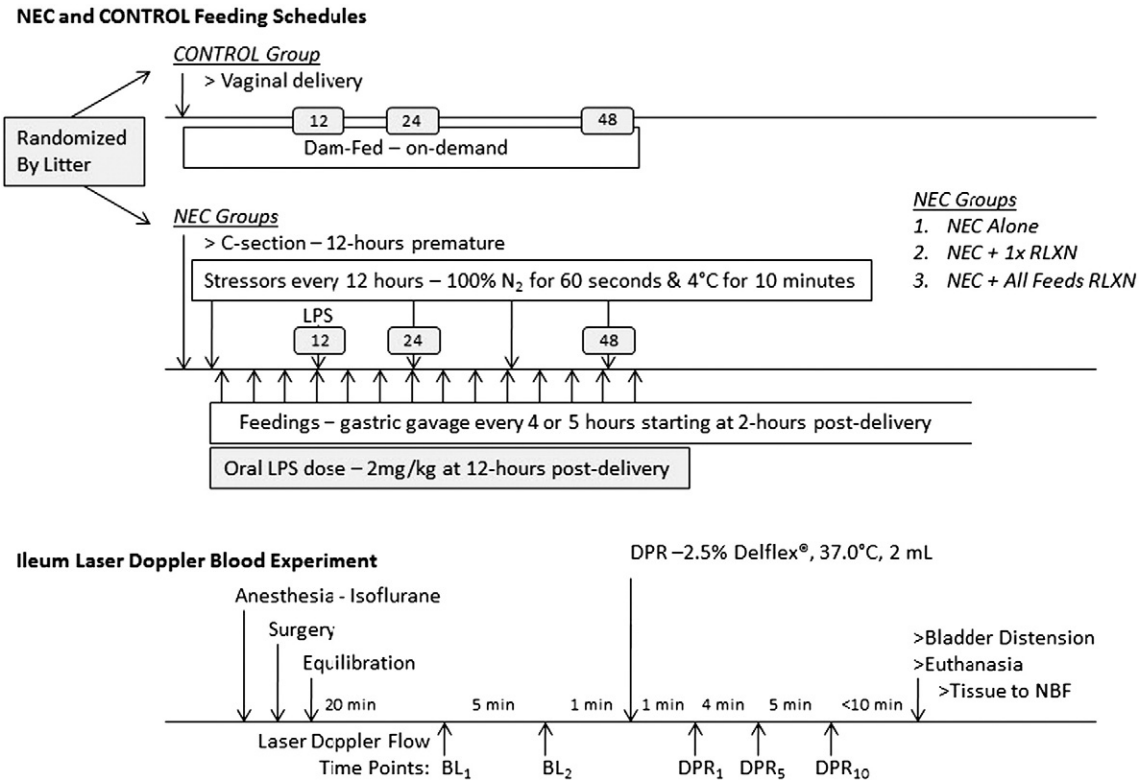

Fig. 1. Experimental timeline. Experimental timeline. DPR, 2.5% Delflex; BL, baseline time point; NBF, 10% neutral buffered formalin; RLXN, rat relaxin-1 at 0.25 ng/feed.