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Similac Special Care and Elecare cause Neonatal Gut injury in Mice

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Research Abstract Title

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IRB Number: IACUC Protocol

Describe role of Submitting/Presenting Trainee in this project (limit 150 words):

I was involved in hypothesis driven conception of the project along with my primary mentor. I have conducted the animal experiments myself, including feeding per protocol. I have conducted the experiments: PCR, H&E NEC Scoring, TUNEL with the help of other members of the team. I drafted, edited the abstract with my primary mentor, Dr. Sampath.

Background, Objectives/Goal, Methods/Design, Results, Conclusions limited to 500 words

Background:
Necrotizing Enterocolitis (NEC) is the most common gastrointestinal emergency in neonates. Infants fed formula milk are more likely to develop NEC compared to breast milk; attributed to gut injury and microbial dysbiosis. Despite these risks, formula use is prevalent in NICUs across the country when breast milk is unavailable. The incidence or severity of NEC associated with different preterm formula has not been reported. In this study, we examined neonatal gut injury caused by Elecare (E), Neosure (N), and Similac special care (S) in a mouse model. Additionally, we examined whether *Lactobacillus rhamnosus* GG (LGG), a probiotic, known to protect against human NEC, reduces formula-feeding induced gut injury.

Objectives/Goal: 1. To quantify the effects of E, S and N on gut inflammation, and severity of intestinal injury in neonatal mice. **2.** To demonstrate the protective effect of LGG treatment on formula-fed induced gut injury. **3.** To determine the effects of different human preterm formulae on gut dysbiosis in mice.

Methods/Design:
Formula-fed (FF) C57Bl6 pups were gavage-fed 0.15 mL of either E, N or S formula fortified to 26 Kcal/oz, 5X/day from day 8-10. FF pups were sacrificed on day 11 and compared to littermate, breastfed controls. Intestinal lysates were used to assess inflammation, pro-inflammatory signaling (MAPK and NFKβ) and apoptosis. A validated scoring tool was used to quantify intestinal injury and 16sRNA sequencing will be

used to characterize the gut microbiome. In the second arm of the study, FF pups were pre-treated with LGG from day 5-7, followed by formula feeding from day 8-10.

Results:

Neonatal mice fed E and S showed significant gut injury, while N-fed pups showed minimal or no injury **(Fig 1).** Analysis of ileal protein lysates showed activation of the MAPK, p38, and the transcription factor NFKB in the FF pups, especially in S and E groups **(Fig 2)**. TUNEL staining showed that the apoptotic index increased 0.24% in controls vs. 2.14% and 2.34% respectively in E and S groups **(Fig 3)**. LGG pre-treatment decreased the severity of intestinal injury caused by S (p=0.026) **(Fig 4)**. We are currently investigating alterations in gut microbiota signatures between the three FF groups.

Conclusions:

In neonatal mice, preterm formula, S and E caused significant gut injury, compared to N. Gut injury in E and S were associated with increased proinflammatory signaling and apoptosis. LGG pre-treatment attenuated S-induced injury. Ongoing work will clarify whether changes in the microbiome signatures contribute to the phenotype.

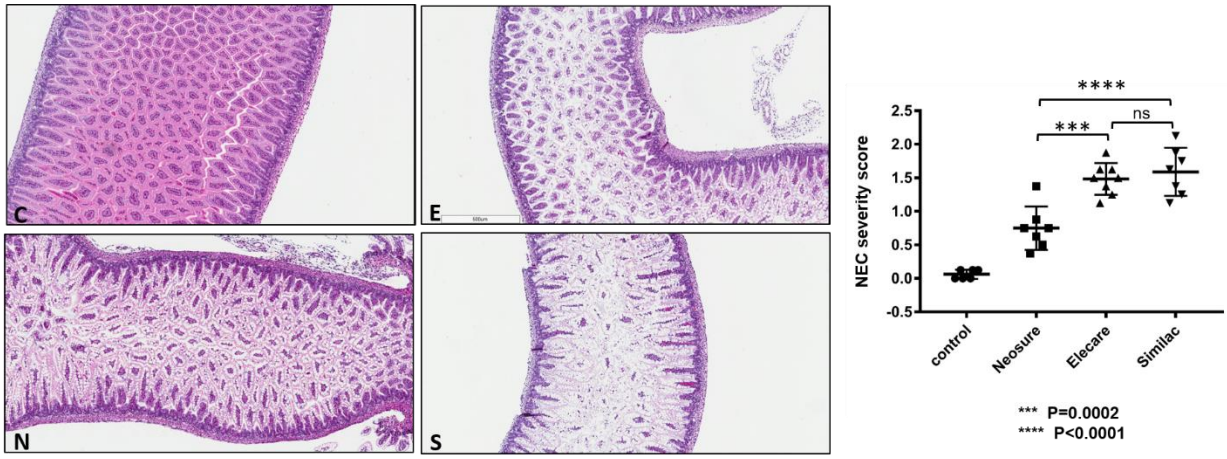

Fig 1. Intestinal injury is significantly more in FF pups compared to breast fed controls. Hematoxylin & Eosin staining of intestinal tissues from FF pups and controls were examined and a validated scoring tool was used to calculate injury scores. Elecare (E) and Similac (S) formula fed pups demonstrated more injury than the Control (C) and Neosure group (N) ($p<0.001$). $n \geq 6$-7 pups in each group.

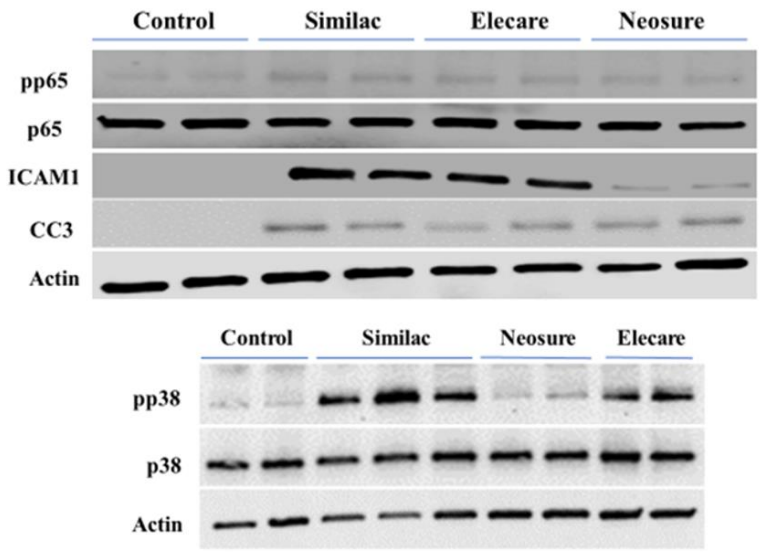

Fig 2. Increased proinflammatory signaling seen in Elecare and Similac pups compared to Control and Neosure pups. Western Blot showed increased protein expression of phosphorylated p65, p38, CC3 and ICAM1 in the Elecare and Similac pups compared to the Control and Neosure groups. (n=4-6/group).

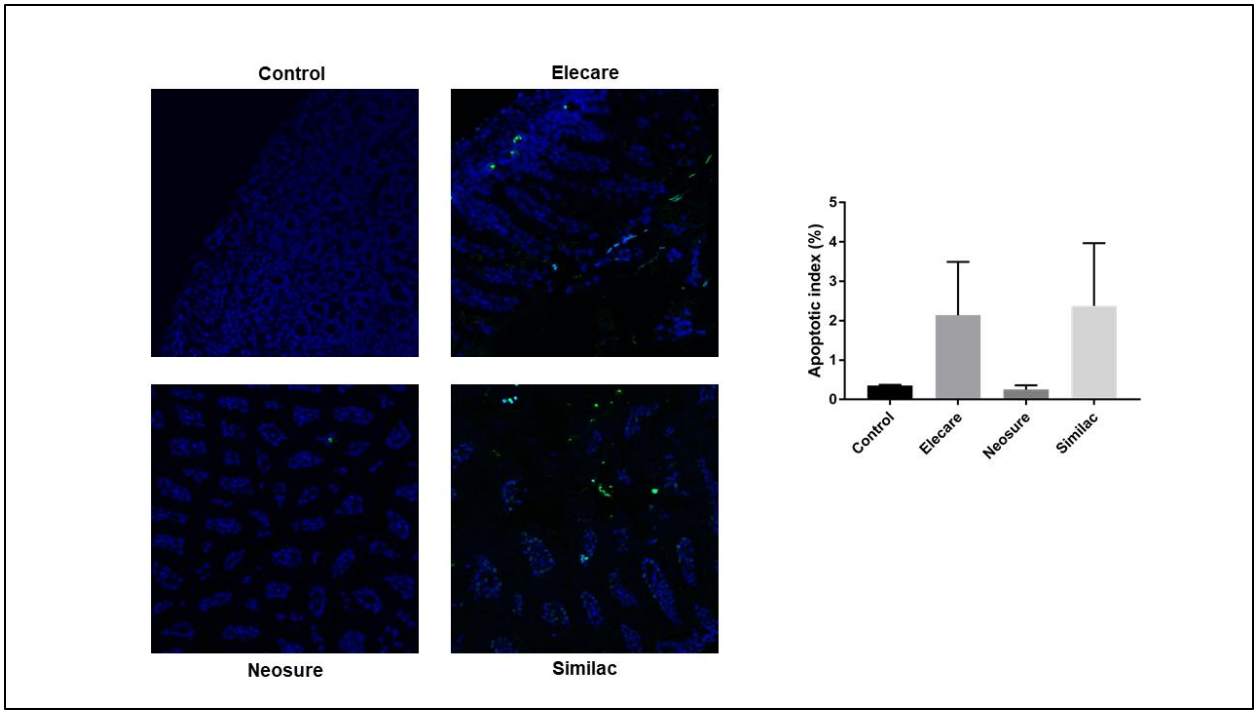

Fig 3. Increased intestinal apoptosis was noted in the intestinal tissues of the Elecare and Similac pups in comparison to the Control and Neosure pups. Intestinal apoptosis was quantified using TUNEL assay of ileal homogenates. TUNEL+ cells (green) indicating apoptosis was increased in Elecare (2.14%) and Similac (2.34%) groups compared to Control (0.24%) and Neosure (0.25%) fed pups.

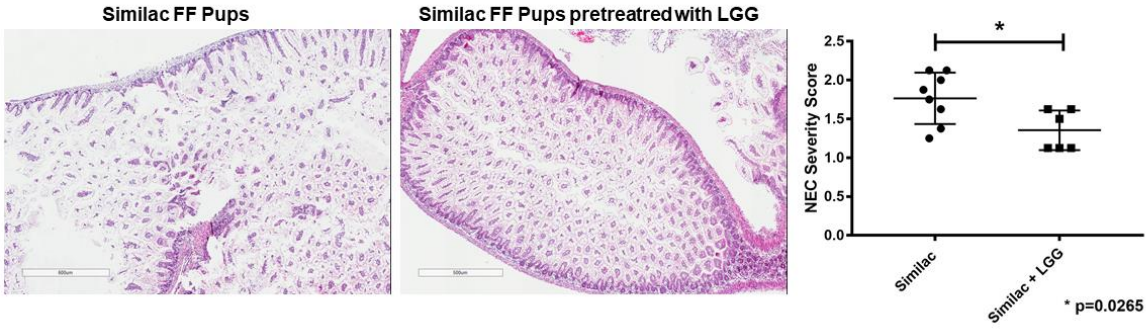

Fig 4. Intestinal Injury seen in Similac fed pups is decreased with high dose LGG pretreatment. In the second arm of the study FF pups were pretreated once daily with 0.1 ml of LGG (10^8 CFU/ml) from day 5-7, followed by FF with Similac from day 8-10 and were sacrificed on day 11. Hematoxylin & Eosin staining of intestinal tissues from FF pups and FF pups that received LGG were examined and a validated scoring tool was used to calculate injury scores. The FF pups that were pretreated with LGG showed less severe injury on staining.