Neutrophil Extracellular Traps in Necrotizing Enterocolitis

***Hala Chaaban, *Jeffrey Eckert, †Ravi Keshari, *Kathryn Burge, *Aarthi Gunasekaran, †Florea Lupu**

** University of Oklahoma Health Sciences Center; † Oklahoma Medical Research Foundation*

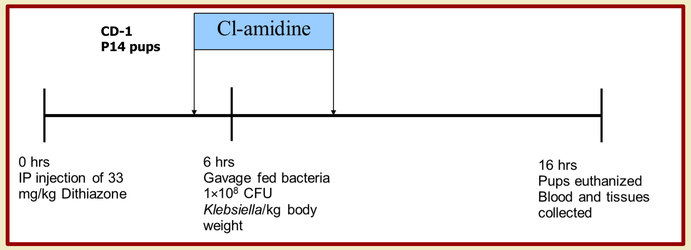
BACKGROUND

- Necrotizing enterocolitis (NEC) continues to be one of the major causes of morbidity and mortality in preterm infants.
- We previously showed extensive neutrophil extracellular trap formation (NETs) in intestinal tissues from infants with NEC.
- NETs is a novel cell death process in which activated neutrophils expel their nuclear content in the form of web-like structures.
- Although, NETs has been shown to exhibit antimicrobial functions by trapping and killing pathogens, NET-associated factors, specifically histones, cause "collateral damage" to the host.

OBJECTIVE

- To determine the role of NET formation in experimental NEC.
- We hypothesize that NET inhibition would decrease mortality, pro-inflammatory cytokine releases, and organ injury in NEC.

METHODS



Klebsiella/ Dithizone NEC Model

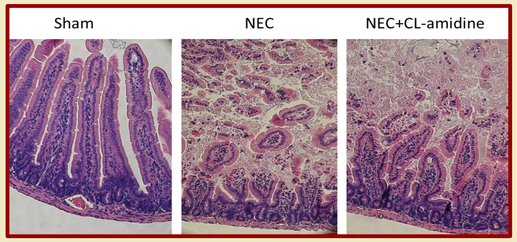
- NEC was induced using the Dithizone/Klebseilla (DK) method.
- P14-16 CD-1 mice were injected i.p. with 33 mg/kg dithizone. Six hours after injection, mice were enterally infected with 1×10^8 CFU *Klebsiella* per kg.
- Cl-amidine (a PAN Peptidylarginine deiminase inhibitor), was given 15 min before oral *klebsiella* and 3 hours after.
- Cl-amidine has been shown to irreversibly inhibit NET formation in multiple murine models.

METHODS CONT'D

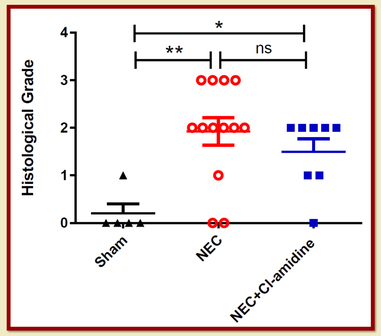
- Effect inhibiting NET formation by Cl-amidine (80 mg/kg) on incidence and severity of NEC
  - Survival between the groups
  - H&E Intestinal histological injury scores by a blinded pathologist
  - Systemic pro-inflammatory and anti-inflammatory cytokines (IL1β, IL-10, TNF-α, IL-6, GRO-α) in plasma were measured using XMAP procartaplex® multiplex immunoassays.

RESULTS

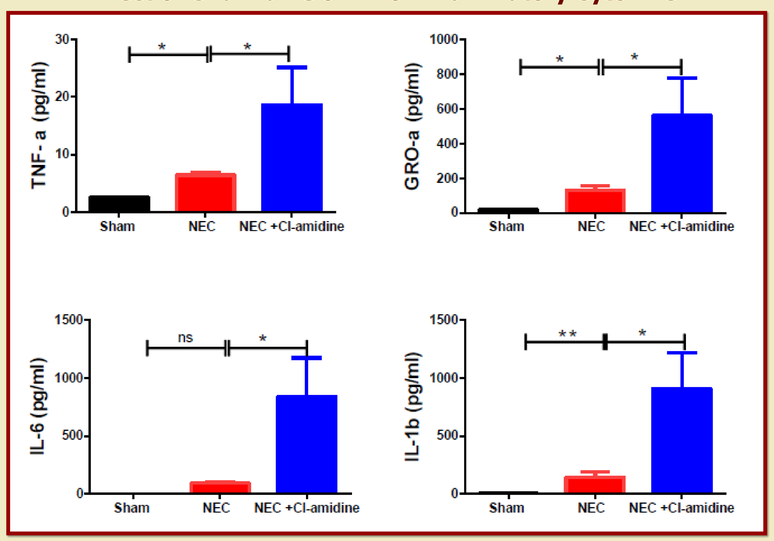
Effect of Cl-amidine on NEC Severity and Histological Injury Scores



NEC injury score was not statistically different between the groups (1.8 ±0.44 in NEC +Cl-amidine group vs 2.0 ± 0.41 in NEC group, (P=0.71).

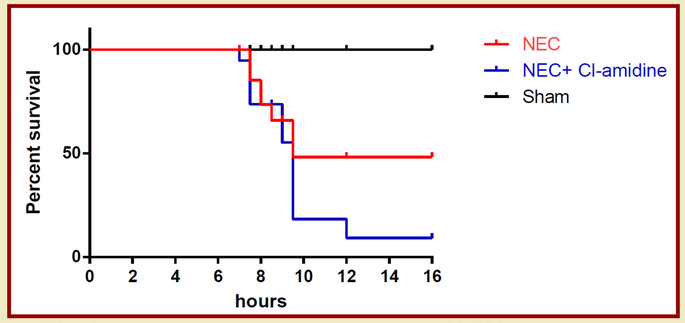


Effect of Cl-amidine on Pro-inflammatory Cytokine



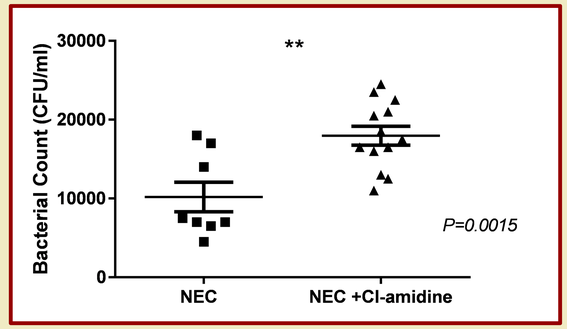
RESULTS CONT'D

Effect of Cl-amidine on Mortality in DK NEC model



NEC+ Cl-amidine group had higher mortality (90.78% in NEC+ Cl-amidine (n=22) vs 51.7% in NEC (n=20),P< 0.0001)

Effect of Cl-amidine on Bacteremia in DK NEC model



Total CFU was significantly higher in the NEC+Cl -amidine group (17,962 + 1191 CFU/ml, n=13) compared to NEC group (10,188 + 1868 CFU/ml, n=12), P=0.0015.

CONCLUSIONS

- NET inhibition using Cl-amidine in a DK NEC model increased mortality, systemic inflammatory response, and bacteremia.
- NET formation could be one of the protective innate immune mechanisms that decreases bacterial translocation.
- Further studies are needed to determine the effect of NET inhibition in other models of NEC
- Further studies are needed to determine the role of delayed NET/ histones inhibition in NEC models

College of Medicine
Pediatrics

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