INDUCED NECROTIZING ENTEROCOLITIS BY ISCHEMIA IN ANIMAL MODELS

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Abstract

Research into necrotizing enterocolitis has evolved recently from the gross and microscopical level to the biochemical and genetic level. For this new type of research there are many animal models used mostly in rats and mice that use ischemia, hypothermia and feeding protocols, all associated or used in individual matter, as an inducing factors for necrotizing enterocolitis.

Animal models of necrotizing enterocolitis suggest both indirect and direct participation of ischemia in tissue damage, among many other factors.

Key words: *animal model, ischemia, necrotizing enterocolitis*, *colon*

Abbreviations

NEC - necrotizing enterocolitis
SMA – superior mesenteric artery
PAF – platelet activating factor
I/R – ischemia/reperfusion

NEC is the most common disease of infancy, afflicting 5% of infants where birth-weight is less than 1500 grams. This disease is manifested by intestinal necrosis that often leads to the death of the patient.

In the United States of America, there are more then 2,000 newborns diagnosed with NEC each year. The mortality of this disease is up to 20-50% resulting in more than 1,000 deaths each year.[1]

Risk factors may roughly be grouped into four main categories: prematurity, transient ischemia of the intestine, local/systemic inflammation predisposing the bowel to injury and therapeutic interventions.[9]

Several agents with the potential to alter intestinal hemodynamics have been considered as mechanisms responsible for NEC-related ischemia including platelet activating factor (PAF) inducible nitric oxide synthase, leukotrienes, prostaglandins and cytokines.[9]

The concept of ischemia as a preliminary cause of tissue damage in NEC generally assumes that this damage is mediated by a profound compromise of parenchymal O_2 delivery; hence, blood flow and tissue O_2 delivery fall below the "critical " level necessary to maintain mitochondrial pO_2, leading to cell demise.[1]

The most salient and distinguishing feature of the newborn intestinal circulation are its very low resting vascular resistance and hence high rate of blood flow when compared with older subjects.

There are two major and important factors that contribute to the control of the vascular resistance within the newborn intestine. The principal constrictor stimulus in the newborn intestine circulation is the peptide endothelin-1 or ET-1, as evidenced by the significant vasodilatation that occurs when ET-1 decreases.[1]

ET-1 is produced in endothelial cells in a constitutive fashion and exerts its constrictor effect by binding to ETa receptors on adjacent vascular smooth muscle, thus acting in a paracrine fashion.[1]

The constriction generated by ET-1 is sustained and profound and a significant increase in ET-1 production generates in the absence of counterbalancing vasodilator stimuli, leads to tissue hypoxia. Although constitutively produced, ET-1 production can be increased by a wide range of stimuli, including reduced blood flow rate, hypoxia and inflammatory cytokines. [1]

The principal dilator stimulus in newborn intestine is nitric acid NO. The NO, relevant to vascular regulation is generated during the reduction of L-arginine to L-citrulline by the endothelial isoform of nitric oxide synthase (eNOS). eNOS is a constitutive enzyme but its activity can be increased by chemical agonists and mechanical stimuli, particularly flow rate.[1]

Perhaps of greatest importance to regulation of the newborn intestinal circulation is that developmental regulation of eNOS occurs during perinatal life. The eNOS expression within the mesenteric artery is low during fetal life, increases after birth in association with feeding, then decreases with subsequent maturation.[1]

The low vascular resistance characteristics of newborn intestine reflects substantial generation of eNOS-derived NO when compared with ET-1, a circumstance that mirrors the increased expression of eNOS within the newborn intestinal circulation. This balance favoring dilation facilitates an increased basal rate of blood flow and hence O_2 delivery to the newborn intestine and is designed to meet the substantial oxidative demand of newborn intestine.[1]

Clearly, ischemia can cause intestinal damage: alternatively however, it is possible that intestinal damage, caused by factors other than ischemia, reduces the need for perfusion and reduces intestinal blood flow.[1]

Ischemia is certainly not the sole basis for NEC-related tissue damage, while it is very likely that ischemia occurs at some time before complete tissue destruction. Unfortunately, existing data from human and animal studies fail to provide significant insight into this issue.

Touloukian et al.[2], described 25 infants with NEC, 18 of which were <2500 grams who had evidence of significant birth asphyxia The authors suggest that asphyxia induces a

massive sympathetic discharge that generated intestinal ischemia leading to NEC.

Alward et al.[3], confirms that massive postnatal asphyxia induces mucosal destruction in newborn piglets, evidence that appeared to support the asphyxia-ischemia hypothesis.

Epidemiological studies of NEC failed to find correlation between birth asphyxia or between postnatal hypoxia (another potent sympathetic discharger) and NEC. It was also demonstrated that sustained stimulation of extrinsic sympathetic nerves in newborn intestine caused only transient reduction in flow rate or oxigen uptake.[1]

Recent studies analyzed superior mesenteric artery SMA blood flow velocity in fetal life and after birth.

Rhee et al.[4], evaluated antenatal SMA flow in growth-retarded fetuses, a group of population that historically demonstrated a high incidence of NEC. Doppler ultrasonography was used to measure the SMA blood flow velocity and to calculate a pulsatility index, from which an indirect estimate of vascular resistance was calculated. Forty percent of these fetuses demonstrated compromised SMA flow velocity and manifest a pulsatility index suggestive of increased intestinal vascular resistance; however the subsequent incidence of NEC in these infants was not different from the remaining 60% of infants whose fetal SMA hemodynamic profile was normal.

Kempley et al.[5], reported an increase of SMA flow velocity in preterm infants, rather than a decreased, for stage II NEC when compared with age-matched, unfed controls. No correlation was noted between SMA flow velocity before the onset of NEC-like symptoms and the incidence of the disease in these infants.

It is important to stress that the Doppler technique measures blood flow velocity (mm/sec) a term that is only indirectly related to the volume of blood flow (ml/min); it is the latter term that is relevant to O_2 transport physiology.[1] Furthermore, measurements of SMA hemodynamics provide little insight into downstream intramural microvascular events. Substancial compromise of downstream flow must occur before SMA flow velocity is affected. These data acknowledge the fact that global reduction of intestinal blood flow does not precede the development of NEC.[1]

Animal models of NEC suggest both indirect and direct participation of ischemia in tissue damage, but do not specifically address the question of timing. The rat pup model of NEC, perhaps the best accepted model to date generates disease by repetitive exposure of formula fed pups to hypoxia and hypothermia.[1]

The protocol used in our laboratory for both the mouse and the rat model is based on feeding patterns at 2 to 3 hours intervals associated with stress induced by exposure to nitrogen (hypoxia) and hypothermia. All pups used for this animal model are delivered by C-section on day 21.5 for the rat model and 20 for the mouse protocol so the factor that is added to stress induced by hypoxia, hypothermia and feeding patterns, is prematurity.

The following pictures present some of the results obtained with this protocol.

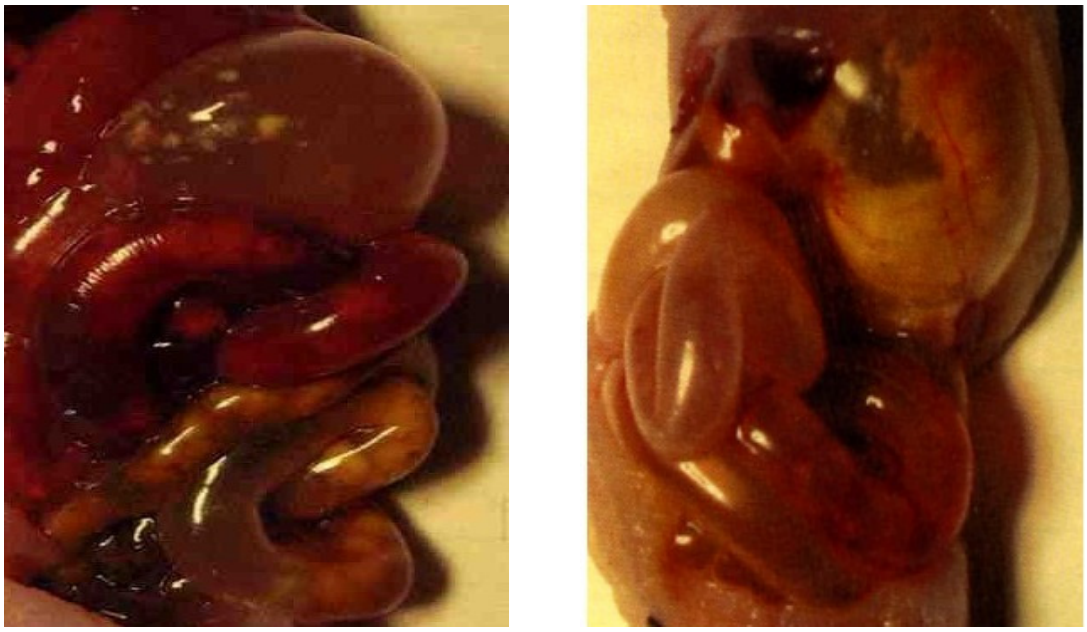

Figure 1 The presence of abdominal distention, bloody stools and characteristic aspects of the colon.

Based on data from larger animals it is likely that both hypoxia and hypothermia compromise intestinal perfusion in the rat pup model, however, artificial feeding of the pups is requisite for the development of NEC, suggesting that ischemia per se is not sufficient to generate tissue damage to this model.[1]

A piglet model of NEC recently developed, utilizes direct reduction of intestinal perfusion via cerclage of the superior mesenteric artery (SMA), but requires co infusion of lipopolysaccharide (LPS), into the gut lumen to generate significant tissue damage. [1]

The sequence of the insult, LPS then ischemia or vice versa, does not affect the final degree of tissue damage; however, both are necessary. Therefore, while it is clear that reduction of flow through the mesenteric artery by 60% for 6 hours is required for tissue damage in this model, the relative role of this ischemia with respect to luminal LPS remains unclear.

Nonetheless, the etiology of NEC remains elusive and no specific treatment or prevention approaches have been successful yet.

Great hope is nowadays given to the protective action of heparin binding epidermal growth factor (HB-EGF) on the bowel discovered and still under investigation by Besner[10] in our laboratory.

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