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Near-Infrared Spectroscopy Measurement of Abdominal Tissue Oxygenation is a Useful Indicator of Intestinal Blood Flow and Necrotizing Enterocolitis in Premature Piglets

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Abstract

Purpose—A major objective of necrotizing enterocolitis (NEC) research is to devise a non-invasive method of early detection. We hypothesized that abdominal near-infrared spectroscopy (A-NIRS) readings will identify impending NEC in a large animal model.

Methods—Piglets were prematurely delivered and received parenteral nutrition followed by enteral feedings. Serial A-NIRS readings were obtained for five days, and animals were monitored for NEC. Separately, A-NIRS readings were obtained in healthy piglets to validate the correlation of A-NIRS with splanchnic oxygen delivery.

Results—Three of twenty-nine piglets developed NEC. Eleven piglets without NEC died prematurely. Fifteen piglets remained healthy, had normal histologic assessment of their intestines, and served as controls. A-NIRS readings within twelve hours of birth were significantly lower in animals that developed NEC compared to healthy littermates (4% vs 33%, $p=0.02$). For all time-points measured, ANIRS readings were significantly lower in NEC group compared to controls (21% vs 55%, $p<0.001$). A-NIRS readings correlated with both decreased pulse oximetry readings during apneic episodes ($r=0.96$) and increased superior mesenteric artery flow in response to glucagon-like peptide-2 ($r=0.67$).

Conclusion—A-NIRS is capable of detecting alterations in intestinal oxygenation and perfusion in neonatal piglets and may allow early detection of neonates at risk for NEC.

Keywords

Necrotizing enterocolitis; NEC; Near infra-red spectroscopy; NIRS; A-NIRS; StO₂; premature; piglets

Necrotizing enterocolitis (NEC) prevails as the most common gastrointestinal emergency of the newborn, affecting one to three children per 1000 live births. Despite diligent pursuit,

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our understanding of NEC pathology remains limited. Some of the main factors involved are prematurity, enteral feeding, intestinal ischemia, and possibly carbohydrate malabsorption and bacterial overgrowth. Due to its insidious progression, NEC can currently only be detected once clinical signs manifest. Thus, substantial research is currently focused on interventions to prevent the development of the disease. A major objective of NEC research is to devise non-invasive methods and biological markers to detect the disease in its early stages.

Near-infrared spectroscopy (NIRS) can be used as a non-invasive method of measuring local tissue hemoglobin oxygen saturation, (StO_2), at both the capillary bed and the post-capillary venule. It is currently applied to the extremities and abdomen of adults to determine the extent of shock and effectiveness of resuscitation. NIRS is also used in the neonatal intensive care units (NICU), cardiac intensive care units and operating rooms to monitor cerebral oxygenation. Previous studies that applied NIRS to the abdomen of neonates demonstrated that neonates with an acute abdomen have lower cerebro-splanchnic NIRS ratios. In addition, NIRS measurements of the abdomen have also been shown to respond to systemic hemoglobin desaturation during apneic episodes in three infants. Together these studies suggest that abdominal NIRS (A-NIRS) measurements in neonates may identify changes in bowel oxygenation and perfusion.

Since the early histologic observations of NEC, bowel ischemia has been widely hypothesized to provide critical insult in NEC pathology. Several theories exist regarding the timing of ischemic insult to the bowel. For example the diving reflex theory postulates that intestinal ischemia is instigated during parturition. The ischemic etiology is further supported by studies demonstrating that abnormal flow velocity of the superior mesenteric artery (SMA) predicts not only feeding intolerance but NEC itself. These results further strengthen the possibility that measurements of bowel oxygenation may reflect the early onset of NEC as it is occurring just beneath the surface of the neonatal abdomen.

SMA blood flow has shown to be increased by glucagon-like peptide-2 in neonatal pigs. Glucagon-like peptide-2 (GLP-2) is a 33-amino acid peptide synthesized by intestinal endocrine L-cells in response to enteral feeding. Administration of GLP-2 to TPN-fed piglets resulted in increased perfusion to visceral organs within the SMA distribution, namely the duodenum, jejunum, and pancreas. Other gastrointestinal benefits associated with GLP-2 include enhancement of mucosal barrier function, increased nutrient uptake, and prevention of intestinal mucosal atrophy. GLP-2 has shown clinical benefit in patients suffering from short bowel syndrome via improvement of both intestinal absorption and nutritional status. With intestinal ischemia as a potential therapeutic target for necrotizing enterocolitis, GLP-2 may prove to be a useful tool in combination with NIRS measurements for the management of this disease.

A unique large animal model of spontaneous necrotizing enterocolitis in premature piglets has recently been developed which exhibits clinically relevant features of NEC as seen in human infants. In this piglet model the incidence of NEC is dependent on prematurity, enteral formula versus breast milk feeding, and the presence of bacteria. Moreover the model exhibits the hallmark clinical signs of NEC in infants, including abdominal distention, diarrhea, bacterial overgrowth as well as intestinal pathology, such as inflammation, hemorrhagic vascular congestion, pneumatosis intestinalis, and necrosis. The clinical signs of NEC in this model do not occur prior to oral feeds, correlating with the fact that NEC seldom occurs prior to oral feeds in the neonatal intensive care unit (NICU). In addition, each piglet litter allows for animals with NEC to be studied alongside healthy littermates exposed to similar perinatal conditions.

The aims of these studies were to determine the correlation of abdominal NIRS measurements with NEC using an experimental model of NEC, and to verify that abdominal NIRS measurements accurately reflect changes in intestinal blood flow.

1. Methods

1.1 Necrotizing Enterocolitis Experiment

Cesarean Section Delivery and Perinatal Care—All procedures were approved by the Animal Care and Use Committee of the Baylor College of Medicine (#AN-4121). Pregnant Yorkshire pigs at 105–107 days gestation (term=115 days) underwent cesarean section without induction of parturition as previously described. Briefly, sows were sedated with ketamine 15mg/kg and xylazine 1mg/kg IV and anesthesia was maintained with isoflurane gas 0.5–4% in O₂. A lower abdominal midline laparotomy incision was made and each piglet was removed through separate hysterotomy incisions. Following standard neonatal resuscitation, each piglet had an umbilical artery catheter (3.5 Fr) and orogastric feeding tube (8 Fr) inserted. The piglets were placed in a protective jacket, housed individually in warmed cages, and received daily intravenous injections of enrofloxacin 5 mg/kg/day.

Nutritional Support—The timeline (Figure 1) and nutritional support in the study is as previously described. Briefly, the pigs were given continuous parenteral nutrition (Baxter Healthcare, Deerfield, IL; Ajinomoto, Tokyo, Japan) at an initial rate of 5 ml/kg/h and gradually increased to a rate of 10 ml/kg/h to maintain slightly positive energy balance. After 72 hr of parenteral nutrition, orogastric feeds were initiated. A cow's milk-based formula (Advance Liqui-Wean®, Milk Specialties Co., Dundee, IL) was given by gavage every three hours to provide 120 ml/kg/day.

NIRS readings and NEC Monitoring—A 3.5 cm NIRS probe was placed 1 cm lateral to the umbilicus, and connected to an InSpectra StO₂® (Hutchinson Technologies, Minnesota, USA) tissue oxygenation monitor to measure between a depth of 1.8 and 3.2 cm below the skin. NIRS readings were measured for a period of 1–5 minutes until a steady state was evident. Abdominal StO₂ measurements were performed at the same anatomical location every 12 h for the first 72 h after delivery. In the first 72 hr after delivery, piglets were observed for signs of NEC such as lethargy, abdominal distention, and bloody stools. Arterial blood gas samples were obtained via umbilical catheter daily to assess oxygenation status. After the initiation of enteral feeding, abdominal StO₂ measurements were taken at 3 hour intervals and piglets were monitored for clinical signs of NEC.

Euthanasia and Tissue Collection—At the conclusion of the study (day 5), the piglets were euthanized with pentobarbital-phenytoin sodium (Beuthanasia®, Schering-Plough, Kenilworth, NJ). Immediately thereafter, the abdominal cavity was exposed and the splanchnic organs examined for macroscopic signs of NEC. Tissue samples were collected from liver, stomach, small intestines and colon in three regions (proximal, medial, and distal) for histology. Piglets that had clinical and pathological findings consistent with NEC were assigned to the NEC group. Piglets that did not exhibit clinical symptoms and whose bowel were grossly and histologically normal were assigned to the control group.

1.2 GLP-2 Infusion Experiment

NIRS values reflect tissue oxygen delivery that is dependent on oxygen content and blood flow. To evaluate the ability of abdominal-NIRS to preferentially detect changes in blood flow in the splanchnic bed of piglets, A-NIRS readings in response to a GLP-2 infusion was determined. Healthy, two-day-old term piglets (n=10) were implanted with an internal

jugular, silastic catheter (3.5 Fr) and an ultrasonic flow probe (3–4 mm; Transonics, Ithaca, NY) on the superior mesenteric artery by laparotomy as previously described. The piglets were allowed to recover for 7 days while being fed parenterally. On day 10 of life, animals were anesthetized using isoflurane (1–4% in oxygen), with the placement of a 3.5 mm A-NIRS probe and a hind-foot peripheral pulse oximeter. Serial measurements of pulse rate, peripheral oxygen saturation, SMA flow rate, and A-NIRS readings were recorded at 1 minute intervals. Sterile normal saline was infused via jugular catheter for 10 minutes, and a 25 µg/kg intravenous bolus of GLP-2 peptide analog (NNC 103-0066, Novo Nordisk, Denmark) was then administered for 20 minutes. After 30 min piglets were euthanized with an intravenous injection of pentobarbital/phenytoin sodium. The position of the ultrasonic flow probe around the SMA was confirmed at necropsy.

1.3 Apnea Experiment

To evaluate the ability of A-NIRS to detect changes in oxygen content in the splanchnic bed of piglets, A-NIRS readings in response to an apneic response was determined. Healthy, five-day-old term piglets (n=3) were anesthetized using isoflurane (1–4% in oxygen), with the placement of a 3.5 mm ANIRS probe and a hind-foot peripheral pulse oximeter. Apnea was induced by increasing the anesthetic to 5% isoflurane and reversed with a return to 1% anesthetic. Measurements were taken in 3 second intervals at baseline, as respirations ceased, and as spontaneous respirations resumed. All measurements were continued until the animals were euthanized.

1.4 Histology

Tissue samples were placed into 4% buffered formalin for 24 hours, dehydrated in alcohol, embedded in paraffin and sectioned (4 µm). Hematoxylin and eosin stained sections were assessed under a light microscope for vascular congestion, mucosal architecture, and necrosis margins by a pathologist blinded to the experimental groups.

1.5 Statistical Analysis

Data were analyzed using Minitab® statistical software (Minitab Inc. State College, PA). Pearson's correlation coefficients were employed for Doppler flow and NIRS using Origin® software program (Microcal Software Inc., Northampton, MA). Comparison between groups was performed by ANOVA with post-hoc analysis or two-tailed t-test as appropriate. Results are expressed as means and standard errors of the mean, and a p-value < 0.05 was considered statistically significant.

2. Results

2.1 Necrotizing Enterocolitis

Twenty-nine piglets were delivered from three litters. Fifteen piglets had small and large intestine that appeared uniformly pink in color with no evidence of inflammation, necrosis, or pneumatosis. These piglets were assigned to the control group. In contrast, three piglets exhibited inflamed, congested bowel, patchy necrosis, and pneumatosis which increased in severity from proximal to distal intestine (Figure 2). These 3 piglets were assigned to the NEC group. Eleven piglets died prior to oral feeds, presumably due to lung immaturity or sepsis. These 11 piglets had no evidence of clinical or histologic bowel pathology and were assigned to the “premature death” group for separate analysis.

For control and NEC pigs, variables such as birth weight, arterial blood gas values, hemoglobin, and body temperature were compared as shown in Table 1. Of these variables, only hemoglobin level was noted to be significantly different (p < 0.01) between the control and NEC groups. In the NEC group, one piglet exhibited bloody stools and vomiting, two

piglets exhibited lethargy and vomiting, and no piglets developed abdominal distension. All histologic samples in this group demonstrated an inflammatory infiltrate with obliteration of microvilli consistent with NEC (Figure 2). When compared to the control group, average A-NIRS values were significantly lower in the NEC group (Figure 3). The average A-NIRS reading throughout all time points for control animals was $55\% \pm 19\%$ versus $21\% \pm 20\%$ for those with NEC ($p < 0.001$). Furthermore, A-NIRS readings of the control group were significantly higher than those of the NEC group at 10 of 14 time points. This relationship was found between control and NEC groups as early as the first 12 hours after birth, $33\% \pm 5\%$ versus $4\% \pm 3\%$, respectively ($p = 0.02$). Of note, the average A-NIRS reading throughout all time points for piglets in the “premature death” group was significantly lower ($23\% \pm 7\%$) than that of the control pigs ($p < 0.001$), and in particular at 3 of 5 time points (12, 48, and 60 hours from birth). While these excluded animals did not manifest signs and symptoms of NEC, they apparently had physiologic perturbations that were identified by the A-NIRS readings.

2.2 GLP-2 Infusion

A-NIRS was able to detect increases in splanchnic tissue oxygenation as a result of increased splanchnic blood flow. The average baseline A-NIRS was $52\% \pm 22\%$, peripheral SaO_2 $93\% \pm 6\%$, and SMA blood flow 46 ± 18 ml/min. Within 6 minutes of GLP-2 analog infusion, SMA flow increased by 140% to 109 ± 31 ml/min. In parallel, GLP-2 induced a selective increase in A-NIRS to $64\% \pm 17\%$, without any effect on the peripheral SaO_2 ($93\% \pm 6\%$, Figure 4). Furthermore, a linear relationship was observed between SMA flow and abdominal StO_2 ($r = 0.67$, $p < 0.001$).

2.3 Apnea

A-NIRS could detect changes in splanchnic tissue oxygenation related to decreased blood oxygen content. With 1% inhaled isoflurane anesthesia, all three animals exhibited a stable average peripheral SaO_2 of $95\% \pm 2\%$ and A-NIRS reading of $76\% \pm 6\%$. When inhaled anesthetic was increased to 5%, respirations ceased, peripheral SaO_2 decreased to $47\% \pm 16\%$, and A-NIRS decreased to $36\% \pm 7\%$. As a result of this induced apnea, both SaO_2 and A-NIRS had a relative decrease of approximately 50%. An average compensatory increase in heart rate of 37 ± 17 beats per minute was seen during these periods of desaturation. Upon return to 1% isoflurane anesthesia and resumption of respiration, a return to baseline SaO_2 and A-NIRS were observed (Figure 5).

3. Discussion

Therapeutic interventions to rapidly diagnose and prevent NEC continue to be aggressively pursued. Previous studies of abdominal NIRS measurement have provided hints that this technology may be useful in detecting the early onset of NEC in infants. The recent development of a clinically relevant model of necrotizing enterocolitis provides an exciting opportunity to study the etiology and diagnosis of the disease prospectively in a large animal model.

Pulse oximetry is limited as it only measures oxygenation at the pre-capillary arteriole. In contrast, NIRS measures oxygenation at both capillary bed and post-capillary venule (StO_2). Our data suggest that NIRS probes placed over the abdomen of neonatal piglets was able to preferentially detect changes in oxygenation and blood flow in the splanchnic bed, specifically in the small bowel. In healthy piglets apnea decreased blood oxygen content with a consequential decrease in both peripheral SaO_2 and A-NIRS readings. A simultaneous increase in SaO_2 and A-NIRS resulted as ventilation was restored. In contrast, with the increase in SMA blood flow induced by GLP-2 there was a parallel increase in A-

NIRS values without a change in peripheral SaO_2 . This suggests an increase in splanchnic oxygen delivery without an effect on systemic oxygenation and further confirms that A-NIRS readings are specific for the splanchnic bed. In addition, the linear relationship between GLP-2 induced SMA blood flow and A-NIRS StO_2 suggest that GLP-2 may have a role in the amelioration of the ischemic events surrounding NEC.

Pigs that developed NEC had significantly lower A-NIRS values than healthy controls. This difference was evident as early as twelve hours after delivery, suggesting that A-NIRS may be able to identify piglets at risk for NEC well in advance of the onset of disease. If this observation is validated in clinical studies, A-NIRS will provide a unique, non-invasive way to identify neonates at highest risk for NEC and allow intensification of studies aimed at prevention of the disease. Also of interest was that in contrast to healthy controls, NEC piglets demonstrate a downward trend in A-NIRS values prior to enteral feeding and a dramatic increase in A-NIRS once fed. These changes in A-NIRS readings within the NEC group may represent intestinal ischemia-reperfusion injury, and provide further support for such a theory of NEC pathogenesis, and may prompt earlier cessation of feeds.

Piglets that died prior to enteral feedings from a cause unrelated to NEC also had low initial ANIRS values, despite dying of non-intestinal causes. Diversion of blood flow from the splanchnic bed is an early response to stress. Investigating the role of A-NIRS in identifying early signs of stress may be revealing.

This study is limited by the small number of animals that progressed to develop NEC (10%). Eleven of 29 animals could not be classified as NEC or healthy controls as they died prior to the initiation of enteral feeds and did not have pathologic signs of NEC. A confounding observation in this study was the significantly lower hemoglobin levels between the NEC and control groups. NIRS measurements have not been shown to vary with hemoglobin levels. However, a lower hemoglobin may reduce the oxygen carrying capacity of the blood and further predispose the bowel to the effects of ischemia. Furthermore, intermittent A-NIRS measurements may have missed valuable information in between measurements. Intermittent observations were made due to technical limitations with the device and to avoid undue animal restraint. Smaller devices are now available that will permit continuous monitoring. Nevertheless, this large animal model allowed us to effectively correlate ANIRS findings with NEC pathology.

In conclusion, this study suggests that transcutaneous abdominal NIRS monitoring of the splanchnic bed is plausible in the neonatal pig and offers the opportunity for non-invasive monitoring of animals at risk for NEC. A-NIRS readings correlate with changes in blood oxygen content and blood flow in the splanchnic bed. Low A-NIRS readings within the first day of life may identify neonates at risk for NEC.

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Figure 1.
Timeline of parenteral and enteral nutrition for necrotizing enterocolitis study

Figure 2.

a. Gross examination of the abdominal viscera of a healthy control piglet. b. Gross examination of NEC piglet organs shows hyperemic, congested bowel with areas of necrosis in the distal small intestine. c. Histologic examination of normal small intestine of control piglet. d. Histologic examination of small intestine of NEC piglet shows inflammatory infiltrate, engorged vasculature, and the absence of intact microvilli.

Figure 3. Abdominal StO₂ measurements show significantly lower A-NIRS values for NEC vs. control piglets at multiple time points (* indicates $p < 0.05$).

Figure 4.

Response of superior mesenteric artery (SMA) blood flow, abdominal StO₂, and peripheral SaO₂ to GLP-2 analog infusion in healthy piglets (n=10). SMA flow increases by $140\% \pm 7\%$ upon GLP-2 analog infusion. Abdominal StO₂ mirrors this change in SMA flow with an increase of $13\% \pm 1\%$ while peripheral SaO₂ remains unchanged.

Figure 5.

Apnea-induced changes in StO₂, SaO₂, and heart rate of healthy piglets (n=3). Abdominal StO₂ and peripheral SaO₂ exhibit a similar proportional decline during apneic episodes with a compensatory increase in heart rate. Both StO₂ and SaO₂ rebound with similar slopes.

Table 1

Baseline laboratory data and vital signs of piglets

	Healthy (n=15)	NEC (n=3)
Weight (g)	1015 ± 62	927 ± 135
pH	7.47 ± 0.03	7.46 ± 0.03
pCO ₂ (mmHg)	42 ± 3	44 ± 4
pO ₂ (mmHg)	76 ± 6	68 ± 12
HCO ₃ ⁻ (mmol/L)	31 ± 1	30 ± 2
O ₂ sat (%)	93 ± 3	92 ± 4
Temp (F)	98.7 ± 1.0	98.7 ± 1.6
Hemoglobin *	7.1 ± 0.5	5.7 ± 0.7

* indicates p<0.01