0031-3998/87/2104-0422$02.00/0
PEDIATRIC RESEARCH

Vol. 21, No. 4, 1987
Printed in U.S.A.

Intestinal Ischemic Injury following Mild Hypothermic Stress in the Neonatal Piglet

PETER A. SCHNEIDER, STANLEY R. HAMILTON, AND DAVID L. DUDGEON

Division of Pediatric Surgery, The Department of Surgery and The Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT. The core body temperature of unanesthetized neonatal piglets was lowered 4 ± 1° C for 4.5 h. Although systolic blood pressure, arterial blood gases, and pH were similar in hypothermic and control animals, grossly evident small intestinal injury occurred in 94% of hypothermic piglets but in none of the controls ($p < 0.01$). The histopathology of the intestinal lesions in the experimental animals was characteristic of ischemic injury and ranged from superficial mucosal necrosis to transmural infarct. The lesions were more frequent in the mid- and distal small bowel and involved the muscularis propria only in the distal small bowel. The location and histopathologic characteristics of the bowel lesions in these animals support the concept that mild hypothermia could be a pathogenetic factor in the ischemic bowel lesions of human neonatal necrotizing enterocolitis. (*Pediatr Res* 21: 422–425, 1987)

Abbreviation

NNEC, neonatal necrotizing enterocolitis

NNEC is a devastating human intestinal disease which primarily affects stressed premature infants (1). The pathologic features of NNEC are those of ischemic bowel injury and are confined mainly to the distal small intestine and proximal colon (2–5). The etiology of NNEC is not clear; however, hypoxia appears to play an important role in the pathogenesis of a similar ischemic lesion in animals (6, 7).

Hypothermia is a frequent complicating environmental stress for the newborn premature infant and has been postulated as an etiologic factor in the development of intestinal ischemic injury in newborn animals (6). Furthermore, hypothermia with an induced drop in core body temperature of 4 ± 1° C for 30 min has been shown previously to produce a selective decrease in intestinal blood flow in the anesthetized neonatal piglet (8). The most significant decrease in gastrointestinal blood flow in the hypothermic piglet occurred in the distal small intestine. This area is also most commonly affected by NNEC in infants. Reduced blood flow, however, does not necessarily result in structural damage to the intestine, *i.e.* ischemic injury. Therefore, in the present study, we examined the pathologic findings in the gastrointestinal tract following prolonged exposure of unanesthetized neonatal piglets to the same level of hypothermia as in our previous studies on blood flow.

Received August 12, 1985; accepted November 13, 1986.
Correspondence David L. Dudgeon, M.D., The Johns Hopkins Hospital, 601 N. Broadway, CMSC 7-116, Baltimore, MD 21205.
Supported by NIH Biomedical Research Support Grant RR5378.

MATERIALS AND METHODS

Three pregnant Duroc-Berkshire sows were obtained 1 wk before delivery from The Johns Hopkins Research Farm, Freeland, MD. Three litters of piglets, 30 animals, were delivered spontaneously without complications. Approximately 12–24 h following birth the piglets were anesthetized with light halothane anesthesia and underwent placement of a polyethylene artery catheter in the right femoral artery through a groin incision using sterile technique. The animals were allowed to recover for 12 h to resume feeding and stooling prior to hypothermia.

The hypothermia apparatus consisted of an enclosed plexiglass chamber divided into five compartments. The temperature within the apparatus was lowered and maintained for 4.5 h to produce a 4 ± 1° C decrease in rectal temperature, as in our previous study (8). Piglet weight at the initiation of hypothermic stress was 1.2 ± 0.4 kg with a range of 0.8 to 1.8 kg. Fourteen animals served as controls and were not cooled but were confined in a box of similar dimensions with circulating room air for 4–5 h. Systolic blood pressure was monitored in all animals via the right femoral artery catheter and a Sanborn 1700 Transducer/Recording system. Arterial pO_2, pCO_2, and pH were monitored at regular intervals (after 30 min, 1½, 2½, 3½, and 4½ h of hypothermia) with samples drawn through the arterial catheter. Volume replacement, ml for ml, with 5% Dextrose in normal saline was given. Following the hypothermic stress, all animals were returned immediately to the sow to continue nursing. Examinations for stool blood were performed daily with a guaiac reagent test.

Animals were sacrified with intraperitoneal and intracardiac injections of sodium pentothal given 24 to 48 h after the hypothermic stress. The entire gastrointestinal tract was removed, opened longitudinally along the antimesenteric border, examined grossly, and immediately fixed in 10% buffered formalin.A representative specimen, including any area with gross evidence of hemorrhage and/or ulceration, was taken in a standardized fashion for histologic study from each of the following anatomic areas: the mid-esophagus; fundus of the stomach; mid-duodenum; proximal, mid- and distal small intestine; cecum; and proximal and distal colon.

Coded histopathologic sections were evaluated by a gastrointestinal pathologist (S.R.H.) for evidence of intestinal injury. Because the lesions had the features characteristic of ischemic bowel injury, the grading system modified from that previously reported by Parks *et al.* (9) was used. Sections of the small intestine were graded on the basis of ischemic injury to the villous epithelium, crypt epithelium, and the contiguous lamina propria, as shown in Table 1. Where applicable, injury to the muscularis propria of the small intestine was also graded. The esophagus, stomach, and colon were evaluated on a scale of 0–4+ for mucosal injury since no ischemic changes were found in the deeper layers. Mucosal necrosis in the latter sites was graded

Table 1. *Histologic grading system for small intestinal ischemic injury*

Villi	
0	No ischemic change
1+	Loss of epithelium with congestion, edema, and hemorrhage in lamina propria of occasional villous tips
2+	Majority of villous tips affected
3+	Majority of the tips and midportion of some of the villi affected
4+	Tips, mid-, and lower portion of a majority of the villi affected
Crypts	
0	No ischemic change
1+	Loss of epithelium with congestion, edema and hemorrhage in lamina propria of occasional crypts
2+	Scattered crypts affected
3+	Majority of crypts affected
4+	All crypts affected
Muscularis propria	
0	No ischemic change
1+	Areas of congestion, hemorrhage and edema in inner layer
2+	Moderately extensive changes involving inner layer
3+	Extensive changes involving inner and outer layer
4+	Complete transmural necrosis (infarct)

1+ if cells were frequently sloughed from the mucosa with mild disruption of mucosal architecture, 2+ for moderate disruption, 3+ for severe disruption, and 4+ for complete disruption of the full thickness of the mucosa. A grade of 0 was used if no histopathologic changes are identified.

After the code was broken, the incidence of ischemic changes in the experimental and control groups was compared with a χ^2 analysis using a 2 × 2 contingency table. The paired Student's t test was used for evaluation of mucosal injury within each bowel wall area. Differences in severity of mucosal injury in the various gastrointestinal tract segments of the experimental animals were compared by the two-tailed Kruskal-Wallis test for one-way analysis of variance (10). This test was also used to determine differences in severity of injury to the deeper layers of each region of the small intestine.

RESULTS

A core body temperature reduction of 4 ± 1° C for 4.5 h was produced in piglets and resulted in no significant differences of the arterial blood pressure, blood gases, or pH between the experimental and control groups.

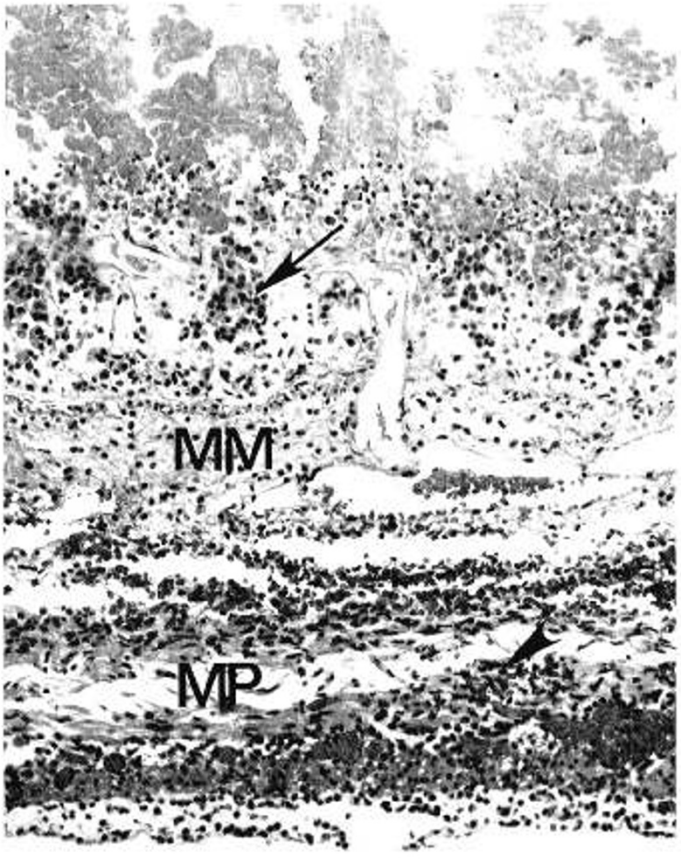

Fig. 1. Histopathologic section of distal small bowel in experimental piglet killed 48 h after cessation of hypothermic stress. Transmural ischemic injury is present with coagulative necrosis of the mucosa including muscularis mucosae (*MM*), desquamation of necrotic crypt epithelium (*arrow*), fresh hemorrhage, edema, and infiltration of the muscularis propria (*MP*) by neutrophils (*arrowhead*). The grading was 4+ villous injury, 4+ crypt injury and 4+ muscularis propria injury. This ischemic specimen is from the same experimental piglet as Figures 2 and 3 (hematoxylin and eosin, ×210).

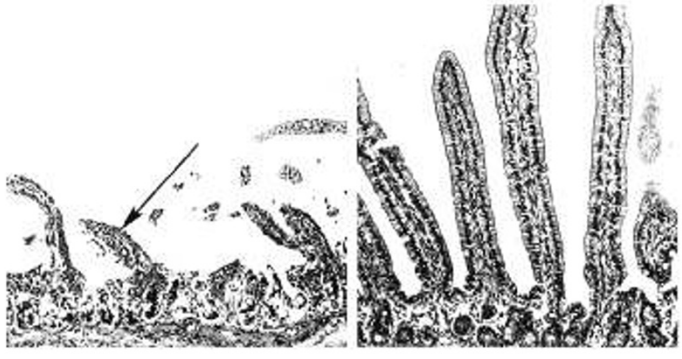

Fig. 2. Histopathologic section of mid-small bowel from experimental piglet (*left panel*) and control piglet (*right panel*). Ischemic injury in the experimental piglet is manifested by loss of villous and superficial crypt epithelium with collapse of the lamina propria, resulting in short denuded villi (*arrow*). The lamina propria shows congestion and edema. The grading was 4+ villous injury and 2+ crypt injury. The experimental piglet is the same animal as shown in Figures 1 and 3 and illustrates the greater severity of the lesions in the distal small bowel (hematoxylin and eosin, both ×185).

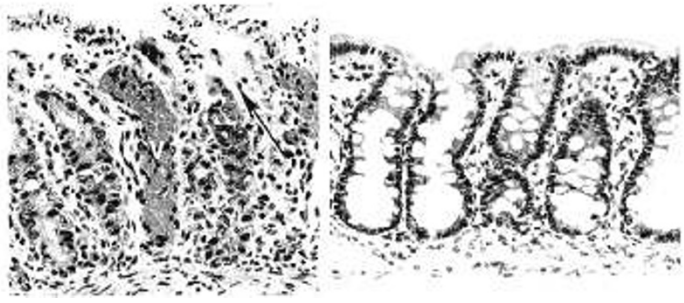

Fig. 3. Histopathologic section of mucosa of proximal colon from experimental piglet (*left panel*) and control piglet (*right panel*). Ischemic injury in the experimental piglet is manifested by necrosis of surface epithelium and superficial lamina propria (*arrow*), marked congestion of a dilated venule (*V*) with edema and focal fresh hemorrhage, and crypt epithelial injury and regeneration with decreased numbers of goblet cells and nuclear atypia. The grading was 1+ mucosal injury. The experimental piglet is the same animal as shown in Figures 1 and 2 (hematoxylin and eosin, ×275).

Table 2. *Incidence and mean histopathologic grade of small intestinal injury in piglets after mild hypothermic stress*

	Control ($n = 14$)		Experimental ($n = 16$)	
	Incidence %	Mean grade (SD)	Incidence %	Mean grade (SD)
Villi				
Proximal small bowel (PSB)	14	0.2 ± 0.4	44*,[a]	1.0 ± 0.3†
Mid-small bowel (MSB)	29	0.3 ± 0.5	94[c]	2.1 ± 0.3†‡,[d]
Distal small bowel (DSB)	29	0.5 ± 0.8	94[c]	2.4 ± 0.3†,[d]
Crypts				
PSB	0	0	19[a]	0.4 ± 0.2†
MSB	0	0	25[b]	0.7 ± 0.4†,[e]
DSB	0	0	38[c]	0.9 ± 0.4†,[e]
Muscularis propria				
PSB	0	0	0	0
MSB	0	0	13	0.3 ± 0.2†
DSB	0	0	25[b]	0.4 ± 0.3†

* Differences in incidence between experimental and control groups: [a] $p < 0.05$, [b] $p < 0.025$, [c] $p < 0.01$.

† Differences in mean grade between experimental and control groups (individual bowel wall layers): $p < 0.01$.

‡ Differences in mean grade of mid or distal small bowel compared with proximal small bowel: [d] $p < 0.01$, [e] $p < 0.05$.

Abnormal clinical findings were limited to the experimental group in which 50% of the piglets developed bloody diarrhea and lethargy; however, no animals died of intestinal ischemic injury and/or sepsis during the 48 h following their hypothermic stress.

Gross bowel lesions of ischemic bowel disease, *i.e.* mucosal and mural hemorrhage and/or ulceration, occurred in 94% of the experimental group and in none of the control animals. The histopathology of these lesions ranged from transmural infarct with hemorrhagic necrosis (Fig. 1) to superficial mucosal necrosis (Fig. 2). The most frequent and prominent microscopic lesions were located in the mid- and distal small intestine.

The results of the small intestinal histopathologic grading are summarized in Table 2. The incidence of mid- and distal small bowel villous injury in stressed piglets was significantly greater than in the control animals ($p < 0.01$). Within the experimental group, the incidence and severity of mid- and distal small bowel mucosal injury was significantly greater than in the proximal small bowel and all other segments of the gastrointestinal tract ($p < 0.01$ for villous injury and $p < 0.05$ for crypt injury). A significant degree of hypothermic tissue injury, as compared with controls, was noted in all layers of the various bowel segments except the muscularis propria of the proximal small bowel (Fig. 3).

In contrast to the small bowel, ischemic injury in the colon of the experimental group was sporadic and confined to the mucosa. When present, lesions were most frequent in the proximal colon (50%) and cecum (44%); however, the incidence and severity of these lesions were not statistically significant when compared to control animals.

DISCUSSION

NNEC is a form of ischemic bowel disease with varying degrees of severity that include transmural necrosis and/or perforation accompanied by peritonitis and sepsis (1–4). Selective intestinal ischemia attributed to neonatal stress such as hypoxia or shock (7, 12) has been suggested as a pathogenetic mechanism. In an experimental model using rats, combined acute cold stress and hypoxia were shown to produce intestinal ischemia (6) although the amount of the induced stress was not controlled. Furthermore, deep hypothermia in young pigs has been found to produce a decrease in cardiac output with an even larger decrease in visceral perfusion attributed to a left-to-right shunt. Piglet age may be an important factor in the vascular response to hypothermia since older animals do not show this selective ischemia (13). We have previously reported that a controlled 4° C decrease in core body temperature for 30 min produced a significant decrease in distal small intestinal blood flow in anesthetized neonatal piglets (8). The present study utilized the same level of hypothermia for a 4-h period in unanesthetized neonatal piglets. Intestinal lesions with the histopathologic characteristics of ischemic injury have resulted from this prolonged hypothermic stress. These grossly and histopathologically evident lesions occurred predominantly in the distal small intestine, the same site as the hemodynamic alterations attributed to hypothermia in our previous study (8). Furthermore, these areas of ischemic intestinal damage are also in a comparable location to the intestinal ischemic injury identified in infants with NNEC.

This model was not intended as a model of NNEC in humans and did not duplicate all of clinical aspects of NNEC. In particular, we did not produce a lethal injury due to bowel perforation and sepsis such as that noted in clinical NNEC. This may be related to findings in previous studies of intestinal ischemic injury in animals which suggested an important protective role for mother's breast milk (14). Our piglets received breast milk both before and after hypothermic stress. These maternal breast milk feedings did not prevent intestinal ischemic injury but may have altered progression of the injury.

This study demonstrates intestinal ischemic damage in the unanesthetized neonatal piglet as a result of clinically mild hypothermic stress. These findings lend support to the concept that mild to moderate hypothermia could be a pathogenetic factor leading to the ischemic intestinal lesions of NNEC in infants.

Acknowledgments. The authors thank Mr. Jerry Harris and Ms. Ann Sonaga for their assistance in this project.

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