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Intraluminal intestinal microdialysis detects markers of hypoxia and cell damage in experimental necrotizing enterocolitis

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Abstract

Background/purpose: Necrotizing enterocolitis (NEC) represents one of the gravest complications in premature infants and carries significant morbidity and mortality. There is a great need for improved diagnostic methods to reduce the severity and incidence of NEC. The aim of the study was to investigate if intraluminal microdialysis can detect intestinal ischemia in newborn rats with induced experimental NEC.

Methods: The studies were performed on 1-day-old Sprague-Dawley rat pups. Experimental NEC was induced using hypoxia/reoxygenation treatment. Microdialysis catheters were rectally inserted and placed in the rectosigmoid part of the colon. Microdialysate levels of glucose, lactate, pyruvate, and glycerol were measured. Intestinal specimens were collected at the end of the experiments for microscopic evaluation.

Results: Intraluminal microdialysis revealed signs of intestinal hypoxia and cellular damage, with a marked increase of lactate and glycerol. Microscopic evaluation confirmed intestinal damage in the NEC group.

Conclusion: Intraluminal microdialysis can detect intestinal hypoxic stress and mucosal cell membrane decay in a rat model of NEC. Intestinal intraluminal microdialysis is easily accessible through the rectum and may be a useful noninvasive complement to other methods in the assessment of NEC.

Necrotizing enterocolitis (NEC) is the most serious gastrointestinal disorder in newborn infants with an incidence of 10% to 15% in those with very low birth weight (<1500 g) [1-4]. More than 90% of the infants who develop NEC are born premature, and the risk is inversely related to gestational age and birth weight [2]. The disease is characterized by inflammation of the bowel that can progress to bowel necrosis and multiple organ failure. Despite decades of research, NEC remains a major cause of death in neonates with a mortality rate approaching 40% in very low birth weight infants [2,4], and the pathogenesis of NEC still remains elusive. Intestinal

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ischemia is thought to be an important component of the pathogenesis of NEC and results in variable degrees of ischemic necrosis of the small and large intestines, ranging from mild ischemia of the intestinal mucosa to transmural necrosis of the bowel wall. Findings from intestinal samples support an ischemic basis for the disease, with histopathologic findings such as mucosal disintegration, villus degeneration, and intestinal wall necrosis.

Experimental NEC has previously been induced with hypoxia/reoxygenation treatment in newborn rats [5-7] or a combination of hypoxia/reoxygenation treatment and cold stress [5,6,8-13]. The induction of intestinal necrosis was verified by histopathologic findings similar to what can be seen in patients with NEC.

Recent studies on intestinal ischemia with the microdialysis technique in adult animals [14-18] showed a typical metabolic response to anaerobic metabolism, reduced glucose levels, and production of lactate leading to an elevated lactate/pyruvate ratio, accompanied by increased glycerol levels because of cell membrane phospholipid degradation caused by ischemia-induced phospholipase activation. Markers of metabolism such as glucose, lactate, pyruvate, and markers of cell deterioration such as glycerol and glutamate can be measured through microdialysis and provide a direct indicator of anaerobic metabolism and cell injury.

Using microdialysis, different locations have been used to measure these metabolic events. In studies of intestinal ischemia, intraperitoneal or serosal measurement close to the affected site has been used extensively [19-22]. An intramural approach has also been used, but the inflammatory reactions around the probe were severe, affecting metabolism and subsequent measurements [14].

The intestinal mucosa is most vulnerable to ischemic events, leading to intraluminal release of the mentioned metabolites [16]. The intraperitoneal (serosal) compartment reflects the metabolism of the outer layers of the gut wall [20]. Because the muscularis layer is less vulnerable to ischemic stress than the mucosa, detection of anaerobic metabolites on the serosal side reflects a later stage of the ischemic insult [16]. This suggests that the intraluminal compartment is the best location for detecting biomarkers of anaerobic metabolism and cell decay at an early stage.

The aim of this study was to evaluate the possibility of detecting signs of intestinal ischemia and mucosal cell damage by using intraluminal microdialysis in newborn rat pups with an experimental model of NEC induced by hypoxia/reoxygenation.

1. Materials and Methods

1.1. Animals

Time-pregnant Sprague-Dawley rats were obtained on day 15 of gestation. The rats were purchased from Charles River (Charles River GmbH, Sulzfeld, Germany). They were housed in the animal department at the Uppsala Biomedical Center and kept at +22°C, with a 12-hour light-dark cycle, and fed standard pellet food and water ad libitum.

On day 21, the rat pups were delivered vaginally. The rat pups were randomized to either induced experimental NEC (n = 11) or to serve as controls (n = 8).

The study was approved by the regional animal ethics committee and performed in accordance with the Guide for the Care and use of Laboratory Animals, published by the National Research Council in 1996.

1.2. Induction of NEC

The rat pups were treated on the first day after delivery. They were stressed with asphyxia, breathing 100% carbon dioxide for 10 minutes, followed by breathing 100% oxygen for another 10 minutes. The rats were then returned to their mother's cages and allowed ad libitum nursing of maternal milk.

1.3. Surgical procedure

On the second day, all animals were anesthetized with thiobutabarbital sodium (Inactin®; Sigma-Aldrich, Sweden AB, Stockholm, Sweden), 40 mg/kg body weight intraperitoneally. They were placed on a heated operating table.

1.4. Microdialysis

All measurements were performed using a CMA 20 Elite microdialysis catheter (cutoff, 20 kDa; 10-mm membrane length; CMA Microdialysis AB, Stockholm, Sweden). The microdialysis catheters were connected to microinjection pumps (CMA 102 and CMA 402; CMA Microdialysis AB) and perfused with an isotonic Ringer solution with a flow rate of 0.7 μL/min.

The microdialysis catheter was rectally inserted 10 mm, reaching the rectosigmoid part of the colon. Initially, in situ stabilization was allowed for 30 minutes. Microdialysate samples were then collected every 30 minutes for a total of 90 minutes.

Samples were immediately placed on ice. Analyses of glucose, L-lactate, pyruvate, and glycerol were done using an enzymatic colorimetric technique on a CMA 600 Microdialysis Analyser (CMA Microdialysis AB). The CMA 600 Analyser was automatically calibrated at start-up and recalibrated every sixth hour using standard calibration solutions from the manufacturer (CMA Microdialysis AB). Quality controls at 2 different concentrations for each analyte were performed every weekday. Total imprecision coefficient of variation was less than 10% for all analytes.

1.5. Morphology

Intestinal specimens were taken from the ileum at the end of the experiments. The specimens were fixed in 4%