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Oxidant-Induced Increases in Mucosal Permeability in Developing Piglets

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ABSTRACT. Reactive oxygen metabolites have been implicated in the pathogenesis of mucosal injury induced by ischemia-reperfusion in adult animals, with recent interest centering on the capacity of polymorphonuclear neutrophil-derived oxidants to mediate this injury. A role for oxidants has also been postulated in the etiology of neonatal necrotizing enterocolitis. Based on evidence that the intrinsic capacity of the neonatal piglet intestine to detoxify hydrogen peroxide (H_2O_2) is minimal relative to that of older piglets, we characterized the changes in mucosal permeability induced by luminal perfusion with H_2O_2 and hypochlorous acid at concentrations that can be produced physiologically by activated neutrophils (0.05 mmol/L, 0.1 mmol/L, and 0.5 mmol/L), in the distal ileum of 1-d- and 1-mo-old piglets. Mucosal permeability was quantitated by measurement of blood-to-lumen clearance of 51-labeled chromium EDTA. Luminal perfusion with either H_2O_2 (0.05 mmol/L and 0.1 mmol/L) or hypochlorous acid (0.1 mmol/L and 0.5 mmol/L) significantly increased mucosal permeability in newborn piglets but did not affect mucosal permeability in 1-mo-old animals. Perfusion with 0.5 mmol/L H_2O_2 significantly increased mucosal permeability over control values in both age groups, but injury in the newborn intestine was significantly greater than that observed in 1-mo-old animals. Thus, as predicted by the reduced intrinsic capacity of the mucosa of neonatal piglets to detoxify H_2O_2, the ileum of newborn piglets is more vulnerable to oxidant-induced mucosal injury than is the ileum of older animals. (*Pediatr Res* 28: 28–30, 1990)

Abbreviations

H_2O_2, hydrogen peroxide
HOCl, hypochlorous acid
^{51}Cr-EDTA, 51-labeled chromium EDTA
SOD, superoxide dismutase

A considerable quantity of experimental data supports the hypothesis that reactive oxygen metabolites mediate the microvascular and mucosal permeability changes encountered after reperfusion of ischemic intestine in adult animals (1–7). The potential for reactive oxygen metabolites to induce injury is dependent upon several factors. First, a source of these toxic oxygen species must exist. The two major sources of oxygen-derived free radicals in adult intestine are xanthine oxidase (8) and the NADPH-oxidase associated with phagocytic leukocytes (neutrophils, eosinophils, and macrophages) (9). Xanthine oxidase and activated phagocytes can produce large quantities of cytotoxic oxidants including superoxide, H_2O_2, and possibly hydroxyl radicals (10). Another determinant of oxidant-mediated tissue injury is the antioxidant capacity of the tissue. Cellular enzymatic defense mechanisms include SOD, which dismutates the superoxide anion to H_2O_2 and oxygen; and catalase and glutathione peroxidase, which detoxify H_2O_2 (11–13). Finally, the ability of oxidants to induce tissue damage must be considered because free radicals differ in stability, tendency to attack specific substrates (lipids, proteins, DNA, RNA), and ability to diffuse from sites of generation and traverse biologic membranes (14, 15). For example, H_2O_2 can rapidly cross plasma membranes because of its small size and neutral charge (16), but it diffuses relatively large distances because of its slow reaction with organic substrates.

In neonatal intestine, a role for reactive oxygen metabolites has been postulated (17–20) in the pathogenesis of necrotizing enterocolitis. Recently, it has been demonstrated that the intestinal mucosa of neonatal piglets contains no xanthine oxidase, but a significant number of resident phagocytic cells are present that have the capacity, when activated, to produce oxidants such as H_2O_2 and HOCl (21). It also appears that the neonatal piglet intestine is predisposed to H_2O_2-induced cell injury due to the absence of catalase activity, low glutathione peroxidase activity, and high basal SOD activity. Because the product of SOD-catalyzed dismutation of superoxide is H_2O_2 and oxygen, one would expect higher fluxes of H_2O_2 within the neonatal piglet enterocyte than in that of older animals. Thus, the objective of our study was to determine whether the neonatal intestine is more susceptible to mucosal injury induced by physiologic levels of H_2O_2 and HOCl, as predicted by the decreased mucosal antioxidant capacity of newborn ileum (22–24).

MATERIALS AND METHODS

Hampshire/Yorkshire piglets of either sex, ages 1-d-old (n = 17, 1.2 ± 0.2 kg) and 1-mo-old (n = 10, 7.4 ± 2.1 kg) were studied. One-d-old piglets were never nursed (n = 15) or were fasted for 16 h (n = 2) before the experiment. One-mo-old animals were fasted for 24 h.

Surgical procedure. After intramuscular injection with ketamine hydrochloride (20 mg/kg), and xylazine (2 mg/kg), the animals were anesthetized with i.v. pentobarbital sodium (15 mg/kg) and anesthesia was maintained throughout the experiment with additional doses of pentobarbital sodium (5 mg/kg) as needed.

The animals were artificially ventilated (Harvard Apparatus intermediate ventilator, South Natick, MA) via a tracheostomy at a tidal volume and respiratory rate to maintain normal arterial blood gases and pH (Instrumentation Laboratory Model 1304 pH/blood gas analyzer, Lexington, MA). Cannulas were inserted

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into the left carotid artery to monitor systemic blood pressure and into the right jugular vein for administration of pentobarbital and maintenance of hydration. Body temperature was maintained at 38°C.

The abdomen was opened through a midline incision and the renal vessels were ligated to prevent urinary excretion of ^{51}Cr-EDTA (New England Nuclear, Boston, MA). Loops of ileum, approximately 15 cm in length, were isolated, cannulated at both proximal and distal ends, and flushed with warm lactated Ringer solution. The intestine and abdominal contents were subsequently covered with plastic wrap to prevent evaporative water loss.

Experimental protocol. The cannulated ileal segment was perfused with warm lactated Ringer solution at a rate of 1 mL/min. ^{51}Cr-EDTA was injected i.v., such that plasma cpm were at least 25 000/mL (100–150 μCi/kg). Thirty min were allowed for tissue equilibration of the ^{51}Cr-EDTA, after which 0.5 mL of plasma and the luminal perfusate were collected during each 20-min interval. Solutions of H_2O_2 and HOCl (0.05 mmol/L, 0.1 mmol/L, 0.5 mmol/L) were prepared in lactated Ringer solution immediately before each experiment. After 60 min of perfusion with lactated Ringer solution, ileal loops were perfused for an additional 180 min with either lactated Ringer solution (n = 2 loops for 1-d- and n = 3 loops for 1-mo-olds), H_2O_2 (n = 5 loops for each concentration, except n = 2 for 0.05 mmol/L in 1-mo-olds), or HOCl (n = 5 loops for each concentration, except n = 3 for 0.05 mmol/L in 1-mo-olds). ^{51}Cr-EDTA activity in plasma and in luminal perfusate was measured in a LKB CompuGamma spectrometer (model 1282, LKB Instruments, Inc., Gaithersburg, MD). At the completion of the experiment, the ileal loops were removed, rinsed with isotonic saline, patted dry, and weighed. The animals were killed with an i.v. injection of sodium pentobarbital.

Calculation of ^{51}Cr-EDTA clearance. The plasma-to-lumen clearance of EDTA was calculated as follows:

$$\text{Clearance} = \frac{(\text{perfusate cpm/mL})(\text{perfusion rate})(100)}{(\text{plasma cpm/mL})(\text{wt of ileal segment in g})}$$

where clearance is expressed in $\text{mL}\cdot\text{min}^{-1}\cdot 100\ \text{g}^{-1}$.

Data analysis. All values are expressed as mean ± SEM. Clearance values were evaluated with age group and perfusate (for a given concentration) as the main effects by use of a repeated measures (over time) ANOVA. If the ANOVA revealed differences within or between age groups, the significance of individual data points was evaluated by use of Duncan multiple range tests (SAS Institute Inc., Cary, NC). Differences were considered significant at $p < 0.05$.

RESULTS

Within age groups, perfusion of the ileal lumen with lactated Ringer solution for 240 min did not significantly alter ileal mucosal permeability measured using blood-to-lumen clearance of ^{51}Cr-EDTA in either 1-d- or 1-mo-old piglets. Likewise, in ileal loops from 1-mo-old piglets, mucosal permeability was not significantly increased over control with any concentration of HOCl or with H_2O_2 (except for a small, but significant increase with the highest concentration of 0.5 mM H_2O_2). In contrast, perfusion of the ileal lumen of 1-d-old piglets with 0.1 mmol/L and 0.5 mmol/L concentrations of H_2O_2 and HOCl (Fig. 1*A* and *B*) resulted in significant increases in ^{51}Cr-EDTA clearances compared with control values. Although luminal perfusion with 0.05 mmol/L H_2O_2 demonstrated a trend toward increased permeability in 1-d-old intestine (Fig. 1*C*), it did not reach statistical significance.

Evaluation of between-group differences showed that luminal perfusion with 0.5 mmol/L H_2O_2 and HOCl (Fig. 1*A*) and 0.1 mmol/L H_2O_2 (Fig. 1*B*) led to significantly greater EDTA clearance in newborn intestine compared with that in 1-mo-old animals (for an equivalent concentration of perfusate). Perfusion

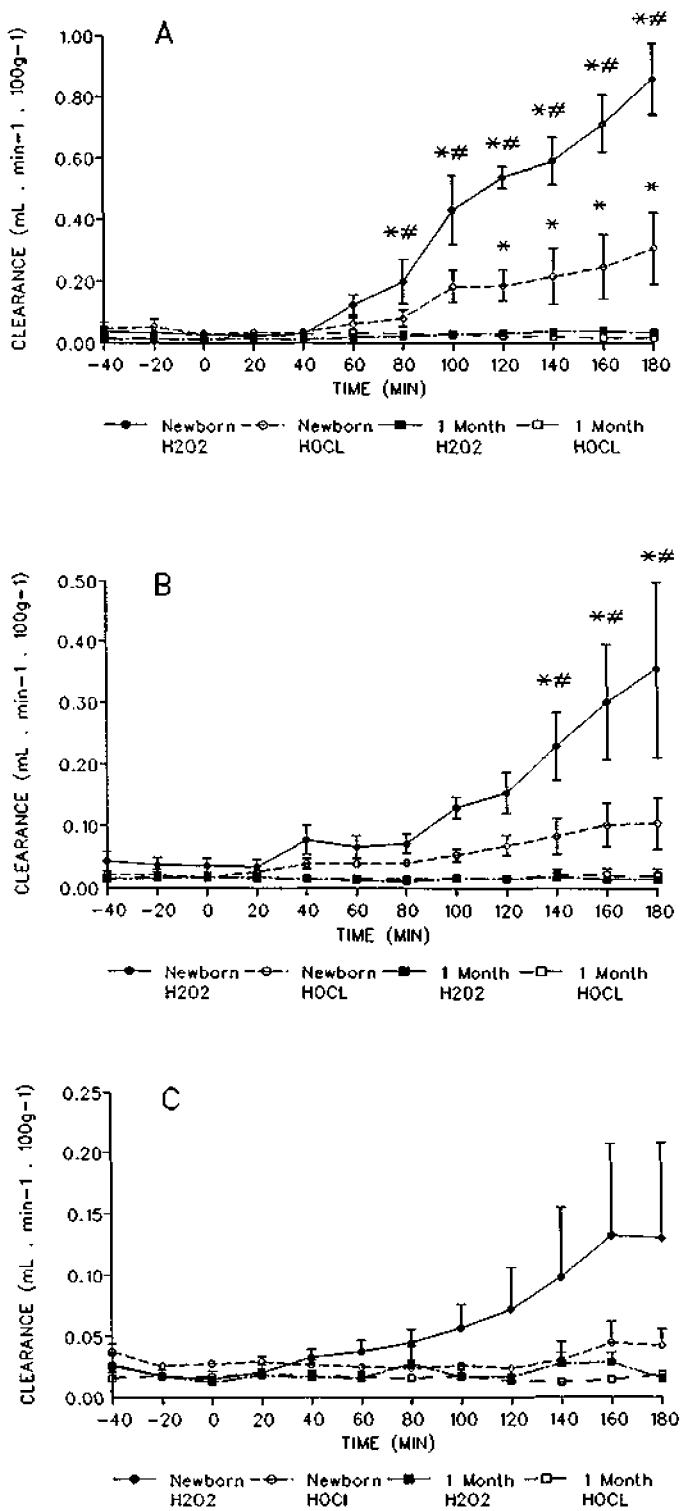

Fig. 1. ^{51}Cr-EDTA clearance during luminal perfusion with 0.5 mmol/L (*A*), 0.1 mmol/L (*B*), and 0.05 mmol/L (*C*) H_2O_2 and HOCl in piglet ileum. Control clearance during perfusion with lactated Ringers solution was measured for the first 60 min, followed by H_2O_2 or HOCl perfusion beginning at time = 0 min. * indicates $p < 0.05$ for 1-d- *vs* 1-mo-old values and # indicates $p < 0.05$ for H_2O_2 *vs* HOCl perfusion.

with 0.1 mM HOCl caused no significant differences in mucosal permeability in 1-d- and 1-mo-old animals.

Comparison of equimolar concentrations of H_2O_2 and HOCl (Fig. 1*A*, 0.5 mmol/L and *B*, 0.1 mmol/L) illustrates that luminal perfusion with H_2O_2 led to significantly greater injury to newborn intestine than did HOCl. There was no significant difference in