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Does maternal nicotine exposure during gestation increase the injury severity of small intestine in the newborn rats subjected to experimental necrotizing enterocolitis

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Abstract

Background/Purpose: The aim of this study was to evaluate the effects of maternal nicotine exposure during gestation on injury severity of small intestine in the newborn rats subjected to hypoxia-reoxygenation and cold stress.

Methods: A total of 21 Sprague-Dawley pregnant rats were divided into 3 equal groups. The groups were labeled as group 1, control group; group 2, hypoxia-reoxygenation group; and group 3, nicotine-hypoxia-reoxygenation group. The rats of group 3 were exposed to nicotine via subcuticular injection for the last week of gestation (2 mg/kg/d). Newborn rats were collected immediately after birth to prevent suckling of maternal milk (40 rat pups in group 1, 43 rat pups in group 2, and 41 rat pups in group 3). Litters in groups 2 and 3 were stressed twice daily with asphyxia followed by cold (4°C for 10 minutes) stress to induce hypoxic intestinal injury which is relevant to human necrotizing enterocolitis. Breathing 100% CO_2 for 10 minutes in a chamber followed by 10-minute 100% O_2 breathing was the asphyxia model repeated twice daily. After hypoxia-reoxygenation and cold stress, newborn rats were returned to their mother's cages. This protocol was repeated for the following 2 days, and the rat pups were decapitated on the third day. Using this protocol of asphyxia and cold stress, all of neonatal rats developed clinical and pathological signs of hypoxia-induced intestinal injury. The entire gastrointestinal tract was removed and examined macroscopically. A 2-cm section of distal ileum from each animal was taken for histopathological and biochemical examinations. Histological changes in ileal architecture were scored and graded from 1 to 5. The remaining intestinal tissues of the animals were used for lipid peroxidation analysis.

Results: Typical signs of hypoxia-induced intestinal injury were observed in the 2 experimental groups (groups 2 and 3) macroscopically. There were more grades 3 and 4 injuries in group 3 ($P < .05$). The malondialdehyde levels were elevated in groups 2 and 3 ($P < .001$). The malondialdehyde levels of the group 3 were also significantly higher than group 2 ($P < .01$).

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Conclusions: Maternal nicotine exposure during gestation results in higher grade histological injury in newborn rats subjected to hypoxia-reoxygenation and cold stress.

Necrotizing enterocolitis (NEC) is the most common gastrointestinal disease of premature infants [1], has a multifactorial etiology and an incompletely defined pathogenesis, and predominantly affects neonates with severe necrotizing injury to the intestine because the underlying clinical circumstances are not uniform. Necrotizing enterocolitis may represent a syndrome, with common findings and a variety of etiologies. Advanced cases of NEC may cause multisystem organ failure [2] with a higher mortality rate. Necrotizing enterocolitis remains a leading cause of morbidity and mortality in neonatal intensive care units.

It has been suggested that the major risk factors for NEC are prematurity, formula feeding, intestinal hypoxia, and bacterial colonization which promote an inflammatory cascade that results in the pathology associated with this disease [3,4]. Oxygen-derived free radicals and inflammatory mediators including tumor necrosis factor, platelet-activating factor, and leukotrienes play an important role in the genesis of NEC [5-8].

Hypoxia, a risk factor for NEC, delays gastric emptying and the intrinsic intestinal rhythm in the experimental studies [9,10]. Hypoxia also decreases spontaneous small intestinal contractions in the adult mouse [11], pig stomach [12], and isolated human intestine [13]. After hypoxia, neonatal feeding intolerance [14,15] and an increased risk of NEC, heralded by intestinal mucosal injury, bacterial translocation, and dysmotility [16], are documented.

It was demonstrated that maternal hypoxia during the last third of gestation significantly decreased fetal rabbit gastrointestinal motility which might contribute to neonatal NEC [17]. As in previous studies in sheep and monkeys, nicotine significantly increased maternal arterial pressure and decreased uterine blood flow [18-20]. Administration of nicotine during pregnancy has been reported to produce fetal hypoxia [18-22] and decreased birth weight [23-26].

With these knowledge, we conducted an experimental study to evaluate the effects of maternal nicotine exposure during gestation on the degree of intestinal injury in the newborn rats subjected to hypoxia-reoxygenation and cold stress.

1. Materials and methods

1.1. Animals

The study was performed on adult pregnant time-dated Sprague-Dawley rats and their newborn pups. The protocol was approved by the Animal care and Use Committee of University of Kahramanmaraş Sütçü İmam. Animals were kept in separate cages and maintained under standard conditions.

1.2. Experimental groups

A total of 21 Sprague-Dawley pregnant rats (weighing 180-200 g) were divided into 3 equal groups. The groups were labeled as group 1, control group; group 2, hypoxia-reoxygenation group; and group 3, nicotine-hypoxia-reoxygenation group. The rats of group 3 were exposed to nicotine via subcuticular injection for the last week of gestation (2 mg/kg/d in 2 equal doses). Newborn rats were collected immediately after birth to prevent suckling of maternal milk which is a protective factor for NEC. Dead-born rats, 5 in group 1, 6 in group 2, and 7 in group 3, were excluded from the study. There were 40 rat pups in group 1 (weighing 4.9-5.3 g), 43 rat pups in group 2 (weighing 5-5.3 g), and 41 rat pups in group 3 (weighing 4.8-5.1 g).

1.3. Animal model of NEC

All rat pups in groups 2 and 3 were stressed twice daily with asphyxia followed by cold (4°C for 10 minutes) stress to induce hypoxic intestinal injury which is relevant to human NEC. Breathing 100% CO_2 for 10 minutes in a chamber followed by 10-minute 100% O_2 breathing was the asphyxia model repeated twice daily. This protocol was a modification of the method of Okur et al [27]. After hypoxia-reoxygenation and cold stress, the rat pups were returned to their mother's cage and kept in a normothermic environment (21°C-23°C). They were allowed to receive breast milk after but not before the above procedure. After repeating the protocol for 3 days, rat pups were decapitated, and the entire gastrointestinal tract was removed and examined macroscopically for typical signs of hypoxic intestinal injury relevant to human NEC (intestinal discoloration, intestinal hemorrhage, ileal distention, and/or ileal stenosis). Using this protocol of asphyxia and cold stress,

Table 1 Histopathological evaluation

Groups	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Group 1 (n = 40)	40	–	–	–	–
Group 2 (n = 43)	–	24	16	3	–
Group 3 (n = 41)	–	12	21	8	–

Group 3 different from group 2, $P < .05$.