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Experimental intestinal injury induced by intrauterine ischemia in rats

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Abstract: Objective The intestine is one of the most seriously injured organs during neonatal asphyxia. But a model for studying the perinatal intestinal injury caused by asphyxia is absent. This paper described a model of intestinal damage produced by reversible intrauterine ischemia in rats. **Methods** The model of acute reversible intrauterine ischemia was established by clamping the arteries and veins on one side of the uterus and ovaries in pregnant Wistar rats (E21) for 20 minutes. The fetal rats on the occluded side of the uterus were used as the Ischemia group and the rats on the other side were used as the Control group. After 20 minutes of vascular occlusion, the uteri were opened and the pups were removed. In each group, 18 pups were sacrificed at 0, 24, 48 and 72 hrs after ischemia, respectively. The intestinal mucosal damage index (IMDI) was evaluated. **Results** After ishemia, the pups in the Ischemia group were cyanotic or pale, listless, and hypopneic, while the control pups manifested normal. The intestinal mucosa of controls were not damaged. In the Ischemia group, intestinal damage reached a peak at 48 hrs post-ischemia, with an IMDI that was significantly increased above controls (3.40 ± 0.16 vs 0.00 ± 0.00, $P < 0.01$). At 72 hrs post-ischemia, the changes of intestinal tissues had largely recovered and the IMDI decreased to 0.60 ± 0.21. **Conclusions** The animal model of asphyxia induced perinatal intestinal injury can be established by clamping the arteries and veins on one side of the uterus and ovaries in pregnant rats. [**Chin J Contemp Pediatr, 2005, 7(1):12-14**]

Key words: Asphyxia; Intestines; Ischemia; Models, animal; Rat

大鼠宫内缺血后肠道损伤模型的建立

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[**摘 要**] **目的** 肠道是新生儿窒息后受损最严重的器官之一。但目前国内外尚无一经典模型用于窒息后肠道损伤的研究。本研究采用可逆性宫内缺血制作了大鼠窒息后肠道损伤模型。**方法** 钳夹足月孕鼠(E21)一侧子宫和卵巢动静脉 20 min,以结扎侧的胎鼠为窒息组,对侧胎鼠为对照组。剖宫产取出胎鼠,复苏,代乳鼠代乳。分别饲养 0,24,48,72 h 后处死(每组每时点 18 只),取出肠组织行病理观察,评估肠黏膜损害指数(IMDI)。**结果** 对照鼠娩出后表现正常;缺血鼠娩出后出现皮肤青紫、呼吸减弱、四肢活动减少等窒息改变。缺血鼠病理学改变在缺血后 48 h 最重,IMDI 明显上升(3.40 ± 0.16),对照组肠粘膜几乎没有损害(0.00 ± 0.00)。缺血后 72 h 肠道损伤明显恢复,IMDI 降至 0.60 ± 0.21。**结论** 钳夹足月孕鼠一侧子宫、卵巢动静脉可以制成窒息后肠道损伤的动物模型。 [**中国当代儿科杂志,2005, 7(1):12-14**]

[**关 键 词**] 窒息;肠道;缺血;动物模型;大鼠
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Neonatal asphyxia occurs in the uterus and also during delivery with a high morbility and mortality, may lead to damage of the heart, brain, kidney and gastrointestinal tract [1]. When systemic perfusion decreases by 10%, the intestinal blood flow will decrease by 40%, so the gastrointestinal tract is one of the most frequently injured organs[2]. But a model for studying the perinatal intestinal injury is absent, which results

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in a limitation on its research. Based on the model of experimental intrauterine growth retardation produced by transient period of uteroplacental ischemia in pregnant rats[3], the rats model of perinatal intestinal injury induced by intrauterine ischemia was established.

Materials and methods

Animal model

Sixty-three Wistar rats, 53 females and 10 males, were provided by the Animal Center of the Second Affiliated Hospital of China Medical University. The average weights of female and male rats were 270 ± 30 g and 300 ± 20 g, respectively. The blood flow to the two sides of the uterus is symmetric. If the vascular supply of the uterus is occluded on one side, then the pups in the occluded side can be used as an Ischemia group, while the pups in the unoccluded side can be used as a Control group. Pregnant Wistar dams (E21) were anesthetized with ether, and the arteries and veins of the uterus and ovary were exposed. The vessels supplying blood to one side of the uterus and ovary were occluded with arterial clamps for 20 minutes. After 20 minutes of occlusion, the uteri were opened and the pups were removed. Immediate resuscitation was performed by clearing the respiratory tract, stimulating respirations, oxygen inhalation, and placing the pups in a controlled environment with temperature of 36-37℃ and humidity of 40%-60%. A total of 144 pups were enrolled in this study, 72 in the Ischemia group and 72 in the Control group. The pups were nursed by foster dams, and were sacrificed at 0, 24, 48 and 72 hours after delivery ($n = 18$ at each time point). Fifty mm of intestinal tissue from each subject was removed and fixed in 10% formalin for hematoxylin and eosin staining.

Evaluation of intestinal mucosal damage index (IMDI)

Formalin fixed intestinal tissue was embedded in paraffin; sliced into 5 μm sections and stained with hematoxylin and eosin. The IMDI was employed to evaluate the extent of ischemic injury. Five high power fields randomly selected from each sample were evaluated in a blind by a pathologist. The extent of injury was scored as follows: 0 score, normal intestinal mucosal villi. 1 score, development of subepithelial Gruenhagen's space, usually at the apex of the villus, often with capillary congestion. 2 scores, extension of the subepithelial space with moderate lifting of epithelial layer from the lamina propria. 3 scores, massive epithelial lifting from the sides of villi. A few tips may be denuded. 4 scores, denuded villi with lamina propria and dilated capillaries exposed. Increased cellularity of lamina propria may be noted. 5 scores, digestion and disintegration of lamina propria; hemorrhage and ulceration[4].

Statistical analysis

All data were expressed as $\bar{x} \pm s$. Group differences were analyzed with the t test and analysis of variance (SPSS 10.0).

Results

Manifestations after asphyxia

After clamping the vessels supplying blood to one side of the uterus for 20 minutes, the pups in the Ischemia group were cyanotic or pale, listless, and hypopneic. The mortality of asphyxiated rats was about 30%. Nearly all the control pups survived except 5 (6.5%) that died from blood loss.

Histological changes and IMDI

The intestinal tissues of control pups well developed. At 24 hours post-ischemia, the lamina propria of asphyxiated pups were hyperemic, and the sub-epithelial space was expanded with moderate lifting of the epithelial layer from the lamina propria. Intestinal mucosal injury was the most severe at 48 hours post-ischemia. The numbers of villi were obviously reduced, villi tips were partially denuded, villous capillaries were dilated, and the cellularity of the lamina propria was increased. Compared with controls, the IMDI of the Ischemia group at 48 hours post-ischemia was significantly increased (3.40 ± 0.16 vs 0.00 ± 0.00). At 72 hours post-ischemia, the changes of intestinal tissues

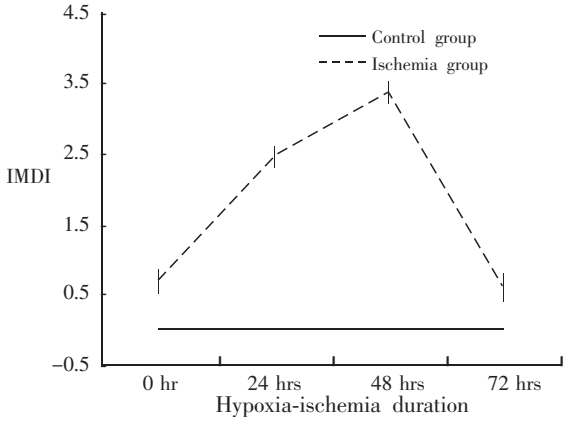

Figure 1 **Changes of IMDI after intrauterine hypoxia-ischemia** ($n = 18$, $\bar{x} \pm s$)

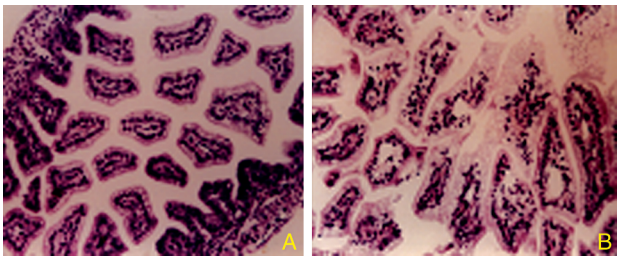

Figure 2 Histological changes of intestinal tissues (hematoxylin and eosin staining, 400 ×). **A** Intestinal tissues well developed in the normal newborn rat. **B** At 48 hrs post-ischemia, the structure changes were obvious, denuded villi with lamina propria and dilated capillaries exposed, with increased cellularity of lamina propria.

had largely recovered, with an IMDI decreased to 0.60 ±0.21, although the number of villi was less than in the control pups. The intestinal mucosae of controls were not damaged (Figures 1 and 2).

Discussion

Neonatal asphyxia can lead to damage of multiple organs. The intestine is one of the first organs involved. About 80% - 90% of neonatal asphyxia happens in uterus or during laboring[5]. This study established a model of transient intrauterine ischemia to investigate intestinal injury in perinatal asphyxia.

The model was used to study intrauterine growth retardation (IUGR)[3]. It was modified and used in the study of intestinal injury after asphyxia, because perinatal hypoxia-ischemia is thought the main cause of neonatal asphyxia, which is consistent with the clinical etiology. In this model , marked intestinal histological changes were observed. The changes of gastric mucosa were observed in a similar model[6].

There were some difficulties during the establishment of the animal model: Firstly, the maturity degree of fetus must be assessed accurately so as to determine a suitable operation time. Secondly, the newborns are tiny with body weights of only 4-5 g, which makes it hard to care for and resuscitate babies, resulting in a difficulty in obtaining samples. Thirdly, the mother rats could not feed their babies after surgery, so the problem of raising the babies had to be solved.

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