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新生小鼠缺氧缺血性脑损伤模型的制作研究 *

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摘要 目的:通过对动物模型的制作模拟新生儿围产期缺氧缺血性脑损伤,研究其脑组织病理变化,为新生儿缺氧缺血性脑损伤的病理生理的研究以及进一步有效的治疗提供实验基础。**方法:**将 40 只 7 d 新生昆明小鼠分四组,分别为正常组(A 组)、单侧颈总动脉结扎组(B 组)、单侧颈总动脉结扎+ 缺氧组(C 组)和双侧颈总动脉结扎组(D 组)。单侧颈总动脉结扎组(B 组)行右侧颈总动脉结扎;单侧颈总动脉结扎+ 缺氧组(C 组)行右侧颈总动脉结扎后将其置于 20 ℃ 的恒温 50 mL 密闭容器中,分不同的时间将其取出;双侧颈总动脉结扎组(D 组)行双侧颈总动脉结扎,各组术后均送回母鼠身边继续母乳喂养,三天后再作病理检测。**结果:**行单侧颈总动脉结扎加缺氧 60 min 时,小鼠结扎侧皮质及海马区出现病理改变,随着缺氧时间延长(90 min、100 min、120 min)病变范围逐渐扩大,病理改变越明显。**结论:**本实验显示单侧颈总动脉结扎同时缺氧一定时间可以导致小鼠脑组织损伤,脑细胞发生病理改变,且皮层及海马区域的神经细胞对缺氧缺血最为敏感,从而为进一步研究新生儿缺氧缺血性脑损伤提供了较为可靠的模型。

关键词:缺氧缺血性脑损伤;动物模型;小鼠;病理

中图分类号:Q95-3,R741.02 **文献标识码:**A **文章编号:**1673-6273(2014)01-57-05

The Production Research of Neonatal Mice Model of Hypoxic-Ischemic Brain Damage*

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ABSTRACT Objective: To investigate the pathological changes of brain tissue by building he animal model simulated neonatal perinatal hypoxic ischemic brain damage, and study neonatal hypoxic ischemic brain injury in the pathophysiology to provide an experimental basis for further effective treatment. **Methods:** 40 postnatal 7 d neonatal Kunming mice were divided into four groups: normal group(A group), unilateral common carotid artery ligation group (group B), unilateral common carotid artery ligation plus hypoxia group (group C) and bilateral common carotid artery ligation group (D group). Unilateral carotid artery ligation group (group B) underwent right carotid artery ligation; Unilateral common carotid artery ligation plus hypoxia group(group C) underwent right carotid artery ligation and put them into the 50 mL container in the constant temperature of 20℃ and the mice taken out at different time. Bilateral common carotid artery ligation group (D group) underwent bilateral carotid artery ligation, and each group after operation were returned to the maternal side to continue breastfeeding, three days later for histologic detection. **Results:** Unilateral common carotid artery ligation plus hypoxia 60min, cortex and hippocampus ligation side in mice brain appeared pathological changes, with prolonged hypoxia (90 min, 100 min, 120 min) lesions scope gradually expanded, more obvious pathological changes. **Conclusion:** Unilateral carotid artery ligation and hypoxia for a certain period of time can cause mice brain injury, brain cells occur pathological changes, and cortical and hippocampal region of the neural cells to hypoxia and ischemia are the most sensitive, so as to further study of neonatal hypoxic-ischemic brain injury provides a more reliable model.

Key words: Hypoxic ischemic brain damage; Animal model; Mice; Pathology

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前言

新生儿缺氧缺血性脑病(hypoxic-ischemic encephalopathy, HIE)是新生儿期常见危急重症之一,其引起的脑损伤是导致脑瘫、癫痫、智力低下等中枢神经系统疾病乃至死亡的主要原因[1,2],其发病机制还不清楚,对缺氧缺血性脑损伤尚无根本性有效的防治措施。因此,深入探究其发病机制是有效治疗该病的前提条件。本文对制作新生小鼠缺氧缺血性脑损伤模型的过程及脑组织的病理观察作详细研究和阐述,从而为新生儿缺氧缺血性脑损伤的病理生理的研究以及进一步有效的治疗提供实验基础。

1 材料与方法

1.1 实验动物

新生 7 d 昆明小鼠 40 只,雌雄不限,体重平均 5± 0.45 g,新生小鼠由母鼠母乳喂养。由辽宁中医药大学实验动物中心提供。

1.2 材料

解剖显微镜、电子秤、50 mL 密闭容器、动物手术器材(止血钳、手术刀、手术剪、镊子、玻璃分针、酒精灯、手术缝合针线等)。

1.3 方法

1.3.1 分组 正常组(A 组)、单侧颈总动脉结扎组(B 组)、单侧颈总动脉结扎+缺氧组(C 组)和双侧颈总动脉结扎组(D 组)。各组在体重、数量上均无统计学差异。

1.3.2 制备方法 B 组:将小鼠用医用胶布仰卧固定于手术台上,颈部常规消毒,2%利多卡因局麻后,在解剖显微镜下,颈部正中切口,长约 0.5 cm,切开皮肤后用眼科镊分离颈前肌群及气管,再用玻璃分针向右侧轻拉暴露颈动脉三角,靠近右侧气管旁,可见颈总动脉搏动,旁有迷走神经伴行,轻轻分离颈总动脉后,用电凝器将颈总动脉凝断,从而阻断血流。最后用 4 号带针缝合线缝合切口[3-5]。整个手术过程中,保持周围环境温度在 30℃ 左右。术后将小鼠放回母鼠身边继续母乳喂养。

C 组:将术后小鼠放置 1 h,待完全清醒后放入 20℃ 的恒温 50 mL 密闭容器中[6],分别缺氧 30 min(C1 组)、60 min(C2 组)、90 min(C3 组)、100 min(C4 组)、120 min(C5 组),缺氧后放回母鼠身边继续母乳喂养。

D 组:双侧颈总动脉结扎组采用同 B 组相同的手术方式对另一侧颈总动脉进行结扎。术后亦将小鼠放回母鼠身边继续母乳喂养。

1.4 HE 染色

在术后第三天,将各组存活的小鼠断头处死,快速完整分离取出鼠脑,再置于 4%多聚甲醛溶液中固定 24 h,根据小鼠脑立体定位图谱,将固定的脑组织从前囟后以视交叉为中心前后 3 mm 进行取材,将取好的组织块置于 0.01 mol·L^{-1} PBS 缓冲液中 2 h,置换标本中的固定剂。梯度乙醇脱水、浸蜡、包埋、切片(用 LEICA SM2400 型石蜡切片机做连续冠状切片), 片厚 4 μm,56℃ 烤片 1 h,HE 染色,中性树胶封片,光镜下观察病理改变[7,8]。

2 结果

A 组小鼠的脑组织结构层次明显, 皮质及髓质界限清楚。神经细胞核清晰可见,核仁清楚,胞浆丰富。B 组和 C1 组小鼠脑组织镜下未见明显异常。单侧颈总动脉结扎联合缺氧(60 min、90 min、100 min、120 min) 后的小鼠脑组织切片可见右侧大脑皮层、海马等区域神经细胞明显减少,脑组织层次结构欠清晰,皮质及髓质界限不清,皮质及海马区域神经细胞核固缩明显,着色加深,核结构呈现不规则状,胞体缩小呈三角形,且结扎侧较为未结扎侧明显。

表 1 各组死亡率比较

Table 1 Comparison of mortality

	Group A	Group B	Group C					Group D
			Group C1	Group C2	Group C3	Group C4	Group C5	
Number	5	5	5	5	5	5	5	5
Death number	0	1	0	0	0	0	0	5
Mortality(%)	0	20	0	0	0	0	0	100

缺氧时间与病理改变:将小鼠单侧颈总动脉结扎后,随着缺氧时间的延长,其结扎侧脑组织的病理改变随之逐渐加重。

3 讨论

缺氧缺血性脑病是严重威胁人类健康疾病,因为神经细胞较其他类型细胞对缺血缺氧更为敏感。新生儿缺氧缺血性脑病引起脑损伤,细胞死亡形式以凋亡为主,而凋亡是一种持续时间长进展较慢的可逆过程,及时阻断细胞凋亡,减少和避免产生神经系统后遗症积极地干预治疗。针对这一特点及早挖掘围产期脑损伤患儿神经发育的潜力促进神经功能的恢复。

随着各个领域对动物脑瘫模型的研究,有关动物脑瘫模型的制作方法不断被创新及完善,国内研究的脑瘫鼠模型大都分三种:第一种方法是脂多糖(LPS)等神经毒素诱导的动物模型[9];第二种方法是缺血缺氧的动物模型:①将临产的孕鼠双侧子宫动脉夹闭,维持一定时间后取出胎鼠,致使新生乳鼠在母体子宫内缺氧缺血制成脑瘫模型;②将幼鼠置于极度缺氧环境中一定时间制成脑瘫模型,或者将幼鼠单侧颈总动脉阻断、结扎或(和)切断,再将动物置于缺氧环境中一定时间制作脑瘫模型[10-14]; 第三种方法是注射神经毒素联合缺血缺氧制成的动物模型[15]。

表 2 脑组织病理形态学改变

Table 2 Brain tissue pathological morphology change

	Hypoxia Time				
	Group C1 (30 min)	Group C2 (60 min)	Group C3 (90 min)	GroupC4 (100 min)	GroupC5 (120 min)
Hypoxia ischemia injury after 72 hours of HE staining	Compared with the normal age mice, on both sides of the brain tissues showed no significant abnormality	On the right side of the brain cortex and hippocampus regions visible nuclear structure is not clear, some nuclear pyknosis, left is not obvious	On the right side of the brain cortex and hippocampal regions of little visible nuclear pyknosis, deeper colored nuclear structure, irregular shape, microscopic nuclear pyknosis cell count more than the left, the group than in group C2 significantly	On the right side of the brain cortex and hippocampal regions showed large amounts of nuclear pyknosis, deeper colored nuclear structure, irregular shape, microscopic nuclear pyknosis cell count more than the left, the group than that in group C2, C3	Bilateral cortical and hippocampal regions of visible large nuclei pyknosis, deeper colored nuclear structure, irregular shape, shrinking cell body is triangular, the most obvious change group

本研究发现单纯结扎小鼠单侧颈总动脉组(B 组)和单侧颈总动脉结扎+ 缺氧组(C1 组)小鼠脑组织的病理形态改变不明显,因小鼠的脑血流与人类相似,颈内动脉和椎动脉在大脑底部形成 Willis 环,考虑与侧支循环建立形成代偿有关。另外,结扎双侧颈总动脉组(D 组)术后的小鼠在 72 h 内的死亡率为 100%,考虑原因可能为小鼠年龄小,体重轻,耐受性较差,双侧结扎颈总动脉后,侧支循环建立的较慢或无法建立。本实验采用的方法在传统的 Rice 缺氧缺血方法的基础上改良, 将小鼠行单侧颈总动脉阻断, 后置于 37℃ 的恒温 50 mL 密闭容器中缺氧。研究结果表明,与正常同龄新生小鼠相比,C2 组、C3 组和 C4 组脑组织病理形态改变明显, 尤其是皮层及海马区域的神经细胞对缺氧缺血最为敏感。随着缺氧时间的延长,小鼠脑组织病变侧神经细胞核固缩的明显且异常细胞数量逐渐增加。对比模型小鼠脑组织切片的左右脑半球病理改变,说明缺氧缺血处理后(缺氧 60 min、90 min、100 min、120 min)主要引起颈总动脉结扎侧脑组织的病理形态改变,且右侧(颈总动脉结扎侧)较左侧改变明显。综上所述,本实验建立了新生小鼠缺氧缺血性脑损伤的动物模型,且可重复性较高,为下一步深入研究缺氧缺血性脑损伤的机理及相应的早期干预治疗奠定基础。本实验方法操作简单、效果肯定、可重复率高,可更广泛地应用于新生儿 HIE 的动物实验研究。

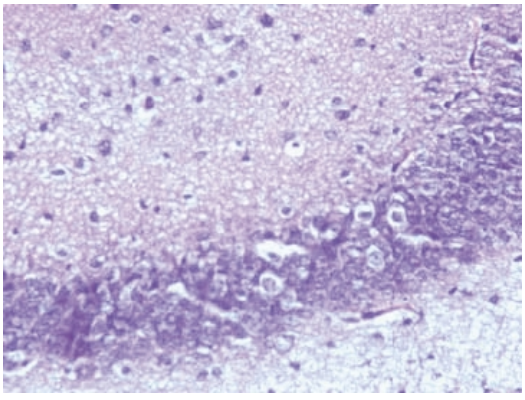

图 1 缺氧 120min 海马× 40(核固缩较重)

Fig.1 Hypoxia 120min hippocampus × 40 (nuclear pyknosis heavy)

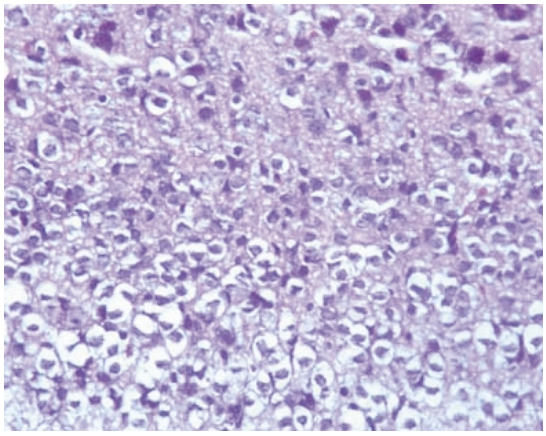

图 2 缺氧 120 min 皮层× 40(核固缩较重)

Fig.2 Hypoxia 120 min cortex × 40 (nuclear pyknosis heavy)

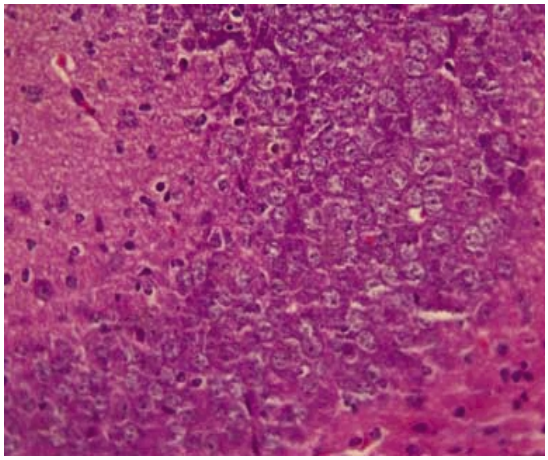

图 3 缺氧 100min 海马× 40

Fig.3 Hypoxia 100min hippocampus× 40

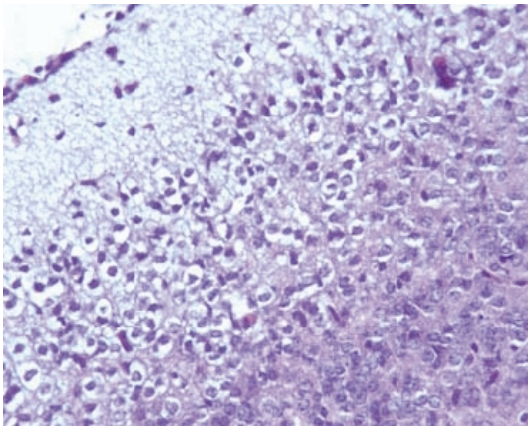

图 4 缺氧 100min 皮层× 40

Fig.4 Hypoxia 100min cortical × 40

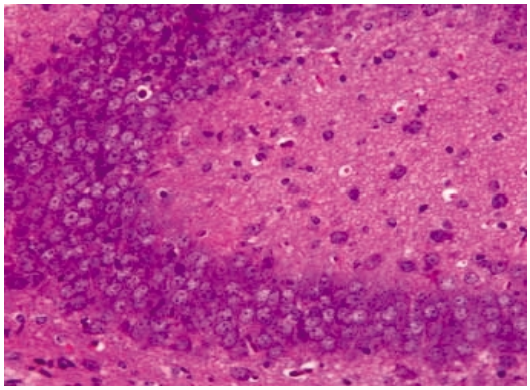

图 5 缺氧 90min 海马× 40

Fig.5 Hypoxia 90min hippocampus× 40

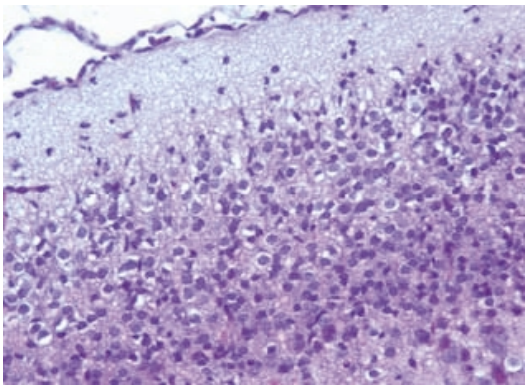

图 6 缺氧 90min 皮层× 40

Fig.6 Hypoxia 90min cortical × 40

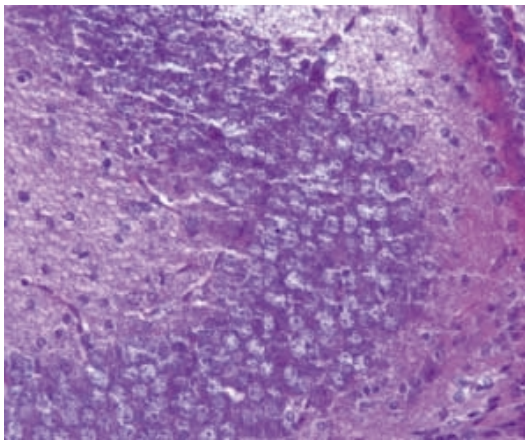

图 7 缺氧 60min 海马× 40

Fig.7 Hypoxia 60min hippocampus× 40

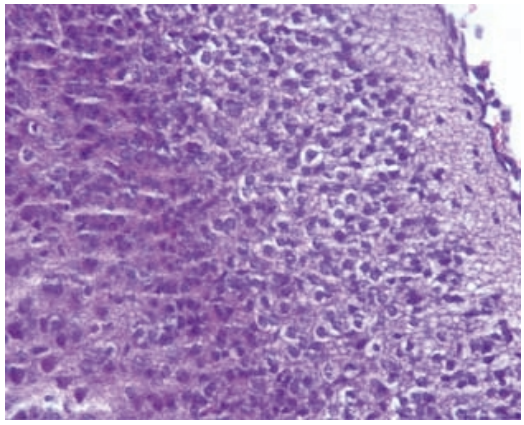

图 8 缺氧 60min 皮层× 40

Fig.8 Hypoxia 60min cortical × 40

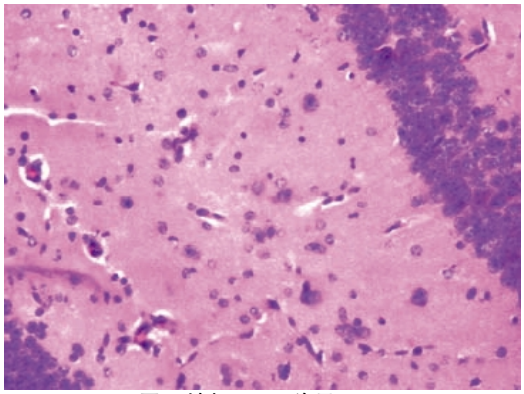

图 9 缺氧 30min 海马× 40

Fig.9 Hypoxia 30min hippocampus× 40

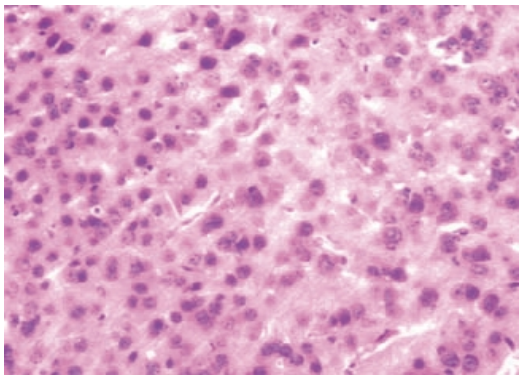

图 10 缺氧 30min 皮层× 40

Fig.10 Hypoxia 30min cortical × 40

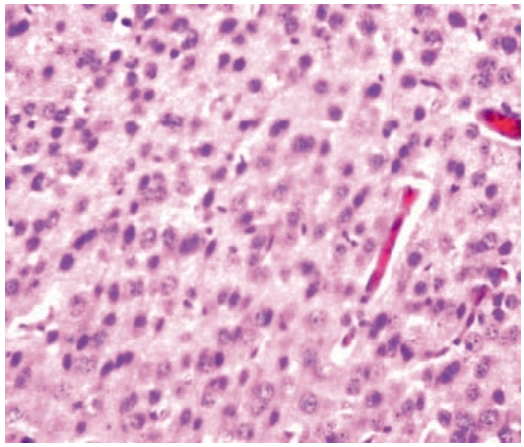

图 11 正常对照组 皮层× 40

Fig.11 Normal control group cortical x 40

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