

紫绀缺氧未成熟心肌对缺血-再灌注损伤耐受性的兔实验

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摘要:【目的】构建幼兔缺氧模型, 研究紫绀缺氧未成熟心肌对缺血-再灌注损伤的耐受性。【方法】新生幼兔 28 只, 分 2 组, I 组(缺氧组) II 组(对照组)。I 组置于常压低氧饲养舱中喂养 7~9 d, 构建慢性缺氧模型。在 Langendorff 缺血-再灌注离体心脏模型上, 观察 2 组再灌注末心肌损伤程度及心功能恢复情况, 从分子生物学和超微结构角度, 初步探讨其机制。【结果】缺氧幼兔心肌大量中小线粒体增生, 超氧化物歧化酶(SOD)活性下降, 腺苷三磷酸(ATP)含量减少。与 I 比较, 再灌注末 II 组心肌 SOD 下降幅度和丙二醛(MDA)上升幅度增大(27.3±4.9)% 比(14.1±8.7)% ;(66.79±12.8)% 比(36.5±10.1)% ;心肌 ATP 下降更为明显(39.0±3.5)% 比(5.1±5.7)% ;再灌注后冠状窦流量恢复率(CFR)、心肌收缩力恢复率也不如对照组, 超微结构破坏程度更为显著。【结论】缺氧紫绀幼兔心肌对缺血-再灌注损伤耐受性下降, 长期缺氧使未成熟心肌抗氧化酶活性下降, ATP 和糖原消耗, 对再灌注后氧自由基损伤更敏感。

关键词: 未成熟心肌; 缺氧动物模型; 心肌缺血-再灌注损伤; 氧自由基; 兔/外科学

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Experiment on the Tolerance of the Hypoxic Immature Myocardium to Ischemia - Reperfusion Injury in Rabbits

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Abstract: 【Objective】To establish hypoxic model of neonatal rabbits and, study the tolerance of the hypoxic immature myocardium to ischemia - reperfusion injury. 【Methods】28 neonatal New Zealand rabbits were randomized into 2 groups, group I (hypoxic group) and group II (control group). Group I was fed in a normobaric hypoxic chamber for 7~9 d to develop a cyanotic hypoxic model. Using Langendorff isolated heart ischemia-reperfusion model, the degree of ischemia-reperfusion injury and recovery of myocardium function were observed. The mechanisms of different results between hypoxic and normoxic neonatal rabbits were explored at molecular biological and ultra-structural level. 【Result】In group I, numbers of small mitochondrias accumulated in myocardial cytoplasm, superoxide dismutase (SOD) reserve capacity and ATP content in myocardium decreased. Compared with group II, the degree of SOD decreasing rate and malonydealdehyde (MDA) increasing rate during ischemia-reperfusion in group I were significantly increased (27.3±4.90)% vs (14.1±8.7)% ;(66.79±12.8)% vs (36.5±10.1)% respectively and ATP decreased more obviously (39.0±3.5)% vs (5.1±5.7)%. After reperfusion, coronary sinus flow recovery rate (CFR) and myocardial contractility recovery rate in group I were also significantly lower than that in group II. The alteration of myocardium ultrastructure exhibited more seriously damage in group I than in group II. 【Conclusion】The hypoxic cyanotic neonatal rabbits are less tolerant to ischemia - reperfusion in-

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jury. Long-time hypoxia depresses myocardial antioxidants reserve capacity, depletes energy reserve, and the heart is more vulnerable to free radical injury during reperfusion.

Key words: immature myocardium; hypoxic animal model; myocardial ischemia - reperfusion injury; oxygen free radicals; rabbits/surgery

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临床上,紫绀型先天性心脏病的心肌保护效果不如非紫绀型先天性心脏病,本实验针对此类心肌的代谢特点,使用缺氧幼兔模型,研究缺氧因素对未成熟心肌缺血再-灌注损伤耐受性的影响并初步探讨其机制,为进一步提高心肌保护效果提供指导。

1 材料与方法

1.1 实验分组

新生3~5 d幼兔28只,体重(96.8±5.4)g,随机分为2组:I组(缺氧组,n=14)和II组(对照组,n=14)。参考Bitar等^[1]的方法将I组幼兔置于体积分数10% O₂常压低氧饲养舱中喂养7~9 d,构建慢性缺氧模型。缺氧组幼兔喂养3 d左右出现口唇、耳朵及趾端紫绀,并逐渐加重,1周后紫绀明显。对照组另置于体积分数21% O₂的常氧环境下喂养7~9 d。实验时2组平均体重分别为(119.2±7.9)g(147.3±10.2)g(P<0.05)。

1.2 离体缺血-再灌注损伤模型

20 g/L戊巴比妥钠2 mL/kg和肝素300 U/kg腹腔注射,待动物麻醉后,正中劈开胸骨,暴露心脏大血管,距主动脉起始部3~4 mm迅速取下心脏,投入盛有0~4℃冰盐水的平皿中,将心脏移至Langendorff灌流装置中,用37℃ Krebs-Hensleit 碳酸缓冲液(KHB液)进行逆灌(灌注压力60 mmHg),平衡5 min后,灌ST-Thomas II号冷停搏液(0℃)2 min,心脏在低温14~18℃下缺血60 min,间隔30 min再灌一次冷停搏液,然后将心脏置于37℃恒温,以氧合血再灌注液恢复心肌灌注(恒压60 mmHg)30 min。氧合血再灌注液配置:取供血兔动脉血,按血:KHB液=1:1混合,以含体积分数21% O₂、74% N₂、5% CO₂的混合气体进行氧合5 min。测得氧分压(133±22)mmHg。

1.3 幼兔缺氧模型检验指标

从缺氧和无缺氧幼兔中各取6只,进行动脉血

气、心肌代谢以及超微结构测定。

1.4 离体心脏缺血-再灌注前后检验指标

每组剩余幼兔各8只,进行缺血-再灌注前后心肌代谢、功能及超微结构检测。

1.4.1 心肌丙二醛(MDA)、超氧化物歧化酶(SOD)和腺苷三磷酸(ATP)测定 缺血前、再灌注末各取右室和左室心肌少许测定MDA、SOD和ATP含量,分别计算各幼兔缺血-再灌注后MDA、SOD、ATP上升率或下降率 $\pi = (d_2 - d_1) / d_1$ 。

1.4.2 心肌结构、功能检测 收集缺血前、再灌注后10 min、30 min从冠状窦流出的灌注液并准确记量,按心脏重量计算出冠状窦流量(CSF)(mL/(min·g⁻¹)),计算冠脉流量恢复率(CFR);利用BL-410生物信号采集预处理系统检测缺血前、再灌注末心肌收缩力改变,计算心肌收缩力恢复率;每组随机取2只幼兔的左室前壁的全层心肌,透射电镜下观察再灌注末超微结构;实验结束后取剩余心肌按(1-干质量/湿质量)×100%计算心肌含水量。

1.5 统计学处理

实验采用随机和单因素研究方法,用SPSS9.0统计软件建立数据库,所得数据用均数±标准差表示,采用方差分析,组间比较用q检验,检验水准α取0.05。

2 结果

2.1 幼兔缺氧模型检验

I组缺氧幼兔动脉血气显示低氧血症、代谢性酸中毒、红细胞压积(HCT, φ_{HCT})升高等变化,心肌MDA含量与对照组比较差异无统计学意义(P>0.05),但SOD和ATP含量下降(P<0.05)(表1)。缺氧组幼兔心肌细胞超微结构发生变化,细胞轻度水肿、肌丝走向稍紊乱,线粒体增生,数目增多成堆积状,体积大小不一,可见较多的小线粒体存在,胞质内糖原颗粒分布减少(图1)。

表 1 两组幼兔动脉血气和心肌代谢的比较

Table 1 Comparison of arterial blood gas and myocardial metabolism between two groups

Group	Arterial blood gas					Myocardial metabolism		
	pH	pO ₂ /(mmHg)	SaO ₂ /(%)	φ _{HCT} /(%)	c _{HCO₃} /(mmol·L ⁻¹)	z _{SOD} /(U/g)	z _{MDA} /(nmol·g ⁻¹)	z _{ATP} /(μmol/g)
I	7.31 ± 0.02 ¹⁾	48.5 ± 5.6 ¹⁾	75.2 ± 6.2 ¹⁾	50.6 ± 4.1 ¹⁾	18.9 ± 3.3 ¹⁾	3.83 ± 0.88 ¹⁾	277.3 ± 38.3 ²⁾	4.16 ± 0.29 ¹⁾
II	7.38 ± 0.04	85.7 ± 9.3	94.7 ± 4.4	32.7 ± 3.9	24.1 ± 2.5	5.26 ± 0.59	283.9 ± 27.4	4.46 ± 0.40

1) Compared with II group, P < 0.05; 2) Compared with II groups, P > 0.05

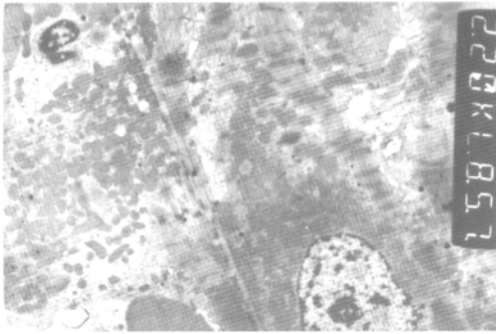


图 1 幼兔缺氧模型缺血前心肌超微结构

Fig. 1 Myocardium ultrastructure for hypoxic neonatal rabbits model before ischemia

The hyperplastic mitochondria accumulated in cytoplasm and their size was different. There were number of small mitochondria existed. Cytoplasm glycogen granule decreased (× 2 200)

2.2 I、II 组离体心脏缺血 - 再灌注前后代谢、功

能及超微结构改变

再灌注末, I 组心肌 SOD、ATP 含量明显低于 II 组, 而 MDA 含量显著高于 II 组 (P < 0.05), 缺血 - 再灌注期间 I 组 SOD 下降率、MDA 上升率以及 ATP 下降率明显高于 II 组 (P < 0.05) (表 2)。再灌注后 I 组心肌收缩力恢复率低于 II 组 (P < 0.05) (表 3)。I 组再灌注各时点冠状窦流量恢复率明显低于 II 组 (P < 0.05), 再灌注末心肌含水量高于 II 组, 差异无统计学意义 (P > 0.05) (表 4)。再灌注末 I 组细胞水肿程度加重, 线粒体结构破坏、嵴排列紊乱、外膜完整性消失, 基质密度下降, 核质边聚, 糖原颗粒分布稀少, 心肌毛细血管内皮破坏, 管腔出现细胞碎片 (图 2); II 组心肌超微结构改变较轻, 细胞中度水肿, 线粒体及其它细胞器结构尚完整 (图 3)。

表 2 两组心肌缺血 - 再灌注后 SOD、MDA、ATP 含量的变化

Table 2 The change of SOD (U/g), MDA (nmol/L) and ATP (μmol/g) during ischemia-reperfusion in two groups (x̄ ± s)

Group	Before ¹⁾ (d ₁)			30 min ²⁾ (d ₂)			π/%		
	SOD	MDA	ATP	SOD	MDA	ATP	SOD	MDA	ATP
I	3.56 ± 0.19	341.1 ± 59.4	3.59 ± 0.31	2.58 ± 0.13 ³⁾	571.6 ± 121.7 ³⁾	2.20 ± 0.28 ³⁾	-27.3 ± 4.90 ⁵⁾	66.79 ± 12.8 ⁵⁾	-25.1 ± 5.7 ⁵⁾
II	4.21 ± 0.36	346.1 ± 60.3	4.29 ± 0.27	3.59 ± 0.17 ⁴⁾	470.2 ± 74.4 ⁴⁾	3.21 ± 0.26 ⁴⁾	-14.1 ± 8.7	36.5 ± 10.1	-39.0 ± 3.5

1) Before myocardial ischemia; 2) 30 min after myocardial reperfusion; 3) Compared with d₁ in group I, P < 0.05; 4) Compared with d₁ in group II, P < 0.05; 5) Compared with group II, P < 0.05

表 3 两组缺血前、再灌注末心肌收缩力恢复率比较

Table 3 Compared with the myocardial contractility recovery rate in two groups during ischemia-reperfusion

Group	Myocardial contractility		
	Before ¹⁾ (d ₁)	30 min ²⁾ (d ₂)	Recovery rate π/%
I	4.46 ± 0.50	2.90 ± 0.35 ³⁾	65.1 ± 5.6 ⁵⁾
II	5.71 ± 1.23	4.95 ± 1.19 ⁴⁾	86.4 ± 6.7

1) Before myocardial ischemia; 2) 30 min after myocardial reperfusion; 3) Compared with d₁ in group I, P < 0.05; 4) Compared with d₁ in group II, P < 0.05; 5) Compared with group II, P < 0.05

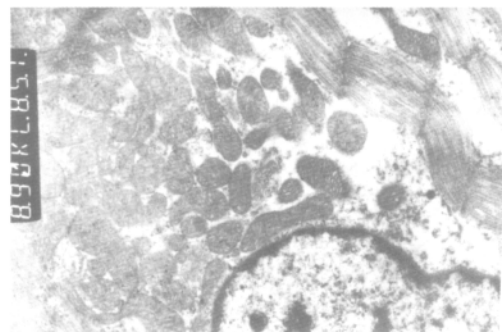


图 2 缺氧组 (I 组) 再灌注末心肌超微结构

Fig. 2 Myocardium ultrastructure for hypoxic group (group I) in the end of reperfusion

The myocytic mitochondria were prominently destructed with altered cristal pattern and discontinuous exo-membrane (× 8 900)

表4 两组缺血-再灌注后各时点冠状窦流量恢复率以及再灌注末心肌含水量的比较

Table 4 Compared with the CFR and water content after reperfusion in two groups

Group	q_{CSF}			CSF recovery rate		Water content
	mL/(min · g ⁻¹)			π (%)		φ_w (%)
	Before ¹⁾ (d_1)	10 min ²⁾ (d_2)	30 min ³⁾ (d_3)	10 min ²⁾ ($\pi = d_2/d_1$)	30 min ³⁾ ($\pi = d_3/d_1$)	30 min ³⁾
I	3.63 ± 0.63 ⁴⁾	2.63 ± 0.51	2.57 ± 0.51	72.4 ± 5.6 ⁶⁾	70.6 ± 5.7 ⁶⁾	74.9 ± 1.50 ⁷⁾
II	4.51 ± 1.25 ⁵⁾	3.83 ± 0.86	3.60 ± 0.70	86.1 ± 6.5	81.8 ± 11.2	72.3 ± 2.44

1) Before myocardial ischemia; 2) 10 min after myocardial reperfusion; 3) 30 min after myocardial reperfusion; 4) Compared between d_1 , d_2 and d_3 in group I, $P < 0.05$; 5) Compared between d_1 , d_2 and d_3 in group II, $P < 0.05$; 6) Compared with group II, $P < 0.05$; 7) Compared with II groups, $P > 0.05$

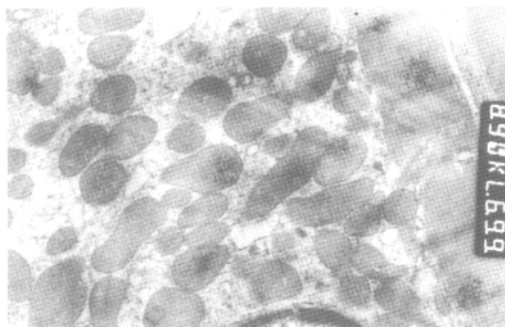


图3 对照组(II组)再灌注末心肌超微结构表现

Fig. 3 Myocardium ultrastructure for control group (group II) in the end of reperfusion

The degree of morphological injury was not severely, middle swollen mitochondria with almost normal structure remained ($\times 8900$)

3 讨论

实验中,新生幼兔在低氧环境下喂养约一周后出现典型缺氧症状,皮肤、黏膜紫绀,生长发育迟缓;动脉血气显示细胞压积上升,低氧血症和代谢性酸中毒;心肌内源性氧自由基清除剂超氧化物歧化酶 SOD 含量下降,ATP 储备减少。这些特征与紫绀型心脏病的临床表现和心肌代谢改变相符合^[2]。电镜显示缺氧模型心肌线粒体出现代偿性变化,大量的中小型线粒体聚集、增生;肌丝走向紊乱,糖原颗粒分布减少。这种超微结构变化更接近文献报道的法乐四联症心肌超微结构特点^[3]。由于存在这些相似点,该模型的实验结果对紫绀型先天性心脏病的心肌保护研究有一定的参考价值。

本实验结果显示缺氧未成熟心肌缺血-再灌注后心功能恢复较无缺氧幼兔差。再灌注后,缺氧组心肌氧自由基产物 MDA 含量升高更明显,能量储备下降率更高,心肌超微结构改变更严重。有作者认为缺氧幼兔对缺血有更好耐受性,Baker^[4]等在类似的缺氧幼兔心脏缺血模型上以携氧 KHB 液再灌注,发现缺氧幼兔乳酸脱氢酶释放量和心肌收

缩功能的恢复均优于无缺氧幼兔。上述两种方法中,结论的差异与再灌注液成分有关。Baker 组采用晶体液再灌注,由于晶体不能有效的携带氧,能量底物不足,再灌注期间,心肌未能有效的利用能量底物进行有氧代谢和合成高能磷酸化合物,实际上仍是缺血过程的继续,结果反映缺血期间心肌功能受损程度。我们在实验中采用氧合血再灌注,氧合血含有丰富的能量物质并且可有效的供氧,再灌注时心肌得以恢复能量合成,进行细胞结构的修复,实验结果不仅反映了缺血对心肌的影响,同时也体现了再灌注后心肌恢复情况和再灌注损伤的程度,该过程更接近于临床实际。

大量的研究表明,缺血心肌再灌注时亦导入新的致伤因素,称再灌注损伤,再灌注损伤的主要机制与氧自由基有关^[5]。本实验显示,缺氧本身虽未造成术前心肌 MDA 升高,但使心肌 SOD 储备下降,对氧自由基损伤更为敏感。另外,长期的缺氧使心肌线粒体增生,结构发生改变,再灌注时,功能缺损的线粒体可能更难以适应骤然升高的氧含量,从而加重再灌注期间的“氧反常”现象,造成更严重的再灌注损伤,细胞超微结构受破坏程度也更为明显。同时,长期缺氧还导致心肌高能磷酸化合物合成减少,糖原储备下降,使心肌缺血期间能量匮乏更加严重,也影响了再灌注后心肌功能的恢复。

综上所述,长期缺氧造成幼兔未成熟心肌细胞结构受损,特别是线粒体结构功能的变化,心肌抗氧自由基酶活性下降,能量储备减少,缺氧幼兔对缺血-再灌注损伤更为敏感。本实验未能发现缺氧对心肌缺血-再灌注损伤的保护作用。

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及耻骨直肠悬带,特别是不要游离远侧的尿道,以防术后尿失禁。本组未发生一例尿失禁,在术中保留耻骨前列腺韧带及耻骨直肠悬带、靠近前列腺尖部横断尿道、紧靠前列腺结扎进入前列腺的血管以保留神经束、将背侧血管复合体及直肠前筋膜分别与尿道前后壁缝合再与膀胱吻合等一系列措施,保存了良好的尿道的稳定性、足够长的膜部尿道及外括约肌以及其神经支配,可以避免此并发症的发生。

过去常有一错误观念认为进入耻骨后间隙后,在膀胱远侧即可显露前列腺,盲目地缝扎背血管复合体,造成术中出血多,显露不清,无目的的钳夹,术后并发症必然较高。实际上在盆内筋膜切开之前,是不能显露前列腺的,因为膀胱逼尿肌纤维向下延伸覆盖了膀胱颈及前列腺前方并融合形成前列腺纤维肌肉基质^[7-9]。而且,在前列腺外侧面有肛提肌纤维覆盖;所以,切开盆内筋膜及紧贴前列腺推开肛提肌是显露前列腺的关键,然后,从侧方可扪及前列腺侧方及尖部,前列腺较小时可扪及导尿管。用腹腔镜用的“雪橇”针带 1 号 Dexon 线或较尖的直角钳,在靠近前列腺尖部横行穿过背血管复合体与前列腺间的平面,缝扎或钳夹、结扎、切断背血管复合体即可显露前列腺尖及膜部尿道,可以非常满意的控制术中出血。控制出血后就能仔细分离及保存膜部尿道及其周围支持组织结构、神经支配,最大限度保存排尿功能。

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