

二氢杨梅素对间歇低氧大鼠脑组织内质网应激诱导细胞凋亡的影响

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【摘要】 目的 观察二氢杨梅素(DHM)对间歇低氧大鼠脑组织内质网应激诱导细胞凋亡的影响。**方法** 选择 96 只 Wistar 雄性大鼠,按随机数字表法分为正常对照组、5% 间歇低氧组及 DHM 组,每组再分为制模后 2、4、6、8 周 4 个亚组,每个亚组 8 只大鼠。5% 间歇低氧组向实验箱内依次注入 30 s 氮气、40 s 空气和 50 s 空气,使箱内氧浓度在 5%~21% 之间变化,每 2 min 为 1 个循环,反复进行,每天 7 h;正常对照组大鼠向实验箱内持续注入空气,使箱内氧浓度为 21%。DHM 组大鼠于制模前 30 min 每日晨起 07:00 灌胃 DHM 250 mg·kg⁻¹·d⁻¹,正常对照组和 5% 间歇低氧组不进行任何处理。实验 2、4、6、8 周取材,于透射电镜下观察皮质神经细胞内质网的超微结构;采用免疫组化法检测各组皮质脑组织细胞 C/EBP 同源蛋白(CHOP)的蛋白表达水平;采用原位末端缺刻标记试验(TUNEL)检测各组皮质神经细胞的凋亡情况。**结果** 透射电镜下可见:正常对照组神经元形态无异常,内质网丰富,形态完整,无扩张肿胀及脱颗粒改变;5% 间歇低氧组内质网出现脱颗粒、扩张及肿胀,甚至出现融合,随低氧时间延长内质网损伤越严重;DHM 组神经细胞内质网的损伤较 5% 间歇低氧组减轻。正常对照组各时间点神经细胞 CHOP 的蛋白表达及凋亡指数均较低,皮质神经细胞阳性细胞较少。与正常对照组比较,5% 间歇低氧组及 DHM 组神经细胞 CHOP 蛋白表达和凋亡指数升高,于制模型 2 周开始出现统计学差异[CHOP(A 值):19.15±0.92、14.99±0.71 比 10.51±0.28;凋亡指数:0.398±0.026、0.283±0.053 比 0.058±0.014,均 $P<0.05$],持续到制模后 8 周[CHOP(A 值):58.44±2.45、40.99±1.60 比 10.42±0.29;凋亡指数:0.826±0.034、0.645±0.039 比 0.058±0.016,均 $P<0.05$];DHM 组上述指标均较 5% 间歇低氧组明显降低(均 $P<0.05$)。**结论** DHM 可通过抑制间歇低氧大鼠脑组织内质网应激诱导的神经细胞凋亡,进而发挥神经保护作用。

【关键词】 二氢杨梅素; 间歇低氧; 内质网应激; 细胞凋亡; C/EBP 同源蛋白

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Effect of dihydromyricetin on apoptosis induced by endoplasmic reticulum stress in rats with intermittent hypoxia

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【Abstract】 Objective To investigate the effect of dihydromyricetin (DHM) on endoplasmic reticulum stress induced apoptosis in rats with intermittent hypoxia. **Methods** A total of 96 Wistar male rats were randomly divided into normal control group, 5% intermittent hypoxia group, and DHM group according to random number table method. Each group was further divided into four subgroups at 2, 4, 6, and 8 weeks after modeling with 8 rats in each subgroup. In the 5% intermittent hypoxia group, 30 seconds nitrogen, 40 seconds air and 50 seconds air were injected into the experimental chamber successively, so that the oxygen concentration in the chamber varied between 5%–21%, every 2 minutes was a cycle, repeated for 7 hours a day; normal control group rats continued to inject air into the experimental chamber, the oxygen concentration in the chamber was 21%. DHM group rats were injected with DHM 250 mg·kg⁻¹·d⁻¹ from 07:00 every morning before 30 minutes modeling, while normal control group and 5% intermittent hypoxia group were given no treatment. The samples were collected at experiment 2, 4, 6, and 8 weeks for index detection. The ultrastructure of endoplasmic reticulum of cortical nerve cells was observed under transmission electron microscope; The protein expression level of C/EBP homologous protein (CHOP) in cortical brain cells of each group was detected by immunohistochemistry; The apoptosis of cortical nerve cells in each group was detected by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL). **Results** Under transmission electron microscopy: the morphology of neurons in the normal control group was complete, no abnormalities, abundant endoplasmic reticulum, no expansion, swelling, and degranulation changes. In the 5% intermittent hypoxia group, the endoplasmic reticulum appeared degranulation, dilation, swelling, and even fusion and the injury became more serious with the extension of

hypoxia time. The damage of endoplasmic reticulum of nerve cells in DHM group was less than that in 5% intermittent hypoxia group. CHOP protein expression and apoptosis index of nerve cells were lower in normal control group at all time points, and there were fewer positive cortical nerve cells. Under light microscope, apoptotic cells are brownish yellow or brown. CHOP protein expression and apoptosis index of nerve cells in 5% intermittent hypoxia group and DHM group were significantly increased compared with normal control group, and statistical differences began to appear after 2 weeks of model building [CHOP (A value): 19.15 ± 0.92 , 14.99 ± 0.71 vs. 10.51 ± 0.28 , apoptotic index: 0.398 ± 0.026 , 0.283 ± 0.053 vs. 0.058 ± 0.014 , all $P < 0.05$], and lasted until 8 weeks after modeling [CHOP (A value): 58.44 ± 2.45 , 40.99 ± 1.60 vs. 10.42 ± 0.29 , apoptotic index: 0.826 ± 0.034 , 0.645 ± 0.039 vs. 0.058 ± 0.016 , all $P < 0.05$], while above indexes in DHM group were significantly decreased compared with 5% intermittent hypoxia group (all $P < 0.05$). **Conclusion** DHM can play a neuroprotective role by inhibiting the apoptosis of nerve cells induced by endoplasmic reticulum stress in intermittently hypoxic rats.

【Key words】 Dihydromyricetin; Intermittent hypoxia; Endoplasmic reticulum stress; Apoptosis; C/EBP homologous protein

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阻塞性睡眠呼吸暂停低通气综合征 (obstructive sleep apnea hyponeasynndrome, OSAHS) 一种是呼吸系统睡眠障碍性疾病,随着肥胖症、糖尿病等疾病发病率的升高,OSAHS 目前已成为临床常见疾病^[1]。OSAHS 所致的间歇性缺氧可对全身多系统造成损害^[2-3]。其中,神经系统作为 OSAHS 的重要靶器官,长期间歇性缺氧可导致认知功能障碍^[4]。动物实验证实,细胞凋亡是 OSAHS 所致神经系统损伤的重要发病机制^[5]。

内质网应激是诱导凋亡的重要通路之一,本课题组前期的研究已证实,间歇缺氧可引起内质网应激,并参与肺部的损伤^[6]。目前,间歇低氧对神经系统内质网应激诱导细胞凋亡的研究甚少,鲜见二氢杨梅素 (dihydromyricetin, DHM) 干预的相关文献报道。本实验通过复制间歇低氧大鼠模型模拟 OSAHS 的发病特点,同时给予 DHM 药物干预,观察大鼠皮质脑组织内质网应激标志性蛋白 CHOP 的蛋白表达及神经细胞凋亡情况,探讨 DHM 对间歇低氧大鼠皮质脑组织内质网应激诱导细胞凋亡的影响。

1 材料与方法

1.1 实验动物及分组: 将 96 只雄性 Wistar 大鼠 (体质量 170 g 左右) 按随机数字表法分为正常对照组、5% 间歇低氧组和 DHM 组,每组 32 只;再将大鼠按制模后 2、4、6、8 周进行观察。

1.2 动物模型复制: 5% 间歇低氧组向实验箱内依次注入氮气、空气和空气,时间分别为 30 s (流速为 10 L/min)、40 s (流速为 10 L/min)、50 s (流速为 5 L/min),充入氮气 80 s 后使箱内氧浓度降至 5%,充入 40 s 空气后使箱内氧浓度升至 21%,再充入 50 s

空气使箱内氧浓度保持在 21%,以上 2 min 为 1 个循环,反复进行,每天 7 h。正常对照组向实验箱内持续注入氧浓度为 21% 的空气。DHM 组大鼠于制模型前每日 07:00 给予 DHM $250 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ 灌胃,30 min 后置于实验箱内,实验过程与 5% 间歇低氧组相同;正常对照组和 5% 间歇低氧组不进行任何处理。

1.3 伦理学: 本实验中动物处置方法符合动物伦理学标准,并经本院科研部门审核批准 (审批号: LAEC-NCST-2020006)。

1.4 检测指标及方法

1.4.1 大脑皮质组织超微结构观察: 于制模后 2、4、6、8 周用水合氯醛麻醉大鼠,生理盐水灌注固定,立即置于冰上取大脑皮质组织,分别进行预固定、固定、脱水、包埋、切片等处理后进行染色,电镜下观察脑皮质组织超微结构变化。

1.4.2 采用免疫组化法检测各组大鼠脑皮质组织 C/EBP 同源蛋白 (C/EBP homology protein, CHOP) 的蛋白表达情况: 将用 4% 多聚甲醛水溶液固定的大鼠脑皮质组织经脱水、透明、包埋等处理后切片 (片厚 $4 \mu\text{m}$);用链霉素抗生物素蛋白-过氧化物酶二步法染色、脱蜡、高压修复,滴加 1:500 的 CHOP 抗体,再滴加二抗后显色,最后脱水、透明、封片。于制模后 2、4、6、8 周随机选取 3 张切片,每张切片选取 5 个不同视野。应用 Motic 医学图像分析系统进行处理,以吸光度 (absorbance, A) 值表示 CHOP 阳性细胞的蛋白表达量。

1.4.3 皮质神经细胞凋亡情况观察: 将免疫组化制备的切片经脱蜡、水化,用 3% 过氧化氢处理,磷酸缓冲盐溶液漂洗,加入 $20 \mu\text{L}$ 原位末端缺刻标记试

[illegible]

亡越多,说明 OSAHS 通过引起神经细胞凋亡进而造成神经系统损伤,这与国外的研究结果一致^[10]。

细胞凋亡广泛存在于多细胞生物体内,是细胞的程序性死亡,有经典的死亡受体途径及线粒体凋亡途径调节,此外,还有近年来新发现的凋亡途径为内质网应激^[11]。内质网是重要的细胞器之一,在蛋白质合成、折叠、运输等过程中发挥重要作用,当受到缺氧、饥饿、感染等外界因素作用时,错误折叠的蛋白质积聚于内质网内,诱发内质网应激,参与癌症、代谢性疾病等多种疾病的发生^[12]。CHOP 是内质网应激的标志性因子,同时也是参与凋亡的重要信号蛋白,CHOP 蛋白的高表达,可诱发细胞出现凋亡^[13]。本课题组前期通过复制大鼠慢性间歇低氧模型进行研究,结果显示,肺组织内 CHOP 的蛋白表达增加,表明其参与了肺组织的损伤^[14]。在对慢性间歇性缺氧幼鼠的研究表明,间歇性缺氧可使海马神经元 CHOP 的蛋白表达及神经元凋亡增加,进而引起脑损伤导致认知功能下降^[15]。本研究结果显示:5% 间歇低氧组皮质神经细胞内质网出现脱颗粒、扩张及肿胀,甚至出现融合,随低氧时间的延长内质网损伤越严重;5% 间歇低氧组大脑皮质神经细胞 CHOP 的蛋白表达及凋亡指数均升高,内质网出现脱颗粒、扩张及肿胀,甚至融合,随低氧时间延长内质网损伤越严重,说明间歇低氧诱发了大脑皮质神经细胞的内质网应激,进而促进神经细胞的凋亡。

DHM 是黄酮类化合物,具有抗炎、抗氧化、清除自由基、抗菌、抗凋亡等的作用^[16-17]。国外有研究显示,DHM 可通过降低内质网氧化应激反应进而减少细胞凋亡,发挥细胞保护作用^[18]。在对辐射后神经系统损伤的研究表明,DHM 通过减少神经细胞的凋亡及氧化应激反应等机制对神经细胞发挥保护作用^[19]。本课题组前期的研究已经证实,DHM 可通过改善突触可塑性减轻神经系统的损伤^[20]。但 DHM 对间歇低氧神经系统损伤中内质网应激的影响未见报道,本研究通过复制间歇低氧模型,透射电镜结果显示:与 5% 间歇低氧组比较,DHM 组神经细胞内质网损伤减轻。免疫组化结果显示:与 5% 间歇低氧组比较,DHM 组神经细胞 CHOP 的蛋白表达及凋亡指数明显降低。说明 DHM 可通过抑制间歇低氧大鼠皮质脑组织内质网应激诱导的神经细胞凋亡,进而发挥神经保护作用。

本研究仅局限于动物实验,与临床 OSAHS 患者尚有一定的区别,但可说为以后临床研究打下基础,

为临床 OSAHS 脑损伤的治疗提供新的靶点。

利益冲突 所有作者均声明不存在利益冲突

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