·基础研究·

高压氧对成年大鼠脑梗死后神经干细胞增殖及分化的影响

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【摘 要】 **目的** 探讨高压氧(HBO)干预对大脑中动脉栓塞(MCAO)模型大鼠神经干细胞(NSCs)增殖及分化的影响。**方法** 采用随机数字表法将 72 只成年 SD 雄性大鼠分为高压空气组、常压氧组、高压氧组及模型组。将上述各组大鼠制成 MCAO 模型大鼠,模型组大鼠于制模后未给予任何特殊处理,高压空气组、常压氧组、高压氧组于制模 2 h 后分别给予高压空气、常压氧及高压氧干预,每天治疗 1 次。分别于制模后 2 d、3 d、7 d 及 14 d 时采用 Western-blot 法检测各组大鼠脑缺血侧海马神经巢蛋白(Nestin)、胶质纤维酸性蛋白(GFAP)及微管相关蛋白-2(MAP-2)含量。**结果** 制模后 3 d、7 d、14 d 时高压氧组脑缺血侧海马 Nestin 蛋白表达均较其它各组明显增高($P<0.05$),并于制模后 3 d 时达到峰值(0.55 ± 0.04);高压氧组制模后 7 d 时 MAP-2 表达(1.23 ± 0.10)及制模后 14 d 时 MAP-2 表达(0.80 ± 0.04)均较其他各组显著升高($P<0.05$);高压氧组 GFAP 表达在制模后不同时间点均显著低于其它各组水平($P<0.05$)。**结论** 高压氧干预可促进 MCAO 模型大鼠脑缺血侧海马 NSCs 增殖及向神经元分化,同时还能抑制 NSCs 向星形胶质细胞分化。

【关键词】 高压氧; 脑缺血; 神经干细胞; 增殖; 星形胶质细胞

The effect of hyperbaric oxygen on neural stem cell proliferation and differentiation after cerebral ischemia

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【**Abstract**】 **Objective** To study the effect of hyperbaric oxygen (HBO) on the proliferation and differentiation of neural stem cells (NSCs) in rats after middle cerebral artery occlusion (MCAO). **Methods** Seventy-two adult, male, Sprague-Dawley rats were randomly divided into a control (CON) group, a hyperbaric air (HBA) group, a normobaric oxygen (NBO) group and a hyperbaric oxygen (HBO) group. All were subjected to MCAO. Rats in the CON group did not receive any treatment; those in the other groups were treated with HBA, NBO or HBO daily beginning 2 hours after the operation. Western blotting was applied to detect the expression of nestin, MAP2 and GFAP at 2, 3, 7 and 14 days after the MCAO. **Results** The expression of nestin in the HBO group was significantly higher than in the other groups on the 3rd, 7th and 14th days. It peaked at day 3 but remained high until day 14. Similarly, expression of MAP2 was significantly higher than in the other groups at least until day 14. GFAP expression was significantly lower than in the other groups. **Conclusion** HBO can increase neural stem cell proliferation and neuronal differentiation, and inhibit the proliferation of astrocytes.

【**Key words**】 Hyperbaric oxygen; Cerebral ischemia; Neural stem cells; Proliferation; Astrocytes

成年个体大脑中新生的神经细胞能够存活较长时间,它们不仅对神经组织具有支持保护作用,还与学习、记忆可塑性关系密切[1-2]。环境变化、生理或病理刺激、神经活性改变等均对成年个体脑室管膜下区(subventricular zone,SVZ)及海马颗粒下层(subgranular layer zone,SGZ)神经发生具有影响作用[3-5],其中海马 SGZ 区神经干细胞(neural stem cells,NSCs)增殖与分化是神经重塑的重要基础,对患者空间学习及认知功能改善具有重要意义[6]。高压氧(hyperbaric oxygen,HBO)作为一种安全有效、改善细胞微环境的治疗手段,其对干细胞增殖及分化的影响作用日益受到临床重视。本研究拟通过观察 HBO 干预对成年大鼠脑缺血侧海马组织中 NSCs 增殖与分化的影响,进而为临床采用 HBO 治疗脑卒中患者提供理论依据。现报道如下。

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材料与方法

一、实验动物

共选取健康雄性清洁级 Sprague-Dawley(SD)成年大鼠 72 只(由华中科技大学同济医学院动物房提供),大鼠体质量 250 ~ 280 g;主要实验仪器包括 02-Y900-002 型动物氧舱(烟台产)、moorLAB 多通道激光多普勒血流仪(北京产)等;主要实验试剂包括辣根过氧化物酶标记巢蛋白(nestin)抗体(1:200)、胶质纤维酸性蛋白(glial fibrillary acidic protein, GFAP)抗体(1:200)、大鼠微管相关蛋白-2(microtubule-associated proteins 2, MAP-2)抗体(1:1000)等。

二、分组及干预

采用随机数字表法将 72 只大鼠分为高压空气组(20 只)、常压氧组(20 只)、高压氧组(20 只)及模型组(12 只)。选用线栓法将上述大鼠制成大脑中动脉栓塞(middle cerebral artery occlusion, MCAO)模型[7]。采用激光多普勒血流仪监测大鼠大脑中动脉血流速度,如血流速度较栓塞前降低 > 70% 及 Zea-Longa 评分 > 1 分[8]提示制模成功。除模型组大鼠外,余各组大鼠均于大脑中动脉栓塞 2 h 后开始相应干预。

高压氧组大鼠制模后给予 HBO 治疗,首先洗舱(氧流量 10 L/min)15 min,待舱内氧浓度达到 95% 以上时升压(氧流量 5 ~ 8 L/min),于 15 ~ 20 min 内舱内压力达到 2.5 个绝对大气压水平;稳压(保持氧浓度 95% 以上) 期间大鼠持续吸氧 60 min,随后舱内压力于 20 ~ 30 min 内匀速降至正常大气压水平出舱,每天治疗 1 次。高压空气组干预步骤同上,只是将氧气换为空气。常压氧组大鼠制模后置于氧舱内并持续通纯氧,在 15 min 使舱内氧浓度升高至 95% 以上,治疗时间同上。模型组大鼠制模后未给予特殊处理。

三、观察指标

各组分别于制模后 2 d、3 d、7 d 及 14 d 时各取 5 只大鼠断头取脑,于冰面上快速分离海马组织,加入裂解液提取蛋白并转移至离心管中,将裂解液置于凝胶加样缓冲液中,沸水加温至变性。具体 Western-Blot(WB)检查步骤参见 Kim 等[9]介绍的方法;采用 Band-Scan 软件分析制模后不同时间点各组大鼠脑缺血侧海马 Nestin、GFAP 及 MAP-2 蛋白含量。

四、统计学分析

本研究所得计量资料以($\bar{x} \pm s$)表示,采用 SPSS 18.0 版统计学软件包进行数据分析,多组间比较采用多因素方差分析,组间两两比较采用 SNK 法,$P < 0.05$ 表示差异具有统计学意义。

结　果

高压氧组大鼠脑缺血侧海马 Nestin 蛋白表达在制模后第 3 天时达到峰值(0.55 ± 0.04),制模后第 14 天时 Nestin 蛋白表达(0.42 ± 0.04)仍处于较高水平,并且该组大鼠 Nestin 蛋白表达在制模后 3 d、7 d 及 14 d 时均显著高于其它各组水平($P < 0.05$);高压氧组大鼠脑缺血侧海马 MAP-2 表达于制模后第 7 天时达到峰值(1.23 ± 0.10),制模后第 14 天时 MAP-2 表达(0.80 ± 0.04)仍处于较高水平($P < 0.05$),并且该组大鼠 MAP-2 表达在制模后 7 d、14 d 时均显著高于其它各组水平($P < 0.05$);制模后 7 d、14 d 时高压氧组大鼠脑缺血侧海马 GFAP 表达均显著低于其它各组水平($P < 0.05$),具体结果见表 1、图 1。

表 1　制模后不同时间点各组大鼠脑缺血侧海马 Nestin、MAP-2 及 GFAP 蛋白表达结果比较($\bar{x} \pm s$)

组别	只数	Nestin	MAP-2	GFAP
模型组				
制模后 2 d 时	5	0.32 ± 0.04	0.28 ± 0.09	0.39 ± 0.09
制模后 3 d 时	5	0.14 ± 0.05	0.18 ± 0.09	0.60 ± 0.07
制模后 7 d 时	5	0.10 ± 0.04	0.11 ± 0.05	0.55 ± 0.05
制模后 14 d 时	5	0.14 ± 0.02	0.14 ± 0.03	0.77 ± 0.18
高压空气组				
制模后 2 d 时	5	0.38 ± 0.03	0.57 ± 0.04	0.13 ± 0.07
制模后 3 d 时	5	0.47 ± 0.04	0.60 ± 0.07	0.10 ± 0.06
制模后 7 d 时	5	0.35 ± 0.05	0.58 ± 0.04	0.16 ± 0.01
制模后 14 d 时	5	0.20 ± 0.02	0.37 ± 0.08	0.50 ± 0.09
常压氧组				
制模后 2 d 时	5	0.45 ± 0.05	0.11 ± 0.02	0.29 ± 0.06
制模后 3 d 时	5	0.33 ± 0.04	0.28 ± 0.03	0.35 ± 0.06
制模后 7 d 时	5	0.15 ± 0.05	0.35 ± 0.05	0.43 ± 0.05
制模后 14 d 时	5	0.17 ± 0.05	0.42 ± 0.03	0.75 ± 0.09
高压氧组				
制模后 2 d 时	5	0.41 ± 0.03	0.32 ± 0.02	0.16 ± 0.01
制模后 3 d 时	5	0.55 ± 0.04[a]	0.84 ± 0.08	0.20 ± 0.02
制模后 7 d 时	5	0.45 ± 0.04[a]	1.23 ± 0.10[a]	0.13 ± 0.06
制模后 14 d 时	5	0.42 ± 0.04[a]	0.80 ± 0.04[a]	0.17 ± 0.07

注:与模型组、高压空气组及常压氧组比较,[a]$P < 0.05$

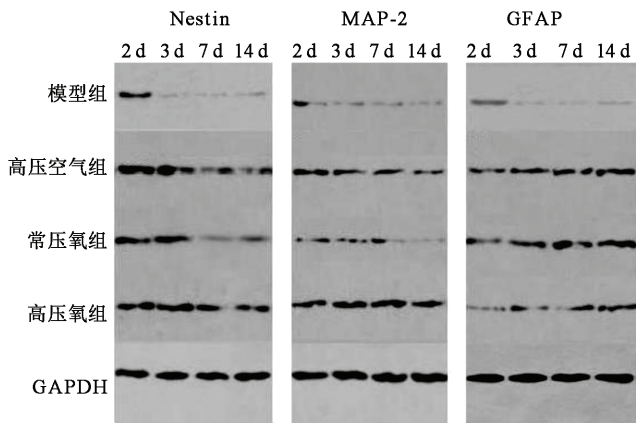

图 1　制模后不同时间点各组大鼠 Nestin、MAP-2 及 GFAP 蛋白电泳图比较

讨 论

NSCs 最早被 Reynolds 等[10]发现存在于哺乳动物室管膜下区(SVZ)及海马齿状回颗粒下区(SGZ)。有研究证实,缺氧、缺血性损伤能促进成年大鼠体内 NSCs 增殖、分化,从而缓解因损伤导致的神经元减少,但因自然条件下 NSCs 增殖数量较少,且 80% 以上的新生神经元在脑卒中后 6 周内死亡[11-15],可见仅靠自然条件下机体 NSCs 对损伤的反应性增殖是无法达到修复神经损伤的目的,故如何采取有效措施促进 NSCs 增殖对改善受损神经功能具有重要意义。

随着 HBO 被广泛应用于临床各个领域,其对机体微环境的改善作用越来越受到重视。相关文献报道,HBO 能通过氧合作用促进受损脑组织内 NSCs 分化及迁移[14];HBO 预处理能减轻手术损伤后脑水肿并加速神经功能恢复[16]。王晓莉等[17]研究证实,HBO 能抑制新生大鼠缺氧、缺血性脑损伤后 NSCs 死亡;同时还发现 HBO 能通过 Wnt3 蛋白促进 NSCs 增殖[18]。Yang 等[19]证实 HBO 能促进新生大鼠缺氧、缺血性脑损伤后内源性 NSCs 迁移到脑皮质并分化为成熟神经细胞。目前关于 HBO 对成年大鼠脑梗死后干细胞增殖及分化影响的报道较少,基于该背景,本研究通过观察 HBO 治疗后成年大鼠海马组织内 Nestin、GFAP 及 MAP-2 蛋白含量变化,从而探讨 HBO 对成年大鼠脑梗死模型 SGZ 区 NSCs 增殖及分化的影响。

Nestin 即巢蛋白,是一种在细胞质中表达的中间丝蛋白,通常作为神经干细胞的公认标记物被广泛应用于基础研究中[20-22]。本实验结果发现,高压氧组 Nestin 蛋白表达量于制模后第 3 天时达到峰值,并持续至制模后第 14 天时,与 Wang 等[18]研究结果类似,如 Wang 等[18]通过制作新生大鼠缺血缺氧模型,于制模 3 h 后给予 HBO 干预,通过动态观察发现大鼠 SVZ 区 NSCs 增殖、Wnt3 蛋白表达增强,且二者具有相关性。上述结果均表明,HBO 可促进 NSCs 增殖,且较高压空气、常压氧等具有更好的疗效,这为临床采用 HBO 治疗脑卒中患者提供了理论支持。

NSCs 在增殖同时也可按照一定时间及空间模式进行分化,通常 NSCs 可分化为 MAP-2 神经元和 GFAP 星形胶质细胞。MAP-2 即微管相关蛋白-2,与 MAP-4 和 tau 蛋白同属于结构性微管相关蛋白家族,主要在中枢神经系统神经元胞体和树突中表达[23],可由 NSCs 分化而来。本研究结果表明,高压氧组 MAP-2 蛋白表达于制模后第 7 天时达到峰值,且持续至制模后第 14 天时,并且该组大鼠 MAP-2 蛋白表达在制模后 3 d、7 d、14 d 时均显著高于高压空气组及常压氧组水平,上述结果提示 HBO 治疗不仅能促进 NSCs 增殖,同时还能加速其向 MAP-2 细胞分化。

胶质纤维酸性蛋白(GFAP)是一种由星形胶质细胞特异表达的中间丝细胞骨架蛋白,决定了星形胶质细胞的结构及功能[24],可由 NSCs 分化而来。多数学者认为,中枢神经损伤(如脑缺血、脊髓损伤等)后,大脑星形胶质细胞形态、增殖状态等均会发生改变,进而形成星形胶质细胞瘢痕;胶质瘢痕形成一方面能保护未受损脑组织,抑制炎症扩散,改善细胞外环境[25-26],另一方面也形成了物理屏障,阻碍了轴突生长,容易引发氧化还原反应并产生炎症介导因子,从而导致更多组织受损[25,27-29]。本研究结果表明,高压氧组脑缺血侧海马 GFAP 表达明显低于其它各组水平($P<0.05$),提示 HBO 在促进 NSCs 增殖、向 MAP-2 分化的同时,并没有增加 NSCs 向胶质细胞的分化,减少了胶质瘢痕形成的可能性。Zhang 等[30]也观察到 HBO 干预能抑制星形胶质细胞生成,并认为 HBO 是通过下调 BMP-4 基因表达来抑制星形胶质细胞生成。

综上所述,本研究结果表明,HBO 干预能显著促进脑梗死成年大鼠海马 SGZ 区 NSCs 增殖、向成熟神经元分化,同时还能抑制 NSCs 向星形胶质细胞分化,这可能是高压氧治疗缺氧缺血性脑损伤的重要机制之一。

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· 外刊撷英 ·

Subthalamic deep brain stimulation and cognition in parkinson′s disease

BACKGROUND AND OBJECTIVE While bilateral, subthalamic deep brain stimulation (STN DBS) is an established treatment for motor symptoms of Parkinson′s disease (PD), the effect of this intervention on cognition remains controversial. This study assessed the effect of this treatment on cognition within the first six months post-surgery.

METHODS This prospective study included patients with PD who underwent bilateral STN DBS implantation, and were followed for at least 36 months after surgery. Before and after surgery, all patients were assessed with the Unified Parkinson′s Disease Rating Scale (UPDRS), for levodopa equivalent daily dose (LEDD) and with neuropsychological testing. Global cognitive function was assessed using the Mini Mental Status Exam (MMSE). Patients were excluded if they had a preoperative MMSE score of less than 25, a repositioning of electrodes within three years, staged bilateral surgery or missing follow-up MMSE scores.

RESULTS Thirty-six patients were enrolled. The mean change in MMSE scores from baseline to six months was greater than the change from six months to 36 months ($P=0.015$). Baseline LEDD ($P=0.005$) and the axial subscore of the off-UPDRS motor score ($P=0.023$) were significantly related to the change in MMSE score during the first six months.

CONCLUSION This study of patients with Parkinson′s disease, undergoing sub-thalamic deep brain stimulation, found a rapid decline in global cognitive function six months after surgery as compared to the period of six months to three years.

【摘自：Kim H, Jeon BS, Yun JY, et al. Initial cognitive dip after sub-thalamic deep brain stimulation in Parkinson′s disease. J Neurol, 2013, 260: 2130-2133.】