·基础研究·

局灶性脑缺血成年大鼠高压氧干预后神经干细胞增殖及分化研究

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【摘 要】 **目的** 观察高压氧(HBO)干预对成年大鼠急性局灶性脑缺血后海马齿状回颗粒下区(SGZ)神经干细胞增殖及向神经元分化的影响。**方法** 选取 48 只成年雄性 SD 大鼠,按随机数字表法分为模型组、高压氧组、高压空气组和常压氧组,每组 12 只。各组大鼠均进行线栓法 MCAO 制模,除模型组制模后不接受任何干预外,其余 3 组均于线栓插入后 2 h 分别给予高压氧,高压空气和常压氧干预,每日 1 次。采用免疫荧光双重标记制模成功后第 2、3、7 和 14 天脑梗死侧 SGZ 区增殖的神经干细胞($BrdU^+/nestin^+$)及其分化的神经元($BrdU^+/DCX^+$),并在荧光显微镜下计数。**结果** 制模成功后第 2 天,高压氧组 SGZ 区 BrdU/nestin 和 BrdU/DCX 的共标细胞数分别为(2340.45 ± 1109.59)个和(5520.66 ± 1103.09)个,分别与常压氧组和模型组同时间点相同细胞比较,差异均有统计学意义($P<0.05$);制模成功后第 3 和第 7 天,高压氧组的 BrdU/nestin 和 BrdU/DCX 共标细胞数均显著高于高压空气组、常压氧组和模型组,差异均有统计学意义($P<0.05$),且制模成功后第 14 天,高压氧组的 BrdU/DCX 共标细胞数亦显著高于高压空气组、常压氧组和模型组,差异均有统计学意义($P<0.05$)。**结论** 高压氧干预可显著促进成年大鼠梗死侧 SGZ 区神经干细胞的增殖和向神经元分化。

【关键词】 高压氧; 局灶性脑缺血; 神经干细胞; 增殖; 神经元

Neural stem cell proliferation and differentiation in focal brain ischemia adult rats after hyperbaric oxygen intervention

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【**Abstract**】 **Objective** To investigate the effect of hyperbaric oxygen intervention at different time on the neural stem cell proliferation and differentiation in dentate gyrus subgranular zone (SGZ) of adult rats after acute focal cerebral ischemia. **Methods** Totally 48 Sprague-Dawley male adult rats were randomly divided into a middle cerebral artery occlusion (MCAO) group, a hyperbaric oxygen group, a hyperbaric air group and a normobaric oxygen group, each of 12. A middle cerebral artery occlusion (MCAO) model was induced to all rats using the modified Zea-Longa's method of intraluminal filament occlusion, Except the MCAO group, the other 3 groups received corresponding hyperbaric oxygen, hyperbaric air and normobaric oxygen intervention once a day two hours after the suture insertion. The rats were sacrificed for double-label immunofluorescent analysis at 2 days, 3 days, 7 days and 14 days after brain ischemia. $BrdU^+/nestin^+$ labeled the proliferated neural stem cells, and $BrdU^+/DCX^+$ labeled its differentiated derivates, early neurons, in SGZ of ischemic hippocampus dentate gyrus. Also, the cell number was calculated under the fluorescence microscope. **Results** Two days after brain ischemia, the numbers of BrdU/nestin and BrdU/DCX cells in SGZ in the hyperbaric oxygen group were (2340.45 ± 1109.59) and (5520.66 ± 1103.09) respectively, which had increased significantly, compared with the hyperbaric air group and normobaric oxygen group ($P<0.05$). Three and 7 days after brain ischemia, the numbers of BrdU/nestin and BrdU/DCX cells in SGZ in the hyperbaric oxygen group had shown significant increase compared with the other 3 groups ($P<0.05$). Fourteen days after brain ischemia, the numbers of BrdU/DCX cells in SGZ in the hyperbaric oxygen group had significantly increased compared with the hyperbaric air group, normobaric oxygen group and the MCAO group ($P<0.05$). **Con-**

DOI:10.3760/cma.j.issn.0254-1424.2015.010.003

基金项目:国家自然科学基金(81070986)

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clusion Hyperbaric oxygen promotes the proliferation and differentiation of neural stem cells in ischemic SGZ.

【**Key words**】 Hyperbaric oxygen; Focal cerebral ischemia; Neural stem cells; Proliferation; Neuron

研究证实,成年哺乳动物大脑中的神经干细胞可维持生命过程中的神经发生,对认知功能、脑损伤修复具有重要作用[1-2]。然而,衰老、长期压力刺激或者神经系统病变等生理病理因素均可限制神经干细胞的增殖和神经再生,影响正常的机体功能[1]。有研究证实,高压氧(hyperbaric oxygen,HBO)可促进梗死脑内源性干细胞的增殖、迁移和分化,并显著改善大鼠的运动、记忆和认知功能[3-5]。本研究对局灶性脑缺血再灌注急性期成年大鼠进行了 2 周的 HBO 干预,旨在观察 HBO 对梗死侧海马齿状回颗粒下区(subgranularzone,SGZ)神经干细胞增殖及其向神经元分化的影响。

材料与方法

一、实验动物及分组

选取 8 周龄无特定病原体(specific pathogen free,SPF)级雄性 Sprague-Dawley(SD)大鼠 48 只,体重 250 ~ 290 g,由华中科技大学同济医学院附属同济医院动物房提供。按照随机数字法将其分为模型组、高压氧组、高压空气组和常压氧组,每组大鼠 12 只,所有大鼠均置于实验室恒温条件下饲养,自由饮食和摄水,自然昼夜节律光照。

二、模型的制备

选用线栓法对 4 组大鼠行右侧大脑中动脉栓塞再灌注(middle cerebral artery occlusion-reperfusion,MCAO)[6-7],暴露颈内动脉、颈外动脉和颈总动脉,分离并结扎颈外动脉和颈总动脉。沿颈总动脉向颈内动脉方向插入线栓,从动脉分叉处计算深度为(1.7 ± 0.2)cm。栓塞 80 min 后抽出线栓,永久结扎颈总动脉分叉处,消毒,缝合切口。使用激光多普勒血流仪(moorLAB,北京)监测大鼠大脑中动脉血流速度,较栓塞前下降超过 70%,且 Zea-Longa 评分达 1 ~ 3 分,提示 MCAO 模型制作成功。

三、高压氧干预方法

4 组大鼠均于线栓插入 2 h 后进行干预。高压氧组:纯氧洗舱(02-Y900-002 型动物氧舱,烟台产)15 min,待氧监测仪显示舱内氧浓度达 95% 以上时,升压至 0.15 MPa(2.5 ATA)并稳压 60 min,随后匀速减压 25 min 左右,降至正常大气压水平。高压空气组:除用空气加压外,其余步骤与高压氧组相同。常压氧组:纯氧洗仓后,15 min 内使舱内氧浓度达到 95% 以上,不加压,持续治疗 60 min 后匀速减压(方法同高压氧组)。以上干预均为每天 1 次,模型组不给予任何干预。

四、5-溴-2-脱氧尿嘧啶核苷注射

5-溴-2-脱氧尿嘧啶核苷(5-bromo-2-deoxyuridine,BrdU)注射[8]:各组大鼠均于制模当天和第 2 天行 BrdU腹腔内注射(BrdU 溶于生理盐水,终浓度为 20 mg/ml,按照 50 mg/kg 体重剂量注射),每日 3 次,每次间歇 4 h,标记增殖的细胞。

五、脑组织处理和免疫荧光组织化学双重标记

脑组织处理:4 组大鼠均于制模成功后第 2、3、7 和 14 天按随机数字表法均抽取 3 只大鼠,采用 0.9% 生理盐水和 4% 多聚甲醛灌注取脑,用 15% 蔗糖及 30% 蔗糖溶液梯度脱水至脑组织沉底。冰冻切片机切片,从前囟 -2.16 ~ -5.76 mm 开始,每 360 μm 取一张,每张片厚 30 μm。切片存于 -20 ℃ 盛有冻存液(含 30% 乙二醇、30% 蔗糖的磷酸盐缓冲液)的 24 孔板中。

免疫荧光双标:10% 驴血清 +0.3% Triton-X100 + 0.01 M PBS 常温封闭 1 h。用一抗孵育液巢蛋白(nestin,1∶100,小鼠抗大鼠)/双皮质激素(Doublecortin,DCX,1∶300,山羊抗大鼠) +5% 驴血清 +0.3% Triton-X100 +0.01 M 磷酸盐缓冲液(phosphate buffered saline,PBS)孵育过夜(4 ℃)后,加入 Alexa Fluor 488 标记的驴抗小鼠二抗工作液(1∶200) +10% 驴血清 + PBS,置于室温环境避光孵育 2 h,然后采用 PBS 漂洗 3 次,每次 10 min,最后采用甘油封片剂封片,置于激光扫描共聚焦显微镜下,使用 FV10-ASW Viewer 图像采集系统记录荧光照片。

六、细胞计数

采用单盲法计数切片中整个梗死侧 SGZ 区的共定位细胞(细胞核为红色荧光,细胞质为绿色荧光)数目。每隔 12 张切片为一组,取 2 张染色,于每张切片的 SGZ 区随机取 5 个视野计数,统计每张的 5 个视野平均细胞数目,乘以每张脑片厚度,再乘以脑片数,所得数字计为整个 SGZ 区 5 个视野的细胞总数。使用 Image J 1.45s 版图像分析软件统计荧光共定位细胞数目。

七、统计学方法

采用 SPSS 18.0 版软件统计分析,比较制模成功后第 2、3、7、14 天各组梗死侧 SGZ 区 $BrdU^+/nestin^+$、$BrdU^+/DCX^+$ 的细胞数,采用多组均数比较的方差分析,多重比较采用最小显著性差异法检验(Least-Significant Difference,LSD),方差不齐用 Tamhane′s T2 检验。所有数据以($\bar{x} \pm s$)表示,以 $P < 0.05$ 为差异有统计学意义。

结　果

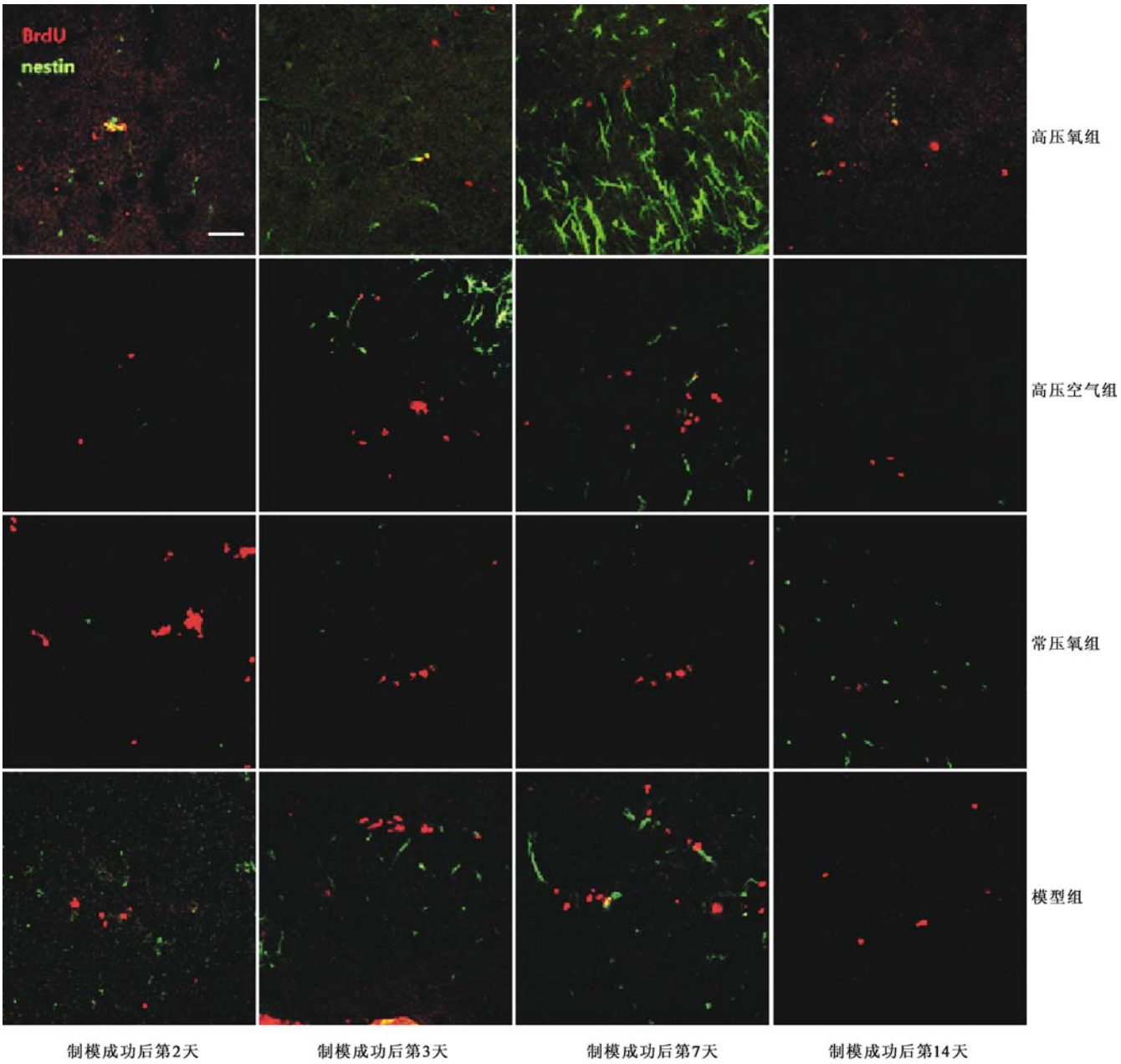
一、高压氧干预对梗死侧 SGZ 区 BrdU/nestin 共标的细胞数目的影响

制模成功后第 2 天，高压氧组的 BrdU/nestin 共标细胞数与常压氧组和模型组比较，差异均有统计学意义（$P<0.05$）；制模成功后第 3 天，高压氧组的 BrdU/nestin 共标细胞数达到峰值，并于制模成功后第 7 天减少，上述 2 个时间点，高压氧组的 BrdU/nestin 共标细胞数均显著高于高压空气组、常压氧组和模型组，差异均有统计学意义（$P<0.05$）。制模成功后第 14 天，4 组均未见到荧光共标的细胞，详见表 1 和图 1。

表 1　4 组大鼠不同时间点梗死侧 SGZ 区 BrdU/nestin 共标细胞数比较（个，$\bar{x}\pm s$）

组别	只数	制模成功后第 2 天	制模成功后第 3 天	制模成功后第 7 天	制模成功后第 14 天
高压氧组	12	2340.45 ± 1109.59	3000.35 ± 224.50	840.48 ± 84.85	0
高压空气组	12	1020.76 ± 84.85	780.65 ± 339.41[a]	60.11 ± 84.85[a]	0
常压氧组	12	540.66 ± 424.26[a]	180.91 ± 146.97[a]	60.69 ± 84.85[a]	0
模型组	12	480.01 ± 449.00[a]	840.23 ± 339.41[a]	180.65 ± 146.97[a]	0

注：与高压氧组同时间点比较，[a]$P<0.05$



注：红色为 BrdU，绿色为 nestin

图 1　4 组大鼠不同时间点梗死侧 SGZ 区 BrdU/nestin 免疫荧光双标共聚焦图像（免疫组化染色，×40）

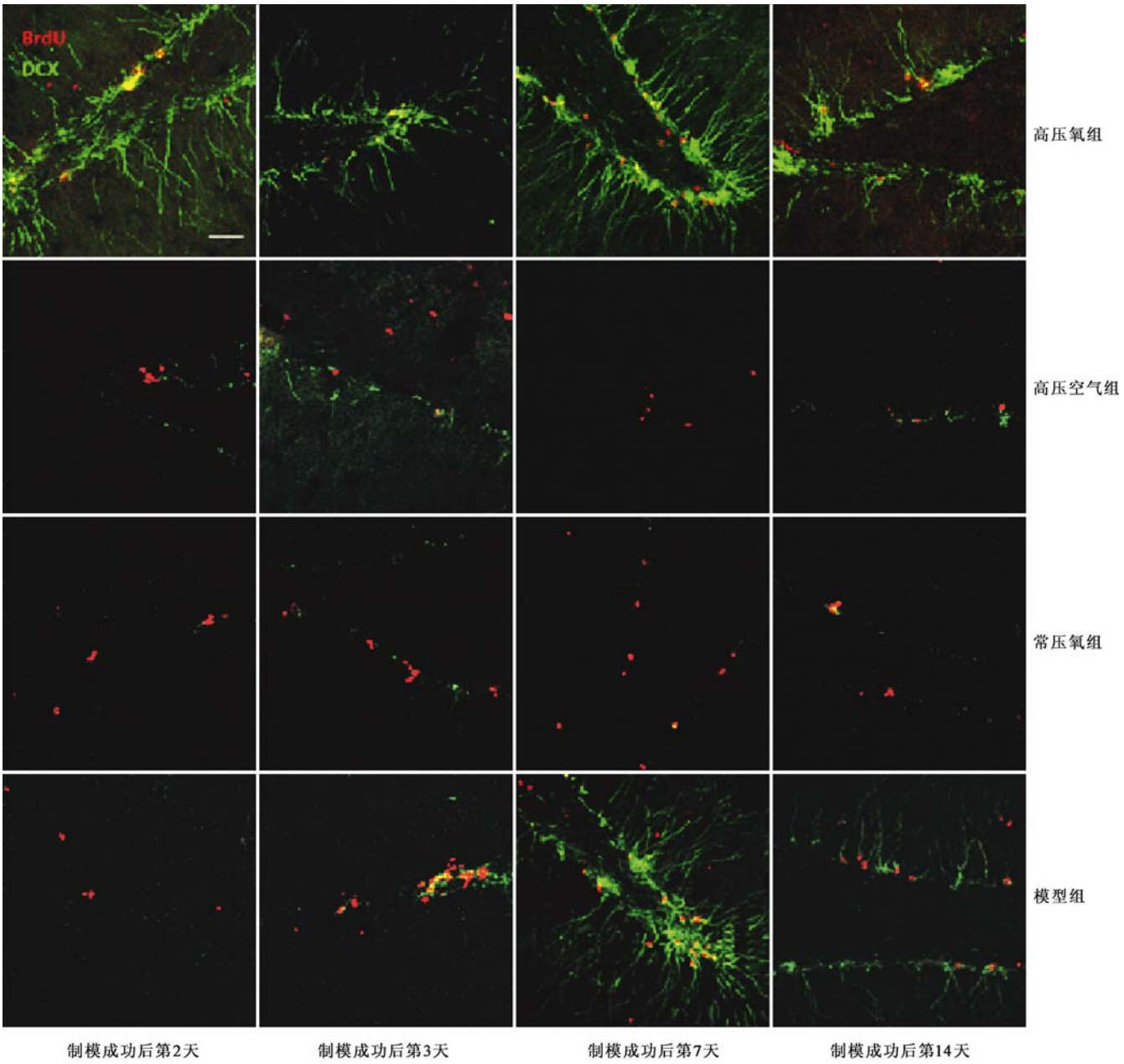
二、高压氧干预对梗死侧 SGZ 区的 BrdU/ACH 共标的细胞数目的影响

制模成功后第 2 天，高压氧组的 BrdU/DCX 共标细胞数，与常压氧组和模型组比较，差异均有统计学意义（$P<0.05$）；制模成功后第 3 天，高压氧组的 BrdU/DCX 共标细胞数开始增加，于制模成功后第 7 天达到峰值，于制模成功后第 7 天减少，上述 3 个时间点，高压氧组的 BrdU/DCX 共标细胞数目逐均显著高于高压空气组、常压氧组和模型组，差异均有统计学意义（$P<0.05$），详见表 2 和图 2。

表 2　4 组大鼠不同时间点梗死侧 SGZ 区 BrdU/DCX 共标细胞数比较（个，$\bar{x}\pm s$）

组别	只数	制模成功后第 2 天	制模成功后第 3 天	制模成功后第 7 天	制模成功后第 14 天
高压氧组	12	5520.66 ± 1103.09	6900.09 ± 1557.69	7740.88 ± 2649.59	3780.57 ± 509.12
高压空气组	12	3360.45 ± 1703.41	3300.23 ± 593.97[a]	3600.35 ± 1018.23[a]	1380.31 ± 339.41[a]
常压氧组	12	1620.82 ± 529.91[a]	2220.12 ± 84.85[a]	1560.63 ± 861.16[a]	1440.19 ± 673.50[a]
模型组	12	360.71 ± 509.12[a]	4260.55 ± 885.89[a]	3240.42 ± 1694.93[a]	1980.17 ± 818.29[a]

注：注：与高压氧组同时间点比较，[a]$P<0.05$



注：红色为 BrdU，绿色为 DCX

图 2　4 组大鼠不同时间点梗死侧 SGZ 区 BrdU/ACH 免疫荧光双标共聚焦图像（免疫组化染色，×40）

讨 论

缺血性脑卒中是临床常见病、多发病,严重危害着人们的身体健康,其中年轻的成年人(<50 岁)占 10% ~14%[9-10],给社会和家庭造成了巨大的负担,因此,探索成年个体脑缺血的治疗手段及其可能的机制,具有重大的现实意义。大量的动物实验研究表明,高压氧对治疗中枢神经系统缺血缺氧性损伤有很好的疗效[11-12];且早期高压氧干预可减轻急性脑缺血后的运功及记忆认知功能障碍[3-5,13-14]。因此,本研究选取急性局灶性脑缺血成年大鼠作为研究对象,探讨高压氧干预的可能机制。

有研究发现,高压氧可通过上调脑缺血缺氧模型新生大鼠 β-catenin/Ngn1 信号通路,或增加 Wnt3 信号通路的活动来促进内源性干细胞的增殖、分化及神经再生[15-16]。海马 SGZ 区是成年哺乳动物神经系统中的三大干细胞生发区域之一[17],与机体的学习、记忆和认知等功能密切相关,虽然缺血缺氧性损伤会刺激该区域的神经干细胞增殖、迁移及向神经元和胶质细胞的分化,但其增殖及分化能力有限,且分化的神经元存活不到 1/5[18-21]。

本研究中,课题组以 BrdU/nestin 荧光共标细胞表示增殖的神经干细胞,BrdU/DCX 荧光共标细胞表示由增殖的神经干细胞分化而来的神经元,经过不同干预后发现,高压氧组大鼠梗死侧 SGZ 区的共标神经干细胞和神经元数目即明显高于模型组和常压氧组同时间点,差异均有统计学意义($P<0.05$)。Malek 等[22]在对局灶性脑缺血沙鼠的研究中也证实了类似的观点,即高压氧和高压空气均可减轻缺血性神经元损伤,但其具体机制尚需进一步阐明。制模成功后第 14 天,高压氧组梗死侧 SGZ 区的神经干细胞和神经元数目与高压空气组、常压氧组、对照组同时间点比较,差异均有统计学意义($P<0.05$)。这与 Lee 等[13]的研究结果一致。还有研究发现,不仅在脑缺血急性期,即使在脑缺血慢性期,高压氧治疗也可促进 SGZ 区的神经再生,并改善其功能[23]。上述研究均支持重复多次应用高压氧可影响神经干细胞增殖和神经再生的观点。

本研究还发现,高压氧组的大鼠梗死侧 SGZ 区神经干细胞数从制模成功后第 2 天(脑缺血后第 2 天)即显著增加,于制模成功后第 3 天达到高峰,这与 Wei 等[5]的结果类似。本研究结果提示,高压氧可促进神经干细胞增殖高峰提前出现,对脑缺血早期可能具有保护作用[24]。在本研究中,高压氧组新生神经干细胞于制模成功后第 2 天即有向神经元的分化,至第 7 天达到高峰,对比各时间点的神经元数课题组发现,高压氧促进神经干细胞分化为神经元的趋势维持至少为14 d。

本研究通过免疫荧光化学方法证明,2.5 ATA 的高压氧干预 2 h,可促进 SGZ 区的神经干细胞增殖和向神经元分化,促使神经干细胞增殖高峰提前出现,并使神经再生的时间延长,这可能是改善脑缺血后的功能障碍的机制之一,这与本课题组前期的 Western Blot 分析结果一致[25]。

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(修回日期:2015-08-17)
(本文编辑:阮仕衡)

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Exercise and patellar cartilage in women with mild knee osteoarthritis

BACKGROUND AND OBJECTIVE Knee osteoarthritis (OA) is characterized by a loss of, and degeneration of, hyaline cartilage. This study investigated the effects of 12 months of supervised aerobic/step aerobic exercise program on patellar cartilage.

METHODS This 12-month, randomized, controlled trial included postmenopausal women with knee pain on most days, and grade one to two Kellgren-Lawrence radiographic tibiofemoral joint OA. The subjects were randomized to one of two experimental arms; an aerobic/step aerobic training group or a non-training control group. The exercise group performed 55-minute sessions of an aerobic and step aerobic jumping exercise program three times per week, progressing in intensity for 12 months. The control group was asked to maintain their usual activities. Daily activity of all participants was measured with an accelerometer. Cartilage measurements were made through MRI, with the secondary outcomes including muscle force, muscle power and cardiorespiratory fitness.

RESULTS At 12-month follow-up, the full patellar cartilage T2 relaxation time values had improved in the exercise group, suggesting improved cartilage quality ($P=0.018$). Positive effects were noted in the lateral and medial segments of the joint, as well as in the total deep zone ($P=0.013$). While the exercise group showed better improvement in pain, OA symptoms, and quality-of-life scores, the difference between the exercise and control groups did not reach statistical significance.

CONCLUSION This randomized, controlled, high-impact exercise trial involving postmenopausal women with mild osteoarthritis of the knee found that T2 relaxation time decreased, indicating improved patellar cartilage quality, after 12 months of exercise.

【摘自:Koli J, Multanen J, Kujala UM, et al. Effects of exercise on patellar cartilage in women with mild knee osteoarthritis. Med Science in Sports Exer, 2015, 47(9): 1767-1774.】