·基础研究·

高压氧治疗对血管性痴呆大鼠学习记忆能力的影响

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【摘要】 **目的**　探讨高压氧治疗(HBOT)对血管性痴呆(VD)大鼠学习记忆能力的影响。**方法**　选择无特定病原体(SPF)级 SD 大鼠 60 只,雌雄不拘,按照随机数字表法将其分为正常组、假手术组、VD 组、HBOT 组,每组 15 只。采用改良四动脉方法建立 VD 模型,正常组不予特殊处理,假手术组手术中不灼烧椎动脉也不夹闭颈总动脉,HBOT 组在 VD 组基础上进行 HBOT 7 d。术后 7 d,分别对 4 组大鼠行 Morris 水迷宫实验检测其学习、记忆能力。**结果**　与正常组比较,假手术组、VD 组、HBOT 组的平均逃避潜伏期较长($P<0.05$)。假手术组、VD 组、HBOT 组的平台象限穿越次数均少于正常组,平台象限游动距离均较短,其占总游程的百分比均低于正常组($P<0.05$)。与假手术组比较,VD 组和 HBOT 组的平均逃避潜伏期较长($P<0.05$)。VD 组、HBOT 组的平台象限穿越次数均少于假手术组,平台象限游动距离均较短,其占总游程的百分比均低于假手术组($P<0.05$)。与 VD 组比较,HBOT 组平均逃避潜伏期[(25.73±6.20)s]较短,平台象限穿越次数[(9.51±2.25)次]较多,平台象限游动距离[(494.72±208.26)次]较长,其占总游程的百分比[(28.71±5.32)%]较高,差异有统计学意义($P<0.05$)。**结论**　HBOT 可有效改善 VD 大鼠的学习记忆能力。

【关键词】 高压氧治疗;　血管性痴呆;　Morris 水迷宫;　学习;　记忆

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Hyperbaric oxygen therapy can improve the learning capacity and memory in vascular dementia

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【**Abstract**】 **Objective**　To explore the influence of hyperbaric oxygen therapy (HBOT) on the learning and memory ability using a rat model of vascular dementia (VD). **Methods**　Sixty Sprague-Dawley rats were selected and divided into a normal group, a sham operation group, a VD group and an HBOT group, each of 15 rats using a random number table. The improved four-vessel method was used to establish a VD model in all except the rats in the normal group, but for the sham operation group, the vertebral artery was not subjected to firing and the arteria carotis communis was not clipped. The HBOT group was then given HBOT for 7 days. The Morris water maze test was used to evaluate their learning and memory ability of all 4 groups. **Results**　Compared to the normal group, the mean escape latency of the sham operation group, the VD group and the HBOT group was significantly longer in each case ($P<0.05$). Their platform quadrant crossing times were also significantly less, and their quadrant swimming distance as a proportion of their total swimming distance was significantly smaller ($P<0.05$). Compared with the sham operation group, the mean escape latency of the VD and HBOT groups was significantly longer ($P<0.05$), the times of crossing platform quadrant were less and their quadrant swimming distance was a smaller proportion of their total swimming distance. As compared with the VD group, the mean escape latency of the HBOT group was significantly shorter [(25.73±6.20) s], the average platform quadrant crossing times were significantly more [(9.51±2.25) times] and the platform quadrant swimming distance of the HBOT group was significantly longer [(494.72±208.26)], representing a significantly larger proportion of the total swimming distance [(28.71±5.32)%] ($P<0.05$). **Conclusion**　HBOT can effectively improve learning and memory in vascular dementia, at least in rats.

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血管性痴呆(vascular dementia,VD)是由多种脑血管因素导致的脑组织累积性损伤引起的智能损害综合征。VD 患者临床表现为不同程度的智力障碍,性格、情感、记忆障碍和局灶性神经系统症状及体征,病程呈阶梯式进行性发展。随着脑血管病发病率的增高,VD 的发病率也逐年增加,给患者家庭和社会带来了沉重的负担,故探索合理、有效的治疗方案非常重要。本研究通过观察高压氧治疗(hyperbaric oxygen therapy,HBOT)对 VD 模型大鼠学习记忆能力的影响,旨在为临床上采用高压氧(hyperbaric oxygen,HBO)治疗 VD 进一步提供理论依据。

材料与方法

一、实验动物

选择无特定病原体(specific pathogen free,SPF)级 Sprague-Dawley 大鼠(SD)大鼠 60 只,雌雄不拘,体重 250~300 g,由中国人民解放军军事医学科学院实验动物中心提供[许可证号:SCXK-(军)2002-001]。动物房环境温度 18~20 ℃,相对湿度 40%,每小时通风换气 8 次,每日喂食标准饲料,自由饮用清洁自来水。适应性饲养 7 d 后进行实验。

二、实验分组

按照随机数字表法将其分为正常组、假手术组、VD 组、HBOT 组,每组 15 只。术中、术后死亡的大鼠予以剔除,并补足相应的大鼠数目。

三、VD 模型制作

在“四动脉法”基础上加以改良,建立 VD 模型[1-3]。大鼠术前 12 h 禁食,术前 4 h 禁水,用 3%戊巴比妥钠(45 mg/kg)腹腔注射麻醉,取仰卧位固定,剪毛备皮消毒后沿头顶部纵中线切开,钝性分离肌肉,暴露双侧第一颈椎,用电灼针灼烧椎动脉,青霉素钠局部用药抗感染,缝合皮肤。24 h 后,大鼠取仰卧位,颈部剪毛备皮消毒后于颈部正中切开皮肤,皮下组织钝性分离出双侧颈总动脉,用动脉夹夹闭双侧颈总动脉20 min后再通,术中避免损伤交感神经和迷走神经,青霉素钠局部用药抗感染,分层缝合伤口,造模完成,放回笼中保温饲养。正常组不予特殊处理,假手术组手术步骤、用药同 VD 组,但术中不灼烧椎动脉也不夹闭颈总动脉,HBOT 组在 VD 组基础上进行 HBOT 7 d。

四、HBOT

将 HBOT 组大鼠放置于上海产 DWC150-300 型透明纯氧动物实验舱内,纯氧洗舱 5 min,排出舱内空气,匀速加压 10 min 使舱内压达到 0.2 MPa,稳压停留 45 min,稳压过程中给予 2 L/min 持续纯氧通风,气体等压交换,舱内氧浓度达 90%以上,减压 15 min 出舱。每日 1 次,连续 7 d,每日进仓时间均为上午 9 点。

五、Morris 水迷宫行为学测试

术后 7 d,采用中国产 DMS-2 型 Morris 水迷宫对各组大鼠进行学习记忆能力的测定。实验室保持安静,窗户用不透光窗帘遮挡,水池周围参照物保持不变。在水池里注入清水,用墨汁染黑池水。

1.定位航行试验:将水池等分为右上、右下、左下、左上 4 个象限,并分别命名为第一至第四象限,平台放于第四象限(左上)中间,平台沉没于水面下 2 cm。从每个象限的中点面向水池池壁将鼠放入水中,每日上午、下午在每个象限各测试 4 次,每次测试 120 s。记录大鼠分别在各个象限找到平台所需的时间(即潜伏期),计算平均值。若大鼠≥120 s 未找到平台,则由操作者将鼠牵引至平台上休息 30 s,其潜伏期记为120 s;若大鼠在 120 s 内找到平台,如实记录时间,让大鼠在平台上休息 30 s,即为 1 次完整训练,连续训练6 d。由系统自动记录、保存大鼠的运动轨迹、游泳时间、游泳距离、找到平台的潜伏期等信息。

2.空间探索试验:7 d 后,撤除平台,选定第二象限的相同入水点将大鼠放入水中,测其 120 s 内穿越原始平台位置的次数、在各象限的游动距离及平台象限游程占总游程的百分比。

六、统计学方法

采用 SPSS 17.0 版统计学软件进行数据处理,计量资料采用($\bar{x}\pm s$)形式表示,组间比较采用 t 检验,$P<0.05$表示差异有统计学意义。

结 果

与正常组比较,假手术组、VD 组、HBOT 组的平均逃避潜伏期较长($P<0.05$)。假手术组、VD 组、HBOT 组的平台象限穿越次数均少于正常组,平台象限游动距离均较短,其占总游程的百分比均低于正常组($P<0.05$)。与假手术组比较,VD 组和 HBOT 组的平均逃避潜伏期较长($P<0.05$)。VD 组、HBOT 组的平台象限穿越次数均少于假手术组,平台象限游动距离均较短,其占总游程的百分比均低于假手术组($P<0.05$)。与 VD 组比较,HBOT 组平均逃避潜伏期较短,平台象限穿越次数较多,平台象限游动距离较长,其占总游程的百分比较高,差异有统计学意义($P<0.05$)。详见表 1。

表 1　各组大鼠逃避潜伏期、平台象限穿越次数、平台象限游动距离及其占总游程百分比的比较($\bar{x}\pm s$)

组别	只数	平均逃避潜伏期(s)	平台象限穿越次数(次)	平台象限游动距离(cm)	占总游程百分比(%)
正常组	15	16.82±4.31	13.24±3.17	596.84±230.15	36.44±8.01
假手术组	15	22.97±5.03[a]	11.90±4.59[a]	527.47±193.73[a]	33.87±6.53[a]
VD 组	15	30.41±9.15[ab]	7.08±3.36[ab]	341.51±187.38[ab]	22.59±4.70[ab]
HBOT 组	15	25.73±6.20[abc]	9.51±2.25[abc]	494.72±208.26[abc]	28.71±5.32[abc]

注:与正常组比较,[a]$P<0.05$;与假手术组比较,[b]$P<0.05$;与 VD 组比较,[c]$P<0.05$

撤除平台后大鼠的游泳轨迹图显示,正常组、假手术组、HBOT 组大鼠寻找平台的方式为探索式,具有明显的目的性和范围性。VD 组大鼠寻找平台时表现出毫无目的的状态,寻找方式为随机式、边缘式。

讨　论

VD 患者每 5.3 年增加 1 倍,其患病率仅次于阿尔兹海默病[4]。VD 的发病机制可能为脑多发性梗死时脑动脉狭窄或闭塞,引起脑组织灌注量减少,神经细胞兴奋性降低,导致脑代谢率下降,进而出现思维过程缓慢、认知功能下降[5-6]。有研究报道,学习记忆功能障碍可能是由脑缺血再灌注损伤诱发多种病理生理性改变、进而导致神经细胞发生凋亡所引起[7]。

与记忆功能有关的脑区有海马、纹状体、前额叶皮质,其中海马区在学习记忆过程中发挥着重要作用。有研究发现,HBOT 后大鼠脑组织中超氧化物歧化酶(superoxide dismutase,SOD)水平升高,丙二醛(malondialdehyde,MDA)水平降低,海马存活神经元数量增多,提示 HBO 可通过拮抗氧自由基、减少神经细胞凋亡数目等方式发挥对缺血缺氧性脑损伤的保护作用[8-9]。脑源性神经营养因子(brain-derived neurotrophic factor,BDNF)对中枢神经系统内多种类型神经元的生长、发育、分化、维护和再生都具有显著作用[10]。有研究利用微透析技术观察局灶性脑缺血再灌注鼠模型时发现,HBO 早期干预可改善脑部纹状体的代谢,减少兴奋性氨基酸的毒性作用,保护缺血脑组织[11]。在实验中还发现 HBO 可明显抑制神经肽 $Y_{1\text{-}36}$ 免疫活性物(immunoreactive neuropeptide $Y_{1\text{-}36}$, ir-$NPY_{1\text{-}36}$)升高,减少血管收缩,改善脑的供氧供血情况,减轻脑损伤[12]。有研究报道,HBO 可上调胶质细胞源性神经营养因子(glial cell line-derived neurotrophic factor,GDNF)和神经生长因子(nerve growth factor,NGF)的表达水平,有利于保护神经并促进神经修复[13]。

本研究采用改良四动脉法进行 VD 造模,此法可导致大鼠脑部严重缺血,损害其海马等与智能相关部位的功能。脑干部分由于有脊髓前动脉的供血,尚能维持正常的生理状态,缺血后生理指标稳定,病理改变充分、明确,无明显肢体运动功能障碍,是目前国际公认的 VD 造模方法之一[2]。本研究结果显示,VD 组、HBOT 组大鼠的学习记忆能力低于正常组及假手术组,HBOT 组大鼠的学习记忆能力显著优于 VD 组($P<0.05$)。撤出平台后,正常组、假手术组、HBOT 组大鼠寻找平台具有明显的目的性和范围性,VD 组对平台所在象限及空间位置尚未形成记忆,提示 HBOT 可有效改善 VD 大鼠的学习记忆能力。

HBO 对 VD 的治疗作用可能与以下机制相关:①HBO 可以提高血氧分压、血氧含量,提高血氧弥散半径[14];②HBO 能调节血管舒缩功能,减轻脑水肿,降低颅内压,改善组织微循环,增强微循环血液流变功能,促进侧支循环建立[15];③HBO 可以改善缺血半暗带神经元细胞的有效氧供,促进其恢复功能[16];④HBO 可以减少神经元凋亡数目,减轻脑损伤;⑤HBO 对神经营养因子可产生保护及促修复作用。

本研究发现,HBOT 对提高 VD 大鼠的学习记忆能力有积极作用。假手术组大鼠的多项指标较正常组差,提示手术创伤对其造成了影响,其原因尚待进一步研究证实。

参　考　文　献

[1] Pulsinelli WA, Brierley JB. A new model of bilateral hemispheric ischemia in the unanesthetized rat[J]. Stroke, 1979, 10(3): 267-272.

[2] 曾贵刚,李峻,彭海东,等.大鼠血管性痴呆动物模型的研究进展[J].中国比较医学杂志,2012,22(3):50-55. DOI:10.3969/j.issn.1671.7856.2012.003.011.

[3] 陈翔,高风超,田新英.血管性痴呆动物模型的研究现状[J].医学综述,2013,19,(13):2379-2382. DOI:10.3969/j.issn.1006-2084.2013.13.025.

[4] Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association[J]. Stroke, 2011, 42(9): 2672-2713. DOI: 10.1161/STR.0b013e3182299496.

[5] Levine DA, Langa KM. Vascular cognitive impairment: disease mechanisms and therapeutic implications[J]. Neurotherapeutics, 2011, 8(3): 361-373. DOI: 10.1007/s13311-011-0047-z.

[6] 朱燕平,寇雪莲,何松彬,等.高压氧联合复方海蛇胶囊对血管性痴呆大鼠学习记忆能力行为学的影响[J].中国康复医学杂志,2013,28(1):18-22. DOI:10.3969/j.issn.1001-1242.2013.01.005.

[7] 陈浩,毕竹梅,崔宁,等.不同时间窗高压氧对脑缺血-再灌注大鼠模型学习记忆功能的影响[J].中国老年学杂志, 2007,27(15):1447-1449.

[8] 冯娟娟,何予工,梁维娣,等.脑缺血后立即高压氧治疗对脑缺血再灌注损伤大鼠脑梗死体积的影响[J].中华物理医学与康复杂志,2014, 36(2): 141-143. DOI: 10.3760/cmaj.issn.0254-1424/2014.02.017.

[9] 陈晶,陈燕惠,吕红艳,等.缺氧缺血新生大鼠脑组织超氧化物歧化酶、丙二醛的变化及高压氧的保护作用[J].中国儿童保健杂志,2012,20(12):1094-1097.
[10] 张禹,潘树义,李航,等.高压氧对脑损伤大鼠 BDNF 表达的影响[J].中国医药导报,2012,9(17):19-21.
[11] Badr AE, Yin W, Mychaskiw G, et al. Effect of hyperbaric oxygen on striatal metabolites: a microdialysis study in awake freely moving rats after MCA occlusion[J]. Brain Res, 2001, 916(1-2): 85-90.
[12] 巨容,封志纯.高压氧对缺氧缺血性脑损伤的保护作用及机制研究概述[J].中国新生儿科杂志,2007,22(3):186-188.
[13] 张祥根,姜正林,王国华,等.高压氧治疗创伤性脑损伤的效用及机制研究[J].中国应用生理学杂志,2012,28(1):42-46.
[14] 徐江,黄晓琳.高压氧治疗脑缺血再灌注损伤的疗效、时间窗及机制[J].神经损伤与功能重建,2007,2(3):168-70.
[15] Gao-Yu C, Cong-Yina D, Li-Jun Z, et al. Effects of hyperbaric oxygen preconditioning on energy metabolism and glutamate level in the peri-infarct area following permanent MCAO[J]. Undersea Hyperb Med, 2011, 38(2): 91-99.
[16] Carson S, McDonagh M, Russman B, et al. Hyperbaric oxygen therapy for stroke: a systematic review of the evidence[J]. Clin Rehabil, 2005, 19(8): 819-833.

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·外刊撷英·

Risk of pre-stroke antiplatelet therapy

BACKGROUND AND OBJECTIVE Intravenous tissue plasminogen activator (tPA) is known to improve outcomes after ischemic stroke. Many patients receive antiplatelet therapy before ischemic stroke, with the concern that those individuals might face an increased risk of bleeding when treated with tPA. This study assessed the risks and benefits associated with pre-stroke antiplatelet therapy among patients with ischemic stroke who received intravenous tPA.

METHODS Data were obtained from 85,072 adult patients with ischemic stroke who received intravenous tPA. Of these, 38,844 had been receiving antiplatelet therapy before hospital admission. The participants were followed for outcomes including symptomatic intracranial hemorrhage (sICH), in-hospital mortality, discharge ambulatory status and modified Rankin scale score (mRS).

RESULTS After risk adjustment, the prior use of antiplatelet agents was found to be associated with a higher onset of sICH (OR 1.18), with a small, absolute increased risk of 0.68%. Prior antiplatelet therapy was not associated with a significantly greater risk of in-hospital mortality. Those receiving antiplatelet therapy had higher ratios of being discharged to home, of independent ambulation and of better mRS scores at discharge.

CONCLUSION This study of patients with acute ischemic stroke who received tPA found that those receiving pre-stroke antiplatelet therapy were at increased risk of symptomatic intracranial hemorrhage, but also had better functional outcomes than those who had not received antiplatelet therapy.

【摘自:Xian Y, Federspiel JJ, Grau-Sepulveda M, et al. Risks and benefits associated with pre-stroke antiplatelet therapy among patients with acute ischemic stroke treated with intravenous tissue plasminogen activator. JAMA Neurol, 2016, 73(1): 50-59.】

Cerebrolysin and stroke recovery

BACKGROUND AND OBJECTIVE Ischemic stroke is the second most common cause of death worldwide, and the third leading cause of loss of disability-adjusted life years. Cerebrolysin, a neuropeptide preparation consisting of low molecular weight neuropeptides and free amino acids, has been shown by animal studies to have neuroprotective properties. This study analyzed the efficacy and safety of cerebrolysin during post-stroke recovery.

METHODS Adult patients with ischemic supratentorial strokes were recruited for randomization. The subjects received either cerebrolysin, at 30 mL per day, or a placebo, once daily for 21 days, beginning at one to three days after stroke onset. The primary efficacy criterion was the change in the Action Research Arm Test (ARAT) of upper limb motor function from baseline to day 90. Secondary outcome measures were changes from baseline in gait velocity, fine motor function, global neurologic state, level of disability or dependence in activities of daily living, aphasia, neglect, quality of life and depression.

RESULTS For the 205 patients included in the study, the ARAT scores improved from 10.1 at baseline to 40.7 on day 90 in the cerebrolysin group, and from 10.7 to 26.5 in the placebo group($P<0.0001$). A multivariate assessment of global status, as assessed by 12 outcome measures, demonstrated superiority of the treatment group as compared to the placebo group ($P<0.0001$).

CONCLUSION This study of patients with ischemic stroke found that treatment with cerebrolysin, beginning 24 to 72 hours after stroke onset, had a beneficial effect on functional and global outcome.

【摘自:Muresanu DF, Heiss WD, Hoemberg V, et al. Cerebrolysin and recovery after stroke (cars): randomized, placebo controlled, double-blind, multicenter trial. Stroke, 2016, 47(1): 151-159.】