常压氧与高压氧对成年大鼠脑缺血再灌注损伤后微血管新生影响的比较 *

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摘要 目的：研究常压氧与高压氧对成年大鼠脑缺血再灌注损伤后微血管新生影响的差异。方法：将成年 SD 雄性大鼠随机分为三组：假手术组（SS 组）、常压氧治疗组（NBO 组）、高压氧治疗组（HBO 组），每组又随机分为 3、7、10 天三个亚组。采用线栓法对 NBO 组和 HBO 组大鼠进行大脑中动脉栓塞（MCAO），缺血 1.5 小时后拔出栓子再灌注，NBO 组进行常压氧治疗，HBO 组进行高压氧治疗。大鼠分别在 3、7、10 天麻醉处死，取脑组织切片，血管内皮生长因子（VEGF）、VEGF 受体 -1（FLT-1）和 CD34 免疫组化染色，光镜观察取图和统计分析。结果：NBO 各组与 SS 各组相比，VEGF、FLT-1 和 CD34 阳性细胞数目均明显增多（P＜0.05）；HBO 各组与 NBO 各组比较，7 天、10 天组 VEGF、FLT-1 和 CD34 阳性细胞数目均显著增多（P＜0.05）。结论：HBO 治疗较 NBO 治疗对成年大鼠微血管的新生更有促进作用。

关键词 高压氧 脑缺血 再灌注损伤 中动脉栓塞 血管新生

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Comparison of Normobaric Oxygen and Hyperbaric Oxygen on Angiogenesis in the Brain of Adult Rats with Cerebral Ischemia-Reperfusion Injury*

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ABSTRACT Objective: To investigate the difference between normobaric oxygen (NBO) and hyperbaric oxygen (HBO) on angiogenesis in the brain of adult rats with cerebral ischemia-reperfusion injury. Methods: Adult male Sprague-Dawley (SD) rats were randomly divided into three groups: the sham surgery (SS) group, the NBO group and the HBO group. Each group was randomly divided into three subgroups: the 3 days group, the 7 days group and the 10 days group. Middle cerebral artery occlusion (MCAO) was performed to the NBO and HBO groups to develop the animal model. 1.5 hours later, reperfusion was made. Then, the NBO group received NBO treatment, and the HBO group received HBO treatment. All the rats were sacrificed respectively on day 3, 7, 10; Cerebral samples were collected and prepared to sections. The sections were treated with immunohistochemical staining with the following antibody: Vascular endothelial growth factor (VEGF), VEGF receptor-1 (Flt-1), CD34. The changes in ischemia region were detected under the optical microscope , and the pictures were taken. Results: Compared with those in the SS group, numbers of VEGF and Flt-1 and CD34 positive cells increased markedly in the NBO group (P＜0.05). The numbers of VEGF and Flt-1 and CD34 positive cells increased significantly on day 7 and day 10, following HBO treatment compared with those in the NBO group (P＜0.05). Conclusions: HBO therapy is better to promote angiogenesis in the brain of adult rats with ischemia-reperfusion injury than NBO treatment.

Key words: HBO; Cerebral ischemia; Reperfusion injury; Middle cerebral artery occlusion ;Angiogenesis

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前 言

脑血管病在临床上有高患病率、高复发率、高致残率、高死亡率的 " 四高 " 特点，已成为严重危害人类健康的主要疾病之一[1]；而缺血性脑血管病的死亡率为脑血管病的主要部分，约占 75%[2]。缺血性脑病又会发生再灌注损伤，因而称其为缺血再灌注损伤[3]。并且随着我国人口老龄化，缺血再灌注损伤所带来的经济和社会负担将日益加重，因此，如何预防和治疗缺血再灌注损伤，已成为当今生命科学领域研究的热点。

高压氧作为一种无创疗法对缺血再灌注损伤的疗效已得到医学上的肯定[4-7]；也有文献报道常压氧治疗缺血再灌注损伤亦有一定疗效[8]；但也有文献报道常压氧治疗缺血再灌注损伤有一定疗效，而高压氧却没有疗效[9]。本实验通过制作成年大鼠大脑中动脉栓塞（Middle cerebral artery occlusion, MCAO）模型，观察 NBO、HBO 治疗后微血管的变化，为临床治疗脑缺血再灌注损伤提供理论依据。

1 材料和方法

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1.1 实验动物及分组

清洁级成年雄性 SD 大鼠，体重 250-280 g，均购于第四军医大学实验动物中心。随机分为 3 组：假手术组（SS 组）、常压氧治疗组（NBO 组）、高压氧治疗组（HBO 组），每组又随机分为 3、7、10 天三个亚组，每个亚组各 10 只大鼠。SS 组为正常大鼠。

1.2 仪器和试剂

上海 DWC450-1150 型高压氧动物实验舱，Finesse325 切片机，OlympusBX51 显微镜，尼龙线栓（北京沙东生物技术有限公司），CD34 兔多克隆抗体，VEGF 兔多克隆抗体，Flt 兔多克隆抗体，SABC 免疫组化试剂盒（均为武汉博士德生物有限公司）。

1.3 制作 MCAO 模型

NBO、HBO 组大鼠采用线栓法制作 MCAO 模型[10-11]。2%戊巴比妥钠麻醉 SD 大鼠后（40 mg/Kg 体质量），酒精消毒颈部，切开皮肤，分离暴露右侧颈总动脉、颈外动脉、颈内动脉，依次结扎颈总和颈外动脉，将尼龙线栓缓慢插入颈内动脉 20-22 mm，1.5 h 后拔出线栓再灌注。

1.4 HBO 治疗

HBO 组大鼠在模型制作后 4h 第一次进行 HBO（0.28 MPa[12], 60 min）治疗，之后 1 次 /d，三个亚组分别治疗 3、7、10 天。

1.5 NBO 治疗

NBO 组大鼠在模型制作后 4h 进行第一次 NBO（0.10 MPa，纯氧，60 min）[13]治疗，之后 1 次 / d，三个亚组分别治疗 3、7、10 天。

1.6 组织切片

2%戊巴比妥钠麻醉大鼠，依次经心脏灌注生理盐水 150 ml、4%多聚甲醛液 400 ml 后，迅速取脑，4%的多聚甲醛后固定 24 h。常规脱水后石蜡包埋，选择视交叉平面行 4 μm 冠状切片。

1.7 免疫组织化学染色

采用三步法免疫组织化学染色。一抗：VEGF 兔多克隆抗体，CD34 兔多克隆抗体，Flt 兔多克隆抗体，均 1:50 稀释。二抗：生物素标记羊抗兔 lgG，1:100 稀释，再用 SABC，1:100 稀释，最后 DAB 显色，苏木精复染。

1.8 组织学观察

每张切片在梗死周边区随机选取 8 个视野× 400 倍显微镜，分别观察每个视野内 VEGF、SD34、Flt-1 阳性细胞数，并取其平均值。

1.9 统计学处理

采用 SPSS 11.0 统计软件进行统计学分析，结果用均数± 标准差（$\bar{x}\pm s$）表示，组间比较采用方差分析，$P<0.05$ 为差异有统计学意义。

2 结果

2.1 CD34 阳性表达

SS 组 CD34 阳性细胞表达较少，散在分布于脑组织的各个区域，染色较淡，多呈细条索状，少量圆型和椭圆型。NBO 组和 HBO 组 CD34 阳性细胞主要表达于皮层区和梗死周边，多呈条索状，少量呈圆形和椭圆形，数量增多，染色深，体积明显增大，梗死区及对侧非梗死区阳性细胞表达很少。HBO 组 CD34 阳性细胞表达较 NBO 组同时间点，数目均有所增多，各亚组的差异均有统计学意义（$P<0.05$）（见表 1 和图 1）。

表 1 各组大鼠梗死周边区 CD34 阳性细胞表达的比较（$\bar{x}\pm s$）

Table1 Comparison of the positive cells of CD34 in the peripheral of infarction area in each group（$\bar{x}\pm s$）

Group	Number	3 d	7 d	10d
SS Group	10	3.1± 0.39	3.7± 0.74	3.5± 0.51
NBO Group	10	11.3± 3.56[a]	13.7± 2.68[a]	8.3± 3.55[a]
HBO Group	10	13.4± 4.31[ab]	28.6± 6.45[ab]	33.7± 4.87[ab]

注：与 SS 组同时间点比较 [a]$P<0.05$；与 NBO 组同时间点比较 [b]$P<0.05$。

Note: compared with SS group in the same time [a]$P<0.05$; compared with NBO group in the same time [a]$P<0.05$.

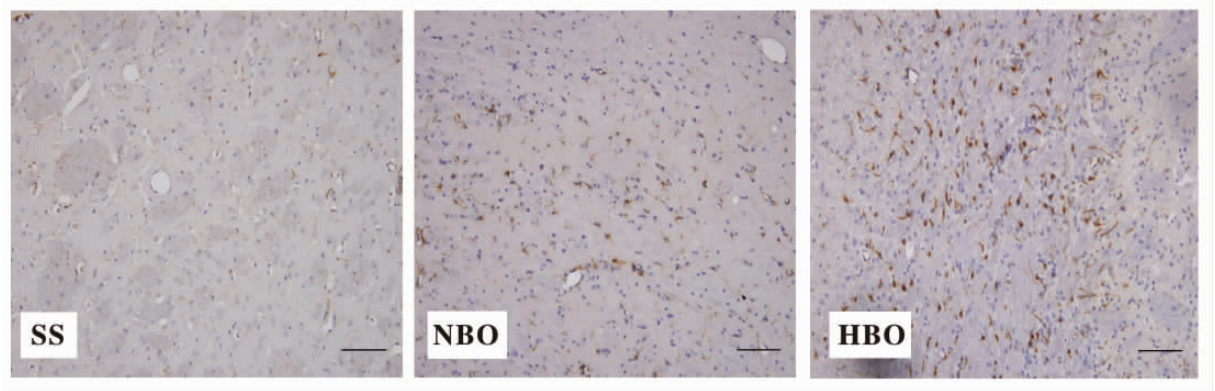

图 1 各组 7 天时 CD34 免疫组化染色结果（× 400，标尺 =50μm），染色成棕黄色的细胞为阳性细胞。

Fig. 1 The immunohistochemical staining results with CD34 antibody (× 400, scale=50μm) , the brown cells is positive cells.

2.2 VEGF 阳性表达

SS 组 VEGF 阳性细胞表达非常少，散在分布，染色浅。HBO 和 NBO 组 VEGF 阳性细胞表达大量增加，多呈圆形和椭圆形，大小不一，染色较深，主要分布在梗死周边区，其次是皮层区，梗死区及对侧非梗死区表达较少。HBO 组与 NBO 组相同时间点相比，阳性细胞染色加深且数目均有所增加，各亚组的差异均有统计学意义（P＜0.05）（见表 2 和图 2）。

表 2 各组大鼠梗死周边区 VEGF 阳性细胞表达的比较（$\bar{x}\pm s$）

Table 2 Comparison of the positive cells of VEGF in the peripheral of infarction area in each group（$\bar{x}\pm s$）

Group	Number	3 d	7 d	10d
SS group	10	4.6± 0.45	4.8± 0.67	4.5± 0.23
NBO group	10	13.4± 4.33[a]	16.6± 5.89[a]	15.3± 2.57a
HBO group	10	25.3± 7.21[ab]	40.5± 6.28[ab]	43.6± 5.49[ab]

注：与 SS 组同时间点比较 [a]P＜0.05；与 NBO 组同时间点比较 [b]P＜0.05

Note: compared with SS group in the same time [a]P＜0.05; compared with NBO group in the same time [a]P＜0.05

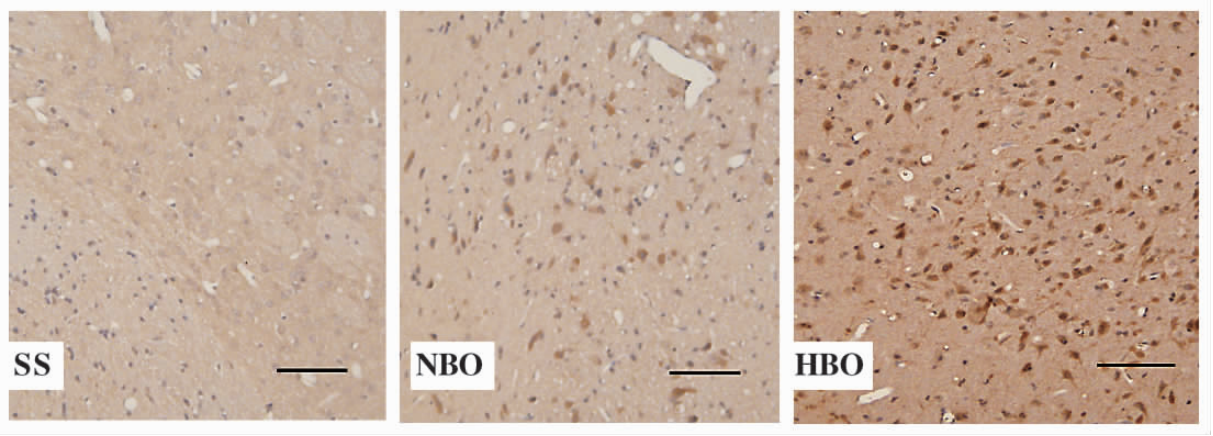

图 2 各组 7 天时 VEGF 免疫组化染色结果（× 400，标尺 =50μm），染色成棕黄色的细胞为阳性细胞。

Fig. 2 The immunohistochemical staining results with VEGF antibody (× 400, scale=50μm) , the brown cells is positive cells.

2.3 Flt-1 阳性表达

SS 组 Flt-1 阳性细胞表达少，散在分布。NBO 组 Flt-1 阳性细胞表达量增加，主要分布在梗死周边和皮层区，7d 亚组数量明显增多，多呈椭圆形和圆形，与 SS 组相比，其数量差异有统计学意义（P＜0.05）。HBO 组与 NBO 组相同时间点相比，阳性细胞染色加深且数目均有所增加，各亚组的差异均有统计学意义（P＜0.05）（见表 3 和图 3）。

表 3 各组大鼠梗死周边区 Flt-1 阳性细胞表达的比较（$\bar{x}\pm s$）

Table 3 Comparison of the positive cells of Flt-1 in the peripheral of infarction area in each group（$\bar{x}\pm s$）

Group	Number	3 d	7 d	10d
SS group	10	3.1± 0.26	3.9± 0.53	3.3± 0.65
NBO group	10	16.7± 4.72[a]	17.4± 4.30[a]	16.5± 4.61[a]
HBO group	10	25.6± 4.67[ab]	36.8± 5.58[ab]	37.7± 4.95[ab]

注：与 SS 组同时间点比较 [a]P＜0.05；与 NBO 组同时间点比较 [b]P＜0.05。

Note: compared with SS group in the same time aP＜0.05; compared with NBO group in the same time [a]P＜0.05.

3 讨论

增加氧供已成为脑缺血再灌注损伤的一种重要治疗方式。依据供氧方式的不同，氧疗分为常压氧疗和高压氧疗。高压氧相对于常压氧来说，不仅仅是量的不同，它有严格的定义：① 必须是氧分压超过 100KPa(必须超过常压纯氧水平)；② 是在高气压环境下吸入高浓度氧气(因为在 5 个大气压下吸入空气也可达到 100KPa 的氧分压，所以定义必须明确高气压下吸入低浓度氧不能称高压氧)；③ 对多种疾病可产生特殊疗效的氧气才能称高压氧[14]。血液是提供大脑细胞生存所需葡萄糖和氧气的唯一途径[15]，因此，促进大脑微血管新生，对缺血再灌注损伤是一种非常有价值的治疗方法。血管新生需要很多因子调

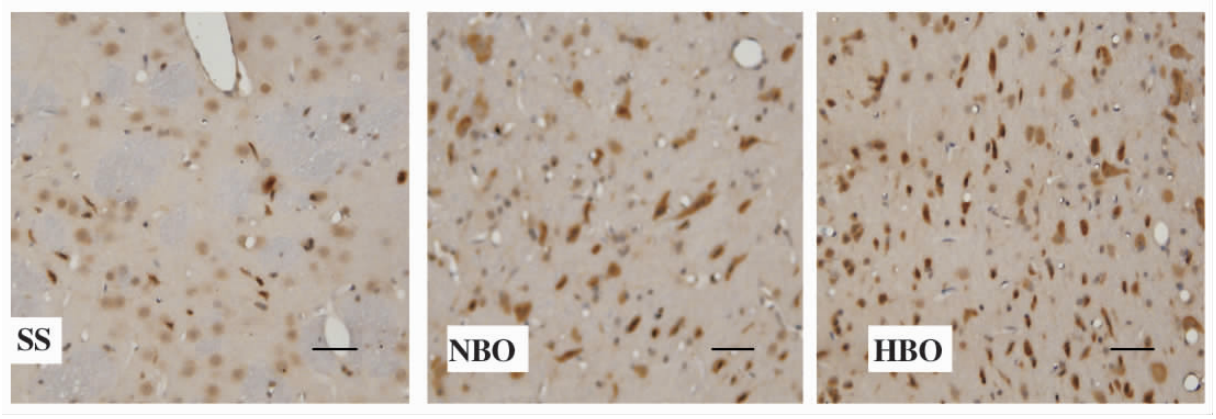

图 3 各组 7 天时 Flt-1 免疫组化染色结果 (免疫组织化学染色 × 400),染色成棕黄色的细胞为阳性细胞。

Fig. 3 The immunohistochemical staining results with Flt-1 antibody (SS, NBO, HBO× 400) , the brown cells is positive cells.

节,其中 VEGF 结合它的受体导致了内皮细胞的活化、增殖、转移、侵入和存活,是主要的调节者[16-17]。CD34 是一种新型的微血管标记物,特异性最高,优于内皮细胞的其他标记物[18],主要表达于毛细血管内皮细胞,大血管内皮细胞表达较少[19-20]。因此,本实验采用 VEGF 及其受体 -1 (Flt-1) 和 CD34 作为标记物。

本实验结果显示,NBO 各组大鼠较同时间点 SS 组大鼠 CD34、VEGF、Flt-1 阳性细胞表达都明显增强,具有统计学意义。HBO 各组大鼠较同时间点 NBO 组大鼠 CD34、VEGF、Flt-1 阳性细胞表达也都明显增强,具有统计学意义。阳性细胞表达都在梗死周边区和皮层区,梗死区和对侧大脑阳性细胞表达较少,可能是因为梗死区组织损坏较严重,不利于血管新生,对侧大脑未受缺血刺激,因而阳性细胞表达较少。

综上所述,HBO 较 NBO 治疗大鼠脑缺血再灌注损伤具有更显著地效果,但其需要高压氧舱、专业技术人员等条件。在缺乏上述条件的情况下应用操作简便、安全易行的 NBO 治疗亦有一定效果。但具体机制尚不明确,需待进一步研究。

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