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硕 士 学 位 论 文

迷走神经刺激联合高压氧治疗对脑缺血损伤的神经保护作用及机制实验研究

Experimental Study of Neuroprotective Effect on Combination of Vagus Nerve Stimulation and Hyperbaric Oxygen Treatment against Cerebral Ischemic Injury and Its Related Mechanism

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摘 要

目的：建立大鼠短暂性和永久性脑缺血模型，通过迷走神经刺激、高压氧治疗及两者联合治疗等对其进行干预，观察其神经行为学评分、脑梗死体积、脑肿胀程度、脑组织病理学变化及脑内炎症细胞因子表达情况等，探讨两者联合治疗是否具有更显著的神经保护效应，及其可能存在的效应机制。

方法：根据脑缺血时间不同设置短暂性脑缺血组（T-MCAO 组）及永久性脑缺血组（P-MCAO 组），每组再根据干预措施不同进一步分成 4 个亚组，即联合组（T-VNS+HBO 组/P-VNS+hBO 组,给予迷走神经刺激和高压氧治疗）、迷走神经刺激组（T-VNS 组/P-VNS 组，单纯行迷走神经刺激）、高压氧组（T-HBO 组/P-HBO 组，单纯行高压氧治疗）和对照组（T-Con 组/P-Con 组，无任何治疗措施）等。将雄性 SD 大鼠 176 只随机平均分配到每个亚组，22 只/亚组。T-MCAO 组采用线栓法将大脑中动脉闭塞 2h 后再灌注，建立短暂性脑缺血模型；P-MCAO 组采用线栓法将大脑中动脉闭塞后不灌注，建立永久性脑缺血模型。造模成功后，针对不同亚组给予相应的治疗措施，并于缺血 24h 后观察其神经行为学评分、脑梗死体积、脑肿胀程度、脑组织病理学变化及脑内炎症细胞因子表达情况等。

结果：T-MCAO 组内 4 亚组两两比较，结果示：①T-VNS+HBO 组神经行为学评分、脑梗死体积较 T-VNS 组、T-HBO 组和 T-Con 组均明显改善（$P<0.05$），镜下缺血灶边缘区神经细胞坏死及组织水肿程度明显减轻，脑内促炎细胞因子 TNF-α 和 IL-1β 表达水平明显降低（$P<0.05$），抑炎细胞因子 IL-10 水平明显升高（$P<0.05$），但是脑肿胀程度的改善只较 T-Con 组有统计学意义；②T-VNS 组和 T-HBO 组的神经行为学评分、脑梗死体积都较 T-Con 组有显著改善（$P<0.05$），镜下缺血灶边缘区神经细胞坏死及组织水肿程度也有较明显减轻，脑内促炎细胞因子 TNF-α 和 IL-1β 表达水平明显降低（$P<0.05$），抑炎细胞因子 IL-10 水平明显升高（$P<0.05$），但是脑肿胀程度的改善程度无统计学意义（$P>0.05$）；③T-VNS 组和 T-HBO 组之间的所有指标的差异都无统计学意义（$P>0.05$），镜下病理组织学表现相似。

P-MCAO 组内 4 亚组两两比较，结果示：①P-VNS+HBO 组神经行为学评分、

脑梗死体积较 P-VNS 组、P-HBO 组和 P-Con 组均明显改善（P<0.05），镜下缺血灶边缘区神经细胞坏死及组织水肿程度明显减轻，脑内促炎细胞因子 TNF-α 和 IL-1β 表达水平明显降低（P<0.05），抑炎细胞因子 IL-10 水平明显升高（P<0.05），但是脑肿胀程度的改善只较 P-Con 组有统计学意义；②P-VNS 组和 P-HBO 组的神经行为学评分、脑梗死体积都较 P-Con 组有显著改善（P<0.05），镜下缺血灶边缘区神经细胞坏死及组织水肿程度也有较明显减轻，脑内促炎细胞因子 TNF-α 和 IL-1β 表达水平明显降低（P<0.05），抑炎细胞因子 IL-10 水平明显升高（P<0.05），但是脑肿胀程度的改善程度无统计学意义（P>0.05）；③P-VNS 组和 P-HBO 组之间的所有指标的差异都无统计学意义（P>0.05），镜下病理组织学表现相似。

对 T-MCAO 组和 P-MCAO 组内干预相同的亚组进行组间比较，结果示：①与 P-VNS+HBO 组相比，T-VNS+HBO 组的神经行为学评分明显升高（P<0.05），脑梗死体积明显减小（P<0.05），镜下缺血灶边缘区神经细胞坏死及组织水肿程度减轻，脑内促炎细胞因子表达水平明显降低（P<0.05），抑炎细胞因子表达水平明显升高（P<0.05）,但脑肿胀程度并无明显改善（P>0.05）；②与 P-VNS 组相比，T-VNS 组的神经行为学评分明显升高（P<0.05），脑梗死体积明显减小（P<0.05），镜下缺血灶边缘区神经细胞坏死及组织水肿程度减轻，脑内促炎细胞因子表达水平明显降低（P<0.05），抑炎细胞因子表达水平明显升高（P<0.05）,但脑肿胀程度并无明显改善（P>0.05）；③与 P-HBO 组相比，T-HBO 组的神经行为学评分明显升高（P<0.05），脑梗死体积明显减小（P<0.05），镜下缺血灶边缘区神经细胞坏死及组织水肿程度减轻，脑内促炎细胞因子表达水平明显降低（P<0.05），抑炎细胞因子表达水平明显升高（P<0.05）,但脑肿胀程度并无明显改善（P>0.05）；④与 P-Con 组相比，T-Con 组的神经行为学评分明显升高（P<0.05），脑梗死体积明显减小（P<0.05），镜下缺血灶边缘区神经细胞坏死及组织水肿程度减轻，脑内促炎细胞因子表达水平明显降低（P<0.05），抑炎细胞因子表达水平明显升高（P<0.05）,但脑肿胀程度并无明显改善（P>0.05）。

结论：迷走神经刺激与高压氧治疗均可通过调控炎症反应对大鼠脑缺血损伤产生神经保护效应，并且该调控和保护效应在暂时性脑缺血模型比永久性脑缺血模型更显著。两者联合干预时可通过炎症调控效应的叠加产生更为显著的神经保护效应，并且该效应同样在暂时性脑缺血模型比永久性脑缺血模型更显著。

关键词：迷走神经刺激　高压氧　脑缺血　炎症　细胞因子　神经保护　机制

Abstract

Objective: To establish rat transient and permanent cerebral ischemia model and to give them vagus nerve stimulation , hyperbaric oxygen treatment and combined treatment of both. After the intervention, we observed their neurobehavioral score, infarct volume , brain swelling, brain histopathology and the expression of inflammatory cytokines in brain to detect whether combination therapy with vagus nerve stimulation and hyperbaric oxygen treatment have more significant neuroprotective effects , and to explore its potential mechanisms.

Methods: According to the different cerebral ischemia time, we setted transient cerebral ischemia group (T-MCAO group) and permanent cerebral ischemia group (P-MCAO group). And then everyone of them was divided further into four sub-groups respectively according to the different interventions, which included combined treatment group (T-VNS + HBO group / P-VNS + HBO group) , which were given vagus nerve stimulation and hyperbaric oxygen therapy, vagus nerve stimulation group (T-VNS group / P-VNS group), which were given simply vagus nerve stimulation, hyperbaric oxygen group (T-HBO group / P-HBO group), which were given simply hyperbaric oxygen therapy, and control group (T-Con group / P-Con group), which were not given any treatment. 176 male Sprague-Dawley rats were randomly assigned to each subgroup, 22 / subgroups. Transient Cerebral ischemia model of T-MCAO group was produced by intra-arterial filament occlusion of the right MCA (Middle Cerebral Artery) for 2 hours, and then reperfusion was achieved by withdrawal of the filament. Permanent Cerebral ischemia model of P-MCAO group was produced by intra-arterial filament occlusion of the right MCA, but no reperfusion was achieved. After succeful modeling, everyone of different subgroups were given appropriate therapic methods respectively. At 24 hours after ischemia, we observed neurobehavioral score, infarct volume, brain swelling, brain histopathology and the expression of inflammatory cytokines in brain.

Results: There were some results of pairwise comparison for 4 subgroups in T-MCAO group to be shown. ① Compared with T-VNS group, T-HBO group and T-Con group, T-VNS+HBO group's neurobehavioral score, infarct volume were significantly improved ($P < 0.05$), and necrosis and tissue edema were significantly

reduced in ischemic lesion marginal-zone under the microscope, and the expression levels of brain proinflammatory cytokine TNF-α and IL-1β were significantly lower (P <0.05), the expression levels of brain antiinflammatory cytokines IL -10 were significantly increased (P <0.05). However, its degree of brain swelling were not more significantly improved than T-VNS group and T-HBO group's but T-Con group's. ② Compared with T-Con group, T-VNS and T-HBO group's neurobehavioral score, infarct volume were significantly improved (P < 0.05), and necrosis and tissue edema were significantly reduced in ischemic lesion marginal-zone under the microscope, and the expression levels of brain proinflammatory cytokine TNF-α and IL-1β were significantly lower (P <0.05), the expression levels of brain antiinflammatory cytokines IL -10 were significantly increased (P <0.05). However, both of their degree of brain swelling were not more significantly improved than T-Con group's. ③ There was not any significant difference between T-VNS group and T-HBO group 's all indicators (P> 0.05), which included neurobehavioral score, infarct volume, brain swelling and the expression of inflammatory cytokines in brain. And the microscopic histopathology performance of them were similar to each other.

There were some results of pairwise comparison for 4 subgroups in P-MCAO group to be shown. ① Compared with P-VNS group, P-HBO group and P-Con group, P-VNS+HBO group's neurobehavioral score, infarct volume were significantly improved (P < 0.05), and necrosis and tissue edema were significantly reduced in ischemic lesion marginal-zone under the microscope, and the expression levels of brain proinflammatory cytokine TNF-α and IL-1β were significantly lower (P <0.05), the expression levels of brain antiinflammatory cytokines IL -10 were significantly increased (P <0.05). However, its degree of brain swelling were not more significantly improved than P-VNS group and P-HBO group's but P-Con group's. ② Compared with P-Con group, P-VNS group and P-HBO group's neurobehavioral score, infarct volume were significantly improved (P < 0.05), and necrosis and tissue edema were significantly reduced in ischemic lesion marginal-zone under the microscope, and the expression levels of brain proinflammatory cytokine TNF-α and IL-1β were significantly lower (P <0.05), the expression levels of brain antiinflammatory cytokines IL -10 were significantly increased (P <0.05). However, both of their degree of brain swelling were not more significantly improved than P-Con group's. ③ There was not any significant difference between P-VNS group and P-HBO group 's all indicators (P> 0.05), which included neurobehavioral score, infarct volume, brain

swelling and the expression of inflammatory cytokines in brain. And the microscopic histopathology performance of them were similar to each other.

There were some results of comparison between T-MCAO group and P-MCAO group's subgroups, which were given the same treatment.

① Compared with the P-VNS+HBO group , T-VNS+HBO group's neurobehavioral scores were significantly increased ($P < 0.05$), and infarct volume was significantly reduced ($P < 0.05$), and necrosis and tissue edema were significantly reduced in ischemic lesion marginal-zone under the microscope, and the expression levels of proinflammatory cytokine in brain were significantly decreased ($P < 0.05$), and the expression levels of antiinflammatory cytokines were significantly increased ($P < 0.05$)，but brain swelling was not improved. ② Compared with the P-VNS group , T-VNS group's neurobehavioral scores were significantly increased ($P < 0.05$), and infarct volume was significantly reduced ($P < 0.05$), and necrosis and tissue edema were significantly reduced in ischemic lesion marginal-zone under the microscope, and the expression levels of proinflammatory cytokine in brain were significantly decreased ($P < 0.05$), and the expression levels of antiinflammatory cytokines were significantly increased ($P < 0.05$), but brain swelling was not improved. ③ Compared with the P-HBO group , T-HBO group's neurobehavioral scores were significantly increased ($P < 0.05$), and infarct volume was significantly reduced ($P < 0.05$), and necrosis, and tissue edema were significantly reduced in ischemic lesion marginal-zone under the microscope, and the expression levels of proinflammatory cytokine in brain were significantly decreased ($P < 0.05$), and the expression levels of antiinflammatory cytokines were significantly increased ($P < 0.05$), but brain swelling was not improved. ④ Compared with the P-Con group , T-Con group's neurobehavioral scores were significantly increased ($P < 0.05$), and infarct volume was significantly reduced ($P < 0.05$), and necrosis and tissue edema were significantly reduced in ischemic lesion marginal-zone under the microscope, and the expression levels of proinflammatory cytokine in brain were significantly decreased ($P < 0.05$), and the expression levels of antiinflammatory cytokines were significantly increased ($P < 0.05$)，but brain swelling was not improved.

Conclusions: Both Vagus nerve stimulation and hyperbaric oxygen therapy can regulate the inflammatory response in order to produce neuroprotective effect for cerebral ischemic injury in rats, and the effect of regulation and nerve-protection on

transient cerebral ischemia model are greater than permanent cerebral ischemia model. When Combined Vagus nerve stimulation with hyperbaric oxygen therapy to treat cerebral ischmia, it have more significant effects of neuroprotective and inflammation regulation, and the effects on transient cerebral ischemia model are also greater than permanent cerebral ischemia model.

Keywords: vagus never stimulation; hyperbaric oxygen; cerebral ischemia; inflammation; cytokines; nouroprotective; mechanism

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