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The effect of heliox treatment in a rat model of focal transient cerebral ischemia

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ABSTRACT

Manipulation of inhaled gases during ischemia/reperfusion is a potential novel therapy for acute stroke. We previously found that treatment with a mixture of 70%/30% helium/oxygen (heliox) or 100% oxygen protects the brain against acute focal ischemia–reperfusion injury. This study evaluates the potential neuro-protective effects of delayed heliox treatment and its dose response effects in a rat transient focal cerebral ischemia model. Adult male rats were subjected to 2-h middle cerebral artery occlusion and then assigned to 1 of 4 inhaled gas exposure groups: I: 70%/30% nitrogen/oxygen (control); II: 70%/30% helium/oxygen administered immediately after occlusion; III: 70%/30% helium/oxygen administered after a 30–60 min delay; or, IV: 40%/30%/30% nitrogen/helium/oxygen administered immediately after occlusion. Outcome measurements included infarct size and neurological deficit score. Mean infarct sizes from groups I to IV were 228, 35, 109, and 124 mm^3 respectively ($p = 0.012$). Only group II had significantly smaller infarct size compared to the control group ($p = 0.008$). In addition, only Group II had a significantly lower neurological deficit score at 24 h post ischemia when compared to the control group ($p < 0.001$). Since heliox reduced infarct size and improved neurological deficit scores if initiated immediately after onset of ischemia, it may be a useful adjuvant to other stroke therapies.

Manipulation of inhaled gases during ischemia constitutes a potential novel therapy for acute stroke. We previously found that a mixture of 70%/30% helium/oxygen (heliox) protects the brain against acute focal ischemia–reperfusion injury [24]. Helium is a colorless, odorless, tasteless, and nontoxic gas at room temperature. It is non-reactive with body tissues and relatively insoluble in body fluids [9]. Normobaric hyperoxia [7,12,13,25,26], helium [3,24], xenon [2] and argon [15] all induce a reduction of cerebral ischemic injury. This study evaluates the therapeutic window and dose response of heliox in a rat transient focal cerebral ischemia model.

The Animal Care Committee of Saint Louis University approved all procedures. Adult male Sprague-Dawley rats weighted 287–329 g (Harlan, Indianapolis, IN) were anesthetized with 4% isoflurane mixed with 100% O_2 for induction, then maintained with 1.5% isoflurane in 70%/30% nitrogen/oxygen while spontaneously breathing through a face mask of concentric rings of Plexiglas. The inner cylinder conveyed gas for inhalation while the outer cylinder was under negative pressure to prevent re-breathing of exhaled gas. These rats were subjected to right middle cerebral artery occlusion (MCAO) with a filament for 2-h and a 1-h reperfusion [24]. During surgery, MCAO and the first hour of reperfusion, the rat was placed on a heating plate, and rectal temperature was monitored and maintained at 37 °C by a regulated heating plate. Oxygen saturation was monitored constantly with a pulse oximeter (Nonin Medical, Plymouth, MN) and was 96 ± 1.4% ($M \pm SD$) throughout the procedure. Regional cerebral blood flow was monitored with a laser Doppler probe (1 mm diameter) glued to the right parietal bone to monitor regional cerebral blood flow velocity (MoorLab, Devon, UK).

At the time of MCAO, the rats were randomly assigned to one of five groups: control, immediate heliox, 30 min delay heliox, 60 min delay heliox, and immediate partial heliox groups. Since there was no significant difference between the 30 and 60 min delay heliox treatment groups, they were combined as a single delayed treatment group. Thus, for analyses we had four treatment groups (I) 70%/30% nitrogen/oxygen control ($n = 8$); (II) immediate 70%/30% helium/oxygen ($n = 6$); (III) delayed 70%/30% helium/oxygen ($n = 8$); and (IV) immediate 40%/30%/30% nitrogen/helium/oxygen ($n = 9$). The delayed treatment group (Group III) inhaled 70%/30% nitrogen/oxygen, until the heliox treatment was initiated at 30 or 60 min post ischemia onset. Rats continued breathing the assigned gas mixture during the first hour of reperfusion in all groups. At the end of 1 h of reperfusion, anesthesia was terminated and the rats began to breathe room air.

Transient focal cerebral ischemia was induced by a modification of the intraluminal filament model in the rat [16]. A 3 cm parasagittal incision was made over the right common carotid artery (CCA).

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The right external carotid artery (ECA) was ligated, transected, and rotated 180° proximally. The pterygopalatine artery was ligated. The proximal CCA and distal internal carotid artery (ICA) were clamped transiently with aneurysm clips. A length of 30 mm filament (diameter 0.259 mm) with an enlarged tip of 0.345 mm was inserted from the ECA into the lumen of the ICA. The filament was advanced about 20 mm from CCA bifurcation until it blocked the origin of the MCA, which was confirmed by reduction in regional cerebral blood flow to 26 ± 12% ($M \pm SD$) baseline level. After 2 h MCAO, reperfusion was achieved by withdrawing the filament from the ICA. The neck and skull incisions were closed with a suture.

Neurological deficits were recorded at both 3 and 24 h post MCAO using Hunter's neurological scores [11]: 1, failure to extend left forepaw fully; 2, decreased grip of left forelimb while tail pulled; 3, spontaneous circling or walking to contralateral side; 4, walks only when stimulated with depressed level of consciousness; 5, unresponsive to stimulation.

Rats were euthanized at 24 h after MCAO. Brains were removed, cut into 2 mm slices in a brain matrix, and stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC, Sigma, St. Louis, MO) for 15 min at 37 °C [5]. Brain slices were refrigerated in 4% paraformaldehyde in 0.1 M phosphate buffered saline. Photographs were captured digitally (MicroImager II, Q-Imaging Corp., Burnaby, BC, Canada) and saved in the computer with Northern Eclipse software (Empix Imaging, Inc. Mississauga, ON, Canada). Infarct lesion area, right and left hemisphere were measured by a blinded investigator using the imaging analysis capabilities of the Northern Eclipse software. Area was then used to calculate to volume from known section thinness. Brain edema was estimated as an edema index (ipsilateral hemisphere volume divided by contralateral hemisphere volume), and an edema corrected infarct volume was calculated by the infarct lesion volume divided by the edema index [28].

Significant differences were determined statistically using the non-parametric Kruskal–Wallis test for comparisons across groups followed by the Mann–Whitney U test for multiple pair-wise comparisons with each group being compared to the control group. Because of small sample size, the Hunter's neurological scores were dichotomized: scores of 1 and 2 were considered a good outcome while scores of 3–5 were considered a poor outcome. Neurological scores at 3 and 24 h were then compared between each treatment group and control group with Fisher's exact test. Comparisons were considered significantly different when $p < 0.05$ (2-tailed) and $p < 0.01$ (2-tailed) for multiple comparisons. All statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago IL).

The ischemic infarct was visualized as the unstained area on the TTC stained sections (Fig. 1A). The infarct volume of each rat is shown in a scatter dot blot (Fig. 1B). The mean infarct volume from groups I to IV were 228, 35, 109, and 124 mm^3 respectively with a significant difference in groups ($p = 0.012$). Although delayed and partial helium treatments trended towards reduction of infarct size, only the immediate heliox treatment group had a significantly smaller infarct volume compared to the control group ($p = 0.008$).

At 3-h post MCAO all but one rat exhibited spontaneous circling or walking to the side contralateral to the lesion (neurological score of 3; Fig. 1C). The exception was a rat in group II which exhibited a decreased grip of the left forelimb and received a neurological score of 2. At 24 h post MCAO, all rats in group II had improved their neurological scores by 1 or 2 points, but the majority of rats in the other groups remained unchanged from the 3 h time point post MCAO. Exceptions were: one rat worsened 1 level in group I, one rat improved 1 level in group III, and one rat improved 2 levels in group IV. However, only group II had significantly less deficit at 24 h post MCAO when compared to control ($p < 0.001$).

These results confirm our previous findings [24] that in a transient focal cerebral ischemia rat model immediate treatment with 70%/30% heliox both decreases the infarct size and improves neurological outcome while partial helium treatment of 40%/30%/30% nitrogen/helium/oxygen or delayed 70%/30% heliox showed only a trend towards a beneficial effect.

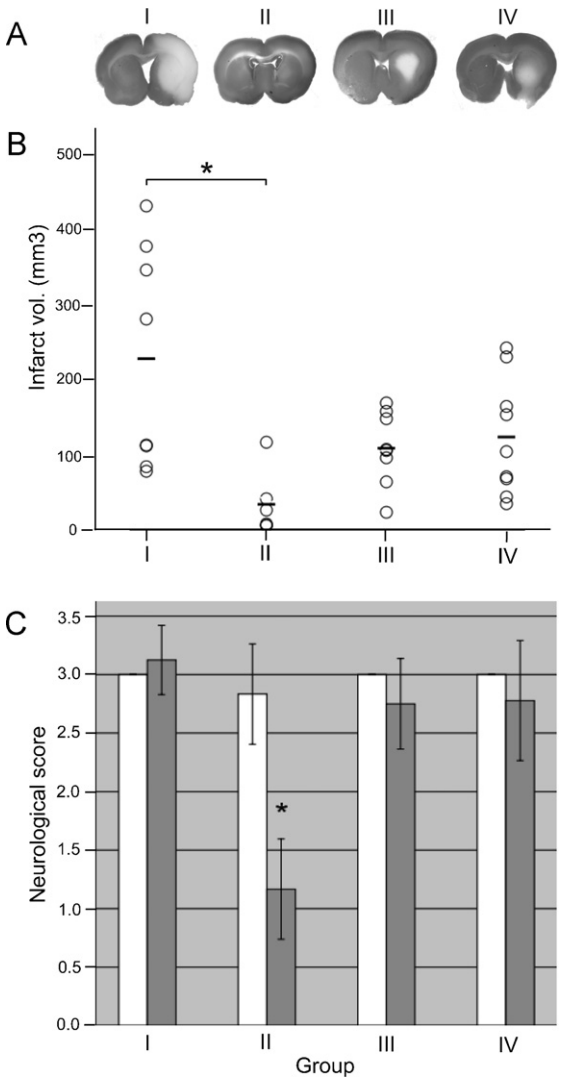

Fig. 1. Neurological outcome of heliox treatments in focal cerebral ischemia. (A) Representative sections of ischemic rat brains after various treatments. Infarcts appear as the unstained area after processing with 2% 2,3,5-triphenyltetrazolium at 24 h post MCAO in control (I), 70%/30% heliox immediate treatment (II), 70%/30% heliox delayed treatment (III) and 40%/30%/30% nitrogen/helium/oxygen treatment (IV). (B) A scatter dot plot illustrates the infarct volume of individual rats (circles) and means of the group (horizontal lines). The difference was statistically significant among the groups ($p = 0.012$), but only Group II had significantly smaller infarct size compared to Group I control rats ($^*p = 0.008$). (C) Neurological scores had no statistic difference among groups at 3 h post MCAO (white columns). At 24-h post MCAO (gray columns), the difference between rats in the heliox immediate treatment (II) and control group (I) was significant ($^*p < 0.001$).

Numerous past studies have investigated helium for ischemic injury. Although most of these studies utilized a 70%/30% mixture of heliox, the treatment regiments investigated varied substantially. For example, in a cardiac ischemia model, 25–30 min of pulsed conditioning treatment with heliox reduced myocardial infarction size [6,10,18]. Moreover, David and colleagues [3] found in a focal cerebral ischemic model that helium treatment delayed for 1 h post MCAO reduced ischemic infarction size and improved neurological outcome when treatment was administered at 25 °C, but not at a 33 °C. This effect may have been due to the effect of inhaled gas on body temperature. The current study did not show a significant benefit of delayed heliox treatment administered at a temperature