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RESEARCH LETTER

Postresuscitation Ventilation With a Mixture of Argon and Hydrogen Reduces Brain Injury After Cardiac Arrest in a Pig Model

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Survival with good neurological recovery after cardiac arrest (CA) remains disappointingly low, 3% to 18%, with a wide variation among countries.[1]

In a preclinical pig model of prolonged untreated CA and cardiopulmonary resuscitation (CPR), we have recently demonstrated that a 4-hour postresuscitation ventilation with 70% argon in oxygen improved neurologic recovery and ameliorated brain injury in comparison with standard ventilation.[2] Potential mechanisms of argon protection include oxygen-like properties, antiapoptotic effects on the molecular pathways involved in cell survival, and prevention of mitochondrial permeability transition pore opening.[2,3] Thus, a 2-phase randomized controlled clinical trial on argon ventilation after CA is currently ongoing (NCT05482945).

Molecular hydrogen (H_2) has also demonstrated protection against ischemia–reperfusion injury in animal models, exerting antioxidant, antiapoptotic, and anti-inflammatory effects.[3] In a rat model of CA, postresuscitation inhalation of 1.3% H_2 was beneficial in promoting neurological recovery and suppressing neuronal degeneration and microglial activation.[4] A randomized controlled clinical trial showed that supplementing standard ventilation with 2% H_2 for 18 hours after CA increased 90-day survival without neurological deficits.[5]

The aim of this study was to investigate whether ventilation with a mixture of argon and H_2 would reduce brain injury in a porcine model of CA and CPR.

Data are available from the corresponding author upon request. The study was approved by the institutional review board and governmental institution (Ministry of Health 657/2020-PR) and followed the Animal Research: Reporting of In Vivo Experiments guidelines. CA was ischemically induced in 24 pigs (40±2 kg) and left untreated for 12 minutes before starting 5 minutes of CPR (Figure [A]), as previously described.[2] Animals were randomized, using no transparent envelopes, to 4-hour postresuscitation ventilation with: 70% nitrogen–30% oxygen (control, n=12) or 68% argon-2% H_2–30% oxygen (argon and hydrogen, n=12). Hemodynamics and myocardial function were monitored. Pigs were then observed up to 96 hours for functional survival (according to the overall performance category). Brains were then removed from the skull, fixed in 10% buffered formalin and then embedded in paraffin.[2] Damaged neurons were investigated

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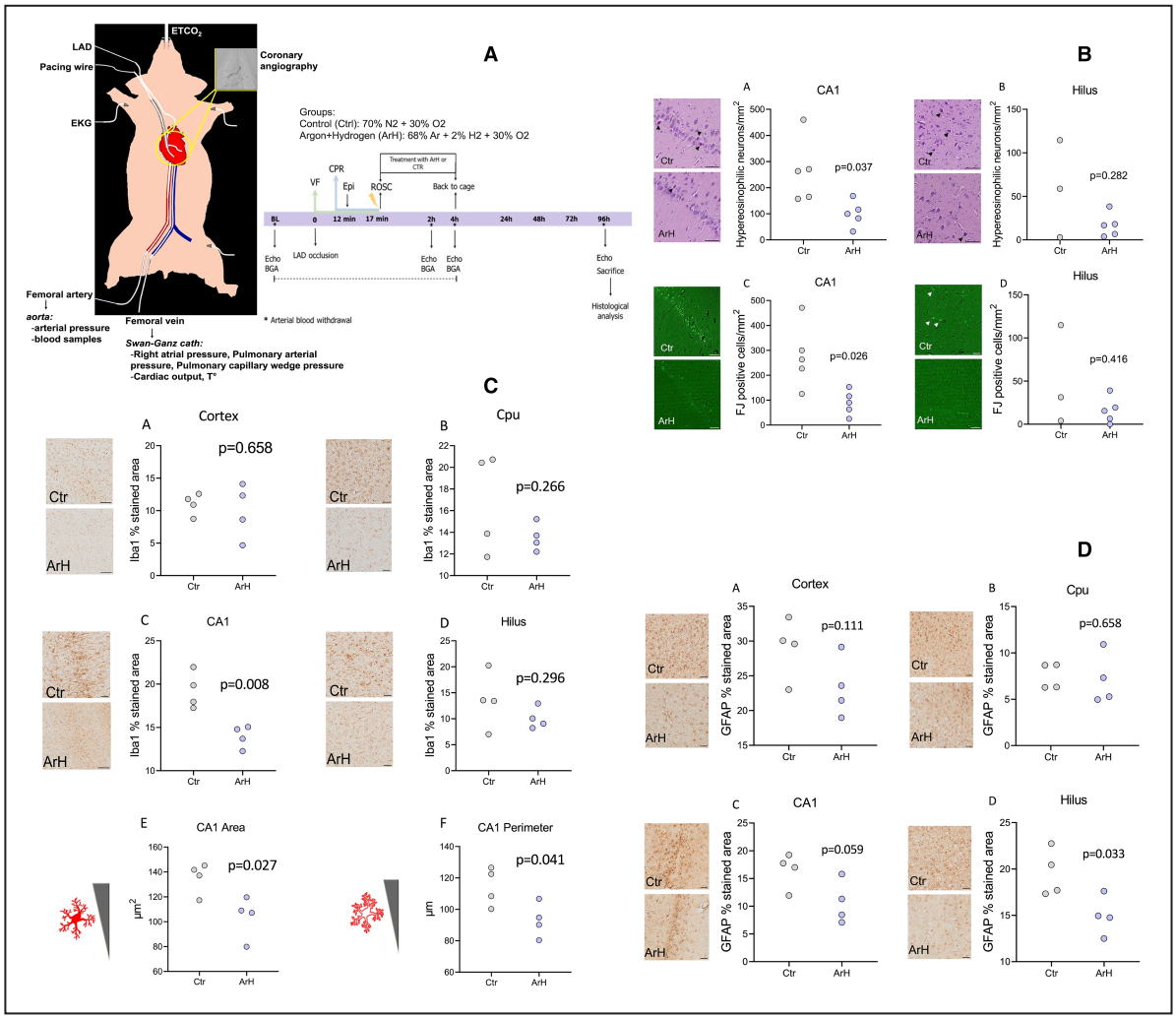

Figure. Ventilation with argon and hydrogen reduces brain injury after cardiac arrest and cardiopulmonary resuscitation.

A. Schematic representation of the animal preparation and experimental design, as described by Fumagalli et al.[2] Under general anesthesia, a cuffed tracheal tube was placed, and animals were mechanically ventilated and $EtCO_2$ monitored. To measure aortic pressure, a fluid-filled 7F catheter was advanced from the right femoral artery into the thoracic aorta. To measure right atrial pressure, core temperature, and cardiac output, a 7F pentalumen thermodilution catheter was advanced from the right femoral vein into the pulmonary artery. To induce myocardial ischemia, a 6F balloon-tipped catheter was inserted from the right common carotid artery and advanced into the aorta, then into the LAD with the aid of image intensification. To induce VF, a 5F pacing catheter was advanced from the right subclavian vein into the right ventricle. CPR included chest compression with the LUCAS 2 (Physio-Control Inc), mechanical ventilation with oxygen, epinephrine (30 μg/kg) administration after 2 minutes, and defibrillation with a single biphasic 150-J shock (MRx, Philips Medical Systems). If resuscitation was not achieved, CPR was resumed and continued for 1 minute before a subsequent defibrillation. Additional epinephrine doses were administered at minute 7 and 12 of CPR. CPR was continued until successful resuscitation or for a maximum of 15 minutes. After ROSC, animals were monitored during the 4 hours of treatment and then returned to the cage for 96 hours. More details in Fumagalli et al.[2] **B**, Representative image of hematoxylin–eosin (HE) staining in CA1 and hilus areas. Compared with control, ventilation with ArH reduced hypereosinophilic neurons in CA1 region of hippocampus (A) and in hilus (B). Representative image of degenerating neurons (Fluoro-Jade) staining in CA1 and hilus. Compared with Ctr, ArH reduced neurodegeneration in CA1 region of hippocampus (C) and in hilus (D). **C**, Representative micrographs of IBA1 and quantification of immunostaining after CA/CPR in cortex (A), Cpu (B), CA1 hippocampal region (C), and hilus (D). Compared with Ctr, ArH resulted in decreased Iba1 immunoreactivity in CA1. Quantitative analysis of the morphology of Iba1-positive cells after CA/CPR in CA1 region of the hippocampus: compared with Ctr, ArH reduced the mean area (E) and perimeter (F) of Iba1-positive cells. **D**, Representative micrographs of GFAP and quantification of immunostaining after CA/CPR in cortex (A), Cpu (B), CA1 hippocampal region (C), and hilus (D). Compared with Ctr, ArH resulted in decreased GFAP immunoreactivity in cortex (A) and hilus (D). Data are mean±SD. Permutation *t* test for small sample sizes vs Ctr. Bar =50 μm. ArH indicates argon and hydrogen; BAG, arterial blood gas analysis; BL, baseline; CA, cardiac arrest; CPR, cardiopulmonary resuscitation; Cpu, caudate-putamen; Ctr, control; Echo, echocardiography; Epi, epinephrine administration; $EtCO_2$, end-tidal CO_2; FJ, Fluoro-Jade; GFAP, glial fibrillary acidic protein; H_2, molecular hydrogen; IBA1, ionized calcium-binding adaptor molecule 1; LAD, left anterior descending coronary artery occlusion; OPC, overall performance category; ROSC, return of spontaneous circulation; and VF, ventricular fibrillation.

on 8-μm coronal sections with hematoxylin–eosin and Fluoro-Jade staining. Immunohistochemistry was performed on sections incubated overnight at 4 °C with anti-IBA1 (ionized calcium binding adaptor molecule 1; 1:200) and anti-GFAP (glial fibrillary acidic protein; 1:2000) antibodies. Permutation *t* test for small sample sizes was used for comparison between groups; a $P<0.05$ was considered statistically significant.

No differences between the 2 groups were observed in either hemodynamics, myocardial function, end-tidal CO_2, and blood gas analyses at baseline and postresuscitation or duration of CPR and number of defibrillations delivered before resuscitation (data not shown). Eighteen pigs were resuscitated and subjected the study ventilation (control n=10; argon and hydrogen n=8), which was successfully conducted in all animals with no adverse effects. The percentage of animals that survived for 96 hours with a complete neurological recovery (overall performance category =1–2) was 50% in the control group versus 63% in the argon and hydrogen group ($P=0.06$).

Argon and hydrogen ventilation significantly reduced damaged neurons (hypereosinophilic neurons with pyknotic nucleus) compared with control ventilation ($P=0.037$; Figure [B]) in the CA1 region of the hippocampus, whereas no difference was observed in the hilus. This result was further confirmed after quantification of positive Fluoro-Jade cells in the same brain areas (argon and hydrogen versus control, $P=0.026$ in the CA1; Figure [B]).

Brain neuroinflammation was also mitigated by ventilation with argon and hydrogen. Indeed, treatment with argon and hydrogen significantly reduced Iba-1 immunoreactivity in the CA1 of the hippocampus compared with controls ($P=0.008$; Figure [C]). The morphological analyses of Iba-1 positive cells in the CA1 also showed significantly smaller area ($P=0.027$) and perimeter ($P=0.041$) after argon and hydrogen compared with control (Figure [C]). Furthermore, CA/CPR caused an increase in GFAP immunoreactivity, that was significantly reduced in the hilus by argon and hydrogen ventilation ($P=0.033$ versus control; Figure [D]).

This small study demonstrated that the new inhalatory mixture of 68% argon +2% H_2 in oxygen reduced both neuronal degeneration and neuroinflammation after CA. In addition, withstanding the limitation of not having compared ventilation with argon + hydrogen versus argon and H_2 separately, the protective effect of combined argon and hydrogen observed at histopathology appeared to be more pronounced than that observed in an earlier study with the same model, in which animals received only 70% argon in oxygen.[2] Thus, the combination of argon and H_2 might represent a promising new, inexpensive intervention to mitigate post-CA brain injury, taking advantage of the possibility to exploit the different protective mechanisms played concurrently by argon and H_2. More specifically, the argon and hydrogen ventilation appears to combine the antiapoptotic effects and mitochondrial preservation mediated by argon with the hydroxyl radical scavenging activity of H_2, together with its indirect ability to induce antioxidation systems and decrease expression of proinflammatory factors.[2–5] Future studies are now needed to confirm these initial observations and also to confirm the effects on functional recovery (likely not observed in this study because of the small sample size). A comparison of the effects of a single gas versus different combinations of argon and H_2 is also needed to find the optimal inhalatory strategy for neuroprotection after CA.

ARTICLE INFORMATION

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Disclosures

None.

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