Depletion of Vascular Nitric Oxide Contributes to Poor Outcomes after Cardiac Arrest

To the Editor:

Sudden cardiac arrest (CA) is a leading cause of death worldwide. Despite advances in cardiopulmonary resuscitation (CPR) methods, including the introduction of the automatic electrical defibrillator and targeted temperature management, only 10–20% of adult out-of-hospital CA (OHCA) victims survive to hospital discharge, and up to 60% of survivors have moderate to severe cognitive deficits. Poor outcomes after successful resuscitation from CA are a result of post-CA syndrome, which includes neurological and cardiovascular dysfunction and systemic inflammatory reaction (1). On a pathophysiological level, the ischemia–reperfusion injuries associated with post-CA syndrome involve microcirculatory dysfunction, vascular leakage with ensuing edema, and an increase in platelet and leukocyte adhesion to the activated/injured endothelium.

Intravascular hemolysis releases cell-free hemoglobin (Hb) into plasma (2). Oxygenated plasma (cell-free) Hb (Oxy-Hb) depletes vascular nitric oxide (NO) via a dioxygenation reaction to form methemoglobin and nitrate, an inert NO metabolite. Endothelial-derived NO plays a key role in maintaining vascular homeostasis. Depletion of vascular NO by plasma Oxy-Hb promotes vasoconstriction, thrombosis, and inflammation, thus impairing tissue perfusion (2). Because CPR with chest compression has been reported to cause hemolysis (3), we investigated the potential role and prognostic capacity of hemolysis and plasma NO consumption in patients resuscitated from OHCA, as well as in a murine model of CA and CPR.

We enrolled blood samples of adult (>18 yr) post-OHCA patients ($n = 21$) at Beth Israel Deaconess Medical Center and Massachusetts General Hospital (Table 1). Cardiac arrest of traumatic etiology was excluded, as were patients considered a protected population (i.e., prisoners, pregnant patients). Plasma samples were collected within 12 hours after return of spontaneous circulation. We also enrolled blood samples of healthy adult volunteers ($n = 8$) and preoperative cardiac surgery patients ($n = 3$) as control subjects at Massachusetts General Hospital. To assess degree of hemolysis, plasma cell-free Hb was measured with a QuantiChrom Hemoglobin Assay Kit (BioAssaySystems). Plasma NO consumption was determined with a NO chemiluminescence analyzer (Sievers 280i; GE) by measuring the decay of NO concentration after the addition of a known amount of NO donor to plasma samples, as previously described (4). Cerebral performance category was assessed in patients with OHCA at the time of hospital discharge. The institutional review boards at Beth Israel Deaconess Medical Center and Massachusetts General Hospital approved the study, and all subjects or their legally authorized surrogate provided written informed consent.

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Table 1. Characteristics of Included Human Subjects

	OHCA Patients ($n = 21$)	Control Subjects ($n = 11$)
Demographics		
Age, yr	64 (51–79)	53 (46–66)
Sex, F, n (%)	8 (38.1)	3 (27.3)
Arrest characteristics		
Initial rhythm, n (%)		
Shockable	10 (47.6)	—
Nonshockable	11 (52.4)	—
Total downtime, min*	14 (8–28)	—
Treatments		
Targeted temperature management, n (%)	16 (80)	—
Outcomes		
Favorable neurological outcome at hospital discharge (CPC = 1, 2), n (%)	7 (33.3)	—
Mortality, n (%)	14 (66.7)	—

Definition of abbreviations: CPC = cerebral performance category; OHCA = out-of-hospital cardiac arrest.

Continuous variables are presented as median (25th–75th percentile).

* $n = 20$ because of a missing data.

Post-OHCA patients had higher levels of plasma Hb and NO consumption compared with control subjects (Figures 1A and 1B). Although the levels of plasma Hb did not differ between post-CA patients with favorable (cerebral performance category = 1 or 2) and poor (cerebral performance category = 3, 4, or 5) neurological outcomes, plasma NO consumption was markedly increased in patients with poor neurological outcomes compared with patients with favorable outcomes (Figures 1C and 1D). These observations suggest that higher levels of plasma NO consumption are associated with severity of neurological impairment after OHCA.

Plasma NO consumption can be inhibited by inhaled NO because methemoglobin, which is produced by oxidation of plasma Oxy-Hb from inhaled NO, does not scavenge NO (5). Inhaled NO improves neurological outcomes and survival after experimental CA/CPR in multiple species (6, 7). To determine the role of plasma NO consumption after CA/CPR, we measured plasma Hb and NO consumption in sham-operated mice and mice subjected to experimental CA/CPR that breathed air or 40 ppm NO in air after return of spontaneous circulation. Similar to the post-OHCA patients, plasma Hb and NO consumption were markedly increased in post-CA mice that breathed air. Although inhaled NO did not affect the degree of hemolysis, breathing NO at a dose and timing shown to improve neurological outcomes after CA/CPR in this murine model (6) inhibited the increase of plasma NO consumption in post-CA mice (Figures 1E and 1F). These results suggest that inhibition of vascular NO consumption may mediate the beneficial effects of NO inhalation after CA/CPR.

Vascular NO consumption by plasma Oxy-Hb has been implicated in the pathogenesis of a variety of human diseases, including vaso-occlusive crisis in sickle cell anemia (8), adverse effects of transfusion of Hb-based oxygen carrier (9),

Figure 1. Plasma hemoglobin (Hb) levels (upper panels) and plasma nitric oxide (NO) consumption levels (lower panels). (A and B) Plasma Hb and NO consumption levels in control subjects ($n = 10$ for plasma Hb because of missing data) and patients with out-of-hospital cardiac arrest (OHCA). (C and D) Plasma Hb and NO consumption levels in patients with OHCA who had favorable neurological outcomes (cerebral performance category = 1 or 2) and who had poor neurologic outcomes (cerebral performance category = 3, 4, or 5) at hospital discharge. (E and F) Plasma Hb and NO consumption levels in sham-operated mice, and post-CA mice breathing either air or 40 ppm NO in air. Mann-Whitney tests were used to test statistical difference in human data, as the data were not normally distributed. One-way ANOVA with the Holm-Sidak's multiple comparisons *post hoc* test was used to test statistical difference in mice data, as the data were normally distributed. Data are presented as mean $\pm$ SEM. CPR = cardiopulmonary resuscitation.

and acute kidney injury after prolonged cardiopulmonary bypass (4). Our results suggest that vascular NO consumption is associated with poor neurological outcome after CA/CPR, and that inhibition of plasma NO consumption may be therapeutic. These observations also suggest that plasma NO consumption could be used in the future to categorize post-CA patients to guide therapy; patients with increased plasma NO consumption are likely to benefit from treatments that decrease NO consumption (e.g., inhaled NO). Of note, vascular NO may be consumed by other mechanisms. For example, we have recently reported that S-nitrosoglutathione reductase (GSNOR) activity is increased in plasma of post-OHCA patients and in plasma and multiple organs in mice subjected to experimental CA/CPR (10). Catabolism of GSNO, an important reservoir of NO, by GSNOR depletes NO bioactivity and protein S-nitrosothiol levels. Pharmacological inhibition or genetic deletion of GSNOR restored S-nitrosothiol levels and improved outcomes in post-CA mice. Further studies are warranted to determine the effect of vascular NO consumption on post-CA outcomes. ■

Kei Hayashida, M.D., Ph.D.
Yusuke Miyazaki, M.D., Ph.D.
Binglan Yu, Ph.D.
Michael G. Silverman, M.D.
Riccardo Pinciroli, M.D.
Lorenzo Berra, M.D.
Rajeev Malhotra, M.D., M.S.
Massachusetts General Hospital
Harvard Medical School
Boston, Massachusetts

Michael W. Donnino, M.D.
Beth Israel Deaconess Medical Center
Harvard Medical School
Boston, Massachusetts

Fumito Ichinose, M.D., Ph.D.*
Massachusetts General Hospital
Harvard Medical School
Boston, Massachusetts

*Corresponding author (e-mail: fichinose@mgh.harvard.edu).

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CO₂ Oscillation during Cardiopulmonary Resuscitation: The Role of Respiratory System Compliance

To the Editor:

We read with interest the manuscript by Grieco and colleagues entitled “Intrathoracic Airway Closure Impacts CO₂ Signal and Delivered Ventilation During Cardiopulmonary Resuscitation” (1).

The authors conducted a very elegant clinical investigation corroborated by focused experiments in the Thiel cadaver model, describing a peculiar oscillatory capnographic pattern during cardiopulmonary resuscitation (CPR). From the results, it has been speculated that a low oscillating CO₂ during chest

compressions is a result of the presence of airway collapse, which obstructs the airflow. However, as mentioned by the authors in the discussion, individual differences in the compliance of the respiratory system (Cpl_{rs}, including the lungs and the chest wall) could have a great prominence in explaining this finding. For a given pressure applied to the sternum during a chest compression, the gas volume displacement will be directly proportional to the compliance of the respiratory system. Therefore, patients with a lower airway opening index (AOI) may have had lower Cpl_{rs}. This would also explain the correlation between alveolar ventilation and AOI, because greater ventilation was likely in patients with higher compliance. Interestingly, a greater AOI was observed in patients with a shockable rhythm, who are likely to have a shorter duration of cardiac arrest compared with those with nonshockable rhythms and thus a lesser deterioration in Cpl_{rs} (2).

We wonder about the correlation between Cpl_{rs} measured during regular tidal ventilation and AOI.

Although small airway closure might affect the measurement of compliance, this is unlikely during regular tidal ventilation. We accept that a formal “static” measurement of Cpl cannot be obtained during chest compression, but a good estimate would be possible from the delivered V_T, given that a constant inspiratory pressure was applied. Indeed, a reliable value for Cpl_{rs} can be obtained in the Thiel cadavers.

Respiratory system compliance could also explain the beneficial effects of positive end-expiratory pressure (PEEP). Ventilation resulting from chest compression occurs between FRC and residual volume (3). At this level of lung volume, the pressure–volume curve of normal subjects is less compliant than it is above FRC (4). The application of PEEP would increase the end-expiratory lung volume and cause the chest compressions to “ventilate” the respiratory system in a more compliant part of the pressure–volume curve, leading to greater V_T and greater alveolar ventilation.

Finally, the “circulatory” component of CPR, that is, forward blood flow generated by chest compression, affects end-tidal CO₂ and might potentially affect the subsequent AOI calculation. Indeed, ventilation and hemodynamics are deeply interconnected in the pathophysiology of CPR, as $\dot{Q}$ is the major determinant of CO₂ transport to the lungs. Because $\dot{Q}$ may vary during CPR in relationship to chest compression quality and/or patient’s condition, for example, development of stoned heart for prolonged resuscitation and Cpl_{rs} modification, the evolving AOI may change over time.

In conclusion, although we believe that the work by Grieco and colleagues does not completely tease out the relative contributions of compliance versus airway closure in the generation of CO₂ oscillation, their findings have tremendous clinical relevance, particularly for the potential benefits of PEEP during CPR, and highlight the importance of more studies on the topic. ■

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Emanuele Rezoagli, M.D.
University of Milan-Bicocca
Monza, Italy

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