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HYDROGEN INHALATION IS SUPERIOR TO MILD HYPOTHERMIA IN IMPROVING CARDIAC FUNCTION AND NEUROLOGICAL OUTCOME IN AN ASPHYXIAL CARDIAC ARREST MODEL OF RATS

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ABSTRACT—**Background:** Non-shockable rhythms represent an increasing proportion of reported cases of out-of-hospital cardiac arrest but with an associated poor prognosis. In the present study, we investigated the effects of hydrogen inhalation on cardiac and neurological function after cardiopulmonary resuscitation and compared the therapeutic benefit with hypothermia in an asphyxial rat model of cardiac arrest. **Methods:** Cardiopulmonary resuscitation was initiated after 5 min of untreated asphyxial cardiac arrest. Animals were randomly assigned to three experimental groups immediately after successful resuscitation: ventilation with 2% hydrogen/98% oxygen under normothermia (H_2 inhalation), ventilation with 2% nitrogen/98% oxygen under normothermia (Control), and ventilation with 2% nitrogen/98% oxygen under hypothermia (TH). Mixed gas inhalation continued for 1 h while hypothermia continued for 2 h. Animals were observed up to 96 h for assessment of survival and neurologic recovery. **Results:** No statistical differences in baseline measurements were observed among groups and all the animals were successfully resuscitated. Serum cardiac troponin T and S100B measured during earlier post-resuscitation period were markedly reduced in both H_2 inhalation and hypothermic groups. However, significantly better left ventricular ejection fraction, cardiac work, and neurological deficit score were observed in the H_2 inhalation group. Ninety-six hours survival rate was significantly higher in the H_2 inhalation group (75.0%), either compared with TH (45.8%) or compared with Control (33.3%). But there was no statistical difference between TH and Control. **Conclusions:** Small amounts of inhaled hydrogen were superior to mild hypothermia in improving cardiac function and neurological outcome in this asphyxial rat model of cardiac arrest.

KEYWORDS—Asphyxia, cardiac arrest, hydrogen, hypothermia, myocardial function, neurological outcome

INTRODUCTION

Sudden cardiac arrest remains a major public health issue and is the most common direct cause of death in Western and developing societies (1, 2). Despite efforts to improve outcomes from cardiac arrest, including updated cardiopulmonary resuscitation (CPR) Guidelines over the past 50 years, overall survival rate remains less than 10% (3, 4). Among patients who achieved restoration of spontaneous circulation (ROSC), two-thirds died during the subsequent days due to significant myocardial dysfunction and neurologic disability and fewer than 30% of survivors returned to a normal functional life-style (4, 5).

The two most prevalent causes of cardiac arrest are ventricular fibrillation (VF) and asphyxiation, representing clinical cardiac arrest from respective cardiac and respiratory etiologies (6). During the last two decades, the incidence of VF as the presenting rhythm has declined significantly. In contrast, the incidence of pulseless electrical activity (PEA) or asystole as presenting rhythms has increased in reported cases of out-of-hospital cardiac arrest (7, 8). In addition to this rise in non-shockable rhythms is the fact that these rhythms are associated with a poor prognosis, with a survival to discharge rate less than 5% (9, 10).

Among all post-resuscitation care interventions recommended, therapeutic hypothermia (TH) is the most persuasive that has proven benefits for both the brain and the heart (11, 12). Although TH is accepted as the gold-standard method to improve neurological outcome and survival in patients resuscitated from shockable rhythms (13), its effect remains controversial for victims with non-shockable rhythms (14–16). As asphyxia differs in cardiac arrest course, neurologic deficit, and myocardial damage compared with VF cardiac arrest (17–19), the development of alternative approaches with or without TH is an unmet medical need in ameliorating the prognosis of these patients.

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Since the discovery that hydrogen has antioxidant and anti-apoptotic properties that protect the brain against ischemia-reperfusion injury by selectively neutralizing hydroxyl radicals (20), the beneficial effect of hydrogen has been studied in VF model of cardiac arrest (21–24). In a rat model of VF and CPR, Hayashida et al. (22) reported that inhalation of mixed gas comprising 2% hydrogen and 98% oxygen for 2 h at the beginning of CPR improved brain and cardiac function. Using the same animal model, the authors observed that inhalation of mixed gas comprising 1.3% hydrogen and 26% oxygen after ROSC for 2 h also improved neurological recovery and survival (23). Huang et al. (24) demonstrated that Intraperitoneal injection of 100% hydrogen after ROSC improved neurological recovery and 72-h survival in a rabbit model of VF. However, asphyxial cardiac arrest differs significantly from dysrhythmic cardiac arrest with regard to pathophysiologic mechanisms and response to therapy (17–19). The effectiveness of hydrogen inhalation in non-shockable rhythms therefore still needs to be validated.

In the present study, we evaluated the effects of hydrogen inhalation on cardiac and neurological function after CPR and compared the therapeutic benefit with post-resuscitation TH in an asphyxial rat model of cardiac arrest. The null hypothesis (H0) was that hydrogen inhalation had no effect on survival or cardiac function or neurological outcome after resuscitation. The alternative hypothesis (Ha) was that inhalation of hydrogen would improve survival, cardiac function, and neurological outcome after asphyxial cardiac arrest.

MATERIALS AND METHODS

This prospective, randomized animal study was approved by the animal investigation committee of Third Military Medical University. All animals received humane care in compliance with the Principles of Laboratory Animal Care and Guide for the Care and Use of Laboratory Animals. Seventy-two healthy Sprague-Dawley rats of both sexes (36 male and 36 female) weighing between 217 and 337 g (10–12 weeks age) were used for this study.

Animal preparation

All animals were fasted overnight except for free access to water. Anesthesia was initiated with an intraperitoneal injection of pentobarbital (45 mg/kg). Additional doses of 10 mg/kg were administered if signs of stress occurred such as increases in heart rate or respiratory frequency or spontaneous movements. After placing on a surgical board in the supine position, the tracheas of the animals were intubated through a tracheotomy with a 14-gauge cannula and mechanically ventilated with a tidal volume of 0.65 mL/100 g at an FiO2 of 0.21 (ALC-V8, Alcott Biotech Co Ltd, Shanghai, China). A PE-50 catheter was advanced from the right femoral artery for measurement of arterial pressure and blood sampling. Another PE-50 catheter was advanced through the left external jugular vein and into the right atrium for measurement of right atrial pressures. Arterial pressure, right atrial pressure, and a conventional lead II ECG were continuously measured by a multiparameter monitor (Model 90369, Spacelabs, Snoqualmie, WA). Core temperature was monitored by a thermocouple probe (TH-212, Bjhocy Science and Technology Co Ltd, Beijing, China) that was placed into the esophagus and maintained by a heating lamp throughout the experiment to ensure appropriate temperature management. The left femoral vein was also cannulated with an additional PE-50 catheter to allow for administration of fluids and drugs. All catheters were flushed intermittently with saline solution containing 2.5 IU/mL heparin.

Experimental procedures

After collection of baseline data, a single dose of vecuronium (0.1 mg/kg i.v.) was administered to induce respiratory paralysis and immobilize the animals. Two minutes later, the mechanical ventilator was disconnected and endotracheal tube was clamped to induce asphyxia. Cardiac arrest was defined as systolic blood pressure <20 mm Hg with either PEA or asystolic rhythm, occurring approximately 3 min after asphyxia.

CPR, including chest compression and ventilation, was begun after 5 min of untreated arrest. Mechanical chest compression was delivered by a pneumatically driven compressor at a stroke rate of 200/min with a depth of 25% to 30% of the anterior posterior diameter of the animal's chest. Coincident with the start of precordial compression, animals were mechanically ventilated at a frequency of 80/min with a tidal volume 0.6 mL/100 g and an FiO_2 of 0.98. A dose of epinephrine (0.02 mg/kg) was injected 1 minute after the start of CPR. CPR was continued until spontaneous pulse was observed in arterial tracing and mean arterial pressure was reached above 60 mm Hg for at least 5 min.

Animals were randomly assigned to three experimental groups (n = 24 each, 50% in sex ratio) immediately following ROSC and monitored in an intensive care setting for 4 h: ventilation with 2% hydrogen/98% oxygen under normothermia (37°C, H_2 inhalation), ventilation with 2% nitrogen/98% oxygen under normothermia (37°C, Control), and ventilation with 2% nitrogen/98% oxygen under hypothermia (33°C, TH). For all animals, mechanical ventilation was continued with mixed gas for 1 h and then with room air for another 3 h. For animals subjected to TH group, surface cooling was initiated immediately after ROSC with the aid of ice packs and an electrical fan. Once the target temperature reached 33.0°C, it was maintained over the first 2 h of post-resuscitation and then gradually returned to 37.0°C over a rewarming period of 2 h (25). For those animals assigned to H_2 inhalation and Control group, core temperature was maintained at 37.0 ± 0.3°C until the end of the experiment.

All catheters, including endotracheal tube, were removed and wounds were surgically sutured 4 h after resuscitation. Animals were then returned to their cages with a room temperature maintained at 20°C to 24°C and observed for 96 h. At the end of the experiment, the animals were euthanized by a lethal intraperitoneal injection of sodium pentobarbital (150 mg/kg).

Measurements

ECG and blood pressure were continuously recorded with a PC-based data acquisition system supported by WINDAQ hardware and software (DATAQ Instruments Inc, Akron, OH). Cardiac function was noninvasively measured at baseline and at hourly intervals after resuscitation with an echocardiograph system (DC-6, Mindray Medical International Limited, Shenzhen, China). Left ventricular ejection fraction (LVEF) served as quantitative measurements of myocardial contractile function and cardiac work (CW) was used as measure of power of left ventricle.

Arterial blood samples were drawn at baseline, 2 and 4 h after ROSC. Blood gases were measured with the aid of a blood analyzer (i15, Edan Instruments Inc, Shenzhen, China). Serum concentration of cardiac troponin T (cTnT) and S100B that were quantified with an enzyme-linked immunoassay (Elisa Kit, Cusbio Biotech Co Ltd, Wuhan, China) according to the manufacturer's instructions served as biomarkers of cardiac and cerebral injury (26).

Neurological deficit score (NDS) was examined every 24 h and confirmed by two investigators blinded to the treatment. Consciousness and breathing, cranial nerve reflexes, motor function, sensory function, and coordination were scored according to an NDS system (0 to 500 scale; 0 no observed neurological deficit, 500 death or brain death) that was developed to evaluate neurological outcome after global cerebral ischemia for rats (27).

Statistical analyses

Continuous data are presented as mean ± SD. Normal distribution of the data was confirmed using the Kolmogorov–Smirnov test. Hemodynamic and biochemical variables were compared by a mixed-effects model for repeated measures analyses, followed by ANOVA with Bonferroni correction for *post hoc* comparisons. NDSs were compared among groups using a Mann–Whitney nonparametric test. Survival data was expressed as a percentage and tested with Chi-square test. Survival curves were obtained with a Kaplan–Meier analysis and compared between groups with a log-rank test. A $P < 0.05$ was regarded as statistically significant.

RESULTS

Baseline and resuscitation

Baseline physiological measurements did not differ significantly among the three groups (Table 1). There were also no significant differences in baseline arterial blood gases, LVEF and CW among groups. Cardiac arrest was successfully induced in all animals and PEA was changed to VF during CPR in six animals (one in Control, three in TH, and two in H_2 inhalation group). Spontaneous circulations were restored after delivering

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Table 1. **Baseline physiological variables (mean ± SD)**

Variable	Control (n = 24)	TH (n = 24)	H_2 inhalation (n = 24)
Body weight, g	267.3 ± 41.2	278.5 ± 29.1	272.4 ± 32.3
Preparation time, min	45.6 ± 1.2	46.9 ± 1.0	47.7 ± 0.8
Heart rate, bpm	428.0 ± 40.3	411.2 ± 31.7	420.4 ± 43.0
Mean arterial pressure, mm Hg	130.3 ± 9.7	125.8 ± 12.7	131.5 ± 7.5
Temperature, °C	36.9 ± 0.1	37.1 ± 0.2	36.9 ± 0.1

bpm indicates beat per minute; TH, hypothermia.

a 2-J defibrillation shock for animals with VF. There were no significant differences in asphyxial time to induce cardiac arrest, CPR time, and total epinephrine dosage (Table 2). The resuscitation success rates were 100% in the three investigated groups and all animals survived the 4-h post-resuscitation monitoring period.

Post-resuscitation hemodynamic, cardiac function, and cerebral injury

For hypothermic animals, the target core temperature was obtained within 12.7 ± 3.2 min and was returned to 37.0°C after 2-h rewarming (Fig. 1). The heart rate of hypothermic animals was significantly lower than other two groups after induction of hypothermia (Fig. 2A), while the mean arterial pressure (MAP) was considerably higher in the H_2 inhalation group 3 h after ROSC compared with TH and Control groups (Fig. 2B). However, no significant differences were noted at any time point for arterial gas analyses among groups (Table 3).

Post-resuscitation myocardial function, as evaluated by the changes of LVEF and CW, was severely impaired when compared with the baseline values in the Control group (Fig. 3). Significant improvement in LVEF was observed in both H_2 inhalation and TH groups during the entire post-resuscitation monitoring period (Fig. 3A), but the improvement was significantly greater in H_2 inhalation group after 2 h of resuscitation. At the same time, CW was significantly lower in animals treated with hypothermia during the first 3 h after ROSC compared with Control and TH groups. On the contrary, CW was preserved in animals treated with hydrogen and was significantly higher compared with the other two groups, except measurement at 4 h in the TH group (Fig. 3B).

After resuscitation, an obvious increase in serum cTnT and S100B levels was observed in all groups in contrast to their pre-arrest values (Fig. 4). Compared with Control, these biomarkers were significantly lower in both TH and H_2 inhalation groups measured at 2 and 4-h post-resuscitation. At the same time, cTnT level in H_2 inhalation group was significantly lower compared with TH (Fig. 4A), but no statistical difference in S100B was observed between H_2 inhalation and TH groups (Fig. 4B).

Neurological outcome and survival

No statistical difference was observed for NDS between TH and Control groups during the observational period. However, NDS was significantly better in the H_2 inhalation group compared with the other two groups (Table 4).

Eighteen animals in the H_2 inhalation group survived to 96 h, this contrasted with 11 and 8 animals in TH and Control groups (Fig. 5). The survival rate was significantly higher in H_2 inhalation group (75.0%), either compared with TH (45.8%) or compared with Control group (33.3%). But there was no statistical difference between TH and Control.

Effects of sex in the neurological outcome and survival

There were no statistical differences between female and male animals in baseline physiological measurements, except a significantly lower body weight for female animals (see Table, Supplemental Digital Content 1, at http://links.lww.com/SHK/A374). Success rate of resuscitation, post-resuscitation hemodynamic, cardiac function, and cerebral injury were also no significant differences between females and males within groups. NDSs were relative lower and 96-h survival rates were relative higher for female animals in all of the three investigated groups, but did not achieve statistical significance.

DISCUSSION

The present study demonstrates that a shorter duration of hydrogen inhalation after ROSC significantly improved neurological outcome and survival compared with mild hypothermia in a rat model of asphyxial cardiac arrest.

The physiological effects of hypothermia are thought to be multifactorial, including the suppression of free radicals, enzymes, and excitotoxic and inflammatory reactions, in addition to the direct physical protection of membranes (28). In the present study, hypothermia alleviated the cardiac and brain injuries during the early post-resuscitation period, but 96-h neurological outcome and survival were not significantly improved for asphyxial cardiac arrest. This is consistent with previous studies that investigated the effects of hypothermia

Table 2. **Variables of asphyxial cardiac arrest and resuscitation (mean ± SD)**

	Control (n = 24)	TH (n = 24)	H_2 inhalation (n = 24)
Asphyxial time to cardiac arrest, s	168.2 ± 31.1	181.7 ± 28.6	167.5 ± 27.3
CPR time, s	80.3 ± 25.1	82.3 ± 18.6	85.21 ± 16.7
Total dose of epinephrine, ug	9.0 ± 2.0	7.0 ± 1.5	9.0 ± 2.0
Successful resuscitation, %	100	100	100

CPR indicates cardiopulmonary resuscitation; TH, hypothermia.

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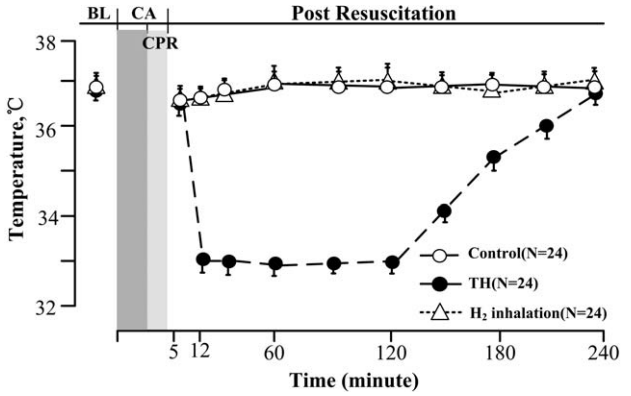

FIG. 1. **Esophagus temperature measurement before and after resuscitation (mean ± SD).** BL indicates baseline; CA, cardiac arrest; CPR, cardiopulmonary resuscitation. n = 24 at each time point in each group.

TABLE 3. **Arterial blood gas analyses at baseline, 120 (PR120), and 240 min (PR240) after resuscitation (mean ± SD)**

	Baseline	PR120	PR240
PH			
Control (n = 24)	7.43 ± 0.02	7.39 ± 0.02	7.41 ± 0.03
TH (n = 24)	7.42 ± 0.02	7.34 ± 0.05	7.40 ± 0.02
H_2 inhalation (n = 24)	7.42 ± 0.04	7.48 ± 0.03	7.41 ± 0.02
PaO_2, mm Hg			
Control (n = 24)	82.7 ± 8.7	79.9 ± 6.4	89.8 ± 4.3
TH (n = 24)	78.2 ± 9.6	82.2 ± 7.9	88.5 ± 7.8
H_2 inhalation (n = 24)	83.3 ± 17.5	89.8 ± 16.1	89.4 ± 6.7
$PaCO_2$, mm Hg			
Control (n = 24)	43.6 ± 4.3	40.1 ± 3.9	39.1 ± 2.6
TH (n = 24)	41.9 ± 3.6	43.0 ± 5.9	38.6 ± 4.3
H_2 inhalation (n = 24)	45.6 ± 5.7	41.5 ± 6.1	37.6 ± 4.5
SaO_2, %			
Control (n = 24)	95.8 ± 2.0	96.4 ± 2.1	97.3 ± 1.6
TH (n = 24)	95.0 ± 1.9	92.6 ± 3.8	94.3 ± 5.1
H_2 inhalation (n = 24)	96.0 ± 1.9	96.2 ± 1.9	96.8 ± 2.0

TH indicates hypothermia.

using cardiac arrest animal model of asphyxia (29, 30), but opposite to studies using animal model of VF (22, 25). The discrepancy could be explained by the difference in severity of brain and heart damage between the two most frequently used animal models of cardiac arrests. Vaagenes et al. (17) reported that asphyxial cardiac arrest had worse morphologic brain damage than VF cardiac arrest and might have different responses to cerebral resuscitation treatments. Tsai et al. (18) observed more diffuse myocardial injuries and more severe mitochondrial damages in asphyxial than VF cardiac arrest with the same pulseless duration. Wu et al. (19) demonstrated the more severe cardiac dysfunction caused by asphyxial cardiac arrest associated with shorter survival time. The beneficial effects of TH on the heart and brain were reflected by the reduced cTnT and S100B levels measured during the early post-resuscitation period compared with normothermic controls. LVEF was greatly increased during the first 2 h after ROSC, but the difference became unapparent 3 h later. As an adverse effect, CW was significantly dropped during the cooling period due to the decreased heart rate (29, 30). Even though it returned to pre-arrest level after rewarming to normothermia, the sustained depression of ejection fraction was remained and overall hemodynamic function not mitigated. As a consequence, the neurological outcome and survival rate were not significantly improved.

The duration of hypothermia may affect the efficacy of TH. In a rat model of VF, Ye et al. (25) have demonstrated that a 2 hours duration of mild hypothermia that was induced immediately after ROSC, improved myocardial and cerebral functions, and survival as well as, or better than, prolonged duration of hypothermia of 5 and 8 h. In the current study, we chose a 2-h duration for hypothermia, followed by 2 h of rewarming as reported by Ye et al. (25). Although the optimal duration of TH that was derived from VF may not be applicable to asphyxial model, but its effect is minimal since Jia et al. (29) demonstrated that there was no significant difference in mean duration of survival time between groups when the core temperature was maintained between 32 and 34°C for 12 h. The risk–benefit ratio of induced hypothermia, which was clearly favorable in shockable rhythms, therefore was altered when cardiac arrest was induced by asphyxia.

As a novel selective hydroxyl radical and peroxynitrite scavenger, hydrogen is cost-effective and has low toxicity with little drug–drug interaction (31). These features could make hydrogen a treatment option that could be implemented during CPR and/or after ROSC. Hydrogen has been demonstrated to benefit neurological outcome and function to an extent comparable to hypothermia in VF model of cardiac arrest (22, 23). In a rat model of asphyxial cardiac arrest, Huo et al. (32) found hydrogen-rich saline treatment after resuscitation improved survival and neurological outcome. But whether hydrogen

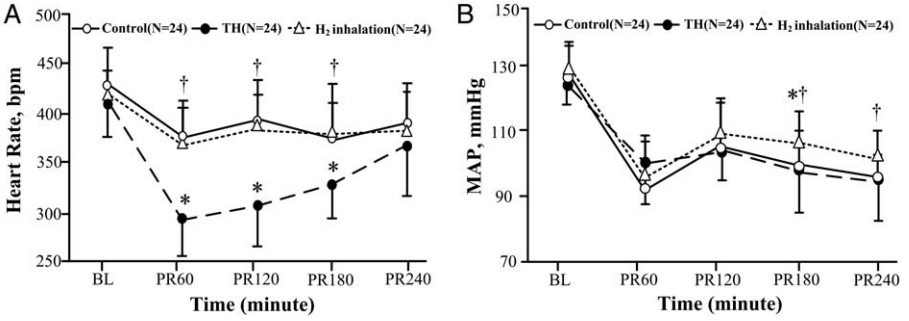

FIG. 2. **Comparisons of heart rate (A) and mean arterial pressure (B) at baseline, 60, 120, 180, and 240 min after resuscitation among groups (mean ± SD).** * $P < 0.05$ versus Control group; † $P < 0.05$ versus hypothermic (TH) group. BL indicates baseline; PR, post resuscitation. n = 24 at each time point in each group.

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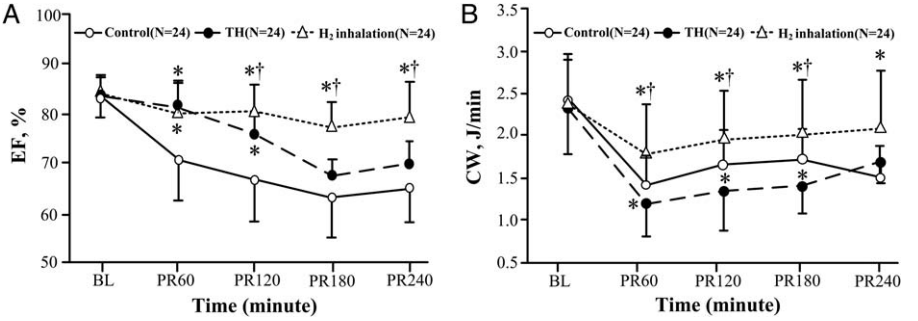

FIG. 3. **Comparisons of left ventricular ejection fraction (LVEF) (A) and cardiac work (CW) (B) at baseline, 60, 120, 180, and 240 min after resuscitation among groups (mean ± SD).** * $P < 0.05$ versus Control group; † $P < 0.05$ versus hypothermic (TH) group. BL indicates baseline; PR, post-resuscitation. n = 24 at each time point in each group.

inhalation was more efficient than TH for non-shockable rhythms is still uncertain. In the current study, inhaling 2% hydrogen for 1 h immediately after ROSC was demonstrated to be protective and, in some aspects, superior to TH for asphyxial cardiac arrest. The serum astroglial protein S100B was significantly decreased in the H_2 inhalation group, to an extent comparable to TH group during earlier post-resuscitation period. The circulatory cTnT level was also dramatically dropped in animals treated with hydrogen, not only significantly lower than Control group, but also lower than TH group. Heart rate was considerably higher during the first 3 h post-resuscitation in the H_2 inhalation group compared with animals treated with hypothermia. Meanwhile, MAP in H_2 inhalation group was also significantly higher 3 h after ROSC compared with the other two groups. Both LVEF and CW were preserved and returned to its baseline level 1 h after ROSC in H_2 inhalation group. The improved hemodynamic stability and myocardial function may explain, at least in part, the improved neurological outcome and survival.

Our finding that hydrogen was superior to hypothermia on reducing post-resuscitation myocardial damage was in agreement with previous animal studies. In isolated perfused hearts, Hayashida et al. (33) demonstrated that inhalation of hydrogen at incombustible levels during ischemia and reperfusion reduced infarct size without altering hemodynamic parameters, thereby preventing deleterious left ventricular remodeling. Furthermore, inhalation of hydrogen also prevented the increases in left ventricular end-diastolic pressure and serum IL-6 at 2 h after ROSC in rat model of VF and CPR (22), which was not observed in animals treated with hypothermia (34). These evidences suggest hydrogen may be a good candidate for post-cardiac arrest care as a safe and effective therapy with minimal side effects.

The protective mechanism of hydrogen is primarily through selective reactive oxygen species (ROS) attenuation (20, 31). ROS is massively produced in the brain after ischemia/reperfusion, and oxidative damage to brain tissues has been regarded as a fundamental mechanism of brain injury after cardiac arrest. ROS-triggered injury cascades are exacerbated by reduced cardiac output and local circulatory impairment due to altered blood–brain barrier permeability (35, 36). Since molecular hydrogen is permeable to cell membrane, the inhaled hydrogen can be rapidly transported and reach the ischemic tissues in a timely fashion. Hydrogen specifically neutralizes detrimental ROS such as hydroxyl radicals (•OH) and peroxynitrite ($ONOO^-$) but does not affect superoxide ($\bullet O2^-$) and hydrogen peroxide (H_2O_2) that having physiological roles (31). Additionally, hydrogen also reduces ROS through indirectly increasing the activities of antioxidant enzymes, including superoxide dismutase and catalase (37). The reduction of oxidative stress leads to various effects including anti-inflammatory and anti-apoptotic responses via changes in gene expression, signal transduction, and mitochondrial membrane potential (20–23, 37, 38). As a result, outcomes of post-cardiac arrest syndrome characterized by systemic ischemia/reperfusion injury and systemic inflammation are greatly improved.

We recognize several limitations in our study. First, the small rodent used in the study has different rates of metabolism and

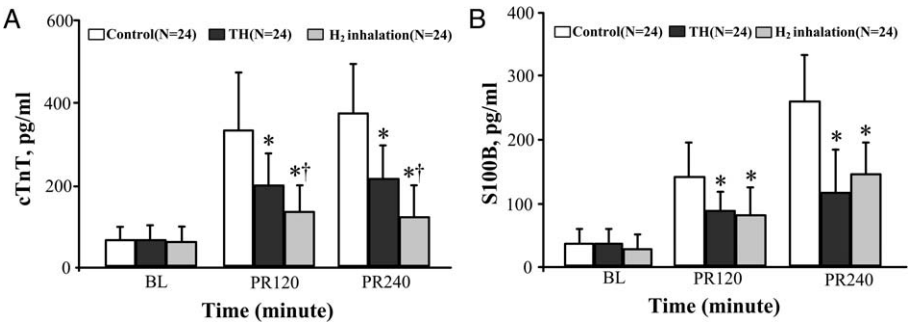

FIG. 4. **Serum level cardiac troponin T (cTnT) (A) and S100B (B) measured at baseline, 120 and 240 min after resuscitation (mean ± SD).** * $P < 0.05$ versus Control group; † $P < 0.05$ versus hypothermic (TH) group. BL indicates baseline; PR, post-resuscitation. n = 24 at each time point in each group.

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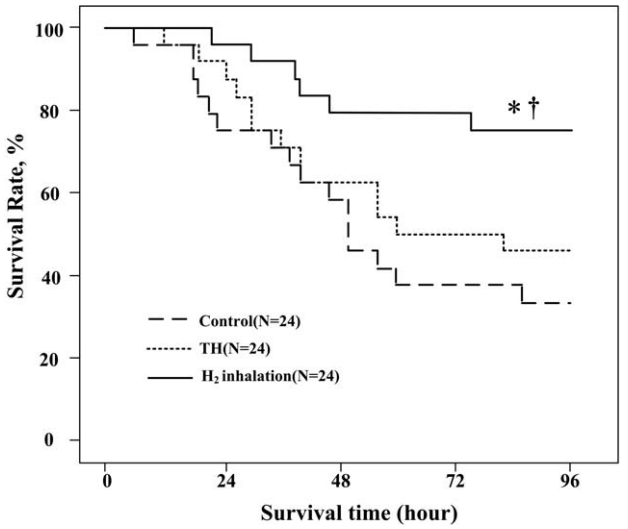

FIG. 5. **Kaplan–Meier analysis of cumulative survival at 96-h post-resuscitation.** * $P < 0.05$ versus Control group; † $P < 0.05$ versus hypothermic (TH) group. n = 24 in each group.

TABLE 4. **Neurological dysfunction score (mean ± SD)**

	Control (n = 24)	TH (n = 24)	H_2 inhalation (n = 24)
At 24 h	345.4 ± 115.2	307.3 ± 92.8	200.6 ± 84.5*,†
At 48 h	359.2 ± 161.7	329.0 ± 155.6	200.2 ± 168.8*,†
At 72 h	373.8 ± 192.3	317.3 ± 195.3	167.3 ± 195.3*,†
At 96 h	365.2 ± 210.8	311.0 ± 210.9	145.6 ± 213.6*,†

TH indicates hypothermia.
*$P < 0.05$ versus Control group.
†$P < 0.05$ versus TH group.

physiology. Thus, the results cannot be extrapolated to large animal or human and other etiology of cardiac arrest. Second, we observed that inhalation of 2% hydrogen with 98% oxygen for 1 h immediately after ROSC significantly improved neurological outcome and survival, but the optimal duration, together with optimal concentration of hydrogen/oxygen of the treatment, is still unclear. Third, we did not compare the effectiveness among different methods of hydrogen application, such as inhalation of hydrogen gas, oral hydrogen water, and hydrogen saline injection. Fourth, it was difficult to compare the cardiac function measured by echo in TH group with the other groups with normothermia because TH markedly reduced the heart rate. Therefore, whether combining hydrogen ventilation with hypothermia would provide a greater degree of cardiac protection is uncertain. Fifth, although we showed that hydrogen was superior to hypothermia for cardiac protection, but the potential mechanism still needs to be elucidated.

CONCLUSIONS

In this asphyxial rat model of cardiac arrest and resuscitation, small amount of inhaled hydrogen for 1 h was superior to 2 h mild hypothermia in improving cardiac function and neurological outcome. Hydrogen may be a good candidate for post-resuscitation intervention in cardiac arrest, especially caused by asphyxia and respiratory arrest.

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