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ORIGINAL RESEARCH

Cerebral Blood Flow–Guided Manipulation of Arterial Blood Pressure Attenuates Hippocampal Apoptosis After Asphyxia-Induced Cardiac Arrest in Rats

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BACKGROUND: In most post–cardiac arrest patients, the autoregulation mechanism of cerebral blood flow (CBF) is dysregulated. We examined whether recovery of CBF by adjusting mean arterial pressure mitigates post–cardiac arrest neuronal damage.

METHODS AND RESULTS: Wistar rats that underwent 8-minute asphyxia-induced cardiac arrest and resuscitation were computer-randomized to norepinephrine or control groups. The CBF was measured at the dorsal hippocampal CA1 region of the left hemisphere. In the norepinephrine group, the mean arterial pressure was adjusted to recover CBF to 80% to 100% of baseline. Twenty-four hours following resuscitation, neurological outcomes were assessed, and brain tissues and blood samples were harvested for neuronal apoptosis and injury assessment. Thirty resuscitated rats were randomized into 2 groups, each containing 12 rats that completed the experiments. Norepinephrine infusion effectively prevented posthyperemia hypoperfusion and recovered CBF to pre-arrest baseline levels; a moderate positive linear correlation between mean arterial pressure and CBF during this period was also observed (P<0.001). There were no significant between-group differences in neurological recovery. In the norepinephrine group compared with the control group, upregulated cleaved caspase-3 protein expression in brain tissue determined by Western blot was reduced (P=0.02) and the densities of apoptotic cells in hippocampal CA1 and CA3 regions determined by terminal deoxynucleotidyl transferase-mediated dUTP biotin nick-end labeling were decreased (P<0.001). No significant differences in serum neuron-specific enolase or S100β levels were detected between the 2 groups.

CONCLUSIONS: CBF recovery demonstrated neuroprotective effects by reducing activation of cerebral apoptosis and number of apoptotic neurons. However, these effects did not significantly improve clinical neurological function, necessitating further investigation.

Key Words: apoptosis ■ blood pressure ■ cardiac arrest ■ cerebral auto-regulation ■ cerebral blood flow ■ neurological outcome

Globally, out-of-hospital cardiac arrest (CA) strikes an estimated 44 people per 100 000 annually.[1] The prognosis following out-of-hospital CA remains dismal, with only 8.6% of patients[2] able to recover neurological function. Brain injury is a major component of the post-CA syndrome,[3] contributing to 68% of deaths following out-of-hospital CA.[4] Guidelines[5,6] recommend a protocolized treatment algorithm to improve neurological recovery and suggest maintaining mean arterial pressure (MAP) >65 mm Hg[6] or systolic blood pressure >90[6] or 100 mm Hg[5] during the early postresuscitation phase.

Keeping blood pressure above a certain threshold is aimed to provide adequate cerebral blood flow

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CLINICAL PERSPECTIVE

What Is New?

- This rat model of cardiac arrest revealed a pattern of initial cerebral hyperemia followed by persistent hypoperfusion during the immediate postresuscitation period.
- Because of the dysregulated cerebral autoregulation, the postresuscitation cerebral blood flow could be recovered to the baseline level by adjusting the blood pressure through norepinephrine infusion.
- The recovered cerebral blood flow might benefit neurological recovery by reducing neuronal apoptosis.

What Are the Clinical Implications?

- For postresuscitation patients, physicians may use norepinephrine infusion to maintain patients' baseline blood pressure, which might be beneficial for recovering cerebral blood flow and neurological status.
- Newer devices may be needed to measure cerebral blood flow in clinical practice in order to adjust blood pressure in a personalized manner, instead of a "one-size-fits-all" algorithm.

Nonstandard Abbreviations and Acronyms

CA	cardiac arrest
CBF	cerebral blood flow
CPR	cardiopulmonary resuscitation
FiO_2	inspired oxygen fraction
MAP	mean arterial pressure
NSE	neuron-specific enolase
ROSC	return of spontaneous circulation
TUNEL	terminal deoxynucleotidyl transferase-mediated dUTP biotin nick-end labeling

(CBF), which is physiologically stabilized by cerebral autoregulation in light of the constantly fluctuating MAP. However, CBF is dysregulated after CA.[7] In most post-CA patients, cerebral autoregulation is reportedly either absent or right-shifted during the immediate postresuscitation phase.[8] Therefore, a universal blood pressure goal may not provide adequate CBF for all patients; this is also recognized by the guidelines.[5,6]

CBF promotion has been suggested to improve neurological recovery in a dog CA model.[9] Induced hypertension also demonstrated protective effects against histopathologic damage in hippocampal areas.[10] Hemodynamic-directed cardiopulmonary resuscitation (CPR) has been shown to improve survival in a swine CA model.[11] In the current study, we examined whether CBF-guided MAP adjustment to recover pre-CA CBF levels could mitigate neuronal damage in a rat model of asphyxia-induced CA.

MATERIALS AND METHODS

This animal study was approved by the Institutional Animal Care and Use Committee (approval number 20150352) and conducted according to the *Guide for the Care and Use of Laboratory Animals* by the US National Institutes of Health. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Animal Preparation

The animal experiments were performed using an established rat model of asphyxia-induced CA and CPR.[12] In brief, 14-week-old male Wistar rats were anesthetized with intraperitoneal pentobarbital (45 mg/kg). Tracheal intubation with mechanical ventilation was initiated with a tidal volume of 0.6 mL/100 g body weight, a frequency of 100 breaths per minute, and an inspired oxygen fraction of 21%. To measure CBF, a stationary laser Doppler probe (OxyFlo™; Oxford-Optronix, Oxford, UK) was inserted through a small cranial window created over the left hemisphere. The insertion site was marked on the dura with the stereotaxic coordinate anteroposterior −3.5 mm and lateral 2.0 mm from the bregma. The rats were then fixed in the supine position to a customized stereotaxic frame. The OxyFlo™ probe was inserted 2 mm from the dura into the dorsal hippocampal CA1 region.[13] The right femoral artery and left jugular vein were cannulated to obtain arterial blood pressure and blood samples and administer medications, respectively. The rectal temperature was maintained at 36.5 to 37.5°C.

Asphyxia-Induced CA

CA was induced 15 minutes after surgical preparation (Figure 1) by clamping the endotracheal tube. CA was defined as MAP ≤20 mm Hg. CPR was started at 8 minutes after asphyxia with 1 dose of intravenous epinephrine (0.01 mg/100 g), 1 dose of sodium bicarbonate (1 mEq/kg), and manual chest compressions (200/min). During CPR, the ventilatory inspired oxygen fraction was increased to 100%. The return of spontaneous circulation (ROSC) was defined as a return of supraventricular rhythm with a MAP ≧40 mm Hg for 10 minutes. Rats were excluded if ROSC did not occur within 6 minutes of CPR. After ROSC, the rats were mechanically ventilated for 150 minutes with an initial

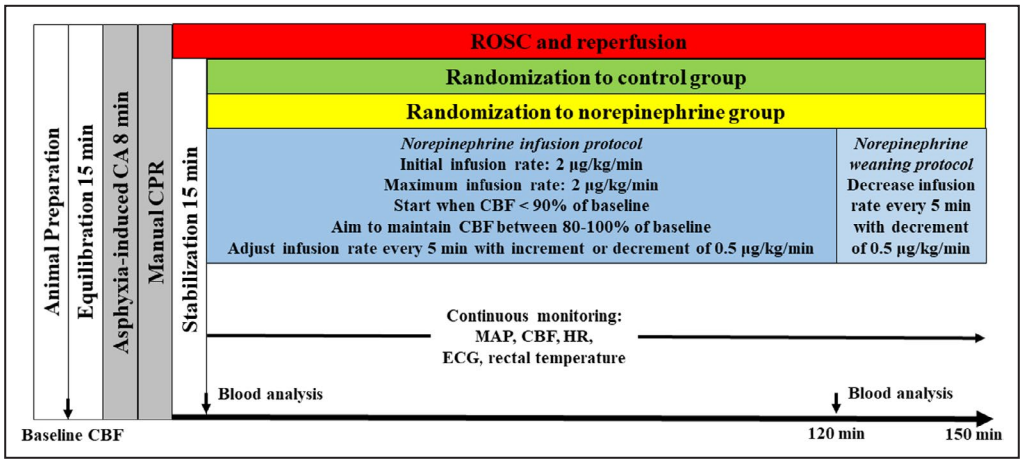

Figure 1. Experimental procedure and measurements during baseline state, asphyxia, CA, CPR, and reperfusion.
CA indicates cardiac arrest; CBF,cerebral blood flow; CPR, cardiopulmonary resuscitation; HR, heart rate; MAP, mean arterial pressure; and ROSC, return of spontaneous circulation.

inspired oxygen fraction of 100% for 15 minutes and an inspired oxygen fraction of 50% for the duration of the experiments.

CBF-Guided MAP Adjustment

The rats with sustained ROSC for at least 15 minutes were computer-randomized in blocks of 4 into norepinephrine and control groups. The baseline CBF was measured 15 minutes before asphyxia. The CBF was recorded in blood perfusion units and expressed as a percent of baseline values. In the norepinephrine group, norepinephrine infusion was initiated after post-ROSC 15 minutes if the CBF was <90%. The initial infusion rate was 2 μg/kg per minute and adjusted every 5 minutes with increments or decrements of 0.5 μg/kg per minute to maintain CBF between 80% and 100%. The maximum infusion rate was set at 2 μg/kg per minute.[14] At post-ROSC 120 minutes, if norepinephrine was still being infused, the infusion rate was reduced every 5 minutes with a decrement of 0.5 μg/kg per minute. MAP, CBF, and norepinephrine infusion rate were recorded every 5 minutes. Arterial blood analyses (epoc® Blood Analysis System, Siemens Healthineers, Erlangen, Germany) were conducted at post-ROSC 15 and 120 minutes.

Outcome Measures of Survival and Neurological Recovery

After the 150-minute experiment, the rats were returned to the incubator. At post-ROSC 24 hours, the survival status of each randomized rat was recorded; neurological recovery was assessed by neurological function scores for rats (Table S1).[15] Rats were then euthanized with intraperitoneal pentobarbital (250 mg/kg). Brain tissues of the rats surviving for 24 hours were harvested for Western blotting or terminal deoxynucleotidyl transferase-mediated dUTP biotin nick-end labeling assay; right atrium blood samples were collected from these rats for enzyme-linked immunosorbent assay. The right hemispheres were washed in physiologic saline, immediately frozen in liquid nitrogen, and stored at –80°C, and the left hemispheres were immediately fixed in 4% formaldehyde in 0.1 mol/L phosphate buffer.

Western Blotting

The right hemispheres were used for Western blotting. The primary antibody, caspase-3 (#14220, Cell Signaling Technology, Danvers, MA), was used. GAPDH (#60004-1-Ig, Proteintech Group, Rosemont, IL) was used as a loading control. Signals from immunoblots were quantified using ImageQuant™ LAS 4000 (GE Healthcare Life-Sciences, Marlborough, MA).

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Biotin Nick-End Labeling Assay

The left hemispheres were used for terminal deoxynucleotidyl transferase-mediated dUTP biotin nick-end labeling (TUNEL) assay with a DeadEnd™ Fluorometric TUNEL System (Promega, Madison, WI) according to the manufacturer's instructions. TUNEL was performed on 5-μm-thick paraffin-embedded sections. 4′,6-Diamidino-2-phenylindole was used to stain cell nuclei. The apoptotic cells were examined by confocal fluorescence microscopy (EVOS FL Auto Imaging System, Thermo Fisher

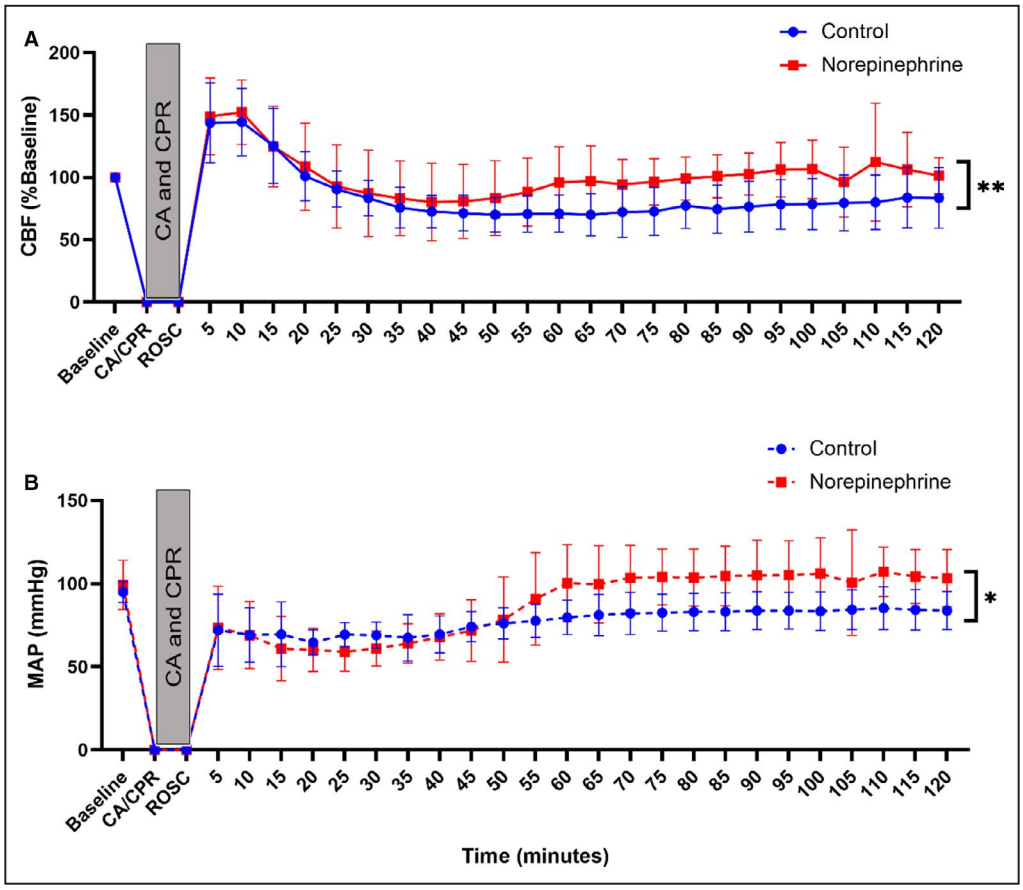

Figure 2. Changes in CBF and MAP after asphyxia-induced CA in rats with or without norepinephrine infusion.
A, Norepinephrine group vs control group; *P*=0.008. **B**, Norepinephrine vs control group; *P*=0.03. Data are presented as the mean±SD. CA indicates cardiac arrest; CBF, cerebral blood flow; CPR, cardiopulmonary resuscitation; MAP, mean arterial pressure; and ROSC, return of spontaneous circulation.

Scientific, Waltham, MA). In each sample, fields were randomly selected in the hippocampal CA1 and CA3 regions for quantitative comparisons.[16] Each field was photographed under microscopy at a 520-nm wavelength (green TUNEL) and a 430-nm wavelength (bluish-violet 4′,6-diamidino-2-phenylindole). PhotoImpact X3 (Corel, Ottawa, Canada) was used to merge the 2 images for the final counting analysis with ImageJ software (Version 1.52a, National Institutes of Health, Bethesda, MD). The densities of TUNEL-positive cells in the aforementioned regions were measured at ×100 final magnification, with an area of each microscopic field of ≈0.48 mm^2 (apoptotic cells/mm^2).

ELISA

ELISA kits of Neuron-specific enolase (NSE) (#E-EL-R005) and S100β (#E-EL-R0868) (Elabscience Biotechnology, Houston) were used to determine the serum NSE and S100β concentrations according to the manufacturer's instructions. Absorbance was measured on a microplate (SpectraMax Plus 384 Microplate reader, Molecular Devices, San Jose, CA).

Statistical Analysis

Twelve animals per group were necessary to demonstrate a mean difference of 20% in injured neuronal cells with a power of 80% at the 5% level and a SD of 20% (MedCalc, version 19.0.7, MedCalc Software, Ostend, Belgium). Continuous data were expressed as mean±SD and compared using 1-way ANOVA with post hoc Tukey's test. Time-based measurements, including MAP and CBF, were compared with 2-way repeated measurement ANOVA (using the factors treatment and time) with Bonferroni's post-tests. The correlation between MAP and CBF was evaluated by generalized estimating equations and the Pearson correlation. A 2-tailed $P<0.05$ was considered significant. ANOVA tests were conducted with GraphPad Prism Version 8.2.1 (GraphPad Software, La Jolla, CA). Generalized estimating equations and the Pearson correlation were conducted using SAS 9.4 software.

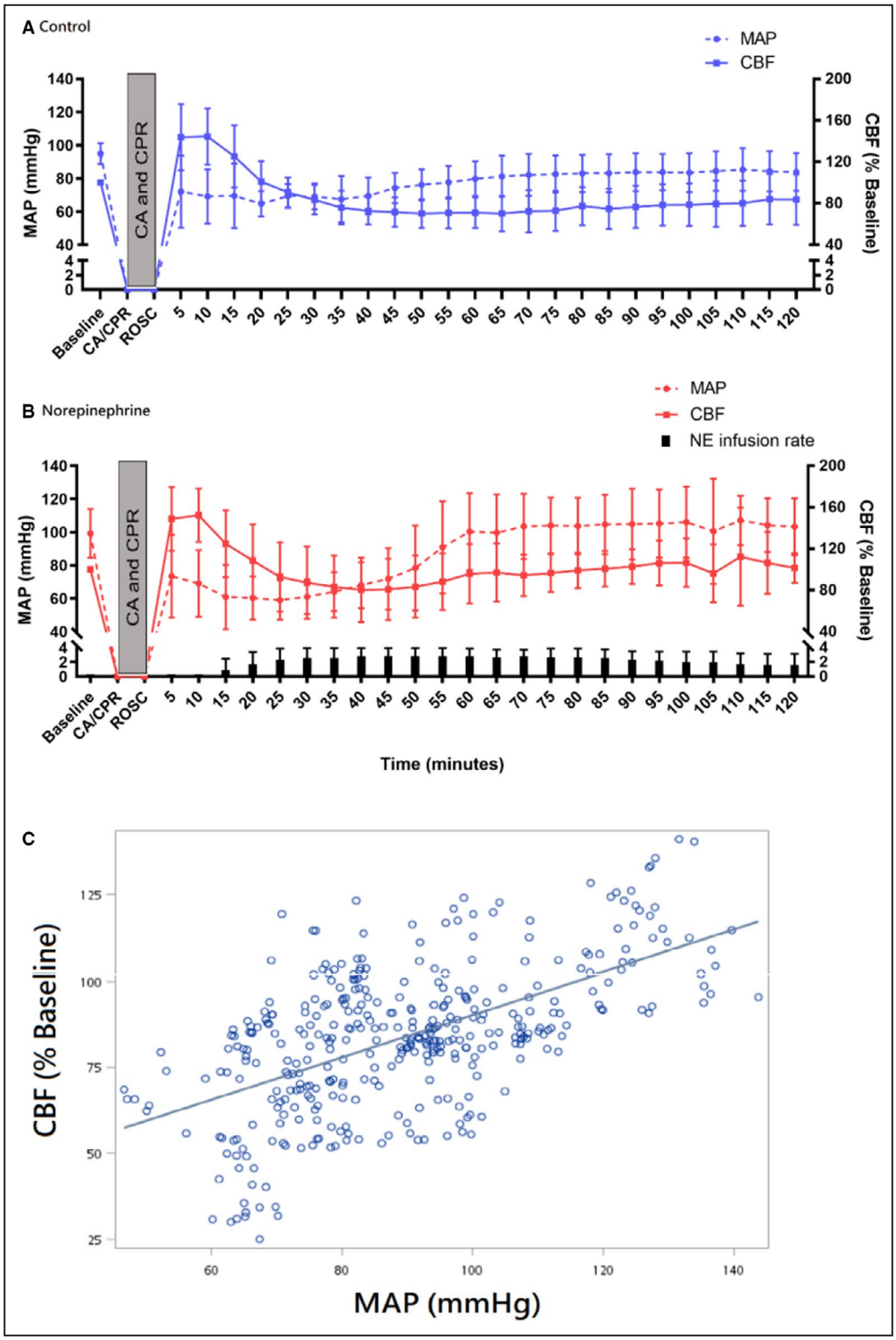

Figure 3. The correlation between CBF and MAP after asphyxia-induced cardiac arrest in rats with or without NE infusion.

A, Control group. **B**, NE group. Data are presented as the mean±SD. **C**, Scatterplot of MAP and corresponding CBF after post-ROSC 40 minutes. Pearson's ρ=0.58, *P*<0.001. CA indicates cardiac arrest; CBF, cerebral blood flow; CPR, cardiopulmonary resuscitation; MAP, mean arterial pressure; NE, norepinephrine; and ROSC, return of spontaneous circulation.

RESULTS

Baseline Characteristics and Resuscitation Variables

A total of 41 rats were included (Figure S1). Four rats, which did not receive any surgical procedures, were selected for the naïve group as control for Western blotting and TUNEL. Seven rats did not achieve ROSC within 6 minutes. Thirty resuscitated rats were randomized into 2 groups. In each group, only 12 rats completed the experimental procedures. Among the baseline characteristics and test results, only blood gas pH and P_{CO_2} at post-ROSC 120 minutes differed significantly (Table S2).

CBF and MAP

Early post-ROSC hyperemia (CBF >100% baseline) was detected in both groups (Figure 2A). Maximum CBF occurred at post-ROSC 10 minutes (control group, 144±26%; norepinephrine group, 152±25%). Post-ROSC CBF fell below the baseline at post-ROSC 25 minutes, after which the CBF remained at lower levels than baseline in the control group (77±6%), while in the norepinephrine group, the CBF recovered to 96±9% (norepinephrine versus control group, P=0.008).

In the control group, the minimum MAP occurred at post-ROSC 20 minutes (65±7 mm Hg versus baseline, 95±6 mm Hg) and then stayed at ≈77±8 mm Hg (Figure 2B). In the norepinephrine group, the minimum MAP occurred at post-ROSC 25 minutes (59±11 mm Hg versus baseline, 97±14 mm Hg), and then gradually recovered to 94±16 mm Hg (norepinephrine versus control group, P=0.03).

As shown in Figure 3A and 3B, both CBF and MAP were plotted by group to observe the correlation between these 2 variables. In the control group (Figure 3A), CBF first showed hyperemia and then hypoperfusion although MAP (≈80% baseline) did not change dramatically during this period. In the norepinephrine group (Figure 3B), the timing of initiating infusion varied greatly, as the infusion was started only when CBF fell below 90% (time to infusion: post-ROSC 27±15 minutes). Also, the duration of infusion at the maximum rate varied greatly, as CBF was maintained between 80% and 100% (time to decreasing infusion rate, post-ROSC 64±26 minutes). The cumulative dose of norepinephrine was 153.9±36.9 μg/kg before post-ROSC 120 minutes. These results suggested that the severity of cerebral autoregulation dysfunction may be highly variable. A scatterplot of the MAP and corresponding CBF after post-ROSC 40 minutes in both groups (Figure 3C) demonstrated a moderate positive linear correlation (generalized estimating equations

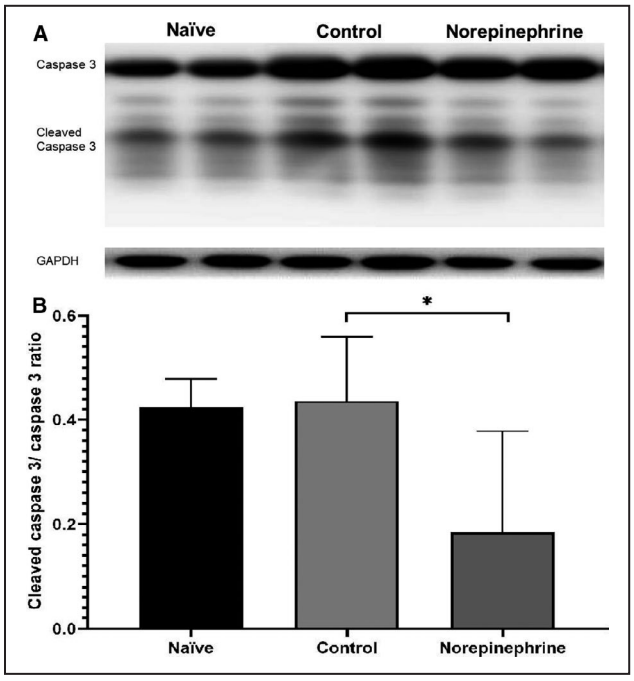

Figure 4. Hippocampal cleaved caspase-3 protein expression after asphyxia-induced cardiac arrest in rats. The cleaved caspase-3 protein expression in brain hippocampal tissues after cardiac arrest and resuscitation was determined by Western blotting. **A**, Representative blots. **B**, Quantification of Western blotting. Data are presented as the mean±SD; P=0.02.

β=0.46; 95% CI, 0.36–0.57; P<0.001; Pearson's ρ=0.58, P<0.001).

Survival and Neurological Outcomes

All rats completing the experimental procedures survived for 24 hours without significant between-group differences in neurological recovery (Table S2).

Attenuated Apoptosis by Recovering CBF

Compared with the control group, the norepinephrine group had reduced upregulation of cleaved caspase-3 protein expression (P=0.02) (Figure 4). The TUNEL assay demonstrated that the densities of apoptotic cells in hippocampal CA1 and CA3 regions were significantly decreased in the norepinephrine group (P<0.001) (Figure 5).

Analysis of Blood NSE and S100β Concentrations

No significant differences in serum NSE and S100β concentrations were detected between norepinephrine and control groups (NSE, norepinephrine versus control, 0.29±0.07 ng/mL versus 0.35±0.14 ng/mL; S100β, norepinephrine versus control, 3.28±2.23 pg/mL versus 2.55±1.03 pg/mL).

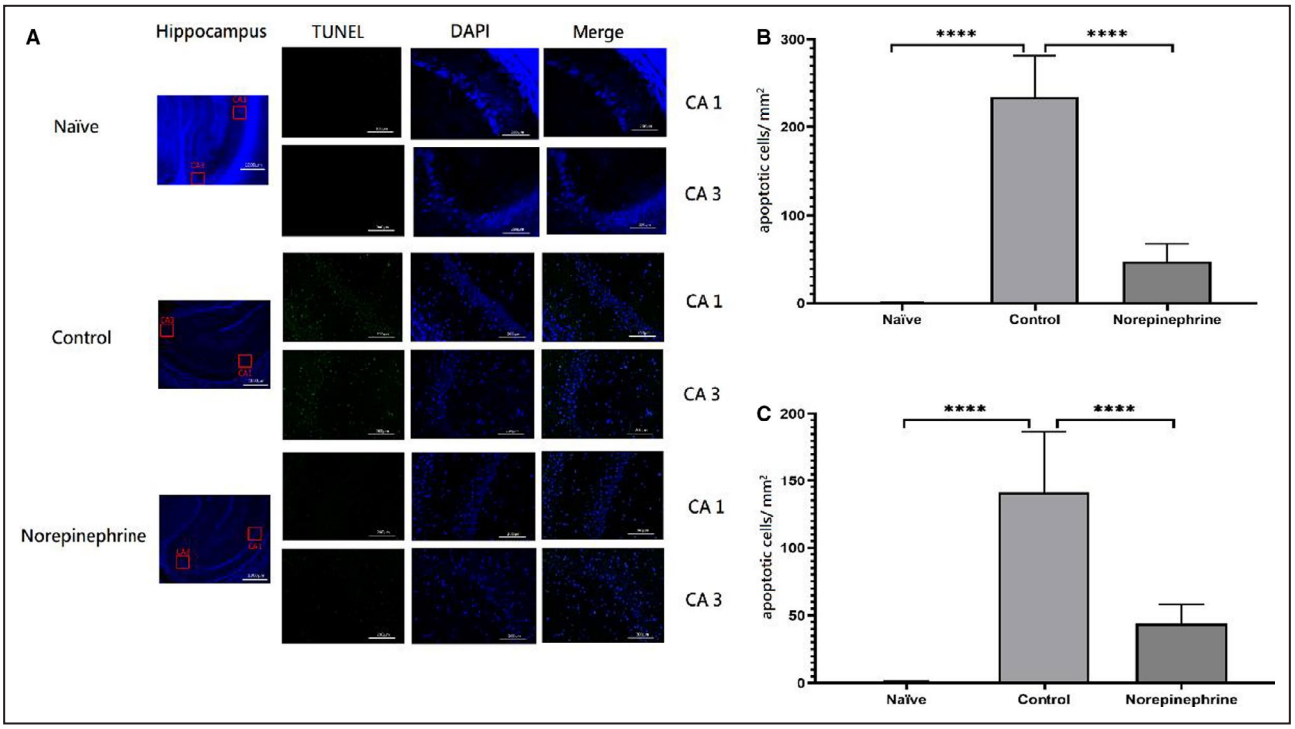

Figure 5. Hippocampal apoptosis after asphyxia-induced cardiac arrest in rats.
A, Brain sections were stained for TUNEL (green) and DAPI (bluish-violet). Confocal microscopy was used to image CA1 and CA3 regions at ×100 magnification. The red rectangles indicate the selected CA1 and CA3 regions for quantification. **B**, Comparisons of densities of apoptotic cells in the CA1 region. Naïve vs control group, $P<0.001$; norepinephrine vs control group, $P<0.001$. **C**, Comparisons of densities of apoptotic cells in the CA3 region. Naïve vs control group, $P<0.001$; norepinephrine vs control group, $P<0.001$. Data are presented as the mean±SD. DAPI indicates 4′,6-diamidino-2-phenylindole; and TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP biotin nick-end labeling.

DISCUSSION

Main Findings

This rat CA model revealed a pattern of initial cerebral hyperemia followed by persistent hypoperfusion during the immediate post-ROSC period. The post-ROSC CBF could be recovered to the baseline level by adjusting the MAP through norepinephrine infusion. A moderate positive linear correlation between MAP and CBF was observed, suggesting that cerebral autoregulation may be impaired during the early post-ROSC period. Finally, the recovered CBF might benefit neurological recovery by reducing neuronal apoptosis.

Post-ROSC CBF Pattern

The post-ROSC CBF pattern revealed by the OxyFlo™ probe in our study was consistent with previous studies using magnetic resonance imaging.[17] We selected hippocampal CBF as the guide to adjust MAP because the hippocampus area was most susceptible to ischemic injuries, especially in the CA1 and CA3 regions.[18,19] The initial hyperemia may be caused by the transient surge of circulating catecholamine concentrations.[3] Following hyperemia, microcirculatory disturbances may result in cerebral areas with different levels of perfusion deficits, the so-called no-reflow phenomenon.[20,21] The no-reflow phenomenon may be worsened by impaired cerebral autoregulation, with loss of the ability to increase CBF leading to further ischemic injuries.[8,22]

CBF-Guided MAP Manipulation

In a rat model of asphyxia-induced CA, Hachimi-Idrissi et al used norepinephrine infusion to maintain MAP at 140 mm Hg (≈160% of baseline) for 1 hour.[23] Rats undergoing induced hypertension, and treated with mild hypothermia concurrently, however, did not exhibit better neurological recovery at post-ROSC 72 hours but did have better survival at post-ROSC 28 days, compared with normotensive and normothermic rats.[23] In contrast, in a dog model of ventricular fibrillation–induced CA, Safar et al demonstrated that induced hypertension (MAP, 140 mm Hg), when combined with hemodilution and mild hypothermia, had mitigated histological injuries and improved neurological outcomes, compared with the normotensive, normothermic, and hypocapnic group.[9] Therefore, promoting CBF may improve outcomes. However, in these studies,[9,23] the net effects of

induced hypertension could not be evaluated. In contrast to the results of these previous studies targeting a high MAP,[9,23] our results demonstrate that a pre-CA MAP may be sufficient to recover the pre-CA CBF level.

In our study, the P_{CO_2} was significantly higher in the norepinephrine than in the control group at post-ROSC 120 minutes (Table S2) despite that the ventilator settings, including tidal volume and respiratory rate, were the same in both groups. The mildly elevated P_{CO_2} might also contribute to the increased CBF in the norepinephrine group.[24] Nonetheless, the positive linear correlation between MAP and CBF not only suggested disruption of cerebral autoregulation but also indicated that the CBF may still be regulated mainly by MAP during the post-ROSC period. Using transcranial Doppler ultrasonography, Sundgreen et al noted that 44% and 28% of post-ROSC patients had absent (ie, CBF changed along with the MAP without a clear plateau phase) or right-shifted cerebral autoregulation, respectively.[8] In the present study, we could not verify which type of cerebral autoregulation dysfunction the rats developed because the MAP in the norepinephrine group only fluctuated within a small range. However, the widely varying initiation time, duration of maximum infusion rate, and cumulative dosage of norepinephrine indicate that the severity of cerebral autoregulation dysfunction may also be heterogeneous. Therefore, guided by real-time CBF, adjusted MAP may be more closely tailored to individual needs than a one-size-fits-all goal.[9,23]

In a pilot trial, Jakkula et al randomized patients into groups with low-normal (65–75 mm Hg) or high-normal (80–100 mm Hg) MAP for 36 hours following ROSC.[25] They found that different MAP levels did not affect the NSE concentration at post-ROSC 48 hours. If the severity of cerebral autoregulation dysfunction is heterogeneous, a universal MAP target and treatment duration may not provide optimal CBF for all patients. For example, a right-shifted cerebral autoregulation curve in patients with long-standing hypertension[26] may necessitate higher MAP to maintain adequate CBF,[27,28] thereby suggesting that a universal low-normal MAP may be insufficient in these patients.[25] In contrast, the linear correlation between MAP and CBF (ie, absent cerebral autoregulation) observed in some patients[8] suggests that the high-normal MAP may render these patients vulnerable to a dangerous increase in CBF, leading to worsening intracranial hypertension and poor neurological recovery. Interestingly, Jakkula et al observed that near infrared spectroscopy–measured cerebral oxygenation was similar between low-normal and high-normal groups.[25] Meng et al suggested that the area sampled by near infrared spectroscopy may be too superficial to detect regions vulnerable to ischemic insults following CA, such as the hippocampus.[29] Iordanova et al[7] indicated there were knowledge gaps in CBF monitoring and cerebral goal-directed therapies for post-CA management, which necessitated a new device to facilitate CBF-guided MAP manipulation in clinical practice.[25]

Recovering CBF Attenuated Apoptosis

As an obligate glucose consumer, the brain would deplete its glucose storage within 5 minutes following circulatory cessation.[30] Although ROSC can recover neuronal ATP levels to at least 70% of pre-CA levels, it does not completely restore cell function and delayed neuronal death ensues in the brain regions susceptible to ischemia–reperfusion injuries.[31] Both neuronal necrosis and apoptosis have been reported after CA. However, the relative contribution of each cell death pathway remains unknown.[32] Our study did not find significant differences in levels of neuronal necrosis markers (ie, serum NSE and S100β) between norepinephrine and control groups. Instead, we found that CBF recovery significantly mitigated neuronal apoptosis in the CA1 and CA3 regions in the norepinephrine group.

The primary determinant of the impact of CBF on post-ROSC outcomes is the coupling between the CBF and brain metabolism during this critical period. It has been assumed that the reduced CBF in the post-ROSC period just reflects the decreased metabolic requirements of injured brain cells or the decreased number of viable cells, compared with the pre-CA state, and accordingly, artificially increased MAP and CBF may not be necessary. However, by using a microdialysis probe, Hifumi et al[33] found that the lactate/pyruvate ratio progressively increased in post-ROSC patients with unfavorable neurological recovery, suggesting that metabolic requirements may not be adequately met in these patients, which could consequently be resolved through CBF recovery.

Therefore, concerning the coupling between the CBF and metabolism, recovering CBF alone is only half of the picture; it is also important to determine whether the neurons are still sufficiently viable to utilize the blood flow. For our study, the goal of CBF, to reach 80% to 100% of the pre-CA level, was selected empirically; the CBF was supposed to be higher than in the hypoperfusion state in the control group and lower than the pre-CA level to avoid injuries caused by increased intracranial pressure. It is unknown whether CBF higher than baseline leads to better outcomes. By inserting microdialysis probes into the hippocampal CA1 region in a rat CA model, Hosmann et al demonstrated the serial metabolic changes in lactate/glucose/glutamate

concentrations and lactate/pyruvate ratio after ROSC.[34] Among these indicators, Hosmann et al suggested that glutamate concentration may be an early marker of cerebral ischemia.[34] Accordingly, the metabolic changes detected by the cerebral microdialysis tube to adjusted CBF in real time warrant further investigation.

Study Limitations

First, the benefits of CBF-guided MAP manipulation were determined based on the reduction of histopathological injuries rather than survival or neurological outcomes. Because the severity of cerebral autoregulation dysfunction varies, more animals may be needed to maximize the statistical power to examine differences in clinical outcomes. Second, the target of CBF and treatment duration by norepinephrine infusion were empirically selected according to our pilot studies. Further dose–response analyses are needed to identify the optimal treatment protocol. Third, the mild hypercapnia noted in the norepinephrine group may also be neuroprotective.[35] Whether or not the neuroprotective effects of hypercapnia were independent of enhanced CBF should be further examined. Fourth, the nonsignificant comparison of activated caspase 3 between the naïve and control groups (Figure 4) was quite unexpected. The possible explanation may be the result of a lack of statistical power (ie, the limited number of rats in the naïve group), or involvement of activated caspase 3 in some nonapoptotic function.[36] However, we did not have appropriate evidence to corroborate these assumptions. Despite this limitation, the interpretation of the comparison results between control and norepinephrine groups might not be influenced. Fifth, our study was performed using healthy animals without any known comorbidities. The impact of underlying diseases, such as hypertension, on the effects of recovering CBF is unknown. Further studies in disease models are needed to clarify this issue.

CONCLUSIONS

The cerebral autoregulation mechanism may be impaired after ROSC. CBF-guided MAP manipulation with norepinephrine infusion effectively restored post-ROSC CBF to pre-CA levels, demonstrating neuroprotective effects by reducing activation of cerebral apoptosis and number of apoptotic neurons. However, these effects did not significantly improve clinical neurological function, necessitating further investigation. Considering the variable levels of cerebral autoregulation dysfunction in clinical practice, CBF-guided MAP manipulation may be more appropriate than a one-size-fits-all treatment algorithm.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Materials

Tables S1–S2
Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Definition of neurological function score.

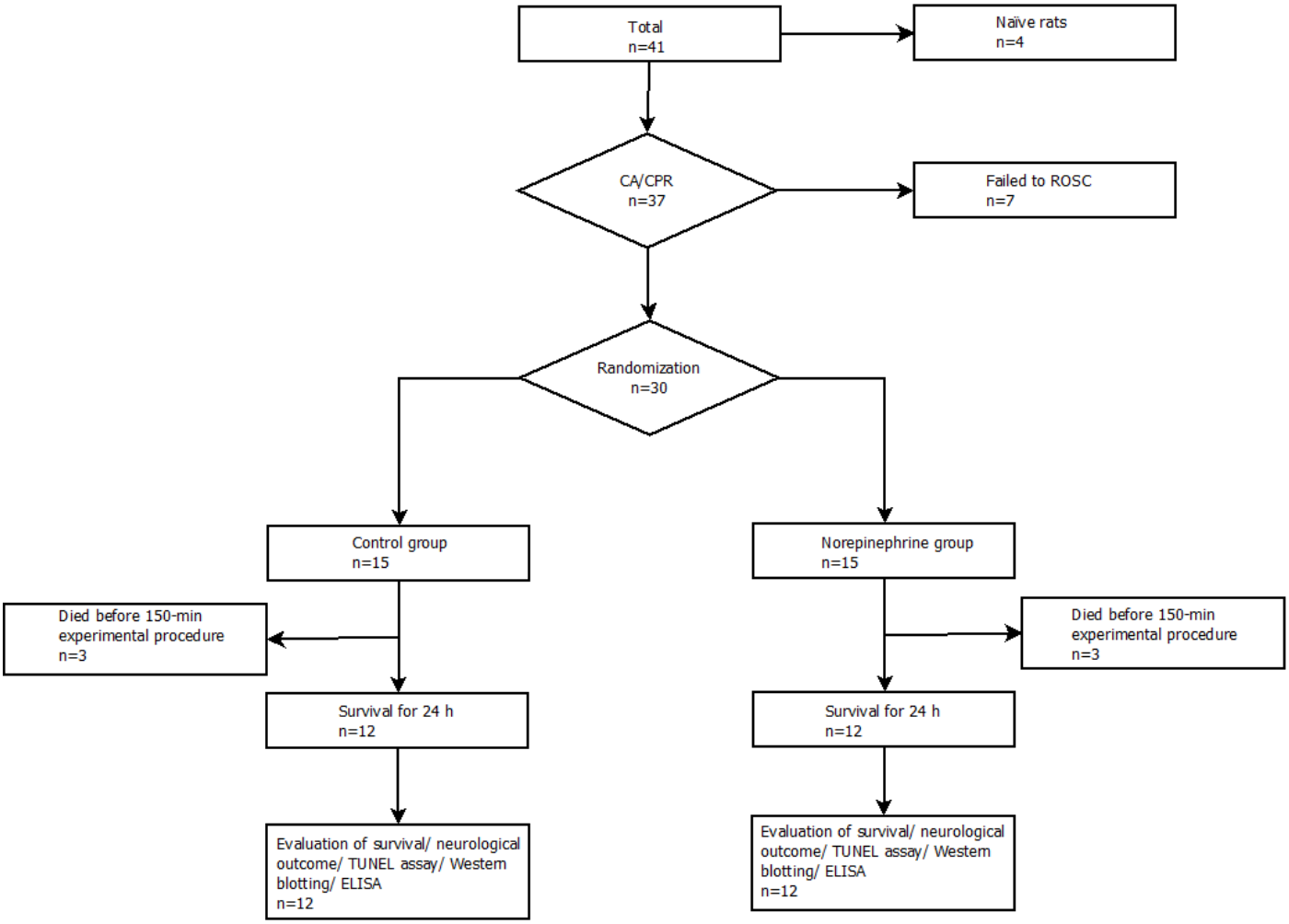
Level of consciousness	Corneal reflex	Respirations	Righting reflex	Coordination	Movement/ activity	Score
No reaction to pinching of tail	No blinking	Irregular breathing pattern	No turning attempts	No movement	No spontaneous movement	0
Poor response to tail pinch	Sluggish blinking	Decreased breathing frequency, normal pattern	Sluggish turning	Moderate ataxia	Sluggish movement	1
Normal response to tail pinch	Normal blinking	Normal breathing frequency and pattern	Turns over quickly and spontaneously	Normal coordination	Normal movement	2

Table S2. Baseline characteristics, resuscitation variables and outcomes of included animals.

	Naïve (n=4)	Control (n=12)	Norepinephrine (n=12)	*p* value
Body weight, g	455 (21)	488 (35)	465 (33)	0.16
Cardiac arrest time, s	NA	253 (26)	241 (42)	0.45
CPR time, s	NA	103 (85)	138 (104)	0.40
Body temperature, ℃				
Baseline	NA	36.5 (0.8)	36.7 (0.6)	0.48
60 mins following ROSC	NA	36.9 (0.5)	37.0 (0.4)	0.57
120 mins following ROSC	NA	37.0 (0.4)	37.1 (0.4)	0.39
Blood analysis at 15 mins following ROSC				
pH	NA	7.16 (0.06)	7.12 (0.07)	0.23
PCO_2, mmHg	NA	71.2 (8.3)	78.6 (12.6)	0.12
PO_2, mmHg	NA	98.1 (26.3)	89.9 (47.0)	0.62
$HCO3^-$, mEq/L	NA	25.1 (2.3)	25.6 (3.0)	0.67
Haematocrit, %	NA	39.7 (2.0)	40.6 (2.1)	0.31
Glucose, mg/dL	NA	374.3 (34.6)	391.2 (56.3)	0.40
Lactic acid, mg/dL	NA	57.3 (16.4)	59.7 (13.9)	0.71
Blood analysis at 120 mins following ROSC				
pH	NA	7.34 (0.04)	7.29 (0.06)	0.03
PCO_2, mmHg	NA	56.0 (9.0)	65.7 (8.4)	0.02
PO_2, mmHg	NA	181.7 (55.5)	168.5 (66.0)	0.63
$HCO3^-$, mEq/L	NA	30.2 (3.3)	31.2 (2.3)	0.39
Haematocrit, %	NA	40.9 (3.1)	43.3 (3.7)	0.12
Glucose, mg/dL	NA	178.5 (19.9)	216.1 (55.7)	0.06
Lactic acid, mg/dL	NA	4.8 (1.2)	5.9 (3.8)	0.39
Neurological function score	NA	9.9 (0.3)	9.8 (0.4)	0.56

Data are expressed as mean (standard deviation). CPR, cardiopulmonary resuscitation. ROSC, return of spontaneous circulation.

Figure S1. Flow diagram of the experimental group randomization and testing procedures.



CA= cardiac arrest, CPR = cardiopulmonary resuscitation, ELISA = enzyme-linked immunosorbent assay, ROSC = return of spontaneous circulation, TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labelling.