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Review

Neuroprotective Approaches for Brain Injury After Cardiac Arrest: Current Trends and Prospective Avenues

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With the implementation of improved bystander cardiopulmonary resuscitation techniques and public-access defibrillation, survival after out-of-hospital cardiac arrest (OHCA) has increased significantly over the years. Nevertheless, OHCA survivors have residual anoxia/reperfusion brain damage and associated neurological impairment resulting in poor quality of life. Extracorporeal membrane oxygenation or targeted temperature management has proven effective in improving post-cardiac arrest (CA) neurological outcomes, yet considering the substantial healthcare costs and resources involved, there is an urgent need for alternative treatment strategies that are crucial to alleviate brain injury and promote recovery of neurological function after CA. In this review, we searched PubMed for the latest preclinical or clinical studies (2016–2023) utilizing gas-mediated, pharmacological, or stem cell-based neuroprotective approaches after CA. Preclinical studies utilizing various gases (nitric oxide, hydrogen, hydrogen sulfide, carbon monoxide, argon, and xenon), pharmacological agents targeting specific CA-related pathophysiology, and stem cells have shown promising results in rodent and porcine models of CA. Although inhaled gases and several pharmacological agents have entered clinical trials, most have failed to demonstrate therapeutic effects in CA patients. To date, stem cell therapies have not been reported in clinical trials for CA. A relatively small number of preclinical stem-cell studies with subtle therapeutic benefits and unelucidated mechanistic explanations warrant the need for further preclinical studies including the improvement of their therapeutic potential. The current state of the field is discussed and the exciting potential of stem-cell therapy to abate neurological dysfunction following CA is highlighted.

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Background

Sudden cardiac arrest (CA) is among the leading causes of mortality and severe morbidity across the world.[1-3] Over the recent years, the survival rate of CA patients has significantly increased following improvements in emergency networking and the broad application of cardiopulmonary resuscitation (CPR) and defibrillation technology.[2,4,5] In the United States, the respective rates of survival to hospital discharge for out-of-hospital cardiac arrests (OHCA) and in-hospital cardiac arrests (IHCA) are 12% and 25%, yet only 8% of OHCA survivors have favorable neurological outcomes.[6]

While resuscitation with the return of spontaneous circulation (ROSC) increases overall chances of survival, secondary neurological injuries can occur as a consequence of reperfusion following successful resuscitation. After CA, the brain is subjected to both ischemic and reperfusion injuries, which in turn mediate unfavorable neurological outcomes. This ischemia-reperfusion

injury post-CA-ROSC elicits multiple processes that lead to cell death: excitotoxicity, acid toxicity, ionic imbalance, oxidative/nitrative stress, pathological protease cascade inflammation, apoptosis, and spreading depolarization[5,7] and have been implicated as the targets of currently used and future neuroprotective strategies that may improve patient outcomes.

Despite the heterogeneous etiologies of CA, the terminal consequence is multi-organ ischemia, particularly in the brain. Improvement of survival and clinical outcomes after CA is dependent on prompt recognition and timely initiation of high-quality CPR and advanced cardiovascular life support (ACLS) with subsequent post-resuscitation care such as extracorporeal CPR[8] and targeted temperature management (TTM),[9] leading to improved brain recovery.

TTM is the sole international guideline-recommended therapy for preventing hypoxic-ischemic brain damage in CA patients.[10,11] We have recently reviewed the current state of artwork in the field of TTM, summarizing preclinical and clinical trials, current recommendations, and future directions, including new cooling methods under investigation and advancement in high-quality patient-centered care.[12] Despite the proven benefits of timely intervention, neuroprotective drugs, and hypothermic treatments on survival, most CA survivors suffer significant neurologic deficits; therefore, there remains an urgent need for the advent of novel neuroprotective therapies guided by the pathways of neurological injury in CA.

In addition to TTM, several other approaches involving the therapeutic administration of gases, pharmacological drugs, or stem cells have shown promise in preclinical studies, with some being tested in clinical trials. These neuroprotective strategies target the CA pathophysiology concerning excitotoxicity, mitochondrial dysfunction, oxidative stress, and inflammation. Stem cells have shown promising preclinical results in improving neurological recovery after CA. Cell-based therapy for ischemia-reperfusion injury involves the delivery of stem cells derived from various sources, including adipose tissue, bone marrow, peripheral blood, and the central nervous system. Although many strategies appear effective in preclinical CA models, there is no conclusive evidence that these interventions can significantly enhance survival with favorable neurological outcomes in clinical subjects.

This review summarizes and discusses the present (2016–2023) preclinical and clinical knowledge of therapies used to confer neuroprotection post-CA. We will discuss gas administration strategies, pharmacological treatments organized by their targeted pathways, and lastly discuss stem-cell-based therapeutics. We discuss the inconsistency in the results of preclinical data from the studies using various experimental animal models, various drug combinations and the possible caveats and future recommendations. We highlight the need for the optimization of existing therapies with respect to dosing, timing, and route of administration. We reveal the need for utilization of large animals like pigs and transgenic animals to test the therapeutic benefits and dissect the detailed molecular mechanisms of the therapy, respectively. Lastly, as there is a lack of clinical translation of stem cell therapy in CA, we discuss the current state of the art in preclinical stem cell therapy and existing methods being used to augment their therapeutic benefits in CA. We also discuss the potential pitfalls of current preclinical stem cell therapy in CA-induced brain injury and explore molecular pathways wherein modulation could enhance therapeutic efficacy.

Materials and methods

A literature search was conducted of articles indexed in PubMed between 2016 and 2023 using combinations of keywords including "cardiac arrest," "neuroprotection," "gas," "pharmacological approach," and "stem cells." *In vitro* studies, preclinical animal studies, and clinical trials relevant to global cerebral ischemia associated with CA were reviewed. Articles were selected for review if they described mechanisms of neuroprotection and neurologic outcomes.

Models and outcomes

Commonly used preclinical CA models, replicating the etiology, therapeutic strategies, and neurological outcome measures observed in clinical practice, are induced through methods such as asphyxiation, ventricular fibrillation, or injection of potassium chloride followed by CPR in mice, rats, rabbits, and swine. Neurological outcomes are assessed by mortality, neurological deficit score (NDS), cognitive tests, and sensorimotor functional testing. These models allow researchers to gain an understanding of CA pathophysiology and potential therapeutic strategies with mechanisms to improve outcomes.

Neuroprotective approaches in CA-induced brain injury

Gas administration

Gases, as therapeutic agents, have excellent diffusivity and easily permeate the cell membrane, targeting various organelles including mitochondria and the nuclei. Recently, it has been shown that inhaled gases like nitric oxide (NO), carbon monoxide (CO), carbon dioxide (CO_2), hydrogen (H_2), noble gases, and hydrogen-sulfide (H_2S) have neuroprotective properties post-resuscitation in both preclinical and clinical settings (Table 1).

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Table 1. Application of gases and their isoforms in cardiac arrest induced brain injury

Gases	Model	Species	Route/dose/time	Observed results	Ref no.
Nitric oxide (NO)	7.5-minute KCl	Mouse	Intravenous, 6 mg/kg, 15 min after ROSC	(1) SPL-334.1 attenuated GSNOR activity and elevated the number of SNO proteins (2) Improved neurological functional score and survival (3) Attenuated fluoro-jade B cells in the cortex, striatum, and hippocampus (4) Attenuated BBB damage	13
	10-minute VF	Rat	Inhaled gas, 20 ppm, for 5 h after ROSC in addition to MTH	(1) Combination of NO and MTH improved NDS (2) Decreased lactate and TNF-α release	14
	7- or 8-minute ACA	Rat	Intravenous, 4 μmol in 0.5 mL plasmalyte-A, over 5 min beginning 5 min after ROSC	(1) Reduced neurologic disability and improved survival (2) Reduced ROS generation (3) Maintained ATP generation and increased brain mitochondrial S-nitrosation	15
	10-minute VF	Pig	Inhaled gas, 80 ppm, during CPR	(1) Elevated mean aortic pressure, diastolic BP, cerebral perfusion pressure, and cerebral blood flow (2) Increased mitochondrial complex I oxidative phosphorylation in cortex and hippocampus	16
	Pilot study (20 adult IHCA patients)	Human	Inhaled gas, 40 ppm, for 24 h after ROSC	(1) Higher rates of survival to discharge (2) No improvement in favorable neurologic outcomes	17
	Phase 1 study (82 adult OHCA patients)	Human	Intravenous, nitrite (25 or 60 mg) given as an IV push in 2.5 mL, during CPR	(1) Increased cGMP and NO_2-CLA levels (2) cGMP and NO_2-CLA levels are associated with improved clinical outcomes	18
Carbon-monoxide (CO)	6-minute ACA	Rat	Intravenous, 10 mg/kg/h for 30 min (5 mg/kg), 30 min after ROSC	(1) Improved survival and NDS (2) Decreased number of damaged neurons in CA1 region (3) Increased brain mitochondrial activity and biogenesis (4) Reduced serum S100β levels	19
	6-minute ACA	Rat	Intravenous, 4 mg/kg, after ROSC	(1) Increased 3-day survival and neurologic deficit (2) Ameliorated neuronal apoptosis and necrosis (3) Reduced ROS and prevented cytochrome C release (4) Increased parkin and PINK1-mediated mitochondrial autophagy	20
	4.5-minute ACA	Pig	Novel extracorporeal releasing system, target 7%–13% blood carboxyhaemoglobin, after ROSC	(1) Fast rise in regional oxygen saturation and improved cerebral perfusion (2) Improved activity in median nerve somatosensory-evoked potential (3) Reduced histopathologic cerebral damage by reducing apoptosis and inflammation	21
Carbon-dioxide (CO_2)	6-minute ACA	Rat	Ventilation with 12% CO_2 starting at 15 min after ROSC for 105 min	(1) Improved neurological functional score (2) Reduced brain tissue malondialdehyde and serum NSE and S100β levels (3) Reduced markers of autophagy and apoptosis	22
	12-minute VF	Pig	Ventilation to maintain end tidal CO_2 45–50 mm Hg, immediately after CPR for 4 h	(1) Improved mean arterial pressure (2) No improvement in neurological recovery (3) Reduced neuronal degeneration in frontal cortex	23

Table 1. Continued

Gases	Model	Species	Route/dose/time	Observed results	Ref no.
Hydrogen (H_2)	5-minute ACA	Rat	Inhaled gas, 2% H_2, for 1 h after ROSC	(1) Improved EEG characteristics (2) Improved neurological outcome and survival	24
	5-minute ACA	Rat	Inhaled gas, 2% H_2, for 1 h after ROSC	(1) Improved NDS and 96-h survival rate (2) Reduced serum S100β and cardiac troponin T levels	25
	9- and 11-minute ACA	Rat	Inhaled gas, 60% H_2, for 1 h before and after ROSC	(1) Improved the success rate of resuscitation (2) Showed a tendency to reduce seizure incidence and improve NDS	39
	4-minute VF	Rat	Intraperitoneal, 5 mL/kg H_2 rich saline, beginning of CPR	(1) Improved survival and neurological functions (2) Decreased levels of oxidative products, as well as the increased levels of antioxidant enzyme	26
	Prospective intervention study (4 cardiogenic and 1 septic comatose CA)	Human	Inhaled gas, 2% H_2, for 18 h	(1) Reduced markers of oxidative stress in cardiogenic CA patients (2) Reduced inflammatory cytokines in septic CA patient	27
	RCT (73 adult OHCA patients)	Human	Inhaled gas, 2% H_2, for 18 h	(1) Improved CPC and mRS scores (2) Improved 90-day survival rate	28
Noble gases	12-minute VF	Pig	Ventillation, 50% or 70% Ar, for 4 h after ROSC	(1) Improved neurologic alertness score and NDS (2) Reduced neuronal degeneration and microglial activation (3) Decreased circulating markers of brain injury	33
	RCT 110 comatose adult OHCA patient	Human	Inhaled xenon, maintain end-tidal xenon concentration to 40%, immediately after ROSC for 24 h in combination with MTH	(1) Resulted in lesser white matter damage in MRI (2) No differences in neurological outcomes	34
	9-minute VF	Rat	Ventilation, 70% Ar, 1 h after ROSC for 1 h in addition to MTH	(1) Ar abolished neuroprotective effect of MTH (2) Combination of MTH and Ar increased histopathological damage	40
Hydrogen sulfide (H_2S)	6-minute VF	Rat	Inhaled gas, 40 ppm or 80 ppm, for 1 h after ROSC	(1) Improved neurological outcomes and survival (2) Mitigated brain edema and maintained BBB integrity	36
	5-minute VF	Rat	Intravenous, DATS@MION-PEG-LF (10 mg/kg), immeadiately aftr ROSC	(1) Improved cerebral functions and survival (2) Protected ischemic brain by reducing apoptosis and oxidative stress	37
	6-minute VF	Rat	Intravenous NaHS, 14 μmol/kg·d, 1 h after ROSC	(1) Improved survival (2) Upregulated neuroprotective factors BDNF and Trkβ	38

KCl, potassium chloride; ROSC, return of spontaneous circulation; GSNOR, S-nitrosoglutathione reductase; SNO, S-nitrosothiol; BBB, blood-brain barrier; VF, ventricular fibrillation; MTH, mild therapeutic hypothermia; NDS, neurological deficit score; TNF-α, tumor necrosis factor-α; ACA, asphyxia cardiac arrest; ROS, reactive oxygen species; ATP, adenosine triphosphate; CPR, cardiopulmonary resuscitation; BP, blood pressure; IHCA, in-hospital cardiac arrest; OHCA, out-of-hospital cardiac arrest; cGMP, cyclic guanosine 3',5'-monophosphate; NO_2-CLA, nitrated conjugated-linoleic acid; PINK-1, PTEN-induced kinase 1; EEG, electro-encephalogram; CA, cardiac arrest; CPC, cerebral performance category; mRS, modified Rankin Scale; RCT, randomized controlled trial; MRI, magnetic resonance imaging; Ar, argon; DATS@MION-PEG-LF, mesoporous iron oxide nanoparticle as the carriers of diallyl trisulfide, with polyethylene glycol and lactoferrin; NSE, neuron-specific enolase; BDNF, brain-derived neurotrophic factor; Trkβ, tropomyosin receptor kinase β.

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Nitric oxide

The neuroprotective effects of NO are mediated by the activation of guanylyl cyclase and protein S-nitrosylation which regulates NO bioavailability.

Preclinical evidence

Improving NO bioavailability by inhibiting S-nitrosoglutathione reductase (GSNOR) in a 7.5-minute potassium chloride (KCl)-induced CA mouse model promotes neuroprotection.[13] Pharmacological inhibition with the GSNOR-specific inhibitor SPL-334.1 (intravenous, 6 mg/kg, 15 minutes after ROSC) or genetic deletion of GSNOR in $GSNOR^{-/-}$ mice improved survival, and neurological outcomes which was associated with attenuation of ischemic brain injury, restored S-nitrosylated protein levels in the brain, reduced oxidative stress and preserved blood–brain barrier (BBB) integrity.

Inhaled NO at a dose of 20 parts per million (ppm) for 5 hours after ROSC enhanced the effects of mild therapeutic hypothermia (MTH) in a 10-minute rat ventricular fibrillation-induced CA model,[14] while also yielding an improvements in NDS on days 4–7 after CA that was associated with a decrease in lactate and tumor necrosis factor-α (TNF-α) release in the brain.

Based on the neuroprotective effects of nitrite, a nitric oxide precursor in *in vitro* oxygen-glucose deprivation/reperfusion (OGD/R) of neurons, Dezfulian et al.[15] reported that intravenous (IV) infusion of sodium nitrite (4 μmol in 0.5 mL plasmalyte-A) beginning 5 minutes after ROSC and administered for 5 minutes in 7- and 8-minute asphyxia CA rat models significantly reduced neurologic disability and improved survival. This therapeutic benefit was associated with reduced reactive oxygen species (ROS) generation, maintained adenosine triphosphate (ATP) generation, and increased brain mitochondrial S-nitrosation. In a lipopolysaccharide-infusion and 10-minute ventricular fibrillation piglet model of CA, treatment of NO at a dose of 80 ppm during CPR resulted in superior systemic hemodynamics, regional cerebral blood flow, and cerebral mitochondrial complex I.[16]

Clinical evidence

In a pilot study including 20 adult IHCA patients receiving inhaled NO at a dose of 40 ppm immediately after ROSC for 24 hours and 199 IHCA controls,[17] patients receiving NO had higher rates of survival to discharge compared to controls, yet no difference in the favorable neurologic outcome was observed. In a secondary analysis of phase I clinical trials including 82 adult OHCA patients, IV nitrite (25 mg or 60 mg) administration during resuscitation resulted in increased cyclic guanosine 3',5'-monophosphate (cGMP) and nitrated conjugated-linoleic acid (NO_2-CLA) levels, which are responsible for nuclear factor erythroid 2–related factor 2 (Nrf2)-dependent antioxidant gene expression and inhibiting nuclear factor kappa B (NF-κB) regulated pro-inflammatory signaling. The elevated cGMP and NO_2-CLA levels were thus associated with improved clinical outcomes in the patients.[18]

Carbon monoxide

Despite being considered a toxic gas, CO exerts neuroprotective effects in ischemic-reperfusion injury owing to its anti-inflammatory, antioxidant, and anti-apoptotic properties.

Preclinical evidence

As an alternative to gaseous CO, discovery of small molecule CO-releasing molecules (CORMs) allows selective delivery and local release of CO. In a 6-minute asphyxia-induced rat CA model, IV treatment of CORMs 30 minutes after ROSC at 10 mg/kg/h over 30 minutes (5 mg/kg) improved neurological outcomes post-CA and resuscitation through maintenance of mitochondrial biogenesis and subsequently mitochondrial function.[19] In a 6-minute asphyxia-induced CA model utilizing aged rats (20–22 months), IV infusion of 4 mg/kg CORM-3 after ROSC increased the 3-day survival rate from 25% to 70.83% and increased NDS.[20] This effect was associated with the attenuation of CA-induced neuronal apoptosis and necrosis in the cerebral cortex, with the improved cerebral mitochondrial function via reduced ROS, reversed mitochondrial membrane potential depolarization, prevention of cytochrome C release, and increased mitochondrial autophagy.

In a 4.5-minute asphyxia-induced CA pig model, low-dose CO targeting 7%–13% carboxyhemoglobin (CO-Hb) using a CO-permeable silicone module (PDMSXA-1.0 unit) placed into the arterial line with an oxygenator resulted in reduced cerebral damage and improved neurologic function.[21] CO treatment was associated with a fast rise in regional oxygen saturation, improved activity of median nerve somatosensory-evoked potentials, and diminished post–CA cerebral perfusion differences. Furthermore, CO treatment leads to significantly reduced histopathological injury in hematoxylin-eosin and Iba1 staining.

Carbon dioxide

While higher levels of CO_2 cause brain edema, mild to moderate levels of hypercapnia are considered neuroprotective for global cerebral ischemic injury.

Preclinical evidence

In a 6-minute asphyxia-induced CA rat model, ventilation with 12% CO_2 starting at 15 minutes after ROSC for 105 minutes was associated with significantly higher neurological recovery as assessed by the neurological functional score at 24 hours post-ROSC.[22] The higher neurological recovery was associated with

reduced brain tissue malondialdehyde (a marker of oxidative stress), serum neuron-specific enolase (NSE), and S100β levels in the 12% CO_2 group. Cell death in the hippocampal region associated with memory (CA1 and CA3) and the expression of autophagy-related proteins associated with neuronal degradation were significantly lower in the 12% CO_2 group. In a 12-minute ventricular fibrillation model of CA in pigs, hypercapnic ventilation (end-tidal CO_2 45–50 mm Hg) immediately after CPR for 4 hours was associated with improved mean arterial pressure.[23] Although hypercapnia did not improve neurological recovery in pigs post-CA, neuronal degeneration in frontal cortex was significantly mitigated compared to normocapnia.

Hydrogen

Molecular hydrogen, i.e., hydrogen gas, has been widely reported to have anti-inflammatory, antioxidant, and anti-apoptotic properties by selectively neutralizing hydroxyl radicals.

Preclinical evidence

In a 5-minute rat model of asphyxia CA, treatment with a low (2%) dose of hydrogen for 1 hour after ROSC improved post-resuscitation survival and neurological outcome.[24,25] Additionally, therapeutic benefits were associated with improved post-resuscitation electroencephalogram (EEG) characteristics[24] and reduced serum S100β and cardiac troponin T levels.[25] Alternately, in a 4-minute ventricular fibrillation CA rat model, treatment with hydrogen-rich saline at a dose of 5 mL/kg intraperitoneally during the beginning of CPR significantly improved survival rate and neurological function, which was associated with attenuated endoplasmic reticulum stress, decreased levels of oxidative products, and increased levels of antioxidant enzymes.[26]

Clinical evidence

In their single-center, open-label, single-arm, prospective intervention study including five adult comatose post-CA patients, Tamura et al.[27] revealed that inhalation of 2% hydrogen for 18 hours alleviated oxidative stress markers in plasma, which showed the feasibility and safety of inhaled H_2 gas for patients with post-cardiac arrest syndrome. In a multicenter, double-blind, placebo-controlled, randomized study performed at 15 institutions in Japan including 73 adult OHCA patients, inhalation of 2% H_2 had positive therapeutic benefits. A cerebral performance category (CPC) score of 1 or 2 at 90 days was achieved in 56% and 39% of patients in the H_2 and control groups, respectively, but was not significantly different between the groups.[28] The median modified Rankin Scale (mRS) score was 1 and 5 in the H_2 and control groups, respectively. An mRS score of 0 was achieved in 46% and 21% of patients in the H_2 and control groups, respectively. The 90-day survival rate was 85% and 61% in the H_2 and control groups, respectively.

Noble gases

Noble gases xenon (Xe) and argon (Ar) show neuroprotective effects against hypoxic-ischemic injury in mouse hippocampus *in vitro*.[29] The administration of 0.5 atmospheres of Xe or Ar reduced injury by up to 96% in mice hippocampal brain slices subjected to OGD/R. The therapeutic efficacy of Xe has been extensively verified in preclinical CA models and has already been translated clinically.[30] Ar is more abundantly available at a significantly lower cost while lacking narcotic effects and boasting potent neuroprotective properties.[29]

Preclinical evidence

While MTH remains the only neuroprotective treatment recommended by current guidelines, combination therapy of noble gas and MTH may enhance the neuroprotective effects in the context of brain injury after CA. The combination of 70% Ar and MTH (33°C) significantly reduced cell death in 90-minute OGD/R model of rat cortical neuronal cells in *in vitro* and decreased brain infarct size in an *in vivo* rat model of neonatal asphyxia.[31] In asphyxiated neonatal pigs, treatment with 50% Ar and MTH (33.5°C) starting at 2 hours for 24 hours augmented brain protection as evidenced by improved brain energy metabolism, faster EEG recovery, and reduced cell death.[32]

In a 12-minute ventricular fibrillation model of pig CA, initiating ventilation immediately after ROSC with 70% Ar for 4 hours significantly improved survival and neurological recovery as evidenced by improved neurological alertness score and NDS. Ar treatment also led to the improvement of cardiac function as evidenced by reduced left ventricular infarct size.[33] The improvement in neurological recovery after Ar treatment was associated with reduced neuronal degeneration in the cortex and microglial activation in the hippocampus. The benefits of Ar-treatment in CA pigs are further supported by reduced elevation of circulating biomarkers of brain injury and less kynurenine pathway activation.

Clinical evidence

In a randomized, single-blind, phase II, clinical trial including 110 adult comatose adult OHCA patients, MTH (33°C) in combination with inhaled Xe initiated immediately after ROSC to maintain the end-tidal Xe concentration to 40% for 24 hours resulted in less white matter damage compared to hypothermia alone as measured by fractional anisotropy in diffusion tensor magnetic resonance imaging (MRI).[34] However, there was no difference in neurological outcomes or mortality at 6 months.

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Hydrogen sulfide

H_2S is a signaling gas molecule that has beneficial effects in *in vitro* and *in vivo* models of ischemia/reperfusion neuronal injury. H_2S protected OGD/R induced injury in neuroblastoma cell lines (SH-SY5Y) via activation of mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase-3, and nuclear factor erythroid 2[35] pathways.

Preclinical evidence

In a 6-minute ventricular fibrillation rat CA model, inhalation of 40 ppm or 80 ppm H_2S for 1 hour after ROSC improved survival and neurological outcomes.[36] This therapeutic benefit was associated with attenuation of brain edema and preservation of BBB integrity. In a 5-minute ventricular fibrillation rat CA model, H_2S was targeted to the brain and heart using mesoporous iron oxide nanoparticles (MION) as a carrier of diallyl trisulfide (DATS) with polyethylene glycol (PEG) and lactoferrin (LF). IV injection of DATS@MION-PEG-LF (10 mg/kg), immediately after ROSC showed neuroprotective effects as evidenced by improved cerebral function and survival.[37] The observed therapeutic benefits were associated with anti-apoptotic, anti-inflammatory, and antioxidative mechanisms. Modulating the endogenous H_2S level in the 6-minute ventricular fibrillation rat CA model by IV injection of 1 mL sodium hydrosulfide (NaHS) at 14 μmol/kg·d, 1 hour post-ROSC, increased the rat survival,[38] upregulating neurotrophic factors including brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase β in the brain tissue.

Contradictories, limitations, and perspectives

Despite positive therapeutic effect of low dose H_2 (2%) in rodent and clinical CA subjects, inhalation with a high (60%) dose of hydrogen gas 1 hour before and after ROSC was reported to have a tendency without a statistical difference to improve the success rate of resuscitation, reduce the seizure incidence, and increase NDS score at 3 days after ROSC in 9- and 11-minute asphyxia rat CA model.[39] In contrast to the positive therapeutic effects of combinatory therapy of noble gas and MTH, Brücken et al.,[40] reported that combining 70% Ar with MTH (33°C for 6 h) did not augment MTH effects but rather worsened the functional outcome in the 9-minute ventricular fibrillation CA rats when given for 1 hour with a delay of 1 hour following CA-ROSC. These contradictions may be due to the model variations between global ischemia and reperfusion injury after CA[40] and hypoxic-ischemic encephalopathy[31,32] with distinct pathophysiology.

In addition to inhaled forms of these neuroprotective gases, injectable forms like IV nitrite, CORM-3, and NaHS have proven to be equally effective in ameliorating neurological dysfunctions post-CA. Although inhaled gases and their isoforms have emerged as a promising therapeutic approach with potential neuroprotective effects in the context of CA, the clinical translation faces challenges due to inconsistent results among various species and CA models. The clinical translation of gases like CO and CO_2 is difficult because of the concerns regarding their toxicity and precise regulation of gas delivery. Inhalation of 2% hydrogen provided positive results in clinical setting in terms of survival and neurological outcomes, while the studies included small sample size and uncertainty regarding optimal dosing. The mechanism underlying neuroprotective effects of gases remains unelucidated. It is crucial to better design preclinical studies and evaluate in large animals and translate the efficacy in large scale randomized controlled trials (RCTs).

Despite the current challenges, gases and their isoforms are the promising target for improving outcomes in CA. Gases like NO, CO, CO_2, H_2S, H_2, and noble gases have shown promising neuroprotective effects in the context of CA. These gases exerted significant beneficial effects via mechanisms such as modulation of oxidative stress and inflammation, preservation of BBB integrity, attenuation of cell death, improvement of mitochondrial function, upregulation of neurotrophic factors, and activation of signaling pathways (Figure 1). Some small sized clinical studies utilizing gases like NO, H_2, and Xe are promising and need further investigations in large scale RCTs. The combination of gases with MTH has shown potential synergistic effects, although conflicting results exist in different models, warranting the need of further studies exploring the optimum dosing and underlying mechanisms before their clinical translation.

Pharmacological approach

Pharmacological approaches reducing oxidative stress

In the brain after CA, the generation of ROS and subsequent oxidative stress contribute to brain tissue damage and neurological dysfunction because of the associated apoptosis, inflammation, brain edema, autophagy, and other pathological events. Thus, several antioxidants are being investigated in experimental and clinical CA studies (Table 2).

Drugs reducing free radical generation

Preclinical evidence

Niacin has been recognized as a key player in neuroprotection in animal models of ischemia/reperfusion. Selenium (Se) is an essential trace element and an important component of antioxidant enzymes, such as glutathione peroxidase (GPx). In an OGD model of rat cortical neurons, the combination of niacin and Se synergistically attenuated neuronal injury.[41] This was associated

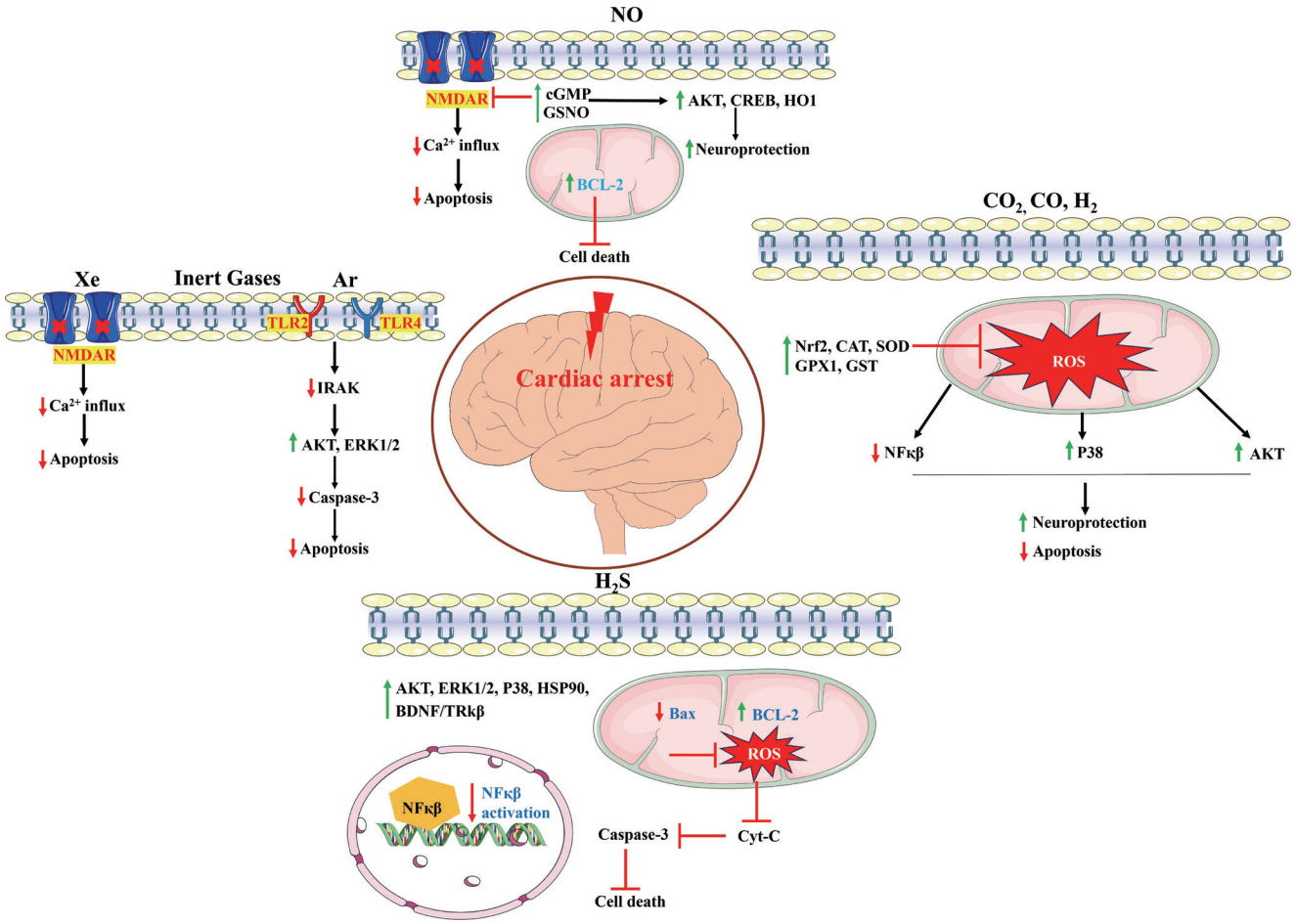

Figure 1. Schematic representation for the reported neuroprotective mechanisms of gases in CA. Red arrows indicate downregulatory effects, whereas green indicates the upregulatory effects for respective signaling mechanisms. Gases suppress oxidative stress, inflammation, and apoptosis while upregulating the neuroprotective mechanisms in the CA brain. CA, cardiac arrest; NO, nitric oxide; CO, carbon monoxide; CO_2, carbon dioxide; H_2, hydrogen; H_2S, hydrogen-sulfide; Xe, xenon; Ar, argon; ROS, reactive oxygen species; NMDAR, *N*-methyl-D-aspartate receptor; cGMP: cyclic guanosine 3',5'-monophosphate; GSNO, S-nitrosoglutathione; BDNF, brain-derived neurotrophic factor; SOD, superoxide dismutase; TLR, toll-like receptor; AKT, protein kinase B; CREB, cyclic adenosine 3,5-monophosphate-response element-binding protein; HO1, heme-oxygenase-1; IRAK, interleukin-1 receptor associated kinase; Nrf2, nuclear factor erythroid 2–related factor-2; CAT, catalase; GPX1, glutathione peroxidase; GST, glutathione S-transferase; NFκB, nuclear factor kappa B; ERK, extracellular signal-regulated kinase; P38, P38 kinase; hsp90, heat shock protein 90; TRκβ, tyrosine kinase β.

with the activation of the glutathione redox cycle and increased glutathione peroxidase activity while reducing hydrogen peroxide levels. In a 6-minute ventricular fibrillation model of rat CA, combination therapy of clinically relevant doses of niacin through an orogastric tube (360 mg/kg) and Se via tail vein (60 μg/kg), 30 minutes after ROSC attenuated neuronal apoptosis in the hippocampus and improved neurologic outcome as evidenced by the improved 7-day NDS.[41] These therapeutic benefits were associated with the upregulation of antioxidant pathways such as ROS reduction, Parkinson protein 7 (PARK7/DJ-1) preservation, and subsequent Akt-nuclear factor erythroid 2–related factor 2 (Akt-Nrf2) signaling upregulation.

BMX001 is a manganese porphyrin compound with potent antioxidant properties by attenuating ROS formation. In an 8-minute KCl-induced CA mice model, intraperitoneal injection of BMX001 (0.1 mg/kg) immediately after resuscitation followed by subcutaneous injections twice per day for 3 days at a dose of 0.2 mg/kg/day significantly improved body weight loss, spontaneous activity decline, neurologic deficits, and decreased rotarod performance with reduced cortical neuronal death.[42] The results were consistent in the 8-minute KCl-CA rat model with BMX001 in the same study that treated rats having a better body-weight recovery and increased rotarod latency with a low percentage of hippocampus neuronal death.

Dimethyl malonate (DMM) is a competitive inhibitor of succinate dehydrogenase, an important enzyme responsible for extensive ROS generation after ischemia reperfusion. In a 6-minute ventricular fibrillation rat CA model, succinate dehydrogenase was inhibited by immediate IV infusion of DMM at a dose of 6 mg/kg/min for 51 minutes, which promoted ROSC and neurological

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Table 2. Application of pharmacological agents in cardiac arrest induced brain injury

Drug	Model	Species	Route/dose/time	Observed results	Ref no.
Niacin and selenium	6-minute VF	Rat	Niacin through an orogastric tube (360 mg/kg) and selenium via a tail vein (60 μg/kg), 30 min after ROSC	(1) Improved neurological outcomes (2) Attenuation of apoptotic neurons in the hippocampus (3) Upregulation of antioxidant pathways	41
Edaravone	5-minute VF	Rat	Intravenous, 3 mg/kg, after ROSC	(1) Improved survival and neurological deficits (2) Decreased MDA and increased SOD activity (3) Decreased pro-apoptotic and increased antiapoptotic gene expression	45
Melatonin	5-minute ACA	Rat	Intraperitoneal, 20 mg/kg/day, 4 times before CA and 3 times after CA	(1) Improved survival and neurological deficits (2) Prevented autophagy-like death of Purkinje cells (3) Reduced ROS and enhanced SODs levels	47
	6 minute-ACA	Rat	Oral gavage, 100 mg/kg body weight/day, 12 consecutive days either before or after CA	(1) Improved neurological outcomes. (2) Increase mitochondrial-binding hexokinase II (HKII) expression (3) Suppression of protein acetylation	48
Vitamin C	8-minute VF	Rat	Intravenous, 200 mg/kg, 5 min after ROSC for half an hour	(1) Improved survival and NDS (2) Improved hemodynamics and myocardial functions (3) Enhanced buccal microcirculation (4) Attenuated pro-inflammatory cytokines and ROS	46
Ruthenium red (RR)	8-minute ACA	Rat	Intravenous, 2.5 mg/kg, at the time of CPR	(1) Decreased CA1 neuronal injury (2) Attenuation of mitochondrial structural disruption and Zn2+ accumulation	51
BMX001	8-minute KCl	Mouse/ rat	1st dose: intraperitoneal, 0.1 mg/kg for mice and 0.225 mg/kg for rats, subcutaneous twice per day for 3 days in mice (at 0.2 mg/kg/day) and 7 days in rats (at 0.45 mg/kg/day)	(1) Improved body weight loss (2) Improved spontaneous activity, neurologic deficits, and rotarod performance (3) Reduced neuronal cell death	42
ERK inhibitor, PD98059	5-minute VF	Rat	Intravenous, 0.3 mg/kg, immediately after ROSC	(1) Improved the survival rates and NDS (2) Reduced production of ROS and increased SOD activity (3) Reduced the number of apoptotic neurons	55
	6-minute VF	Rat	Intravenous, 0.15 mg/kg or 0.3 mg/kg PD98059, within 1 min after ROSC	(1) Improved the survival and NDS (2) Inhibited mitochondria-dependent apoptosis and autophagy (3) Inhibited activation of ERK signaling pathway	56
	7-minute VF	Rat	Intravenous, 0.3 mg/kg, after ROSC	(1) Improved survival rate and NDS (2) Mitigated cerebral ischemia-reperfusion injury	57
Dimethyl malonate (DMM)	6-minute VF	Rat	Intravenous, 6 mg/kg/min, for 51 min	(1) Promotion of ROSC and neurological performance in tape removal assay (2) Inhibition of neuronal apoptosis and excessive hyperpolarization of MMP (3) Reduction in the generation of ROS	43
Oxcarbazepine	5-minute ACA	Rat	Intravenous, 200 mg/kg, 10 min after ROSC	(1) Increased the survival rate and improved neurological deficit (2) Protected cerebellar Purkinje cells from ischemia (3) Reduced in 4-hydroxynonenal and increased SOD1 and SOD2 levels	58
Compound 147	8.5-minute KCl	Mouse	Intravenous, 2 mg/kg, 24 h before surgery and 30 min after ROSC	(1) Improved survival (2) Improved neurological score and open field test score	59
	9-minute KCl	Mouse	Intraperitoneal, 2 mg/kg, 1 day before and intravenously 15 min after ROSC	(1) Restored neurological functions (2) Inhibited neuronal apoptosis and ER stress (3) Upregulation of ATF6 and Nrf2 signaling pathways	60

Table 2. Continued

Drug	Model	Species	Route/dose/time	Observed results	Ref no.
Metformin	10-minute ACA	Rat	Intravenous, 100 mg/kg in 2 mL saline over 15 min, immediately after ROSC	(1) Improved 72 h survival and neurologic function (2) Protected mitochondrial function with a reduction in apoptotic brain injury (3) Potentiated earlier normalization of brain electrophysiologic activity	89
	6-minute ACA	Rat	Intraperitoneal, 200 mg/kg/day, pretreatment for 2 weeks	(1) Increased NDS (2) Improved 7-day survival (3) Decreased neuronal cell apoptosis and attenuated oxidative stress	90
	9-minute ACA	Rat	Intragastric, 30 mg/mL and 200 mg/kg, pretreatment for 2 weeks	(1) Improved neurologic outcome and survival (2) Reduced neuronal cell death (3) Suppressed the activation of microglia and autophagy activation	91
Thiamine	8-minute KCl	Mouse	Intravenous 50 mg/kg 2 min before CPR, followed by daily intraperitoneal for 10 days	(1) Increased survival and neurological functional score (2) Prevented histological neurological injury in fluoro-jade B staining	61
	9-minute VF	Pig	10 mg/kg starting at 20 min after ROSC and every 12 h over 48 h	No effect in improvement of functional neurological outcome or serum levels of NSE	102
	RCT (37 adult OHCA patients)	Human	Intravenous, 100 mg every 8 h for 7 days starting 3.5 h after hospital admission	Did not improve survival and neurological outcomes	103
CDP-choline (citicoline)	RCT (80 pediatric IHCA CA patients)	Human	Intravenous, 10 mg /kg /12 h, for 6 weeks	(1) Improved seizure frequency and duration (2) Improved GCS and mRS scores (3) Decreased intensive care unit stay and mortality rate	52
Ubiquinol	RCT (48 resuscitated adult OHCA patients)	Human	Enteral, 300 mg every 12 h for 7 days	(1) Elevated plasma Co-enzyme Q10 levels (2) Did not change the levels of S100β, NSE, and lactate (3) Did not improve mortality and neurological status	54
Polyethylene glycol-20k	6-minute VF	Rat	Intravenous, 10% solution in saline, 10% estimated blood volume, at the beginning of CPR	(1) Improved survival and NDS (2) Improved myocardial function and buccal microcirculation	84
	6-minute VF	Rat	Intra-aortic, 10% weight/blood volume, 1.8 mL, after 4 min of precordial compression for 3 min	(1) Improved survival and NDS (2) Improved coronary perfusion and sublingual microcirculation	85
	8-minute VF	Rat	Intravenous, 10% blood volume of a 10% PEG solution for 2 min, beginning of precordial compression	(1) Reduced brain edema, decreased S100β and NSE levels (2) Improved microcirculation dysfunction	86
Hypertonic Saline	8-minute KCl	Mouse	Intravenous hypertonic saline (7.5%) infusion 30 min after ROSC for 24 h	(1) Attenuated water content in caudoputamen and cortex (2) Attenuated BBB dysfunction via perivascular Aquaporin-4	87
	5-minute ACA	Rat	Intravenous, 10% hypertonic saline at a rate of 2 mL/h, 30 min before CA	(1) Improved neurological functions (2) Decreased apoptotic hippocampal neuronal cells (3) Reduced expression of apoptosis proteins Bax and Caspase-3	68
Glibenclamide	10-minute ACA	Rat	Intraperitoneal, loading dose of 10 μg/kg and 4 maintenance doses of 1.2 μg every 6 h, after ROSC	(1) Improved survival and neurological outcome (2) Reduced brain edema	92
	10-minute ACA	Rat	Intraperitoneal, loading dose of 10 μg/kg and 4 maintenance doses of 1.2 μg every 6 h, 15 min after ROSC	(1) Improved neurological outcomes (2) Prevented water diffusion abnormality in the brain (3) Increased neuronal survival and decreased glial activation	93

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Table 2. Continued

Drug	Model	Species	Route/dose/time	Observed results	Ref no.
	8-minute ACA	Rat	Intraperitoneal, loading dose of 10 μg/kg followed by a maintenance dosage of 1.6 μg/kg every 8 h for 24 h, 10 min after ROSC	(1) Improved aggregate NDS and survival (2) Improved coma recovery, arousal, and brainstem function (3) Improved somatosensory evoked potential recovery	94
	8-minute ACA	Rat	Intraperitoneal, loading dose of 10 μg/kg followed by a maintenance dosage of 1.6 μg/kg every 8 h for 24 h, 10 min after ROSC	(1) Improved NDS and shortened time of severe neurological deficit (2) Prevented neuroinflammation via TLR4/NLRP3 in microglia	95
	10-minute ACA	Rat	Intraperitoneal, loading dose of 10 μg/kg and 4 maintenance doses of 1.2 μg every 6 h, 15 min after ROSC	(1) Alleviated neurocognitive deficit and neuropathological damage (2) Prevented NLRP3-mediated neuroinflammation via SUR1-TRPM4	96
Rosuvastin	8-minute ACA	Rat	Intraperitoneal, 2 mg/kg/day, pretreatment for 7 days	(1) Improved survival and neurological outcomes (2) Attenuated apoptosis of neural cells (3) Reduced brain edema (4) Decreased serum NSE levels	44
LPC-DHA	10-minute ACA	Rat	Intravenous, 6 mg/kg (0.5 mL) 1 min after ROSC over 1 min	(1) Improved neurological functions in neuronal reflex test and motor co-ordination test (2) Alleviated neuronal cell death, activation of astrocytes, and expression of inflammatory genes	64
Bradykinin	6-minute ACA	Rat	Intraperitoneal, 150 μg/kg, 48 h after ROSC	(1) Increased NDS score (2) Increased neuronal autophagy and inhibited apoptosis	65
Dichloroacetate	6-minute ACA	Rat	Intraperitoneal injection, 80 mg/kg, 15 min after ROSC	(1) Increased 3-day survival time and reduced neurologic deficit (2) Attenuated cellular apoptosis (3) Reduced expression of TNF-α and IL-1β	62
Valproic acid	8-minute ACA	Rat	Intravenous, 300 mg/kg over 20 min, 5 min post-ROSC	(1) Prevented delayed seizure and improved 72-h survival (2) Upregulated the expression of HSP70 (3) Activated an anti-apoptotic signaling pathway to attenuate the expression of cleaved caspase 9	66,67
Flufemic acid	10-minute KCl	Mouse	Intraperitoneal, 12.5 mg/kg once daily for 1 week starting at 1 h after ROSC	(1) Improved survival and neurological outcome (2) Reduced neuropathological injuries, attenuated brain edema and BBB damage, and increased M2 activation of microglia	70
MAGL inhibitor, JZL184	6-minute VF	Rat	Intraperitoneal, 16 mg/kg, immediately after ROSC	(1) Ameliorated cerebral microcirculation dysfucntion (2) Reduced brain edema and BBB permeability (3) Reduced NSE, S100β, and IL-6, and increased serum IL-10	75
HMGB1 binding heptamer peptide (HBHP)	8-minute ACA	Rat	Intravenous, 5 mg/kg, 30 min after resuscitation	(1) Improved 7-day survival rate and NDS (2) Preserved neuronal survival and inhibited activation of microglia and astrocytes	79
HMGB1 inhibitor glycyrrhizin	10-minute VF	Rabbit	Intravenous, 4 mg/kg, 5 min after ROSC	(1) Improved tissue perfusion and attenuated shock (2) Reduced cerebral CD4+ and CD8+ T-cell infiltration	80
Dendrimer N-acetyl cysteine (D-NAC)	7-minute ACA	Rat	Intraperitoneal, 10 mg/kg in 0.2 mL saline, 30 min post-ROSC	(1) Improved survival rate and NDS (2) Enhanced hippocampal neuronal survival	50
Heparin	8-minute ACA	Rat	Intravenous, 0.5 mg/kg, immediately after CPR	(1) Improved NDS and and spatial learning and memory test (2) Reduced number of TUNEL+ cells and Ib1+ microglial cells (3) Reduced expression of pro-inflammatory cytokines (4) Elevated expression of neurotrophic factors (5) Elevated expression of the ERK/CREB pathway and PTN/syndecan-3 pathway molecules	69

Table 2. Continued

Drug	Model	Species	Route/dose/time	Observed results	Ref no.
NLRP3-inflammasome inhibitor MCC950	8.5-minute KCl	Mouse	Intraperitoneal, 10 mg/kg daily for 3 consecutive days, 15 min after the ROSC	(1) Improved survival and functional recovery (2) Reduced IL-1β levels	81
	10-minute ACA	Rat	Intraperitoneal, 10 mg/kg, 10 min after the ROSC	(1) Improved neurological outcomes (2) Alleviated neuro-pathological damage (3) Reduced infiltration of leukocytes	82
	6-minute VF	Rat	Intraperitoneal, 10 mg/kg, immediately after the ROSC	(1) Improved survival and neurological functions (2) Reduced the levels of pro-inflammatory cytokines (3) Preserved cerebral microcirculation and reduced cerebral edema	83
Resolvin D1	8-minute VF	Pig	Intravenous, 0.3 and 0.6 μg/kg, 5 min after ROSC	(1) Improved post-resuscitation neurological dysfunction (2) Decreased serum levels of injury biomarkers (3) Alleviated tissue inflammation and oxidative stress	73
PHA-543,613	8-min ACA	Rat	Intraperitoneal, 12 mg/kg, at the moment of ROSC	(1) Improved neurological functional recovery in Morris's water maze test (2) Decreased CA1 Neuronal death and inflammation	74
DL-3-n-butylphthalide (NBP)	6-minute ACA	Rat	Intravenous, 10 mg/kg twice a day for 3 consecutive days, 10 min after ROSC	(1) Improved neurological function up to 72 h after (2) Reduced apoptosis and inflammatory response	98
Orexin-A	7-minute ACA	Rat	Intranasal, 50 μM, 10 μL for 3 times every 30 s, 30 min post-ROSC	(1) Early arousal from the coma (2) Stabilized EEG and NDS with anti-inflammatory effects	78
Steroids	Retrospective observational study (145,644 adult CA patients)	Human	Patients received steroids during CPR	(1) Improved survival to admission and survival-to-discharge (2) Improved 1-year survival rates	71,72
	RCT (512 adult IHCA patients)	Human	Intravenous, vasopressin (20 IU) and methylprednisolone (40 mg) for a maximum of 4 doses, as soon as possible	(1) Did not improve long-term survival and neurological outcomes (2) Increased likelihood of ROSC	104
Tocilizumab	RCT (80 adult OHCA patients)	Human	Intravenous, 8 mg/kg, for 1 h	(1) Reduced C-reactive protein and leukocyte levels (2) Did not improve survival and neurological outcomes	105
XueZhikang (XZK)	6-minute VF	Rat	Gavage, 20 mg/kg/d or 200 mg/kg/day, pretreatment for 2 weeks	(1) Improved neurological function and 72-h survival (2) Reduced inflammatory cytokines (3) Suppressed TLR4/NF-κB signaling pathway	97
Pomelo peel oil (PPO)	7-minute VF	Rat	Intravenous, 10/20/40 mg/kg, within 10 min after ROSC	(1) Improved NDS and survival (2) Reduced histological brain injury (3) Attenuated necroptosis markers	99
Ginsenoside-Rg1	5-minute ACA	Rat	Intraperitoneal, 40 mg/kg, 1 h after ROSC and once daily for 14 days	(1) Mitigated the spatial learning and memory impairment (2) Improved electrophysiology of CA1 area (3) Attenuated inflammation, neuronal apoptosis	100
Epinephrine	RCT (8014 adult OHCA patients)	Human	Parenteral, 10 mg, 10 syringes with 1 mg epinephrine given every 3 to 5 min	(1) Improved 30-day survival (2) Did not improve favorable neurological outcome	101
Calcium	RCT (391 adult OHCA patients)	Human	Intravenous/intraosseous, 5 mmol, 2 doses after each epinephrine treatment	(1) Worsened survival and neurological outcomes (2) Detrimental effect in sustained ROSC	106

VF, ventricular fibrillation; ROSC, return of spontaneous circulation; MDA, malondialdehyde; SOD, superoxide dismutase; ACA, asphyxia cardiac arrest; CA, cardiac arrest; ROS, reactive oxygen species; NDS, neurological deficit score; KCl, potassium chloride; ERK, extracellular signal-regulated kinase; MMP, matrix metalloproteinase; ER, endoplasmic reticulum; ATF6, activating transcription factor 6; Nrf2, nuclear factor erythroid 2-related factor 2; CPR, cardiopulmonary resuscitation; RCT, randomized controlled trial; NSE, neuron-specific enolase; OHCA, out-of-hospital cardiac arrest; IHCA, in-hospital cardiac arrest; GCS, Glasgow Coma Scale; mRS, modified Rankin Scale; PEG, polyethylene glycol; BBB, blood-brain barrier; IL, interleukin; ERK, extracellular signal-regulated kinase; EEG, electroencephalogram.

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performance as assessed by tape removal assay.[43] The improvements in neurological function after CA was attributed to the inhibition of neuronal apoptosis, inhibition of excessive matrix metalloproteinase (MMP) hyperpolarization, reduction in ROS generation, and stabilization of hypoxia-inducing factor-1α structure.

Rosuvastatin is a potent β-hydroxy β-methylglutaryl-CoA reductase inhibitor, a cholesterol-lowering drug with potent antioxidative, neuroprotective effects via mechanisms including ROS generation. In an 8-minute asphyxia-induced rat CA model, pretreatment with rosuvastatin at a dose of 2 mg/kg/day intraperitoneally for 7 days markedly improved 7-day survival and neurological outcomes which were associated with the attenuation of the neural cell apoptosis, reduction in the brain edema in both the cortex and hippocampus, and a decrease in NSE levels.[44]

Drugs scavenging free radicals

Preclinical evidence

Edaravone is a free radical scavenger with potent antioxidant properties that has been studied for its potential neuroprotective role in CA. Edaravone improved post-resuscitative survival time and NDS in a 5-minute ventricular fibrillation rat CA model when injected IV at a dose of 3 mg/kg immediately after ROSC.[45] This was associated with decreased brain malondialdehyde level, increased superoxide dismutase activity, decreased pro-apoptotic gene expression of caspase-8, caspase-3, and Bax, and increased antiapoptotic Bcl-2 expression at 12, 24, 48, and 72 hours.

Vitamin C is another potent antioxidant that scavenges and attenuates oxidative/nitrosative stress-driven free radicals in a cerebral ischemia-reperfusion injury rat model. In an 8-minute ventricular fibrillation CA rat model, IV administration of vitamin C (200 mg/kg) 5 minutes after ROSC for half an hour improved the survival rate and NDS.[46] This improvement in survival and neurological function was associated with improved hemodynamics and myocardial functions, enhanced buccal microcirculation, inhibition of pro-inflammatory cytokines interleukin-6 (IL-6) and TNF-α, syndecan-1, and hyaluronic acid in plasma, decreased ROS production, and inhibition of MAPK pathway phosphorylation.

Melatonin is a molecule synthesized in the pineal gland and is involved in sleep regulation and cyclical bodily activities. In a 5-minute asphyxia CA rat model, melatonin (20 mg/kg, intraperitoneal, once daily for 4 days before CA, and at 30 min, 6 h, and 1 day after CA) treatment significantly improved the survival rate and reduced neurological deficits versus controls. Melatonin prevented autophagy-like death of Purkinje cells via upregulation of melatonin binding G protein-coupled receptor melatonin receptor-2 (MT2). It reduced ROS, and enhanced copper/zinc superoxide dismutase (SOD1) and manganese superoxide dismutase (SOD2) protein expression, which were dependent on MT2.[47] Additionally, in a 6 minute-asphyxia rat CA model, pre- and post-treatment with melatonin administrated by oral gavage at 100 mg/kg body weight/day for 12 consecutive days either before or after CA improved neurological outcomes when assessed by NDS and Morris water maze test. The neurological functional improvement was mediated by increased mitochondrial-binding hexokinase II expression, protein acetylation suppression, and improvement in mitochondrial function in the hippocampus.[48]

Dendrimer *N*-acetyl cysteine (D-NAC) is the conjugation of *N*-acetyl cysteine (NAC) containing antioxidant and anti-inflammatory properties to dendrimers, allowing controlled delivery and enhanced therapeutic benefits. NAC acts as a potent free radical scavenger. In a study utilizing an 8-minute ventricular fibrillation rat CA model, He et al.[49] reported that IV administration of NAC 5 minutes after ROSC alleviated myocardial dysfunction and improved survival. Intraperitoneal injection of D-NAC (10 mg/kg in 0.2 mL saline) at 30 minutes post-ROSC in a 7-minute rat asphyxia CA model resulted in improved gross neurological score at 48 hours, survival, and increased neuronal cell survival in hippocampal CA1 and CA3 regions.[50]

Progressive Zn^{2+} accumulation is responsible for the deleterious events occurring after ischemia, including mitochondrial dysfunction, swelling, and structural changes. Ruthenium red (RR) with known potential of quenching ROS blocks mitochondrial Ca^{2+} uniporter, the primary route for Zn^{2+} entry. RR attenuated the mitochondrial depolarization, swelling, and the changes in synaptic activity in the OGD/R model of mouse hippocampal slices. RR administered IV (2.5 mg/kg) at the time of CPR decreased CA1 neuronal injury after 8-minute rat asphyxia CA which was associated with attenuation of mitochondrial structural disruption and Zn^{2+} accumulation.[51]

Clinical evidence

CDP-choline (citicoline) is a natural precursor of the cell membrane constituent, phosphatidylcholine with potent antioxidant effect in ischemic brain injury. In an RCT including 80 IHCA pediatric patients (40 citicoline and 40 control group), treatment with citicoline at a dose of 10 mg/kg/12-h IV immediately after ROSC and for 6 weeks exerted neuroprotective effects.[52] Citicoline treatment is associated with decreased seizure frequency and duration, higher Glasgow coma score, and improved mRS score. In addition, there was a decrease in pediatric intensive care unit length of stay and mortality in citicoline-treated patients.

Ubiquinol, also known as coenzyme Q10, is an essential mitochondrial co-factor with potent antioxidant property in the

context of ischemic brain injury, which is significantly lower and is associated with increased mortality.[53] In a randomized, double-blind, placebo-controlled trial of 48 successfully resuscitated adult OHCA patients, enteral ubiquinol (300 mg) every 12 hours for up to 7 days significantly elevated plasma coenzyme Q10 levels at 24, 48, and 72 hours. However, there was no difference in the levels of NSE, S100B, lactate, cellular and global oxygen consumption, neurological status, and in-hospital mortality between the ubiquinol and placebo groups.[54]

Drugs enhancing degradation of free radicals

Preclinical evidence

Extracellular signal-regulated kinase (ERK) belongs to the family of MAPKs and regulates the pathogenesis of CA. The role of ERK inhibitor PD98059 has been investigated in the context of CA. In a 5-minute ventricular fibrillation rat CA model, IV injection of PD98059, a selective inhibitor of MAPKs preventing activation of downstream ERKs at a dose of 0.3 mg/kg immediately after ROSC improved survival and NDS.[55] The therapeutic effects of PD98059 are accompanied by inhibition of oxidative stress while elevating the levels of antioxidant enzyme SOD and reducing apoptotic neurons. In a 6-minute ventricular fibrillation rat CA model, IV injection of PD98059 at a dose of 0.15 mg/kg or 0.3 mg/kg within 1 minute after ROSC improved survival and NDS.[56] These effects are mediated by the attenuation of mitochondrial permeability transition pores and cytochrome C-release. PD98059 further attenuated mitochondria-dependent apoptosis and autophagy which is mediated by the inhibition of the ERK1/2 signaling pathway.[56] In a 7-minute rat ventricular fibrillation CA model, IV injection of PD98059 (0.3 mg/kg) after ROSC significantly improved survival rate and NDS.[57] PD98059 treatment alleviated the endoplasmic reticulum stress (ERs) induced apoptosis via downregulation of "protein kinase R-like ER kinase-activating transcription factor 4-C/EBP homologous protein" signaling pathway and mitigated cerebral ischemia-reperfusion injury.[57]

Oxcarbazepine is a carbamazepine analog commonly used as an antiepileptic drug. In the 5-minute asphyxia rat CA model, IV injection of oxcarbazepine at a dose of 200 mg/kg 10 minutes after ROSC significantly increased the survival rate and improved neurological deficits.[58] Oxcarbazepine protected cerebellar Purkinje cells from ischemia and reperfusion injury induced by CA, which was associated with reduction in 4-hydroxynonenal (an end-product of lipid peroxidation) and increased or maintained the endogenous antioxidant enzymes (SOD1 and SOD2).

Compound 147 is a small molecule that upregulates the pro-survival activating transcription factor 6 (ATF6) signaling in neurons and possesses pro-survival effects. In an 8.5-minute KCl-induced mouse CA model, administration of compound 147 at a dose of 2 mg/kg via the tail vein 24 hours before surgery and 30 minutes after ROSC improved survival and better neurological functions by increased neurologic score and increased traveled distance in open field assessed at day 3 after ROSC.[59] In a 9-minute KCl-induced CA mouse model, administration of compound 147 at a dose of 2 mg/kg intraperitoneally 1 day before and intravenously 15 minutes after ROSC restored neurological function and reduced dead neurons.[60] Compound 147 inhibited neuronal apoptosis and ER stress, with reduction of TUNEL-positive neurons, elevated expression of Bcl-2, and down expression of cleaved caspase-3, caspase-12, C/EBP homologous protein. In addition, compound 147 treatment alleviated the ROS generation while augmenting anti-oxidative mechanisms such as up-regulated SOD activity, enhanced Nrf-2 and heme-oxygenase-1 (HO-1) expressions.

Thiamine, also known as vitamin B, is a cofactor of pyruvate dehydrogenase (PDH) whose activity is impaired in CA brain secondary to increased ROS generation. In an 8-minute KCl-induced mice CA model, IV thiamine at a dose of 50 mg/kg administered in the volume of 50 μL 2 minutes before CPR, followed by daily intraperitoneal injection thiamine increased 10-day survival, improved neurological functional score, and prevented histological neurological injury in fluoro-jade B staining.[61] The beneficial effects of thiamine were accompanied by improved oxygen consumption in mitochondria, restored thiamine pyrophosphate levels, and increased PDH activity.

In a 6-minute asphyxia CA rat model, Wang et al.[62] evaluated the effect of PDH activation by dichloroacetate (DCA), a pyruvate dehydrogenase kinase inhibitor. Intraperitoneal DCA injection at a dose of 80 mg/kg of DCA at 15 minutes post-ROSC increased 3-day survival time and reduced neurologic deficits. It also attenuated cellular apoptosis, neuronal damage, and the expression of TNF-α and interleukin-1β (IL-1β) in the brain. DCA treatment significantly increased ATP production and PDH activity, and decreased blood glucose, lactate, and brain pyruvate levels.

Pharmacological approaches reducing cell death

The interruption of blood flow in the brain during CA elicits multiple cascades of events leading ultimately to cell death. Cerebral ischemia triggers cell death via intrinsic mitochondrial release of cytochrome C and extrinsic binding of cell death ligands with specific cell surface receptors, resulting in activation of caspases.

Preclinical evidence

Lysophosphatidylcholine containing docosahexaenoic acid (LPC-DHA) is a form of omega-3 fatty acid, which is significantly de-

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creased in post-CA plasma in rats and humans.[63] In a 10-minute asphyxia rat CA model, IV injection of 6 mg/kg LPC-DHA (0.5 mL) 1 minute after ROSC over 1 minute significantly improved neurological functions as assessed by neuronal reflex test and motor coordination test at 72 hours after ROSC.[64] Supplementation of LPC-DHA normalized brain levels of LPC-DHA and alleviated neuronal cell death, activation of astrocytes, and expression of various inflammatory and mitochondrial dynamics genes.

Bradykinin, an active component of the kallikrein-kinin system is a peptide with physiological roles like vasodilation, blood pressure control, inflammation, and pain perception. The neuroprotective and cardioprotective roles of bradykinin in the context of CA are being studied in preclinical CA models. In the 6-minute rat asphyxia CA model, intraperitoneal injection of bradykinin (150 μg/kg) 48 hours after ROSC increased neurological function at 3 days after ROSC as assessed by NDS.[65] Bradykinin treatment increased neuronal autophagy, inhibited the expression of the brain injury marker S100β and apoptosis-related protein caspase-3 thus reducing the degree of brain injury caused by ROSC after CA which was mediated by activation of the AMP-activated protein kinase (AMPK)/mTOR signaling pathway.

Valproic acid (VPA) is an antiepileptic medication with neuroprotective properties in various preclinical models of brain injury. In an 8-minute asphyxia-induced rat CA model, IV VPA (300 mg/kg initiated 5 min post-ROSC and infused over 20 min) in combination with MTH (33°C±1°C) prevented delayed seizures and improved 72-hour survival.[66] Furthermore, the MTH/VPA group upregulated heat shock protein 70, activating an anti-apoptotic signaling pathway to attenuate the expression of cleaved caspase-9.[67]

Anti-oxidative drugs play a crucial role in mitigating neuronal cell death by counteracting the harmful effects of ROS. In rodent CA models, the anti-oxidants including the combination therapy of niacin and selenium,[41] BMX001,[42] DMM,[43] rosuvastatin,[44] PD98059,[55-57] D-NAC,[50] and DCA [62] attenuated cellular apoptosis, and neuronal damage. Anti-oxidant compound 147[60] and edaravone[45] decreased apoptotic proteins caspase-8, caspase-3, and Bax while increasing the levels of antiapoptotic protein Bcl-2 in rodent CA models. Hyperosmotic agent 10% HS[68] and heparin with anti-inflammatory effects,[69] also attenuated programmed cell death of neuronal cells in rodent models of asphyxia CA.

Pharmacological approaches reducing inflammation

Hypoxic ischemia during CA leads to brain tissue damage and triggers an inflammatory response characterized by activation of immune cells and the release of inflammatory cytokines which exacerbates brain damage. Anti-inflammatory therapeutic approaches for CA reduce the production or antagonize the action of pro-inflammatory cytokines.

Steroidal and non-steroidal anti-inflammatory drugs

Preclinical evidence

Flufenamic acid (FFA) is a non-steroidal anti-inflammatory drug that selectively inhibits transient receptor potential M4 (TRPM4) channel protein, a novel target for ameliorating BBB disruption and neuroinflammation. In a 10-minute KCl-induced CA mice model, blocking TRPM4 via intraperitoneal injection of FFA (12.5 mg/kg) once daily for 1 week starting at 1 hour after ROSC was associated with improved survival and neurological outcome.[70] FFA-treated mice exhibited statistically higher neurological function scores and better performance in the rotarod test, the open field, and the Morris water maze test 3 days after ROSC. The improved neurological function in FFA-treated mice was associated with reduced neuropathological injuries, attenuated brain edema, reduced leakage of IgG and Evans blue dye, restored tight junction protein expression, and increased microglia/macrophage change from pro-inflammatory to anti-inflammatory phenotype.

Clinical evidence

Studies have indicated that glucocorticoid supplementation during CPR, in conjunction with vasopressors, improves outcomes in instances of CA.[71] In a retrospective observational population-based study in adult CA patients (2,876 in the steroid and 8,628 in the nonsteroid group), patients receiving steroids had significantly higher rates of survival to admission, survival to discharge, and 1-year overall survival.[71] After propensity score matching in the above observational study, Tsai et al.[72] included 5,445 CA patients both in steroid and nonsteroid groups and showed that 1-year mortality rate was significantly lower in the steroid group than in the nonsteroid group. Steroid use during hospitalization was associated with survival to admission and survival to discharge, regardless of age, gender, and underlying diseases.[72]

Cytokines modulating drugs

Pharmacological agents mitigating the levels of pro-inflammatory cytokines IL-1, IL-6, and TNF-α while augmenting the levels of anti-inflammatory cytokines TGF-β and IL-10, have been administered to treat CA.

Preclinical evidence

Resolvin D1 is a bioactive lipid that exerts anti-inflammatory and pro-resolution effects in brain injury. In an 8-minute ventricular fibrillation porcine model of CA, IV administration of resolvin D1, at doses of 0.3 and 0.6 μg/kg 5 minutes after ROSC

significantly improved post-resuscitation neurological dysfunction (assessed by NDS) and markedly decreased serum levels of injury biomarkers NSE and S100β.[73] Resolvin D1 treatment reduced the level of tissue inflammation and oxidative stress markers like TNF-α, IL-6, and malondialdehyde (MDA) while increasing the level of SOD in the brain and heart.

PHA-543,613 is an agonist of the α-7 nicotinic acetylcholine receptor (α7nAChR) which comprises the molecular basis for interactions between the nervous and the immune systems and was shown to effectively allay the release of inflammatory factors like TNF-α, IL-1β, and IL-6. In the brain of the 8-minute asphyxia CA rat model, α7nAChR expression is reduced in the hippocampus and cortex.[74] Activation of α7nAChR with a drug pyridine-5-carboxamide (PHA-543,613) which acts as a potent and selective agonist for the α7nACh, at a dose of 12 mg/kg intraperitoneally at ROSC, enhanced neuroprotection with a decrease in escape latency and an increase in time spent in the target quadrant in the Morris water maze test. This neuroprotection of PHA-543,613 was associated with decreased NSE levels, decreased inflammatory mediators (IL-1β, TNF-α, high mobility group box-1 [HMGB1]), and increased surviving neuronal cells in the hippocampus. In contrast, blocking a7nAChR with methyllycaconitine attenuated these beneficial effects.

JZL184 is a selective inhibitor for monoacylglycerol lipase, which is an enzyme associated with inflammation. In a 6-minute ventricular fibrillation-induced CA rat model, intraperitoneal injection of JZL184 (16 mg/kg) immediately after ROSC had significant neuroprotective effects with amelioration of cerebral microcirculation, mitigation of brain edema, attenuation of BBB permeability, decreased serum levels of NSE, S100β, and IL-6, and increased serum IL-10 level.[75]

Heparin is a commonly used anticoagulant drug with known anti-inflammatory properties. The application of heparin as a neuroprotective agent in brain injury has been expanding in recent years. In an 8-minute rat asphyxia CA model, IV heparin (0.5 mg/kg) immediately after CPR attenuated CA-CPR-mediated cerebral ischemia-reperfusion injury.[69] The NDS was remarkably improved, and the animal performed better with spatial learning and memory testing in the Morris water maze. Heparin treatment was associated with a reduced number of inflammatory and apoptotic cells. Furthermore, the relative expression of pro-inflammatory cytokines (IL-1β, TNF-α) was significantly reduced while the expression of neurotrophic factors (NF200, BDNF, NGF) was significantly elevated. These neuroprotective effects were accompanied by the elevated expression of the ERK/CREB pathway and PTN/syndecan-3 pathway molecules.

Orexin is a neuropeptide produced in a restricted group of neurons in the hypothalamus and regulates arousal. Intraventricular orexin treatment is associated with improved arousal and early EEG entropy in rats after 7-minute asphyxia-induced CA in rats.[76] In an 8-minute asphyxia rat CA model, the orexin pathway was implicated in neurological recovery.[77] Intraperitoneal administration of the dual orexin receptor antagonist, suvorexant (30 mg/kg) shortly after resuscitation and at 10- and 20 hours post-CA led to sustained neurological deficits. In a 7-minute asphyxia rat CA model, intranasal orexin-A (ORXA) treatment 30 minutes post-ROSC at a dose of 10 μL of 50 μM given 3 times every 30 seconds facilitated early arousal from coma, stabilized quantitative EEG, and improved NDS with anti-inflammatory effects.[78]

In addition, antioxidants including vitamin C[46] and DCA[62] also decreased the expression of pro-inflammatory cytokines TNF-α and IL-1β in the CA brain.

HMGB1 inhibitors

HMGB1 binding heptamer peptide (HBHP) blocks HMGB1 signaling, which is the key ignition molecule for toll-like receptor-4 (TLR4). HMGB1-TLR4 signaling promotes inflammation and mediates inflammation injury in the ischemic brain.

Preclinical evidence

In the 8-minute asphyxia CA rat model, HMGB1 was shown to be significantly elevated in the serum.[79] Blocking HMGB1 activation by IV HBHP treatment at a dose of 5 mg/kg 30 minutes after resuscitation improved 7-day survival rate and increased NDS. This effect was associated with the preservation of neurons and inhibition of microglia and astrocyte activation. In a 10-minute ventricular fibrillation rabbit CA model, IV injection of HMGB1 inhibitor glycyrrhizin at a dose of 4 mg/kg, 5 minutes after ROSC improved tissue perfusion and attenuated shock, thereby reducing neurological dysfunction.[80] Neuroprotection following glycyrrhizin was associated with reduced cerebral CD4+ and CD8+ T-cell infiltration 2 hours post-CA, but was not related to BBB preservation.

Nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 (NLRP3) inhibitors

Preclinical evidence

MCC950 is the selective inhibitor of NLRP3 inflammasome which regulates inflammatory and immune responses. In an 8.5-minute KCl-induced mouse CA model intraperitoneal injection of MCC950 at a dose of 10 mg/kg once daily for 3 days injected 15 minutes after ROSC improved survival and neurological function.[81] MCC950 suppressed interleukin-1β mRNA levels and preserved immune homeostasis in the spleen. In a 10-minute as-

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phyxia rat CA model, intraperitoneal MCC950 treatment at a dose of 10 mg/kg (24.72 μmol/kg) at 10 minutes post-ROSC significantly reduced infiltration of leukocytes, improved neurologic outcomes, and alleviated neuro-pathological damage.[82] In a 6-minute ventricular fibrillation rat CA model, intraperitoneal injection of MCC950 at a dose of 10 mg/kg immediately and 15 minutes after ROSC improved both survival and neurological function.[83] The therapeutic effect was associated with the reduction of IL-1β levels, preservation of cerebral microcirculation, and reduction of cerebral edema.

Pharmacological approaches with pleiotropic mechanisms

Pharmacological approaches with pleiotropic mechanisms are those drugs that can target multiple pathways including inflammation, apoptosis, BBB, and oxidative stress in the CA brain.

Hyperosmotics and edema reducing agents

Cerebral edema due to cellular swelling is a fatal pathological consequence of ischemic brain injury in the post-CA brain and as such, targeting cellular swelling is a key clinical goal in an effective therapeutic strategy. Hypersosmotics reduce cerebral edema by creating osmotic gradient.

Preclinical evidence

Polyethylene glycol-20k (PEG-20k), a cell impermeant and a hydrophilic compound that can draw water from brain tissue and reduce edema, has been investigated for its potential role in the management of brain edema. In a 6-minute ventricular fibrillation rat CA model, PEG-20k (10% solution in saline, 10% estimated blood volume) administered at the beginning of CPR by continuous IV infusion improved post-resuscitation neurological functions as assessed by NDS, buccal microcirculation, and survival.[84] Intra-aortic PEG-20k (10% weight/blood volume, 1.8 mL) administered after 4 minutes of precordial compression by continuous arterial infusion for 3 minutes with an infusion pump in a 6-minute ventricular fibrillation rat CA model improved survival and NDS.[85] In an 8-minute ventricular fibrillation rat CA model, PEG-20k administered at the beginning of precordial compression by continuous IV infusion (10% blood volume of a 10% PEG solution in saline) for 2 minutes with an infusion pump improved cerebral microcirculation and reduced brain edema and injury.[86]

Hypertonic saline (HS) is commonly used for the management of cerebral edema and to reduce acutely increased intracranial pressure. Osmotherapy with hypertonic saline has been shown to attenuate cerebral edema following experimental CA and CPR. In an 8-minute KCl-induced CA in mice, IV HS (7.5%) infusion 30 minutes after ROSC to achieve serum osmolality of approximately 350 mOsm/L, for 24 hours attenuated cerebral edema and damage to the BBB.[87] Intravenous hypertonic saline (10%) infusion at a rate of 2 mL/h for 30 minutes before CA is neuroprotective in a 5-minute asphyxia rat CA model.[68] Treatment with HS significantly improved neurological function and decreased apoptosis of CA-induced hippocampal neuronal cells via downregulation of Bax and caspase-3 pro-apoptotic proteins, while enhancing the expression of anti-apoptotic protein Bcl-2.

Drugs targeting oxidative stress and inflammation were reported to have potent efficacy in reducing brain edema after CA. rosuvastatin, an anti-oxidative agent reduced brain swelling in rats after asphyxia CA.[44] Treatment with several anti-inflammatory drugs including FFA,[70] JZL184,[75] and MCC950[83] was also associated with a reduction in BBB leakage after CA.

Hypoglycemic drugs

Preclinical studies

Metformin is a hypoglycemic agent for treating type 2 diabetes mellitus that has potent neuroprotective functions. In an experimental model of oxidative stress induced by OGD/R *in vitro*, metformin provides neuroprotection through the inhibition of oxidative stress and apoptosis.[88] In a 10-minute asphyxia-CA rat model, one-time IV metformin treatment immediately after ROSC (100 mg/kg in 2 mL saline over 15 min) improved 72-hour survival and neurologic function, protected mitochondrial function with a reduction in apoptotic brain injury without activating AMPK, and potentiated earlier normalization of brain electrophysiologic activity.[89] Metformin pretreatment is neuroprotective in the context of CA. In a 6-minute asphyxia CA rat model, metformin pretreatment (200 mg/kg/day, intraperitoneal) for 2 weeks prior increased NDS, improved 7-day survival, decreased neuronal cell apoptosis, and attenuated oxidative stress.[90] In a 9-minute asphyxia CA rat model, metformin pretreatment (intragastric, 200 mg/kg for 2 weeks) significantly improved neurologic outcomes and survival rates.[91] Metformin pretreatment reduced neuronal death and suppressed activation of microglia which was accompanied by augmented AMPK phosphorylation and autophagy activation in affected neuronal tissue.

Glibenclamide (GBC) is a second-generation sulfonylurea used to treat type 2 diabetes mellitus and is being investigated as a neuroprotective agent against CA-induced brain injury. In a 10-minute asphyxia CA rat model, intraperitoneal administration of GBC improved survival and neurological outcomes. The effect was comparable to TTM (33°C for 4 h starting 15-min post-ROSC) therapy. The therapeutic efficacy of GBC was associated with a reduction of neuronal injury and inhibition of microglia and astrocyte activation.[92] MRI studies demonstrate the

neuroprotective effects of GBC in CA/CPR in attenuating cerebral edema.[93] Moreover, GBC administered at a loading dose of 10 μg/kg intraperitoneally 10 minutes post-ROSC, followed by a maintenance dosage of 1.6 μg/kg q8h for 24 hours improved mortality, coma recovery, arousal, brainstem function, and somatosensory evoked potential (SSEP) recovery after 8-minute asphyxia CA in rats.[94] Wang et al.[95] further reported that GBC improved neurological functions with decreased neuroinflammation through TLR4/NLRP3 inflammasome activation in microglia. GBC alleviated neurocognitive deficit and neuropathological damage in a 10-minute rat asphyxia CA rat model by exerting anti-inflammatory effects via inhibition of microglial NLRP3 inflammasome activation by blocking sulfonylurea receptor 1-transient receptor potential M4 and this effect was independent of its role in preventing brain edema.[96]

Plant (herbal) extracts

Certain herbal extracts contain biologically active substances like flavonoids and statins which are known to possess anti-inflammatory and neuroprotective properties. Several groups have investigated the therapeutic efficacy of herbal extract in preclinical CA animal models.

Preclinical studies

XueZhikang (XZK) is an extract of red yeast rice containing natural statins, unsaturated fatty acids, flavonoids, and other biologically active substances. In a 6-minute ventricular fibrillation rat model of CA, XZK pretreatment at doses 20 mg/kg/day or 200 mg/kg/day by gavage once daily for 2 weeks improved neurological function and 72-hour survival rate and was associated with a reduction of inflammatory cytokines through inhibition of TLR4/NF-κB signaling pathway.[97]

DL-3-n-butylphthalide (NBP) is a neuroprotective agent, originally isolated from celery seeds and has been shown to possess therapeutic efficacy in ischemic brain by suppressing neuroinflammation and maintaining the BBB. In rat models of 6-minute asphyxia-induced CA, IV NBP (10 mg/kg) infusion 10 minutes after resuscitation and delivered twice a day for 3 consecutive days improved neurological function up to 72 hours after CA.[98] NBP treatment reduced neuronal damage alongside ultrastructural mitochondrial damage, reduced apoptosis through the Jun N-terminal kinases and p38 mitogen-activated protein kinases (JNK/p38) pathway, and suppressed inflammatory response through the nuclear factor kappa light chain enhancer of activated B cells (NF-κB pathway).

In a 7-minute ventricular fibrillation rat model of CA, Wang et al.[99] showed that IV infusion of pomelo peel oil at doses 10, 20, and 40 mg/kg within 10 minutes post-ROSC increased NDS and enhanced survival. This was associated with a reduction of histological brain injury and the attenuation of necroptosis marker (TNF-α, RIPK1 [receptor-interacting serine/threonine-protein kinase 1], RIPK3 [receptor-interacting serine/threonine-protein kinase 3], p-MLKL/MLKL [phosphorylated mixed lineage kinase domain-like protein/mixed lineage kinase domain-like protein]) expression.

In a 5-minute asphyxia CA rat model, intraperitoneal injection of ginsenoside-Rg1, an active ingredient of the herbal medicine ginseng at 40 mg/kg 1 hour post-ROSC, and once daily for 14 days mitigated the spatial learning and memory impairment in the Morris water maze test and compromised basal synaptic transmission and long-term potentiation at the Schaffer collateral of hippocampal CA1 area during *in vivo* electrophysiology.[100] Ginsenoside-Rg1 inhibited hippocampal neuroinflammation by alleviating microglia and astrocyte activation, and overexpression of related pro-inflammatory cytokines IL-1β and TNF-α. In addition, ginsenoside-Rg1 attenuated neuronal apoptosis, dendritic spines formation, and synaptic ultrastructure defects associated with the upregulation of the key synaptic regulatory proteins.

Others/epinephrine

Clinical evidence

Epinephrine is a commonly used medication in CA as a part of ACLS protocols. Epinephrine increases systematic blood pressure, thus elevating coronary or cerebral perfusion which is essential for ROSC. In a randomized, double-blind PARAMEDIC2 (Prehospital Assessment of the Role of Adrenaline: Measuring the Effectiveness of Drug Administration in Cardiac Arrest) trial including 8,014 adult OHCA patients administered parenteral epinephrine (10 mg in total, 10 syringes each with 1 mg epinephrine given every 3 to 5 min), the use of epinephrine improved 30-day survival.[101]

Contradictories, limitations, and perspectives

Although several pharmacological agents targeting the oxidative stress, brain edema, inflammation, and cell death in the context of CA have been extensively studied and resulted in promising results across various preclinical studies, very few have been translated into the clinical setting and majority have failed to exert significant beneficial effects in CA patients. Unlike its benefits in rodent CA model, thiamine treatment at a dose of 10 mg/kg starting at 20 minutes after ROSC and every 12 hours over 48 hours had no effect in improvement of functional neurological outcome or serum levels of NSE in a 9-minute ventricular fibrillation pig CA model.[102] Thiamine showed no differ-

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ence in survival and neurological functions in an RCT including 37 adult OHCA patients when given IV at a dose of 100 mg every 8 hours (starting at 3.5 h from hospital admission) for 7 days.[103] Although there was a slightly larger proportion of patients with a better CPC score in the thiamine group, the study was limited by the small sample size.

Administration of vasopressin and methylprednisolone did not provide observable therapeutic benefits for long-term survival compared to placebo in a multicenter, randomized, double-blind, placebo-controlled trial of 512 adult patients while steroid treatment was associated with an increased likelihood of ROSC.[104]

In an investigator-initiated, randomized, placebo-controlled, double-blinded, single-center, clinical phase II trial including 80 admitted adult OHCA patients randomly assigned 1:1 to placebo or IL-6 receptor antagonist tocilizumab, infusion of tocilizumab at a dose of 8 mg/kg for 1 hour significantly reduced the systemic inflammation as C-reactive protein and leukocyte levels.[105] Tocilizumab reduced myocardial injury as documented by reductions in creatine kinase myocardial band and troponin T. However, there were no differences in survival or neurological outcomes between the groups.

Drugs like ubiquinol,[54] steroids,[104] and tocilizumab[105] have failed to improve survival or favorable neurological outcomes in CA RCTs. The use of exogenous calcium, with a longstanding practice in treating CA, has conflicting results in terms of efficacy in an RCT including 391 adults with OHCA. Administration of 2 doses of 5 mmol calcium chloride IV or intraosseous (1st dose immediately after 1st and 2nd dose after 2nd epinephrine treatment) demonstrated detrimental effects on sustained ROSC, survival, and neurological outcomes, although not reaching statistical significance.[106] Based on this negative data, the trial was discontinued early. In a large-scale RCT, although epinephrine treatment improved the 30-day survival of OHCA patients, there was no significant difference in the rate of favorable neurologic outcomes between these groups.[101]

The most promising results of the pharmacological agents came from preclinical studies using rodent models of CA. The majority of these drugs have not been tested for their efficacy in large animal models and human subjects. Studies testing the effect of dose-response and combinatory approaches for pharmacological agents are rare in both preclinical and clinical settings. In the clinical setting, most studies evaluating efficacy of pharmacological agents are limited by the sample size and study design. The results from retrospective and observational studies can have biases and confounding factors impacting the reliability of the results. Considering the limitations in current preclinical and clinical investigations of pharmacological drugs for CA, there is a critical need to carefully design experiments tailored to assess the effects of various doses of pharmacological drugs. These studies should evaluate the safety and therapeutic effectiveness of these drugs, considering the unique challenges posed by CA. Combinatory pharmacological approaches yielding synergistic interactions of multiple medications targeting distinct pathophysiological mechanisms of CA may enhance the overall prognosis and treatment outcomes. Despite current challenges and limitations, pharmacological agents are a promising approach for improving neurological outcomes in CA (Figure 2). Further investigation addressing the existing contradictions and limitations is necessary for advancement of pharmacological treatment in CA.

Stem cell-based strategies

Major cell types being investigated for their neuroprotective effects in the context of ischemic brain injury post-CA include mesenchymal stem cells (MSCs) and neuronal stem cells (NSCs). Owing to their paracrine pleiotropic benefits like angiogenesis, neurogenesis, anti-apoptosis, and anti-inflammation in the ischemic brain (Figure 3), stem cells have been investigated as a therapeutic agent for the treatment of ischemic brain injury post-CA (Table 3).

Mesenchymal stem cells

MSCs are self-renewing multipotent stem cells with the potential to differentiate into adipocytes, chondrocytes, and osteocytes.

Preclinical evidence

In rat models of 5-minutes[107] or 6-minutes[108] of asphyxia CA-induced brain injury, IV transplantation of 1×10^6 bone marrow MSCs (BMSCs) 1 hour after ROSC improved neurological severity score. BMSCs migrated to the hippocampus and increased the levels of insulin-like growth factor 1 (IGF-1) *in situ*.[107] BMSCs also attenuated the levels of inflammatory mediators such as IL-1β and TNF-α while upregulating levels of the anti-inflammatory cytokine IL-10 in the brain after CA.[108] In a rat 6-minute ventricular fibrillation CA model, IV injection of 2.5×10^6 induced pluripotent stem cell (iPSC)-derived MSCs immediately after CPR improved the 24-hour survival rate which was associated with immunomodulatory effects in the brain by increasing arginase-1 and CD14+ M2 microglia, while decreasing CD86 and iNOS+ M1 microglia.[109] In an 8-minute ventricular fibrillation swine CA model, IV pre-treatment 1.5 and 3 days prior to CA with human embryonic stem cell-derived MSCs at a same dose of 2.5×10^6/kg was associated with a reduction in cerebral dysfunction.[110] MSC administration significantly decreased pyroptosis-related proteins (NLRP3, cleaved caspase-1) and pro-

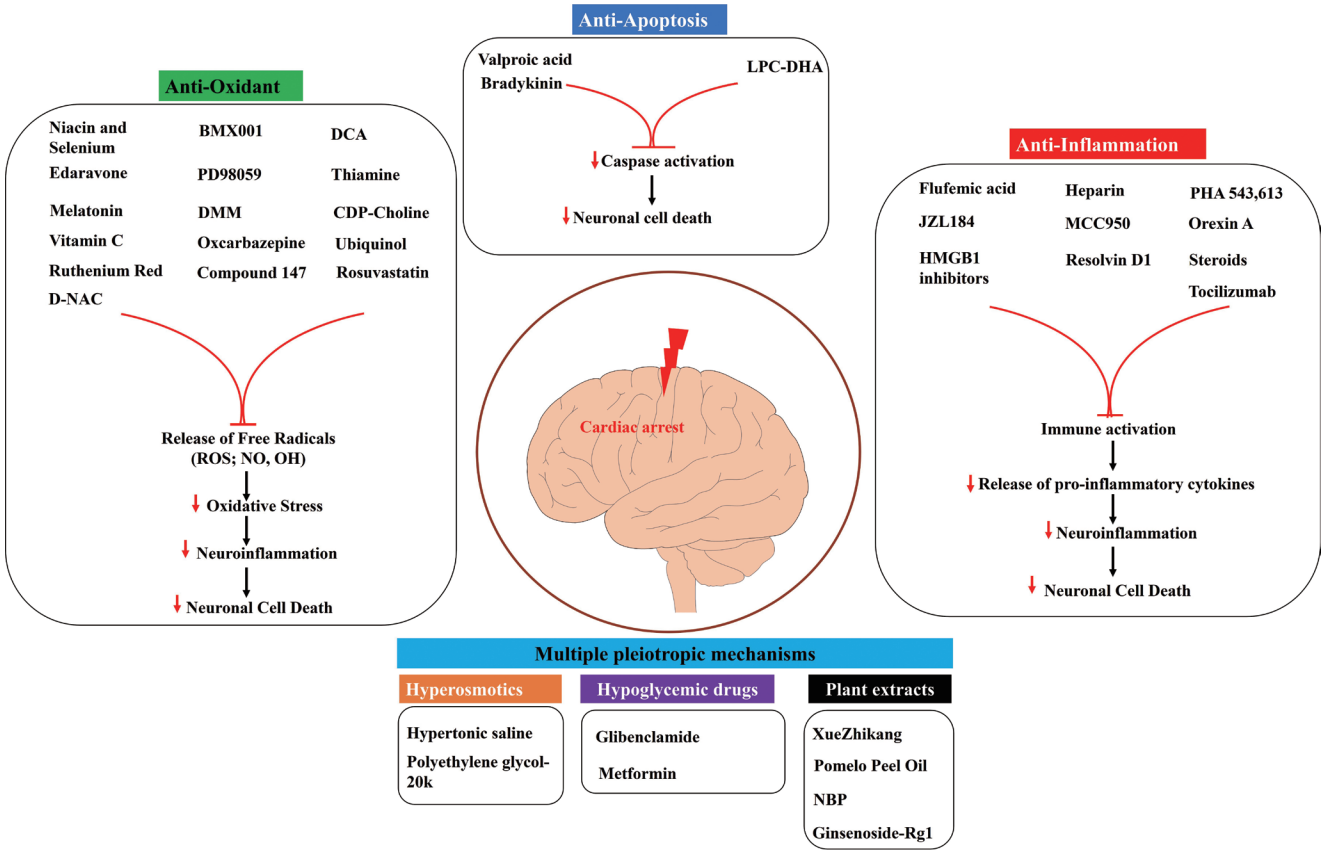

Figure 2. Possible neuroprotective mechanisms of pharmacological agents used for cardiac arrest. Drugs included beneath the box represent respective pathophysiological processes such as anti-inflammation, anti-apoptosis, antioxidant, and multiple pleiotropic mechanisms which suppress the ongoing pathophysiology to confer neuroprotection thus mitigating neuronal cell death. LPC-DHA, lysophosphatidylcholine containing docosahexaenoic acid; DCA, dichloroacetate; DMM, dimethyl malonate; D-NAC, dendrimer *N*-acetyl cysteine; ROS, reactive oxygen species; NO, nitric oxide; OH, hydroxyl radical; HMGB1, high mobility group box-1; NBP, DL-3-n-butylphthalide.

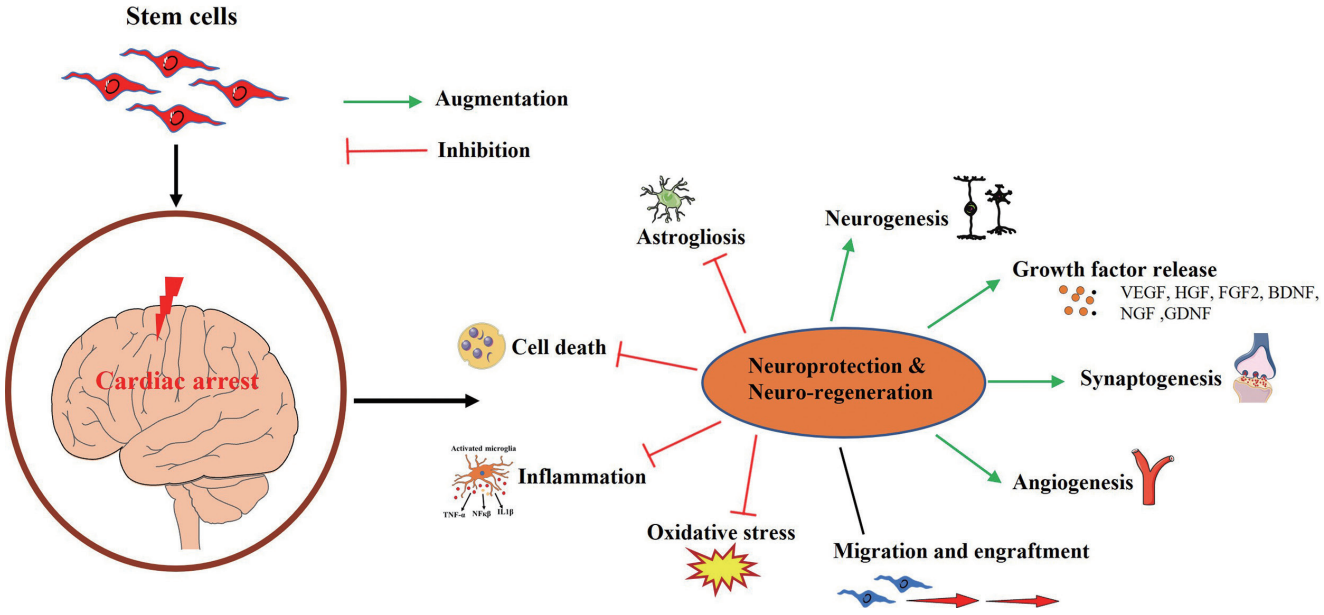

Figure 3. Diagram illustrating the proposed mechanisms of therapeutic efficacy for exogenous stem cell therapy in cardiac arrest. Stem cells can provide beneficial therapeutic effects via directly differentiating into functional neurons and by possessing pleiotropic paracrine effects such as angiogenesis, neurogenesis, synaptogenesis, anti-fibrosis, anti-apoptosis, and anti-inflammation through secretion of growth factors. VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; FGF, fibroblast growth factor; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; GDNF, glial cell line-derived neurotrophic factor.

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Table 3. Application of stem cells in cardiac arrest induced brain injury

Stem cells	Model	Species	Route/dose/time	Observed results	Ref no.
Mesenchymal stem cells (MSCs)	5-minute ACA	Rat	Intravenous, 1×10^6 cells, 1 h after ROSC	(1) Reduced neurological severity (2) Reduced serum S100β levels (3) Upregulated IGF-1 in hippocampus	107
	6-minute ACA	Rat	Intravenous, 1×10^6 cells, 1 h after ROSC	(1) Improved NDS (2) Decreased serum S100β level (3) Attenuated IL-1β, TNF-α while upregulated IL-10	108
	6-minute VF	Rat	Intravenous, 2.5×10^6 cells, immediate after ROSC	(1) Improvement of 24-h survival rate (2) Increased Arg-1 and CD14+ M2 microglia (3) Decreased CD86 and iNOS+ M1 microglia	109
	8-minute VF	Pig	Intravenous, 2.5×10^6 cells/kg, 1.5, and 3 days before CA	(1) Reduction in cerebral dysfunction (2) Decreased pyroptosis-related proteins (NLRP3, cleaved caspase-1) (3) Decreased pro-inflammatory cytokines (IL-1β, IL-18)	110
Modified MSCs	8-minute ACA	Rat	Intravenous, 3×10^6 BDNF and VEGF modified MSCs, 2 h after ROSC	(1) Improved NDS (2) Reduced brain edema, serum S100β levels (3) Reduced pycnotic cells in H&E staining and TUNEL positive cells (4) Enhanced angiogenesis	111
Neural stem cells (NSCs)	8-minute ACA	Rat	Intra-cerebroventricular, 2.0×10^5 cells, 3 h after ROSC	(1) Improved NDS and sub-NDS (2) Enhanced neuronal survival in the hippocampal CA2 region (3) Augmented endogenous NSC proliferation and migration (4) Upregulated Wnt signaling pathway in SVZ	102
	8-minute ACA	Rat	Intranasal, 2.0×10^5 cells, 3 h after ROSC	(1) Improved NDS and shortened time of severe neurological deficit (2) Reduced microglial activation and neuroinflammatory response (3) Downregulated TLR4/NLRP3 pathway related proteins	95
	12-minute KCl	Rat	Intra-hippocampal into both hippocampi, 2 μL each of 5×10^7 cells/mL suspension, 1 week after ROSC	(1) Migration and persistence of transplanted NPCs in CA1 region at 6 weeks (2) NPCs differentiation into neuronal phenotype	113
NSCs+MSCs	8-minute ACA	Rat	Intra-cerebroventricular, 1×10^6 cells, 20 min after ROSC	(1) Improved NDS (2) Promoted survival of neuronal cells (3) Release of extracellular vesicles loaded with miR-133 (4) Upregulated JAK1 and AKT-GSK-3β-Wnt pathway	114

ACA, asphyxia cardiac arrest; ROSC, return of spontaneous circulation; IGF-1, insulin-like growth factor 1; NDS, neurological deficit score; TNF-α: tumor necrosis factor-α; IL, interleukin; VF, ventricular fibrillation; CA, cardiac arrest; NLRP3, nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; NDS, neurological deficit score; H&E, hematoxylin and eosin; NSC, neuronal stem cell; SVZ, sub-ventricular zone; TLR4, toll-like receptor-4; KCl, potassium chloride; NPC, neural progenitor cell; JKA1, Janus kinase 1.

inflammatory cytokines (IL-1β, IL-18) compared to the control group.

To optimize the therapeutic efficacy of MSCs in CA, Zhou et al.[111] overexpressed BDNF and vascular endothelial growth factor (VEGF) using a lentiviral vector and examined their efficacy for the treatment of brain lesion and neurofunctional deficits in an 8-minute asphyxia CA rat model. BDNF and VEGF-modified MSCs were superior to their naïve counterpart in reducing neurological deficits, hippocampal neuronal death, and augmenting angiogenesis in the CA-ROSC brain.

Neural stem cells

NSCs are multipotent cells that can differentiate into neurons, astrocytes, and oligodendrocytes.

Preclinical evidence

A recent study by Wang et al.[112] depicted that in a rat model of 8-minute asphyxia CA, intracerebroventricular delivery of 2×10^5 human NSCs 3 hours after ROSC improved neurological function as assessed by NDS. NSC transplantation was associated with enhanced neuronal survival in the hippocampal CA2 region and augmentation of endogenous NSC proliferation and migration, which may be attributed to the activation of the Wnt/β-catenin pathway in the sub-ventricular neurogenic niche. It was further verified that NSCs migrate to the injured hippocampus when administered intranasally 3 hours after CA. NSCs regulated microglial activation and the neuroinflammatory response via TLR4/NLRP3 signaling, exerting multiple neuroprotective effects such as improved neurological function and shortened time of severe neurological deficits.[95] In a KCl-induced 12-minute rat CA model, transplanted embryonic neural progenitor cells (NPCs) transplanted 1 week after CA migrated and retained in the injured hippocampal CA1 region 6 weeks after transplantation whereupon they differentiated into the neuronal phenotype.[113]

In an 8-minute asphyxia rat model, intracerebroventricular injection of co-cultured NSCs and MSCs (1×10^6 cells/well, 1 μL/min [5 μL]) administered 20 minutes post-ROSC[114] revealed that in rats treated with BMSCs+NSCs, the NDS value was significantly higher, indicating a facilitated recovery from CA-induced cerebral injury via the release of extracellular vesicle-loaded miR-133b and subsequent regulation of Janus kinase 1 and AKT-GSK-3β-Wnt pathways. At 24 hours, the number of NeuN-positive cells was elevated and the number of TUNEL+ cells was decreased in the cerebral cortex and the hippocampal CA1 region, and the increase was most significant upon treatment with BMSCs+NSCs. Although cell-based therapies have gained popularity as a therapeutic approach to mitigate ischemic brain injury post-stroke, a relatively small number of preclinical studies targeting CA by stem cells have been carried out. To date, there are no clinical studies for treating CA patients with stem cells.

Optimizing stem cell therapy for CA

Clinical trials with stem cell therapy in stroke have been shown to be safe, but only with minimal therapeutic benefits in treating ischemic brain injury.[115,116] This warrants the optimization of stem cell-based therapy for neurological diseases including CA. We have recently reviewed the current state of the art to optimize stem cell therapy in ischemic brain injury.[117] Metabolic glycoengineering (MGE), the process of manipulating cellular glycosylation pathways, is one among various strategies to optimize stem cell therapy. Building on the *in vitro* findings that modifying the glycan structures in human NSCs using the Ac5ManNT-Prop monosaccharide analog improves the differentiation of human neural stem cells (hNSCs) and their adhesion to extracellular matrix components,[118,119] Du et al.[120] recently reported that MGE improved hNSC viability and differentiation, demonstrated multifaceted benefits by regulating cell survival, immunomodulation, synaptic plasticity, and further enhanced the therapeutic efficacy of NSC therapy.

Limitations and perspectives

Recent research from Wang et al.[95] has demonstrated superior neuroprotection of NSCs compared to GBC and highlighted the further enhanced therapeutic efficacy by MGE to boost neural repair,[120] demonstrating neuroprotective potential of stem cell therapy following CA. While a few preclinical studies utilizing MSCs and NSCs have demonstrated neuroprotective potential following CA, most studies targeting stem cell therapy in CA have been performed in rodent models. There are currently no reports of clinical translation of stem cell therapy in CA.

The efficacy of stem cell therapy depends on its capacity to influence complex pathways involved during the progression of the disease.[121] While stem cell therapy holds immense promise, the underlying neuroprotective mechanisms for early paracrine effects and late reconstructing and neuro-regenerative effects are complicated and remain to be fully elucidated. Early interventions prioritize neuroprotection by releasing paracrine signaling molecules, while later stages focus on the neuro-repair and replacement of damaged neural tissue. Crucial factors, such as the type of cells utilized, the timing of administration, the method of delivery, and the expected therapeutic outcomes, all play a substantial role in the success of stem cell therapy in the context of CA.

While preclinical studies have shown potential benefits in reducing brain damage and improving neurological outcomes, translating these results to effective clinical treatments is complex. Challenges include determining the optimal type of stem cells, delivery methods, timing, and dosages, which are important but have not been fully addressed in preclinical CA studies. Refinement in the study design considering these factors is highly warranted in future CA research. Testing the therapeutic efficacy in large animal models and unraveling the underlying molecular mechanism will be helpful for the advancement of stem cell therapy in CA. The long-term effects and potential risk of stem cell therapy have also not been well established in CA. Studies investigating long-term safety and efficacy of stem cell therapy in CA are imperative before their translation to clinical trials. Continued research addressing these challenges is thus essential towards future stem cell therapy in CA patients.

Conclusions

The pathophysiology of ischemic brain injury following CA involves the alteration of multiple pathways that lead to excitotoxicity, mitochondrial dysfunction, oxidative stress, and neuroinflammation. Although some gases, pharmacological agents, and stem cell therapies have shown neuroprotective effects after CA-ROSC in preclinical studies, overall, these outcomes have not been substantially translated in clinical settings. As most of the pharmacological interventions for CA-ROSC provide neuroprotection through targeting certain altered pathways, optimal therapeutic efficacy has not yet been obtained by these methods. Thus, searching for, and optimizing the efficacy of a candidate drug by combination approach targeting the multiple pathways altered after CA-ROSC is of utmost need.

Very few clinical studies utilizing gases and pharmacological agents are available with inconsistent results, warranting the need for more systematic preclinical studies, with large animals to be beneficial to test the therapeutic efficacy of the drug. Stem cells could serve as an alternative to pharmacological and gas-

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eous treatment as they exert paracrine neuroprotective effects by modulating diverse pathways in the CA-ROSC. Unlike some gas-mediated or pharmacological approaches, current reports of clinical evidence and trials for stem cell therapy in CA are still lacking. However, evidence of very few preclinical studies utilizing NSC and MSCs without sufficient detailed molecular mechanisms of the therapeutic mode of action (MOA) is preventing their translation in the clinical setting. More preclinical studies using optimized stem cell therapy with systematic evaluation of transplanted cell fate and MOA in large animal models are warranted for clinical translation of stem cell therapy of CA. Despite promising results in animal models, more research investigating the long-term efficacy and safety of stem cell therapy in CA is imperative to establish the efficacy and safety in humans towards their translation to clinical trials.

Bridging the gap between preclinical and clinical settings requires time-dependent intervention considerations owing to the dynamic and time-sensitive nature of post-CA pathophysiology which possesses challenges, leading to data variability across the studies. Standardizing protocols for defining the optimal time window is essential, with a consensus on the importance of early intervention, particularly in the immediate post-resuscitation period. The specific time window for neuroprotective therapy following CA can vary, but it is generally understood that earlier intervention is crucial pending further large animal and clinical studies verification. The most critical period is often within the first few hours after resuscitation for initiating certain neuroprotective treatments to be most effective.

The lack of reproducibility of positive preclinical results necessitates a strategic approach to designing preclinical animal studies that mirroring anticipated human studies across multiple laboratories. Unlike the Stroke Therapy Academic Industry Roundtable (STAIR) and Stroke Preclinical Assessment Network (SPAN) providing valuable frameworks to enhance the validity and reproducibility of preclinical stroke research, there are few initiatives and collaborative efforts involving researchers, clinicians, and organizations aiming to improve preclinical methodologies and translational outcomes of CA. Forming a preclinical consortium in resuscitation science, to standardize methodology including rigorous study design and transparent reporting of preclinical animal studies following ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines enhances the potential for future clinical translation.

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Conflicts of interest

The authors have no financial conflicts of interest.

Author contribution

Conceptualization: XJ. Study design: XJ. Methodology: XJ. Data collection: SM. Investigation: SM. Writing—original draft: SM. Writing—review & editing: SM, XJ. Funding acquisition: XJ. Approval of final manuscript: SM, XJ.

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