Mechanisms of Injury in Hypoxic-Ischemic Encephalopathy: Implications to Therapy

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ABSTRACT

Cardiac arrest survivors commonly suffer ischemic brain injury, and understanding the mechanisms of injury is essential to providing insight for effective therapies for brain protection. Injury can occur at the time of the cardiac arrest and is dependent not only on the duration but also the degree of impaired circulation. Injury can be ongoing even after the return of spontaneous circulation, giving the clinician an additional window of opportunity to treat and protect the injured brain. This section will review the molecular basis of injury with cardiac arrest and will elucidate the different mechanisms of injury between cardiac arrest, pure respiratory arrest, and arrest secondary to toxins (e.g., carbon monoxide). The rationale for multiple postarrest therapies, such as hypothermia and induced hypertension, will also be reviewed.

KEYWORDS: Hypoxia, ischemia, cardiac arrest, encephalopathy

Animal studies have shown that in sudden cardiac arrest, brain oxygen stores and consciousness are lost within seconds, and glucose and adenosine triphosphate stores are lost within 5 minutes.[1,2] Beyond this time, secondary brain injury occurs, and a full neurological recovery becomes less likely. In animal models, cellular death from apoptosis has been demonstrated within several minutes after the onset of global ischemia.[3] However, in other animal models, including cats and monkeys, circulatory arrest for up to 1 hour can be followed by electrophysiological and functional recovery under certain circumstances.[4,5]

In patients who have a primary respiratory event, especially without a concomitant cardiac arrest, the brain may become dysfunctional but not severely or permanently damaged. Patients who suffer an arrest secondary to carbon monoxide poisoning may have a similar pattern of injury and recovery, depending upon the degree of myocardial suppression and systemic hypotension that occurs as a result of the carbon monoxide poisoning. The mechanisms of injury are different in these patient populations, as is the prognosis for neurological recovery. Understanding which patients are likely to recover well on their own will help the clinician to spare these patients from unnecessary and potentially dangerous therapies.

CARDIAC ARREST PATHOPHYSIOLOGY

During states of decreased or absent cerebral blood flow, multiple mechanisms contribute to injury on a microscopic level (Table 1). As has been shown in models of focal and global ischemia, with impaired cell membrane function there is leakage of intracellular potassium into the extracellular space and an influx of calcium into cells. Lactic acid and hydrogen ions (H^+) are released into the local environment, as is glutamate, an excitotoxic neurotransmitter. Acidosis abounds on a local level, contributing to further cell injury and edema. There is secondary activation of several destructive enzymes,

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Table 1 Derangements in Cardiac Arrest

During Circulatory Arrest	After Circulatory Arrest
Potassium extracellular	Potassium intracellular
Calcium influx	Calcium outflow
Lactic acidosis, hydrogen ions	Free radical formation
Glutamate release	Glutamate release
Release of proteases, lipases, nucleases	Nitric oxide release
Flow arrest	Impaired microcirculation

including lipases, proteases, and nucleases, which break down neuronal tissue. With the return of spontaneous circulation, reoxygenation injury can occur, with free radical formation, iron, nitric oxide, further glutamate release, and renewed calcium shifts.[6] Furthermore, with the reestablishment of blood flow, edema and microhemorrhages can occur with the impaired function of damaged capillaries, further contributing to brain swelling.

Despite the reestablishment of an adequate systemic pulse and blood pressure, the circulation in the cerebrovasculature may remain significantly impaired. Immediately following the return of spontaneous circulation in cardiac arrest patients there appears to be a short-lived period of hyperemia, followed by a more protracted period of global and multifocal hypoperfusion (the "no-reflow phenomenon").[7] Much of the impaired blood flow occurs on the microcirculatory level, as the capillary flow is impeded by red blood cell sludging, disseminated intravascular coagulation (DIC), endothelial cell swelling, and increased interactions between white blood cells and the endothelium.[8] The local environment is also changed by imbalances of nitric oxide, adenosine, and endothelin. Thus, even though the systemic circulation may be reestablished, the cerebrovascular flow on a microcirculatory level may remain quite impaired, causing ongoing ischemic damage.

SPECIFIC AREAS OF BRAIN INJURY

Specific brain regions appear to be most commonly affected with events causing poor systemic circulation. These regions are affected either because they lie in watershed vascular areas or because the neurons in those locations have a higher metabolic rate and oxygen/glucose demand, making them less forgiving of periods of relative ischemia. The neurological syndromes that occur in cardiac arrest survivors can be partially explained by the focal areas of brain injury. The CA1 pyramidal neurons of the hippocampus are quite commonly damaged with prolonged ischemia, with resulting impairment in memory functioning.[9] Cerebellar Purkinje cell injury may result in ataxia, commonly manifesting as a gait disturbance from axial instability. Other commonly affected neurons include the thalamic reticular neurons, the medium-sized striatal neurons, and the pyramidal neurons in layers 3, 5, and 6 of the neocortex.[10] With more prolonged periods of ischemia, arterial border zone regions can be appreciated macroscopically on neuroimaging. Patients with carbon monoxide poisoning appear to have preferential injury to the basal ganglia, specifically the globus pallidus, and may have subsequent parkinsonism and cognitive dysfunction.

Patients with a prolonged period of hypoxia followed by a global ischemic event appear to be susceptible to preferential injury to the subcortical white matter, in what appears to be a primary myelinolytic process. Ginsberg et al[11] performed a neuropathological evaluation of patients who underwent a prolonged period of hypoxia, followed by hypotension and elevated venous pressure. They postulate that injury occurs preferentially in the subcortical matter in situations in which there is a significant period of alveolar hypoventilation, progressive acidosis, and severe metabolic disturbances in the periarrest period and may occur because of cerebral edema, elevated venous or cerebrospinal fluid pressure, or disturbances to the regional vasculature, which lies in the watershed areas of the brain. More recently, magnetic resonance imaging (MRI) studies have noted a similar pattern of injury primarily involving the white matter in patients undergoing diffusion-weighted imaging following a primary respiratory arrest followed by a cardiac decompensation.[12]

In animal studies, a cardiac arrest model is performed under highly controlled circumstances, and the time of poor perfusion can be rigidly defined. Unfortunately, it remains challenging in humans to get a reliable indication of a patient's "down time." Bystanders are notoriously inaccurate in their estimation of the number of minutes a patient was felt to be unresponsive, and documentation by emergency systems personnel, even under the best of circumstances, is often inaccurate in describing the number of minutes a patient is resuscitated in different hemodynamic states. Because of this, reported periods of ischemia and anoxia are often inaccurate and unreliable.

HISTOLOGICAL CHANGES

Histologically, in the initial hours following an ischemic event, there may be little change consisting mostly of "cloudy swelling" of the nuclear region of neurons and loss of a basophilic appearance to the nucleus. After 8 to 12 hours, the classic "red neurons" begin to appear, with progressive eosinophilia of the cytoplasm. These represent shrunken cells, with pyknotic nuclei containing coarse nuclear chromatin with an eosinophilic cytoplasm.[13,14]

HYPOXIC VERSUS ISCHEMIC BRAIN INJURY

Although the term "hypoxic-ischemic encephalopathy" is widely used in reference to any "code" event, patients who suffer a purely hypoxic event without a concomitant cardiovascular collapse have different patterns of dysfunction, degrees of injury, and chances for recovery than those who suffer a cardiac arrest. Some general rules apply to patients who suffer an isolated hypoxic event. They are typically younger patients, with less baseline atherosclerotic disease, including of the cerebrovascular tree. The event is typically caused by airway obstruction, such as caused by epiglottitis, anaphylaxis, trauma, or drug intoxication. With a hypoxic event, the cerebral blood flow (CBF) actually increases, secondary to intact cerebral autoregulatory mechanisms in the setting of an elevated carbon dioxide level and falling pH. With cardiac arrest, by definition the CBF decreases. With a hypoxic event with preserved systemic circulation, glucose and other nutrients are still supplied to the brain, but not with cardiac arrest. With a systemic circulatory collapse, the local environment of the brain is exposed to metabolic waste products, such as lactate and hydrogen ions, which may lead to secondary injury to neuronal tissue. With an isolated hypoxic event, the circulation is preserved, and these toxic metabolites are washed from the local environment. Ischemia is associated with significant elevations in the levels of glutamate, which is excitotoxic; this does not appear to be the case with pure hypoxic events. Ischemia has been associated with increased expression of heat shock proteins 72 and 32, which serve as additional markers of cellular injury. This does not occur with pure hypoxia.[15,16]

It has been demonstrated in both animals and humans that pure hypoxic injury does not lead to severe brain injury, even in the setting of prolonged and/or extreme hypoxia, so long as the systemic circulation is preserved. Hypoxia likely induces changes in the function of the neuron, without necessarily causing death of the neuron. Thus, patients who suffer a hypoxic event may also be poorly responsive and even comatose but may have a much better chance for survival with good neurological recovery than those with cardiac arrest. The neuronal dysfunction induced by hypoxia appears to occur on the synaptic level, with a selective gamma-aminobutyric acid-ergic deficiency, leading to an increased frequency of myoclonus and seizures. The time course for recovery from a purely hypoxic event closely mirrors the time for synaptic regeneration, which is approximately 2 weeks. Therefore, with a comatose patient who has suffered a purely hypoxic event and who does not have additional ancillary data (e.g., neuroimaging, somatosensory evoked potentials) pointing to a likely poor outcome, it is reasonable to continue supportive care in anticipation of a possible awakening to a good neurological outcome.

Miyamoto and Auer[17] evaluated a rat model of pure hypoxic injury, exposing rats to hypoxia at a PaO_2 of 25 mm Hg for 15 minutes, while the $PaCO_2$ was maintained in the normal range. Acidosis was controlled with an intravenous (IV) infusion of sodium bicarbonate, and the mean arterial blood pressure was fixed at levels above 30 mm Hg. In animals exposed to pure hypoxia without hypotension, there was some slowing of electroencephalogram (EEG) frequencies with an increased amplitude, but histologically these animals showed no areas of brain necrosis. However, in animals exposed to hypoxia plus ischemia, there invariably was ischemic brain injury seen histologically, and the degree of injury was directly related to the degree of hypotension. In a separate experiment, these authors showed that the degree of ischemic damage incurred with a middle cerebral artery occlusion in rats was mitigated by increasing levels of normobaric oxygenation, suggesting that hyperoxygenation may be protective during ischemic injury.

Although it is obviously impossible to perform a controlled human study of brain hypoxia and ischemia, there have been several interesting observations reported in case series. In a series of 22 patients from Yale New Haven Hospital who were comatose (some of whom had extensor posturing), with prolonged hypoxia ($pO_2 < 20$ mm Hg) but without hypotension, successful treatment of the underlying medical condition leading to hypoxia resulted in recovery to the morbid state in 13 of the 22 patients.[18] Rie and Bernad[19] reported three patients with prolonged hypoxia ($pO_2 < 45$ mm Hg) for 1 to 8 days but with no concomitant systemic hypotension. These patients ultimately died of sudden cardiac arrest, and on autopsy none revealed changes suggestive of ischemic damage.

Thus, in attempting to provide information to families regarding prognosis for survival for patients in a comatose state, it is important for the clinician to consider the circumstances leading to and surrounding the patient's state. Comatose patients who have suffered a cardiac arrest with systemic hypotension may have significantly worse chances for good neurological recovery compared with patients with pure hypoxia, who may have a similarly poor clinical examination in the acute period but may have better chances of a meaningful, and perhaps full neurological recovery. In evaluating the comatose patient with pure hypoxia in whom the prognosis is uncertain, it may be useful to seek additional data in the form of neuroimaging (e.g., MRI with diffusion-weighted imaging), EEG, or somatosensory evoked potentials.

CARBON MONOXIDE POISONING

Oxygen binding to hemoglobin is blocked by carbon monoxide (CO). The subsequent hypoxia induces

release of nitric acid from endothelial cells and platelets, and this forms peroxynitrate, a free radical. In the brain, this leads to mitochondrial dysfunction, capillary leakage, and apoptosis. It is logical then that the areas of brain that are most commonly affected are those with a high metabolic rate and oxygen demand, such as the basal ganglia, hippocampus, and cerebellar Purkinje cells. The degree of injury directly correlates with the level and duration of exposure. With higher levels of CO poisoning, myocardial suppression can also occur, thus leading to further brain injury with systemic hypotension (please see above section).

The mainstays of treatment with CO poisoning are hyperbaric oxygen and intensive care of systemic complications, such as hypotension and cardiac dysrhythmias. The proposed mechanism for effectiveness of hyperbaric oxygen is the accelerated dissociation of CO to heme[20] and increased levels of oxygen dissolved in blood.[21] Weaver et al[22] performed a double-blind, randomized trial of hyperbaric oxygen compared with normobaric oxygen therapy in acute CO poisoning. The trial was stopped after an interim analysis showing that patients treated with hyperbaric oxygen had less frequent cognitive dysfunction compared with the patients treated with normoxia (25.0% versus 46.1%, $p = 0.007$). This effect persisted at 12 months of follow-up as well (4% versus 15%, $p = 0.04$).

IMPLICATIONS TO THERAPY

Neuroprotective Agents

Numerous agents have been investigated for neuroprotection following stroke and cardiac arrest (Table 2). Unfortunately, none have shown significant efficacy, despite good theoretical justification for their use. Barbiturates provide theoretical benefit by decreasing the cerebral metabolic rate and acting as free radical scavengers. However, a common side effect of barbiturates, especially when given in high intravenous doses, is hypotension, and this may paradoxically worsen neurological outcomes by further impairing the cerebral circulation after an arrest. Monsalve et al[23] performed a nonrandomized trail of 53 patients treated with thiopental during resuscitation, followed by phenobarbital. Of survivors, there appeared to be better cerebral outcomes in the barbiturate-treated group, but they also noted worse outcomes in patients with ischemic heart disease, with a higher rate of hemodynamic deterioration in this group. The Brain Resuscitation Clinical Trial I Study Group[24] performed a large (262 patients) randomized trial of thiopental in comatose survivors of cardiac arrest and found no benefit.

Table 2 Failed or Unproven Neuroprotective Agents

Barbiturates—thiopental, phenobarbital
Calcium channel blockers—lidoflazine
Benzodiazepines—diazepam
Magnesium
NMDA receptor antagonists
AMPA receptor antagonists
Estrogen
Caspase inhibitors
Lamotrigine
Immunosuppressants—cyclosporin A, FK506
Transgenic expression of superoxide dismutase
Brain-derived neurotrophic factor

Calcium channel blockers were also evaluated as neuroprotective agents in cardiac arrest survivors after animal models had shown promise, theoretically by treating the abnormalities in calcium homeostasis that occur after an ischemic injury.[25] This study enrolled 520 patients in a blinded, placebo-controlled, randomized fashion and unfortunately also did not find a significant benefit for this agent. Although this agent was felt to have relatively minimal cardiovascular suppressant effects, one could postulate that the detrimental effects of even small decreases in blood pressure might have offset any benefit achieved on a molecular level of blocking calcium entry to cells.

Other agents evaluated in clinical trials include magnesium and diazepam. Longstreth et al[26] performed a double-blind, placebo-controlled, randomized trial of 300 patients, who received (from paramedics in the field) IV magnesium, IV diazepam, both, or placebo. Unfortunately, neither agent, either alone or in combination, improved neurological outcomes for cardiac arrest survivors versus placebo-treated patients. A potentially significant limitation of this study, however, may have been imbalances of baseline characteristics of patients in the different groups.

Additional agents have shown promise in animal models but not yet in human studies. These include N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists, aiming to block the effects of the excitatory neurotransmitter glutamate.[27,28] Other agents currently under investigation include estrogen,[29] caspase inhibitors,[30] lamotrigine (which may also block the effects of glutamate toxicity),[31] immunosuppressants cyclosporin A and FK506,[32] transgenic expression of superoxide dismutase,[33] and even intraventricular delivery of brain-derived neurotrophic factor.[34] All of these agents remain investigational at this point, and no recommendations can be made regarding their use in human subjects at this time.

Fibrinolytic Therapy

A promising new area of research in resuscitation of cardiac arrest patients is the use of thrombolytic therapy

in the acute setting. Under the premise that the great majority of patients who suffer a cardiac arrest do so because of a coronary thrombosis (or less likely a pulmonary embolus), thrombolytic therapy given acutely may increase the chances for survival by treating the underlying cause more rapidly, thereby theoretically decreasing the overall period of arrest and perhaps the mortality rate as well. But the use of thrombolytics makes sense not just on a cardiovascular basis but also on a cerebrovascular level as well. As the microcirculation is clogged by DIC, red blood cell sludging, and microthrombosis, this may also be a useful secondary target for acute thrombolytic therapy directed at the coronary circulation.

In a prospective study, Böttiger et al[35] treated 40 out-of-hospital cardiac arrest patients not resuscitated within 15 minutes of cardiopulmonary resuscitation (CPR) with a bolus of 5000 U of IV unfractionated heparin and 50 mg of recombinant tissue plasminogen activator. This was repeated if the return of spontaneous circulation was not achieved after 30 minutes. These patients were compared with 50 historical controls (the ethics committee did not approve randomization of unconscious patients) who received standard resuscitation with CPR alone. The patients treated with thrombolysis had a higher rate of return of spontaneous circulation (68% versus 44%, $p = 0.026$). There appeared to be a higher survival rate at 24 hours (38% versus 22%) and patients ultimately discharged from the hospital (15% versus 8%) in the group receiving thrombolysis, although these results did not reach significance due to the small number of patients in the study. There were two patients who required transfusions of red blood cells in the days ensuing, secondary to gastric ulcer bleeding. There were no bleeding complications related to the CPR. There was no report of ultimate neurological outcomes in this study, but thrombolytic therapy in the acute cardiac arrest setting appears to be safe and effective, and a randomized clinical trial is underway.[36]

Additionally, endothelin(a) receptor antagonists have shown promise in animal models,[37] and this may provide additional aid with cerebral microperfusion by decreasing endothelial cell swelling and leukocyte-endothelial cell interactions.

Induced Hypertension

Again focusing on the "no reflow" phenomenon and impaired cerebral microcirculation following a cardiac arrest, research in the area of induced hypertension and hemodilution has sought to improve neurological outcomes by providing adequate tissue perfusion. Long used in the subarachnoid hemorrhage population to improve blood flow in patients with cerebral vasospasm, hypertension and hemodilution may also provide an avenue of treatment for cardiac arrest patients as well. One concern of using vasopressive agents in cardiac arrest patients, however, is the risk of exacerbating coronary ischemia in patients with myocardial infarction as their cause of arrest, and thus this technique may not always be effective or safe.

Induced hypertension has not yet been evaluated in humans, but several animal models have shown promise. Hachimi-Idrissi et al[38] showed a beneficial effect for both survival and neurological deficits in a rat model of combined mild hypothermia and induced hypertension, and this effect was persistent at 4 weeks. Safar et al[39,40] showed a similar effect in a dog model exposed to mild hypothermia and induced hypertension, but the effect was mitigated by a delay in therapy or significant hemodilution, presumably due to the decreased oxygen carrying capacity. Again, no controlled human studies have been performed, but in our cardiac arrest patients we suggest maintaining the mean arterial pressure > 90 in patients who are felt safe to tolerate such measures, given the theoretical benefit of improved cerebral perfusion.

Induced Moderate Hypothermia

Hypothermia for the neuroprotection of cardiac arrest is discussed in detail elsewhere in this issue; thus, we will only briefly mention the proposed mechanisms of action. Hypothermia decreases the cerebral metabolic rate and oxygen demand, thereby reducing cerebral edema. It appears to decrease excitotoxicity, free radical formation, and destructive enzymatic processes, including proteases, lipases, and nucleases. It may also stabilize lipoprotein membranes. Colbourne and colleagues demonstrated in a rodent model that hypothermia attenuated injury in the hippocampal CA1 region by effects on the AMPA receptor GluR2 subunit.[41] Ischemia induces down-regulation of GluR2 mRNA in CA1 selectively, and this effect was reduced with hypothermia given after 1 hour of ischemia for 2 days, with complete recovery to control levels after 7 days.

The major clinical trials of hypothermia in human cardiac arrest survivors were published in 2002,[42,43] leading to the subsequent endorsement of therapeutic moderate hypothermia by the International Liaison Committee on Resuscitation.[44] This technique is not without its risks, however, including an increased risk of infection, coagulopathy, and cardiac dysrhythmias. It should also be emphasized that hyperthermia following cardiac arrest is likely detrimental, and thus strict temperature control to normothermia should be the goal in patients following treatment with induced hypothermia, or for those who are ineligible for induced hypothermia.

Multiple other avenues of therapeutic options exist for the future. Gene therapy and stem cell research have garnered much attention in ischemic stroke therapy[45] and may someday be applied to cardiac arrest

patients. Similarly, hyperglycemia has been linked with worse outcomes in patients with ischemic stroke, and as the same likely applies to patients with cardiac arrest, future avenues of study involve not only tight glucose control postarrest but also the effect of insulin and insulin-derived growth factor in hypoxic-ischemic injury.[46]

CONCLUSION

Survivors of cardiac arrest may commonly suffer neurological injury, which can be devastating to the patient and family. The mechanisms of injury, as well as the prognosis, vary depending on the nature of the arrest: worse outcomes are associated with prolonged periods of hypoperfusion, but perhaps better outcomes can be expected with isolated respiratory arrest. Injury may be incurred: (1) at the time of the initial event, with local toxicity from lactic acid, hydrogen ions, and glutamate; (2) at the time of reperfusion with free radical formation and renewed calcium shifts; or (3) in the minutes to hours after the return of spontaneous circulation, due to impaired CBF on a multifocal and microcirculatory level. Multiple methods show promise in prevention of poor neurological outcome, including multiple neuroprotective agents, thrombolysis, and induced hypertension, but to date only induced moderate hypothermia has been clinically proven and widely accepted as standard of care. A key to future improvements will be improved speed of application, which will hopefully come with an emphasis on improved development and delivery of resuscitation techniques.

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