Protecting the brain during cardiac surgery: Does understanding the molecular mechanisms of cerebral injury provide insight into neuroprotection strategies?

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Abstract

Introduction
Although cardiothoracic surgeons are aware of the deleterious effects of prolonged exposure to cardiopulmonary bypass on brain function and structure, how to best protect the brain during the sensitive time of interruption of normal cerebral blood flow remains controversial.

Short communication

The complex functional anatomy of the brain on the one hand, and the multifactorial mechanisms of neural injury on the other hand, has resulted in a significant gap in translating the current basic science understanding of the molecular mechanisms of ischemic brain injury to effective clinical strategies and outcome assessment. Here, the goal is to address the current understanding of the mechanisms underlying brain injury after cardiopulmonary bypass and relevant strategies of neuroprotection.

Conclusion

By identifying the key players in neuronal injury following hypothermic circulatory arrest, their potential as therapeutic targets can be evaluated in novel neuroprotective strategies.

Introduction

Neurological injury after cardiac surgery
One of the most important innovations in cardiac surgery over the last 50 years has been the development of cardiopulmonary bypass (CPB). Although CPB has unquestionably saved lives by allowing complex surgery on the heart, it has been the cause of significant morbidity, particularly of the central nervous system. Hypothermic circulatory arrest (HCA) has been used for some 40 years as a means of interrupting normal perfusion of the brain and preventing subsequent cerebral ischemic injury during various cardiovascular surgical procedures. The optimal implementation of HCA has yet to be determined, including the maximal duration and depth of cooling. HCA for longer than 60 min is associated with a high risk of neurological damage. In adults, this is usually manifested by specific cognitive or motor deficits, whereas in neonates and infants it includes seizures or choreoathetoid movements. HCA also disrupts autoregulation of the cerebral vasculature, which may lead to over perfusion, resulting in increased intracellular pressure. In addition to postoperative central nervous system complications, CPB in cardiac procedures is associated with a significant risk of stroke, presumably caused by macroembolic injury. Although postoperative cognitive dysfunction is common following cardiac surgery, the incidence is highly variable, ranging from 45% to 88% at hospital discharge and 15%-36% up to 6 weeks postoperatively. Severe focal neurological injuries occur in up to 28% of patients with stroke and transient ischemic attack, which are common sequelae of cardiac surgery with a frequency of up to 80% postoperatively. The statistics dictate the urgent need for novel treatment modalities, particularly for those that can be offered as a preventive or neuroprotective therapy before cardiac surgery.

The growing concern about the subtle cerebral sequelae after use of HCA has led to a surge in experimental animal model in recent years. Experimental studies have demonstrated that prolonged HCA can lead to neuronal cell injury or death, probably as a consequence of a number of different pathways triggered by ischemia. After global ischemic insult, certain neuronal subpopulations are known to die whereas others do not. This selective vulnerability occurs in the adult and neonatal brain and reflects heightened sensitivity of specific neuron groups to ischemic injury. Neurons in the hippocampus, cerebellum, striatum, amygdala, lateral thalamic nucleus and third to fifth layers of the neocortex are selectively vulnerable to ischemia in adults. Neurological damage in these areas may explain, in part, impairment of memory, cognition and motor function seen in adults after cardiac arrest. Recent work indicates that some selectively vulnerable neurons in adults and neonates die after ischemia by a process called apoptosis (programmed cell death). In apoptosis, cell death is orchestrated, involving the activation of specific genes and enzymes, through which cells neatly commit suicide, breaking up into membrane-packaged bits for removal by resident macrophages. Cell death by necrosis, on the other hand, is uncontrolled, involving energy failure, catalysis and membrane rupture.
spilling cellular contents to elicit inflammation and secondary injury. Apoptosis plays a role in neuronal cell death after hypoxia–ischemia, brain trauma and neurodegenerative diseases, although its role relative to necrosis remains unclear. The evidence suggests that either focal or global cerebral ischemia can lead to neuronal injury by apoptosis, as well as by necrosis in a time-dependent process. In an effort to characterise acute brain injury after HCA, neurological injury was assessed in a juvenile porcine model after 75 min of HCA at 18°C (ref. 15). An increase in neurological injury was accessed in a tertiary acute brain injury after HCA, mechanisms of cerebral injury provide insight into neuroprotection strategies. OA Case Reports 2013 Jan 31;2(1):8.

Apoptosis plays a role in neuronal injury and is believed to play a part in the cerebral injury, its role has generally been identified through plain histological techniques. These snapshots do not permit a clear delineation of the timeline of apoptosis. As such, because its role is not clear, therapies have yet to be designed for the specific purpose of inhibiting apoptosis. A clearer understanding of the structural and functional consequences of HCA will be pivotal in clinical decision-making, including when to initiate circulatory arrest and the appropriate interval. The aim of this article is to discuss whether understanding the molecular mechanisms of cerebral injury provide insight into neuroprotection strategies to protect the brain during cardiac surgery.

**Short Communication**

The author has referenced some of its own studies in this short communication. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

**Mechanisms of neuronal injury**

Neuronal death is normal during development of the nervous system, but it is abnormal in brain and spinal cord disease and injury. The available evidence indicates that the survival of neurons and their death are highly regulated and finely orchestrated dynamic events that depend on a number of internal and external factors. Two types of cell death have been described; cell necrosis resulting from injury and causes inflammation and apoptosis, observed normally in development and now identified as programmed cell death. They are generally considered to be distinct forms of cell death, but there is mounting evidence supporting an apoptosis–necrosis cell death continuum. In this continuum, neuronal death can result from varying contributions of coexisting apoptotic and necrotic mechanisms, resulting in some of the distinctions between apoptosis and necrosis becoming blurred. Today, it is believed that apoptosis may contribute to the neuronal degeneration in neurological injuries, such as cerebral ischemia and trauma.

Necrosis can result from acute oxidative stress characterised by passive cell swelling, rapid energy loss and generalised disruption of internal homeostasis with lysis of the nucleus, intra-nuclear organelles and plasma membranes leading to the release of intracellular components that induce a local inflammatory response that in turn, result in oedema and injury to neighbouring cells. Morphologically, cell death is characterised by swelling of organelles and rupture. Necrotic cell death is characterised by inflammation and widespread damage. Apoptosis is a process of cell suicide, the mechanisms of which are encoded in the chromosomes of all nucleated cells. Although the capacity to carry out apoptosis appears to be inherent in all cells, the susceptibility to apoptosis varies markedly and is influenced by external and cell-autonomous events. Apoptosis is regulated by complex molecular signalling systems resulting in an orderly, energy-dependent enzymatic breakdown into characteristic molecular fragments, DNA, lipids and other macromolecules. In contrast to those observed in cell necrosis, the morphological changes that occur during developmental cell death include cell shrinkage, membrane blebbing, chromatin condensation and DNA fragmentation. Earlier studies showed that one of the biochemical hallmarks of apoptosis is DNA cleavage at inter-nucleosomal linker regions, resulting in ladder formation of DNA of 180–200 bp or multiples thereof.

Several families of proteins and specific biochemical signal-transduction pathways regulate cell death. Predominant factors in cell death and cell survival include fas receptor, Bcl-2 and Bax, cytochrome c, caspases, p53 and extracellular signal-regulated protein kinases. Some forms of cell death require gene activation, RNA synthesis and protein synthesis, whereas other forms are transcriptionally–translationally independent and are driven by post-translational mechanisms such as protein phosphorylation and protein translocation. The precise signalling cascade starting from the detection of the signal at the cell surface to the events that occur in the nucleus in apoptosis is not well established, with several grey zones in most...
suggested pathways. Following an appropriate stimulus, the first stage or ‘decision phase’ of apoptosis is the genetic control point of cell death. This is followed by the second state or ‘execution phase’, which is responsible for the morphological changes of apoptosis. The decision phase or genetic control appears to be mediated by two genes Bcl-2 and p53, while the execution phase appears to result from the activation of caspases. It has become apparent that the Bcl-2 family of proteins constitutes a critical intracellular checkpoint within a distal common pathway of programmed cell death.

Apoptosis is controlled genetically, and two genes, Bcl-2 and p53. Proteins encoded by the Bcl-2 gene family are major regulatory components of the apoptotic pathway and are found on the mitochondrial membrane and endoplasmic reticulum. Proteins such as Bcl-2 and Bcl-xL prevent apoptosis (death repressor), whereas Bcl-2-associated x proteins (Bax), such as Bax, Bad, Bak and Bcl-xs, promote apoptosis (death inducers). The susceptibility of an individual cell to apoptosis-triggering stimuli is largely determined by its genetic content, metabolic state and developmental/proliferative state, as well as its distinct receptor and signal transduction pathways, the ratio of death inducer to death repressor proteins seems to exert a rheostat-like control over whether an individual cell will ultimately survive or die. Recent studies show that the ratio of Bcl-2 to Bax is an important determinant in the susceptibility to apoptosis, such as during hypoxia, which lead to an increased expression of Bax protein leading to the altered ratio of these two proteins. More specifically, when Bax was over expressed in cells, apoptotic death in response to a death signal was accelerated, earning its designation as a cell death agonist. Bax may potentiate apoptosis by caspase activation, membrane pore-forming properties and cytochrome c release from mitochondria and by heterodimerization with Bcl-2. When Bcl-2 was over expressed, it heterodimerized with Bax and death was repressed. The central events in apoptosis are proteolysis and mitochondrial inactivation. Cellular disruption results from activation of a family of cysteine proteases called caspases. The evidence suggests that there may be several apoptotic pathways that may depend on the cell type and the inducing agent and that most of these pathways may converge at the caspases step. Caspases are cysteine proteases that cleave certain proteins after specific aspartic acid residues. They are activated through self-cleavage and some activate others, thus acting in a proteolytic cascade that eventually leads to the death of the cell.

There is now evidence for the activation of ‘immediate early genes’ during apoptosis in neural cells; enhanced expression of the transcriptional factors c-jun and c-fos and increased levels of c-jun mRNA have been observed in neural apoptosis. Excessive extracellular glutamate triggers delayed neuronal death by promoting the influx of calcium into cells by activating N-methyl-D-aspartate (NMDA) or non-NMDA glutamate receptors. Glutamate receptor activation, in turn, stimulates expression of rapidly induced transcriptional activators known as the immediate-early genes. These genes initiate a complex cascade of events that transduce extracellular signals into alterations of cellular functions by regulating target gene expression (late-response genes). The immediate-early gene c-fos is rapidly and transiently induced in neurons within the hippocampal formation of the brain after seizures, hypoxia and global ischemia through glutamate-mediated NMDA and non-NMDA receptor activation. The protein product of c-fos mRNA, FOS, modulates the transcription of several late-response genes, such as p53, heat-shock protein, Bcl-x, tyrosine hydroxylase and opioid peptides. Some of the late-response genes expressed after c-fos induction are associated with apoptosis, whereas others enhance cell survival. Although it is not known whether c-fos expression is involved with cell survival or cell death, the appearance of nuclear-associated FOS protein is a useful indicator of severely stressed neurons.

**Discussion**

Protecting the brain during cardiac surgery

The tolerance to cerebral ischemia under normothermia is only a few minutes. Hypothermia is essential for cerebral protection during HCA, although HCA can be prolonged only with limitation. Hypothermia reduces cerebral metabolic activity, oxygen demand and prevents the release of neurotransmitters and delays the onset of fatal biochemical cascade. Although reduced, brain metabolism is not suppressed adequately and remains relatively high at 18°C in traditional HCA protocols. The idea that lower temperature during prolonged HCA is more neuroprotective derives from experimental studies that show more complete suppression of metabolism and electrophysiological activity at more profound levels of hypothermia, better functional recovery in survival models, and fewer conventional histological changes in the brain. Our experimental findings have shown that profound hypothermia to 10°C reduced neurological injury during 75 min of HCA in an acute porcine model compared to less profoundly cooled (18°C) animals. These data support the general consensus that deliberate hypothermia appears to be a reliable method of neuroprotection against injuries related to cerebral ischemia from any cause.

Pre-ischemic conditioning has long been recognised as a powerful method to protect the brain against ischemic injury. The protective effects of preconditioning may result from the attenuation of the mitochondrial permeability transition, prevention of apoptosis, and the activation of a family of proteins that is important for cell survival. The mechanism by which preconditioning reduces neurological injury is not fully understood, but it is likely to involve the activation of transcription factors such as c-fos.

**Short Communication**

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means to induce tolerance against cerebral ischemia and to reduce neuronal death and functional damage after an ischemic injury. Traditional preconditioning regimens including brief episodes of ischemia or hyperthermia are not appropriate for the clinical use. Recent studies suggest that a single dose of the macrolide antibiotic erythromycin can induce tolerance against cerebral ischemia in vivo via pharmacologic preconditioning. In addition, preliminary findings have shown that a single injection of a clinically accepted dose of erythromycin 6–24 h before ischemia reduced neuronal death in the hippocampus and parietal neocortex. Erythromycin is a highly promising candidate for clinical application as a tolerance-inducing drug in conditions with high risk for cerebral ischemia, such as during surgery. First, this is attributed to the preliminary data showing that erythromycin has a significant ability to affect cerebral ischemic tolerance. In addition, erythromycin has been effectively used long-term in clinical practice with few side effects. Both observations make it an excellent candidate for a novel regimen of ischemic-tolerance induction with limited side effects. Despite erythromycin’s ability to affect cerebral ischemic tolerance, the molecular mechanisms that underlie this pharmacological tolerance induction have yet to be identified. Sustained preconditioning regimens have been associated with de novo protein synthesis via adapted gene transcription, suggesting that erythromycin may act at the transcriptional level.

Conclusion
Recent interest in preventing brain injury during heart surgery has focused on intraoperative and perioperative neuroprotection and neuromonitoring. Several strategies have been adopted as ‘standard of care’, particularly for the infant brain during congenital heart surgery, although the strength of evidence supporting these practices is unclear. A reliable neuroprotective strategy has yet to be established, which is primarily related to a lack of understanding of the underlying mechanisms of the neural injury. Recent technological advances allow us to elucidate the neurophysiological and molecular events that lead to neurological injury following insult. Ultimately, by identifying the key players in neuronal injury following HCA, their potential as therapeutic targets can be evaluated in novel neuroprotective strategies. The underlying premise is that a better understanding of the molecular mechanisms of neuronal cell death can lead to new therapeutic approaches for the prevention of neuro-degeneration and neurological disabilities through translational research efforts and will expand the field of cell death biology.

Abbreviations list
CPB, cardiopulmonary bypass; HCA, hypothermic circulatory arrest; NMDA, N-methyl-D-aspartate.

References

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