REJUVENATION RESEARCH

Volume 15, Number 2, 2012

http://www.ncbi.nlm.nih.gov/pubmed/22533424

http://online.liebertpub.com/doi/abs/10.1089/rej.2011.1272

Vascular and Neuronal Ischemic Damage in Cryonics Patients

Benjamin P. Best
Cryonics Institute, Clinton Township, Michigan

ABSTRACT

Cryonics technology seeks to cryopreserve the anatomical basis of mind so that future medicine can restore legally dead cryonics patients to life, youth, and health. Most cryonics patients experience varying degrees of ischemia and reperfusion injury. Neurons can survive ischemia and reperfusion injury more than is generally believed. But blood vessels are more vulnerable, which can impair perfusion of vitrifying cryoprotectant solution intended to eliminate ice formation in the brain. Forms of vascular and neuronal damage are reviewed, along with means of mitigating that damage. Recommendations are also made for preventing such damage.

INTRODUCTION

The goal of cryonics is cryopreservation of the anatomical basis of mind in the belief that future medicine will be able to restore well-preserved legally dead persons (cryonics patients) to life and youth. Specifically, it is believed that future medicine will be able to cure all disease and repair damage due to aging, the dying process, the cryopreservation process, and some amount of ischemia/reperfusion injury that occurs after legal death.[1]

Ischemia is the damage inflicted on blood vessels and body tissues due to absence of blood flow. Cryonics patients can suffer from *warm ischemia* if there is no immediate cooling when the heart stops at the time of legal death, and can suffer *cold ischemia* if much time is spent packed in ice pending cryoprotectant perfusion. Ischemic damage to blood vessels causes them to leak, resulting in edema and impaired cryoprotectant perfusion.

Because cryonics can only be performed on people who are legally dead, some pre-mortem ischemic damage frequently occurs, but more ischemic damage typically occurs post-mortem. How much ischemic damage short of complete decomposition allows for the possibility of future restoration of a person with memories and personal identity intact is the subject of considerable questioning among those who believe that cryonics may work. Attention is focused on the brain, specifically the blood vessels, neurons, and connections between neurons. How well are these components preserved under the typical post-mortem conditions of a cryonics patient?

Under the best conditions, a terminal cryonics patient is pronounced dead immediately after cardiac arrest, and a cryonics standby team waiting by the bedside immediately begins cooling and restoration of circulation. After cooling to at least 10ºC the patient is perfused with cryoprotectants to prevent ice formation in the brain, and is then cooled to liquid nitrogen temperature for long-term storage. If the cryonics standby does not occur close to a cryonics facility the patient is shipped in ice to the cryonics facility, experiencing 12 to 72 hours of cold ischemia before cryoprotectant perfusion. Few cryonics patients receive the ideal treatment of local standby. It is of great interest to practicing cryonicists to find means to minimize ischemic damage in non-ideal cryonics cases, and to understand the prospects for future reconstruction of patients who have suffered warm and cold ischemic damage.

BLOOD VESSELS

If circulation is restored in less than a few minutes after cardiac arrest, blood vessels and tissues benefit from the restoration of gas exchange and nutrient, and are able to recover. But if circulation is restored after a certain delay, blood vessels and tissues are damaged even more than they would have been without restoration of circulation. Instead of oxygen reviving the tissues, oxygen in delayed reperfusion causes free radical damage, which can initiate other forms of *reperfusion injury*.

Cardiocytes are much more resistant to ischemic damage than neurons,[2] but the principle of reperfusion damage is the same for both cell types. After an hour of ischemia, 3 hours of reperfusion kills over 60% of cardiocytes, whereas after an additional 3 hours of ischemia (rather than reperfusion), only about 15% of the cardiocytes die.[3] The reperfusion injury is completely abolished by antioxidant treatments, demonstrating that reperfusion injury is initially due to oxidative damage.[4] Like antioxidants, spin-trapping agents protect against the initial stages of reperfusion injury.[5] A probe for hydrogen peroxide and superoxide shows a slow rise in ROS (Reactive Oxygen Species) under ischemia, but a dramatic burst of ROS upon initiation of reperfusion.[6] Similar experiments on rabbit hearts demonstrate a peak in ROS at 10-20 seconds of reperfusion following 10 to 30 minutes of ischemia, with no ROS observable after 5 or more minutes of reflow.[7]

Many of the oxidants released on reperfusion were generated during the ischemic period. Reducing the length of ischemia can reduce the level of oxidants produced during reperfusion.[6] Endothelial cells are rich in mitochondria, and it is mitochondria which are the source of most of the superoxide produced in reperfusion.[8,9] Endothelial cell NADPH oxidase[10] and xanthine oxidase[11] are also significant sources of superoxide during reperfusion. Superoxide reacts with nitric oxide resulting in peroxynitrite, which is more damaging than the superoxide.[12] During reperfusion, abnormally high amounts of superoxide converts almost all available nitric oxide to perxoynitrite — regarded as the agent causing most of the damage to brain capillary endothelial cells.[13] Methylene blue can protect against reperfusion injury by reducing nitric oxide (and hence peroxynitrite) formation.[14]

After free radicals, the second wave of blood-vessel damaging agents in ischemia and reperfusion injury are inflammatory cytokines. Tumor Necrosis Factor-alpha (**TNF- a**) and InterLeukin-one-beta (**IL–1ß**) appear within hours of ischemia, followed by neutrophils within

4 to 6 hours of ischemia.[15] Inflammatory cytokines promote further free radical production while activating PolyMorphoNuclear leukocytes (PMN leukocytes, of which neutrophils are the most abundant) and endothelial cells.[16] Activated leucocytes and endothelial cells secrete adhesion molecules causing the leucocytes to stick to blood vessel walls, and infiltrate into the ischemic tissue between 6 to 12 hours after reperfusion.[17]

Within an hour of reperfusion, leucocytes adhering to blood vessel walls can completely occlude the vessels, leading to a "no reflow" phenomenon.[18] The "no reflow" effect can be considerably reduced by the monoclonal antibody IB4, which inhibits neutrophil adhesion to the endothelium.[19] Red blood cell aggregation near the exit of capillaries pushes leukocytes against endothelial cells, thereby increasing leukocyte adhesion.[20] Leukocyte adhesion (and reperfusion damage) is higher in older animals.[21]

Swelling of endothelial cells also contributes to the "no reflow" phenomenon. Within a half-hour of ischemia, rat microvessel diameter was reduced to 80% and after an hour was further reduced to 76% of the original diameter.[22] Experimental middle cerebral artery occlusion has shown blood flow reduction to 71% of control after a one hour occlusion and reduction to 22% of control after a four hour occlusion.[23]

Permeability of brain capillaries (the Blood-Brain Barrier, BBB) increases progressively in ischemia and reperfusion, ultimately leading to brain edema. BBB permeability to the amino acid leucine (a small molecule) increases within 15 minutes of reperfusion injury following an hour of ischemia.[24] Superoxide, nitric oxide, and glutamate interact to contribute to the early stages of BBB permeability increase.[25,26,27] Arachidonic acid also increases BBB permeability, but not in the absence of lipid peroxidation.[28] Antioxidant enzyme pre-treatment substantially reduces edema in the early stages of reperfusion.[29] In the inflammatory stage of ischemia/reperfusion injury, the cytokine TNF- a requires the presence of ICAM-1 (InterCellular Adhesion Molecule-1) to increase vascular permeability.[30]

Edema following reperfusion has been shown to increase for warm ischemic times up to 3 hours, but not beyond 3 hours.[31] Rats subjected to ischemia without reperfusion showed progressively greater brain edema due to sodium entry across the BBB after 12 hours, but albumin did not begin crossing the BBB until after 2 days of ischemia.[32] From 3 to 48 hours following reperfusion there is an increase in Matrix MetalloProteinases (MMPs) which attack capillary walls to increase permeability and edema.[33] MMPinhibitors have been used to protect the BBB against ischemia/reperfusion injury.[34,35,36,37,38,39]

Treatments to oppose edema formation have also included antagonism of VEGF (Vascular Endothelial Growth Factor, also known as vascular permeability factor),[40] AVP (Argenine VasoPressin) antagonism[41], erythropoietin, [42] hyperbaric oxygen,[43] and tPA (tissue-type Plasminogen Activator) inhibition.[44] Although tPA can increase perfusion by its thrombolytic action, it can also erode the BBB and be cytotoxic.[45] A variety of other agents have been used to protect the BBB.[46]

NEURAL TISSUE

There is a widespread myth that neurons die after a few minutes of ischemia. It would be more accurate to say that neurons subjected to reperfusion injury after a few minutes of ischemia are started on a path that ultimately leads to cell death, but this process can take many hours.

The myth of neuron death after a few minutes of ischemia is based on the fact that people do not survive if circulation is not restarted within a few minutes of cardiac arrest.[47] A study of nearly seventeen thousand out-of-hospital human cardiac arrest patients found that only about one in 500 patients were living after one month if the delay between a call and ambulance arrival was between 8 and 12 minutes.[48]

Ultimate survival is not the same as immediate death, however. And the ultimate survival of unhealthy human adults who experience cardiac arrest under uncontrolled conditions is an end-point loaded with confounding variables. 46% of healthy mice subjected to 3 minutes of cardiac arrest survived at least 72 hours after reperfusion, while 40% died in the first 24 hours.[49]

In 1976, after dogs were subjected to 12 minutes of cardiac arrest, their circulation was restored using elevated blood pressure, norepinephrine, heparin, and hemodilution with dextran 40. The dogs suffered no neurological damage.[50] Increased cerebral perfusion pressure alone can extend the tolerable ischemic period to 12 minutes.[51] These experiments illustrate that neurons are not irreversibly destroyed by a few minutes of ischemia, and that resistance to recirculation, not neuron viability, is responsible for the fact that a few minutes of ischemia followed by reperfusion results in failure to survive without neurological damage under normal conditions.

Neurons are relatively tolerant of ischemia compared to blood vessels. Only 36% of neurons were dying after one hour of ischemia followed by two hours of reperfusion.[52] In rats not subjected to reperfusion injury, only 15% of neurons were necrotic after 6 hours of normothermic ischemia.[53]

Protective measures can be taken to reduce neuron death due to ischemia and reperfusion. Calcium (Ca^{2+}) influx into neurons due to the glutamate excitotoxicity of ischemia/reperfusion can be blocked with nimodipine.[54] TNF- a-receptor blockers can protect neurons from ischemic apoptosis,[55] other receptor agonists/antagonists are also effective,[56] and anti-apoptotic proteins can protect cells against ischemia/reperfusion injury.[57]

HYPOTHERMIA

Cooling is the most powerful weapon against ischemic damage. In general, each 10ºC reduction in temperature between 0ºC and 40ºC reduces metabolic rate by one-third to one-half,[1] although there are discontinuities in Arrhenius behavior associated with chilling sensitivity and other specific temperature-dependent activation energies (E_a).[58] Experiments with dogs that have been quickly cooled with blood washout solution have shown that cooling from 30ºC to 10ºC can extend the tolerable period of cardiac arrest without neurological damage from 5 minutes to as much as 120 minutes.[59] Rats subjected to 2 hours of cerebral ischemia showed neuroprotective

benefits from hypothermia even when begun up to 4 hours after reperfusion.[60] Hypothermia also protects the blood-brain barrier by depressing activity of proteases.[61]

But a few degrees of cooling can reduce ischemic damage to a far greater extent than would be predicted by the reduction of metabolic rate.[62] The first few degrees of cooling reduces cerebral tissue inflammatory cytokines to one-third the normothermic value, with further reduction by the use of anaesthetic.[63] In a study of 137 control and 136 hypothermic patients it was found that reduction of body temperature from 37ºC (normal human body temperature) to 32-34ºC for a 24 hour period following cardiac arrest increased 6 month survival from 41% (control) to 55% (hypothermic) and reduced 6 month brain damage from 61% (control) to 45% (hypothermic).[64]

Cold ischemia is not warm ischemia in slow motion. Unlike cold ischemia, warm ischemia inhibits nitric oxide synthase and results in production of eicosanoid vasoconstrictors during reperfusion.[65] Unlike warm ischemia, cold ischemia is associated with an increase in chelatable iron, which opens the Mitochondrial Permeability Transition Pore (MPTP), leading to apoptosis or (more often) necrosis,[66] although there is a limited potential for perfusion solution additives to reduce damage resulting from long periods of cold ischemia.[67] Other forms of injury induced by cold are *cold shock*[68] and *chilling injury*.[69]

CONCLUSIONS

Under optimal conditions, immediately after pronouncement of death a cryonics patient is placed in an ice bath, and circulation is restarted with a mechanical chest compressor. Once cooled to near ice water temperature, the patient is perfused with vitrifying cryoprotectant solution, cooled under computer control to nearly -196ºC, and then transferred to liquid nitrogen for long-term storage. Unfortunately, only a minority of cryonics patients receive this treatment. More often the patients suffer greater amounts of warm and cold ischemia. Terminal cryonics patients should move to be near a cryonics perfusion/storage facility and arrange for a standby cryonics team in order to receive such reduced ischemic injury.

Neuron viability is not retained in current cryonic preservation practice, which is based on the hope that preservation of *structure* will be adequate for restoration of *function* by future medicine. The fact that neurons can survive such long periods of ischemia and reperfusion — and the fact that hypothermia can substantially improve preservation — bodes well for the preservation of the anatomical basis of mind even after hours of warm ischemia and days of cold ischemia. Nonetheless, efforts are being made to preserve potential neuron viability by finding vitrifying agents with reduced toxicity, and by the use of storage temperatures above liquid nitrogen temperature to eliminate cracking from thermal stress.

Blood vessels may not be essential for future reconstruction and revival of a cryonics patient, but blood vessels need to be able to deliver vitrifying cryoprotectant to all parts of the brain if ice formation is to be prevented in the brain of a cryonics patient cooled to cryogenic temperatures. Ischemia, reperfusion injury, and subsequent edema can mean that some blood vessels are blocked in an ischemic cryonics patient, resulting in ice formation in parts of the brain not saturated with vitrification solution. Although some theorists have speculated that cryonics

patients with frozen brain tissue may someday be reconstructed with nanotechnology,[70,71] it is safer to eliminate or minimize such damage.

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