
The Role of Pharmacology in Spinal Cord Protection During Thoracic Aortic Reconstruction

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Surgery of the thoracic aorta continues to have a significant risk of neurologic complication. Several strategies to minimize this risk are emerging. Pharmacologic protection from these complications continues to be researched, but at this point few medications are being used clinically. This article reviews the pathophysiology of ischemic spinal cord injury and summarizes the investigational pharmacology that may prevent these serious complications.

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Neuroprotective drugs remain minimally used during thoracic aortic reconstruction, and no single agent has emerged that prevents neurologic impairment after thoracic aortic clamping in these procedures. Technical strategies, such as drainage of cerebral spinal fluid, hypothermia, and distal perfusion, are commonly used for this purpose, while drug therapy trails behind.¹ Despite the lack of emergence of any reliable agents for neuroprotection, an extensive body of literature promises possible protective therapies in thoracoabdominal aortic reconstruction.

This summary reviews mechanisms of spinal cord injury during aortic reconstruction, specifically addressing the similarities and differences between ischemia/reperfusion injury of neurons and other organs. The potential delivery routes for pharmacologic neuroprotective therapy will be addressed. Finally, drug therapies that have shown promise in comparative animal models for reducing neurologic dysfunction following spinal cord ischemia will be reviewed and compared.

Injury

Operations on the aorta above the renal arteries have a significant risk of temporary or permanent spinal cord dysfunction. Depending on the location of the aortic lesion, the rates of paraplegic complications can range from 1% to 21%.²⁻⁸ Interruption of peridiaphragmatic aortic blood flow increases the risk of spinal cord ischemia because the major blood supply to the anterior spinal cord often occurs from this level (i.e., T8 to L1).¹ The great anterior medullary artery of Adamkiewicz is usually the largest of 8 to 10 unpaired anterior medullary arteries that supply the anterior spinal artery. Whether transient or permanent, the lack of perfusion of the anterior spinal artery or one of its main tributaries can lead to paraplegia following surgery.

Regardless of the end organ, once blood flow to metabolically active tissue is interrupted, the oxygen demand becomes higher than the oxygen supply. Although spinal cord neurons maintain normal metabolism, normal mitochondrial oxidative phosphorylation stops within minutes of ischemia, with a resultant decrease in adenosine triphosphate (ATP) stores.⁹ Without ATP to drive energy-dependent membrane pumps, intracellular electrolyte homeostasis is compromised. Intracellular hypercalcemia activates pathologic cytoplasmic enzymes that damage deoxyribonucleic acid (DNA) and essential proteins and increases reactive oxygen species and excitatory amino acids.¹⁰ Intracellular hypernatremia pulls water into the cell, leading to cytotoxic edema. Anaerobic metabolism ensues with the produc-

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tion of lactate, making the cells relatively acidotic. The electrolyte disturbances directly inactivate normal cellular biochemical reactions, including glyceraldehyde-3-phosphate dehydrogenase and sodium channels (most importantly the Na⁺/K⁺ ATPase). Clearance of toxic metabolites becomes limited, while reactive oxygen species production increases drastically via nitric oxide synthase, phospholipase activity, xanthine oxidase, and the Fenton and Heber-Weiss reactions.¹¹ The increased concentration of free radicals damages DNA and structural elements in addition to compromising cellular membrane integrity.¹²

A process unique to damaged neurons further exacerbates this injury. In response to injury, excitotoxic neurotransmitters, mostly glutamate and aspartate, are released from the neurons.^{11,12} These substances activate N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-kainate receptor subtypes. Activation of these receptors increase metabolic demand similar to a normal neuronal discharge, which some injured neurons cannot tolerate. Excitotoxicity leads to further intracellular sodium accumulation, exacerbating edema and acidosis. All phases of injury are worsened by excitotoxic perturbation of electrolyte abnormalities, membrane channel dysfunction, and increased production of reactive oxygen species.

Subsequent to the initial ischemic events, inflammatory cells are recruited and activated locally. The injured neurons release inflammatory signals that activate microglia and neutrophils. Mediators include intercellular adhesion molecule, P-selectin, interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF).^{11,12} Neutrophils are recruited within the first 24 hours during the early phase of leukocyte accumulation, amplifying the inflammatory process by the release of more cytokines, chemokines, and reactive oxygen species. These substances injure the ischemic and nonischemic parenchyma and endothelium. The capillaries become more porous, causing increased tissue edema. With the accumulation of fluid within the inflexible dural sac, local pressure increases leading to a compartment syndrome. After the first 24 hours, macrophages begin to accumulate at the injury site to phagocytose debris and necrotic tissue. At times, the oxidative and lysosomal enzymes remove debris, causing more damage to injured cells.¹¹ Irrevers-

ible injury ultimately leads to active neuronal termination, termed apoptosis, which correlates with permanent neurologic dysfunction. The role of T-cell activation is a process that is beginning to be understood, which may be significant in ischemia and reperfusion injury, but has not yet been shown in spinal cord injury.

Parenchymal vasculature reacts to both the direct injury to the endothelium and the secondary inflammation. These reactions can include vasospasm, thrombosis, hemorrhage, and capillary leak. The dysfunction spiral worsens as an increase in cellularity and edema cause compartment syndrome and more ischemia. The sum of all these injuries leads to cellular processes of apoptosis and concurrent functional impairment. Once the neuron is dead, reparation is no longer possible, and deficits will persist permanently. At this point in the injury, little to nothing can be done to reverse the process.

Route of Delivery

Pharmacologic intervention can be delivered through several routes, each of which has advantages and limitations. Delivery can take 4 forms: (1) intravenous (IV), (2) retrograde venous, (3) selective arterial, and (4) intrathecal. Systemic IV delivery requires only IV access, and no further intervention is required. It is by far the least invasive route of delivery possible. However, it is nonselective in site of action, resulting in delivery of the drug to the whole body. Systemic drug levels may need to be higher to maintain adequate concentrations at the local target area. Higher systemic levels increase the possible side effects as well as increasing the degradation of some substances. When targeting the central nervous system, this route is also limited by the ability of the agent to cross the blood-brain barrier.

Retrograde venous infusion requires accessing the spinal cord venous drainage, usually via the hemiazygous vein or inferior vena cava, with the delivery of fluid at a rate high enough to penetrate the spinal cord parenchyma. This process allows local delivery of medication at levels that may be toxic systemically, or be broken down by the kidney, liver, or other enzyme systems. The disadvantage of retrograde delivery is that it requires extra intervention, and critics have questioned whether the potential effects are direct or

secondary to the eventual systemic delivery of the drug. The third intravascular route is direct delivery to excluded collateral arteries. This route provides direct access to the ischemic tissue but can be technically demanding. Finally, intrathecal delivery is possible. Delivery of an agent within the dural sac provides another route of direct delivery of agent discussed here. The agent may act directly on the cord from within the dural sac, but the parenchymal penetration may not be as efficient as desired. In addition, the infusion of a fluid in the closed space may increase cerebrospinal fluid (CSF) pressure and worsen the possible compartment syndrome.

Pharmacologic Intervention

Preserving Blood Flow

The simplest process of avoiding ischemic damage would be the preservation of blood flow during the procedure. Local vasodilatation can recruit small collaterals to increase the collateral blood flow during vascular occlusion. Svensson and colleagues used intrathecal papaverine on patients undergoing thoracoabdominal aneurysm repair.¹³ Using 30 mg papaverine in a 10% dextrose solution before cross clamping, they prevented all early paraplegia, but delayed paraplegia developed in 1 patient. The investigators concluded that this therapy provided spinal cord protection during thoracic aortic operations, particularly during prolonged ischemic periods. They followed this study with another reporting no systemic side effects of papaverine therapy.¹⁴ Interestingly, papaverine had no effect on CSF pressure either. Neither study showed any evidence of negative side effects of the drug nor intrathecal catheter placement.

These studies suggest that local vasodilatation may be protective in ischemic spinal cord injury. However, systemic vasodilatation can actually worsen the injury by limiting spinal cord perfusion pressure. Marini and coworkers used a canine model of subclavian aortic cross clamping for 1 hour to study the effects of using either sodium nitroprusside or nitroglycerin in thoracic aortic cross clamping.¹⁵ These medications are used to control proximal hypertension during the clamping. However, both of these medications proved detrimental to spinal cord perfusion pressure and neurologic outcome. Lowered systemic

pressure decreased perfusion pressure in the spinal cord, leading to further ischemia. A later study by this group showed that CSF drainage was not able to reverse this injury.¹⁶ Local pharmacologic vasodilatation appears to be effective in reducing paraparesis and paraplegia in aortic surgery, but care must be taken to avoid the systemic effects of lowering spinal cord perfusion pressure that can exacerbate neurologic injury following ischemia.

Metabolic Manipulation

Although preservation of oxygen delivery is important, reducing metabolic demand during ischemic periods may be equally important. This reduction consists of controlling 2 phases, as discussed in the "Injury" section. Initial reduction in the metabolism of normal cellular processes can reduce the ischemic insult. Then, prevention of excitotoxicity, with its pathologic magnification of metabolic injury, is a second therapeutic possibility.

Barbiturates and Opiate Antagonists

Barbiturates could have a role in reducing spinal cord injury based on their efficacy in reducing cerebral injury after ischemia by slowing brain metabolism and lowering intracranial pressure. Nylander and coworkers used thiopental (20 mg/kg) before 30 minutes of aortic occlusion in canines to reduce spinal cord injury.¹⁷ The thiopental group had a significantly lower incidence of paraplegia to both control animals and animals receiving mannitol and methylprednisolone. However, Kirshner and colleagues found that only in combination with local hypothermia and superoxide dismutase was thiopental able to improve neurologic deficit in a canine model of thoracic aortic occlusion for 40 minutes.¹⁸ Barbiturates may have some protective effect, but the protection may not be adequate as a single agent.

Similar to barbiturate therapy, opiate antagonism seems to exert some protective effect on spinal cord ischemia and reperfusion injury. The opiate antagonist nalmeferene (0.1 mg/kg IV after reperfusion) provided significant preservation of function and histologic outcomes.¹⁹ Suzuki and coworkers reported similar findings in a feline model of spinal cord ischemia, showing that naloxone can reverse α -aminobutyric acid retarda-

tion of evoked potentials.²⁰ These 2 studies imply that opiate antagonism can preserve spinal cord function, and at least 1 case report backs this finding in humans.²¹ The predictable controversy with this therapy concerns pain control. Even the use of local or intrathecal opiate antagonists create potential pain issues that must be considered before using them clinically.

Reducing Excitotoxicity

As discussed earlier, the major difference between ischemia of neurons and of other tissues is the propensity for excitotoxicity. Limitation of NMDA and AMPA-kainate receptor activation provides another pathway of spinal cord protection. NMDA antagonism may be a significant treatment option in spinal cord protection from ischemic injury.

Riluzole blocks NMDA receptor activation in addition to inhibiting the presynaptic release of glutamate. It also inactivates both sodium and potassium channels. Two studies have shown the efficacy of this therapy in ischemic spinal cord injury. Lang-Lazdunski and coworkers studied riluzole given to rabbits intravenously at 8 mg/kg before infrarenal aortic cross-clamping for 40 minutes.²² They found that riluzole preserved hind limb motor function, while preserving microtubule associated protein-2 expression and neuronal viability. Lips and colleagues reported similar results in a rabbit model after 29 minutes of warm spinal cord ischemia.²³ Using the same dosing as the previous study, they showed improved hind limb motor function in riluzole-treated animals. They also found histologic evidence of preservation of spinal cord neuronal architecture in the riluzole-treated animals compared with ischemic controls. A study in the safety profile of the drug needs to be completed before human testing can begin.

Memantine is another NMDA receptor antagonist that acts noncompetitively. This drug is currently being used in humans for depression and epilepsy in neurodegenerative diseases. It works through the reduction of excitotoxicity and prevention of calcium influx into ischemic neurons. Although safety profiles are established, the outcomes of memantine use have been variable. von Euler and coworkers found no protective neurologic effects in rats with a photothrombosis model of spinal cord ischemia.²⁴ Rimpilainen and

colleagues showed similar results in a swine model of hypothermic circulatory arrest.²⁵ They found no added neuroprotection by administering 5 mg/kg memantine before 75 minutes of warm spinal cord ischemia. Alternately, Ehrlich published more favorable results using memantine in a rabbit model of infrarenal aortic occlusion.²⁶ Both segmental arterial infusion and systemic IV therapy with 20 mg/kg memantine significantly improved motor function, preserved neuronal histologic architecture, and preserved motor evoked potentials after 48 hours of spinal cord reperfusion.

Magnesium sulfate inhibits the activation of the NMDA receptor, inhibits glutamate release, provides a calcium buffer in the mitochondria, antagonizes calcium channels, and enhances recovery of cellular energy metabolism following ischemia. Simpson and coworkers used a canine model of thoracic aortic cross-clamping for 45 minutes to study magnesium treatment with 3 mg/kg before aortic occlusion.²⁷ After 24 hours of reperfusion, the treated animals showed no measurable neurologic injury, while 7 of 8 control animals showed severe injury. Histologic evaluation supported the functional findings with preserved motor neuron viability in treated animals. Lang-Lazdunski and colleagues compared magnesium and riluzole in a rabbit model with 40 minutes of infrarenal aortic occlusion.²² Magnesium alone, at 100 mg/kg before clamping, preserved hind limb function significantly compared with control at 24 and 48 hours of reperfusion. This therapy also preserved cytoarchitecture, but functional and histologic results were not as good as riluzole alone. Combination of these 2 therapies afforded no additive protection.

Cellular Electrolyte Homeostasis

Calcium influx into the ischemic neuron secondary to excitotoxic stimulus is a major part of the pathologic mechanism of ischemic injury. Intracellular hypercalcemia leads to the activation of many of proteases, production of reactive oxygen species, and even apoptosis via caspase activation. The blockade of this calcium influx could lower the metabolic burden, thus decreasing injury. Although early studies using calcium channel blockers produced marginal results, more recent studies have shown more potential.²⁸ Burns and colleagues used a rat model for spinal cord

ischemia and reperfusion to test intrathecal administration of ziconotide, a calcium channel blocker.²⁹ In their experiment, rats undergoing less than 10 minutes of ischemia with continuous intrathecal therapy (300 or 600 ng/kg/h initiated 24 hours before ischemia and continuing an additional 24 hours) showed improved function, correlating with microtubule associated protein-2 preservation. This result seemed to limit neuronal degeneration after 24 hours of reperfusion. The protective effect was lost when the ischemic time was expanded beyond 9 minutes. Calcium channel antagonism may be important for preventing ischemic spinal cord injury, but further study is required to define its role in achieving this protection.

The blockade of other electrolyte channels has been neuroprotective in animal models for cerebral ischemia. Voltage-dependent sodium channel antagonism in cerebral ischemia can reduce neuronal metabolic demand.^{30,31} Sodium channel blockade is hypothesized to delay the hypoxic depolarization due to excitotoxicity. From our laboratory at The University of Virginia, Gangemi and coworkers used a rabbit model of infrarenal aortic cross clamping to study the effects of sodium channel blockade after 45 minutes of warm spinal cord ischemia.³² The studies involved both systemic and retrograde phenytoin infusion to achieve sodium channel blockade. We found that the retrograde delivery of phenytoin at doses of 50 or 100 mg in a saline carrier significantly preserved spinal cord function when compared with systemic treatment or ischemic controls. In summary, both preventing excitotoxicity and limiting its secondary electrolyte shifts can improve functional and histologic outcomes following spinal cord ischemia. The use of NMDA antagonists and electrolyte channel blockers has significant potential for spinal cord protection after aortic occlusion following thoracic aortic reconstruction.

Reduction of Toxic Metabolites

Reduction of reactive oxygen species can limit damage caused by these substances on injured neurons, also limiting the inflammatory recruitment through chemotaxis and diapedesis. Several types of antioxidants have been used, including superoxide dismutase, allopurinol, and deferoxamine. Early reports showed that superoxide dis-

mutase has neuroprotective efficacy after short periods of warm spinal cord ischemia.¹² However, this protection was not observed with longer periods of ischemia.²⁸ After longer periods of spinal cord ischemia, there appeared to be premature drug degradation and a lack of penetration across the neuronal cell membrane. The solution to this problem was the conjugation of superoxide dismutase with polyethyleneglycol, which both increased the serum half-life and improved cellular penetration. Using a rabbit model for spinal cord ischemia, Agee and coworkers, also from our laboratory, used conjugated superoxide dismutase before and during ischemia to improve hind limb motor function.³³ We found a lower incidence of paraplegia in the group receiving the conjugated superoxide dismutase compared with both control and unconjugated superoxide dismutase. This protection was lost when clamp time was increased to an hour, implying that other mechanisms of damage may not be affected by free radical scavenging.³⁴ There appears to be a limit to the benefit of antioxidant therapy after longer periods of ischemia.

Allopurinol is an analogue of hypoxanthine that acts as both a substrate and a potent inhibitor of xanthine oxidase. This enzyme is responsible for the production of at least some of the reactive oxygen metabolites in ischemia and reperfusion. Svensson and coworkers showed that allopurinol did not protect primates from spinal cord dysfunction following ischemia.²⁸ Interestingly, the medication did prevent gastric stress injury in this model, showing that the medication could have effects elsewhere in the body. Qayumi and colleagues were able to show injury amelioration in swine using the combination of allopurinol and deferoxamine.³⁵ Deferoxamine chelates iron, therefore inhibiting the iron catalyzed free radical production and lipid peroxidation. Pigs were given allopurinol doses of 50 mg/kg/d for 3 days preoperatively and a deferoxamine dose of 50 mg/kg IV over 3 to 4 hours that was completed 1 hour before 30 minutes of descending aortic cross-clamping. Treated animals showed that complete neurologic recovery correlated with preservation of histologic parameters. Another study by Qayumi and coworkers showed superior results with deferoxamine in spinal cord ischemia when compared with superoxide dismutase or allopurinol alone.³⁶ Unfortunately, there are reports that deferoxamine only delays the onset of

cellular death in models of myocardial ischemia and reperfusion.³⁷ In addition, there is a report of dose-related toxicity with deferoxamine therapy.³⁸ In summary, decreasing toxic metabolites has not consistently improved spinal cord function following spinal cord ischemia.

Inflammatory Inhibition

Steroids

The classic use of steroids for inflammatory inhibition is becoming increasingly controversial. Even in traumatic spinal cord injury, there are many who question the benefit of what has been called the standard of care. Several studies have shown that glucocorticoid therapy can reduce ischemic damage to the spinal cord. The most important effect seems to come from the reduction of free radical release from activated neutrophils and the secondary lipid peroxidation from these free radicals.

In 1984, Laschinger and coworkers found that IV methylprednisolone (30 mg/kg) improved ischemic spinal injury in dogs.³⁹ Treated animals showed no evidence of dysfunction despite 2 of 3 of control animals having spastic paraplegia. Tetik and coworkers eliminated all functional deficits in rabbits receiving methylprednisolone (30 mg/kg) with vitamins C and D.⁴⁰ They delivered the solution in hypothermic saline via the excluded segment of infrarenal aortic cross clamping in rabbits. In their work, the use of steroids appears to improve function, but the combination of hypothermia and antioxidant therapy makes specific effects difficult to interpret. Kanellopoulos and colleagues showed that methylprednisolone therapy reduced apoptosis in rats undergoing 20 minutes of thoracic aortic occlusion.⁴¹ This study used the 30 mg/kg dose. Although DNA laddering and nonviable neurons were reduced by this therapy, no improvement in functional outcomes could be shown after 48 hours of reperfusion.

Methylprednisolone is one of the few pharmacologic interventions to be used in the clinical realm of spinal cord protection in aortic surgery. Kouchoukos and colleagues have published good results in thoracoabdominal aortic repair using methylprednisolone (7 mg/kg) in conjunction with hypothermic circulatory arrest and cardiopulmonary bypass.⁴² However, it remains difficult

to credit the protection to either temperature or steroid therapy individually because they are used in combination. Steroid therapy appears to improve outcomes in most animal models, but the human study has not consistently shown significant improvement. With growing controversy about steroid use in traumatic injury, further study on this type of drug must be completed before the use of steroids is incorporated into mainstream vascular surgery.

Nonglucocorticoid steroids have also reduced injury, but these compounds tend to have antioxidant properties in addition to inflammatory inhibition. The 21-aminosteroid tirilazad has improved outcomes following infrarenal aortic cross-clamping in rabbits. In another study from our laboratory, Francel and coworkers reported significant reduction in paraplegic rates as well as histologic injury grading after preoperative treatment with 1 mg/kg tirilazad.⁴³ Fowl and colleagues published similar results using the same model.⁴⁴ The only difference was that this group gave hourly IV boluses after the initial preoperative 1 mg/kg, but mean neurologic outcomes were improved in treated groups compared with ischemic controls. These compounds may provide dual mechanisms of neuroprotection that can be used for the spinal cord protection following ischemia.

Prostaglandins

Prostaglandins can also act as inflammatory inhibitors. They actually have an excellent mechanistic profile for treating ischemia and reperfusion injury. First of all, they can act as vasodilators. Second, they inhibit both platelet aggregation and neutrophil activation. Finally, they stabilize lysosomal membranes. Based on these properties, several prostaglandins have been studied in ischemia and reperfusion injury of the spinal cord. Ohtake and coworkers infused prostaglandin E₁ in selected intercostal arteries during ischemia.⁴⁵ They found improved functional scores in dogs that received 50 ng/kg/min during 60 minutes of thoracic aortic cross-clamping. Somatosensory evoked potentials were also significantly preserved in these treated animals. Grabitz and colleagues found further improvement when they combined prostaglandin E₁ (100 ng/kg/min during ischemia and 1 hour after) with superoxide dismutase (1 mg/kg bolus followed by

25 minutes infusion of 0.4 mg/kg/min at reperfusion).⁴⁶ Katircioglu and coworkers showed that administration of prostacyclin (25 ng/kg/min) during ischemia significantly improved neurologic function following 90 minutes of aortic cross-clamping in dogs.⁴⁷ The same group showed that a prostacyclin analogue, iloprost (25 ng/kg/min), significantly improved neurologic recovery while limiting histologic vacuolization and mitochondrial damage compared with control animals.⁴⁸ Different prostaglandins appear to have protective effects in spinal cord ischemia with aortic cross-clamping, so future studies with humans may prove this an effective tool for spinal cord protection.

Other Inflammatory Inhibitors

Inflammatory inhibition can be accomplished by a number of other substances. Three other drugs that act via this pathway are potential interventions that deserve mention. First, activated protein C (APC) has been popularized by its use in sepsis. Its anti-inflammatory characteristics are hypothesized to be secondary to decreased TNF- α elaboration, diminished nuclear factor κ -B expression, and nuclear translocation.⁴⁹ Hirose and coworkers used 20 minutes of balloon occlusion of the rat descending aorta to study the effects of IV APC on ischemic spinal cord injury.⁵⁰ They found that APC, given at 100 μ g/kg, reduced levels of TNF- α , IL-1b, and myeloperoxidase in spinal cord tissue following thoracic aortic cross-clamping. These indicators of inflammatory inhibition were correlated with significant improvement in functional scores over the first 7 hours and survival over the first 3 weeks compared with control. The investigators concluded their findings to be secondary to serine protease inhibition of neutrophil activation rather than anticoagulation-mediated TNF- α reduction.

Second, erythropoietin is emerging as a prominent cytokine that may provide inflammatory inhibition in ischemic spinal cord injury. The exact mechanism of protection is still unknown but is probably anti-inflammatory in nature. Celik and coworkers found 3 different doses of erythropoietin preserved motor function in rabbits undergoing 20 minutes of infrarenal aortic occlusion.⁵¹ Functional preservation was maximized at the dose of 800 U/kg. Erythropoietin therapy also showed significant reduction in ap-

optosis, as determined by TUNEL staining. The mechanism remains undetermined, but the therapy appears to be very promising. Finally, FK506 has shown promise in preventing ischemic spinal cord injury. FK506 works by inhibiting calcineurin, reducing perioxynitrite, and limiting calcium-dependent apoptosis. Lang-Lazdunski and coworkers studied the effects of FK506 (1 mg/kg IV) pretreatment in a rat model of thoracic aortic cross-clamping.⁵² Treated rats had significantly less paraplegia. Histologic studies showed treated animals had less neutrophil infiltration and more mild histologic changes, as graded by neuropathologic scores. All 3 of these drugs have shown positive results that could be built on for future therapy in spinal cord ischemia

Adenosine and Adenosine A_{2A} Agonists

Adenosine

Since the early 1960s, research at The University of Virginia has pursued the physiologic role of adenosine. Dr. Robert Berne pioneered the study and use of adenosine in cardiology at our institution. More recently, our laboratory was one of the first to use adenosine and its analogues to prevent injury from spinal cord ischemia and reperfusion.

Adenosine is an endogenous purinergic autotoxin that is abundant in normal tissue environments. It is present in the central nervous system, playing a regulatory role in neuronal activity. Specifically, adenosine inhibits baseline neuronal activity, which can lower cellular metabolic demand. This potential protective effect may be a part of injury autoregulation of damaged cells. Adenosine levels in tissue can increase at times of hypoglycemia, hypoxemia, or traumatic tissue injury. Cells can also respond to stress with upregulation of specific adenosine receptors that may play a role in ischemic preconditioning. These characteristics give adenosine a pharmacologic profile that has the potential to promote immunomodulatory and cytoprotective effects on the ischemic spinal cord.

To date, four different G-protein coupled subtypes of adenosine receptors have been identified. Following G-protein signal transduction, receptor activation increases cyclic-AMP, which can immediately alter cellular function through changes in intracellular calcium. Each adenosine receptor

subtype produces specific effects that may be both helpful and harmful for use in ischemia and reperfusion injury.

The A_1 receptor plays a role in the regulation of heart rhythm. This is the site of action for the antiarrhythmic indications for the use of adenosine in cardiology. Unfortunately, this same action is the major limitation of the use of systemic adenosine in other capacities due to a tendency for hemodynamic compromise. Protective effects of this receptor on neuronal tissue lie in its ability to decrease neuronal excitability and limit the influx of calcium into the cell. Thus, the A_1 receptor is significant because it may be able to modulate neuronal metabolism, but it also is difficult to use systemically due to its cardiovascular properties.

The A_{2A} receptor is the most significant in the role of protection from ischemia and reperfusion injury. First, it plays a role in vascular tone. Activation of this receptor leads to vasodilatation, which, as discussed previously, can be both helpful and harmful in ischemic injury. This receptor also appears to be responsible for the anti-inflammatory actions of adenosine. A_{2A} receptors are most prominent on leukocytes, platelets, endothelium, and cells with rapidly cycling functions, such as myocytes or neurons. A_{2A} receptor activation inhibits platelet and neutrophil activation, and thereby decreases reactive oxygen species production in addition to inhibiting T cell and macrophage function. The inflammatory inhibition has been shown in both animal and human cells.^{53,54} Inflammatory inhibition by this receptor can be superior to steroids because it can be immediate, not dependent on posttranscriptional anti-inflammatory events. Activation of this receptor may also limit TNF- α production and its pathway neuronal apoptosis induction.^{55,56} This receptor clearly has the most value in modulation of reperfusion injury.

The final two known receptors do not have as much significance regarding reperfusion injury. The A_{2B} receptor acts on mast cells, where its activation leads to degranulation releasing histamine among other substances. This receptor also regulates some processes in endothelial function. The A_3 receptor appears to affect some types of ischemic preconditioning and may also be responsible for allergic responses to adenosine therapy.⁵⁷

Our laboratory has reported promising results with adenosine and its analogues. The first study

from our laboratory on adenosine or its analogues compared systemic adenosine with regional adenosine and systemic hypothermia alone.⁵⁸ To our surprise at the time, the only significant preservation of hind limb motor function came in the groups that received regional adenosine therapy via the occluded aorta. Retrospectively, this finding makes complete sense. Two characteristics of adenosine necessitate local or regional delivery of the drug for spinal cord ischemia. First of all, systemic adenosine, at doses required to improve ischemic spinal cord function, causes hemodynamic instability. Secondly, the adenosine molecule itself is labile at normothermia, so it must be given in a hypothermic carrier. Therefore, adenosine therapy in spinal cord injury requires regional access to the spinal cord parenchyma. This extra intervention can be a drawback but can provide a way to cool locally the cord if desired. This requirement complicates the interpretation of the results of adenosine therapy because its hypothermic adjunct is also potentially protective in spinal ischemia.

Four follow-up studies from our laboratory have used regional adenosine in a saline carrier at 4°C. Local delivery was accomplished in 1 of 3 different routes. Routes of delivery included perfusion through the excluded segment of the cross-clamped infrarenal aorta in rabbits, retrograde delivery through the inferior vena cava in infrarenal aortic clamping in rabbits, and retrograde delivery via the accessory hemiazygous vein in thoracic aortic cross-clamping of swine.⁵⁹⁻⁶² All these studies showed that regional adenosine provided significant functional amelioration of ischemic injury compared with control. The study by Ross and coworkers also showed that hypothermic adenosine preserved significantly more somatosensory-evoked potentials than control.⁶² Despite the fact that groups receiving hypothermic adenosine showed a trend towards improvement over groups receiving regional hypothermia alone, the improvement did not reach statistical significance.

Adenosine A_{2A} Agonism

The study and design of adenosine analogues by our pharmacology and physiology colleagues at the University of Virginia led to the creation of an adenosine A_{2A} receptor agonist called ATL-146e. ATL-146e had already been tested on other organ

systems before it was available for us to study in spinal ischemia reperfusion. It was shown to lessen the injury due to ischemia/reperfusion in the skin, the lungs, the heart, and the kidneys, as well as causing inflammatory inhibition in septic arthritis.⁶³⁻⁶⁹ Interestingly, the drug is now undergoing clinical trials as a coronary vasodilator at doses 50 to 100 times the dose that would be used in the reperfusion setting.^{70,71} Therefore, the safety profile is defined for much higher doses, and systemic delivery of the lower dose of this substance does not cause the systemic hemodynamic lability of adenosine.

Using the infrarenal aortic cross-clamping for 45 minutes in a rabbit model, we showed in a reproducible fashion that ATL-146e prevented the loss of hind limb motor function after spinal cord ischemia and reperfusion. We reported in our initial study that a delivery of the compound at 0.06 $\mu\text{g}/\text{kg}/\text{min}$ starting 15 minutes before reperfusion and continuing for the first 3 hours of reperfusion optimized neurologic functional outcome.⁷² We found that a concentration of 0.12 $\mu\text{g}/\text{kg}/\text{min}$ was associated with cardiovascular collapse and death of animal subjects. We attributed this observation to the loss of A_{2A} receptor specificity at high doses.

The 5 subsequent studies of ATL-146e in this same model have all supported our initial finding of significant improvement in hind limb function.⁷³⁻⁷⁷ The later studies have elucidated parts of the protective mechanism. We found that the drug significantly preserves the cytoarchitecture of neurons following ischemic insult. Treatment preserved both neuronal viability indexes and neurofilament expression.⁷³ We also found that adenosine A_{2A} agonism decreases neuronal apoptosis, as determined by decreasing heat shock protein 70 and poly(ADP-ribose) polymerase expression.⁷⁴ ATL-146e treated animals showed inflammatory inhibition in this model compared with ischemic controls by lowered systemic TNF- α levels and reduced platelet-endothelial cell adhesion molecule (PECAM)-1 staining in spinal cord endothelium.⁷⁶ This study hints at 2 separate processes. Reducing the TNF- α , if spinal cord levels correlate with systemic, may decrease the TNF- α induced apoptosis. In addition, by decreasing PECAM-1, ATL-146e can attenuate platelet adhesion and leukocyte migration across the endothelium. Limiting PECAM-1 expression may play a major role in reduction of early in-

flammatory processes in this ischemic injury. Finally, A_{2A} agonism prevented the upregulation of A_{2A} receptor expression that was present in control animals.⁷⁵ This effect supports the idea of the direct effect of the drug on neurons and receptor expression, as well as the role of this receptor in ischemic preconditioning. Interestingly, this also implies that adenosine therapy during the ischemic period may actually exacerbate injury and natural protection by preconditioning could be restricted.

Our study of adenosine and its analogues for spinal cord protection supports several conclusions about ischemic neurologic injury. First, because ATL-146e is observed to improve neurologic outcome if given during reperfusion, it appears that reperfusion may be as important to the overall injury as the ischemic interval itself. Second, systemic administration of ATL-146e is effective without the requirement of regional spinal cord perfusion. This result implies that localized inflammatory insult of spinal cord injury can be safely attenuated by systemic manipulation. Lastly, ATL-146e has multiple cellular and humoral immunomodulatory effects, not only decreasing neutrophil and platelet adherence at the time of injury but possibly decreasing initiation of TNF- α induced neuronal apoptosis. The neuroprotective activity of purinergic drugs, such as ATL-146e, may allow new insight into therapeutic intervention for debilitating spinal cord injury in which reperfusion injury is the prevalent mechanism.

Ischemic Preconditioning

The concept of ischemic preconditioning is relevant to ischemia/reperfusion injury of any tissue. Positive results from studies of ischemic preconditioning have led to the study of pharmacologic pathways to accomplish the same protective results. Diazoxide, among other drugs, has been used in both cerebral and heart ischemia.⁷⁸⁻⁸² The mechanism of protection afforded by ischemic preconditioning is not completely clear, but 2 separate mechanisms are described in the literature. First, preconditioning appears to dilate the microvasculature and, therefore, improve blood flow before and after the ischemic interval. Second, preconditioning protects ATP-dependent electrolyte channels from dysfunction following ischemic insult. Through one or both of these

mechanisms, the following studies show that preconditioning can ameliorate ischemic damage following aortic occlusion.

Preconditioning has been studied for spinal cord protection in the experimental literature. Ueno and colleagues used two 3-minute ischemic periods followed by 3 minutes of reperfusion before the ischemic time of 15 minutes in rats.⁸³ They reported a significant increase in spinal cord blood flow during the first 3 hours of reperfusion in preconditioned animals. Although they were unable to show any overall difference in neurologic function, spastic paraplegia was less likely to develop in preconditioned animals than in controls. Other groups had similar difficulty showing significant neurologic improvement with varied preconditioning techniques.⁸⁴ Abraham and colleagues were able to show differences in functional outcome in preconditioned rats.⁸⁵ Using either 2 or 5 minutes of preconditioning 48 hours before 10 minutes of occlusion decreased the incidence of neurologic complications compared with controls. Furthermore, Zvara and coworkers studied preconditioning in a rat model with 3 minutes of preconditioning, followed 30 minutes later with 12 minutes of ischemia.⁸⁶ They found that preconditioned animals had improved survival and preservation of both sensory and motor function relative to ischemia-alone animals.

Given the background of preconditioning to reduce ischemic spinal cord injury, the use of diazoxide to mimic this intervention is not surprising. Caparrelli and coworkers used this pharmacologic preconditioning to improve early and late neurologic complications in rabbits receiving 20 minutes of infrarenal spinal cord occlusion.⁸⁷ They implicated the ATP-dependent potassium channel agonism as the mechanism of protection by using an antagonist to the particular channel to block its effects. Ischemic and pharmacologic preconditioning can exert protective effects in animal models of spinal cord ischemia reperfusion but these effects have not yet been proven in the clinical world.

Conclusion

Current and past research in pharmacologic neuroprotection from spinal cord reperfusion injury has yielded important insight into the pathophysiology of paralysis after thoracic aortic recon-

struction, as well as some promising adjuncts to current clinical techniques. The ultimate solution to the problem of spinal cord injury following vascular surgery probably lies in some mixture of several different types of protective mechanisms. This is not limited to pharmacologic intervention but should include other possibilities, such as CSF drainage and distal bypass. Attacking the problem from all directions may optimize the outcomes in patients undergoing these procedures. Once there is a better determination of effective pharmacologic intervention, aortic surgeons can use drugs as adjuncts as part of a multimodality approach to spinal cord protection.

At this point, the pharmacologic protection from neurologic injury during thoracoabdominal aortic surgery remains in its infancy. Few surgeons are using drugs for prevention of spinal cord injury. However, some surgeons do use the principals of traumatic spinal cord injury to prevent this type of injury in aortic surgery. Still, the literature only serves to present a myriad of possible therapies without showing any unquestionable therapy that needs to be adopted. Current studies do show promise, but further study is necessary before pharmacologic protection from spinal cord injury during thoracoabdominal aortic surgery becomes the standard of care.

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