

# Ischemia and Reperfusion Injury: When Cells Almost Die

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## Introduction

Ischemia is defined as inadequate blood supply to a part of the body, usually caused by partial or total blockage of an artery. Reperfusion injury occurs when tissue perfusion and oxygenation are restored to an affected area after an ischemic event.

Ischemia/reperfusion (I/R) injury is a complex cascade of events resulting in devastating effects to the body, sometimes including death. Despite more than 70 years of research, I/R injury is not fully understood. Events such as gastric dilatation-volvulus (GDV), mesenteric torsion, or strangulation of a limb can lead to I/R injury. It is important for all veterinary personnel to understand I/R injury so treatment and prevention can begin as early as possible.

## **The Ischemic Cascade**

The chain of events involved in I/R injury can be broken down into the ischemic cascade (**BOX 1**) and reperfusion injury (**BOX 2**). An ischemic episode involves a series of events called *the ischemic cascade*. Within 5 minutes of the development of ischemia, the electrolyte balance within cells becomes disturbed.<sup>1</sup> The ischemic cascade usually continues for 2 or 3 hours but can last for days, even after perfusion is restored to the affected area.<sup>2</sup> The term *cascade* suggests that events follow a sequential pattern, which is not true of the ischemic cascade. Events can occur simultaneously and do not always occur in a linear pattern.<sup>1</sup>

To fully understand the ischemic cascade, it is important to consider adenosine triphosphate (ATP) and how it functions. ATP is a multifunctional nucleotide (a structural factor of DNA and RNA) that is considered to be the most important nucleotide responsible for transporting energy for metabolism within cells.<sup>3</sup> One of the fastest ways that ATP is produced is by oxidative phosphorylation, implying that ATP production requires oxygen.<sup>3</sup> Despite the importance of ATP, cells do not stockpile ATP.<sup>3</sup> They only

make what they need for a particular time. When ischemia occurs, oxygenation of cells ceases, resulting in anaerobic ATP production, which is less efficient.<sup>3</sup>

When oxygen becomes unavailable to cells, anaerobic glycolysis starts. This can be a lifesaving way for cells to obtain energy; however, this process is extremely wasteful. During the anaerobic process, pyruvic acid and hydrogen atoms combine with nicotinamide adenine dinucleotide (NAD) to form NADH and H<sup>+</sup>.<sup>3</sup> If the buildup of NADH and H<sup>+</sup> becomes too great, the anaerobic process stops, thus terminating energy production to maintain cells.<sup>3</sup> However, NADH and H<sup>+</sup> combine to form lactic acid, which diffuses from cells rapidly so that the process can continue.<sup>3</sup> Although this is not ideal, the body can safely continue this process for several minutes. If the process continues for too long, as in ischemia, lactic acid can build up, indicating worsening illness. As a consequence of lactic acidosis, pH decreases, injuring and inactivating mitochondria. Some researchers think that lactic acid may also interfere with the recovery of aerobic ATP production after ischemia.<sup>3</sup> For all ischemic patients, a lactate level should be obtained. Values <2 mmol/L are normal.

When ATP fails to form, cells become depolarized, allowing calcium and sodium (normal extracellular electrolytes) to enter cells.<sup>4</sup> Potassium, which is normally found in cells, leaks rapidly into the extracellular space.<sup>4</sup> Excessive calcium overexcites cells, creating free radicals and many calcium-dependent enzymes. The extent of ischemic damage is related to the amount of calcium that enters cells and the duration of time that the intracellular calcium level remains elevated.<sup>5</sup> The longer calcium stays in cells, the greater the amount of harmful chemicals will be created.

One of the most important events involving calcium is the conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XO).<sup>6</sup> XO requires oxygen for activation. During ischemia, oxygen is not present, so XO accumulates without getting used. Later, during reperfusion, XO can damage cells.

As the mitochondria break down, they release toxins, causing apoptosis. Apoptosis is the body's way of safely disposing of dead cell parts by autolysis (self-destruction) of cells. Another important event during ischemia is the activation of nuclear factor- $\kappa$ B (NF $\kappa$ B), leading to the production of inflammatory mediators.<sup>1</sup> NF $\kappa$ B becomes activated during stress.<sup>3</sup> NF $\kappa$ B activates inflammatory cytokines and their receptors as well as platelet-activating factor.<sup>1</sup> This allows neutrophils to enter through the vascular endothelium. Activated neutrophils are generally more rigid and stiff because of hypoxia and acidosis, which accompany ischemia. Because of the alteration

of the cell membrane and the high number of neutrophils, capillaries may become plugged or clogged by neutrophils.<sup>7</sup> Even after reperfusion, the redistribution of blood to affected areas may not produce enough force to clear clogs.<sup>7</sup>The full pathway of NFkB is still not understood.<sup>1</sup>

### **Reperfusion Injury**

It would seem that simply reintroducing oxygen into the affected area would be beneficial. In patients with GDV, oxygen is restored when the stomach is decompressed or untwisted, allowing oxygen and blood to flow back into the stomach wall. However, the reintroduction of oxygen into affected areas initiates a complex chain of events. Despite the harsh effects of ischemia alone, they do not cause nearly as much damage as reperfusion does.<sup>1</sup> The longer the onset of the ischemic event, the greater the insult from reperfusion injury.<sup>1</sup>

One of the first events in reperfusion is that oxygen finally binds with XO that has built up during ischemia. XO combines with oxygen and hypoxanthine to form superoxide ( $O_2^-$ ), a radical.<sup>4</sup> Superoxide is not that damaging but can inactivate iron-sulfur-containing enzymes, liberating free iron and generating highly reactive hydroxyl ( $\cdot OH$ ) radicals. Hydroxyl is considered to be a reactive oxygen species (ROS).

Free radicals are radicals that move from where they were created. They are highly reactive and are usually involved in chemical reactions. An ROS is an oxygen-containing molecule that is very chemically reactive even though it may not be classified as a free radical. ROS molecules react quickly with other molecules. If present in high levels, they can damage cellular macromolecules such as DNA and RNA or cause endothelial injury, microvascular dysfunction, and apoptosis (cell death).<sup>8</sup> ROS can form within 10 to 30 seconds after the onset of reperfusion.<sup>4</sup>

During ischemia, neutrophils leak into the endothelium because of the activation of NFkB and XO. Reperfusion accelerates the influx of neutrophils to the affected area.<sup>9</sup> They respond because of the inflammatory response that takes place. Neutrophil activation alone can lead to even more ROS formation.<sup>4</sup> The inflammatory cascade accelerates during reperfusion. In short, neutrophils and macrophages attack reperfused tissues. Inflammatory cytokines are released as neutrophils are activated.<sup>1</sup> They are released by activated monocytes, macrophages, and neutrophils. When the body becomes overwhelmed with inflammatory cells, cytokines can be overproduced,

resulting in massive cytokine influx (hypercytokinemia) into the affected tissue.<sup>1</sup> The exact mechanism behind this phenomenon is not fully understood.<sup>1</sup>

### **Complications**

The ischemic insult sets up the body for a damaging chain of events that is initiated by reperfusion. Although some researchers debate the exact relationship between I/R injury and these events, the end result can be the death of the patient. The most common complications include disseminated intravascular coagulation, systemic inflammatory response syndrome, multiple organ dysfunction syndrome and, if the I/R injury occurred to the muscles, rhabdomyolysis

### **Treatment**

There is no known definitive cure for I/R injury. Many doctors and scientists agree that stopping the cascade at the earliest possible point would produce the best results.

### ***Reactive Oxygen Species Formation***

In the past several years, human and veterinary researchers have conducted several promising studies using *N*-acetylcysteine, which is a powerful scavenger of the hydroxyl radical.<sup>4</sup> While most studies involving rats, rabbits, and mice have produced promising results with this drug, the most recent (2008) veterinary-related study showed that the use of *N*-acetylcysteine failed to decrease I/R injury of the liver in a canine model.<sup>10</sup> One of the most recent studies concluded that *N*-acetylcysteine protects lung tissue from the effects of I/R.<sup>11</sup> It is clear that more research is needed.

Deferoxamine has been studied for more than 20 years in humans and is now being studied in animals. The research in humans and animals has been promising; in a study in 2009, deferoxamine significantly protected rats with ischemic stroke.<sup>12,13</sup> This is because deferoxamine is an iron chelator and, therefore, inhibits hydroxyl radical formation.<sup>14</sup> A recent study showed that the use of *N*-acetylcysteine and deferoxamine, but not their isolated use, prevented an increase in creatinine following an I/R injury event in the kidneys.<sup>15</sup>

Allopurinol can inhibit XO formation and neutrophil infiltration during reperfusion.<sup>16</sup> The best results have been obtained when allopurinol has been used as a pretreatment against I/R injury. Because predicting when I/R injury will occur is almost

impossible, it would be difficult to obtain optimal results on the use of allopurinol in a clinical setting.<sup>16</sup>

### ***Other Options***

Ketamine may also prove to be a cost-effective and safer treatment for I/R injury. Ketamine inhibits *N*-methyl-D-aspartate receptors, reduces neutrophil adhesion, and decreases cytokine production.<sup>17, 18</sup> A study in 2009 concluded that the use of ketamine as an anesthetic reduced intestinal I/R injury in rats.<sup>19</sup> As with lidocaine, ketamine has analgesic properties and is frequently used in veterinary medicine in postsurgical constant-rate infusion analgesic combinations such as morphine–lidocaine–ketamine. Perhaps ketamine could be used similar to lidocaine in postsurgical patients at risk for I/R injury.

The use of colloids (hydroxyethyl starches) may have some benefit in reducing the effects of I/R injury.<sup>14</sup> This is likely because high-molecular-weight colloids can help to decrease microvascular permeability. A 2008 study concluded that hydroxyethyl starches were superior to other colloids when used as a pretreatment for the protection against I/R injury effects.<sup>20</sup>

Therapeutic hypothermia has been shown to help minimize harmful effects of the inflammatory cascade and decrease ROS production during reperfusion.<sup>21</sup> One of the most common I/R injuries in humans follows cardiac arrest, which cuts off oxygen to the heart. In 2007, Dr. Lance Becker at the University of Pennsylvania, showed that cooling the body after cardiac arrest increases the chance of survival by 16%.<sup>21</sup> This prompted the American Heart Association to recommend cooling of every cardiac arrest patient.<sup>22</sup> Since 2007, an injectable ice–salt mixture has allowed emergency personnel to quickly cool humans to help slow or even prevent I/R injury. In the United States paramedics carry cool sodium chloride fluids in their ambulances so when a person has a heart attack they can drop their body temperature right on the scene to decrease I/R injury later.<sup>22</sup>

Therapeutic hypothermia has started to be used in veterinary medicine during surgical procedures that induce a I/R injury effect. The most common use being tried in veterinary teaching hospitals is with cardiac surgery. A 2015 published case report found that therapeutic hypothermia helped to protect the brain during cardiopulmonary bypass surgery. The report went on to state that “for most cardiac surgical procedures, mild to modest (32-36 °C) hypothermia will be sufficient to assure neuroprotection.”<sup>23</sup>

While still in its infancy in veterinary medicine, therapeutic hypothermia is used widely in human surgical cases as well as in the emergency room.

### **Conclusion**

The pathology of I/R injury is extensive and not fully understood, even in humans. More research is needed to help develop tests and treatments for I/R injury. To improve medical and veterinary knowledge, it is important to identify I/R injury and record treatments used.

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## **BOX 1**

### **The Ischemic Cascade**

- Lack of oxygen causes failure of the normal aerobic process for making ATP.
- Cells switch to anaerobic respiration, creating lactic acid.
- Potassium leaks from cells, while sodium and calcium enter cells.
- Calcium converts XDH to XO.
- XO builds up.
- NFκB is activated, activating inflammatory mediators and platelet-activating factor.



- Damaging free radicals are formed.
- Mitochondria break down and start apoptosis.
- Neutrophils start to overwhelm the affected areas.
- Cells die.

## **BOX 2**

### **Reperfusion Injury**

- Oxygen is reintroduced into the affected area.
- XO forms superoxide radicals.
- Superoxide liberates free iron, causing hydroxyl ROS.
- ROS damage macromolecules (i.e., DNA, RNA) and cause endothelial injury, microvascular dysfunction, and apoptosis.
- Neutrophils overwhelm the affected areas.
- The inflammatory process accelerates.
- Excessive cytokines are produced.