Apoptosis: Understanding Programmed Cell Death for the CRNA

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Apoptosis, or programmed cell death, is a physiologic mechanism employed by most multicellular organisms to maintain homeostasis of body tissues. In balance with the production of new cells by mitosis, apoptosis provides for the orderly destruction and removal of cells that are no longer needed by the organism. Apoptosis occurs by complex pathways involving multiple biochemical signals and processes. Dysfunctional apoptotic mechanisms are the pathologic basis for many human diseases, including common disorders of the heart, lungs, brain, and endocrine systems.

Researchers have demonstrated in animal models that neurodegenerative changes after the administration of anesthetic drugs are related to apoptosis. Anesthesia drugs have been found to induce apoptosis, perhaps through the production of reactive oxygen species. Propofol is a drug used in anesthesia that has unique antioxidant qualities that may be beneficial.

The purpose of this article is to review, for nurse anesthesia providers, current information about the process of apoptosis, the role of apoptosis in comorbid diseases, and the implications of the effects of anesthesia drugs on normal apoptotic mechanisms that need to be evaluated as potential sources of risk or benefit to surgical patients.

Keywords: Apoptosis, caspase, programmed cell death.
ly published journals specifically focused on apoptosis. In 2002, Brenner, Horvitz, and Sulston were able to elucidate the genetic influences of PCD, for which they were awarded the Nobel Prize in Physiology or Medicine. The importance of apoptosis in healthcare is evident, with more than 132,000 PubMed (National Library of Medicine) citations for apoptosis in the last decade.

Pathways to Cell Death: Apoptosis and Necrosis

Apoptosis is a form of programmed cell suicide which allows for the orderly removal of unwanted, improperly functioning, or injured cells, in balance with the production of new cells by mitosis. The apoptotic cycle is an energy-dependent process in which a genetically controlled sequence of events regulated by complex and numerous biochemical signals result in the condensation and fragmentation of an individual cell into small vesicles that contain the cellular components (organelles and nuclear fragments). These vesicles are phagocytized by macrophages and neighboring cells, and recycled. One characteristic of apoptosis is that neighboring cells are not damaged during this process.

Apoptosis is differentiated from necrosis, also referred to as passive cell death because necrosis is a nonenergy dependent process. Necrosis is pathologic or accidental cell death that occurs as a result of insult from harmful events such as hypoxia, toxicity, or infection. With necrosis, cell death transpires marked by the presence of inflammation, resulting in damage to adjacent cells; however, in the normal process of apoptosis, cells are usually affected individually. Cell shrinkage in apoptosis is unlike what occurs in necrosis when enzymatic digestion results in deterioration of the cell membrane and leakage of noxious cellular contents into surrounding tissues, resulting in inflammation and widespread damage. In contrast, apoptosis generally does not induce inflammation or scarring. Apoptosis also requires activation of cell signaling, which is usually not a part of the necrotic process. Intracellular adenosine triphosphate (ATP) concentrations may be important in determining which path to cell death is taken. High ATP concentrations favor apoptosis, whereas low ATP shifts the cell toward necrosis. The nature or severity of an insult that precipitates cell death may result in the depletion of ATP or reduced ATP synthesis, thus favoring necrosis.

Phases of Apoptosis

Apoptotic mechanism may be conceptualized as a cascade of biochemical events comprising 4 explicit phases: (1) signaling, (2) control and regulation, (3) execution, and (4) removal of the dead cell. During the signaling phase, a variety of stimuli are capable of triggering apoptotic pathways. These stimuli may include glucocorticoids, toxins, nitric oxide, nutrient deprivation, viral infection, hypoxia, ligand (molecule) receptor activation, ionizing irradiation, or attack by cytotoxic lymphocytes. Activation of ligand-gated receptors on the cell surface (also referred to as “death receptors”) results in changes to these transmembrane channels, triggering the sequences that lead to cell death. Two commonly identified cell surface death receptors are Fas and tumor necrosis factor (TNF). The activation of Fas receptors results in the formation of Fas-associated death domain (FADD), and activation of TNF receptors yields TNF receptor-associated death domain (TRADD). The death domain molecules stimulate caspase activation, which leads to apoptosis.

During the second phase of apoptosis, control and regulation, the cell will either commit to apoptosis or mechanisms are activated that interrupt the cascade of events initiated during the first phase and apoptosis is aborted. Two genes are important regulators at this stage of apoptosis, Bcl-2 and p53. The Bcl-2 gene family regulates apoptosis and exhibits both proapoptotic and antiapoptotic influences. Bcl-2, referred to as a “cellular life or death switch,” will determine which cellular insults will ultimately result in apoptotic progression. Elevated levels of the Bcl-2 gene are known to inhibit apoptosis and are associated with the development of a number of human cancers, such as lymphoma, leukemia, adenocarcinoma, renal and lung cancers, neuroblastoma, and melanoma. The p53 gene is a nuclear phosphoprotein, which suppresses cell proliferation. Deficiencies of the tumor suppressor p53 gene are commonly found in half of human cancers and are also associated with resistance to treatment. When apoptosis is allowed to proceed, the signaling phase activates a series of the caspase family of proteases. Caspases, a proteolytic central component of apoptosis, exist in an inactive form called procaspases. Procaspases must be activated by specific apoptotic signaling pathways in order for apoptosis to continue. Thus activated, caspases are destructive enzymes capable of protein cleavage, leading to the morphologic changes in the apoptotic cell. At least 14 caspases have been identified in mammals, and a number of these are present in the human apoptotic cascade. Caspase activation also facilitates apoptosis by increasing permeability of the mitochondrial membrane, which results in the release of mitochondrial proteins into the cytosol. These proteins bind in-
hibitors of apoptosis proteins (IAPs), allowing cell death to proceed.

Once fully committed to apoptosis, the cell enters into the execution phase. The cell now will undergo an organized degradation of its matrix and organelles by the proteolytic caspases. Caspases will activate cytoplasmic DNase (CAD) that enters the nucleus and affects the breakdown of DNA. Morphologic changes associated with the execution phase include the condensation of chromatin (DNA and histones in the nucleus) and the blebbing and formation of vesicles containing the compacted cellular structures. 12, 16

Following the execution phase, the cell enters the removal phase of apoptosis. The apoptotic bodies present engulfment ligands (so-called “eat me” signals) on their surfaces, which are recognized by scavenger receptor sites in phagocytic cells. 17 Engulfment ligands may consist of lipid, sugar, or protein markers. One phospholipid, phosphatidylserine (PS), is normally sequestered on an inner leaflet of the plasma membrane; however, during apoptosis this lipid is translocated to the outer leaflet, where it becomes accessible to receptors on macrophages. 18 With the macrophage receptors activated, the consumption of the apoptotic bodies is completed.

Figure 1. Morphologic Changes Associated With Apoptosis and Necrosis
Pathways of Apoptosis

Two primary caspase-mediated pathways have been identified (Figure 2): (1) the extrinsic, or death-receptor-mediated, pathway and (2) the intrinsic, or mitochondria-dependent, pathway. The extrinsic pathway is activated when cell surface proteins (ie, Fas or TNF) encounter the ligand activators to form the death domain molecules. Death domain molecules, FADD and TRADD, in turn bind inactive forms of caspase 8 and caspase 10 to generate active forms of these enzymes. Caspase 8 and caspase 10 activate other downstream caspases, which in turn allow the apoptotic disposal of the cell. The extrinsic pathway plays an important role in tissue homeostasis, especially in the maintenance of lymphocytes. Patients with caspase deficiencies or Fas mutations are at risk of autoimmune or immunodeficiency disorders related to defective apoptosis of lymphocytes.

In contrast, the intrinsic apoptotic pathway is regulated at the mitochondrial level and is sensitive to both extracellular stimuli and internal insults such as DNA damage. This pathway is initiated via alterations in mitochondrial membrane potential and increased permeability of the membrane, resulting in the leakage of cytochrome c, a protein important to mitochondrial respiration. In the

![Figure 2. Simplified Diagrams of Extrinsic and Intrinsic Apoptotic Pathways](chart)

TNF indicates tumor necrosis factor; FADD, Fas-associated death domain; TRADD, TNF receptor-associated death domain; CytC, cytochrome C; Apaf-1, apoptosis activating factor 1.
cytosol, cytochrome c binds with apoptosis activating factor (ApaF-1) to form a complex known as an apoptosis, which activates caspase 9. At this point, if the balance between proapoptotic and inhibitory control is tipped in favor of cell death, the caspase cascade is initiated and the cell is destroyed.

Although the extrinsic and intrinsic pathways of caspase activation are the cascades most often described, other pathways may also contribute to PCD. A pathway has been described that involves the exposure of target cell cytoplasm to a serine protease, granzyme B. Granzyme B initiates the caspase cascade through activation of caspase 3. Granzyme B may also initiate the proapoptotic gene Bcl-2, leading to cell destruction. The granzyme B pathway is used by T cells and natural killer cells for the elimination of virus-infected cells from the body. Other pathways have also been suggested, including a caspase-independent extrinsic pathway and intrinsic pathways dependent on other stimuli that are not yet understood.19,20

Apoptosis and Human Disease

Historically, tissue and cellular injury related to certain diseases was associated only with necrosis; however, recent studies have demonstrated that altered cellular regulation and damage by apoptosis are also important in understanding disease processes. Many of our patients have acute or chronic diseases (or both) that are the source of their need for surgical intervention, or they have comorbid conditions that complicate the delivery of anesthesia. Many nurse anesthetists may either be unaware of the role of altered apoptosis in disease or have limited understanding of this important physiological process. As most diseases are now associated with altered apoptosis, it would be a rational choice for the skilled anesthesia provider to have a foundational knowledge of these pathways.

Diseases involving aberrant apoptotic mechanisms may be categorized as related to (1) excessive apoptosis or (2) insufficient apoptosis (Table).3 Numerous common diseases are related to excessive apoptotic activity. Surgical patients presenting with cardiac or pulmonary impairment present specific challenges to the anestheticsian clinician, and an appreciation of the role of upregulated apoptosis in disorders of the heart and lungs is useful. Ischemia of coronary heart disease triggers apoptosis of cardiomyocytes and is a contributing factor in the evolution of myocardial infarction.13,21 Ischemia followed by reperfusion (as in the instrumentation and reopening of an infarct-related coronary artery) is a potent stimulus of cardiac cell death, which persists even after perfusion is reestablished. Other critical factors in cardiac disease appear to be the production of free radicals and depletion of growth factor and energy sources. Apoptotic cardiac insults may precipitate heart failure via the gradual loss of myocytes. The progressive thinning of ventricular tissues and loss of myocytes in heart failure, resulting in ventricular remodeling, is known to have origins in perturbations of apoptosis, although levels of apoptosis in heart failure are low in contrast to ischemia-reperfusion events.22

A focus on the suppression of cardiomyocyte apoptosis has led to interest in the development of cardioprotective drugs. Therapeutic drug interventions that are currently being used or investigated for this purpose include caspase inhibitors, antioxidants, and angiotensin receptor antagonists.13 Nurse anesthetists are familiar with the frequent use of β-adrenergic receptor antagonists in the operating room to control the impact of surgical and anesthesia stress in patients with known or suspected ischemic heart disease. β-Blockers such as carvedilol dramatically prevent myocardial ischemia and reperfusion-related apoptosis by inhibiting caspases and stress-activated protein kinases and by antioxidant mechanisms.23

Respiratory disorders are likewise correlated with increased levels of apoptosis. Respiratory diseases in which excessive apoptosis plays a central role include respiratory infections, bronchial asthma, smoking-induced chronic obstructive pulmonary disease (COPD), acute lung injury, hypoxic lung injury, cystic fibrosis, and interstitial pulmonary fibrosis.24,25 Sphingolipids, key regulators of processes in biological membranes, are known to affect apoptosis in lung tissue. Certain sphingolipids are known to be proapoptotic. The pharmacologic manipulation of

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**Table. Apoptosis and Common Diseases**

COPD indicates chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome.

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<th>Excess cell death (enhanced apoptosis)</th>
<th>Inappropriate cell survival (diminished apoptosis)</th>
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these cell membrane lipids is generating new research that may eventually yield more effective treatments for lung disease than the typical bronchodilator, antibiotic, and anti-inflammatory therapies currently available.26

Excessive apoptosis has also been demonstrated in neurodegenerative problems, including Alzheimer disease, Parkinson disease, Huntington disease, and amyotrophic lateral sclerosis (ALS).27 The progression of human immunodeficiency virus (HIV) to AIDS, marked by the depletion of CD4+ T-helper lymphocytes, leads to immune system compromise. One of the important mechanisms by which the T-helper cells are depleted in HIV is by apoptosis.28 Data also suggest that accelerated rates of apoptosis resulting in up to a tenfold increase in the destruction of β-cells in the pancreas may contribute to the development of type 2 diabetes mellitus. Apoptosis has been identified as the primary mechanism for accelerated pancreatic β-cell death in type 1 diabetes; however, the apoptotic pathways in type 1 diabetes differ from those in type 2. Although β-cell failure in type 1 diabetes may be related to autoimmune-mediated apoptosis, β-cell death in type 2 diabetes is likely related to increased apoptosis stimulated by chronic hyperlipidemia and hyperglycemia.29 Interventions directed at reducing rates of β-cell apoptosis may offer future strategies for treatment of patients with type 2 diabetes. Alterations in apoptosis have also been reported in other physiological abnormalities, such as hemorrhage, stroke, sepsis, osteoarthritis, allograft rejection, and inflammation.3,7,14,30 Understanding the impact of the apoptotic pathways in disease is currently an important stimulus in the search for innovative pharmacologic treatments of these maladies.

The mechanism of disease in the presence of insufficient apoptosis is inappropriate cell survival and proliferation. Diseases of insufficient apoptosis include different types of cancer, autoimmune disorders, and viral infections in which apoptosis is inhibited in order to preserve the host cell for viral replication.31 In some cancers, the apoptosis initiator p53 gene is expressed abnormally, favoring tumor growth and resistance to treatment. Papillomavirus and adenovirus are suspected of being able to encode an inhibitory signal into the p53 gene, thus deferring apoptosis. Similarly, the Epstein-Barr virus has been shown to interfere with Bcl-2 gene activity, resulting in cancers such as Hodgkin lymphoma and posttransplant lymphoma.32 Cancer researchers have long been aware that many chemotherapeutic drugs exert their effects by promoting apoptosis. Genetic defects, however, in intrinsic cellular self-destruction programs often lead to chemoresistance. The apoptosis-resistant characteristics of some cancers have led to interest in the development of drugs that inactivate antiapoptotic mechanisms, thus making cancer cells more susceptible to conventional treatments. Attractive targets for drug intervention are the genes that express signals that inhibit apoptosis.3,33

Anesthesia Drugs and Apoptosis

We may assume that our standard monitoring of patients gives us the means to assess all possible effects of the anesthetic drugs being administered. We may also assume that the effects of anesthetic drugs are completely reversed once they have been removed from the patient or metabolized. We need to reevaluate these assumptions, however. In the practice of nurse anesthesia, CRNAs administer drugs that affect multiple intercellular and intracellular mechanisms, to include receptor activation and the regulation of genes. Recent laboratory and clinical studies have demonstrated that most anesthetic agents are capable of activating the mechanisms that lead to premature cell death.3,6 Other researchers have reported potentially beneficial effects of certain anesthetics on apoptotic pathways and related physiological processes.34-37 A clear understanding of apoptotic pathways will enable the anesthetist to sort through seemingly conflicting research in order to be aware of potential implications stemming from the effects of anesthesia drugs on this important process.

One area of particular concern is in regard to drug-related disruption of the normal apoptotic pathways. For example, neurotoxic effects have been demonstrated in rodent models as a result of anesthesia drugs administered during the developmental period referred to as synaptogenesis.6,38,39 Synaptogenesis may be conceived of as a “growth spurt” of the brain synapses; in humans it takes place from the sixth month of gestation until several years of age.40 Although synapse formation may occur throughout the lifespan of a healthy individual, synaptogenesis in early childhood is characterized by an explosion of activity important to normal neurologic development. Anesthetic drugs that stimulate apoptosis in neural tissues and inhibit synaptogenesis can impair synaptic function, possibly altering neurologic development.39

Research, then, has established that drugs used in anesthesia result in widespread apoptotic neurodegeneration in the developing brains of animal models. Although there are undoubtedly multiple mechanisms by which intravenous and inhalation anesthetic drugs affect different cellular receptors, the 2 principal receptors involved are the N-methyl-D-aspartate (NMDA) receptor and the γ-aminobutyric acid (GABA) receptor. Ketamine and nitrous oxide are 2 known NMDA antagonists. All inhalation agents, as well as the intravenous barbiturates, propofol, etomidate, and benzodiazepines potentiate GABA receptors.41 Studies have revealed that the neuroapoptotic effects of anesthetic drugs are both time- and dose-dependent, such that greater levels of damage occur at higher doses and over increasing lengths of exposure. Peak vulnerability to the apoptotic effects of both NMDA antagonists and GABA mimetics is during the sensitive period of brain growth when the surface area of the brain must be expanded to accommodate new synaptic connections. This potential for long-term neurologic injury
as a result of anesthesia administration to human infants during this critical developmental period is cause for concern.

Older adults may also be at risk of neuroapoptotic effects of anesthetics. The proapoptotic effect of isoflurane has been reported to induce the generation and aggregation of amyloid-β protein in the brain. Alzheimer disease, the most common form of age-related dementia, is a rapidly growing health problem. Amyloid-β-protein production and accumulation are major pathologic hallmarks of Alzheimer disease. Given this relationship, isoflurane-induced apoptosis and a subsequent transient increase in the production of amyloid-β protein in the geriatric patient is yet another concern for the anesthetist. Although the preponderance of research has addressed the detrimental effects of anesthesia on neuroapoptosis, the apoptotic pathways in other cell types are also at risk.

In an effort to understand the proapoptotic effects of anesthetics on cells, researchers are investigating the role of anesthesia drugs in the formation of reactive oxygen species (ROS). Important regulatory molecules, ROS are produced under normal metabolic conditions by aerobic organisms. Although ROS have physiologic importance in their use by macrophages to disable harmful bacteria by phagocytosis, they cause cellular and organ damage when produced endogenously in excess, accumulated from exogenous sources (eg, smoking, pollution), or in the presence of insufficient antioxidant defenses. When the body’s antioxidant resources are overwhelmed by ROS, a condition of oxidative stress is said to exist. Injury from oxidative stress results in the lipid peroxidation of cell membranes, often causing cellular injury or death, related to a wide range of acute and chronic conditions. Inhalation agents have been demonstrated to increase ROS in human tissues, predisposing the patient to a condition of oxidative stress and increased apoptosis. One detrimental effect resulting from this is the profound but transient reduction in blood lymphocytes in the immediate postoperative period, resulting in a period of immunosuppression, thus rendering the patient susceptible to localized infection and even sepsis.

Local anesthetics have also been studied for their effects on apoptosis. In vitro studies have demonstrated the ability of lidocaine, in clinically relevant concentrations, to induce apoptosis via the mitochondrial pathway. Higher concentrations of lidocaine were responsible for cell death by necrosis. This is offered as a possible explanation for lidocaine neurotoxicity. Zink et al reported myotoxic damage related to continuous peripheral nerve blocks with bupivacaine in a pig model. Both apoptotic and necrotic cell death were indentified in muscle cells examined after exposure to bupivacaine. Human studies that further define the role of local anesthetics in altered apoptosis are being conducted.

Fortunately, not all of the news is bad. Anesthetic drugs, in some circumstances, may provide protection against the detrimental proapoptotic effects of ROS. Animal studies have demonstrated potential benefits of volatile anesthetics in protecting the myocardium against ischemia-reperfusion injury, representing a possible therapeutic benefit of this phenomenon. This activity appears to be mediated via a reduction of ROS in heart tissue by inhalation anesthesia during periods of induced ischemia. These agents also appear to protect myocardial cells from the apoptosis death-signaling effects of norepinephrine. Researchers, using in vitro methods, have also demonstrated the ability of isoflurane to reduce neuronal cell death in cortical cells that were subjected to hypoxic conditions, raising interest in the potential benefits of volatile agents for patients with hypoxic brain injury.

The intravenous anesthetic propofol is receiving considerable attention in studies related to ROS and apoptosis. Propofol, with structural similarities to phenol-based free radical scavengers such as butylated hydroxytoluene (BHT) and α-tocopherol (vitamin E), has well-documented antioxidant properties and has been evaluated as a tool for reducing injury from oxidative stress in both animal and human tissues. For example, propofol has been shown to protect lung, heart, hepatic, and vascular endothelial tissues from the negative effects of oxidative stress. Blood components such as erythrocytes also obtained antioxidant benefits in studies with propofol. Hence, propofol does have important cellular and nuclear protective effect for cardiac and lung cells as well as other tissues.

Pharmacologic agents used daily in the administration of anesthesia have both favorable and unfavorable properties with regard to apoptosis. Therefore, the anesthetist is confronted with confusing implications about which pharmacologic techniques may be best in the anesthetic care of patients. Although the volume of research about the effects (good or bad) of anesthetics on apoptosis is growing at a rapid pace, clear recommendations are not yet available to guide the anesthesia provider in the selection of pharmacologic agents in this regard. It appears currently that certain conditions may favor the use of either intravenous propofol or volatile agents, depending on their known effects on ROS and apoptosis. Controlled human clinical trials to further define the apoptotic and antiapoptotic effects of anesthetics are needed to clarify the attendant risks versus benefits of our pharmacologic choices. Long-term follow-up studies of both pediatric and elderly patients would be especially important. In the mean time, a thorough understanding of the pathways of apoptosis will assist anesthesia providers in the correct interpretation of future research and its incorporation into practice. Above all, we must remember that the potent anesthetic agents we administer have multiple cellular effects not currently within our ability to monitor in the operating room.
Summary

The physiologic processes of apoptosis have been reviewed to provide foundational knowledge for nurse anesthetists about this important means by which the body's cells are eliminated. Disturbances of apoptosis are the pathologic basis of many human diseases. Diseases frequently encountered in anesthesia practice that are mediated by apoptotic mechanisms were identified. Included among these are diseases of the heart, blood vessels, lungs, and nervous system, as well as cancers and immune system disturbances. Nurse anesthetists are encouraged to be aware of the possible effects of anesthetic drugs in altering normal apoptosis. Researchers have identified numerous effects of anesthesia drugs, including local anesthetics, on apoptosis, which may affect patients requiring surgery. Practitioners are encouraged to maintain knowledgeable awareness about anesthesia drugs and inhalation agents that may possess apoptotic or antiapoptotic properties.

REFERENCES

43. Culey DJ, Nie Z, Crosby G. General anesthetic-induced neurotoxicity.


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