AANA Journal Course

Update for nurse anesthetists

4

6 CE Credits*

Life in the balance: The role of serpins in disease genesis and prevention

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Complex biological systems are often shaped and maintained by opposing forces. A relevant biological example is the delicate balance between proteases and their inhibitors. Serine proteases contain a serine residue in the active site of the molecule that is essential to the activity of the enzyme. Protease inhibitors limit the activity of proteases in the body. As examples, aprotinin (Trasylol), a serine protease inhibitor, and aminocaproic acid (Amicar), a lysine protease inhibitor, are used to decrease the rate of fibrinolysis and have recently been the subject of considerable controversy in the literature regarding safety and efficacy. This* AANA Journal *course reviews 2 common examples of protease inhibitor disorders, angioedema and a form of emphysema, that are of particular anesthetic relevance.

Key words: **α_1-Antitrypsin deficiency, angioedema, emphysema, serpins.**

Objectives

At the completion of this course, the reader should be able to:

1. Describe the functional importance of the family of naturally occurring proteases.
2. Describe the importance of protease inhibitors in biological function.
3. Understand the pathogenesis of α_1-antitrypsin deficiency.
4. Detail the current therapy for people with α_1-antitrypsin deficiency.
5. Articulate an understanding of the etiology of panacinar emphysema and of angioedema.

Introduction

Since ancient times, many have believed that the universe is shaped and maintained by 2 fundamentally opposing but intertwined forces called *Yin* and *Yang* that interact to achieve an exquisite balance. When *Yin* and *Yang* fall out of balance, disharmony, with potentially catastrophic consequences, follows. This *AANA Journal* course explores how slight imbalances in biologically complex systems can lead to intracellular disharmony with profound systemic effects. This course focuses on 2 iconic examples—pulmonary emphysema and upper airway angioedema—that have clinically significant implications for anesthesia providers. These conditions are discussed in the context of their occurring secondary to imbalances in the mechanics of protease inhibition by the serpins.

Protease, serpins, and their functional importance

Catalysts that initiate and accelerate the speed of a reaction without being modified in the process are termed *enzymes*. Enzymes that are specific to the cleavage of peptide bonds (peptide bonds join amino acids to form proteins) are termed *proteases*. Serine proteases get their name from the serine residue found in the active site of the molecule essential to the activity of the enzyme. Proteins are unbranched chains of amino acids that are functionless until they coil into a specific 3-dimensional architecture through a process known as *folding*. Proteases are found throughout the body and have essential roles in maintaining homeostasis.

Proteases function to hydrolyze proteins (ie, they are proteolytic) and are specific in their action

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depending on the target protein. Table 1 lists a few of the more than 500 proteases known to occur naturally.[1] Thrombin, for example, is a serine protease that targets the arginine-glycine bonds of fibrinogen to form fibrin, releasing fibrinopeptides A and B. Prostate-specific antigen is also a protease, one used to diagnose prostate cancer. Pepsin is a protease added to food following secretion from the gastric mucosa to initiate protein digestion.

There are different families of proteases; the serine proteases contain 3 amino acids, serine, histidine, and aspartate at the active site, referred to as the catalytic triad. When the protein properly folds (Figure 1), the catalytic triad becomes positioned in such a way that the protease can cleave a targeted peptide bond. Different enzymes have different target sites. For example, chymotrypsin cleaves peptide bonds that are next to aromatic residues (eg, tyrosine), trypsin cleaves bonds next to basic residues (eg, lysine), and elastase seems less specific but optimally cleaves bonds located near certain hydrophobic residues (eg, alanine).

The functional importance of proteases links them to virtually every function in the body. Many disease states, including cancer, viral infections, digestive syndromes, aberrations in coagulation, neurodegenerative processes, pulmonary disorders, cardiovascular disease, and connective tissue abnormalities, are linked to protease-inhibitor dysfunction.

There are many protease inhibitors found in the body whose function is not necessarily to prevent, but rather to limit, the activity of proteases in the body. Anesthesia providers have observed an ongoing clinical debate regarding the intraoperative use of certain protease inhibitors used to decrease the rate of blood loss and, thus, impact the need for transfusion in specific surgical procedures (eg, cardiac bypass and scoliosis). Aprotinin (Trasylol), a serine protease inhibitor, and aminocaproic acid (Amicar), a lysine protease inhibitor, are used to decrease the rate of fibrinolysis and have recently been the subject of considerable controversy in the literature regarding safety and efficacy.

One particular family of protease inhibitors, the serpins, includes α_1-antitrypsin (α_1-AT) and C1 inhibitor. α_1-AT protects pulmonary connective tissue from elastase that is released by neutrophils and macrophages; C1 inhibitor controls complement activation, a critical pathway in the body's inflammatory response. Imbalances in protease–protease inhibitor relationships can have significant clinical consequences (Figure 2).

The serpins (short for *serine protease inhibitors*) are conspicuous among the many families of protease inhibitors in that they undergo dramatic conformational change in their structure in preparation for their intended task.[2] Serpins, like any protein in the body, are polymers of amino acids that fold into a unique and complex architecture that is necessary to execute their function (see Figure 1). The utter complexity of serpin structure and the associated shape changes while interacting with their target proteases make them highly vulnerable to even the slightest mutation. Even stress of many types can impart dysfunction.

Pathology in transcription or folding may result in the aggregation of aberrant forms of protein that may lead to a whole host of disease states, including cirrhosis and the Alzheimer disease. The body has the ability to synthesize additional chaperones (see Figure 2) to aid the dispersion and removal of abnormal proteins, but this system can be overwhelmed. Pathophysiologic states related to these malformed proteins fall into a relatively new taxonomy of conditions known as *conformational diseases*.[3]

Table 1. Example of naturally occurring proteases*

Pepsin
Trypsin
Chymotrypsin
Subtilisin
Kallikreins
Cathepsins
Thrombin
Aprotinin (Trasylol)
Aminocaproic acid (Amicar)
Prostate-specific antigen

*** This is only a partial list; there are more than 500 known proteases with many more likely to be discovered in the near future.**

Figure 1. Protein folding

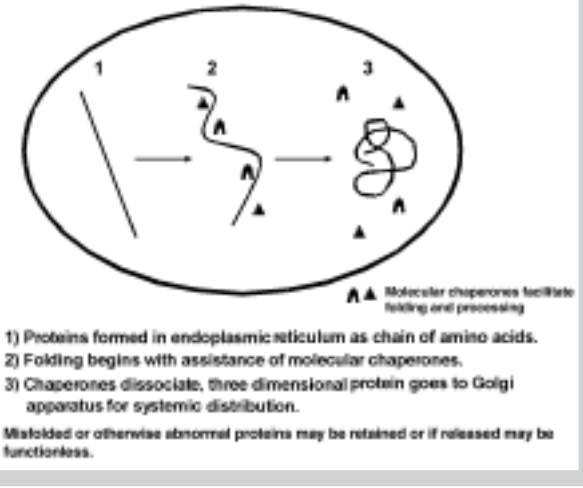
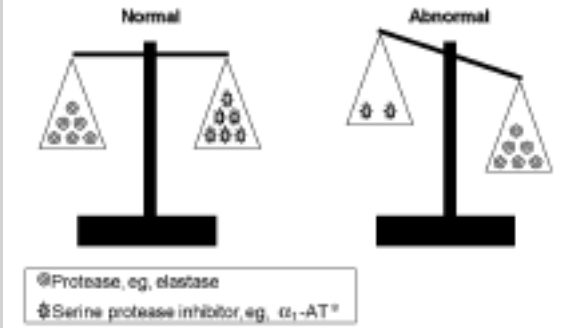


Figure 2. Preventing excessive proteolytic activity



Many processes in the body are in exquisite balance between a protease responsible for a proteolytic process (such as elastase cleansing the alveoli) and a protease inhibitor (such as α_1-AT*) that prevents cell damage from excessive protease activity.

*α_1-antitrypsin

Table 2. Medical management for people with α_1-antitrypsin deficiency

Smoking cessation
Avoidance of air pollutants and secondhand smoke
Vaccination (pneumococcal, influenza, hepatitis A and B)
Daily bronchodilator therapy
Early treatment of respiratory infections
Supplemental oxygen
Pulmonary rehabilitation for people with functional impairment

Emphysema

Emphysema is classically defined as the destruction of pulmonary parenchyma distal to the terminal bronchioles producing permanent enlargement of the acinus secondary to alveolar wall destruction. Emphysema may be further characterized according to the site of parenchymal involvement. Centriacinar emphysema occurs with septal destruction of the respiratory bronchiole and alveolar ducts in the upper zones of the lung, whereas panacinar emphysema involves the homogeneous destruction of the acinus and the enlargement of all respiratory spaces in the lower zones of the lung. α_1-AT deficiency produces the characteristic parenchymal lesion of panacinar emphysema.

The etiology of emphysema has been traditionally ascribed to long-term exposure to tobacco smoke. Additional mechanisms responsible for the development and progression of emphysema include oxidative stress and chronic inflammation. However, it is now widely accepted that an imbalance in the protease–protease inhibitor relationship is a critical pathway in the development of emphysema.

α_1-AT produced in the liver reaches the lung following diffusion from the systemic circulation. Macrophages and epithelial cells of the bronchi also produce α_1-AT.[4] The inadequate pulmonary defense against the protease neutrophil elastase by α_1-AT deficiency or decreases in α_1-AT secondary to a chronic inflammatory response that accompanies cigarette smoking is responsible for the development and progression of emphysema.

The neutrophil and macrophage in emphysema

The neutrophil and macrophage are essential cellular components in host defense, maturing in the bone marrow from the promyeloblast. The neutrophil and macrophage are subsequently released into the circulation, where they are activated and recruited to sites of inflammation or become senescent within hours of their release. During differentiation, the neutrophil and macrophage manufacture and store neutrophil elastase in membrane-bound granules. Recruitment (chemotaxis) follows the release of chemoattractant proteins from areas of chronic inflammation or oxidative stress that accompanies cigarette smoke exposure. Neutrophil migration is augmented by the release of the chemoattractant leukotriene B_4 found in peripheral airways.[5] Released neutrophil elastase stimulates the subsequent release of leukotriene B_4 from alveolar macrophages, reinforcing neutrophil recruitment. Following recruitment and activation, neutrophil elastase is released via exocytosis. The released elastase is normally inactivated by α_1-AT (protease inhibitor) in a 1:1 ratio following release. However, in the presence of chronic inflammation, this protease–protease inhibitor relationship is disrupted. The release of the elastase overwhelms the α_1-AT defenses of the lung, producing uniform destruction of alveolar walls with the loss of elastic recoil following the degradation of elastin.

α_1-AT deficiency

Plasma levels of α_1-antitrypsin are reduced by 10% to 15% in people with α_1-AT deficiency, increasing their vulnerability to alveolar destruction by neutrophil elastase.[6] The clinical presentation of α_1-AT deficiency is the same for people with chronic bronchitis, emphysema, or asthma, and, therefore, the diagnosis of α_1-AT deficiency may not be considered. The recommended medical management for α_1-AT deficiency is outlined in Table 2.

Emphysema in a nonsmoker with α_1-AT deficiency may not manifest for decades. Measurable decline in pulmonary function may not develop until age 50

years or later in nonsmokers but will appear by the third decade of life in smokers. Likewise, the progression and development of emphysema in smokers is not immediate but occurs after a number of years of smoking. This late-onset decline in pulmonary function in people with α_1-AT deficiency and cigarette smokers is postulated to occur because of an additional biological imbalance between the repair and degradation of elastin in the connective tissue.[7]

All tissues have the inherent property of repair following injury influenced by individual genetic factors, age, and general nutrition. Injured elastic fibers can be repaired, restoring the pulmonary architecture, as long as repair occurs at the same rate as injury. However, when elastic fibers are destroyed rather than injured, synthetic replication produces disordered lung architecture with homogeneous destruction of the acinus. The clinical determination of lung injury (altered spirometry and chest radiography) is delayed until the lung architecture is irreversibly disrupted to the point of symptomatic progression (Figure 3).

Therapy for α_1-AT deficiency

Initial therapy for people with emphysema due to α_1-AT deficiency includes smoking cessation, bronchodilators, supplemental oxygen, and preventative vaccination, a medical plan of care typically prescribed for a person with emphysema. Focused treatment involves the infusion of purified pooled human plasma α_1-AT, referred to as augmentation therapy. Currently there are 3 commercially available preparations of purified α_1-AT (Table 3). There have been no reported cases of hepatitis, human immunodeficiency virus infection, or prion transmission with augmentation therapy.[8,9]

The goal of augmentation therapy is to increase and maintain a plasma α_1-AT level exceeding the protective threshold of 11 µmol/L, temper the decline of pulmonary function, and delay mortality. Wewers and colleagues[10] found a weekly intravenous infusion of 60 mg/kg of pooled human α_1-AT increased plasma concentrations above the protective threshold during the weekly dosing interval with increased antielastase activity in recovered bronchoalveolar lavage fluid. An examination of patient-convenient dosing intervals of 120 mg/kg every 2 weeks or 250 mg/kg each month failed to maintain effective plasma concentrations during the specific dosing interval.[11-13]

The clinical effectiveness of augmentation therapy has been examined in observational and cohort studies[14-19] and in 1 small, randomized trial in a total of 56 patients.[13] These studies do not support the use of augmentation therapy in people without clinical evidence of emphysema. The 2003 evidence-based standards endorsed by the American Thoracic Society, the European Respiratory Society, the American College of Chest Physicians, and the American Association of Respiratory Care[20] support selected augmentation therapy for people with moderate airflow obstruction (eg, a forced expiratory volume in the first second of expiration [FEV_1] between 35% and 60% predicted). Augmentation therapy in people with moderate airflow obstruction slowed the decline in FEV_1 and reduced mortality. There is a lack of evidence regarding the benefit of augmentation therapy in people with mild (FEV_1 > 50%-60% predicted) or severe (FEV_1 < 35% predicted) airflow obstruction.[20]

Biotechnology may lead to promising new treat-

Figure 3. Time line of alveolar destruction

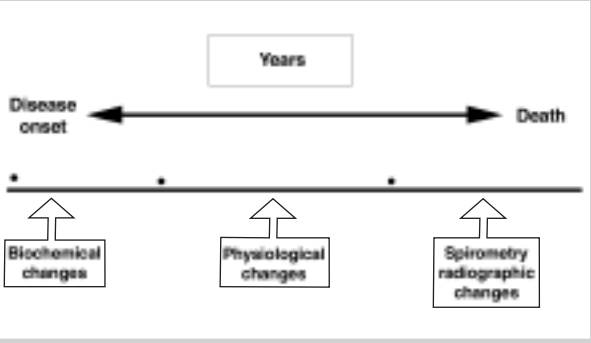


Table 3. Commercial preparations of purified α_1-antitrypsin

Product	Manufacturer	Source
Prolastin	Talecris Biotherapeutics, Research Triangle Park, North Carolina	Pooled human plasma
Aralast	Baxter, Deerfield, Illinois	Pooled human plasma
Zemaira	ZLB- Bering, King of Prussia, Pennsylvania	Pooled human plasma

Table 4. Categories of angioedema based on etiology

- Hereditary angioedema
  Inherited deficiency of C1-esterase inhibitor
- Acquired angioedema
  Often associated with lymphoproliferative diseases
- Angioedema associated with allergic phenomena
  Activation of the complement-immune system
- Angioedema secondary to medications
  Most frequently related to use of angiotensin-converting enzyme inhibitors
- Idiopathic angioedema
  No identifiable cause

ments for α_1-AT deficiency. These new therapies include gene therapy (the introduction of human α_1-AT gene),[21] the blockade of hepatic polymerization of α_1-AT,[22] and the inhibition of neutrophil elastase.[23]

Angioedema

Angioedema is generally grouped into 5 categories, (1) hereditary, (2) acquired, (3) allergy related, (4) medication induced, and (5) idiopathic[24] (Table 4). More than 90% of cases of acute angioedema treated in the emergency department are drug induced, and of those cases, the vast majority occur in people taking angiotensin-converting enzyme (ACE) inhibitors. (A.J. Smally, MD, director, Emergency Medicine, Hartford Hospital, Hartford, Connecticut, oral communication, December 2006). Not uncommonly, the patients have significant upper airway edema, often requiring endotracheal intubation, with death occurring in up to 10% of cases. Of particular interest is the observation that although angioedema related to ACE inhibitors usually (60%-70% of cases) occurs during the first week of therapy, the condition can occur spontaneously in people who have been taking the drug for months or even years.

C1-esterase inhibitor (C1-INH) is yet another member of the serpin family of protease inhibitors. C1 (there are actually a couple of species of C1) is an early step in the complement system, a system with at least 20 known components (likely there are more) that is part of the body's immune response.[25] When C1 is activated, vascular permeability increases and edema ensues; if allowed to progress unabated, the consequent response can, in and of itself, be life threatening. As described earlier, protease inhibitors are activated as a kind of braking or control mechanism for the original protease. When C1-INH is deficient or made inactive, the protease, in this case C1, proceeds unchecked. Uncontrolled C1 activation leads to accumulation of a C2 kinin, a highly vasoactive material, and kallikrein (which ultimately converts to bradykinin, another vasoactive material).

The various categories of angioedema all have at their core abnormalities in quantity or functional quality of C1-INH. Whether hereditary or induced, anything that interferes with bradykinin kinetics (metabolism) can result in angioedema. The ACE inhibitors, because they also block the function of the enzyme kinase II that breaks down bradykinin, can result in the classic presentation of upper airway and facial edema. Accumulation of bradykinin, even in small amounts, can produce the side effect of cough, so commonly seen in people taking ACE inhibitors.

Interventional issues in angioedema

Obviously, at the earliest sign of symptomatology, the drug (and similar drugs) should be stopped. Although there is controversy about whether angiotensin receptor antagonists can be used safely in patients with previous ACE inhibitor–induced angioedema, the general recommendation seems to be to avoid them.[26] In acute episodes, C1-INH concentrate can be administered, if available; it is approved in some countries, although the agent is generally not available in the United States. Fresh frozen plasma obtained from nonafflicted donors contains C1-INH and can be used as a source. Several international pharmaceutical companies are at various stages of developing and marketing bradykinin antagonists and recombinant CI-INH.

When the most dreaded complication of angioedema manifests (airway obstruction due to laryngeal edema), it is essential to achieve a patent airway by whatever approach the provider is most comfortable with. Early intervention is essential, although traditional laryngoscopy can prove nearly impossible. Consideration should be given to fiberoptic intubation; a low threshold for cricothyrotomy or tracheotomy should prevail to prevent total loss of the airway and death.

Summary

The conditions discussed in this *AANA Journal* course, pulmonary emphysema and angioedema, result, in part, from a functional imbalance in the mechanics of protease inhibition by the serpins. We introduced this concept using the metaphor of *Yin* and *Yang*, forces thought by many that interact to achieve an exquisite balance in human life.

Proteases are diffusely located in the body and have essential roles in maintaining homeostasis. The serpin, α_1-AT, exerts influence of such magnitude that aberrations in its expression lead to conditions of significant consideration to anesthesia providers.

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