INTRODUCTION

Human deoxyribonuclease I (DNase I) is an endonuclease that catalyzes the hydrolysis of extracellular DNA and is just one of the numerous types of nucleases found in nature (Horton 2008; Yang 2011). It is the most extensively studied member in the family of DNase I-like nucleases (Keyel 2017; Lazarus 2002; Baranovskii et al. 2004; Shiokawa and Tanuma 2001); the homologous bovine DNase I has received even greater attention historically (Laskowski 1971; Moore 1981; Chen and Liao 2006). Mammalian DNases have been broadly divided into several families initially based upon their products, pH optima, and divalent metal ion requirements. These include the neutral DNase I family (EC 3.1.21.1), the acidic DNase II family (EC 3.1.22.1), as well as apoptotic nucleases such as DFF40/CAD and endonuclease G (Keyel 2017; Lazarus 2002; Evans and Aguilera 2003; Widlak and Garrard 2005). The human DNase I gene resides on chromosome 16p13.3 and contains 10 exons and 9 introns, which span 15 kb of genomic DNA (Kominato et al. 2006). DNase I is synthesized as a precursor and contains a 22-residue signal sequence that is cleaved upon secretion, resulting in the 260-residue mature enzyme. It is secreted by the pancreas and parotid glands, consistent with its proposed primary role of digesting nucleic acids in the gastrointestinal tract. However, it is also present in blood and urine as well as other tissues, suggesting additional functions.

Recombinant human DNase I (rhDNase I, rhDNase, Pulmozyme®, dornase alfa) has been developed clinically where it is aerosolized into the airways for treatment of pulmonary disease in patients with cystic fibrosis (CF) (Suri 2005; Wagener and Kupfer 2012). Cystic fibrosis is an autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene (Kerem et al. 1989; Riordan et al. 1989). Mutations of this gene result in both abnormal quantity and function of an apical membrane protein responsible for chloride ion transfer. The CFTR protein is a member of the ATP-binding cassette transporter superfamily (member ABCC7) and in addition to transporting chloride has many other functions including the regulation of epithelial sodium channels, ATP-release mechanisms, anion exchangers, sodium bicarbonate transporters, and aquaporin water channels found in airways, intestine, pancreas, sweat duct, and other fluid-transporting tissues (Guggino and Stanton 2006). Clinical manifestations of the disease include chronic obstructive airway disease, increased sweat electrolyte excretion, male infertility due to obstruction of the vas deferens, and exocrine pancreatic insufficiency.

In the airways, abnormal CFTR results in altered secretions and mucociliary clearance, leading to a cycle of obstruction, chronic bacterial infection, and neutrophil-dominated inflammation. This bacterial infection and neutrophil-dominated airway inflammation begins early in the patient’s life and, while initially it helps to control infection, the degree of inflammation remains excessive to the degree of infection. Poorly regulated neutrophil-dominated inflammation damages the airways over time due to the release of potent oxidants and proteases. Additionally, necrosis of neutrophils leads to the accumulation of extracellular DNA and actin, increasing the viscosity of mucous and creating further obstruction, and a downward spiral of lung damage, loss of lung function, and ultimately premature death (Fig. 22.1).

rhDNase I has also been studied in a variety of other diseases where extracellular DNA has been postulated to play a pathological role, including prolonged mechanical ventilation due to persistent airway obstruction (Riethmueller et al. 2006),

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†This chapter is dedicated in honor of a lifetime of scientific and clinical contributions by Jeffrey S. Wagener, deceased October 24, 2018.
ventilator-associated pneumonia in infants (Scala et al. 2017), atelectasis (Hendriks et al. 2005), chronic sinusitis (Cimmino et al. 2005), primary ciliary dyskinesia (Desai et al. 1995; ten Berge et al. 1999; El Abiad et al. 2007), other non-CF lung diseases in children (Boogaard et al. 2007a), and empyema (Simpson et al. 2003; Rahman et al. 2011). rhDNase I has also been studied to examine its effectiveness in the presence or absence of antibiotics against biofilm producing strains of Staphylococcus aureus and Staphylococcus epidermidis (Kaplan et al. 2012). rhDNase I exhibits potent antibiofilm and antimicrobial-sensitizing activities at clinically achievable concentrations.

Early in vitro studies in which lung secretions were incubated for several hours with partially purified bovine pancreatic DNase I showed a large reduction in viscosity (Armstrong and White 1950; Chernick et al. 1961). Based on these observations, bovine pancreatic DNase I (dornavac or pancreatic dornase) was approved in the United States for human use in 1958. Numerous uncontrolled clinical studies in patients with pneumonia and one study in patients with CF suggested that bovine pancreatic DNase I was effective in reducing the viscosity of lung secretions (Lieberman 1968). However, severe adverse reactions occurred occasionally, perhaps

Figure 22.1 Cystic fibrosis and rhDNase I. CFTR genetic mutation at birth leads to either reduced or improperly folded CFTR protein, which results in altered ion transport, viscous mucus, and inflammation in the airways. Eventually, this leads to obstruction of the airways, bacterial infection, and further inflammation. After neutrophils arrive to fight the infection, they die and release cellular contents, one of which is DNA. Persistent obstruction, infection, and inflammation leads to structural damage and eventually pulmonary insufficiency and premature death. rhDNase I is aerosolized into the airways where it degrades DNA to lower molecular weight fragments, thus reducing CF mucus viscosity and allowing expectoration, which improves lung function and reduces bacterial infections.
due to allergic reactions to a foreign protein or from contaminating proteases, since up to 2% of trypsin and chymotrypsin were present in the final product (Raskin 1968; Lieberman 1962). Both bovine DNase I products were eventually withdrawn from the market.

In the late 1980s, human deoxyribonuclease I was cloned from a human pancreatic cDNA library, sequenced and expressed recombinantly using mammalian cell culture in Chinese hamster ovary (CHO) cells to reevaluate the potential of DNase I as a therapeutic for cystic fibrosis (Shak et al. 1990). In vitro incubation of purulent sputum from CF patients with catalytic concentrations of rhDNase I reduced its viscoelasticity (Shak et al. 1990). The reduction in viscoelasticity was directly related to both rhDNase I concentration and reduction in the size of the DNA in the samples. Therefore, reduction of high molecular weight DNA into smaller fragments by treatment with aerosolized rhDNase I was proposed as a mechanism to reduce the mucus viscosity and improve mucus clearability from obstructed airways in patients. It was hoped that improved clearance of the purulent mucus would enhance pulmonary function and reduce recurrent exacerbations of respiratory symptoms requiring parenteral antibiotics. This proved to be the case and rhDNase I was approved by the Food and Drug Administration in 1993. Since that time the clinical use of rhDNase I has continued to increase with over 67% of CF patients receiving chronic therapy (Konstan et al. 2010).

**PROTEIN CHEMISTRY, ENZYMEOLOGY, AND STRUCTURE**

The protein chemistry of human DNases including DNase I has been reviewed (Lazarus 2002; Baranovskii et al. 2004). Recombinant human DNase I is a monomeric, 260-amino acid glycoprotein (Fig. 22.2) produced by mammalian CHO cells (Shak et al. 1990). The protein has four cysteines, which are oxidized into two disulfides between Cys101–Cys104 and Cys173–Cys209 as well as two potential N-linked glycosylation sites at Asn18 and Asn106 (Fig. 22.2). rhDNase I is glycosylated at both sites and migrates as a broad band on polyacrylamide gel electrophoresis gels with an approximate molecular weight of 37 kDa, which is significantly higher than the predicted molecular mass from the amino acid sequence of 29.3 kDa. rhDNase I is an acidic protein and has a calculated pI of 4.58. The primary amino acid sequence is identical to that of the native human enzyme purified from urine.

DNase I cleaves double-stranded DNA, and to a much lesser degree single-stranded DNA, nonspecifically by nicking phosphodiester linkages in one of the strands between the 3'-oxygen atom and the phosphorus to yield 3'-hydroxyl and 5'-phosphoryl oligonucleotides with inversion of configuration at the phosphorus. rhDNase I enzymatic activity is dependent upon the presence of divalent metal ions for structure, as there are two tightly bound Ca²⁺ atoms and catalysis, which requires either Mg²⁺ or Mn²⁺ (Pan and Lazarus 1999). The active site includes two histidine

![Figure 22.2](image-url)  
**Figure 22.2** Primary amino acid sequence of rhDNase I. Active site residues are highlighted in blue, cysteine residues that form disulfide bonds are shown in yellow, N-linked glycosylation sites are highlighted in pink, and residues involved in Ca²⁺ coordination are shown in beige. The 22-residue signal sequence that is cleaved prior to secretion is not shown.
residues (His134 and His252) and two acidic residues (Glu78 and Asp212), all of which are critical for the general acid–base catalysis of phosphodiester bonds since alanine substitution of any of these results in a total loss of activity (Ulmer et al. 1996). Other residues involved in the coordination of divalent metal ions at the active site and DNA contact residues have also been identified by mutational analysis and include Asn7, Glu39, Asp168, Asn170, and Asp251 (Pan et al. 1998a; Parsiegla et al. 2012). The two Ca$^{2+}$ binding sites require acidic or polar residues for coordination of Ca$^{2+}$; for site 1 these include Asp201 and Thr203 and for site 2 these include Asp99, Asp107, and Glu112 (Pan and Lazarus 1999). DNase I is a relatively stable enzyme and shows optimal activity at pH 5.5–7.5. It is inactivated by heat and is potently inhibited by EDTA and G-actin. Surprisingly, DNase I is also inhibited by NaCl and has only ca. 30% of the maximal activity in physiological saline.

rhDNase I belongs to the DNase I-like structural superfamily according to SCOP and is also related to the endonuclease-exonuclease-phosphatase family (Andreeva et al. 2008; Dlakic 2000; Wang et al. 2010). The X-ray crystal structure of rhDNase I was initially solved at 2.2 Å resolution and superimposes with the biochemically more widely studied bovine DNase I, which shares 78% sequence identity, with an rms deviation for main chain atoms of 0.56 Å (Wolf et al. 1995). DNase I is a compact α/β protein having a core of two tightly packed six-stranded β-sheets surrounded by eight α-helices and several loop regions (Fig. 22.3). Bovine DNase I has also been crystallized in complex with G-actin (Kabsch et al. 1990) as well as with several short oligonucleotides, revealing key features of DNA recognition in the minor groove and catalytic hydrolysis (Suck 1994).

More recently, the structure of rhDNase I containing a divalent Mg$^{2+}$ and a phosphate in the active site has been solved at 1.95 Å resolution (Parsiegla et al. 2012). The combined structural and mutagenesis data suggest a Mg$^{2+}$-assisted pentavalent phosphate transition state during catalysis of rhDNase I, where Asp168 may play a key role as a catalytic general base. Asn170 is also in close proximity to both the attacking water molecule and the phosphoryl oxygen. His134 and His252 appear to act as general acids in stabilizing the pentavalent transition state. There is also a critical catalytic role for rhDNase I Asn7, a residue that is highly conserved among mammalian DNase I enzymes and members of the DNase I-like superfamily. The Mg$^{2+}$ cation resides at the computationally predicted site IVB (Gueroult et al. 2010) and interacts with Asn7, Glu39, and Asp251 via a complex set of water-mediated hydrogen bond interactions. A comprehensive analysis of the rhDNase I with members of the DNase I-like structural superfamily (Andreeva et al. 2008; Dlakic 2000) such as the apurinic/apyrimidinic endonucleases from human (APE1) (Mol et al. 2000) and Neisseria meningitidis (Nape) (Carpenter et al. 2007), sphingomyelin phosphodiesterase (SMase) from Bacillus cereus (Ago et al. 2006), or the C-terminal domain of human CNOT6L nuclease (Wang et al. 2010) solved in complex with cations or DNA have revealed new insights into the catalytic mechanism of DNA hydrolysis.

Several variants of rhDNase I with greatly improved enzymatic properties have been engineered by site-directed mutagenesis (Pan and Lazarus 1997; Pan et al. 1998a). The methods for production of the variants and the assays to characterize them have been reviewed recently (Pan et al. 2001; Sinicropi and Lazarus 2001). The rationale for improving activity was to increase binding affinity to DNA by introducing positively charged residues (Arg or Lys) on rhDNase I loops at the DNA binding interface to form a salt bridge with phosphates on the DNA backbone. These so-called “hyperactive” rhDNase I variants are substantially more active than wild-type rhDNase I and are no longer
inhibited by physiological saline. The greater catalytic activity of the hyperactive variants is due to a change in the catalytic mechanism from a “single nicking” activity in the case of wild-type rhDNase I to a “processive nicking” activity in the hyperactive rhDNase I variants (Pan and Lazarus 1997), where gaps rather than nicks result in a higher frequency of double strand cleavages.

It is interesting to note that significantly greater activity can result from just a few mutations on the surface that are not important for structural integrity. For whatever reason DNase I is not as efficient an enzyme as it could be for degrading DNA into small fragments. Furthermore the inhibition by G-actin can be eliminated by a single amino acid substitution (see below). Thus, DNase I is under some degree of regulation in vivo. One can only speculate that nature may have wanted to avoid an enzyme with too much DNA degrading activity that could result in undesired mutations in the genome.

**EMERGING BIOLOGY**

■ **Neutrophil Extracellular Traps**

Neutrophils are important effector cells that play a key role in innate immunity. They have well documented ‘first-line defense’ roles in phagocytosis of foreign organisms such as bacteria and fungi. NETosis, the formation and release of neutrophil extracellular traps (NETs) is another defense mechanism that has emerged more recently (Brinkmann et al. 2004). Neutrophils can degranulate, releasing NETs, which are structures of DNA filaments coated with toxic histones, proteases, oxidative enzymes and other proteins that can immobilize and neutralize bacteria in the extracellular environment. NETosis can lead to a cell death process that is distinct from both apoptosis and necrosis, but it can also be independent of cell death (Jorch and Kubes 2017; Honda and Kubes 2018). NETs and NETosis have been broadly studied in biology and their role in a wide variety of diseases is of great interest (Jorch and Kubes 2017; Brinkmann 2018). These have included autoimmune, inflammatory, thrombotic, cancer and other diseases such as SLE, vasculitis, acute pancreatitis, rheumatoid arthritis, Type 1 diabetes mellitus and wound healing, gout, inflammatory bowel disease; vascular occlusion, sepsis, ARDS, metastasis and others (Cools-Lartigue et al. 2014; Martinod and Wagner 2014; Merza et al. 2015; Wong et al. 2015; Loord et al. 2016; Park et al. 2016; Gupta and Kaplan 2016; Jiménez-Alcázar et al. 2017b; Apel et al. 2018; Honda and Kubes 2018). It is particularly significant that DNase I or rhDNase I was almost always used in the biological studies on the above mentioned disease areas to show the effect of NET degradation, most often resulting in beneficial effects. Notably, serum DNase I had been shown to be essential for degradation of NETs (Hakkim et al. 2010); however, other DNase I like nucleases like DNase 1L3 can also degrade NETs (Jiménez-Alcázar et al. 2017b).

While it is clear that NETs and NETosis plays an important role in biology, that role is quite complex. In many cases they likely play a beneficial role, however in others they may play a pathological role. Like many areas of biology and disease, it is likely the right balance as well as the their locality that makes the difference. Furthermore, it is not always clear if they are the cause or result of a given pathology; there is evidence supporting both in thrombosis (Jiménez-Alcázar et al. 2017a). There is much evidence showing that diseases involve a multitude of pathways. For example, there are strong connections between coagulation, inflammation and cancer pathways. It is not surprising that NETs also have connections with these pathways. NETosis is a factor in tumor progression as well as cancer associated thrombosis (Demers and Wagner 2014). Both neutrophils and circulating extracellular DNA have been suggested to play an important role in cancer (Hawes et al. 2015).

With respect to rhDNase I, it is especially noteworthy that NETs play a significant role in CF (Gray et al. 2015; Law and Gray 2017), since this is the major use of rhDNase I clinically. While rhDNase I has a rheological effect in reducing the viscoelasticity in CF sputum, it also has biological effects related to releasing various enzymes and proteins from the NETs upon hydrolysis of the DNA. In the CF lung, NETs may play less of a role as an antibacterial and more of a role in fostering inflammation. rhDNase I likely exerts its anti-inflammatory function due to degradation of NETs.

**PHARMACOLOGY**

■ **In Vitro Activity in CF Sputum**

In vitro, rhDNase I hydrolyzes the DNA in sputum of CF patients and reduces sputum viscoelasticity (Shak et al. 1990). Effects of rhDNase I were initially examined using a relatively crude “pourability” assay. Pourability was assessed qualitatively by inverting a tube of sputum and observing the movement of sputum after a tap on the side of the tube. Catalytic amounts of rhDNase I (50 μg/mL) greatly reduced the viscosity of the sputum, rapidly transforming it from a viscous gel to a flowing liquid. More than 50% of the sputum moved down the tube within 15 min of incubation, and all the sputum moved freely down the tube within 30 min. The qualitative results of the pourability assay were confirmed by quantitative measurement of viscosity using a Brookfield Cone-Plate viscometer (Fig. 22.4). The reduction of viscosity by rhDNase I is rhDNase I concentration-dependent and is associated with reduction in size of sputum DNA as measured by agarose gel electrophoresis (Fig. 22.5).
Additional in vitro studies of CF mucus samples treated with rhDNase I demonstrated a dose-dependent improvement in cough transport and mucociliary transport of CF mucus using a frog palate model and a reduction in adhesiveness as measured by mucus contact angle (Zahm et al. 1995). The improvements in mucus transport properties and adhesiveness were associated with a decrease in mucus viscosity and mucus surface tension, suggesting rhDNase I treatment may improve the clearance of mucus from airways. The in vitro viscoelastic properties of rhDNase I have also been studied in combination with normal saline, 3% hypertonic saline, or nacystelyn, the L-lysine salt of N-acetyl cysteine (King et al. 1997; Dasgupta and King 1996). The major impact of rhDNase I on CF sputum is to decrease spinnability, which is the thread forming ability of mucus under the influence of low amplitude stretching. CF sputum spinnability decreases 25% after 30 min incubation with rhDNase I (King et al. 1997). rhDNase I in normal saline and saline alone both increased the cough clearability index. With the combination of rhDNase I and 3% hypertonic saline, there was minimal effect on spinnability however mucus rigidity and cough clearability improved greater than with either agent alone. The predicted mucociliary clearance did not significantly increase with 3% saline either alone or in combination with rhDNase I. Combining rhDNase I with nacystelyn has an additive benefit on spinnability, but no effect on mucus rigidity or cough clearability (Dasgupta and King 1996). These effects of rhDNase I can be variable in vivo and do not necessarily correlate with the level of DNA in sputum.
sputum from CF patients that clinically responded to rhDNase I contains significantly higher levels of magnesium ions compared with sputum from patients who do not have a clear response (Sanders et al. 2006). Although this response is consistent with the requirement for divalent cations and their mode of action on DNase I (Campbell and Jackson 1980), the mechanism of increased rhDNase I activity by magnesium ions has been attributed to altering the polymerization state of actin such that equilibrium favors increased F-actin and decreased G-actin (see below).

The mechanism of action of rhDNase I to reduce CF sputum viscosity has been ascribed to DNA hydrolysis (Shak et al. 1990). However, an alternative mechanism involving depolymerization of filamentous actin (F-actin) has been suggested since F-actin contributes to the viscoelastic properties of CF sputum and the actin-depolymerizing protein gelsolin also reduces sputum viscoelasticity (Vasconcellos et al. 1994). F-actin is in equilibrium with its monomeric form (G-actin), which binds to rhDNase I with high affinity and is also a potent inhibitor of DNase I activity (Lazarides and Lindberg 1974). DNase I is known to depolymerize F-actin by binding to G-actin with high affinity, shifting the equilibrium in favor of rhDNase I/G-actin complexes (Hitchcock et al. 1976). To elucidate the mechanism of rhDNase I in CF sputum, the activity of two types of rhDNase I variants were compared in CF sputum (Ulmer et al. 1996). Active site variants were engineered that were unable to catalyze DNA hydrolysis but retained wild-type G-actin binding. Actin-resistant variants that no longer bound G-actin but retained wild-type DNA hydrolytic activity were also characterized. The active site variants did not degrade DNA in CF sputum and did not decrease sputum viscoelasticity (Fig. 22.6). Since the active site variants retained the ability to bind G-actin, these results argue against depolymerization of F-actin as the mechanism of action. In contrast, the actin-resistant variants were more potent than wild-type DNase I in their ability to degrade DNA and reduce sputum viscoelasticity (Fig. 22.6). The increased potency of the actin-resistant variants indicated that G-actin was a significant inhibitor of wild-type DNase I in CF sputum and confirmed that hydrolysis of DNA was the mechanism by which rhDNase I decreases sputum viscoelasticity. The mechanism for reduction of sputum viscosity by gelsolin was subsequently determined to result from an unexpected second binding site on actin that competes with DNase I, thus relieving the inhibition by G-actin (Davoodian et al. 1997). Additional in vitro studies characterizing the relative potency of actin-resistant

Figure 22.6 Mechanism of action in CF mucus for rhDNase I. The change in viscoelasticity in CF mucus as a function of DNase I concentration was determined for wild-type rhDNase I, two active site variants that no longer catalyze DNA hydrolysis and four variants that are no longer inhibited by G-actin (Ulmer et al. 1996)
and hyperactive rhDNase I variants in serum and CF sputum have been reported (Pan et al. 1998b).

### In Vivo Activity in CF Sputum

In vivo confirmation of the proposed mechanism of action for rhDNase I has been obtained from direct characterization of apparent DNA size (Fig. 22.7) and measurements of enzymatic and immunoreactive (ELISA) activity of rhDNase I (Fig. 22.8) in sputum from cystic fibrosis patients (Sinicropi et al. 1994a). Sputum samples were obtained 1–6 h post-dose from adult cystic fibrosis patients after inhalation of 5–20 mg of rhDNase I. rhDNase I therapy produced a sustained reduction in DNA size in recovered sputum (Fig. 22.7), in good agreement with the in vitro data.

Inhalation of the therapeutic dose of rhDNase I produced sputum levels of rhDNase I which have been shown to be effective in vitro (Fig. 22.8) (Shak 1995). Similarly, delivery to CF patients as young as 3 months can produce bronchoalveolar lavage fluid levels similar to that of older patients (Wagener et al. 1998). The recovered rhDNase I was also enzymatically active. Enzymatic activity was directly correlated with rhDNase I concentrations in the sputum. Viscocoeasticity was reduced in the recovered sputum, as well. Furthermore, results from scintigraphic studies in using twice daily 2.5 mg of rhDNase I in CF patients suggested possible reductions in pulmonary obstruction and increased rates of mucociliary sputum clearance from the inner zone of the lung compared to controls (Laube et al. 1996). This finding was not confirmed in a crossover design study using once-daily dosing, suggesting that improvement of mucociliary clearance may require higher doses (Robinson et al. 2000). Epidemiologic evaluation of patients changing from once-daily dosing to twice-daily and vice versa has also shown improvement in lung function and fewer pulmonary exacerbations on higher doses, although the clinical indication for a dose change is not clear (VanDevanter et al. 2018).

### Pharmacokinetics and Metabolism

Nonclinical pharmacokinetic data in rats and monkeys suggest minimal systemic absorption of rhDNase I following aerosol inhalation of clinically equivalent doses. rhDNase I is cleared from the systemic circulation without any accumulation in tissues following acute exposure (Green 1994). Additionally, nonclinical metabolism studies suggest that the low rhDNase I concentrations present in serum following inhalation will be bound to binding proteins (Green 1994; Mohler et al. 1993). The low concentrations of endogenous DNase I normally present in serum and the low concentrations of rhDNase I in serum following inhalation are inactive due to the ionic com-
When 2.5 mg of rhDNase I was administered twice daily by inhalation to 18 CF patients, mean sputum concentrations of 2 μg/mL DNase I were measurable within 15 min after the first dose on Day 1 (Fig. 22.9). Mean sputum concentrations declined to an average of 0.6 μg/mL 2 h following inhalation. The peak rhDNase I concentration measured 2 h after inhalation on Days 8 and 15 increased to 3.0 and 3.6 μg/mL, respectively. The mean ± SD is shown with N = 18.

Protein Manufacturing and Formulation

rhDNase I is expressed in mammalian cell culture and purified to homogeneity using a variety of chromato-graphic steps. The development of the formulation of rhDNase I is especially important in that a suitable formulation is required to take into account protein stability, aerosolization properties, solubility, and the sealed container for storage (Shire 1996). rhDNase I (Pulmozyme®, dornase alfa) is manufactured by Genentech, Inc. and formulated as a sterile, clear, and colorless aqueous solution containing 1.0 mg/mL dornase alfa, 0.15 mg/mL calcium chloride dihydrate, and 8.77 mg/mL sodium chloride. The solution contains no preservative and has a nominal pH of 6.3. Pulmozyme® is administered by the inhalation of an aerosol mist produced by a compressed air-driven nebulizer system. Pulmozyme® is supplied as single-use ampoules, which deliver 2.5 mL of solution to the nebulizer.

The choice of formulation components was determined by a need to provide 1–2 years of stability and to meet additional requirements unique to aerosol delivery (Shire 1996). A simple colorimetric assay for rhDNase I activity was used to evaluate the stability of rhDNase I in various formulations (Sinicropi et al. 1994b). In order to avoid adverse pulmonary reactions, such as cough or bronchoconstriction, aerosols for local pulmonary delivery should be formulated as isotonic solutions with minimal or no buffer components and should maintain pH >5.0. rhDNase I has an additional requirement for calcium to be present for optimal enzymatic activity. Limiting formulation components raised concerns about pH control, since protein stability and solubility can be highly pH-dependent. Fortunately, the protein itself provided sufficient buffering capacity at 1 mg/mL to maintain pH stability over the storage life of the product.

Drug Delivery

The droplet or particle size of an aerosol is a critical factor in defining the site of deposition of the drug in the patient’s airways (Gonda 1990). A distribution of particle or droplet size of 1–6 μm is optimal for the uniform deposition of rhDNase I in the airways (Cipolla et al. 1994). Jet nebulizers are the simplest method of producing aerosols in the desired respirable range. However, recirculation of protein solutions under high shear rates in the nebulizer bowl can present risks to the integrity of the protein molecule. rhDNase I survived recirculation and high shear rates during the nebulization process with no apparent degradation in protein quality or enzymatic activity (Cipolla et al. 1994). Ultrasonic nebulizers produce greater heat than jet nebulizers and protein breakdown prevents their use with rhDNase I. Significant advances in nebulizer technology have occurred since the original approval of rhDNase I. Newer nebulizers using a vibrating, perforated membrane do not produce protein breakdown and provide more rapid and efficient delivery of particles in the respirable range (Scherer et al. 2011).

Approved jet nebulizers produce aerosol droplets in the respirable range (1–6 μm) with a mass median aerodynamic diameter (MMAD) of 4–6 μm. The delivery of rhDNase I with a device that produces smaller droplets leads to more peripheral deposition in the smaller airways and thereby improves efficacy (Geller...
et al. 1998). Results obtained in 749 CF patients with mild disease confirmed that patients randomized to the Sidestream nebulizer powered by the Mobil Aire Compressor (MMAD = 2.1 μm) tended to have greater improvement in pulmonary function than patients using the Hudson T nebulizer with Pulmo-Aide Compressor (MMAD = 4.9 μm). These results indicate that the efficacy of rhDNase I is dependent, in part, on the physical properties of the aerosol produced by the delivery system. Nebulizers with vibrating mesh technology (Pari eFlow®) produce similarly small particles, suggesting these may result in further improved efficacy of rhDNase I. Furthermore, “smart” nebulizers are now available that coach the patient on taking a proper breath to improve delivery to the lower airways. Delivery of rhDNase I improves and lung function improves more when these nebulizers are used (Bakker et al. 2011). A randomized trial of efficacy and safety of dornase alfa delivered by the eRapid® nebulizer in CF patients showed comparable efficacy and safety, shorter nebulization times, and higher patient preference when compared to standard jet nebulizer systems such as the LC Plus (Sawicki et al. 2015).

CLINICAL USE

■ Indication and Clinical Dosage
rhDNase I (Pulmozyme®, dornase alfa) is currently approved for use in CF patients, in conjunction with standard therapies, to reduce the frequency of respiratory infections requiring parenteral antibiotics and to improve pulmonary function (Fig. 22.1). The recommended dose for use in most CF patients is one 2.5 mg dose inhaled daily.

■ Cystic Fibrosis
rhDNase I was evaluated in a large, randomized, and placebo-controlled trial of clinically stable CF patients, 5 years of age or older, with baseline forced vital capacity (FVC) greater than or equal to 40% of predicted (Fuchs et al. 1994). All patients received additional standard therapies for CF. Patients were treated with placebo or 2.5 mg of rhDNase I once or twice a day for 6 months. When compared to placebo, both once daily and twice daily doses of rhDNase I resulted in a 28–37% reduction in respiratory tract infections requiring use of parenteral antibiotics (Fig. 22.10). Within 8 days of the start of treatment with rhDNase I, mean forced expiratory volume in 1 s (FEV₁) increased 7.9% in patients treated once a day and 9.0% in those treated twice a day compared to the baseline values. The mean FEV₁ observed during long-term therapy increased 5.8% from baseline at the 2.5 mg daily dose level and 5.6% from baseline at the 2.5 mg twice daily dose level (Fig. 22.11). The risk of respiratory tract infection was reduced even in patients whose pulmonary function (FEV₁) did not improve. This finding may be due to improved clearance of mucus from the small airways in the lung, which will have little effect on FEV₁ (Shak 1995). Supporting this concept is the finding that rhDNase I improves the lung clearance index in 6–18-year-old CF patients with normal lung function (Amin et al. 2011). Alternatively, use of rhDNase I may be altering the neutrophilic inflammatory response that occurs early in the course of CF lung disease, similar to the use of other anti-inflammatory therapies (Konstan and Ratjen 2012). The administration of rhDNase I also lessened shortness of breath, increased the general perception of well-being, and reduced the severity of other cystic fibrosis-related symptoms. Based on these findings, the US Cystic Fibrosis Foundation, in their chronic therapy pulmonary guidelines, strongly recommends the use of rhDNase I in patients 6 years old and older with moderate to severe lung disease and recommends its use in patients with mild lung disease (Flume et al. 2007).
The safety and deposition, but not efficacy, of rhDNase I has been studied in CF patients <5 years old (3 months to 5 years) since therapy may provide clinical benefit for young CF patients with mild disease (Wagener et al. 1998). After 2 weeks of daily administration of 2.5 mg rhDNase I, levels of rhDNase I deposited in the lower airways were similar for children <5 years compared to a group of 5–10-year-olds. Moreover, rhDNase I was well tolerated in the younger age group with an adverse event frequency similar to that in the older age group. To further understand how rhDNase I might alter the progression of lung disease, children with mild CF related lung disease were treated for 2 years in a randomized controlled trial (Quan et al. 2001). Children with a mean age of 8.4 years and an FVC greater than 85% of predicted were treated once daily with either placebo or 2.5 mg rhDNase I. After 96 weeks, lung function was significantly better in the treated group compared with placebo, particularly for tests measuring function of smaller airways. Respiratory exacerbations were also reduced in the treated group.

Clinical trials have indicated that rhDNase I therapy can be continued or initiated during an acute respiratory exacerbation (Wilmott et al. 1996). rhDNase I however does not produce a pulmonary function benefit when used short-term in the most severely ill CF patients (FVC less than 40% of predicted) (Shah et al. 1995; McCoy et al. 1996). Short-term dose ranging studies demonstrated that doses in excess of 2.5 mg twice daily did not provide further significant improvement in FEV1 (Aitken et al. 1992; Hubbard et al. 1992; Ramsey et al. 1993). Patients who have received drug on a cyclical regimen (i.e., administration of rhDNase I 10 mg twice daily for 14 days, followed by a 14-day washout period) showed rapid improvement in FEV1 with the initiation of rhDNase I and a return to baseline following withdrawal (Eisenberg et al. 1997). rhDNase I use improves quality of life as measured by the validated CFQ-R questionnaire (Rozov et al. 2010).

Concomitant therapy with rhDNase I and other standard CF therapies often show additive effects. The intermittent administration of aerosolized tobramycin was approved for use in CF patients with or without concomitant use of rhDNase I (Ramsey et al. 1999). Aerosolized tobramycin was well tolerated, enhanced pulmonary function, and decreased the density of P. aeruginosa in sputum. In combination with rhDNase I a larger treatment effect was noted but did not reach statistical significance. No differences in safety profile were observed following aerosolized tobramycin in patients that did or did not use rhDNase I. Chronic use of azithromycin has also been studied in CF patients chronically infected with P. aeruginosa (Saiman et al. 2005). Similar improvement in lung function and reduction in respiratory exacerbations was seen in patients receiving rhDNase I as those not, suggesting an additive, but not synergistic benefit of the two therapies used together. The combination of hypertonic saline therapy with chronic use of rhDNase I has similar additive benefits (Elkins et al. 2006), in agreement with the previously mentioned in vitro studies. Finally, combining ivacaftor, which potentiates chloride transport in CF patients with the G551D gene mutation, with chronic rhDNase I use produces an additive benefit in both improved lung function and reduced respiratory exacerbations (Ramsey et al. 2011). Notably, there was no evidence of a change in adverse events related to combination therapy in any of these studies.

Following FDA approval of rhDNase I in 1993, a large epidemiologic study of CF patients was initiated (the Epidemiologic Study of Cystic Fibrosis or ESCF), which continued until 2005 (Morgan et al. 1999). This study was designed to evaluate practice patterns in CF patients and has included data from over 24,000 patients. Recent analysis of ESCF data showed that chronic use of rhDNase I is associated with a decreased rate of decline in lung function overtime (Konstan et al. 2011). This reduced rate of decline in lung function is similar to findings with oral ibuprofen (Konstan et al. 1995, 2007) and inhaled corticosteroids (Ren et al. 2008), suggesting that there may be a long-term anti-inflammatory benefit with the use of rhDNase I. This potential anti-inflammatory effect is supported by a randomized trial in 105 CF patients with mild lung disease (FEV1 >80% predicted) (Paul et al. 2004). Based on an initial bronchoscopy and alveolar lavage, patients were divided into two groups, those without airway inflammation (n = 20) and those with. The patients with inflammation were then randomized to treatment with rhDNase I (n = 46) or not (n = 39). Follow-up bronchoscopy and lavage was performed at 18 and 36 months. In the patients treated with rhDNase I, there was no change in inflammation as measured by elastase and IL-8 levels and neutrophil number. Patients not treated with rhDNase I and patients who did not have inflammation at baseline all had worsening neutrophilic inflammation on follow-up. Although this study was not designed to evaluate the rate of lung function decline, treated patients dropped FEV1 by 1.99% predicted per year compared to a 3.26% predicted drop per year in the untreated subjects. Finally, rhDNase I is associated with a 15% reduction in the odds of subsequent year mortality in patients with CF (Sawicki et al. 2012).

**Non-cystic Fibrosis Respiratory Disease**
Although originally considered beneficial for the treatment of non-CF related bronchiectasis (Wills et al. 1996), rhDNase I had no effect on pulmonary function or the frequency of respiratory exacerbations in a randomized controlled trial (O’Donnell et al. 1998).
In another randomized controlled trial of rhDNase I, young children had shorter periods of ventilatory support following cardiac surgery when rhDNase I was instilled twice daily into the endotracheal tube (Riethmueller et al. 2006). Complicating atelectasis was less frequent in the treated group, consistent with numerous case reports suggesting that rhDNase I decreases, and can be used to treat, atelectasis when directly instilled into the airway (Hendriks et al. 2005). This effectiveness in treating atelectasis seems particularly true for newborns with lung disease requiring mechanical ventilation (MacKinnon et al. 2011; Dilmen et al. 2011; Fedakar et al. 2012; Altunhan et al. 2012). Limited benefit has been seen in children with asthma (Puterman and Weinberg 1997) although no benefit has been seen in adults (Silverman et al. 2012), consistent with the lack of neutrophil dominated inflammation in asthma. Finally, while there is increased free DNA in the secretions of infants with respiratory syncytial virus caused bronchiolitis, early suggestions of benefit (Nasr et al. 2001) have not translated into reduced hospitalization or the need for supplemental oxygen (Boogaard et al. 2007b).

**Empyema and Para-Pneumonic Effusion**

In principle rhDNase I may be useful for treating any condition where high levels of extracellular DNA and associated viscoelastic properties are pathological. Pulmonary empyema involves the collection of purulent material in the pleural space and the use of rhDNase I instilled into the pleural space has been reviewed (Simpson et al. 2003; Corcoran et al. 2015). In one large, multicenter clinical trial for the treatment of empyema in adults, twice daily intrapleural administration over 3 days was evaluated in four groups: 5 mg rhDNase, 10 mg tissue plasminogen activator (t-PA), with the combination of both, and a double placebo. Patients receiving the combination therapy had improved fluid drainage and a reduced frequency of surgical referral (Rahman et al. 2011). Additional studies using similar doses have demonstrated similar clinical benefits in children, although determining when and if to perform surgery remains unclear (Piccolo et al. 2015; Gilbert and Gorden 2017). Importantly, it appears that using either t-PA or rhDNase I alone does not produce the benefit of combined therapy.

**Other Medical Conditions**

rhDNase I has also been instilled into the nasal sinuses after surgery for chronic infections (Cimmino et al. 2005; Raynor et al. 2000). While daily use over 28 days of nasal nebulized rhDNase I in patients with CF did not produce a significant change in the sinuses on MRI, there was a significant clinical improvement as measured by quality of life questionnaire (Mainz et al. 2011, 2014).

Children with otitis media develop chronic neutrophil inflammation and an associated increase in NETs. rhDNase I has been proposed for the management of chronic otitis disease in patients not responding well to antibiotics, although no significant clinical trials have been conducted (Thornton et al. 2013).

The effect of rhDNase I has been studied in a small group of advanced head and neck cancer patients with thick, tenacious upper airway secretions while receiving chemoradiation therapy (Mittal et al. 2013). Preliminary results did not show a significant difference versus placebo in quality of life measures, but did show improvement in secondary endpoints of oropharyngeal secretions and DNA concentrations.

**Safety**

The administration of rhDNase I has not been associated with an increase in any major adverse events. Most adverse events were not more common with rhDNase I than with placebo treatment and probably reflect complications related to the underlying lung disease. Most events associated with dosing were mild, transient in nature, and did not require alterations in dosing. Observed symptoms included hoarseness, pharyngitis, laryngitis, rash, chest pain, and conjunctivitis. Within all the studies a small percentage (average 2–4%) of patients treated with rhDNase I developed serum antibodies to rhDNase I. None of these patients developed anaphylaxis and the clinical significance of serum antibodies to rhDNase I is unknown. rhDNase I has also been associated with a slight increased risk of allergic bronchopulmonary aspergillosis in CF patients, although this most likely represents the chronic use of a wet nebulizer and not a complication of rhDNase I (Jubin et al. 2010).

**SUMMARY**

DNase I, a secreted human enzyme whose normal function is thought to be for digestion of extracellular DNA, has been developed as a safe and effective adjunctive agent in the treatment of pulmonary disease in cystic fibrosis patients. rhDNase I reduces the viscoelasticity and improves the transport properties of viscous mucus both in vitro and in vivo. Inhalation of aerosolized rhDNase I reduces the risk of infections requiring antibiotics and improves pulmonary function and the well-being of CF patients with mild to moderate disease. Studies also suggest that rhDNase I has benefit in infants and young children with CF and in patients with early disease who may have “normal” lung function. Additional studies may assess the usefulness of rhDNase I in early-stage CF pulmonary disease and other diseases where extracellular DNA and NETs may play a pathological role.
SELF-ASSESSMENT QUESTIONS

Questions
1. Mutations of the CF gene result in abnormalities of the CF transmembrane conductance regulator protein. How do abnormalities of this protein lead to eventual lung damage?
2. How does rhDNase I result in improved pulmonary function in patients with cystic fibrosis?
3. rhDNase I is strongly recommended by the US Cystic Fibrosis Foundation for use in CF patients with moderate and severe lung disease. What other CF patients may benefit from using this therapy?
4. In addition to improving lung function in CF patients, what benefits have been demonstrated in clinical trials of rhDNase I?
5. What other medical conditions may benefit from treatment with rhDNase I?
6. Why should ultrasonic nebulizers not be used to administer rhDNase I? What other types of nebulizers might be effective for delivering rhDNase I?

Answers
1. Abnormal CFTR protein results in abnormal airway surface liquid, which leads to a vicious cycle of airway obstruction, infection, and inflammation. The chronic, excessive neutrophilic inflammation in the airway results in release of neutrophil elastase, oxidants, and extracellular DNA. These substances result in worsening obstruction and progressive airway damage.
2. rhDNase I cleaves extracellular DNA in the airway of CF patients, resulting in improved airway clearance of secretions. Additionally, rhDNase I may have anti-inflammatory properties, resulting in a slower progression of lung damage and reduced rate of decline in lung function. This anti-inflammatory benefit is more likely present in patients with earlier, less severe lung disease.
3. rhDNase I is also recommended in CF patients over age 6 with mild lung disease. It has also been demonstrated as safe in younger patients, although efficacy has not been studied.
4. rhDNase I use decreases the frequency of respiratory exacerbations. rhDNase I also lessened shortness of breath, increased the general perception of well-being, and reduced the severity of other cystic fibrosis-related symptoms.
5. Although only approved by the FDA for treatment of lung disease in patients with CF, controlled trials have also shown efficacy for treating empyema (in combination with t-PA) and sinus disease in patients with CF.
6. Ultrasonic nebulizers generate heat, which breaks down the protein in rhDNase I. Vibrating permeable membrane nebulizers and “smart” nebulizers do not damage the drug and may improve the effectiveness of rhDNase I.

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