ELSEVIER

European Journal of Pharmacology 451 (2002) 1–10

www.elsevier.com/locate/ejphar

Review

The role of neutrophil elastase in acute lung injury

Kazuhito Kawabata *, Tetsuya Hagio, Shozo Matsuoka

Minase Research Institute, Ono Pharmaceutical Co., Ltd., 3-1-1 Sakurai, Shimamoto, Mishima, Osaka 618-8585, Japan

Received 20 June 2002; received in revised form 22 July 2002; accepted 26 July 2002

Abstract

Beside its physiological function as a powerful host defense, neutrophil elastase is also known as one of the most destructive enzymes in the body. Current notion holds that neutrophil elastase is able to escape from regulation by multiple protease inhibitors at inflammatory sites. Once unregulated, this enzyme disturbs the function of the lung permeability barrier and induces the release of pro-inflammatory cytokines. These actions then cause symptoms that are typical in the pathophysiology of acute lung injury. In this article, we review recent progress in the understanding of the physiological activity of neutrophil elastase and its role in acute lung injury. Evidence in this review that supports the involvement of neutrophil elastase in the pathophysiology of acute lung injury includes: (1) neutrophil elastase levels are increased in both clinical and animal models of acute lung injury; (2) topical or systemic administration of neutrophil elastase produces typical symptoms of acute lung injury both in vitro and in vivo; and (3) inhibition of increased neutrophil elastase activity reduces symptoms of acute lung injury in animal models. A greater understanding of the role of this enzyme in the pathophysiology of acute lung injury will lead to better treatments for this complicated disease.

Keywords: Neutrophil elastase; Lung injury, acute; Respiratory distress syndrome, adult; Permeability; Edema

1. Introduction

Reactive oxygen species and proteases are neutrophil-derived toxic molecules that have long been considered important in the pathophysiology of acute lung injury (Janoff, 1985). Neutrophil elastase (E.C.3.4.21.37) is one such molecule in particular that has received attention as an enzyme capable of degrading almost all extracellular matrix proteins as well as a variety of key plasma proteins (Havemann and Gramse, 1984). In addition to its proteolytic activity, neutrophil elastase induces the release of pro-inflammatory cytokines such as interleukin-6 (Bedard et al., 1993) and interleukin-8 (Nakamura et al., 1992). Under physiological conditions, neutrophil elastase is a powerful host defense and its activity is tightly regulated by endogenous protease inhibitors (Travis and Salvesen, 1983). At inflammatory sites, however, this enzyme appears to remain active due to an imbalance between its own elevated level and that of the endogenous protease inhibitors (Weiss, 1989; Morrison et al., 1990; Kawabata et al., 1996). In lung and its surrounding environment, the unregulated proteolytic activity of neutrophil elastase leads to a variety of changes that are directly and indirectly related to the pathophysiology of acute lung injury.

In this article, we review the findings of recent studies on neutrophil elastase and its role in acute lung injury. First, we survey neutrophil elastase, its natural substrates, target cells, and endogenous protease inhibitors. We then examine the mechanism by which this enzyme is able to escape endogenous protease inhibition at inflammation sites. In the latter part of this article, we focus on: (1) whether neutrophil elastase levels are increased in clinical or animal models of acute lung injury; (2) whether topical or systemic application of neutrophil elastase produces typical symptoms of acute lung injury both in vitro and in vivo; and (3) whether inhibition of increased neutrophil elastase activity reduces symptoms of acute lung injury in animal models. Finally, we propose a general pathophysiological role for neutrophil elastase and sum up with a brief conclusion.

* Corresponding author. Tel.: +81-75-961-1151; fax: +81-75-962-9314.
E-mail address: k.kawabata@ono.co.jp (K. Kawabata).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02182-9

2. Neutrophil elastase and its regulation

2.1. Neutrophil elastase

Neutrophil elastase, a member of the chymotrypsin superfamily of serine proteases, is a 33-kDa enzyme with several isoforms that differ in their extent of glycosylation (Bieth, 1986; Ohlsson and Olsson, 1974). Commercially, the most readily available enzyme is sputum elastase, which is isolated from human sputum and has at least five distinct isozymes (Twumasi and Liener, 1977). This elastase is both immunologically and catalytically indistinguishable from human neutrophil elastase, indicating that it is derived from neutrophils. This enzyme is also found in much smaller amounts in monocytes and mast cells (Lavie et al., 1980; Meier et al., 1985) although its role in these cells is not yet clear. In neutrophils, the concentration of neutrophil elastase exceeds 5 mM and its total cellular amount has been estimated to be up to 3 pg (Liau and Campbell, 1995). Such a high concentration of elastase is tightly regulated by compartmentalization in the azurophil granules (Bieth, 1986). Upon activation, neutrophil elastase is rapidly released from the granules into the extracellular space with some portion remaining bound to the neutrophil plasma membrane (Owen et al., 1995) and is regulated by multiple endogenous inhibitors. It has also been reported that neutrophils contain α_1-protease inhibitor (Du Bois et al., 1991), one of the most abundant endogenous inhibitors in the body (Travis and Salvesen, 1983). Details on neutrophil elastase endogenous protease inhibitors are reviewed later in this article.

2.2. Natural substrates and target molecules of neutrophil elastase

The main intercellular physiological function of neutrophil elastase is the degradation of foreign organic molecules phagocytosed by neutrophils, whereas the main target for extracellular elastase is elastin (Janoff and Scherer, 1968). Although neutrophil elastase has actually been defined as a protease that can degrade elastin fiber, other proteases including protease 3, cathepsin G, macrophage elastase, gelatinase, and cathepsin L and S are able to degrade elastin as well (Lee and Downey, 2001). What makes neutrophil elastase unique and recognized as one of the most destructive enzymes is its ability to degrade almost all extracellular matrix and key plasma proteins (Table 1). In addition to elastin, neutrophil elastase is known to degrade the extracellular matrix proteins, collagen types I–IV, proteoglycan, fibronectin, platelet IIb/IIIa receptor, complement receptor, thrombomodulin, lung surfactant, and cadherins (Havemann and Gramse, 1984; Carden et al., 1998; Ginzberg et al., 2001; Ariel et al., 1998; Abe et al., 1994; Liau et al., 1996). Regarding plasma proteins, neutrophil elastase is able to cleave coagulation and complement factors, immunoglobulin, several proteases, and protease inhibitors (Havemann and Gramse, 1984; Okada et al., 1988), leading to their activation or loss of function. In addition to its proteolytic activity, neutrophil elastase is also known to induce the production of interleukin-6, interleukin-8, granulocyte–macrophage coronary-stimulating factor (Bedard et al., 1993; Nakamura et al., 1992) and mucin from epithelial cells (Sommerhoff et al., 1990), and the release of transforming growth factor-β which binds to extracellular matrix (Taipale et al., 1995). Interestingly, neutrophil elastase-degraded fragments such as those from fibrin (Cepinskas et al., 1997) and laminin (Steadman et al., 1993) are known to be chemotactic toward neutrophils. It has also been shown that neutrophil elastase binds to integrin CR3 (Cai and Wright, 1996) and cleaves intercellular adhesion molecule-1 (Champagne et al., 1998), thereby promoting neutrophil migration from adhesion sites.

Whether the proteolytic activity of neutrophil elastase has any pathophysiological importance is largely unknown. However, as opposed to the classical notion that neutrophil elastase is a pro-inflammatory factor, recent studies suggest that neutrophil elastase down-regulates the inflammatory process. Neutrophil elastase is capable of degrading various pro-inflammatory cytokines such as interleukin-1, tumor necrosis factor (Owen et al., 1997), interleukin-2 (Ariel et al., 1998) and interleukin-6 (Bank et al., 1999) and, as noted

Table 1
Natural substrates and target cells of neutrophil elastase

Class	Proteins/target cells	General function
Matrix proteins	Elastin, collagen (types I–IV), proteoglycan Thrombomodulin, Lung surfactant, cadoherins	loss of function
	Fibronectin	neutrophil chemotaxis
Plasma proteins	Coagulation factors (V, VIII, XI, XIII)	loss of function
	Complement factors (C1s, C2–5, C9) C1-inactivator	activation of complement cascades
	Fibrinogen, α_2-plasmin	neutrophil chemotaxis
Immunoglobulins	IgG, IgM	activation of neutrophil and macrophage
Proteases	Kininogen, collagenase, Gelatinase	activation of enzymes
Protease inhibitor	TIMP[a]	loss of function
Cytokines	IL[b]-1, -2 and -6, TNFα[c]	loss of function
Others	Platelet IIb/IIIa receptor, ICAM-1[d]	loss of function
	Lymphocyte	activation
	Platelet	platelet aggregation
	Epithelial cell	release of IL-6, IL-8 and GM-CSF[e] release of TGFβ[f] and mucin

[a] Tissue inhibitor of metalloprotease.
[b] Interleukin.
[c] Tumor necrosis factor.
[d] Intercellular adhesion molecule-1.
[e] Granulocyte-macrophage coronary-stimulating factor.
[f] Transforming growth factor beta.

above, is also able to release soluble complement receptors that can inhibit complement activation. Furthermore, neutrophil elastase-induced release of transforming growth factor-β, an anti-inflammatory cytokine (Ulich et al., 1991), may be important in the remodeling of inflammation. Conversely, mice lacking the gene for an endogenous protease inhibitor (secretory leukocyte protease inhibitor) exhibit increased elastase activity and tissue inflammation, along with delayed wound healing (Ashcroft et al., 2000). Thus, an artificially elevated elastase activity promotes inflammation and delays wound healing. The pathophysiological relevance of neutrophil elastase-mediated substrate degradation has been reviewed in more detail elsewhere (Lee and Downey, 2001).

2.3. Endogenous protease inhibitors

Body fluids contain high concentrations of multiple protease inhibitors, which both locally, and in circulation, play an important role in protecting against unregulated proteolysis by neutrophil elastase (Table 2). Among these inhibitors, α_1-protease inhibitor, α_2-macroglobulin, and secretory leukocyte protease inhibitor are important in their abundance and characteristics (Travis and Salvesen, 1983; Bieth, 1984). These inhibitors form complexes with neutrophil elastase in 1:1 or, in the case of α_2-macroglobulin, 1:2 molar ratios, thereby inhibiting its enzymatic activity. In the plasma, α_1-protease inhibitor is the major inhibitor of neutrophil elastase with the highest concentration and inhibitory potency, accounting for approximately 92% neutrophil elastase inhibition. The remaining neutrophil elastase inhibition is attributed to α_2-macroglobulin (Ohlsson and Olsson, 1974); however, the mechanism of this inhibition is totally different from that of α_1-protease inhibitor. Whereas α_1-protease inhibitor irreversibly inhibits neutrophil elastase by forming a stable acylenzyme–inhibitor complex through its catalytic site, α_2-macroglobulin stereochemically inhibits neutrophil elastase by trapping it inside itself. Eventually, the trapped neutrophil elastase largely loses its proteolytic activity against macromolecular substrates, but is still able to hydrolyze some native proteins, particularly small substrates, at its unoccupied catalytic site (Travis and Salvesen, 1983). Whether this remaining proteolytic activity has any pathophysiological significance is not clear; however, evidence suggests that part of the tissue degradation in emphysema (Stone et al., 1982) and rheumatoid arthritis (Moore et al., 1999) can be attributed to the proteolytic activity of the α_2-macroglobulin–neutrophil elastase complex. As compared to these two main inhibitors, secretory leukocyte protease inhibitor is a smaller protease inhibitor (10.5 kDa), localized at the cytoplasm of bronchial gland serous cells (Mooren et al., 1982). Due to its smaller size, secretory leukocyte protease inhibitor has easier access to tight spaces as compared to plasma macromolecular inhibitors (α_1-protease inhibitor and α_2-macroglobulin), and a recent study has suggested that secretory leukocyte protease inhibitor plays an important role in protecting against neutrophil elastase-mediated tissue injury in the lower respiratory tract (Owen et al., 1995).

Table 2
Typical endogenous protease inhibitors for neutrophil elastase

	α_1PI[a]	α_2M[b]	SLPI[c]
Molecular weight (kDa)	55	725	10.5
Concentration (μmol/l plasma)	54	3	1.4[d]
Kon (M^{-1} S^{-1})	6×10^7	4×10^7	1.2×10^7

[a] α_1-Protease inhibitor.
[b] α_2-Macroglobulin.
[c] Secretory leukocyte protease inhibitor.
[d] Concentration in bronchial secretion.

2.4. Mechanism by which neutrophil elastase escapes the action of endogenous protease inhibitors

Clearly, the physiological function of endogenous protease inhibitors is to provide an anti-neutrophil elastase screen so that overwhelming neutrophil elastase does not injure host tissue. Given the effectiveness and multiplicity of this anti-neutrophil elastase shield, it seems unlikely that neutrophil elastase mediates extracellular tissue injury. In fact, the calculated half-life of active neutrophil elastase is only about 0.6 ms, and by 3 ms, all neutrophil elastase activity is inhibited in vivo (Travis and Salvesen, 1983). Moreover, the concentration of α_1-protease inhibitor (15–30 μM) is eightfold in excess of its K_i value (3.3×10^{-14} M) for neutrophil elastase (Beatty et al., 1984). However, it was already recognized almost 80 years ago that isolates from purulent fluids recovered from inflammation sites are capable of degrading a variety of native proteins (Opie, 1922).

Current notion holds that neutrophil elastase at inflammatory sites is able to escape from regulation by multiple protease inhibitors in several ways. First, these protease inhibitors have high molecular weights and cannot enter the microenvironment between neutrophils and their substrate tissues due to stereochemical limitations. In fact, it has been shown that proteolysis by neutrophil-associated neutrophil elastase is not fully inhibited by treatment with either high molecular weight endogenous protease inhibitors or plasma, whereas free neutrophil elastase is totally blocked by either treatment. A low molecular weight neutrophil elastase inhibitor is able to block proteolysis even in such a microenvironment (Campbell and Campbell, 1988; Campbell et al., 1982; Weitz et al., 1986). The second blocking mechanism is that α_1-protease inhibitor is inactivated through oxidation of its catalytic center (methionine residue in position 358) by reactive oxygen species derived from activated neutrophils (Matheson et al., 1979). It was subsequently found that this mechanism also inactivates the endogenous protease inhibitors, α_2-macroglobulin and secretory leukocyte protease inhibitor (Weiss, 1989; Kramps et al., 1987). The third mechanism is the impairment of

inhibitory activity against tissue-bound neutrophil elastase (Morrison et al., 1990; Kawabata et al., 1996). This proposed mechanism comes from experiments showing that endogenous protease inhibitors are not fully effective against tissue-bound neutrophil elastase, yet are fully able to inhibit the activity of the free enzyme (Kawabata et al., 1996). Interestingly, in these experiments, the inhibitory activity of endogenous protease inhibitors was related to their molecular weight, suggesting that molecular mass is the main factor for activity impairment in these inhibitors. Low molecular weight neutrophil elastase inhibitors were fully active against tissue-bound and free forms of neutrophil elastase. It has also been shown that endogenous protease inhibitors are not fully effective to inhibit neutrophil elastase bound to the surface of neutrophils (Owen et al., 1995). In addition to the mechanisms mentioned above, a previously proposed mechanism suggests that a locally overwhelming amount of neutrophil elastase shifts the balance in favor of neutrophil elastase attack on tissue; however, until quite recently, there was little experimental support for the existence of this mechanism. Campbell et al. (1999) suggest that a quantum burst of neutrophil granules provides locally a very high concentration of neutrophil elastase that overwhelms the anti-neutrophil elastase screen, resulting in lung damage in patients with α_1-protease inhibitor deficiency. These mechanisms, collectively, may lead to a local imbalance between neutrophil elastase and endogenous protease inhibitors that finally enables neutrophil elastase to escape regulation.

This evidence that the inhibitory activity of endogenous protease inhibitors is impaired at inflammatory sites emphasizes the importance of synthetic neutrophil elastase inhibitors without the size, steric, and mechanistic limitations of endogenous protease inhibitors. As a result, many classes of synthetic neutrophil elastase inhibitors have been developed with the aim of regulating neutrophil elastase activity at inflammatory sites. Details of these synthetic neutrophil elastase inhibitors can be found in some of the recent reviews (William and Norton, 1999; Edwards and Bernstein, 1994).

3. Neutrophil elastase and acute lung injury

Acute lung injury is a syndrome that results from a variety of unrelated insults, including infection by Gram-negative bacteria. The most severe form, adult respiratory distress syndrome, is a high mortality disease that resists most therapies. The pathophysiological characteristics of this disease include interstitial and alveolar edema associated with a large amount of neutrophil infiltration into these spaces (Wiener-Kronish et al., 1990). Recent studies suggest that neutrophil elastase plays an important role in the pathophysiology of acute lung injury. However, to verify this hypothesis, the following must be examined: (1) whether neutrophil elastase levels are increased in clinical or in animal models of acute lung injury; (2) whether application of neutrophil elastase induces typical symptoms of acute lung injury in vitro or in vivo; and (3) whether inhibition of increased neutrophil elastase activity reduces symptoms of acute lung injury.

3.1. Are neutrophil elastase levels increased in clinical and animal models of acute lung injury?

Over the last two decades, large amounts of data have accumulated regarding neutrophil elastase concentration (i.e., neutrophil elastase complexed with α_1-protease inhibitor) and/or, in some cases, neutrophil elastase activity in clinical acute lung injury. An early study using 23 adult respiratory distress syndrome and 55 nonadult respiratory distress syndrome patients demonstrated that neutrophil elastase activity was significantly increased in bronchoalveolar lavage fluid from the patients with adult respiratory distress syndrome (Lee et al., 1981). However, two subsequent smaller studies failed to detect any neutrophil elastase activity in bronchoalveolar lavage fluid (Idell et al., 1985; Weiland et al., 1986). Interestingly, in both studies, an increase in neutrophil elastase–α_1-protease inhibitor complex was detected and, in the case of Idell et al. (1985), a highly positive correlation between the concentration of neutrophil elastase–α_1-protease inhibitor complex and pulmonary function (Aa-PO_2) was observed. More recent studies measuring plasma neutrophil elastase–α_1-protease inhibitor complex have consistently shown that neutrophil elastase is increased in acute lung injury/adult respiratory distress syndrome patients with, in some cases, a positive correlation between the severity of the disease [PaO_2/FiO_2 (Rocker et al., 1989), respiratory index (Donnelly et al., 1995)] and organ failure (Tonz et al., 1995).

Generally, discrepancies are mostly seen in studies measuring bronchoalveolar lavage fluid components. In addition to earlier studies showing that neutrophil elastase activity is not always increased in bronchoalveolar lavage fluid from patients with adult respiratory distress syndrome, a recent study using 38 adult respiratory distress syndrome and 28 nonadult respiratory distress syndrome patients failed to show any positive correlation between neutrophil elastase–α_1-protease inhibitor complex in bronchoalveolar lavage fluid and disease severity as indicated by bronchoalveolar lavage fluid protein concentration and PaO_2/FiO_2 value (Mathy-Hartert et al., 2000). Reasons that may explain these discrepancies include: the diversity of patients' background, the stage of sampling, and the technical difference between systemic and local measurement. For example, in contrast to a plasma measurement, a bronchoalveolar lavage fluid measurement has the advantage of directly measuring neutrophil elastase release in local lung space. However, the results from humans are highly dependent on the specific site of lavage, in contrast with bronchoalveolar lavage fluid measurements from small animals where whole lungs are usually lavaged. In addition to these technical issues, the substrate used in

neutrophil elastase activity measurement should be considered. As has been shown by Wewers et al. (1988), neutrophil elastase recovered in bronchoalveolar lavage fluid from patients with adult respiratory distress syndrome is largely bound to α_1-protease inhibitor or α_2-macroglobulin. Whereas the amount of neutrophil elastase bound to α_1-protease inhibitor is measurable as neutrophil elastase–α_1-protease inhibitor complex, the activity of neutrophil elastase bound to α_2-macroglobulin is not always measurable. This is because the neutrophil elastase–α_2-macroglobulin complex retains proteolytic activity against low molecular weight substrates (Lee et al., 1981) but loses most of its activity against high molecular weight substrates such as elastin (Idell et al., 1985; Weiland et al., 1986). Thus, whether neutrophil elastase activity is detected in bronchoalveolar lavage fluid from patients with adult respiratory distress syndrome is largely attributable to the type of substrate used in each study.

The next question that arises is whether increase neutrophil elastase activity by neutrophil elastase–α_2-macroglobulin complex is correlated with the severity of acute lung injury and/or the proteolytic degradation of intact tissues. Regarding acute lung injury, our data show that neutrophil elastase activity in bronchoalveolar lavage fluid, as detected by chromogenic substrates, was markedly increased in a hamster model of acute lung injury induced by endotoxin inhalation, and that this activity correlates highly with the increase in protein concentration in bronchoalveolar lavage fluid (Kawabata et al., 2000). Similarly, when acute lung injury was induced by intravenous injection of cobra venom factor, plasma neutrophil elastase activity increased rapidly and an increase in lung permeability followed (Hagio et al., 2001) (Fig. 1). It is interesting to note that in endotoxin-induced acute lung injury, neutrophil elastase activity in bronchoalveolar lavage fluid increased but that in plasma did not, whereas in cobra venom factor-induced acute lung injury, the reverse happened. Thus, the target tissue of neutrophil elastase may differ depending on the type of insult leading to acute lung injury. Regarding the proteolytic degradation of intact tissues, we have noted that increased neutrophil elastase activity in bronchoalveolar lavage fluid correlates with desmosine concentration in it in NO_2-exposed hamster models of acute lung injury, both of which also correlate with increased protein concentration in bronchoalveolar lavage fluid (Nakao et al., unpublished results). This evidence suggests that increased neutrophil elastase activity in the neutrophil elastase–α_2-macroglobulin complex correlates significantly with acute lung injury and host tissue degradation.

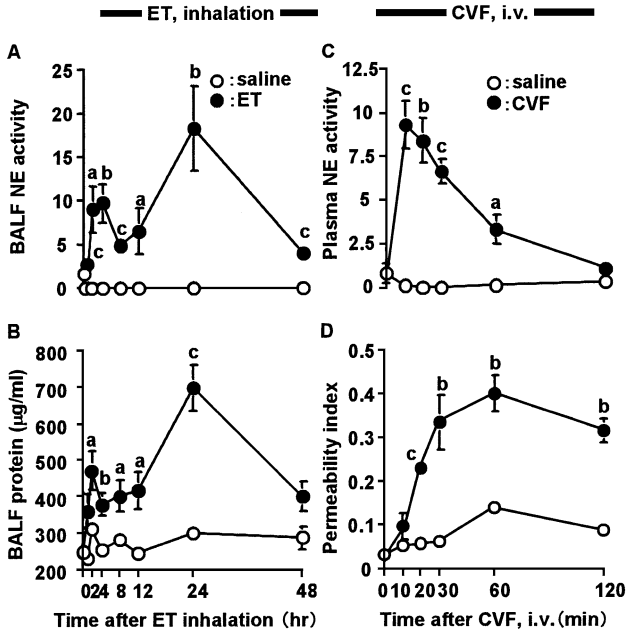

Fig. 1. Changes in neutrophil elastase (NE) activity in hamster models of acute lung injury. Panels A and B show changes in bronchoalveolar lavage fluid (BALF)-NE activity and protein concentration after 30-min endotoxin (ET, 300 μg/ml) inhalation. Each point in panels A and B represents the mean ± S.E.M. for eight to nine animals. Panels C and D show changes in plasma NE activity and lung permeability index after intravenous injection of cobra venom factor (CVF, 10 U/kg) and [^{125}I]-bovine serum albumin. Lung permeability index is expressed as the ratio of radioactivity in lung tissue to that in aortic blood. Each point in panels C and D represents the mean ± S.E.M. for three to four animals. Neutrophil elastase activity was measured with a synthetic substrate, *N*-methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide and is expressed as n mole *p*-nitroanilide release/ml/24 hr. $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$ vs. relevant saline-control group (Student's *t*-test). Modified from Kawabata et al. (2000) and Hagio et al. (2001).

3.2. Does experimental application of neutrophil elastase produces typical symptoms of acute lung injury?

The pathophysiology of acute lung injury is complex and the full spectrum of this condition cannot be entirely attributed to neutrophil elastase. Nonetheless, some acute pathophysiological changes, including interstitial and alveolar edema, intra-alveolar hemorrhage and underlying neutrophil infiltration (Tomashefski, 2000), can be induced by topical neutrophil elastase application. Early studies indicated that intratracheal instillation of neutrophil elastase in hamsters induced hemorrhagic lung edema associated with neutrophil infiltration (Senior et al., 1977) that progressed to emphysema. It was also demonstrated that similar injury was also induced by intravenous injection of pancreatic elastase in hamsters (Schuyler et al., 1978) and in minipigs (Stokke et al., 1986) although the enzyme used in these studies was from pancreas. Interestingly, in the later case, minipigs exhibited progressive respiratory failure with a prompt increase in pulmonary vascular resistance, a decrease in cardiac output, and lung neutrophilia. Thus, pulmonary inflammatory lung edema is induced directly by intratracheal instillation of neutrophil elastase and systemically by intravenous infusion of pancreatic elastase.

More recent studies have focused on the effect of neutrophil elastase either on the organ or cellular level. Using isolated cell-free perfused rabbit lungs, Koch et al.

(1992) showed that elastase most potently increases the capillary filtration coefficient as compared with two other inducers, positive hydrostatic pressure and arachidonic acid. At the cellular level, Suttorp et al. (1993) showed that neutrophil elastase increases the hydraulic conductivity of cultured endothelial cell monolayers, probably by widening intercellular gaps. Another study using cultured epithelial cell monolayers (Peterson et al., 1995) demonstrated that neutrophil elastase increases epithelial cell permeability by charge-dependent mechanisms. Raats et al. (2000) recently suggested that neutrophil elastase increases basement membrane permeability by disrupting the integrity of heparan sulphate proteoglycans. These studies show that neutrophil elastase increases the solute permeability of endothelial and epithelial cells and the basement membrane barrier, all of which are important in the development of interstitial and alveolar edema (Carden et al., 1998).

3.3. Does inhibition of increased neutrophil elastase activity reduces symptoms of acute lung injury?

The development of neutrophil elastase inhibitors has significantly increased our knowledge of the role of neutrophil elastase in acute lung injury. Over the last decade, neutrophil elastase inhibitors have been used in many animal studies. These inhibitors include chloromethylketone (Powers et al., 1977), SC-39026 (Nakao et al., 1987), L-658,758 (Finke et al., 1992), ICI-200,355 (Sommerhoff et al., 1991), and ONO-5046 (Kawabata et al., 1991). Systemic treatment with these inhibitors is consistently effective in different animal models of acute lung injury induced by various insults including endotoxin, reperfusion, thrombin, and phorbol myristate acetate. For example, chloromethylketone ameliorated the increase in [^{125}I]-labeled lung albumin uptake following an intraperitoneal endotoxin plus intravenous *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine challenge in rats (Anderson et al., 1990). Chloromethylketone was also effective against the increase in bronchoalveolar lavage fluid protein after hind limb ischemia reperfusion in rats (Welbourn et al., 1991). Similar efficacy against reperfusion-associated acute lung injury (as indicated by an increase in capillary filtration coefficient) was observed with L-658,758 in isolated rat lung (Carden et al., 1998). In a sheep model of endotoxin-induced lung function, SC-39026 attenuated the increase in lung lymph flow and lung lymph protein clearance (Gossage et al., 1993). A more recent study showed that ICI-200,355 reduced thrombin-induced pulmonary edema in rats with evidence of a decrease in lung neutrophilia (Ahn et al., 1994). Together, results of these studies indicate that treatment with neutrophil elastase inhibitors can reduce the severity of acute lung injury in several animal models.

In addition to the above studies, those using ONO-5046 (LY544349, Sivelestat) may be particularly useful for understanding the role of neutrophil elastase. Sivelestat is highly specific to neutrophil elastase, is systemically potent, and attenuates symptoms of acute lung injury in a variety of animal models, including those induced by systemic and local endotoxin challenge in sheep (Kubo et al., 1994) and guinea pig (Sakamaki et al., 1996), by injection of phorbol myristate acetate (Wang et al., 1999) and triolein (Nakata and Dahms, 2000), and by reperfusion (Kishima et al., 1998). Our results with Sivelestat indicate that this neutrophil elastase inhibitor neither inhibits other proteases, including those in the serine, cystein and matrix metalloprotease families, nor affects many of the inflammatory mediators, such as reactive oxygen species (Iwamura et al., 1993) and cytokines (Table 3), all of which have been implicated in the development of acute lung injury (Wiener-Kronish

Table 3
In vitro profile of Sivelestat

Molecules	Experimental system	Results
Serine protease		
Human neutrophil elastase	synthetic substrate/ purified enzyme	K_i = 46 nM
Hamster neutrophil elastase	synthetic substrate/ neutrophil extract	K_i = 34 nM
Porcine pancreatic elastase	synthetic substrate/ purified enzyme	K_i = 3800 nM
Thrombin, trypsin, plasmin, kallikrein	synthetic substrate/ purified enzyme	IC_{50} > 500 μM
Cystein protease		
Cathepsin B, ICE[a]	native substrate/ semi-purified enzyme	IC_{50}>500 μM
MMPs		
Gelatinase, collagenase, stromelysin	native substrate/ semi-purified enzyme	IC_{50}>500 μM
Macrophage elastase		
P. aeruginosa elastase		
Other enzymes		
Cyclooxygenase I, II	AA[b]/semi-purified enzyme	IC_{50}>300 μM
5,15-Lipoxygenase	AA/purified enzyme	IC_{50}>300 μM
Cytokine production		
IL-1α, TNFβ, IL-6, IL-8	PBMC[c]/ endotoxin	IC_{50}>300 μM
Expression of adhesion molecule		
E-selectin, ICAM-1[d], VCAM-1[e]	HUVEC[f]/TNFα	IC_{50}>100 μM
Production of reactive oxygen species		
H_2O_2	neutrophil/PMA[g]	IC_{50}>200 μM

[a] Interleukin-1 converting enzyme.
[b] Arachidonic acid.
[c] Peripheral blood mononuclear cell.
[d] Intracellular adhesion molecule.
[e] Vascular cell adhesion molecule.
[f] Human umbilical vein endothelial cells.
[g] Phorbol myristate acetate.

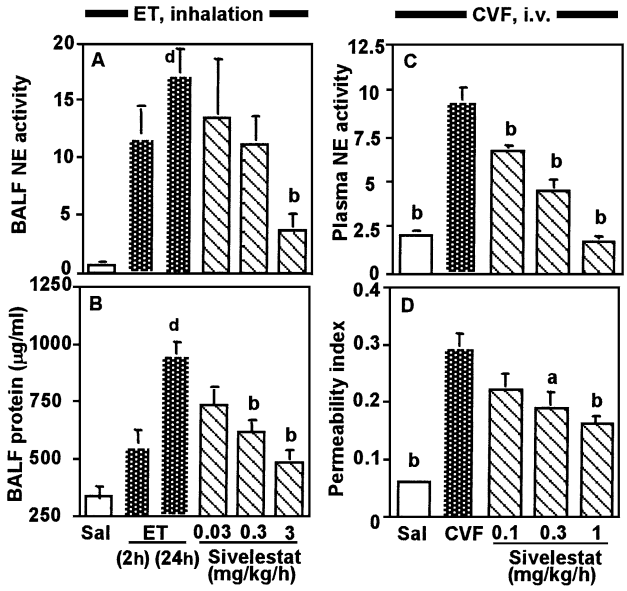

Fig. 2. Effect of neutrophil elastase inhibition (NE) on lung edema in hamster models of acute lung injury. Panels A and B show the effect of Sivelestat on BALF-NE activity and protein concentration 24 h after inhalation of endotoxin (ET). Sivelestat was continuously infused 2–24 h after ET inhalation. Each bar in panels A and B represents the mean ± S.E.M. for 11–12 animals. Panels C and D show the effect of Sivelestat on plasma NE activity and lung permeability index 30 min after intravenous injection of cobra venom factor (CVF) and [^{125}I]-bovine serum albumin. Lung permeability index is expressed as the ratio of radioactivity in lung tissue to that in aortic blood. Sivelestat was continuously infused 90 min before to 30 min after injection of CVF. Each bar in panels C and D represents the mean ± S.E.M. for five to nine animals. $^{d}P<0.001$ vs. saline-inhaled group (Student's *t*-test). $^{a}P<0.05$ and $^{b}P<0.01$ vs. ET-inhaled or CVF-injected group (Dunnett's *t*-test). Modified from Kawabata et al. (2000) and Hagio et al. (2001).

et al., 1990). Furthermore, when continuously infused to hamsters, Sivelestat, at 0.1 mg/kg/h or higher doses, was able to dose-dependently inhibit increased neutrophil elastase activity in peripheral blood activated ex vivo with opsonized zymosan. In hamsters, the inhibition of elevated neutrophil elastase activity in bronchoalveolar lavage fluid by Sivelestat at 0.3 mg/kg/h or higher doses attenuated both the increase in protein concentration and neutrophilia in bronchoalveolar lavage fluid after endotoxin inhalation. Similarly, by inhibiting the elevation of plasma neutrophil elastase activity, Sivelestat ameliorated the increase in lung vascular permeability, as indicated by vascular-lung [^{125}I] albumin ratio as well as by protein concentration in bronchoalveolar lavage fluid (Fig. 2) after cobra venom factor injection. Thus, specific inhibition of increased neutrophil elastase activity can actually reduce the symptoms of acute lung injury, particularly in endotoxin-induced models, even when the infusion was started after the onset of acute lung injury, suggesting that inhibition of neutrophil elastase is therapeutically useful in the treatment of acute lung injury. Furthermore, results from a multicenter, double-blind Phase II study (Ogawa et al., 1997) in 179 patients with systemic inflammatory response syndrome showed that Sivelestat, at 0.2 mg/kg/h, administered for 5 days, effectively reduced acute lung injury as indicated by Mauuray's lung injury score. Using a stratified small size analysis (patients with three or less dysfunctional organs, including lung injury), Sivelestat improved survival rate in the 5-day administration period. Sivelestat is approved in Japan for the treatment of acute lung injury in patients with systemic inflammatory response syndrome.

4. Mechanism by which neutrophil elastase might induce acute lung injury

As mentioned earlier in this article, a substantial amount of evidence supports the current hypothesis that neutrophil elastase plays an important role in the pathophysiology of acute lung injury, although the precise mechanism by which neutrophil elastase contributes to acute lung injury remains speculative. This is because the multisubstrate specificity of neutrophil elastase hampers the identification of its primary target protein in vivo.

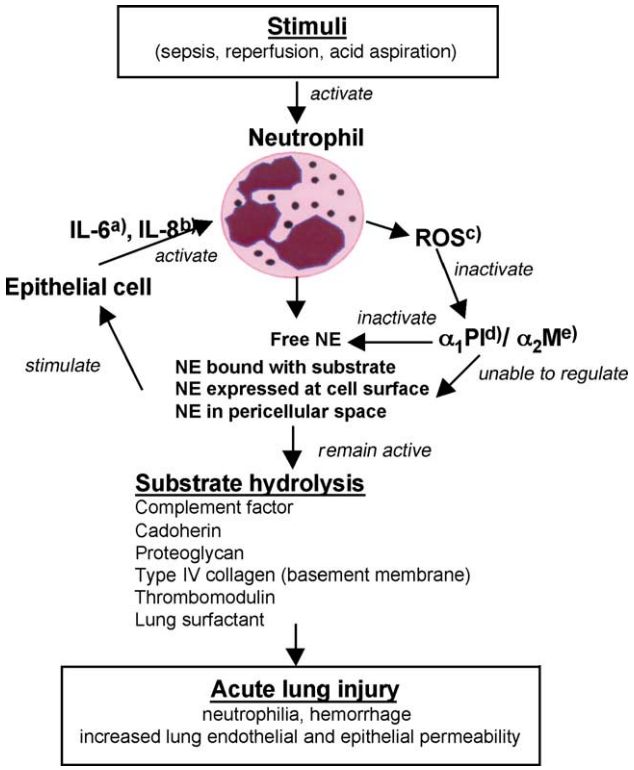

Fig. 3. Proposed mechanism by which neutrophil elastase (NE) induces acute lung injury. Neutrophil elastase is released in response to multiple stimuli and is able to remain active due to several mechanisms. Unregulated neutrophil elastase activity would then hydrolyze several substrates, leading to pathophysiological changes that are characteristics of acute lung injury. Neutrophil elastase may indirectly modulate lung inflammation by stimulating the production of pro-inflammatory cytokines. [a)]interleukin-6, [b)]interleukin-8, [c)]reactive oxygen species, [d)]α_1-protease inhibitor, [e)]α_2-macroglobulin.

Recent studies have suggested that cell junction proteins, such as the cadoherins in endothelial (Carden et al., 1998) and epithelial cells (Ginzberg et al., 2001), thrombomodulin (Miyazaki et al., 1998) and proteoglycans in basement membrane (Raats et al., 2000) and in lung interstitium (Passi et al., 1998), are responsible for the increase in alveolocapillary permeability either by disrupting the function of the lung permeability barrier or by directly damaging it. Neutrophil elastase may also play a critical role in lung inflammation, either by influencing neutrophil chemotaxis and cell adhesion or by inducing the release of inflammatory mediators, such as interleukin-8, granulocyte–macrophage coronary-stimulating factor, and complement factors. While still a speculation, a possibility exists that neutrophil elastase may also contribute to a decrease in respiratory function, not only by inducing alveolar edema but also by damaging alveolar surfactant (Liau et al., 1996). The proposed mechanisms by which neutrophil elastase might contribute to the pathophysiology of acute lung injury are summarized in Fig. 3.

It should also be noted that neutrophil elastase is not the sole factor responsible for lung edema. In fact, Miyazaki et al. (1998) showed that treatment with Sivelestat was less effective to preventing transendothelial cell permeability than transepithelial cell permeability using tumor necrosis factor-α and phorbol myristate acetate-activated neutrophils in isolated perfused rabbit lungs. We have also noted that Sivelestat partially reduces the increase in the lung microvascular permeability index (up to 60%) but totally blocks the increase in bronchoalveolar lavage fluid protein in hamsters injected with cobra venom factor (Hagio et al., 2001). Additionally, Sivelestat did not reduce the acute lung injury induced by air embolism in sheep (Miyahara et al., 1996). Therefore, other factors may also contribute to the increase in alveocapillary permeability in acute lung injury. Additional studies, including the identification of target proteins of neutrophil elastase, would be useful for understanding more precisely the role of neutrophil elastase in the pathophysiology of acute lung injury.

5. Conclusion

Results from the studies discussed in this review indicate that: (1) neutrophil elastase levels are increased in clinical and animal models of acute lung injury, (2) topical or systemic application of neutrophil elastase produces typical symptoms of acute lung injury both in vitro and in vivo, and (3) inhibition of increased neutrophil elastase activity reduces symptoms of acute lung injury. This evidence strongly supports the current hypothesis that neutrophil elastase plays an important role in the pathophysiology of acute lung injury. It is hoped that a better understanding of the role of neutrophil elastase in the pathophysiology of acute lung injury will lead to more efficacious treatment for this complicated and therapy-resistant disease.

References

Abe, H., Okajima, K., Okabe, H., Takatsuki, K., Binder, B.R., 1994. Granulocyte proteases and hydrogen peroxide synergistically inactivate thrombomodulin of endothelial cells in vitro. J. Lab. Clin. Med. 123, 874–881.

Ahn, C.M., Sandler, H., Wegener, T., Saldeen, T., 1994. Effect of ibuprofen on thrombin-induced pulmonary edema in the rat. Pulm. Pharmacol. 7, 393–399.

Anderson, B.O., Brown, J.M., Bensard, D.D., Grosso, M.A., Banerjee, A., Patt, A., Whitman, G.J., Harken, A.H., 1990. Reversible lung neutrophil accumulation can cause lung injury by elastase-mediated mechanisms. Surgery 108, 262–267.

Ariel, A., Yavin, E.J., Hershkoviz, R., Avron, A., Franitza, S., Hardan, I., Cahalon, L., Fridkin, M., Lider, O., 1998. IL-2 induces T cell adherence to extracellular matrix: inhibition of adherence and migration by IL-2 peptides generated by leukocyte elastase. J. Immunol. 161, 2465–2472.

Ashcroft, G.S., Lei, K., Jin, W., Longenecker, G., Kulkarni, A.B., Greenwell-Wild, T., Hale-Donze, H., McGrady, G., Song, X.Y., Wahl, S.M., 2000. Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. Nat. Med. 6, 1147–1153.

Bank, U., Kupper, B., Reinhold, D., Hoffmann, T., Ansorge, S., 1999. Evidence for a crucial role of neutrophil-derived serine proteases in the inactivation of interleukin-6 at sites of inflammation. FEBS Lett. 461, 235–240.

Beatty, K., Matheson, N., Travis, J., 1984. Kinetic and chemical evidence for the inability of oxidized alpha 1-proteinase inhibitor to protect lung elastin from elastolytic degradation. Hoppe-Seyler Z. Physiol. Chem. 365, 731–736.

Bedard, M., McClure, C.D., Schiller, N.L., Francoeur, C., Cantin, A., Denis, M., 1993. Release of interleukin-8, interleukin-6, and colony-stimulating factors by upper airway epithelial cells: implications for cystic fibrosis. Am. J. Respir. Cell Mol. Biol. 9, 455–462.

Bieth, J.G., 1984. In vivo significance of kinetic constants of macromolecular proteinase inhibitors. Adv. Exp. Med. Biol. 167, 97–109.

Bieth, J.G., 1986. In: Mecham, R.P. (Ed.), Regulation of Matrix Accumulation. Academic Press, New York, p. 217.

Cai, T.Q., Wright, S.D., 1996. Human leukocyte elastase is an endogenous ligand for the integrin CR3 (CD11b/CD18, Mac-1, alpha M beta 2) and modulates polymorphonuclear leukocyte adhesion. J. Exp. Med. 184, 1213–1223.

Campbell, E.J., Campbell, M.A., 1988. Pericellular proteolysis by neutrophils in the presence of proteinase inhibitors: effects of substrate opsonization. J. Cell Biol. 106, 667–676.

Campbell, E.J., Senior, R.M., McDonald, J.A., Cox, D.L., 1982. Proteolysis by neutrophils. Relative importance of cell-substrate contact and oxidative inactivation of proteinase inhibitors in vitro. J. Clin. Invest. 70, 845–852.

Campbell, E.J., Campbell, M.A., Boukedes, S.S., Owen, C.A., 1999. Quantum proteolysis by neutrophils: implications for pulmonary emphysema in alpha 1-antitrypsin deficiency. J. Clin. Invest. 104, 337–344.

Carden, D., Xiao, F., Moak, C., Willis, B.H., Robinson-Jackson, S., Alexander, S., 1998. Neutrophil elastase promotes lung microvascular injury and proteolysis of endothelial cadherins. Am. J. Physiol. 275, H385–H392.

Cepinskas, G., Noseworthy, R., Kvietys, P.R., 1997. Transendothelial neutrophil migration. Role of neutrophil-derived proteases and relationship to transendothelial protein movement. Circ. Res. 81, 618–626.

Champagne, B., Tremblay, P., Cantin, A., St Pierre, Y., 1998. Proteolytic cleavage of ICAM-1 by human neutrophil elastase. J. Immunol. 161, 6398–6405.

Donnelly, S.C., MacGregor, I., Zamani, A., Gordon, M.W., Robertson, C.E., Steedman, D.J., Little, K., Haslett, C., 1995. Plasma elastase levels and the development of the adult respiratory distress syndrome. Am. J. Respir. Crit. Care Med. 151, 1428–1433.

Du Bois, R.M., Bernaudin, J.F., Paakko, P., Hubbard, R., Takahashi, H., Ferrans, V., Crystal, R.G., 1991. Human neutrophils express the alpha 1-antitrypsin gene and produce alpha 1-antitrypsin. Blood 77, 2724–2730.

Edwards, P.D., Bernstein, P.R., 1994. Synthetic inhibitors of elastase. Med. Res. Rev. 14, 127–194.

Finke, P.E., Shah, S.K., Ashe, B.M., Ball, R.G., Blacklock, T.J., Bonney, R.J., Brause, K.A., Chandler, G.O., Cotton, M., Davies, P., Dellea, P.S., Dorn Jr., C.P., Fletcher, D.S., O'Grady, L.A., Hagmann, W.K., Hand, K.M., Knight, W.B., Maycock, A.L., Mumford, R.A., Osinga, D.G., Sohar, P., Thompson, K.R., Weston, H., Doherty, J.B. 1992. Inhibition of human leukocyte elastase: 4. Selection of a substituted cephalosporin (L-658,758) as a topical aerosol. J. Med. Chem. 35, 3731–3744.

Ginzberg, H.H., Cherapanov, V., Dong, Q., Cantin, A., McCulloch, C.A., Shannon, P.T., Downey, G.P., 2001. Neutrophil-mediated epithelial injury during transmigration: role of elastase. Am. J. Physiol. 281, G705–G717.

Gossage, J.R., Kuratomi, Y., Davidson, J.M., Lefferts, P.L., Snapper, J.R., 1993. Neutrophil elastase inhibitors, SC-37698 and SC-39026, reduce endotoxin-induced lung dysfunction in awake sheep. Am. Rev. Respir. Dis. 147, 1371–1379.

Hagio, T., Nakao, S., Matsuoka, H., Matsumoto, S., Kawabata, K., Ohno, H., 2001. Inhibition of neutrophil elastase activity attenuates complement-mediated lung injury in the hamster. Eur. J. Pharmacol. 426, 131–138.

Havemann, K., Gramse, M., 1984. Physiology and pathophysiology of neutral proteinases of human granulocytes. Adv. Exp. Med. Biol. 167, 1–20.

Idell, S., Kucich, U., Fein, A., Kueppers, F., James, H.L., Walsh, P.N., Weinbaum, G., Colman, R.W., Cohen, A.B., 1985. Neutrophil elastase-releasing factors in bronchoalveolar lavage from patients with adult respiratory distress syndrome. Am. Rev. Respir. Dis. 132, 1098–1105.

Iwamura, H., Moore, A.R., Willoughby, D.A., 1993. Interaction between neutrophil-derived elastase and reactive oxygen species in cartilage degradation. Biochim. Biophys. Acta 1156, 295–301.

Janoff, A., 1985. Elastase in tissue injury. Annu. Rev. Med. 36, 207–216.

Janoff, A., Scherer, J., 1968. Mediators of inflammation in leukocyte lysosomes: IX. Elastinolytic activity in granules of human polymorphonuclear leukocytes. J. Exp. Med. 128, 1137–1155.

Kawabata, K., Suzuki, M., Sugitani, M., Imaki, K., Toda, M., Miyamoto, T., 1991. ONO-5046, a novel inhibitor of human neutrophil elastase. Biochem. Biophys. Res. Commun. 177, 814–820.

Kawabata, K., Moore, A.R., Willoughby, D.A., 1996. Impaired activity of protease inhibitors towards neutrophil elastase bound to human articular cartilage. Ann. Rheum. Dis. 55, 248–252.

Kawabata, K., Hagio, T., Matsumoto, S., Nakao, S., Orita, S., Aze, Y., Ohno, H., 2000. Delayed neutrophil elastase inhibition prevents subsequent progression of acute lung injury induced by endotoxin inhalation in hamsters. Am. J. Respir. Crit. Care Med. 161, 2013–2018.

Kishima, H., Takeda, S., Miyoshi, S., Matsumura, A., Minami, M., Utsumi, T., Omori, K., Nakahara, K., 1998. Microvascular permeability of the non-heart-beating rabbit lung after warm ischemia and reperfusion: role of neutrophil elastase. Ann. Thorac. Surg. 65, 913–918.

Koch, T., Duncker, H.P., Rosenkranz, S., Neuhof, H., Van Ackern, K., 1992. Alterations of filtration coefficients in pulmonary edema of different pathogenesis. J. Appl. Physiol. 73, 2396–2402.

Kramps, J.A., Van Twisk, C., Dijkman, J.H., 1987. Oxidative inactivation of bronchial antileukoprotease by triggered polymorphonuclear leukocytes. Am. Rev. Respir. Dis. 135, 290, abstract.

Kubo, K., Kobayashi, T., Hayano, T., Koizumi, T., Honda, T., Sekiguchi, M., Sakai, A., 1994. Effects of ONO-5046, a specific neutrophil elastase inhibitor, on endotoxin-induced lung injury in sheep. J. Appl. Physiol. 77, 1333–1340.

Lavie, G., Zucker-Franklin, D., Franklin, E.C., 1980. Elastase-type proteases on the surface of human blood monocytes: possible role in amyloid formation. J. Immunol. 125, 175–180.

Lee, W.L., Downey, G.P., 2001. Leukocyte Elastase. Physiological functions and role in acute lung injury. Am. J. Respir. Crit. Care Med. 164, 896–904.

Lee, C.T., Fein, A.M., Lippmann, M., Holtzman, H., Kimbel, P., Weinbaum, G., 1981. Elastolytic activity in pulmonary lavage fluid from patients with adult respiratory-distress syndrome. N. Engl. J. Med. 304, 192–196.

Liau, T.G., Campbell, E.J., 1995. Nonisotropic enzyme–inhibitor interactions: a novel mechanism for quantum proteolysis by human neutrophils. Biochemistry 34, 16171–16177.

Liau, D.F., Yin, N.X., Huang, J., Ryan, S.F., 1996. Effects of human polymorphonuclear leukocyte elastase upon surfactant proteins in vitro. Biochim. Biophys. Acta 1302, 117–128.

Matheson, N.R., Wong, P.S., Travis, J., 1979. Enzymatic inactivation of human alpha-1-proteinase inhibitor by neutrophil myeloperoxidase. Biochem. Biophys. Res. Commun. 88, 402–409.

Mathy-Hartert, M., Damas, P., Nys, M., Deby-Dupont, G., Canivet, J.L., Ledoux, D., Lamy, M., 2000. Nitrated proteins in bronchoalveolar lavage fluid of patients at risk of ventilator-associated bronchopneumonia. Eur. Respir. J. 16, 296–301.

Meier, H.L., Heck, L.W., Schulman, E.S., MacGlashan Jr., D.W. 1985. Purified human mast cells and basophils release human elastase and cathepsin G by an IgE-mediated mechanism. Int. Arch. Allergy Appl. Immunol. 77 (1–2), 179–183.

Miyahara, T., Kubo, K., Koizumi, T., Kobayashi, T., Sekiguchi, M., 1996. Specific neutrophil elastase inhibitor does not attenuate acute lung injury induced by air embolism in awake sheep. Exp. Lung Res. 22, 613–625.

Miyazaki, Y., Inoue, T., Kyi, M., Sawada, M., Miyake, S., Yoshizawa, Y., 1998. Effects of a neutrophil elastase inhibitor (ONO-5046) on acute pulmonary injury induced by tumor necrosis factor alpha (TNFalpha) and activated neutrophils in isolated perfused rabbit lungs. Am. J. Respir. Crit. Care Med. 157, 89–94.

Moore, A.R., Appelboam, A., Kawabata, K., Da Silva, J.A., D'Cruz, D., Gowland, G., Willoughby, D.A., 1999. Destruction of articular cartilage by alpha 2 macroglobulin elastase complexes: role in rheumatoid arthritis. Ann. Rheum. Dis. 58, 109–113.

Mooren, H.W., Meyer, C.J., Kramps, J.A., Franken, C., Dijkman, J.H., 1982. Ultrastructural localization of the low molecular weight protease inhibitor in human bronchial glands. J. Histochem. Cytochem. 30, 1130–1134.

Morrison, H.M., Welgus, H.G., Stockley, R.A., Burnett, D., Campbell, E.J., 1990. Inhibition of human leukocyte elastase bound to elastin: relative ineffectiveness and two mechanisms of inhibitory activity. Am. J. Respir. Cell Mol. Biol. 2, 263–269.

Nakamura, H., Yoshimura, K., McElvaney, N.G., Crystal, R.G., 1992. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. J. Clin. Invest. 89, 1478–1484.

Nakao, A., Partis, R.A., Jung, G.P., Mueller, R.A., 1987. SC-39026, a specific human neutrophil elastase inhibitor. Biochem. Biophys. Res. Commun. 147, 666–674.

Nakata, Y., Dahms, T.E., 2000. Triolein increases microvascular permeability in isolated perfused rabbit lungs: role of neutrophils. J. Trauma 49, 320–326.

Ogawa, M., Guo, L., Yamaguchi, Y., Tamakuma, S., Hirasawa, H., Siba, T., Nakajima, M., 1997. Low-molecular synthetic neutrophil elastase inhibitor can prevent/improve lung injury caused by various complications. 4th International Congress on the Immune Consequences of Trauma, Shock and Sepsis, 4–8 March, Munich, Germany. Monduzzi Editore S.p.A., Bologna, Italy, pp. 931–934.

Ohlsson, K., Olsson, I., 1974. The neutral proteases of human granulocytes. Isolation and partial characterization of granulocyte elastases. Eur. J. Biochem. 42, 519–527.

Okada, Y., Watanabe, S., Nakanishi, I., Kishi, J., Hayakawa, T., Watorek, W., Travis, J., Nagase, H., 1988. Inactivation of tissue inhibitor of metal-

loproteinases by neutrophil elastase and other serine proteinases. FEBS Lett. 229, 157–160.

Opie, E.L., 1922. Intracellular digestion: the enzymes and anti-enzymes concerned. Physiol. Rev. 2 (255), 252–285.

Owen, C.A., Campbell, M.A., Sannes, P.L., Boukedes, S.S., Campbell, E.J., 1995. Cell surface-bound elastase and cathepsin G on human neutrophils: a novel, non-oxidative mechanism by which neutrophils focus and preserve catalytic activity of serine proteinases. J. Cell Biol. 131, 775–789.

Owen, C.A., Campbell, M.A., Boukedes, S.S., Campbell, E.J., 1997. Cytokines regulate membrane-bound leukocyte elastase on neutrophils: a novel mechanism for effector activity. Am. J. Physiol. 272, L385–L393.

Passi, A., Negrini, D., Albertini, R., De Luca, G., Miserocchi, G., 1998. Involvement of lung interstitial proteoglycans in development of hydraulic- and elastase-induced edema. Am. J. Physiol. 275, L631–L635.

Peterson, M.W., Walter, M.E., Nygaard, S.D., 1995. Effect of neutrophil mediators on epithelial permeability. Am. J. Respir. Cell Mol. Biol. 13, 719–727.

Powers, J.C., Gupton, B.F., Harley, A.D., Nishino, N., Whitley, R.J., 1977. Specificity of porcine pancreatic elastase, human leukocyte elastase and cathepsin G. Inhibition with peptide chloromethyl ketones. Biochim. Biophys. Acta 485, 156–166.

Raats, C.J., Van Den Born, J., Berden, J.H., 2000. Glomerular heparan sulfate alterations: mechanisms and relevance for proteinuria. Kidney Int. 57, 385–400.

Rocker, G.M., Wiseman, M.S., Pearson, D., Shale, D.J., 1989. Diagnostic criteria for adult respiratory distress syndrome: time for reappraisal. Lancet 1 (8630), 120–123.

Sakamaki, F., Ishizaka, A., Urano, T., Sayama, K., Nakamura, H., Terashima, T., Waki, Y., Tasaka, S., Hasegawa, N., Sato, K., Nakagawa, N., Obata, T., Kanazawa, M., 1996. Effect of a specific neutrophil elastase inhibitor, ONO-5046, on endotoxin-induced acute lung injury. Am. J. Respir. Crit. Care Med. 153, 391–397.

Schuyler, M.R., Rynbrandt, D.J., Kleinerman, J., 1978. Physiologic and morphologic observations of the effects of intravenous elastase on the lung. Am. Rev. Respir. Dis. 117, 97–102.

Senior, R.M., Tegner, H., Kuhn, C., Ohlsson, K., Starcher, B.C., Pierce, J.A., 1977. The induction of pulmonary emphysema with human leukocyte elastase. Am. Rev. Respir. Dis. 116, 469–475.

Sommerhoff, C.P., Nadel, J.A., Basbaum, C.B., Caughey, G.H., 1990. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. J. Clin. Invest. 85, 682–689.

Sommerhoff, C.P., Krell, R.D., Williams, J.L., Gomes, B.C., Strimpler, A.M., Nadel, J.A., 1991. Inhibition of human neutrophil elastase by ICI 200,355. Eur. J. Pharmacol. 193, 153–158.

Steadman, R., Irwin, M.H., St John, P.L., Blackburn, W.D., Heck, L.W., Abrahamson, D.R., 1993. Laminin cleavage by activated human neutrophils yields proteolytic fragments with selective migratory properties. J. Leukoc. Biol. 53, 354–365.

Stokke, T., Burchardi, H., Hensel, I., Kostering, H., Kathner, T., Rahlf, G., 1986. Continuous intravenous infusion of elastase in normal and agranulocytic minipigs—effects on the lungs and the blood coagulation system. Resuscitation 14, 61–79.

Stone, P.J., Calore, J.D., Snider, G.L., Franzblau, C., 1982. Role of alpha-macroglobulin–elastase complexes in the pathogenesis of elastase-induced emphysema in hamsters. J. Clin. Invest. 69, 920–931.

Suttorp, N., Nolte, A., Wilke, A., Drenckhahn, D., 1993. Human neutrophil elastase increases permeability of cultured pulmonary endothelial cell monolayers. Int. J. Microcirc. Clin. Exp. 13, 187–203.

Taipale, J., Lohi, J., Saarinen, J., Kovanen, P.T., Keski-Oja, J., 1995. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. J. Biol. Chem. 270, 4689–4696.

Tomashefski Jr., J.F., 2000. Pulmonary pathology of acute respiratory distress syndrome. Clin. Chest Med. 21, 435–466.

Tonz, M., Mihaljevic, T., von Segesser, L.K., Fehr, J., Schmid, E.R., Turina, M.I., 1995. Acute lung injury during cardiopulmonary bypass. Are the neutrophils responsible? Chest 108, 1551–1556.

Travis, J., Salvesen, G.S., 1983. Human plasma proteinase inhibitors. Ann. Rev. Biochem. 52, 655–709.

Twumasi, D.Y., Liener, I.E., 1977. Proteases from purulent sputum. Purification and properties of the elastase and chymotrypsin-like enzymes. J. Biol. Chem. 252, 1917–1926.

Ulich, T.R., Yin, S., Guo, K., Yi, E.S., Remick, D., del Castillo, J., 1991. Intratracheal injection of endotoxin and cytokines: II. Interleukin-6 and transforming growth factor beta inhibit acute inflammation. Am. J. Pathol. 138, 1097–1101.

Wang, H.G., Shibamoto, T., Miyahara, T., Haniu, H., Tanaka, S., Fujimoto, K., Honda, T., Kubo, K., Koyama, S., 1999. Effect of ONO-5046, a specific neutrophil elastase inhibitor, on the phorbol myristate acetate-induced injury in isolated dog lung. Exp. Lung Res. 25, 55–67.

Weiland, J.E., Davis, W.B., Holter, J.F, Mohammed, J.R., Dorinsky, P.M., Gadek, J.E., 1986. Lung neutrophils in the adult respiratory distress syndrome. Clinical and pathophysiologic significance. Am. Rev. Respir. Dis. 133, 218–225.

Weiss, S.J., 1989. Tissue destruction by neutrophils. N. Engl. J. Med. 320, 365–376.

Weitz, J.I., Landman, S.L., Crowley, K.A., Birken, S., Morgan, F.J., 1986. Development of an assay for in vivo human neutrophil elastase activity. Increased elastase activity in patients with alpha 1-proteinase inhibitor deficiency. J. Clin. Invest. 78, 155–162.

Welbourn, C.R., Goldman, G., Paterson, I.S., Valeri, C.R., Shepro, D., Hechtman, H.B., 1991. Neutrophil elastase and oxygen radicals: synergism in lung injury after hindlimb ischemia. Am. J. Physiol. 260, H1852–H1856.

Wewers, M.D., Herzyk, D.J., Gadek, J.E., 1988. Alveolar fluid neutrophil elastase activity in the adult respiratory distress syndrome is complexed to alpha-2-macroglobulin. J. Clin. Invest. 82, 1260–1267.

Wiener-Kronish, J.P., Gropper, M.A., Matthay, M.A., 1990. The adult respiratory distress syndrome: definition and prognosis, pathogenesis and treatment. Br. J. Anaesth. 65, 107–129.

William, A.Z., Norton, P.P., 1999. Inhibitors of human neutrophil elastase as a potential treatment for inflammatory diseases. Expert Opin. Ther. Pat. 9 (7), 851–868.