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In vitro inhibition of human neutrophil elastase by oleic acid albumin formulations from derivatized cotton wound dressings

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Abstract

Human neutrophil elastase (HNE) is elevated in chronic wounds. Oleic acid albumin formulations that inhibit HNE may be applicable to treatment modalities for chronic wounds. Oleic acid/albumin formulations with mole ratios of 100:1, 50:1, and 25:1 (oleic acid to albumin) were prepared and found to have dose response inhibition properties against HNE. The IC_{50} values for inhibition of HNE with oleic acid/albumin formulations were 0.029–0.049 μM. Oleic acid/albumin (BSA) formulations were bound to positively and negatively charged cotton wound dressings and assessed for elastase inhibition using a fiber bound formulation in an assay designed to mimic HNE inhibition in the wound. Cotton derivatized with both carboxylate and amine functional groups were combined with oleic acid/albumin formulations at a maximum loading of 0.030 mg oleic acid + 0.14 mg BSA/mg fiber. The IC_{50} values for inhibition of HNE with oleic acid/albumin formulations bound to derivatized cotton were 0.26–0.42 μM. Release of the oleic acid/albumin formulation from the fiber was measured by measuring oleic acid levels with quantitative GC analysis. Approximately, 35–50% of the fiber bound formulation was released into solution within the first 15 min of incubation. Albumin was found to enhance the rate of elastase hydrolysis of the substrate within a concentration range of 0.3–50 g/L. The acceleration of HNE substrate hydrolysis by albumin required increased concentration of inhibitor in the formulation to obtain complete inhibition of HNE. Oleic acid formulations prepared with albumin enable transport, solubility and promote dose response inhibition of HNE from derivatized cotton fibers under aqueous conditions mimicking the chronic wound.

Keywords: Human neutrophil elastase; Chronic wound dressing; Oleic acid; Albumin formulation; Enzyme inhibition; Inflammatory disease states

1. Introduction

Inhibition of human neutrophil elastase is an important therapeutic target due to the enzyme's involvement in tissue destruction of a number of inflammatory disease states. Elastase is released from neutrophils under inflammatory conditions, and contributes to the pathogenesis of rheumatoid arthritis (Ekerot and Ohlsson, 1984) chronic obstructive pulmonary disease (Shapiro, 2002), adult respiratory distress syndrome (Jaffray et al., 2000), glomerulonephritis (Oda et al., 1997), and chronic and burn wounds (Yager et al., 1997; Grinell and Zhu, 1994). A variety of different types of inhibitors and inhibitor formulations have

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been devised for treatment of these therapeutic targets (Edwards and Bernstein, 1994). However, some of these HNE inhibitors have not been suitable for elastase inhibition in vivo due to protein alkylation.

Chronic, non-healing wounds (Yager et al., 1997) and burn wounds (Grinell and Zhu, 1994) have been shown to have high levels of elastase (36–54 mU/mL wound fluid) which degrade cytokine growth factors, fibronectin, and endogenous levels of protease inhibitors. In addition, it has also been shown that minimal levels of elastase and matrix metalloproteases, which are found in acute wounds, may be required for an appropriate healing response (Hatanaka and Tsuboi, 1991; Yager and Nwomeh, 1999). Thus, it has been postulated that lowering protease levels in the chronic wound to levels normally found in acute wounds may accelerate healing in the chronic wound. Neutralization of high levels of elastase found in the chronic wound by rationally designed wound dressings may be accomplished through either controlled release of an inhibitor or sequestration of elastase from the wound environment (Edwards et al., 2001; Edwards et al., 1999). Oleic acid is a highly selective non-toxic inhibitor of elastase and may be of use in lowering elastase levels in chronic wounds (Ashe and Zimmerman, 1977). However, the low aqueous solubility and transport properties of oleic acid do not make it favorable for release from wound dressing fibers and uptake into wound exudate where protective activity against high elastase levels is required. Albumin can transport a variety of substances including fatty acids, hormones, enzymes, dyes, trace metals, and drugs (Peter, 1975). Our interest has been to use oleic acid/albumin formulations bound to cotton wound dressings in promoting dose dependent lowering of HNE in the chronic wound. This study shows that oleic acid formulated with albumin when bound to derivatized cotton gauze promotes optimal dose dependent elastase-lowering by enabling soluble transport of the inhibitor from the fiber to the enzyme.

2. Materials and methods

Type VII cotton gauze (12 ply, 4 in. × 4 in. USP) was used in the treatments outlined here. Carboxymethylated cotton gauze was prepared as outlined previously (Reinhardt et al., 1957). A solution was made by mixing 25% monochloroacetic acid cooling in an ice bath, and stirring with 50% sodium hydroxide solution. This solution was used to pad a sample of cotton gauze to a 135% wet pickup. The sample was then placed in an oven at 100 °C and dried/cured for 10 min.

Aminized cotton gauze was prepared by immersing six 4 in. × 4 in. gauzes in a 200 mL solution of 30% NaOH, 20% 2-aminoethylhydrogensulfate, and 1% sodium borohydrate. The gauzes were padded to a wet pick-up of 230% and all samples were cured at 125 °C for 12 min. The samples were washed in alternating cycles of cold and hot tap water. The gauzes were rinsed in dilute acetic acid followed by dilute NH_4OH and finally in hot tap water. After washing the samples were dried for 5 min at 85 °C.

2.1. Preparation and characterization of oleic acid/BSA formulations and formulation-coated fibers

A typical 100:1 oleic acid/BSA formulation was typically made in the following manner. Fifty milligrams of fatty acid free bovine serum albumin (FFA-BSA) (Sigma) were dissolved in 600 μL of 50% acetic acid and 200 μL of acetonitrile were added followed by a 300 μL solution of oleic acid (Sigma, 99% pure by capillary GC) in acetonitrile (17 mg/mL). A volume of 1.1 mL of acetonitrile was added in 200 μL increments to help maintain albumin solubility. The solution was freeze-dried and resulted in a fluffy white powder (57 mg, 99% yield, based on oleic acid analysis).

The formulation-bound fibers were prepared as follows; bovine serum albumin (81 mg, Sigma) was dissolved in 900 uL of 50% acetic acid and 400 uL of acetonitrile was added in 200 uL aliquots. Oleic acid (0.178 g) was dissolved in 10 mL acetonitrile. Two milliliters of the oleic acid solution were added to the albumin solution in 200 uL increments with addition of 200 uL of 50% acetic acid. The formulation solution (350 uL) was applied to 50 mg swatches of modified cotton gauze. The fiber samples were lyophilized to dryness.

Assessment of oleic acid in the albumin formulations was completed by extraction of oleic acid from the protein followed by gas phase chromatographic analysis. Approximately, 2–6 mg of albumin

formulation was weighed and placed in 1 mL of a buffer containing 0.1 M sodium phosphate buffer and 0.25 M NaCl at pH 7.6 for 1 h whereupon 1 mL of 0.15 M acetic acid and internal standard, heptadecanoic acid, was added. A Bligh extraction (Bligh and Dyer, 1959) was completed as follows: 7.5 mL of chloroform/methanol (1:2) was added to the formulation and the solution was vortexed three times at 5 s intervals then centrifuged for 5 min at 4000 rpm into a glass tube. The residue was combined with chloroform (2.5 mL), vortexed as above, and centrifuged for 5 min at 4000 rpm whereupon the liquid supernatant was added to a glass tube and 2.5 mL of a 0.88% KCl solution was added. The glass tube was vortexed three times at 5 s intervals and centrifuged for 5 min. The lower chloroform layer was transferred to a brown 12 mL vial and the solvent was evaporated to dryness in an argon stream by heating to 45 °C. The dried oleic acid was silylated with 0.3 mL trimethylsilylimidazole (Pierce, Rockford, IL). It was heated at 45 °C for 30 min and injected into a Varian 3600 CX gas chromatograph (Sugar Land, TX) with a dimethylpolysiloxane column (J & W Scientific), 30 m × 0.32 mm i.d., and helium as a carrier (1.577 mL/min, 16 psi) with a split ratio of 48:1. Quantitation of peak areas was completed with heptadecanoic acid as internal standard using Turbochrom 4.12 software (PE Nelson, Norwalk, CT). Protein concentrations were determined with the Bio-Rad Protein assay (Richmond, CA) with bovine serum albumin as a quantitation standard.

2.2. Assay of oleic acid/albumin formulation and formulation-coated fibers

Human neutrophil elastase inhibition was assessed with free oleic acid, oleic acid/albumin formulations and derivatized cotton gauzes containing bound oleic acid/albumin formulation. The effect of the oleic acid/albumin formulations on elastase inhibition was assessed at oleic acid:albumin ratios of 100:1, 50:1, and 25:1 (mol:mol, oleic acid:albumin). Formulation powder was dissolved in buffer of varying volumes with formulation weight ranges ((0.301 mg oleic acid/0.699 mg BSA)/mg product to (0.097 mg oleic/0.903 mg BSA)/mg product). These weights spanned the range of the formulation mole ratios from 100:1 to 25:1.

The formulation assays with human neutrophil elastase (Athens Res. & Tech., Athens, GA, purity greater than 95% by SDS-PAGE) were conducted in pH 7.6 buffer composed of 0.1 M sodium phosphate, 0.25 M NaCl. Spectrophotometric measurement at 405 nm of the release of *p*-nitroaniline from the enzymatic hydrolysis of *N*-methoxysuccinyl–Ala–Ala–Pro–Val–*p*- nitroanilide (Sigma) (Nakajima et al., 1979) was utilized to assess the enzymatic activity of HNE. The spectrophotometric kinetic assays were performed in a Bio-Rad Microplate Reader (Hercules, CA) with a 96-well-format. The microplate well volume was adjusted to 180 uL with buffer and a 20 μL solution of human neutrophil elastase (6.3 milliunits) was added whereupon 60 uL of a 0.87 mM substrate solution were added to initiate the reaction.

Treated and untreated gauze samples (50 mg) were submerged in 1 mL of buffer. The samples were allowed to incubate for 30 min at room temperature whereupon 34.9 milliunits/100 μL of human neutrophil elastase was added. The fiber samples were allowed to incubate with enzyme at room temperature for 1 h. Two hundred microliter aliquots were removed from each sample and assayed for elastase activity on the Bio-Rad Microplate Reader (Hercules, CA). The enzyme reaction was initiated by adding 60 uL of a 0.87 mM substrate solution.

3. Results

The oleic acid/albumin formulations were applied to three types of cotton gauze fibers to inhibit elastase activity at levels found in the chronic wound. Oleic acid binds naturally with albumin, which serves as a carrier of fatty acids in serum. Albumin formulations of oleic acid were prepared to improve transport and solubility properties of oleic acid upon release from the fibers of the derivatized cotton wound dressings. Mole ratios of oleic acid to albumin were approximately 100:1, 50:1, and 25:1. To assess the homogeneity and charge of the formulation particles, particle size and zeta potential were determined. Formulation particle sizes are listed in Table 1. Formulation particle sizes varied from 896 to 122 nm, and the net charge was positive, neutral, and negative for the 100:1, 50:1, and 25:1 formulations, respectively.

Table 1
Formulation particle size and zeta potential

Molar ratio, oleic:BSA	Concentration (mg/mL)	Particle size (nm)	S.D.	Poly-dispersity	Zeta potential	S.D.
100:1	1	896	63	0.413	7.21	4.34
50:1	1.8	189	24	0.231	−2.52	2.36
22:1	1	122	20	0.226	−3.49	0.91

Particle size and zeta potential were measured on aqueous solutions (1–2 mg/mL) of previously lyophilized formulations of oleic acid and BSA. Measurements were made by dynamic light (laser) scattering at 90° at 25 °C in a 90Plus particle size analyzer, Brookhaven Instrument Corp. The measurements listed in the table refer to mean particle size. Data are the average of five repeated runs for particle size and 10 repeated runs for zeta potential. Instrument specific software was used to analyze the data.

The charge and particle size of the formulation affect the binding affinity of the formulation for the cotton fiber. A net positive charge should have higher affinity for the carboxymethylated cotton fiber and a net negative charge should increase affinity of the formulation for the aminized cotton fiber. Oleic acid/albumin formulations were characterized through the Bligh extraction method (Bligh and Dyer, 1959) and quantitative analysis using gas chromatography techniques. A gas chromatogram of oleic acid and internal standard elution is shown in Fig. 1. Recovery of oleic acid from the formulations was found to be 96 ± 10%.

3.1. Oleic acid/albumin formulations and derivatized cotton wound dressings

Formulation-coated fibers were designed for binding of the fatty acid/protein formulation to negatively and positively charged cotton gauzes containing a carboxylate or amine functionality present on the carboxymethylated and aminized cellulose chain, respectively, (as shown in Fig. 2). Within the pH range of the chronic wound (7.1–7.3), the aminized and carboxymethylated cellulose will assume a positive and negative charge, respectively. The formulation-coated fibers were prepared by co-lyophilization of oleic acid, albumin, and derivatized fiber. Negatively charged side chains of the protein's amino acid composition of glutamates and aspartates residing at the surface of the protein will form salt bridges with the positively charged amine groups of aminized cellulose. Positively charged side chains of the protein's amino acid composition of lysines and arginines residing at the surface of the protein will form salt bridges with the carboxylate groups of the carboxymethylated cellulose.

3.2. Effect of the oleic acid/albumin formulation on elastase activity

The ability of the oleic acid/albumin formulations to inhibit elastase activity is shown in Fig. 3. The dose response relationship of the oleic acid/albumin formulation of a 100:1, 50:1, and 25:1 (mole:mole, oleic acid:albumin) formulation revealed small variations between the formulations as measured by IC_{50} values for all three formulations as shown in Table 2. The 100:1 oleic acid/albumin formulation demonstrated an approximate two-fold improvement in activity over the other two formulations. The results of the formulation dose response relationships are compared with DMSO solubilized oleic acid (IC_{50} of 1.894 nM) which is shown in Fig. 4. Based on a comparison of IC_{50} values shown in Table 2 the formulations (100:1, 50:1, and 25:1) are approximately 15–30 times less potent inhibiting elastase than the oleic acid solubilized in DMSO.

3.3. Albumin enhances the rate of elastase substrate hydrolysis

Albumin enhances elastase activity three-fold as is shown in Fig. 5. Enzymatic hydrolysis of substrate increased within an albumin concentration range of 0.1–0.3 g/L, and the activity profile returns to standard rates within 10–100 g/L albumin. Acceleration of the enzyme hydrolysis rate by the presence of albumin accounts in part for the necessity to use a higher concentration of oleic acid in the albumin formulations.

3.4. Effect of oleic acid–albumin coated fibers on elastase activity

The dose responses of oleic acid/albumin formulations on carboxymethylated, aminized, and neutral

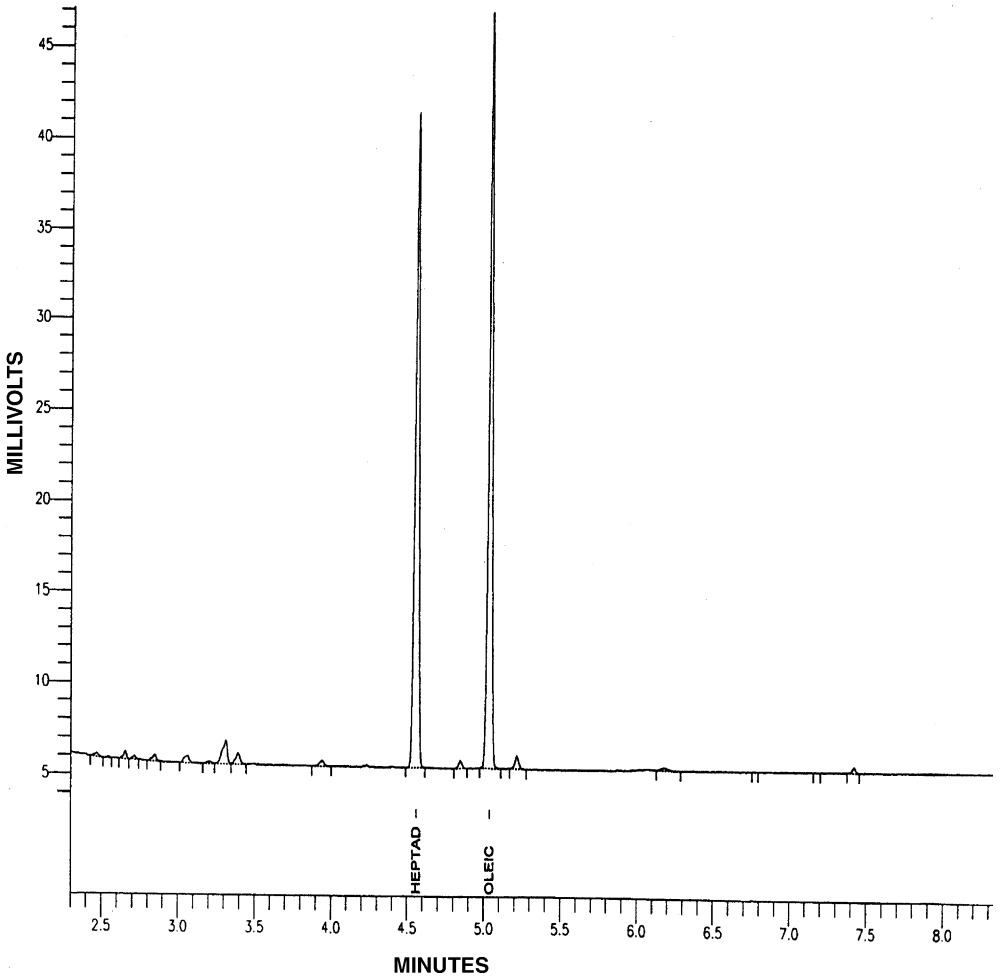

Fig. 1. GC Chromatogram of oleic acid accompanied by the internal standard heptadecanoic acid. Details of the GC chromatography are outlined in Section 2.

cotton fibers are compared in Fig. 6. The results of the dose response of formulation-bound fiber, which compares cellulose with aminized and carboxymethylated cellulose, is based on incubating the fibers in solution for 1.5 h. The IC_{50} values for elastase inhibition by oleic acid/albumin formulation ratios of 25:1, 50:1, and 100:1 are contrasted and shown in Table 2. All formulation ratios of derivatized cotton wound dressings gave comparable IC_{50} values, which were between 0.26 and 0.42 μM. The IC_{50} for the formulations bound to untreated and carboxymethylated cotton gave very similar inhibitory profiles. However, the aminized cotton dressing formulation gave a more potent inhibitory response with the 25:1 ratio. The better inhibitory response for the aminized cotton-bound formulation may relate to improved binding of the formulation to the fiber due to salt bridge formation between the positively charged aminzed fiber and the negatively charged formulation. The net positively charged aminized fiber and the net negatively charged albumin form a salt bridge link which enhances the overall binding of the formulation to the aminized fiber. In the case of the aminized fiber this occurs through the formation of individual salt bridges between the negatively charged amino acid side chains of the protein and the derivatized cotton fiber (struc-

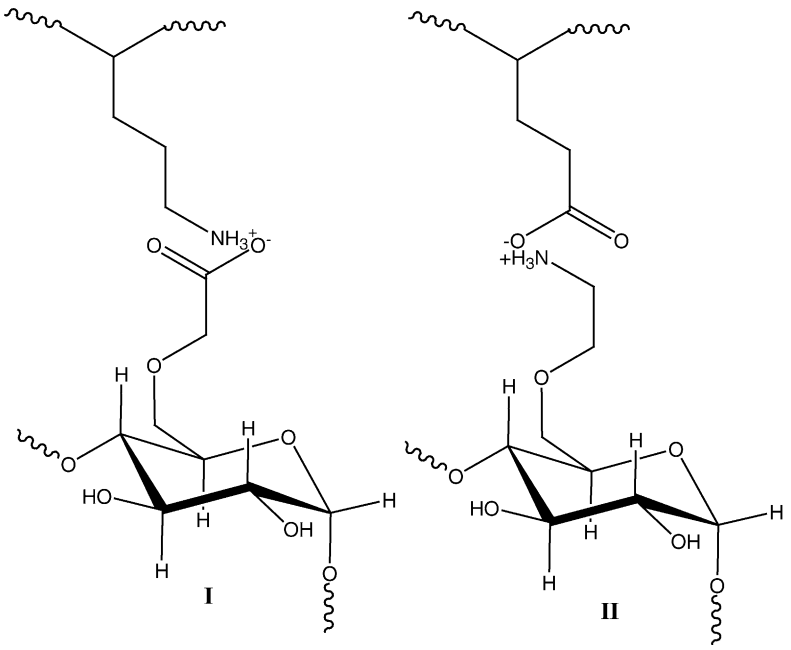

Fig. 2. Structures of carboxymethylated and aminized cellulose chains as viewed with a six-position anhydroglucose unit carboxymethylated or aminized. Diagram of albumin formulation bound to negatively and positively charged groups on the carboxymethylated and aminized cellulose fiber, respectively.

Table 2
A comparison of IC_{50} values for oleic acid/albumin formulations bound to derivatized wound dressings with oleic acid albumin formulations tested directly

Oleic acid/albumin bound to derivatized cotton wound dressing	IC_{50} (μM)*	Oleic acid/albumin formulation	IC_{50} (μM)**
Untreated cotton dressing			
25:1	0.42	25:1	0.049
50:1	0.42	50:1	0.062
100:1	0.35	100:1	0.029
Carboxymethylated cotton dressing			
25:1	0.41		
50:1	0.42		
100:1	0.35		
Aminized cotton dressing			
25:1	0.26		
50:1	0.39		
100:1	0.34		

IC_{50} determined for free oleic acid is 1.89 nM.

* IC_{50} is defined as the concentration of oleic acid contained in the formulation and applied to the cotton wound dressing at 50% of elastase activity.

** IC_{50} is defined as the concentration of oleic acid present in the formulation at 50% elastase activity.

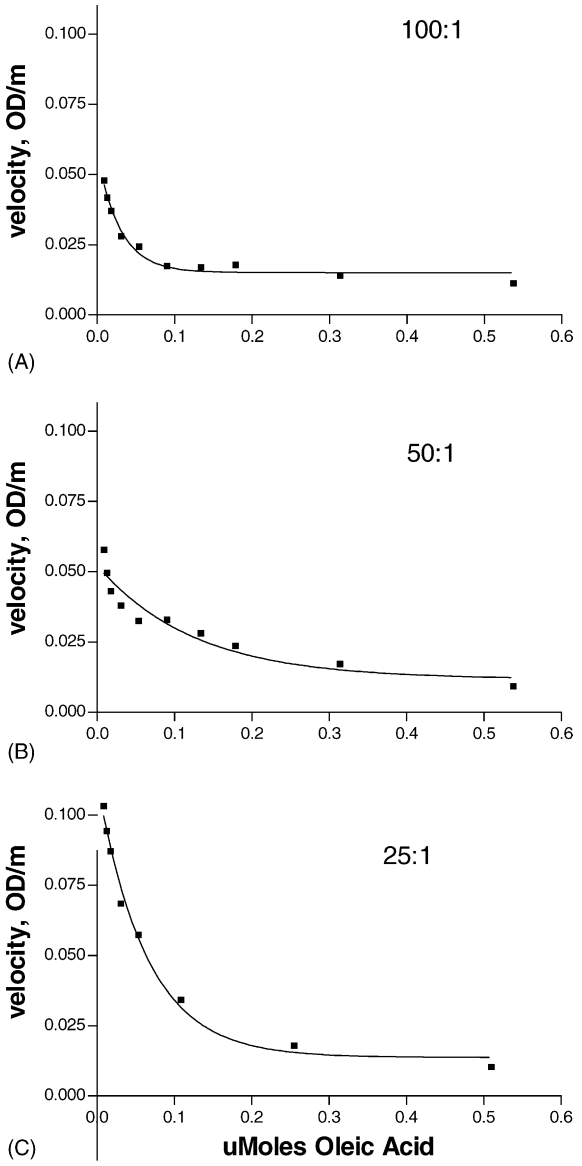

Fig. 3. Dose response curves of the inhibition of human neutrophil elastase (6.9 milliunits) with oleic acid/albumin formulations. The inhibitory activities of the three formulation ratios (25:1, 50:1, and 100:1) are plotted based on oleic acid concentrations present in each formulation. The three dose response curves are a plot of individual initial velocities for each formulation taken from the elastase substrate hydrolysis reaction progress curve. Each point in the three plots represents the slope of individual elastase reaction progress curves run with the formulation ratio at the specified formulation oleic acid concentration in the *x*-axis. Formulations of oleic acid and albumin were prepared at ratios of 100:1 (A); 50:1 (B); and 25:1 (C) (mole:mole, oleic acid:albumin) and assayed as outlined in Section 2.

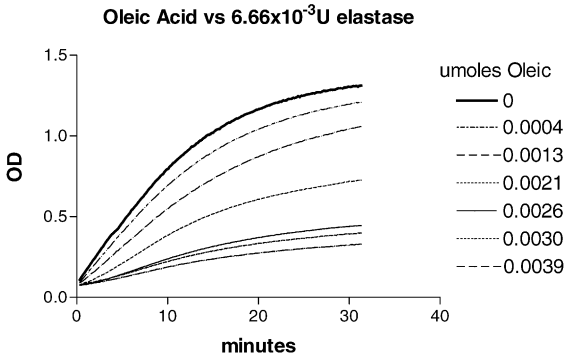

Fig. 4. Reaction progress curve of the dose response of oleic acid inhibiting human neutrophil elastase (6.9 milliunits). The inhibition assay is described in Section 2.

ture II, Fig. 2) creating a salt bridge through which the formulation is bound to the fiber. On the other hand, the carboxymethylated cellulose (CMC) forms a salt bridge with positively charged amino acid group side chains in the albumin protein (structure I, Fig. 1). This explanation is consistent with the zeta potential data in Table 1 where the 25:1 formulation demonstrated a net negative charge and the 100:1 and 50:1 formulations demonstrated a net positive and neutral charge, respectively.

All of the gauze samples extracted elastase from solution. However, the elastase-extraction effect observed with the samples is negligible when compared with the inhibitory effect resulting from derivatization

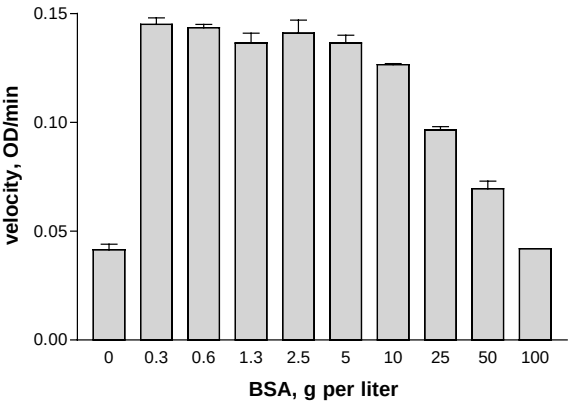

Fig. 5. Dose response of 6.35×10^{-3} U elastase to bovine serum albumin concentrations varying from 1 to 273 nmol.

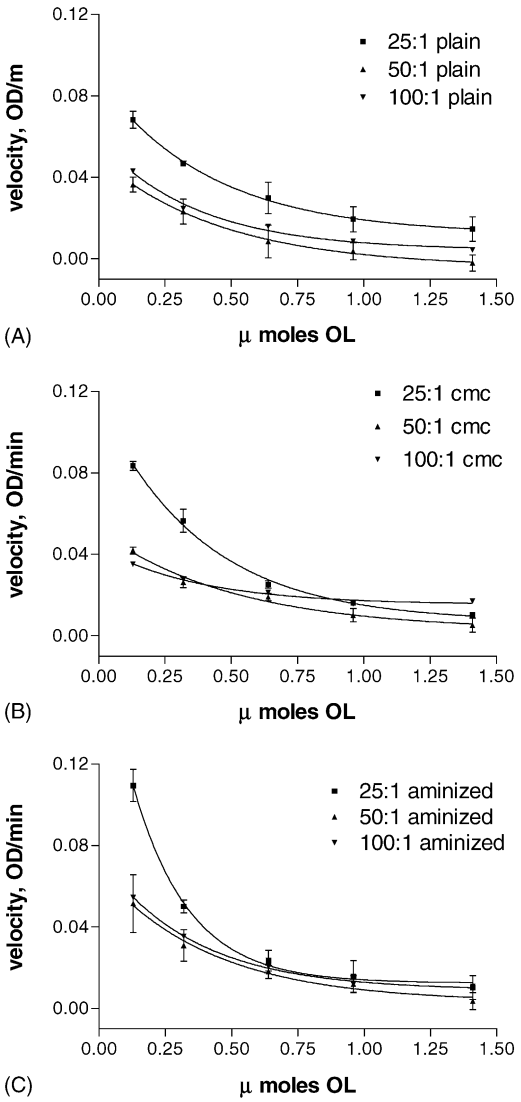

Fig. 6. Dose response of fiber-coated formulations. Oleic acid: albumin formulations were prepared on derivatized cotton gauze as outlined in the materials and methods section. Untreated (A); carboxymethylated (B); and aminized (C) formulation-bound fibers were assayed for HNE inhibition by monitoring HNE substrate hydrolysis.

of the gauze samples with the formulations. We have previously shown that this elastase-extraction effect occurs due to binding of the positively charged elastase to the negatively charged cotton fiber (Edwards et al., 2001).

3.5. Release of oleic acid/albumin formulations from derivatized fibers

An assessment of the release of formulations from the derivatized fibers was evaluated at regular time intervals over a 2 h period. The amount of formulation released from the cotton fibers was monitored by measuring both albumin and oleic acid concentrations at fixed time points following incubation of the fiber bound formulation in phosphate buffer pH 7.6. As shown in Table 1, the particle sizes of the formulations were found to be stable in sodium phosphate buffer. The percent of fiber bound albumin and oleic acid released from the derivatized fibers is shown in Fig. 7. Approximately, 35–50% of the fiber bound formulation was released into solution within the first 15 min of incubation. The amount of formulation released from the fiber, which was assessed measuring the amount of protein and oleic acid, increased over a 2 h period to a level of approximately 60–70% of the original fiber bound formulation.

The activity of one formulation (25:1) bound to the three derivatized cotton fibers was measured versus the amount of oleic acid (Fig. 8B) and albumin (Fig. 8A) released from the fiber. In Fig. 8A, is shown the inhibitory activity of the formulation released from the fiber compared with albumin bound to fiber alone. Fig. 8B demonstrates the released oleic acid concentration measured in solution versus elastase inhibitory activity. IC_{50} values based on enzyme inhibition of released formulation reflected a three- to four-fold increase in potency versus IC_{50} values that were based on the formulation dose applied to derivatized gauze.

4. Discussion

The results of the preparation of the oleic acid formulations and their HNE inhibitory properties illustrate that active elastase levels can be modulated in solution by oleic acid/albumin formulations released from derivatized cotton gauzes. All formulation ratios when released from the fiber give comparable elastase inhibition at similar oleic acid concentrations. However, the 50:1 and 100:1 oleic acid/albumin formulation ratios provide a soluble complex and yield effective inhibition of HNE under conditions that mimic the wound pH and salinity. The lower 25:1

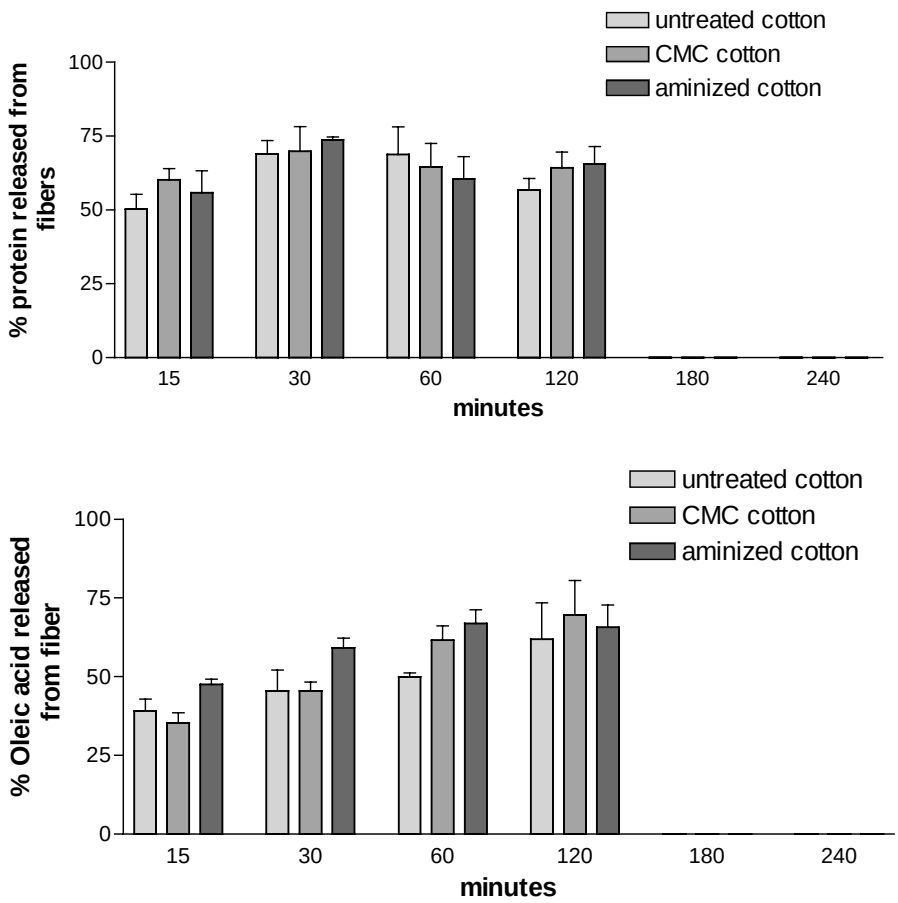

Fig. 7. Cumulative release of protein and oleic acid from derivatized cotton fibers. Fifty milligram amount of each type of derivatized cotton fiber was treated with 8 mg of oleic acid:albumin formulation in 0.1 M phosphate buffer pH 7.6 and lyophilized to dryness. The formulations applied consisted of 2.2 mg of oleic acid and 10.2 mg of BSA per 50 mg pieces of cotton gauze. The fiber bound formulations were incubated in a 1.6 mL phosphate buffer solution. At the defined time intervals, 200 μL aliquots were removed and analyzed for oleic acid and protein as described in Section 2. Both the percent of released protein and oleic acid were calculated based on the values of the prepared formulation.

oleic acid/albumin formulation represents the limit of solubility and inhibitory activity as a stable formulation. The 25:1 ratio requires a higher concentration of albumin and oleic acid to inhibit elastase. However, the 25:1 formulation demonstrated the best activity when attached to aminized cotton.

An increase in albumin concentration in the formulation has the effect of accelerating elastase activity while requiring increased oleic acid concentrations to override the elastase activation properties of the albumin. Previous reports of albumin increasing enzyme activity have been made. The activity of 2-*N*-acetyl-glucosaminidase is increased by the presence of albumin (Von Figura, 1977), and albumin also accelerates activation of plasminogen by tissue-type plasminogen activator (Machovich and Owen, 1997). A possible explanation of the drop in velocity at higher BSA concentrations is that higher concentrations of BSA stabilize the enzyme, and at even higher concentrations, this effect is decreased due to increased aggregation or association of the albumin protein which may in turn hinder the enzymes turnover of available substrate thereby decreasing the initial velocity of elastase hydrolysis. An alternative explanation

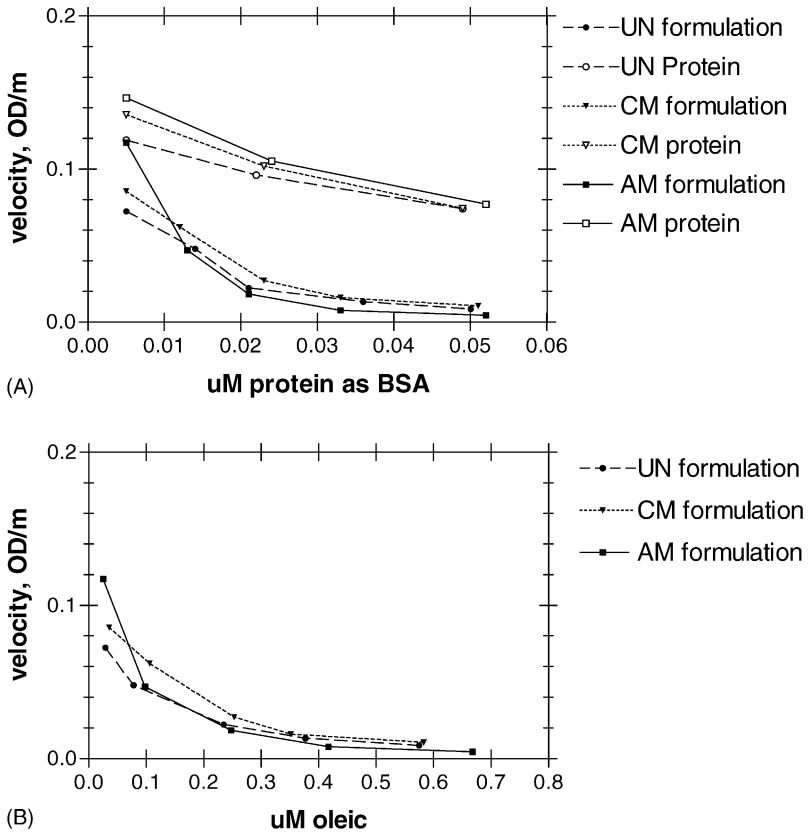

Fig. 8. Plots of rates of enzyme substrate hydrolysis vs. actual amounts of oleic acid:albumin formulation (25:1) released from the derivatized fiber. Formulations were applied to carboxymethylated (CM); aminized (AM); and untreated (UN) cotton. The *x*-axis in plots A and B is the amount of protein (A) and oleic acid (B) released from the derivatized fiber as measured. In plot A, oleic acid:albumin formulation bound to derivatized fiber (formulation) is compared with albumin alone bound to fiber (protein).

is that the higher albumin concentration serves as an additional substrate for elastase hydrolysis and thereby decreases the level of enzyme available for chromophoric peptide substrate analysis.

Oleic acid/albumin complexes and the binding of free fatty acids to albumin have been well studied for their role of lipid transport in serum. The cellular uptake of oleate from albumin has been shown to be both facilitated and passive and the role of albumin in modulating levels of free fatty acid in the serum as opposed to unbound free fatty acids is well documented (Spector et al., 1969; Stump et al., 2001). In this study, we have used albumin to promote the solubility and transport of oleic acid as an enzyme inhibitor. Albumin has three primary binding sites that are involved in the uptake of free fatty acid. The concentration of oleic acid can be increased as much as 500 times above its maximum solubility in salt solutions similar to plasma (Spector et al., 1969). It has been observed that 13.5 ueq of oleate can bind 1 umol of BSA. However, our experience in preparing formulations of 14:1 oleic acid: BSA demonstrated that they are intractable at this ratio and form a viscous colloidal suspension owing to the high protein concentration.

A compromised balance of protease/antiprotease levels prevents healing in the chronic wound. High levels of elastase activity in the chronic wound have been attributed to insufficent levels of alpha-antitrypsin needed to keep HNE proteolysis in check. A few in vivo studies on the use of either alpha-antitrypsin (Wachter and Lezdey, 1992) or elastase formulations with albumin (Martodam et al., 1979) have been

reported. HNE levels employed in this study were 70 mU/mL, and are somewhat higher than levels typically found in the chronic wound (36–54 mU/mL). Numerous potent synthetic inhibitors of HNE are available for evaluation. However, the phamacokinetics of synthetic inhibitors in the wound are not well understood, and prolonged levels of some synthetic inhibitors could be toxic to the environment of the wound. The oleic acid/albumin formulations provide a possible route to modulating elastase levels in the chronic wound with an agent that would be compatible with the protein concentration in the chronic wound. Albumin is the protein found in highest concentration in the chronic wound, and the native free fatty acid composition of albumin is within the range of that used with the oleic acid formulations of this study. It has been noted that median albumin levels in chronic wound fluid of 22 g/L are on average half that of serum levels (Trengove et al., 1996). However, chronic wounds with albumin levels of less than 20 g/L rarely heal (James et al., 2000). Thus, increasing the albumin level in chronic wounds may have an advantageous effect on healing.

Synthetic and protein protease inhibitors formulated as a medicated wound dressing for modulation of protease/antiprotease imbalance require careful consideration of their interaction with the target enzyme as well as other proteins in the wound. For controlled release of an inhibitor from the fibers of a wound dressing to be effective, the following criteria are required: (1) a selective non-toxic protease inhibitor formulated in a wound dressing matrix; (2) release of the inhibitor into the wound environment with duration of effect; and (3) adequate clearance from the wound site. The assessment of a cotton fiber formulation of an elastase inhibitor was previously demonstrated with the peptide *N*-methoxysuccinyl–Ala–Ala–Pro–Val–chloromethylketone (CMK) which was modified on cotton cellulose fibers and assayed as a colloidal system (Edwards et al., 1999). However, a potential disadvantage of the CMK fiber-inhibitor formulation is its ability to alkylate other proteins in the wound since its mode of action is through electrophilic attack of ketone carbonyls. Oleic acid has been shown to be an effective, non-toxic, and selective inhibitor of human neutrophil elastase (Ashe and Zimmerman, 1977) and it has no functional groups that would directly react with proteins. Oleic acid has been used for textile finishing, and it has been studied for its interaction with textiles as a component of human sebum (Weglinski and Obendorf, 1985), which comes in contact with clothes, and adheres to cotton fabrics in the presence of an aqueous environment. However, because of the hydrophobic properties of oleic acid, it is not adequately released from a fiber surface under aqueous conditions as are found in chronic wounds.

Although some reports (Hatanaka and Tsuboi, 1991; Yager and Nwomeh, 1999) have examined the role of HNE in proteolysis of the wound environment, little is known about the potential effect of modulating HNE in the chronic wound with exogenous protease inhibitors. Only a few reports have been published on potential use of protein protease inhibitors, such as alpha-antitrypsin to inhibit high levels of serine proteases in wounds (Wachter and Lezdey, 1992; Wlaschek et al., 1997). This report focuses on utilizing a protein and fatty acid formulation to modulate levels of elastase under conditions where the protein complex would be attached ionically to wound dressing fibers. These findings show that elastase activity may be lowered with oleic acid:albumin formulations in a dose dependent manner. Future studies will focus on the use of other types of albumin carriers available for oleic acid formulations. The effective nature of this type of protein–fatty acid formulation suggests that it may be useful to consider in localized inflammatory disease states where high levels of elastase are contributing to the pathology.

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