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Inhibition of Human Neutrophil Elastase Activity by Encapsulated Serum (Serumsome™) Therapy

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ABSTRACT

In order to inhibit human leukocyte proteolytic activity as a means of arresting the inflammatory response in tissues in vivo, we have designed a novel antiprotease carrier named serumsomes™. Stabilized human serum was added to a flask containing a film of dried, purified lipids (phosphatidylcholine/dicetyl phosphate/cholesterol, 70:20:10) and hand-shaken for 10 min. Equal volumes of human neutrophils, and either serumsomes™ (in stabilized human serum) or stabilized human serum alone were mixed together. Following 2 h of incubation at 37°C, the total elastase content of the neutrophils was reduced to 60 ± 15% and 83 ± 7% of the original activity by serumsomes™ and stabilized human serum, respectively. Analysis of β-glucuronidase activity, a nonproteolytic lysosomal marker enzyme, revealed no diminution of activity during either of these incubations. These experiments demonstrate that human neutrophils are capable of interacting with serumsomes™ in vitro, selectively inhibiting the lysosomal protease elastase. By administering serumsomes™ in vivo, one may potentially preload blood leukocytes with serum antiproteases prior to their migration to inflammatory sites and thus possibly reduce the extent of tissue injury.

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Index Entries: Inhibition, of neutrophil elastase by encapsulated serum; neutrophil elastase, inhibition by encapsulated serum; elastase, inhibition by encapsulated serum of neutrophil; encapsulated serum, inhibition of neutrophil elastase by; serum, inhibition of neutrophil elastase by encapsulated; serumsomes™.

INTRODUCTION

Many lung pathologies involve invasion by foreign particles and infectious agents (*1*). Neutrophils emigrate from the blood to sites of inflammation and can mediate acute tissue injury in the lung and other organs (*2*). Leukocytes, and their lysosomal extracts, are capable of injuring blood vessels, digesting basement membranes, and hydrolyzing elastin (*3*). The availability of antiproteases such as alpha-l-antitrypsin (present in normal serum) (*4*) at sites of lung consolidation may determine the extent of tissue injury. However, in severe inflammation such as that which accompanies Klebsiella and *Staphylococcus aureus* pneumonias, the consolidation may be extensive and allow leukocytes to diapedese inwards, while excluding the influx of serum antiproteases.

We propose that by "preloading" the leukocytes circulating in the blood with antiproteases *prior* to their margination in the lung and other inflammatory sites, one may potentially limit the extent of tissue hydrolysis and destruction that follows, without limiting the leukocyte's phagocytic and bactericidal capabilities. We have previously shown liposomes to be capable of entrapping alpha-1-antitrypsin and soybean trypsin inhibitor (*5*) and to be useful for the introduction of entrapped solutes into human leukocytes (*6, 7*). In this paper, we report on the association (entrapment) of serum (including antiproteases such as alpha-1-antitrypsin and alpha-2-macroglobulin) with a liposome-like carrier that we have named serumsomes™. Evidence is presented for the reduction of human neutrophil elastase activity by serumsomes™ in vitro, illustrating the efficacy of these novel antiprotease carriers.

METHODS

Preparation of Serumsomes™

Stabilized human serum (SHS) (*8*) was added to a flask containing a film of dried, purified lipids (phosphatidylcholine/dicetyl phosphate/cholesterol, 70:20:10) (15 μmol lipid/mL serum) and hand-shaken for 10 min.; the serumsome™ suspension was maintained at room temperature until used.

Preparations of Cell Suspensions and Biochemical Analyses

These were carried out as previously described (*6, 9*).

Incubation of Serumsomes™ with Neutrophils

Equal volumes of neutrophils (5×10^6 cells/mL Dulbecco's PBS) and either serumsomes™ (15 μmol lipid/mL SHS) or SHS alone, were mixed together. Following 2 h of incubation at 37°C, or 2 min at 4°C, the leukocytes were washed free of unassociated serumsomes™ and serum proteins by repeated rapid centrifugations (8000*g*, 3 min) in an Eppendorf microfuge.

RESULTS

Because the uptake of serumsomes™ (with which serum proteins including antiproteases are associated) by the neutrophils was expected, cell pellets were homogenized and assayed for elastase, β-glucuronidase, and LDH. As seen in Table 1, only the neutral protease (elastase) activities were significantly reduced to 57 ± 16% (corrected values, 60 ± 15%) of original levels, whereas the other two hydrolases assayed remained at original levels, following exposure of cells to serumsomes™ at 37°C for 2 h. Elastase is a neutral protease found in neutrophils and is inhibited when serum protease inhibitors have entered the vacuolar system of the leukocyte; LDH and β-glucuronidase are marker enzymes for the cytoplasmic and lysosomal compartments, respectively. Incubations at 4°C had no effect on hydrolase activities. Exposure of the neutrophils to serum at 37°C for 2 h reduced elastase activities to 81 ± 2.5% (corrected = 83 ± 7%) of original activities, while not affecting the cytoplasmic marker, LDH. β-Glucuronidase, present in SHS, showed some association with the neutrophils during incubation at 37°C (Table 1).

TABLE 1
Percentage of Total Enzyme Activities in Human Neutrophil Homogenates

PMN leukocytes exposed to	Total activity,[a] %		Total Activity,[b] %	
	Elastase	β-Glucuronidase	Elastase	β-Glucuronidase
Serumsomes [SHS] (37°C for 2 h) (n = 8)	57 ± 16	99 ± 16	60 ± 15	105 ± 12
Serumsomes [SHS] (4°C) (n = 5)	88 ± 14	97 ± 20	100 ± 16	110 ± 25
SHS alone (37°C for 2 h) (n = 3)	81 ± 3	114 ± 6	83 ± 7	116 ± 12

[a]Total activities determined in freshly obtained neutrophil homogenates; $\bar{x}$ ± SD.
[b]Percentage of total activities; corrected on basis of cellular recovery (LDH) from experiment to experiment; $\bar{x}$ ± SD.

CONCLUSION

This preliminary study demonstrates that the protease activities of neutrophils can be reduced by the leukocyte's exposure to serumsomes™. With further development, this new concept may have far reaching implications in the treatment of acute and chronic inflammatory disorders in the lung and other tissues.

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