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Antibacterial activity of proteolytic enzymes

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Summary

Single clinical isolates of 5 species of bacteria were individually incubated in saline solutions of two proteolytic enzymes at 3 different concentrations for one hour to determine the possible antibacterial activity of these enzymes. The enzymes tested showed higher killing effect against the Gram-negative bacteria than the Gram-positive bacteria. The lethal activity of the enzymes was concentration dependent.

Introduction

Proteolytic enzymes have been used to digest wound debris or coagulum in order to facilitate wound sterilization and healing (Sherry and Fletcher, 1960; Rodeheaver et al., 1974). Another outstanding application of proteolytic enzymes has been in the field of ophthalmology (Colman, 1965). The enzymes have been claimed to have an anti-inflammatory effect and to promote healing in episiotomy patients (Fullgabe, 1957; Albright, 1963). Furthermore, Rodeheaver et al., (1974) have shown that trypsin has antibacterial activity against *Staphylococcus aureus*.

Because of the increasing applications of proteolytic enzymes to clinical medicine, the present study was undertaken to investigate the antibacterial activity of such enzymes against different types of bacteria.

Materials and methods

Recent isolates of bacteria from the clinical laboratory were used for this study. The organisms were cultivated in nutrient broth (Oxoid) until used. They were

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incubated overnight at 37°C, washed in 0.9% saline and suspended in normal saline to give about 10^9–10^{10} colony-forming units per ml.

Different amounts of the tested enzymes, namely trypsin and chymotrypsin (British Drug Houses), 2000–20,000 NF (US National Formulary) units, dissolved in 1 ml of 0.9% sodium chloride were mixed with 1 ml aliquots of the saline suspension of each of the 5 organisms. All suspensions were incubated at 37°C for one hour, and at the end of this time aliquots of the systems were serially diluted 10-fold in 0.9% saline, plated on nutrient agar, and counted after overnight incubation at 37°C. All the results were statistically evaluated using Student's *t*-test with a 95% confidence coefficient as a criterion of significance. The controls were saline alone and were not pH adjusted.

Results and discussion

Means of the colony counts in the reaction systems of the two enzymes against the 5 organisms tested are shown in Tables 1 and 2; all results differed significantly ($P < 0.05$) from control values. It is evident that the two Gram-positive bacteria tested, namely *Staphylococcus aureus* and *Streptococcus pyogenes*, showed almost identical susceptibilities to the two enzymes at the different concentrations used. However, the lethal activity observed was slightly more with chymotrypsin than with trypsin. The bactericidal activity of trypsin at 1000, 5000 and 10000 NF units/ml was 7, 16 and 26%, respectively, against *Staphylococcus aureus*, whereas against *Streptococcus pyogenes* it was 6, 14 and 28% inhibition at the same concentrations. In the case of chymotrypsin against *Staphylococcus aureus*, the lethal effect was 9, 20 and 30% at 1000, 5000 and 10000 units/ml concentrations, respectively, and 10, 22 and 31% against *Streptococcus pyogenes*.

TABLE 1

BACTERICIDAL ACTIVITY OF PROTEOLYTIC ENZYMES AGAINST GRAM-POSITIVE BACTERIA

System concentration (units/ml)	*Staphylococcus aureus* (CFU/ml ± S.E. mean [a])	*Streptococcus pyogenes* (CFU/ml ± S.E. mean [a])
Control (0.9% saline)	$8.32 \pm (0.96) \times 10^{10}$	$7.57 \pm (0.83) \times 10^{10}$
Trypsin, 1000	$7.74 \pm (0.86) \times 10^{10}$ ($P < 0.01$) [b]	$7.12 \pm (0.80) \times 10^{10}$ ($P < 0.01$)
Trypsin, 5000	$6.99 \pm (0.80) \times 10^{10}$ ($P < 0.01$)	$6.51 \pm (0.74) \times 10^{10}$ ($P < 0.01$)
Trypsin, 10,000	$6.16 \pm (0.72) \times 10^{10}$ ($P < 0.01$)	$5.45 \pm (0.65) \times 10^{10}$ ($P < 0.01$)
Chymotrypsin, 1000	$7.57 \pm (0.81) \times 10^{10}$ ($P < 0.01$)	$6.81 \pm (0.73) \times 10^{10}$ ($P < 0.01$)
Chymotrypsin, 5000	$6.66 \pm (0.74) \times 10^{10}$ ($P < 0.01$)	$5.90 \pm (0.66) \times 10^{10}$ ($P < 0.01$)
Chymotrypsin, 10,000	$5.82 \pm (0.62) \times 10^{10}$ ($P < 0.001$	$5.22 \pm (0.60) \times 10^{10}$ ($P < 0.001$)

[a] CFU/ml ± S.E. mean = colony forming units/ml (average of 10–15 determinations) ± standard error of the mean.

[b] Probability values express results of *t*-test between each mean value and that of the control system.

On the other hand, the antibacterial activity of the two enzymes was more pronounced against the three Gram-negative bacteria studied, namely *Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa* (Table 2).

At the concentrations tested, 1000, 5000 and 10000 units/ml, trypsin's killing effect on *Escherichia coli* was 19, 36 and 44%, respectively; and for chymotrypsin it was 24, 43 and 52%. The inhibitory effect on *Proteus vulgaris* in the case of trypsin was 16, 30 and 40% at the 3 specified concentrations, whereas with chymotrypsin, it was 20, 40 and 50%.

The bactericidal activity of chymotrypsin against *Pseudomonas aeruginosa* was 18, 32 and 42% at 1000, 5000 and 10,000 NF units/ml, respectively; with trypsin, it was 14, 26 and 36%.

From these results, it is evident that the Gram-negative bacilli tested (*Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*) were more susceptible to the proteolytic enzymes (trypsin and chymotrypsin) than were the Gram-positive cocci (*Staphylococcus aureus* and *Streptococcus pyogenes*). This could be partly explained in terms of the reported differences in Gram-negative and Gram-positive bacteria (Salton, 1952, 1953), and the higher content of lipoproteins in the former compared to the latter.

The actual mechanism by which the proteolytic enzymes act on bacteria has not been defined. Since it has been established that these enzymes hydrolyze ester or peptide bonds (Colman, 1965), it seems likely that trypsin and chymotrypsin act by

TABLE 2

BACTERICIDAL ACTIVITY OF PROTEOLYTIC ENZYMES AGAINST GRAM-NEGATIVE BACTERIA

System concentration (units/ml)	*Escherichia coli* (CFU/ml ± S.E. mean [a])	*Proteus vulgaris* (CFU/ml ± S.E. mean [a])	*Pseudomonas aeruginosa* (CFU/ml ± S.E. mean [a])
Control (0.9% saline)	$9.47 \pm (1.12) \times 10^9$	$7.66 \pm (0.88) \times 10^9$	$8.88 \pm (1.06) \times 10^9$
Trypsin, 1000	$7.67 \pm (0.82) \times 10^9$ ($P < 0.01$) [b]	$6.43 \pm (0.76) \times 10^9$ ($P < 0.01$)	$7.64 \pm (0.87) \times 10^9$ ($P < 0.01$)
Trypsin, 5000	$6.06 \pm (0.74) \times 10^9$ ($P < 0.001$)	$5.56 \pm (0.64) \times 10^9$ ($P < 0.001$)	$6.56 \pm (0.72) \times 10^9$ ($P < 0.01$)
Trypsin, 10,000	$5.30 \pm (0.64) \times 10^9$ ($P < 0.001$)	$4.60 \pm (0.57) \times 10^9$ ($P < 0.001$)	$5.70 \pm (0.73) \times 10^9$ ($P < 0.001$)
Chymotrypsin, 1000	$7.20 \pm (0.88) \times 10^9$ ($P < 0.01$)	$6.13 \pm (0.67) \times 10^9$ ($P < 0.01$)	$7.29 \pm (0.87) \times 10^9$ ($P < 0.01$)
Chymotrypsin, 5000	$5.40 \pm (0.72) \times 10^9$ ($P < 0.001$)	$4.60 \pm (0.56) \times 10^9$ ($P < 0.001$)	$6.04 \pm (0.72) \times 10^9$ ($P < 0.001$)
Chymotrypsin, 10,000	$4.55 \pm (0.59) \times 10^9$ ($P < 0.001$)	$3.83 \pm (0.43) \times 10^9$ ($P < 0.001$)	$5.16 \pm (0.64) \times 10^9$ ($P < 0.001$)

[a] CFU/ml ± S.E. mean = colony forming units/ml (average of 10–15 determinations) ± standard error of the mean.

[b] Probability values express results of *t*-test between each mean value and that of the control system.

affecting the chemical integrity of the bacteria, attacking the proteinaceous components of their cell walls. The walls of Gram-positive cells do not contain protein. Other possibilities consistent with the data are the reported removal of part of the cell surface of *Micrococcus aureus* upon enzymic treatment and thereby affecting the surface charge (Dyar, 1948), surface alteration of *Mycobacterium tuberculosis* with trypsin (Engelhard and Uyeno, 1957), or even the hydrolysis of the capsid protein at the peptide bonds (Boosman, 1978). Alternatively, the cytoplasmic membrane is a possible target for enzymatic attack. The effect of pH of the enzyme solution was not investigated.

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