CHARACTERIZATION OF A SUBSTANCE IN TRACHEAL EXUDATES WITH ACTIVITY AGAINST PASTEURELLA MULTOCIDA

E. Iglesias, C. Pijuan, E. Hernandez

Facultad de Estudios Superiores "Cuitláhuac" Apdo. Postal 412, Cuautitlán-Izcalli, Edo. de México

Recent studies have described the existence of a substance found in pig tracheal exudates that is highly active against Pasteurella multocida (Pijuan and Ochoa, 1978). These same authors (1978) have postulated that this substance acts as a central role in the defense of the lung against this pathogen, especially in view of the apparent poor activity shown by the same microorganisms against P. multocida (Pijuan et al., 1980). However, little is known about the nature, mechanism and range of action of this substance.

Materials and Methods

Porcine embryo tracheas were collected and cultured as described by Lastar et al. (1978). Culture supernatants were collected again 24 hours later. Proteins were precipitated by salt fractionation with ammonium sulphate, dialyzed against PBS and demineralized with acetone. The resulting fraction was further purified through a sephadex G-50 column. The fluid from the tubes containing fractions showing peaks of absorption at 280 nm, were sterilized by filtration and tested for antimicrobial activity. Antibody content was measured according to the technique of Snell (1974). Molecular weight was established by polacrylamide gel electrophoresis (10% with SDS) with albumin, ysozyme and trypsin as controls. Gels were run for 90 minutes at 20 mA, and stained according to Apple et al. (1980).

To establish the effect of the substance on Pasteurella,ru, the kinetic activity was measured at 15, 30, 60 and 120 minutes by turbidity (600 nm) and viable counts. Treated bacteria (at 15, 30, and 60 minutes) were studied by electron microscopy, both by negative staining and ultrathin-section techniques. A range of gram-positive and gram-negative bacteria were also tested for susceptibility to the purified substance.

Results

The tracheal supernatants showed three separate peaks with antibacterial activity. The second peak, which was the most abundant, was chosen for further study. The activity of the substance was completely destroyed by trypsin, and increased only with heat treatment. By comparing against a glucose curve, it was determined that 20% of the substance was carbohydrate. The molecular weight was determined from SDS-polyacrylamide gel electrophoresis. The mechanism of action was bacteriostatic rather than bactericidal. Electron microscopy showed that the bacteria were shrinking and releasing autolytic enzymes.

Discussion

The results show that the substance is a glycoprotein with a single chain of amino acids and disulfur bonds that are responsible for the activity. It is neither lysozyme (different molecular weight and bacteriostatic activity) nor BSA (similar molecular weight). Therefore, it is probably a new, undescribed substance. The mechanism of action seems to be directed against the cell wall, which shows shrinkage and release of protosomic material. The antibacterial range is wide, especially against respiratory pathogens. However, most enterobacteria and anaerobes seem resistant. The marked susceptibility of Pasteurella multocida is in agreement with the hypothesis that mucobiliary inactivation and clearance constitutes the main non-immune defense mechanism of the pig against this organism.

References


Resumen

Caracterización de una substancia presente en exudados traqueales con actividad contra Pasteurella multocida

Algunos estudios recientes han mostrado que P. multocida es susceptible a la acción microbicida del sobrenadante de cultivos de tracheas embrionarias de cerdo.

Las tracheas se cultivaron en un sistema in vitro y se obtuvo el sobrenadante. Las proteínas fueron fraccionadas con sulfato de amonio y cromatografía de columna. Las fracciones con actividad microbicida fueron caracterizadas por técnicas bioquímicas comunes.

Los resultados muestran que la substancia es un glycoproteinina de una sola cadena con 20% de carbohidratos. Parece tratarse de una nueva substancia, diferente a la lipoquinasa y Beta-lactámica. Tiene un espectro antibacteriano amplio, especialmente contra patógenos respiratorios, aunque la mayoría de los enterobacterias y los anaerobios son resistentes.