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

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Recent studies have described the existence of a substance found in pig embryo tracheal explant cultures, with a strong activity against Pasteurella multocida (Pijoan and Ochoa, 1978). These same authors (1980) have postulated that this substance plays a central role in the defense of the lung against this pathogen, especially in view of the apparent poor activity shown by alveolar macrophages against P. multocida (Pijoan et. al 1980). However, little is known about the nature, mechanism and range of action of this substance.

Material and Methods

Porcine embryo tracheas were collected and cultured as described by Lastra et. al 1978. Culture supernatant collected again 24 hours later. Proteins were precipitated by salt fractionation with ammonium sulphate, dialysis against PBS and dehydration with Acuacide. The resulting fraction was further purified through a sephadex G-50 column. The fluid from tubes containing fractions showing peaks of absortion at 280 nm.were sterilized by filtration and tested for antibacterial activity against P. multocida. Positive fractions were treated with trypsin (1mg /ml) urea (6M) and mercaptoethanol (2 mM), and retested for antibacterial activity. Carbohydrate content was measured according to the technique of Snell (1974). Molecular weight was established by poliacrilamide gel electrophoresis (10% with SDS) with albumin, lysozime and trypsin as controls. Gels were run for 90 minutes at 20 mA, and stained according to Hughes et.al. (1980).

To establish the effect of the substance on Pasteurella, the Kinetic activity was measured at 15, 30, 60 and 120 minutes both 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 decreased 90% with mercaptoethanol and 15% with urea treatment. By comparing against a glucose curve, it was determined that 20% of the substance was carbohydrate. The molecular weight could not be determined definitely with the techniques employed, but proved to be between 10,000 and 50,000 daltons. The kinetics showed little effect on the turbidity of the bacterial suspension, but a marked effect on the viable counts, suggesting that the mechanism of action is bacteriostatic rather than bactericidal. Electron microscopy of treated bacteria showed wrinkling of cellular surface, appearance of half-moon cellular bodies, and release of cytoplasm. Bacteria with marked sensitivity to the substance were Bacillus subtilis, Pasteurella mutocida and Proteus mirabilis. Moderate sensitivity was shown by Micrococcus lisodeikticus, Listeria monocytogenes, Bordetella bronchiseptica, Haemophilus pleuropneumoniae, and Pseudomonas aeruginosa. Resistant species were, Clostridium chauvoei and welchii, E.coli and Klebsiella pneumoniae.

Discussion

The results show that the substance is a glycoprotein with a single chain of aminoacids and disulphur bonds that are responsible for the activity. It is neither lysozime (different molecular weight and bacteriostatic activity) nor Beta Lysine (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 protoplasmic 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 mucociliary inactivation and clearance constitutes the main non-immune defense mechanism of the pig against this organism.

References

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Resumen

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

Algunos estudios recientes han mostrado que P. multocida es susceptible a la accion microbiocida del sobrenadante de cultivos de traqueas embrionarias de cerdo.

Las traqueas se cultivaron en un sistema in vitro y se obtuvo el sobrenadante. Las proteinas fueron fraccionadas con sulfato de amonio y cromatografia de columna. Las fracciones con actividad microbiocida fueron caracterizadas por tecnicas bioquimicas comunes.

Los resultados muestran que la substancia es una glicoproteina de una sola cadena con 20% de carbohidratos. Parece tratarse de una nueva substancia, diferente a la lisozima y Beta-lisina. Tiene un espectro antibacteriano amplio, especialmente contra patogenos respiratorios, aunque la mayoria de las enterobacterias y los anaerobios son resistentes.