THE INTERACTION OF SOME TETRACYCLINES WITH PURIFIED MUCUS GLYCOPROTEINS

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The tetracycline antibiotics have been shown to interact with mucus (Saggers and Lawson, 1966) and it has also been demonstrated that the presence of mucin can affect bioavailability (Braybrooks, Barry & Abbs, 1975). The addition of a number of tetracyclines to sputum has been shown to result in an increase in viscoelastic properties (Marriott & Kellaway, 1975). However, it has also been observed that model mucus gels prepared from either the pooled sputum of diseased patients (Creeth, 1978) or animal models such as the canine tracheal pouch (Reasor et al, 1978) contain considerable quantities of protein contamination in addition to glycoprotein. It is possible that the observed effects could be due to tetracycline interaction with this protein contamination and no data is available on the effect of tetracyclines on the glycoprotein alone.

A rheologically active glycoprotein has been separated from pooled, non-purulent, human bronchitic sputum by extraction with 0.22M potassium thiocyanate followed by gel filtration chromatography on Sepharose 4B. The excluded volume, which contained a protein rich in sugars, was dialysed against distilled, deionised water and then concentrated in an ultra filtration cell until a discrete gel was produced on the membrane. Analytical equilibrium density gradient ultracentrifugation in caesium chloride gave a large peak in the glycoprotein buoyant density region which together with amino acid and sugar analyses indicated that the product was of high purity. Experiments with established mucolytic agents all reduced mucus gel structure.

The effect of tetracycline (I), oxytetracycline (II) and doxycycline (III) on the rheological properties of the purified glycoprotein were evaluated in dilute solution by U-tube viscometry and in the gel state by non-destructive creep compliance testing. An estimate of mucociliary clearance was obtained by measurement of the transport rate on the excised, mucus-depleted upper palate of the frog (*Rana esculenta*). All three tetracyclines exhibited a marked thickening effect on the mucus gels reflected by an increase in the residual shear viscosity (I = 299%, II = 683% and III = 177% at a concentration of 0.5%w/v) and a decrease in the instaneous shear compliance (I = 28%, II = 51% and III = 13% at a concentration of 0.5%w/v). The effect was concentration dependent over the range 0.05 to 1.0%w/v although the relationship was non-linear. A decrease was also observed in the transport rate on the frog palate (I = 25%, II = 40% and III = 7% at a concentration of 0.1%w/v) which indicated that the ciliated epithelium was less able to clear the thickened gel. The intrinsic viscosity also increased in the presence of the tetracyclines which indicates that an expansion in the hydrodynamic volume had taken place. The value of the Huggins' constant was reduced from 0.64 to 0.546 for III, 0.25 for I and 0.21 for II which suggests a change in solvent-solute interaction. The order of increasing activity is therefore, III<I<II and is directly opposite to the order of apparent partition coefficient which has been shown to correlate with the degree of tetracycline binding to serum albumin (Kellaway & Marriott, 1978). However, it is apparent that the thickening of mucus gels produced by the tetracycline antibiotics is due to an effect on mucus glycoproteins and experiments with labelled tetracycline have indicated that binding to the glycoprotein does occur.

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