Some Observations on the Penetration of Antimicrobial Drugs into the Respiration Secretions of Horses

P. M. Dixon, J. R. Thomson, R. Brown, A. Galbraith and *J. A. Bogan*

Veterinary School, University of Glasgow

Effective antimicrobial treatment depends particularly on the ability of the antimicrobial drug to attain therapeutic levels at the site of infection. Unless minimal inhibitory concentrations of the drug are achieved at the infection site, bacterial growth can continue despite in vitro susceptibility of these pathogens to the antimicrobial drug. In chronic broncho-pulmonary infections in man, many studies have shown that antimicrobial concentrations in respiratory secretions (R. S.) are therapeutically more important than serum antimicrobial levels (*Wong* et *al.*, 1975; *Lode* et *al.*, 1980; *Klastersky* and *Thys*, 1983).

With parenteral antibiotic administration in man, variable and often low antibiotic concentrations are found in respiratory secretions. Whilst in acute broncho-pulmonary infections this may not be significant, it is likely to be a reason why in some cases of chronic broncho-pulmonary disease the infection is never fully eradicated or why relapses occur (*Lambert*, 1978).

Parenteral administration of penicillin, ampicillin or a trimethoprimsulfonamide combination are commonly used in the treatment of equine bronchopulmonary infections. Many studies have determined the serum levels attained by these drugs after parentral administration and to a lesser extent the synovial, peritoneal and urine levels attained. However, information is lacking on the penetration of these drugs through the respiratory mucosa i. e. the blood-broncho alveolar barrier and consequently on the antimicrobial levels attained in respiratory secretions after parentral administration. In this study respiratory secretion and plasma levels of penicillin, ampicillin and a trimethoprim-sulfonamide combination were simultaneously measured after parentral administration of these drugs.

A variety of terms are used to describe the mucus secreted by the respiratory tract including mucus, respiratory mucus, bronchial mucus, bronchoalveolar secretions, tracheo bronchial secretions. The term used in this report i. e. respiratory secretions (R. S.) is taken to include the total secretions of respiratory mucus membranes including goblet, serous and mucus cells.

Materials and Methods

Horses free of broncho-pulmonary disease usually have no accumulations of respiratory secretions in their trachea and regular collections of volumes (> 1 ml) adequate for antimicrobial assay is impossible unless tracheal pouches are surgically created. Increased R. S. production is a feature of most chronic broncho-pulmonary diseases in the horse and in such animals, frequent collection of adequate R. S. samples is possible. In this present study adult TB and TB cross horses with chronic obstructive pulmonary disease (COPD) and horses with chronic (greater than 3 month duration) bronchopulmonary disease of unknown aetiology were used, a common factor to all these animals was the excessive production of mucoid or slightly mucopurulent R. S. Animals with very purulent R. S. were not used.

Crystaline sodium benzyl penicillin (Crystopen®, Glaxovet Ltd., Middlesex) at a dosage of 20,000 Iu/kg was dissolved in 20 mls of sterile water and administered by deep intramuscular injection. Ampicillin trihydrate, 15 % w/v in ethyl aleate (Penbritin® injectable suspension, Beecham's Animal Health, Middlesex), was administered intramuscularly at a dose of 10 mg/kg. A combination of trimethoprim 80 mg/ml and sulfadiazine 400 mg/ml (Tribrissen® injectable 48 %, The Wellcome Foundation Ltd, Crewe, Cheshire), was administered at a dose of 15 mg/kg intravenously.

Respiratory secretions were collected from the distal trachea under visual control, using a 1 mm internal diameter plastic catheter (Letro Cath 1155, Vygon, Ecouen, France), through the biopsy channel of a 1 metre long flexible endoscope (Olympus GiF Type K) by manual aspiration with a 50 cc plastic syringe. A venous blood sample taken at the same time was immediately centrifuged. In all animals R. S. and blood samples were collected immediately prior to antimicrobial drug administration, at 30 minutes post administration and then at hourly intervals for 6 hours post administration.

Respiratory secretions are a material of complex and variable composition and the measurement of antibiotic concentrations in theses secretions are subject to greater technical difficulties than other measurement in tissue fluids of more constant composition e. g. serum. (*Lambert*, 1978).

Samples for ampicillin assay were immediately vortexed with an equal volume of trichloracetic acid and the clear supernatant stored at −15 °C until analysed.

Trimethoprim concentrations i/v in plasma and respiratory secretions were measured by the technique of *Schwartz* et *al.* (1969). Sulfadiazine levels were measured as active sulfonamide levels in plasma and respiratory secretions by the technique of *Reider* (1972). Ampicillin levels were estimated by the technique of *Barbhaiyar* et *al.* (1977). Except that equal volumes of 20 % trichloracetic acid was used for precipitation of the respiratory secretions as 10 % TCA did not clear these solutions. Penicillin assays were performed by a tube dilution/inhibition of Oxford staphylococcus. In the initial experiments using this technique whilst adequate plasma penicillin levels were found no penicillin levels were recorded in respiratory secretions. This was thought to be due to penicillinase producing bacteria in the R. S. To counteract this all samples were filtered through salt agar immediately after collection and the tube dilution/inhibition tests were then performed.

Results

In most animals it was possible to collect serial R. S. samples over the 6 hour period. However it was found that some horses, especially those with viscous R. S. where it could take up to 5 minutes to collect the sample, that saliva flowed down through the open glottis and contaminated the R. S. All such samples were discarded. It was found that removal of food and of straw bedding for the duration of the experiment reduced this problem in some cases. On some occasions it was found that horses which previously had large pools of R. S. in their lower trachea had on subsequent collections no visible accumulation of R. S. present. Whether this was due to increased expectoration or coughing due to URT irritation by the endoscope or was due to low volumes of R. S. being replaced was not clear.

Peak R. S. antimicrobial levels were obtained 1 to 2 hours after parenteral administration of the antibiotic. This delay presumably being due to the time required for R. S. production in the distal airways and for its transport to the trachea where it was sampled. Allowing for this time lag the shape of the R. S. antibiotic level curve somewhat paralleled the serum antibiotic curves, although at a much lower level. Ampicillin plasma: R.S. levels (micrograms/ml) at 1 hour were 13.37 : 1.74, at 2 hours 2.26 : 1.89, at 4 hours 1.29 : 1.12 and at 6 hours 0.89 : not detected.

With sulfadiazine, relatively high and prolonged levels were achieved in the R.S., with plasma: R. S. (micrograms/ml) at 1 hour of 71 : 14.4, at 2 hours 66.1 : 25.2, 4 hrs 42.6 : 11.8 and at 5 hours 38.9 : 7.1.

As noted in the materials and methods section, our initial penicillin experiments showed no penicillin activity in R.S. due to penicillinase production during this assay, by bacteria in the R. S., whilst acceptable penicillin levels were recorded in the serum. With salt agar filtration of all samples immediately after collection, penicillin activity was detected in R. S. but it was found that some loss of penicillin activity occurred in the serum with this process. Presumably an equal loss of penicillin activity occurred in both serum and R. S. during this process. The results show that maximal dilution for total staphylcoccus inhibition in plasma was 1/350 at 1 hour whilst the value for R. S. at 1 hour was 1/21. A rapid dropping off of R. S. penicillin activity to negligible levels occurred at about 4 hours. As would be expected with this level of penicillin dosage i/m, serum levels also were short lived.

With trimethoprim persistent technical problems with the R. S. assays allowed satisfactory measurements in only 2 cases. These showed peak R. S. trimethoprim levels to be 30.8 % of plasma levels at 2 hours, but in both animals R. S. trimethoprim levels of over 0.35 microgram/ml which are considered acceptable in man were maintained for over 6 hours, at which stage plasma levels were less than 0.1 microgram/ml or not detectable. This would suggest that trimethoprim has good penetration into R. S. as occurred with sulfadiazine.

Discussion

This preliminary study suggests that some of the commonly used antibiotics achieve low and transient levels in equine R. S. after parenteral administration. It is obvious that the currently used antibiotic dose regimes do not give effective plasma levels 24 hours a day and effective R. S. antibiotic levels are even less effective. Adequate minimum inhibitory concentrations to some pathogens may never occur in R. S. with some antibiotics at currently used dosages.

Sulfadiazine achieved the highest R. S. levels and trimethoprim also appears to achieve relatively high and prolonged R. S. levels whereas the penicillins had lower penetration into the R. S. Another factor to consider with penicillin therapy is the production of penicillinases within the respiratory tract, probably by non-pathogenic respiratory commensal organisms. Even Fleming, the discoverer of penicillin, suggested in 1946 that non pathogenic penicillinase producing bacteria could protect penicillin sensitive pathogens against penicillin therapy (*Fleming*, 1946; cited by *Maddocks* and *May*, 1969). These latter authors also showed that with chronic bronchial infection in man where long term penicillin therapy had been used, that ampicillin therapy gave good serum levels, but negligible levels were found in R. S. due to penicillinase production in the respiratory tract by gram-ve non pathogens. In many of these cases ampicillin treatment was ineffective leading to the concept of "indirect pathogens".

In conclusion this preliminary study suggests the need for more extensive studies on a wider range of antibiotics of their penetration into the R. S. It suggests that sulfadiazine: trimethoprim combination may be more effective than penicillins in this regard. Although penicillins are among the drugs of choice for most acute equine respiratory infections, which are caused by uniformly penicillin sensitive streptococci, further studies should be performed on the value of penicillin usage in chronic broncho-pulmonary disease where penicillinase production in the airways may be significant.

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P. M. Dixon, MVB, PhD, MRCVS
Faculty of Veterinary Medicine, Royal (Dick) School of Veterinary Studies, Department of Veterinary Surgery, Veterinary Field Station Ester Bush, Roslin, Midlothian, England