IN VITRO STUDIES ON TIAMULIN AND KITASAMYCIN AGAINST A RANGE OF ORGANISMS INCLUDING PIG PATHOGENS

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Tiamulin (Squibb Dynamutilin), a semisynthetic derivative of the antibiotic pleuromulin, has been shown to have good activity against a number of bacterial species (Drews et al 1975) with high activity against *T. hyodysenteria* (Taylor 1976 and Kitai et al 1979) and *M.hyopneumoniae* (Goodwin 1979). Kitasamycin (Bayer A.C.Trubrin), a macrolide antibiotic produced by *Streptomyces kitasatoensis Hata*, has antibacterial activity against Gram positive bacteria, spirochaetales and mycoplasma and is indicated as being highly effective in the control and therapy of enzootic pneumonia in pigs and in the prevention and treatment of swine dysentery (Bayer A.C.Trubrin Professional literature). In vitro studies were carried out to compare the antibacterial activity of Tiamulin and Kitasamycin with particular reference to their activities against *T. hyodysenteriae* (swine dysentery) and *M. hyopneumoniae* (enzootic pneumonia).

The organisms used in the study are listed in Table 1. Trypticase Soya Agar, incorporating 5% defibrinated sheep blood, was used for the cultivation and testing of treponemes, anaerobes and aerobes. Chanock medium was used for the cultivation of mycoplasmas, with the incorporation of 1% agar in Chanock medium for the testing procedure. An agar dilution method was used for testing, in which two-fold dilutions of Tiamulin (E.R.Squibb, U.K. Lot No. D230) or Kitasamycin (Toyo Jozo Co., Japan Lot No. TABM-001) were prepared in sterile water and included in sets of media to give final concentrations ranging from 50-0.0015 mcg/ml. Test suspensions of *T. hyodysenteriae* and anaerobes were prepared by removing surface colonies from 48 hour-incubated cultures into 2 ml aliquots of preheated and cooled Robertson's Meat Broth. Suspensions of aerobes were prepared by removing colonies from 24 hour-incubated plates into 2 ml aliquots of Trypticase Soya Broth, supplemented with 5% horse serum. Mycoplasma strains were grown for 48 hours in Chanock medium and the broth suspensions used undiluted. After drying the surfaces of all plates containing dilutions of the antibiotics, suspensions of test organisms were applied to the agar surfaces by means of a Steers-type replicator (Steers et al 1959). Anaerobes and *T. hyodysenteriae* were incubated at 37°C in a 5% carbon dioxide, 95% hydrogen, gas mixture (BBL Gas Pak with cold catalyst). Aerobes were incubated at 37°C and mycoplasmas were incubated at 37°C in closed containers containing dampened tissues. Inoculated plates without antimicrobial agents were incubated as controls for viability of the test organisms.

Results were recorded after 24 hours incubation in the case of the aerobes and after 72 hours incubation in the case of *T. hyodysenteriae*, anaerobes and mycoplasmas. The lowest concentration found to inhibit growth was recorded as the minimal inhibitory concentration (M.I.C.). In the case of *T. hyodysenteriae* M.I.C.'s were recorded as the lowest concentration of antimicrobial agent which completely prevented haemolysis.

Results

The results are presented in Table 1.

Table 1 Summary of Results

Organism		MIC (mcg/ml) Tiamulin	Kitasamycin
S.aureus coag +ve 121[a]	A	0.049	0.390
S.aureus coag +ve 2[a]	A	0.049	0.78
S.aureus coag +ve 1[a]	A	0.195	1.56
Corynebacterium equi 174[a]	A	25	25
Corynebacterium pyogenes[a]	A	0.024	0.39
Corynebacterium ulcerans[a]	A	0.78	0.049
Strep. agalactiae 43[a]	A	0.195	0.39
Strep.suis II[a]	A	3.125	0.195
Strep.lactis 65[a]	A	>50	>50
Strep.zooepidemicus[a]	A	0.39	0.39
Strep.equi 138[a]	A	0.39	0.39
Clostridium perfringens B[a]	An	0.78	1.56
Clostridium perfringens C H2[a]	An	0.78	1.56
Clostridium histolyticum[a]	An	0.195	0.195
Clostridium septicum[a]	An	>50	0.78
Clostridium chauvoei H2[a]	An	0.098	0.195
Campylobacter coli[a]	An	0.39	1.56
Fusobacterium necrophorum[a]	An	0.39	1.56
Treponema hyodysenteriae 2[b]	An	0.049	>50
Treponema hyodysenteriae P289[b]	An	0.049	>50
Mycoplasma bovis 75427[c]	AM	0.098	1.56
Mycoplasma mycoides var capri[d]	AM	0.049	0.39
Mycoplasma bovirhinis[c]	AM	0.006	1.56
Mycoplasma hyopneumoniae JF74[c]	AM	0.098	0.78
Mycoplasma hyopneumoniae[c]	AM	0.098	0.78
Mycoplasma arginini G320[c]	AM	0.006	1.56
Mycoplasma pulmonis[c]	AM	0.195	1.56
Mycoplasma columbinum[c]	AM	0.195	6.25
Mycoplasma columborale[c]	AM	0.195	6.25

a. Clinical isolates supplied by Liverpool Veterinary Investigation Centre, Liverpool, England.
b. Clinical isolates supplied by Leeds Veterinary Investigation Centre, Leeds, England.
c. Clinical isolates supplied by Department of Avian Medicine, University of Liverpool, Leahurst Experimental Station, Neston, Wirral, England.
d, National Collection of Type Cultures NCTC 10137
An = Anaerobic atmosphere A = Aerobic atmosphere
AM = Aerobic atmosphere plus moisture

Discussion

Tiamulin M.I.C.'s against organisms common to this study and previous studies (Drews et al 1975, Taylor 1976, Kitai 1979, Goodwin 1979) are in agreement. Of the twenty-nine organisms tested twenty-six showed equal or greater susceptibility to Tiamulin than to Kitasamycin. All nine mycoplasma species were more highly sensitive to Tiamulin than to Kitasamycin, with the former showing a greater activity ranging from an 8-fold against *M. hyopneumoniae* to a 250-fold greater activity against *M. bovirhinis*. M.I.C. results against two strains of *T. hyodysenteriae* show the greatest differences. Where both strains were susceptible to 0.049 mcg/ml of Tiamulin they were resistant to>50 mcg/ml of Kitasamycin, demonstrating a>1000-fold superiority in vitro by Tiamulin.

References: Drews, J., et al Antimicrobial Agents and Chemotherapy 7, 507-517, 1975. Taylor, D.J., International Pig Veterinary Society Congress, Ames, Iowa 1976. Kitai, K., et al Antimicrobial Agents and Chemotherapy 15, 392-395, 1979. Goodwin, R.F.W., Vet. Record 104, 194-195, 1979. Steers E., et al Antimicrobial Agents and Chemotherapy 9, 307-311, 1959.