

ASSESSMENT OF RESISTANCE DEVELOPMENT TO VALNEMULIN IN ENTEROCOCCUS FAECIUM AND ASSOCIATED CROSS RESISTANCE TO SEVEN OTHER ANTIMICROBIALS

A Pridmore¹, Orysia Chymera¹, J T Holck², P Silley^{1,3}

1) Don Whitley Scientific, Shipley, UK 2) Novartis Animal Health, Greensboro NC, USA 3) MB Consult, Bingley, UK

Introduction

The association of antimicrobial resistance development by human pathogens due to the use of antimicrobials in food animals is a highly controversial issue globally. The use of antimicrobials in food animals has been incriminated by direct exposure to foodborne pathogens (ie Salmonella), as well as indirectly through commensal organisms such as *Enterococcus spp.* This study was carried out to assess whether changes in the valnemulin susceptibility of *E. faecium* strains might occur following repeated sub-culture in the presence of sub-inhibitory concentrations of valnemulin hydrochloride.

Materials and Methods

Three *E. faecium* strains isolated from swine in the USA were selected from a panel of 20 strains of similar origin based upon proven susceptibility to valnemulin and minimal pre-existing resistance to antimicrobials used in human medicine. Valnemulin stock solution was diluted to produce working solutions at concentrations of 0.15 µg/ml and 0.015 µg/ml. Working in triplicate the tubes were inoculated with 0.05 ml of the appropriate standardized inoculum (ie three sets of both valnemulin concentrations for each test strain). The target inoculum density was approximately 5×10^5 cfu/ml. All tubes were incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 1 h. Subsequently, the fixed valnemulin concentrations were prepared on each consecutive day for a total of 14 days. These preparations were re-inoculated with the corresponding incubated broth from the previous culture (ie broth containing a given concentration of valnemulin was used to re-inoculate broth containing the same valnemulin concentration). Each incubated broth from the fixed valnemulin concentration series was also subcultured on to Columbia Blood Agar with stock suspensions prepared for long-term storage at a nominal temperature of -80°C . In addition CBA plates were stored at $5 \pm 3^\circ\text{C}$ until required for MIC testing. At the end of the 14 day period the MIC of valnemulin against the isolates harvested from days 0, 6, 10 and 14 was determined according to standard NCCLS procedures.

Results

Throughout the 14 day experiment, all three *Enterococcus faecium* strains grew vigorously in the presence of Valnemulin hydrochloride at 0.15 µg/ml and 0.015 µg/ml. *Enterococcus faecium* viable count was determined daily in each adjusted suspension used to inoculate the test system. Bacterial density in these suspensions was consistently between 4.0×10^7 cfu/ml and 2.0×10^8

cfu/ml, therefore the final inoculum density in the test preparations fell between 2.0×10^5 cfu/ml and 1.0×10^6 cfu/ml.

The MIC of Valnemulin hydrochloride against each *Enterococcus faecium* “parent” strain was determined in triplicate prior to any exposure to this agent. Following daily exposure to Valnemulin HC1 at either 0.15 µg/ml or 0.015 µg/ml, the MIC against “progeny” strains harvested at 6, 10 or 14 days did not deviate by more than 2 doubling dilutions from these initial values. Furthermore, the MIC of Valnemulin hydrochloride against *E. faecium* strains exposed to this agent for 14 days fell within +/- 1 doubling dilution of that of the parent strain, in all cases.

The antibiotic susceptibility of the original field isolates, and of their valnemulin-exposed progeny, was assessed using a panel of compounds used in the treatment of human *Enterococcus* infections (Vancomycin, quinupristin/dalfopristin, gentamicin), those with a similar mode of action to valnemulin (erythromycin), and representatives of other important therapeutic classes (nalidixic acid, ciprofloxacin and ceftriaxone). For all compounds except ceftriaxone, MIC results against *E. faecium* strains harvested after 6, 10 and 14 days of valnemulin hydrochloride exposure did not deviate by more than 1 doubling dilution from MIC results obtained against the corresponding “parent” strains prior to exposure. For ceftriaxone, changes in MIC were not greater than 2 doubling dilutions from those of the parent strains: these differences fall within the normal range of variability for MIC determinations, and were only observed in strains with initial ceftriaxone MICs of 32 µg/ml and 128 µg/ml.

Discussion

Preliminary studies with tiamulin, a related plueromutilin, have indicated that the stepwise sequential protocol (1) and the fixed concentration subculture protocol (2) give equivalent results. The fixed concentration subculture protocol was selected for the present study because it determines the potential for *in vitro* resistance development at concentrations of antibiotic likely to be encountered in the colon. It is these concentrations to which the GI tract flora will be exposed in the field situation. The present study used concentrations of 0.15 µg/ml and 0.015 µg/ml as a selective pressure. The observations described above demonstrate that repeated exposure of *Enterococcus faecium* strains to Valnemulin hydrochloride at 0.15 µg/ml or 0.015 µg/ml did not affect their susceptibility to this antimicrobial agent in the present study. In addition, repeated daily exposure to valnemulin hydrochloride at 0.15 µg/ml or 0.015 µg/ml did not affect the susceptibility of *Enterococcus faecium* strains to the panel of antimicrobial compounds tested in the present study.

References

1. Brady S M, et al (1988) J. Assoc. Off. Anal. Chem. 71, 295-298.
2. Puntorieri M, et al (1999) International Journal of Antimicrobial Agents 12, 333-339.