

Trans-epithelial transport and intracellular accumulation of macrolide antibiotics

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Introduction

Poultry pathogens sensitive to macrolides are: *Mycoplasma gallisepticum*, *M. synoviae*, *Staphylococcus aureus*, *Ornithobacterium rhinotracheale*, *Brachyspira intermedians* and *B. pilosicoli*, and *Clostridium perfringens*. Macrolides are generally considered to enter cells and be effective intracellularly, but the extent to which this happens varies depending on the particular antibiotic and cell type. The aim of this study was to investigate the uptake and concentration of three macrolide antibiotics in cell types representing enterocytes, epithelial cells and white blood cells.

Materials and Methods

Tylvalosin (in Aivoslin[®] products) was used as the tartrate salt (ECO Animal Health), tylosin as the tartrate salt and tilmicosin, as the phosphate salt (ELANCO Animal Health). Caco2 cells are human cells with the characteristics of mature enterocytes. The differentiated polarised cells form tight junctions, which results in the formation of an apical and basolateral membrane. Caco2 cells were incubated at 37°C with tylvalosin, tylosin or tilmicosin (at 10 µg/ml) in the apical chamber. Medium (in apical and basal chambers) and cells were harvested after 2 and 4 hrs and assayed for antibiotic content. Pig kidney cells (LLC-PK1) were incubated with about 10 µg/ml of the three different macrolides. Cells and medium were harvested at 75 and 120 mins and analysed for macrolide content. Chicken white blood cells were isolated from blood, washed in PBS and re-suspended in medium. Cells were incubated with 10 µg/ml of either tylvalosin or tilmicosin in an Eppendorf tube, on a rotary mixer at 37°C. Cells and medium were harvested at 15, 30 and 60 mins. All samples were analysed using liquid chromatography with tandem mass spectrometry.

Results

At 2 hrs and 4 hrs, tylvalosin was detected at an average concentration of 42.2 µg and 34.4 µg/g of Caco2 cells, respectively. The corresponding values for tilmicosin were 8.6 µg and 16.1 µg/g of cells and the highest value for tylosin was 1.9 µg/g at 4 hrs (fig.1). The concentration of macrolide in the basal chamber was assayed at 4 hrs and compared to that in the apical chamber. The values were 10.48% for tylvalosin, 0.52% for tylosin and 1.12% for tilmicosin, showing trans-epithelial transport. Tylvalosin rapidly entered and accumulated in PK epithelial cells (fig. 2). Tylvalosin reached a maximum cell:medium ratio of 8.9 at 2 hrs, tylosin was not concentrated and tilmicosin had a ratio of 1.7. The concentration of macrolides in chicken white blood cells is shown in fig. 3. All the antibiotics accumulated in these cells - with maximum cell:medium ratios of 19 for tylvalosin, 8 for tilmicosin and 4.7 for tylosin.

Discussion

The data showed the three macrolides varied with respect to speed of uptake and concentration in the different cell types. Tylvalosin was able to rapidly enter and concentrate in all cell types to a greater degree than either tilmicosin or tylosin. The reason for this could be due to the high lipophilicity of the molecule, which is due to the presence of an isovaleryl group (1) Although the three antibiotics share a common macrocyclic lactone ring structure, they were shown to differ in at least one aspect of distribution, relevant to their efficacy in treating clinical disease.

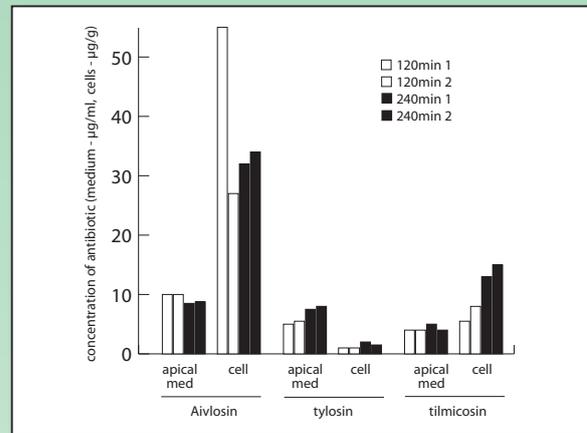


Figure 1. Caco2 cells

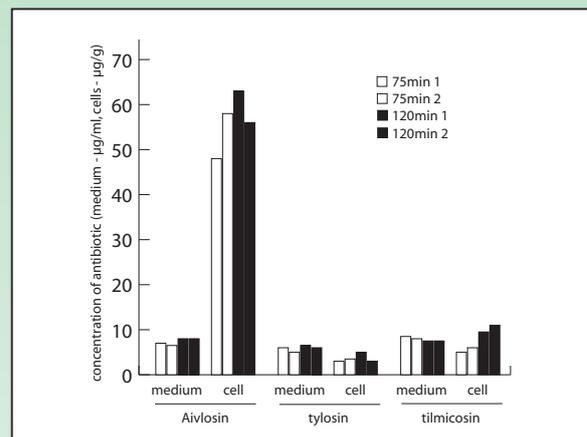


Figure 2. PK epithelial cells

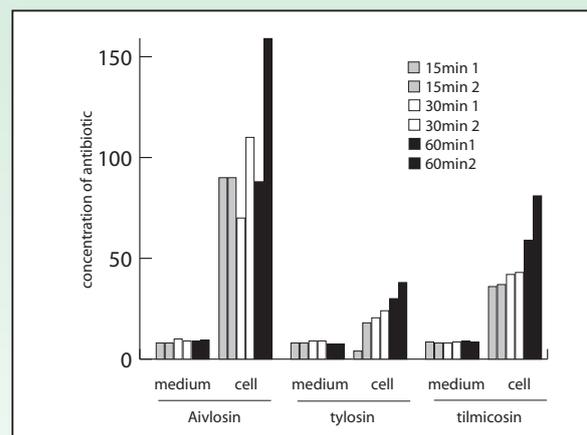


Figure 3. Chicken white blood cells

References

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