Aivlosin and the immune system

Introduction
In addition to assisting the immune system by concentrating within phagocytic cells tylvalosin directly affects the immune system by:

- Driving the differentiation of monocytes to macrophages
- Activating macrophages from the resting state
- Concentrating in lysosomes within the cells

Tissue macrophages are derived from blood monocytes and can exist in either a resting or an activated state. Once in an activated state, the macrophage is more metabolically active, has more lysosomes and has greater phagocytic activity and thus has a greater ability to destroy invading pathogens.

The in vitro studies reported below were conducted in collaboration with the University of Cambridge and demonstrate in vitro the action of tylvalosin on macrophages and the lysosomes within them.

The results from the in vitro studies were confirmed in vivo. Chickens treated with tylvalosin showed a 67% increase in macrophages in lung tissue at necropsy.

Studies have also shown that macrolides can reduce inflammation in tissues by their action on parts of the inflammatory cascade, namely Interleukin-8, and by reducing the release of inflammatory products during cell death.

The beneficial effects of tylvalosin on the immune system are confirmed by the results of clinical studies for both prevention and treatment of chickens naturally infected with Mycoplasma gallisepticum.

In vitro studies

1. Tylvalosin drives the differentiation of monocytes to macrophages

A human monocyte cell line (THP-1) was grown in the presence of tylvalosin. The metabolic activity increased as did the expression of certain surface activation markers, especially DAF which is a protein that helps protect cells against the effects of the complement system (Figure 8).

The metabolic activity induced by tylvalosin was greater than that of other macrolides tested (tylosin and tilmicosin). Various cell lines were used and only the macrophage cell lines showed increased metabolic activity in the presence of tylvalosin.

The increased expression of these surface activation markers in the presence of tylvalosin indicates that tylvalosin drives the differentiation of monocytes to macrophages.

Figure 8: THP-1 cells were treated with or without 1 μg/ml Aivlosin.
2. Tylvalosin activates macrophages from the resting state

A porcine macrophage cell line (3D4/31) was grown in the presence of either tylvalosin or tilmicosin for 48 hours. Cells were stained for a marker protein of porcine tissue macrophages, CD68, a lysosome-associated protein.

The results showed that tylvalosin increased the expression of CD68 more effectively than tilmicosin (Figure 9). Up-regulation of CD68 is an important event that leads to more of this lysosomal protein, and hence more lysosomes being made in the cell.

*Increased lysosome activity is associated with activation of the macrophage*.14

![Figure 9: Tylvalosin increases the production of lysosomal proteins (CD68)](image)

3D4/31 cells, a porcine macrophage cell line, were either treated with 1 μg/ml Tylvalosin, or left untreated. The CD68 protein is shown as green fluorescence. Cells were counterstained to show the actin cytoskeleton (red) and nuclei (blue).

3. Concentrations of tylvalosin in lysosomes

Lysosomes are acidic vesicles containing potent enzymes, which help to destroy invading pathogens. They are contained in large numbers within macrophages and heterophils. Both these cell types are phagocytic, and both are important in the non-specific or innate immune system.

*Tylvalosin concentrates within endosomes and lysosomes in the cell*.15 The combination of tylvalosin with the potent enzymes helps the innate immune system in its battle against invading pathogens.
In vivo studies

1. Tylvalosin has been shown to increase the numbers of macrophages in chicken lungs

Chickens were either medicated with Aivlosin® 625 mg/g Soluble Granules (20 mg tylvalosin/kg bwt) or left unmedicated. Five days later the chicks were euthanased and their lungs fixed then selectively stained. The macrophages (and granulocytes) were stained using a monoclonal antibody (MAC-1) (Figure 10).

Chickens treated with tylvalosin had a 67% increase in macrophage numbers in lung tissue at necropsy\textsuperscript{16}.

2. Reduction of localised inflammation

Macrolides can reduce the secretion of interleukin-8 and hence reduce inflammation\textsuperscript{17}. In addition, macrolides can induce the programmed cell death (apoptosis) of neutrophils which prevents the sudden release of chemicals into the microenvironment and hence reduces inflammation\textsuperscript{18}.

Tylvalosin drives the differentiation of monocytes to macrophages, specifically increases the metabolic activity of macrophages, and increases the production of lysosomal proteins.

Aivlosin® 625 mg/g Solubles Granules and the immune system

In addition to being highly effective against Mycoplasma, tylvalosin has a beneficial effect on the innate immune system thus helping to ensure a rapid recovery.