Introduction

• Resveratrol, a compound found in the skin of grapes, has immunomodulatory activity, stimulating immune function at low concentrations and inhibiting it at high concentrations in human and murine immune cells in vitro, as well as counteracting inflammation and improving immune function in vivo.

• Resveratrol has potential use as a therapeutic agent in humans and animals, helping to bolster immune function in patients with suppressed immunity, and to suppress immunity in patients with immune dysfunction.

• Companion dogs are ideal to evaluate resveratrol in this capacity since humans and dogs share many of the same environmental influences and develop similar diseases spontaneously.

Objectives/Hypothesis

• The objective of our study was to evaluate the in vitro effects of resveratrol on canine leukocyte phagocytic function, oxidative burst, leukocyte cytokine production capacity, and natural killer cell function.

• We hypothesized that resveratrol would demonstrate a dose-dependent effect on immune cell function in dogs in vitro with low concentrations being stimulatory and high concentrations being inhibitory.

Materials and Methods

• Dogs: Whole blood samples from 6 healthy, adult, client-owned dogs were used for each assay.

• Cell viability: Blood was incubated with 120ug/mL (20x concentration) of resveratrol, ethanol or PBS for 4 hours at 37°C, followed by 24 hour incubation with phosphate buffered saline, lipopolysaccharide, lipoteichoic acid and peptidoglycan. Percent live versus dead cells were assessed in each group.

• Incubation: Blood was incubated with high (6ug/mL), intermediate (3ug/mL) and low (1ug/mL) concentrations of resveratrol and a control solution for 4 hours at 37°C.

• Leukocyte phagocytosis: Samples were incubated with FITC-labeled Escherichia coli or a negative control solution for 10 minutes at 37°C. Phagocytic activity was measured via flow cytometry.

• Leukocyte oxidative burst: Samples were incubated with unlabeled opsonized E. coli bacteria, phorbol 12-myristate 13-acetate (PMA) or a negative control solution for 10 minutes at 37°C. Dihydrorhodamine was added as a fluorogenic substrate. Samples were analyzed via flow cytometry.

• Cytokine Production: Whole blood treated with resveratrol was incubated with phosphate buffered saline, lipopolysaccharide, lipoteichoic acid and peptidoglycan) for 24hrs at 37°C. Supernatant was collected and cytokine production will be measured using a canine-specific multiplex bead assay.

• Natural Killer (NK) Cell Cytotoxicity: Whole blood will be treated with resveratol or control solution, peripheral blood mononuclear cells separated, and then incubated with canine thyroid adrenocortical cells (CTAC) for 24 hours at 37°C at NK:CTAC ratios of 1:1, 10:1, 25:1 and 50:1. NK cell cytotoxicity was measured via flow cytometry.

Results

• Cell viability: Cells incubated with 120ug/mL resveratrol showed average cell viability >85% in all groups, indicating no significant adverse effects on cells.

• Phagocytosis: There was no statistically significant difference between ethanol control and resveratrol treated groups in percentage of cells performing phagocytosis and the MFI of cells performing phagocytosis.

• Oxidative Burst: For both E. coli and PMA induced oxidative burst, the mean fluorescent intensity was significantly less for cells treated with resveratrol, and this appears to be a concentration dependent response (Figure 4).

• The MFI for E. coli respiratory burst decreased with increasing concentrations of resveratrol.

• The MFI for PMA respiratory burst decreased with increasing concentrations of resveratrol.

• Leukocyte cytokine production capacity and natural killer cell assays: These assays are currently being performed.

Conclusion and Future Directions

This data suggests that resveratrol has immunomodulatory effects in healthy dogs in vitro, and that these effects are dose-dependent in nature. Further study is warranted in vivo to further define these changes, and leukocyte cytokine production assays and natural killer cell assays are underway.