A novel role for Bouvardin as an inhibitor of canine tumor cell proliferation

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Abstract
Drug therapies are constantly evolving to target various aspects of cancer cell proliferation. Bouvardin has recently been identified in a Drosophila screen as an agent that enhances the effect of ionizing radiation. As a protein synthesis inhibitor, Bouvardin has been shown to enhance the effects of chemotherapeutic agents and radiation in human cancer cells and human cancer xenografts in mice. We proposed to evaluate the sensitivity of a panel of canine tumor-derived cell lines to Bouvardin. A panel of more than 20 cell lines were treated with Bouvardin at concentrations ranging from 10 uM to 0.0625 uM for 72h. Cell proliferation was quantified after 72h using Alamar blue cell viability assay. Bouvardin dose-dependently inhibited canine tumor cell proliferation. A striking difference in sensitivity was observed between and within cell lines of different cancer types, with 50% inhibitory concentrations ranging from <0.0625 uM to >10 uM. Examining the gene expression profiles of the individual cell lines may provide insight into the mechanisms behind the sensitivity or resistance to Bouvardin. These results highlight the future potential for Bouvardin to be used as an effective single agent growth inhibitor or in combination drug therapy with other chemotherapeutic agents.

Goals
The current study aims to evaluate the sensitivity of canine tumor cell lines to Bouvardin. We hypothesize that Bouvardin will variably and dose-dependently decrease cell viability in canine tumor-derived cell lines.

Materials and Methods
A panel of 23 tumor-derived canine cell lines were plated at a density of 2000 cells/well in 96-well plates and grown in Minimum Essential Medium (MEM). The following day, cells were treated with a starting concentration of 10uM Bouvardin with 1:4 serial dilutions. Control cells were grown in MEM alone. After 72 hrs of incubation at 37F, well contents were aspirated and 150uL of Alamar Blue was added to each well. The plates were incubated for 60 minutes and then cell viability was determined using a BioTek plate reader. Data was normalized to the untreated control and 50% inhibitory concentrations (IC50) were determined by constructing 72 hr growth inhibition curves using Prism software.

Results
The 23 cell lines were derived from osteosarcoma, melanoma, hematopoietic, carcinoma, and soft tissue sarcoma tumors. 72 hr growth inhibition curves for seven osteosarcoma cell lines have IC50 values ranging from 0.0625uM to greater than 10uM Bouvardin. All four melanoma cell lines have an IC50 of less than 1uM. Each of the six hematopoietic cell lines has an IC50 of 1uM or below. Four carcinoma cell

lines have IC50 values greater than 1uM, with two greater than 10uM. Finally, the two soft tissue sarcoma lines have an IC50 of 10uM. When treated with Bouvardin, the growth inhibition curves of all 23 cell lines demonstrated a decrease in cell viability. The IC50 values ranged from less than 0.0625uM to greater than 10uM across cell lines.

Summary

Bouvardin inhibits canine tumor cell proliferation in a dose-dependent manner. The range of IC50 values obtained indicates there is a wide variation in sensitivity within and between cancer types. The seven cell lines comprising the osteosarcoma group display the largest variability within cancer type. Differences in sensitivity to Bouvardin treatment are more pronounced between cancer types. Soft tissue sarcomas and carcinomas are the most resistant regardless of dose. Melanoma and hematopoietic lines are most sensitive to Bouvardin treatment. Gene expression profiling of the individual cell lines may help explain the variability seen within in a single cancer type. Due to the success of Bouvardin in decreasing cell viability, the future potential exists for Bouvardin to be used as a single agent therapeutic or in combination with chemotherapeutic agents or radiation.