Investigation of a drug interaction between omeprazole and vinblastine in an *in vitro* canine model

Dominic Baechler, Nick Oldberg, Dayn Godinez, Luke A. Wittenburg
UC Davis School of Veterinary Medicine

UC DAVIS VETERINARY MEDICINE
Students Training in Advanced Research

Background

- **Mast Cell Tumors (MCTs)** are the most common malignant skin tumor found in dogs and represent approximately 20% of all cutaneous tumors.[1]
  - **Vinblastine** is one of the most common chemotherapy agents used to target mast cell tumors in canine patients
  - **Omeprazole**, an antacid, is often given in conjunction with vinblastine to help mitigate increases in gastric acid secretion from parietal cells as a result of excess histamine release from proliferating mast cells.
- **Omeprazole Induces Metabolism of Co-Administered Drugs in Humans**
  - Previous studies have shown that omeprazole can increase elimination of several co-administered drugs in humans by enhancing their metabolism and elimination
  - Omeprazole has been shown to activate the human transcription factor PXR that targets CYP3A metabolizing enzymes and the P-glycoprotein efflux protein.[2-4]
  - Vinblastine is a known to be metabolized by the human Cyp3A4 enzyme which is structurally similar to CYP3A12/26 enzymes in dogs.[5-6]
- **Preliminary Pharmacokinetics Study**
  - The Wittenburg lab previously performed a pharmacokinetic study in 13 canine MCT patients demonstrating nearly 10-fold differences in serum vinblastine concentrations at all time points evaluated.
  - Patients that were co-administered omeprazole with vinblastine had a substantial reduction in the area-under the plasma concentration-time curve (AUC) relative to patients not receiving omeprazole (**Figure 1**).
- **Hypothesis**
  - Omeprazole will activate the canine transcription factor PXR, leading to an increased expression of cytochrome P450 and P-glycoprotein metabolizing enzymes and resulting in faster metabolism of vinblastine *in vitro*.

Aims

1. **Determine concentration-response relationship between omeprazole and canine Pregnane X Receptor (PXR) activity**
2. **Measure CYP and P-gp gene and protein expression changes in the presence of omeprazole**
3. **Examine the *in vitro* metabolism of vinblastine in combination with omeprazole.**

Methods

Aim 1
- Reporter cells from commercially available luminescence-based assay test (Indigo Biosciences) were exposed to various clinically relevant concentrations of omeprazole (0uM, 2.5uM, 25uM, 50uM) and the dose-dependent activation of PXR was measured after a 24-hour incubation period
- Hyperforin was used as a positive control for measurement of canine PXR activity.

Aim 2
- Canine hepatocytes supplied from a commercial vendor were plated on a 96-well, collagen-coated plate and omeprazole was administered at various concentrations (0uM, 25uM, 50uM) for 48-72 hours.
- RNA isolation, cDNA synthesis, and qPCR to determine mRNA levels of Cyp1A1, Cyp1A2, Cyp2B6, Cyp3A4, Cyp3A26, ABCB1 (P-gp).
- Western Blot to analyze altered protein expression of Cyp1A1, Cyp1A2, and Cyp3A1.

Aim 3
- Hepatocytes pre-treated with omeprazole (0uM, 2.5uM, 25uM, 50uM) for 48 hours were exposed to vinblastine for 30 minutes
- Liquid chromatography tandem-mass spectrometry was used to measure the concentrations of vinblastine and in each sample

Results

Pharmacokinetic Study

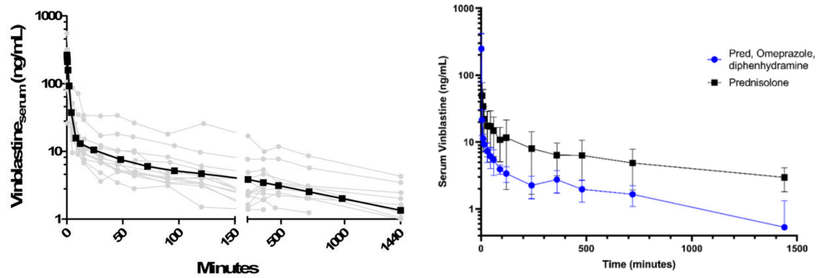

Figure 1. (Left) Serum vinblastine concentration-time curves for 13 canine patients with MCT. The black line represents the mean concentration and light gray lines are individual patients. Substantial interpatient variability is noted in VBL concentrations. **(Right)** When serum VBL concentration data were separated by dogs that either did or did not receive co-administration of omeprazole, a striking decrease in the total systemic exposure was noted in omeprazole-treated patients.

Aim 1: Determine concentration-response relationship between omeprazole and canine PXR activity

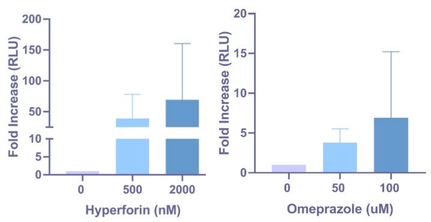

Figure 2. PXR reporter cells treated with clinically relevant concentrations of omeprazole (0um, 25uM, 50uM) for 24 hours had a 5-fold increase in PXR activity relative to the control agonist, Hyperforin. There was no statistical significance observed.

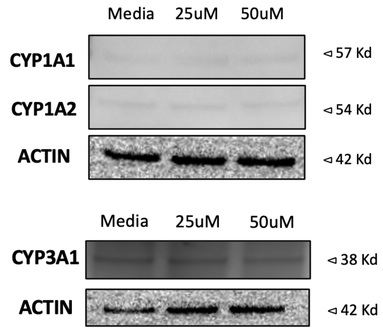

Figure 4. Western blot analysis of canine hepatocytes treated with 0uM, 25uM, and 50uM omeprazole for 48 hours revealed undetectable changes in CYP1A1, Cyp1A2, and Cyp3A1 protein

Aim 2: Measure CYP and P-gp gene and protein expression changes in the presence of omeprazole

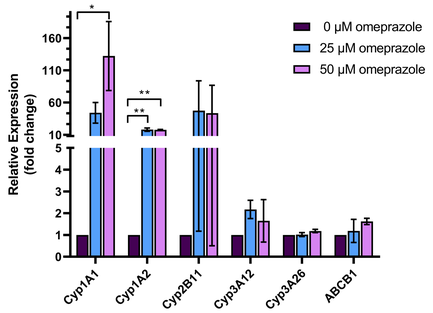

Figure 3. mRNA expression in hepatocytes treated with 50uM omeprazole for 72 hours revealed a significant increase in Cyp1A1 and Cyp1A2. Cells treated with 25uM had a significant increase in Cyp1A2 expression.

Aim 3: Examine *in vitro* metabolism of vinblastine in combination with omeprazole

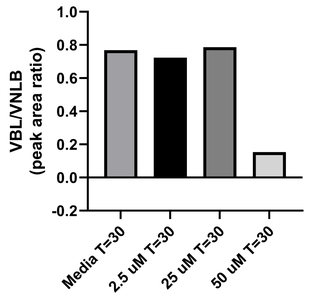

Figure 5. Pretreatment of hepatocytes for 48 hours with omeprazole followed by 30 minute exposure to VBL. LC-MS/MS analysis reveals VBL loss is greater in hepatocytes exposed to 50 uM VBL

Conclusions

Aim 1: Determine concentration-response relationship between omeprazole and canine PXR activity
- Higher concentrations of omeprazole (50uM and 100uM) induced a mild 5-fold increase in PXR activity compared to the 50-fold increase in the positive control, Hyperforin
- Although there was no statistical significance between samples, the increased PXR activity may still be biologically relevant as it may underly the increased expression of Cyp1A1 and Cyp1A2 mRNA that was observed at similar drug concentrations (50uM).
- The modest change PXR activity may also indicate that omeprazole is activating another xenobiotic-activated nuclear transcription factor, such as the Aryl Hydrocarbon receptor.

Aim 2: : Measure CYP and P-gp gene and protein expression changes in the presence of omeprazole
- Hepatocytes incubated with 50 uM omeprazole for 72 hours showed a significant increase in Cyp1A1 and Cyp1A2 mRNA expression.
- Hepatocytes incubated with 25uM omeprazole for 72 hours only revealed a significant difference in the expression of Cyp1A2 mRNA.
- Cyp2B11, Cyp3A12, Cyp3A26, and ABCB1 (P-gp) treated with 50uM omeprazole each had mild elevation in their expression levels that were not statistically significant.
- Western blot data for hepatocytes treated with 0uM, 25uM, and 50uM omeprazole had undetectable changes in Cyp1A1, Cyp1A2, and Cyp3A1 protein expression

Aim 3: Examine *in vitro* metabolism of vinblastine in combination with omeprazole
- The hepatocytes in the 50uM omeprazole treatment group that received vinblastine for 30 minutes had the greatest loss of parent drug. The 0uM, 2.5uM, and 25uM groups appeared to metabolize the vinblastine at equivalent rates.

Future Directions

1. Repeat *in vitro* metabolism assay with multiple concentrations of omeprazole and test varying vinblastine time exposures (3 hours, 6 hours, 12 hours, 24 hours)
2. Enroll canine subjects receiving treatment for mast cell tumors in a prospective clinical trial at the UC Davis VMTH. Each patient will undergo a round of chemotherapy treatment with only vinblastine and another round with co-administration of omeprazole. Blood serum will be collected at several timepoints for both drug-treatment protocols and the area-under the plasma concentration-time curve (AUC) for vinblastine will be measured for each patient.

References

1. *Mast Cell Tumors*. Veterinary Teaching Hospital. (2021, May 18). https://hospital.vetmed.wsu.edu/2021/05/18/mast-cell-tumors
2. Daujat-Chavanieu, M. & Gerbal-Chaloin, S. Regulation of CAR and PXR Expression in Health and Disease. *Cells* 9, doi:10.3390/cells9112395 (2020).
3. Ma, X., Idle, J. R. & Gonzalez, F. J. The pregnane X receptor: from bench to bedside. *Expert Opin Drug Metab Toxicol* 4, 895-908, doi:10.1517/17425255.4.7.895 (2008).
4. Novotna, A. & Dvorak, Z. Omeprazole and lansoprazole enantiomers induce CYP3A4 in human hepatocytes and cell lines via glucocorticoid receptor and pregnane X receptor axis. *PLoS One* 9, e105580, doi:10.1371/journal.pone.0105580 (2014).
5. Martinez, M. N. et al. Challenges in exploring the cytochrome P450 system as a source of variation in canine drug pharmacokinetics. *Drug Metab Rev* 45, 218-230, doi:10.3109/03602532.2013.765445 (2013).
6. Zhou-Pan, X. R. et al. Involvement of human liver cytochrome P450 3A in vinblastine metabolism: drug interactions. *Cancer Res* 53, 5121-5126 (1993).

Acknowledgements

Project funded by the UC Davis Center for Companion Animal Health Grant# 2022-13-F
Financial support provided by the UC Davis SVM Students Training in Advanced Research (STAR) Program and NIH grant.