**OBJECTIVES**

- Define what is drug metabolism and a drug interaction
- Overview of basic Pharmacokinetics
- Explain the cytochrome P-450 system
  - Concept and significance of enzyme inhibition/induction
- Explain the P-Glycoprotein (P-gp) pumps
  - Concept and significance of P-gp inhibition/induction
- Highlighting problematic drug/drug class in terms of drug interactions via case base learning
- Conclusion

**WHAT IS DRUG METABOLISM AND DRUG INTERACTIONS?**

- **Drug metabolism** or ‘biotransformation’ refers exclusively to the chemical alterations of a drug produced by the body
  - Drug activation, deactivation, intermediate metabolite (reactive, active/inactive)
  - Goal of drug metabolism is to alter drugs to a more hydrophilic compound in order to facilitate its excretion or elimination
- **Drug interactions** refer to the alterations of drug disposition ultimately resulting in increased adverse effects or therapeutic failure
CYTOCHROME P450 ENZYME SYSTEM

- Cytochrome (CYP) P450 Enzyme system
  - Member of a superfamily of heme-containing proteins that catalyze phase I metabolism reactions responsible for drug metabolism
  - Located in the smooth endoplasmic reticulum of the hepatocytes, in the villous epithelium of the small intestine and to a lesser extent in the lungs, kidneys, and brain
  - 57 isoenzymes have been identified in humans; each isoenzyme is encoded by 1 gene
  - Major isoenzymes in humans responsible for drug metabolism:
    - 1A2, 2A6, 2C9, 2D6, 2E1, 3A4
CYP ENZYMES NOMENCLATURE

CYP – Arabic Number – Capital Letter – Arabic Number

• CYP: The cytochrome P-450 enzyme system.
• Arabic Number: Family (CYP1, CYP2, CYP3, etc.); must have more than 40% identical amino acid sequence.
• Capital Letter: Subfamily (CYP1A, CYP2C, CYP3A, etc.); must have more than 55% identical amino acid sequence.
• Arabic Number: Polypeptide in a subfamily (CYP1A2, CYP2C9, CYP3A4, CYP2E1, CYP2D6, etc.); identity of amino acid sequences can exceed 90%.

CYP ENZYME SPECIES VARIATIONS

Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction.

Martignoni M, Groothuis GM, de Kanter R. Expert Opin Drug Metab Toxicol. 2006 Dec;2(6):875-94

CLINICAL SIGNIFICANCE OF CYP INDUCTION/INHIBITION

CYP 450 isoenzyme: affects Metabolism

• Substrate – Drug that isoenzyme acts upon; Saturable pathway
  • Induction – Increase enzyme metabolism of substrate
    • Substrate A + Induction agent B → Serum Concentration of substrate A
  • Inhibitor – Decrease enzyme metabolism of substrate
    • Substrate A + Inhibition agent B → Serum Concentration of substrate A
P-GLYCOPROTEIN TRANSPORTER

- P-Glycoprotein (P-gp) is a membrane protein that serves primarily as an efflux pump.
- Found lining the blood-brain barrier, blood-testes barrier, placenta, enterocytes, biliary canalicular cells, and renal tubular epithelial cells.
- P-gp is a product of ATP-binding cassette sub-family B1 (ABCB1), formerly known as the multidrug resistance protein 1 (MDR1).
- Substrate specificity is wide and shares similarities to human CYP 3A4.

P-gp INDUCTION/INHIBITION

- P-gp transporter affects Absorption, Distribution, Excretion.
  - Substrate – Drug that P-gp pump acts upon; Saturable pathway.
  - Inducer – Increase P-gp pump activity on substrate →
    - P-gp Substrate A + Induction agent → Increase efflux of substrate →
      Substrate Concentration
  - Inhibitor – Decrease P-gp pump activity on substrate →
    - P-gp Substrate A + Inhibition agent → Decreased efflux of substrate →
      Substrate Concentration

CASE 1

- Molly, a 27kg, 8 year old F3 golden retriever is diagnosed with B cell lymphoma.
  - Staging: stage 1, with 1 lymph node involved at this time.
  - Medication profile: monthly Heartgard plus & Frontline plus, and fluoxetine 30mg once daily (started > 1yr ago).
  - Treatment protocol:
    - Start CHOP regimen – (Cyclophosphamide, Doxorubicin, Vincristine, Prednisone)
TREATMENT

- 2 weeks into chemotherapy (during doxorubicin cycle), Molly develops areas of fur loss with noted irritation and pigmentation changes
  - Area affected included ventral neck and paws
  - Nail beds and cutaneous folds show signs of infection and are moist
  - Cytology of moist fluid and tape smear reveals a diagnosis of Malassezia dermatitis
- DVM prescribes ketoconazole twice daily for 30 days at a ~8mg/kg dose
  - Prescription states give a 200mg ketoconazole tablet twice daily till recheck

ANTIFUNGALS: AZOLES

Triazoles
- Voriconazole
- Itraconazole
- Fluconazole
- Posaconazole
  - Less affect on mammalian sterol synthesis

Imidazoles
- Ketoconazole
- Clotrimazole
- Miconazole
- Enilconazole
  - Greater affects on mammalian sterol synthesis

- **Indication:** Treatment of fungal infections with a variety of fungal species; Treatment of Cushing’s disease via inhibition of cortisol synthesis
- **MOA:** Effects fungal cell membrane synthesis by interfering with ergosterol production via inhibition of fungal CYP51A
- **Azoles are fungistatic at clinical concentrations, however, – cidal/static activity is also dependent on strain and therapeutic concentrations.

ANTIFUNGALS: AZOLES

<table>
<thead>
<tr>
<th>Drug</th>
<th>CYP Isoenzyme</th>
<th>Spectrum of Activity</th>
<th>P-gp Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Description</td>
<td>Yeasts</td>
<td>Aspergillus</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>+/−</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>No</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>No</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

P450 Inhibitions – ketoconazole > itraconazole > voriconazole > fluconazole
P-gp Inhibition – itraconazole > ketoconazole > voriconazole > fluconazole
AZOLE INHIBITION OF P-gp IN VETERINARY PATIENTS

Biliary excretion of technetium-99m-sestamibi in wild-type dogs and in dogs with intrinsic (ABCB1-1∆ mutation) and extrinsic (ketocanazole treated) P-glycoprotein deficiency


POTENTIAL DRUG INTERACTIONS

<table>
<thead>
<tr>
<th>2C9 SUBSTRATES</th>
<th>3A4 SUBSTRATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>BENZODIAZEPINES</td>
<td>Budesonide</td>
</tr>
<tr>
<td>BUPRENORPHINE</td>
<td>Clopidogrel</td>
</tr>
<tr>
<td>CYCLOSPORINE</td>
<td>CYCLOPHOSPHAMIDE</td>
</tr>
<tr>
<td>CYCLOPHOSPHAMIDE</td>
<td>CYCLOPHOSPHAMIDE</td>
</tr>
<tr>
<td>DEXAMETHASONE</td>
<td>DEXAMETHASONE</td>
</tr>
<tr>
<td>ERYTHROMYCIN</td>
<td>ERYTHROMYCIN</td>
</tr>
<tr>
<td>KETAMINE</td>
<td>KETAMINE</td>
</tr>
<tr>
<td>LANsomPAZOLE</td>
<td>LANsomPAZOLE</td>
</tr>
<tr>
<td>LIDOCAINE</td>
<td>LIDOCAINE</td>
</tr>
<tr>
<td>OMEPRAZOLE</td>
<td>OMEPRAZOLE</td>
</tr>
<tr>
<td>PANTOPRAZOLE</td>
<td>PANTOPRAZOLE</td>
</tr>
<tr>
<td>THEOPHYLLINE</td>
<td>THEOPHYLLINE</td>
</tr>
<tr>
<td>TRAMADOL</td>
<td>TRAMADOL</td>
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<tr>
<td>TRAZODONE</td>
<td>TRAZODONE</td>
</tr>
<tr>
<td>VINLOESTINE/VINCRISTINE</td>
<td>VINLOESTINE/VINCRISTINE</td>
</tr>
<tr>
<td>ZONISAMIDE</td>
<td>ZONISAMIDE</td>
</tr>
</tbody>
</table>

P-gp SUBSTRATES

- CIPROFLOXACIN
- CYCLOSPORINE
- DOXORUBICIN
- ERYTHROMYCIN
- KETAMINE
- LIDOCAINE
- LORAFENIDE
- ONDANSETRON
- OMEPRAZOLE
- PANTOPRAZOLE
- RIFAMPIN
- STEROIDS
- THEOPHYLLINE

*- INHIBITOR; **- INDUCER

MEDICATION PROFILE

Molly, FS Golden Retriever, 27kg, BSA - 0.92m²
- Monthly Frontline Plus & Heartgard Plus
- Fluoxetine 20mg- 1 ½ tablets once daily (30mg dose/d)
- Cyclophosphamide – 50mg/m² – 4mg dose
- Doxorubicin – 30mg/m² – 27.6mg dose
- Vincristine – 0.5mg/m² – 0.46mg dose
- Prednisone – 25mg/m² – 23mg dose
- Ketoconazole – 200mg twice daily (400mg/d)

How many potential drug interactions do you see?

A. 1
B. 2
C. 3
CASE

- Molly returns to the DVM with the owners chief complaint on this visit to be Molly’s increasing anxiety
  - Owner states that fluoxetine is no longer working for her anxiety
  - DVM notes these issues in Molly’s chart and prescribes trazodone to help ease Molly’s anxiety
  - Owner returns to DVM a week later with continuing concerns over Molly’s anxiety and now a mild muscle twitch/tremor
  - States that the anxiety has gotten worse and now Molly is always moving around and seems unable to get comfortable

QUESTION

- Which agent do you feel is the most causative agent for Molly’s increased anxiety?
  - A. Trazodone
  - B. Ketoconazole
  - C. Fluoxetine
  - D. Doxorubicin

SEROTONIN SYNDROME

- Presentation in Humans – typically in a triad of symptoms with variable severity
  - Initial symptoms are usually mild and start with restlessness, tremor, and altered mental status (agitation, confusion, delirium)
  - If the causative issue is not addressed, more life-threatening symptoms can occur including clonus, muscle hypertonicity, and hyperthermia
  - Medication induced serotonin syndrome is the most common cause
  - High risk → Concomitant use of multiple agents that increase endogenous serotonin
    - SSRIs – Fluoxetine, Paroxetine, Citalopram, Sertraline
    - SARIs – Trazodone
    - Opiate-like agonist – Tramadol (2D6 substrate)
    - CYP inhibitors of above agents – Azoles, Erythromycin, SSRIs (inhibit 2D6)
CASE

- Molly exhibits many symptoms that are consistent with serotonin syndrome.
  - Triad - Restlessness, Tremor, and Altered mental status
  - Owner reports of increasing anxiety → altered mental status, possibly confused
  - Molly can not get comfortable and is always pacing → Restlessness
  - Reports of mild tremors were also seen

Which medication is the causative agent?
- Trazodone - Timing of symptoms – prior to trazodone initiation
- Doxorubicin – not the typical adverse effect profile
- Fluoxetine – Molly was symptom free for a long time on this medication… strange for adverse effect to occur now
- Ketoconazole – Causative agent – due CYP inhibition fluoxetine & trazodone

CASE

- Molly returns to the DVM for a recheck,
- Still currently undergoing chemotherapy treatments
- Fungal infection has resolved and ketoconazole has been stopped
- Trazodone was discontinued
  - Anxiety issues returned to normal after completion of ketoconazole and discontinuation of trazodone
- Owners chief complaint for this visit was some loss of fur around the muzzle and legs.
  - Also owner states fleas are getting worst and would like to switch flea control products to a combination product (heartworm/flea control)
  - A skin scrap of affected areas revealed an active demodex infection

CASE

- Treatment
  - Owner asks for combination heartworm preventative and flea control
    - DVM recommends Trifexis for its unique mode of action in flea prevention (Spinosad) and proven heartworm preventative (Milbemycin)
    - DVM initiates extra-label treatment of ivermectin daily
      - Day 1: 100μg/kg PO q24h
      - Day 4: 200μg/kg PO q24h
      - Day 7: 300μg/kg PO q24h
      - Increase by 100μg/kg every third day until target dose of 600μg/kg PO q24h is reached
      - Long treatment duration - 10-33 weeks
CASE

- Molly does well and reaches her target dose of 600μg/kg PO q24h within the a month with no adverse effects
- At the end of the month the owner gives the monthly Trifexis and ivermectin treatment together around 7pm
- By 10 pm Molly was ataxic, hypersalivating and having mild seizures
- The owners rushed her to the emergency room
  - Diagnosed with ivermectin toxicity due to concurrent Spinosad administration

IVERMECTIN/SPINOSAD

IVERMECTIN/SPINOSAD PHARMACOKINETICS

- **Ivermectin**
  - **Metabolism**
    - Highly lipophilic → high volume of distribution
    - Very long half life (~ 2 days)
    - Excreted via bile and feces
  - Undergoes enterohepatic circulation
  - P-gp substrate

- **Spinosad**
  - **Metabolism**
    - Very Long half life - ~10 days
    - P-gp substrate
    - FDA CVM bulletin
      - “Comfortis (Spinosad) and Ivermectin Safety Warning Notification”

IVERMECTIN/SPINOSAD INTERACTION

Pharmacokinetic Interaction of the Antiparasitic Agents Ivermectin and Spinosad in Dogs

- Spinosad & Ivermectin are both P-gp substrates
  - Spinosad has greater affinity to the P-gp
  - Competitively binds to P-gp allowing ivermectin to pass through → ~3.5 fold increase in ivermectin plasma concentrations → ivermectin toxicity

DRUG METABOLISM AND DISPOSITION

OTHER P-gp SUBSTRATES

<table>
<thead>
<tr>
<th>Drug</th>
<th>Toxicity</th>
<th>Dose adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acepromazine, Butorphanol</td>
<td>Profound and prolonged sedation</td>
<td>✷ dose by 25% in heterozygous MDR1 mutation dogs &amp; by 30-50% in homozygous MDR1 mutation dogs</td>
</tr>
<tr>
<td>Enoxaparine, Erythromycin</td>
<td>Neurological toxicity</td>
<td>No dose adjustment known</td>
</tr>
<tr>
<td>Isosorbide</td>
<td>At doses of 300-600 μg/kg can cause neurological toxicity</td>
<td>Avoid use in both heterozygous and homozygous MDR1 mutation dogs</td>
</tr>
<tr>
<td>Loperamide</td>
<td>At doses of 0.1–0.2 mg/kg can cause neurological toxicity</td>
<td>Avoid use MDR1 mutation dogs</td>
</tr>
<tr>
<td>Selamectin, milbemycin, &amp; milbemycin oxide</td>
<td>Dose 10-20 times that of preventative dose can cause neurological toxicity</td>
<td>Avoid use MDR1 mutation dogs</td>
</tr>
<tr>
<td>Vitamin D, Desoxycorticosterone</td>
<td>Dose narrow suppression &amp; GI toxicity</td>
<td>✷ dose by 25% in heterozygous MDR1 mutation dogs &amp; by 50% in homozygous MDR1 mutation dogs</td>
</tr>
<tr>
<td>Cyclosporine, Dapoxetine, Ivermectin</td>
<td>Known P-gp substrate, but appears to be safely tolerated by dogs with the MDR1 mutation</td>
<td>May be P-gp substrate, but appears to be safely tolerated by dogs with the MDR1 mutation</td>
</tr>
<tr>
<td>Metabolite, Ivermectin, fluroxen</td>
<td>Known P-gp substrate in humans, but there is currently no data stating whether they are or are not pumped by canine P-gp. Use caution.</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

- The significance of understanding enzyme induction and inhibition is extremely important when trying to understand a drug interaction or drug sensitivity.
- Both CYP isoenzymes and P-gp are susceptible to the rules of induction and inhibition.
- It is also important to remember that both these biological functions are saturable, so medications with similar substrate specificity can also lead to changes in drug disposition or a drug interaction.
- Therefore to avoid adverse drug events, when adding medications to any patient, it is extremely important for an accurate drug profile to allow for a comprehensive medication review.

P-gp GENOTYPE TESTING

- Washington State University Veterinary Clinical Pharmacology Laboratory
  - Blood sample or cheek swab
  - Blood sample – 2 ml in EDTA tube sent with submission form
  - Cheek swab – order from Washington State University Veterinary Clinical Pharmacology Lab
- Cost ~ $70
- For more information please visit:
REFERENCES