Disclosure

- LCMSMS Grant – ThermoFisher (2012-14)
- Speaker honorarium – Beckman
- Consultations – Preferred Pain Management/Heag/PCLS

Acknowledgement

- Dr. Elizabeth Palavecino
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- Lilly Yee (UWI –Mil.)
- Mark E Hindsdale
- William Nell
- Andrew Vennis
- ThermoFisher scientists
- Sciex scientists
Objectives

1. Antifungals TDM

2. Chiral Analysis:
   - Basic
   - Amphetamines
   - Fluoxetine (LC & CE)
   - Methadone

Voriconazole references


Voriconazole

- Invasive fungal diseases - significant morbidity and mortality in immunocompromised patients
- Voriconazole - a triazole antifungal, the first line treatment of invasive fungal infections
- Voriconazole – high inter-individual variations, non-linear saturation pharmacokinetics, and a narrow therapeutic range
- PO – 200 mg to 300 mg Q12 h 2.5-fold AUC
- Bioavailability - 96%
**Voriconazole PK**

- **T<sub>max</sub>** - 1 – 2 hr, T<sub>1/2</sub> - 6 h, SS – 5 d, V<sub>d</sub> – 4.6 L/Kg
- Metabolism – CYP 2C19 (major determinants of toxicity, Asian with higher frequency), 2C9 and 3A4
- Inhibitory effect on CYP 2C19, 2C9 and 3A4
- May increase calcineurin inhibitors such as CsA by 2 to 3 times
- Excretion – extensive hepatic metabolism with <2% unchanged

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**Voriconazole TDM**

TDM - maximize the efficacy, decrease the risk of toxicity and improve the treatment response in invasive fungal infection

- Trough conc.
- Treatment for > 1 week and more than additional 7 days
- Toxicity
- Drug interaction
- Therapeutic range: 1-5.5 mg/L

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**Voriconazole**

3/10-9/21/15, n=146

<table>
<thead>
<tr>
<th>Conc. mg/L</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.0</td>
<td>37</td>
<td>25</td>
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<tr>
<td>1.0-5.5</td>
<td>79</td>
<td>54</td>
</tr>
<tr>
<td>&gt;5.5</td>
<td>30</td>
<td>21</td>
</tr>
</tbody>
</table>

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A voriconazole patient

- 53 yr old male, haploidentical peripheral blood stem cell transplant for his AML in remission with cells from his son
- Post-transplant complications - intractable hiccups with management leading to serotonin syndrome, chemotherapy induced nausea and vomiting, mucositis, neutropenic methicillin-sensitive staph aureus bacteremia with sepsis, multifocal pneumonia with positive Cytomegalovirus and Aspergillus platelia from Broncho alveolar lavage
- Improved clinically with Ganciclovir and Voriconazole oral doses 500 mg two times daily
- Serum Voriconazole conc. measured by mass spectrometry, showed with corresponding oral doses in next table.

<table>
<thead>
<tr>
<th>Date</th>
<th>Dosage</th>
<th>Conc.</th>
<th>Critical</th>
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<tbody>
<tr>
<td>4/21-13</td>
<td>500 mg two times daily</td>
<td>7.8</td>
<td>critical</td>
</tr>
<tr>
<td>4/21-23</td>
<td>500 mg two times daily</td>
<td>10.7</td>
<td>critical</td>
</tr>
<tr>
<td>4/21-4</td>
<td>500 mg two times daily</td>
<td>7.8</td>
<td>critical</td>
</tr>
<tr>
<td>4/21-7</td>
<td>500 mg two times daily</td>
<td>8.2</td>
<td>critical</td>
</tr>
<tr>
<td>4/21-11</td>
<td>500 mg two times daily</td>
<td>5.9</td>
<td>critical</td>
</tr>
<tr>
<td>5/18-15</td>
<td>300 mg two times daily</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>5/21-15</td>
<td>300 mg two times daily</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>5/27-15</td>
<td>300 mg two times daily</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6/2-15</td>
<td>300 mg two times daily</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>6/8-15</td>
<td>300 mg two times daily</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>6/18-20</td>
<td>300 mg two times daily</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7/30-15</td>
<td>300 mg two times daily</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>7/30-15</td>
<td>300 mg two times daily</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Comments
- Even with critical values, physician continues on given patient a high dose Voriconazole because of invasive aspergillosis.
- After improvement, oral dose lowered to 300 mg twice daily as prophylactic dose.

Posaconazole

- Posaconazole - a novel lipophilic antifungal triazole that inhibits cytochrome P450-dependent 14a-demethylase in the biosynthetic pathway of ergosterol.
- Broad-spectrum against opportunistic, endemic and dermatophytic fungi - organisms such as Candida glabrata, Candida krusei, Aspergillus terreus, Fusarium spp. and the Zygomycetes.
- Oral suspension
- Vd - 5 L/kg, t1/2 - 20 h.
- Not metabolized significantly, primarily excreted in an unchanged
- Although it is inhibitory, cytochrome P3A4 has no effect on 1A2, 2C8, 2C9, 2D6 and 2E1 isoenzymes, and therefore, a limited spectrum of drug–drug interactions can be expected.
- Pharmacokinetic studies in special populations revealed no necessity for dosage adjustment based on differences in age, gender, race, renal or hepatic function.
- Wake Forest patients (n=3 from 8/27-9/11/15) - (0.10 & 0.12) <0.19 and 0.20 mg/L

http://pubchem.ncbi.nlm.nih.gov/compound/Posaconazole#section=Top
Posaconazole references


Distribution of serum posaconazole levels obtained by the Fungus Testing Laboratory, San Antonio, TX, from 26 December 2007 through 30 December 2008.

Voriconazole and Posaconazole LCMSMS assays

- Mix 100 µL aliquot patient samples or calibrator + 300 µL precipitating reagent (Methanolic D3 Voriconazole or D3 Posaconazole)
- Vortex for 1 min for deproteinization
- Centrifuge at 4000 RPM for 10 min
- Mix 10 µL of the supernatant and pipette into 2ml vials and add 990 µL water.
- LCMSMS: C-18 column, and quantitation fragments: Voriconazole - 281 m/z; Pos - 614 m/z
- Reference range: Vor. – 1.0 to 5.5 mg/L; Pos – 0.7 to 3.65 mg/L
- Calibration Ranges: Vor. - 0.1 to 6.2 mg/L; Pos - 0.19 to 5.54 mg/L
- CVs: Vor. - 0.7 to 5.5%; Pos. - 3.5 to 5.5%
- Recent published voriconazole protocols: Ref. 7, 8 (LCMSMS) and 9 (GC/MS)
Objectives

1. Antifungals TDM

2. Chiral Analysis :
   - Basic
   - Amphetamines
   - Fluoxetine (LC & CE)
   - Methadone

Voriconazole monitoring (6 standard and patient)

Posaconazole

[Graphs showing monitoring results for Voriconazole and Posaconazole]
Two enantiomers of a generic amino acid that is chiral

Chiral references

Chiral separation

- TLC
- GC
- HPLC
- Supercritical fluid chromatography
- Capillary electrophoresis
- Capillary electrokinetic chromatography (EKC)
- Micellar EKC
- Microemulsion EKC
- Capillary electrochromatography
- Indirect methods – enantiomers reacting with an enantiopure reagent, pair of diastereomers forms, separation under achiral condition, e.g. GC
- Direct methods – enantiomeric separation in a chiral environment, e.g. HPLC Chirobiotics

Validation of LC-TOF-MS screening for drugs, metabolites, and collateral compounds in forensic toxicology specimens

- LC-TOF-MS – gentle electrospray ionization, accurate mass, and retention data for ID
- Automated SPE, 13 m gradient, resolve isobaric compounds within 15 ppm, and <10 S separation
- Blood, urine postmortem, DUID, DFSA
- Stimulants, benzo., opiates, muscle relaxants, hypnotics, antihistaines, antidepressants, newer synthetic drugs - “designer” “loop holes” “Spice/K2 ” cannabinoids, and cathinone “bath salt”

WF Pain Management Drug Panel

<table>
<thead>
<tr>
<th>Drug</th>
<th>NDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-nor-9-Carboxy-THC</td>
<td>Codeine</td>
</tr>
<tr>
<td>6-Acetylmorphine</td>
<td>Desalflurazepam</td>
</tr>
<tr>
<td>7-Aminoclonazepam</td>
<td>Diazepam</td>
</tr>
<tr>
<td>7-Aminoflunitrazepam</td>
<td>EDDP</td>
</tr>
<tr>
<td>alpha-Hydroxyzolam</td>
<td>Estazolam</td>
</tr>
<tr>
<td>alpha-Hydroxydiazolam</td>
<td>Fentanyl</td>
</tr>
<tr>
<td>alpha-Hydroxytriazolam</td>
<td>Hydrocodone</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>Lorazepam</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>MDA</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>MDEA</td>
</tr>
<tr>
<td>Butalbital</td>
<td>MDMA</td>
</tr>
<tr>
<td>Carisoprodol</td>
<td>Meperidine</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>Meprobamate</td>
</tr>
</tbody>
</table>
### WF Pain Management Drug Panel

<table>
<thead>
<tr>
<th>Drug</th>
<th>Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methadone</td>
<td>Oxycodone</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>Oxydor</td>
</tr>
<tr>
<td>Morphone</td>
<td>ODP</td>
</tr>
<tr>
<td>N-Demethyl-cis-tapentadol</td>
<td>Pentobarbital</td>
</tr>
<tr>
<td>N-Demethyl-cis-tramadol</td>
<td>Phenobarbital</td>
</tr>
<tr>
<td>Norbuprenorphine</td>
<td>Pregabalin</td>
</tr>
<tr>
<td>Noriazaepam</td>
<td>Propoxyphene</td>
</tr>
<tr>
<td>Norfentanyl</td>
<td>Secobarbital</td>
</tr>
<tr>
<td>Norhydroxycodone</td>
<td>Tapentadol</td>
</tr>
<tr>
<td>Normeperidine</td>
<td>Temazepam</td>
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<tr>
<td>Noroxycodone</td>
<td>Tramadol</td>
</tr>
<tr>
<td>Noroxymorphone</td>
<td>Zolpidem</td>
</tr>
<tr>
<td>Norpropoxyphene</td>
<td>Zolpidem phenyl-4-carboxylic acid</td>
</tr>
<tr>
<td>Oxazepam</td>
<td></td>
</tr>
</tbody>
</table>

### Differentiation of Illicit D-Methamphetamine from Over-the-Counter L-Methamphetamine by LC-MS

- Astec® CHIROBIOTIC® V2 column (25 cm x 4.6 mm, 5 µm)
- Mobile phase consisted of 0.04% w/v ammonium trifluoroacetic acid in water:methanol (5:95, v/v)
- Flow rate was set at 1 mL/min, run time was 13.00 min
- Retention times - 10.75 and 11.62 min for D- and L methamphetamine
- Mass spectrometer in ESI+ and MRM modes, 2 transitions 150.0..91.0 and 150.0..119.0.
- Other publications: Ref. 2, 13 and 14.

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Lilly Yee, M.Sc.
Chiral liquid chromatographic analysis and chiral pharmacology of fluoxetine.
University of Wisconsin-Milwaukee

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**S-Fluoxetine**

![S-Fluoxetine](image1)

**R-Fluoxetine**

![R-Fluoxetine](image2)
Interaction between enantiomers and a Chiral Receptor

Rabbit plasma 6 hr post-injection

Conditions: β-cyclodextrin, MeOH/0.3% triethylamine buffer, FR 1ml/min, 40°C and 214 nm
Chromatogram of Plasma sample extract

Conditions: Chirobiotic V, 100 µg/L, Fluoxetine and norfluoxetine std., MP = EtOH/1% triethylamine(9:1), FR 1 mL/min, 265 nm

Enantiomeric separation of fluoxetine and norfluoxetine in plasma and serum samples with higher detection sensitivity capillary electrophoresis, Electrophoresis 1999;20:3432-8

Figure 7. Analysis of an extracted plasma sample from a patient under treatment of depression with Flu. It is after the last intake. Experimental conditions as in Fig. 4.

Findings
- R Flu < S Flu due to increased metabolism
- R Nor-Flu and S NorFlu - variable
Increased use of Heroin and Treatments

Higher purity ~~~ the user
– Ability to snort
  • No risk of AIDS
  • 4 hour "High" @ $10 - $20 for each dose
  • Not an Aggressive "High"
– Ability to smoke
Treatment – Methadone, Buprenorphine, (Prescribed Heroin in Switzerland!!)

Methadone – Chiral pharmacology
• Methadone activity is almost solely to the drug itself rather than the metabolites
• Half life is variable 15-55 hrs
• R methadone – longer $t_{1/2}$ & larger $V_d$, affected by CYP450
• R Methadone active form is 25-50 times more active than S
• However – CYP 2B6 poor metabolizer and S-methadone —— cardiotoxicity
• R methadone – longer $t_{1/2}$ & larger $V_d$, affected by CYP450
• R/S ratios in AM settings – inter-individual variations, 0.5 to 2.5, average <1.0

Methadone Therapeutic and Toxic levels

Chronic administration:
100-200 mg daily  0.83 mg/L (0.57-1.06), 24hrs  0.46 mg/L
$t_{1/2} = 25$ hrs
Lethal Concentrations:

<table>
<thead>
<tr>
<th>Blood</th>
<th>Brain</th>
<th>Liver</th>
<th>Bile</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>avg</td>
<td>1.0</td>
<td>1.0</td>
<td>3.8</td>
<td>7.5</td>
</tr>
<tr>
<td>Range</td>
<td>0.4-1.8</td>
<td>0.5-1.4</td>
<td>1.8-7.5</td>
<td>2.5-18.0</td>
</tr>
</tbody>
</table>
Methadone Metabolism (2003–4)

Protocol

- Mix blood with water and IS solution
- Add NaOH, then extract with butyl acetate, spin, remove organic and dry down
- Mix with MP
- LCMSMS
  - Agilent LC 1100 with chiral AGP column (100 mmX 4.0 mm)
  - Quattro micro from Waters
  - Positive ESI
  - Isocratic, 0.3 mL/min, 25°C, 34 min run
  - Calibration from 0.003 to 2.5 mg/kg blood
Forensic Toxicology Methadone

Preliminary study – n = 10

Methadone deaths due to overdose, higher parent drug, thus R/S ratio higher? * rough estimation*

Blood/plasma ratio – average 0.75

Methadone – postmortem redistribution, heart/femoral blood 0.8 to 1.4 with an average of 1.1
Subclavian/heart blood 0.1 to 2.03
R methadone Vd – higher influx postmortem

EDDP – Blood/EDDP of methadone, urine, same conc. EDDP/methadone higher urine conc. in maintenance than acute overdose
R/S, R methadone to R EDDP lower clearance

Findings – Methadone median R/S = 1.46
EDDP median R/S = 0.80
**Chiral Pharmacogenomics of Methadone Therapy for Drug Addiction**
Shi RZ, Risinger RC, Wong SH
Med. Coll. of Wisconsin, Milwaukee, WI

- N = 35 patients, SS > 4 wk, 10-30 mg
- IRB approval and trough blood draw
- Patient samples genotyped for CYP 2D6, 2C9, 2C19, 3A4 and 3A5, using Pyrosequencing
- Methadone therapy, effectiveness and toxicities evaluated by using opioid dose adequacy scale (ODAS) and a checklist of medication side-effects, supplemented by Beck Depression scale

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**Chiral Methadone Analysis**

- SPE with Clean Screen (UCT)
- Separation of R and S enantiomers of free and total methadone and free and total EDDP was by HPLC on a Chiral-AGP 100 × 3.0 mm (0.5 μm) column followed by analysis on an Applied Biosystems API-4000 tandem mass spectrometer (LC/MS/MS)
Findings

- Total methadone concentrations not well correlated to dose
- Metabolic ratio indicates that s-methadone is preferentially metabolized, or r-methadone has decreased clearance

Conclusions

Enabling Precision/Personalized Medicine?

Antifungals TDM – Voriconazole well accepted, useful for drug-drug interaction, posaconazole – pending, and lack survey-peer comparison needed!

Chiral DAU -
- Clinical applications well established for some DAUs such as amphetamine
- Pending for others – fluoxetine and methadone
- May help to differentiate acute vs chronic ingestion of methadone
- Complementary to Pharmacogenomics

Opportunities for medical laboratory scientists to contribute to R&D