“25-Hydroxy Vitamin D₃: An Endogenous Probe of Hepatic CYP3A Activity”

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Utility of *In Vivo* Enzyme Probes

• Assess drug-enzyme interactions and broadly predict drug-drug interactions (see *FDA Guidance to Industry*):
  – Evaluate inhibition and induction interactions
  – Evaluate source(s) of inter-individual clearance variability:
    • genetic polymorphisms
    • environmental factors that modulate organ enzyme content (enzyme synthesis or degradation)
    • disease, age, gender

• Exogenous (drug) vs endogenous probe?
Vitamin D

- Skin
- Immune cells
- Vasculature
- Colon

Other Tissues

Skin
Immune cells
Vasculature
Colon

CYP2R1

25(OH)D3

CYP27B1

[DBP] 1,25(OH)2D3

Intracrine

Immune cells
Induces cell differentiation

VDR

Kidney
Increases reabsorption of Ca2+ and Pi

Bone
Mineralization and remodeling

Small Intestine
Increases absorption of Ca2+ and Pi, and CYP3A enzyme and activity

Adapted from Deeb et al, 2007
Ratio of Relevant [mRNA] in Paired Human Jejuna/Liver

- ~ 50-fold higher levels of VDR mRNA in human intestine, compared to liver

- CYP3A4, MDR1 and PXR mRNA contents are also higher in the intestine, in contrast to RXRa.

Xu et al., Mol Pharmacol, 2006
LS180 Cells: A Model for Human Enterocytes

- LS180 cells contain relative high expression of hPXR, VDR, CYP3A4 and TRPV6, compared to Caco-2 cells (low PXR and minimal basal CYP3A4)

Very low dose $1,25(OH)_2D_3$ induces all 3 VDR gene targets.

Rifampin is a selective CYP3A4 inducer.

*Emily Zheng et al, Biochem Pharmacol, 2012*
Effect of Oral Vitamin D Supplementation on Oral Atorvastatin Clearance

- Crossover study in healthy elderly adults (n=16);
- During supplementation, people received 800 IU of vitamin D (per day for 6 weeks);
- Atorvastatin administered twice a day, to steady-state; plasma AUC determined for AM dose interval.

<table>
<thead>
<tr>
<th>Targets</th>
<th>Baseline</th>
<th>Vitamin D supplementation</th>
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<tbody>
<tr>
<td>1α,25(OH)₂D</td>
<td>37 ± 17</td>
<td>38 ± 15</td>
</tr>
<tr>
<td>25OHD (ng/ml)</td>
<td>19 ± 18</td>
<td>29 ± 14 *</td>
</tr>
<tr>
<td>Atorvastatin AUC</td>
<td>5,190 ± 4,557</td>
<td>3,250 ± 3,037 *</td>
</tr>
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Parallel $1\alpha,25(OH)_2D_3$ Catabolic Pathways

Oxidative Metabolism of 25(OH)D₃ by CYP3A4

• P450 panel screen revealed CYP3A4 selectivity; moderate intrinsic clearance.
• In HLMs, metabolite formation inhibited by DHB and ketoconazole, and activity correlated with MDZ hydroxylation rate.

Wang et al, Mol Pharmacol, 2012

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Kinetic parameters for the 4-hydroxylation of 25OHD₃ by recombinant CYP3A4 and HLM</th>
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<tbody>
<tr>
<td>Values represent means ± S.D. for triplicate incubations. Total intrinsic CL is the sum of $V_{max}/K_m$ for these two major products [4β,25(OH)₂D₃ and 4α,25(OH)₂D₃].</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4β,25(OH)₂D₃</td>
</tr>
<tr>
<td></td>
<td>$V_{max}$</td>
</tr>
<tr>
<td></td>
<td>pmol · min⁻¹ · pmol⁻¹</td>
</tr>
<tr>
<td>CYP3A4 (b₅ coexpressed)</td>
<td>6.4 ± 0.99</td>
</tr>
<tr>
<td>HLMᵃ</td>
<td>229 ± 62.7</td>
</tr>
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</table>

CL, clearance.  
ᵃ $V_{max}$ is expressed as picomoles per minute per milligram of protein.  
b Calculated on the basis of the CYP3A4 protein level as determined by Western blot (Lin et al., 2002).
Correlation Between $4\beta,25$(OH)$_2$D$_3$ Formation and Midazolam Metabolism

- (A) After treated with rifampin (10 µM), phenobarbital (400 µM), hyperforin (0.5 µM), carbamazepine (50 µM), or vehicles (0.1% v/v) for 48 hr, human hepatocytes (from three different liver donors) were incubated with either midazolam (2 µM) for 30 min or 25OHD$_3$ (2 µM) for 4 hr.

- (B) Correlation between the ratio of plasma $4\beta,25$(OH)$_2$D$_3$/25OHD$_3$ and oral midazolam clearance for pre-RIF and post-RIF treatment periods in healthy volunteers. Open circle: constitutive condition; closed circle: rifampin-induced condition.
Effect of CYP3A Modifiers on 25(OH)D₃ Metabolism and Mineral Homeostasis

6 treatment groups

- Rifampin: CYP3A4 Inducer
- Grapefruit Juice: CYP3A4 Inhibitor (intestinal)
- Clarithromycin: CYP3A4 Inhibitor (intestinal & hepatic)

Study Design

- Blood Draw (plasma & serum)
- 24h Urine (only urine)

Pre-Treatment (1w)
- 200 mL of water once daily
- rifampin, 600 mg, once daily (N = 14)
- 200 mL of Grapefruit Juice once daily
  - 200 mL Grapefruit Juice once a day for 8 days, plus 600 mg rifampin, once daily (N = 14)
- 250 mg clarithromycin, bid (N = 15)
- 250 mg clarithromycin, bid + rifampin, 600 mg, once daily (N = 11)

Treatment (2w)

Washout (2w)

Record Food Intake (5w)

Day #
Phenotype Indices of Enzyme Function

Steady-State Metabolite/Parent Blood Concentration:

- Quantitative and easy to implement – single blood concentration
- However, it is dependent on CL of the metabolite

\[
\frac{C_{m,ss}}{C_{p,ss}} \cdot CL_m = CL_{f \rightarrow m}
\]
- Results suggest that the hepatic CYP3A4 activity controls systemic blood $4\beta,25(OH)_2D_3$ concentration.
- Suppression of 24-hydroxylase by rifampin may be a physiological response to CYP3A4 induction and enhanced $25(OH)D_3$ and $1\alpha,25(OH)_2D_3$ metabolism.
Conclusions

• $4\beta,25(\text{OH})_2\text{D}_3$ formation shows moderate sensitivity as a hepatic CYP3A4 activity probe, responding to individual inductive and inhibition effects as well as dual modulation.

• Chronic treatment of modulator (~14 days) is needed to elicit significant responses and maximum effects may require a longer duration of exposure because of the long half-life of $25(\text{OH})\text{D}_3$.

• May find utility in drug development, along side cholesterol $4\beta$-hydroxylation.
Acknowledgements

University of Washington
Tim Wong
Mackenzie Bergagnini-Kolev
Brian Phillips
Zhican Wang, PhD
Carol Collins, MD
Asthaa Bansal, PhD
Yvonne Lin, PhD

Funding: NIH GM063666
Univ. of WA Health Sciences