Essential Teachings of Steve Tannenbaum as Applied in ADME Sciences

Jinping Gan, Ph.D.
Bristol-Myers Squibb, R&D
Essential Teachings of Steve

- Dedicate to the science
- Be loyal
- Speak English
- Love the Red Sox

Sarkar et al, DMD, 2017 Jul; 45(7): 855–866

Ravindra, et al, JBC in press
Where it all began
Chemical toxicology – Bioactivation

Toxin

Environmental

Occupational

Food

Drug

Endogenous

Target organ

Activated

DNA

Protein

Lipids

Detoxify and excrete

Toxicity
2,6-Dimethylaniline


X axis: in the presence of arachlor induced rat S9

CYP2E1 catalyzes rearrangement

- CYP2E1 but not CYP2A6 catalyzes this rearrangement
- Rat CYP2E1 also active in the rearrangement
- Rat liver CYP2A1 not active in 2,6-DMA metabolism

Therefore, rat liver S9 not active in N-OH-2,6-DMA generation
Whole body autoradiography clearly demonstrated the covalent binding of 3H-2,6-DMA to rat nasal cavity.

Rat nasal cavity has high CYP2A3 (homolog to human CYP2A6) activity

Key lessons

1. The site of bioactivation can be different between species
2. Arachlor-induced rat S9 may not capture all oxidative bioactivation pathways

Bioactivation: can you quantitate it?
Dansylated glutathione for quantitation

Principles
- Tagged GSH forms adducts with reactive intermediates;
- Fluorescence tag provides quantitative information after HPLC separation;
- Mass spectrometry aids identification of adduct structures

Assumptions
1. Fluorescence tag does not alter the reactivity of GSH;
2. Covalent binding of compound has no impact on fluorescence properties of the tag;
3. Tagged GSH doesn’t interfere with metabolic activities of the in vitro activation system.
Probe synthesis and Characterization

Characterization:
- Mass spectrometry
  - MW: 540
- NMR—consistent with structure
- HPLC-UV and fluorescence for purity
  - >99.5% pure
  - λ_ex: 340 nm, λ_em: 525 nm
- Reactivity similar to GSH
- May not be a GST cofactor
- Inhibition to P450s similar to GSH

Dansyl glutathione (dGSH)

Troglitazone dGSH adduct
Montelukast, omeprazole, and lansoprazole spontaneously reacts with thiols to form thiol adducts.
Retrospective analysis

### SAR of Compounds containing 2-Acetylthiophene

![Chemical Structure](attachment:image.png)

<table>
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<th>Compounds</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Formation of GSH (% of parent)</th>
<th>M+H of dGSH adduct</th>
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Target tissue distribution
Examples of QWBA and MALDI-MSI

QWBA
Tumor targeting via Folate Receptor

3D Rendering of Autoradiography
Subcutaneous distribution of Liquid Crystal Formulation

Mass Spec Imaging
Distribution into the Uveal Tract of Eye
3D Rendition of Autoradiographic Images

Wang et al, unpublished data
Liquid microjunction surface sampling in thin tissue slices

- Technology developed at Oak Ridge National Lab* based on Leap Injector Platform
- Direct droplet sampling of tissue slice for LC/MS analysis
- Instrument platform: SepQuant Probe™, Agilent 1290 HPLC, and Thermo Orbitrap Velos mass spectrometer

Metabolite profiles in organs of interest

• Mouse dosed with $^{14}$C-Drug A was sacrificed at different timepoints, and prepared for Quantitative Whole Body Autoradiography (QWBA) images to identify tissues of interest.

• Tissue slices were extracted by droplet sampling using 1:1 methanol/water (5 μL, 5 s) at locations corresponding to the following tissues of interest:
  • liver, kidney, heart (including blood), lung, and GI content

• Extracted samples were directly injected onto a LC-orbitrap for profiling at positive ion ESI mode.

Example photos of pre- and post-extraction in liver
Distribution of Drug A and its Metabolites in Mouse Tissues at one hour after administration of 10 mg/kg $^{14}$CDrug A (single dose, PO)

XIC of Drug A and its metabolites in liver
The extraction window is set at 5 ppm.

Chen et al, unpublished data

Relative abundance of Drug A and its metabolites in different tissues
Targeted delivery
Epothilone-Folate Program: BMS-753493

Folate conjugate
(Kd = 10^{-10})

Folate receptor

Cleavable Linker

Solubilizing Peptide

Folate

BMS-748285

BMS-748285
When $[^3\text{H}]$epothilone folate (BMS-753493) was dosed to dual tumor-bearing mice (FR- and FR+ M109 xenografts at both sides of a mice), accumulation of radioactivity was observed in the FR+ tumor, especially at the later time points.

Tissue distribution of $[^3\text{H}]$BMS-753493 in tumors and major mouse tissues at 24 hr after dosing

QWBA results: $[^3\text{H}]$BMS-753493 preferentially distributed from blood into FR (+) tumor tissue, potentially providing better therapeutic window than the epothilone alone
Advanced Tissue Cultures
For each species (mouse, rat, monkey, human), the preparation step takes about 6-7 days before they can be used for experiment.
Characterization

- omeprazole (CYP1A, 3.3 fold),
- phenobarbital (CYP2B6, 3.2 fold),
- rifampicin (CYP3A, 3.5 fold)

Ly et al, unpublished
Major metabolite of loratadine generated in spheroids

- In humans, 3-OH desloratadine is a major metabolite of loratadine and desloratadine. PM of desloratadine results in 6-fold increase in desloratadine exposure.

But hard to recapitulate using traditional cultures and liver fractions.
Parkinson, A et al. DMD; April 2015, 43 (4) 523-533
Also observed were the glucuronide of desloratadine and 3-OH desloratadine.

Chacko et al, unpublished data
Disposition of a chem tox student

Chemistry

Biology

Chem Tox Ph. D.

Pharmacy

Postdoc

Burt out

Industry Tox or DMPK

Academic
Acknowledgement

- Steve and the Tannenbaum lab
- NEDMDG
- BMS colleagues