Investigative Toxicology: An Exciting Journey in Exploratory Science

Myrtle A. Davis, DVM, PhD, ATS
Executive Director, Discovery Toxicology
Pharmaceutical Candidate Optimization
Bristol-Myers Squibb

Overview of My Career Path
A Career in Investigative Research
A Path for You
Why a Career in Research?

Know what you **enjoy**!

Internships

- Problem Solving
- Writing
- Learning more than Knowing

Coursework

- Discovery!
- Seeing a path to application of knowledge
- Independence
- Goals that matter

Know what you **don’t**!

- Routine
- Lack of Challenge
- Highly Structured Workdays
- Lack of Independence
Human epithelial cell (DNA in blue) with increased numbers of centrosomes (green) amid a sea of normal cells in interphase.

Scanning electron micrograph of an oral squamous cancer cell (white) being attacked by two cytotoxic T cells (red).
Would You Rather................

Be the first to discover a new enzyme.

Apply the key lessons from discovery of an enzyme to a problem in totally different discipline.

Create an assay to measure a new enzyme in a living tissue.

Be part of the team that is responsible for testing an inhibitor of the new enzyme in a clinical trial.

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Why Toxicology?

Basic **science** (or pure **science**) is used to develop information to explain phenomena typically, in the natural world.

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Why Toxicology?

• Toxicology is an **Applied Science**
  • An applied science is a discipline that applies a wide variety of existing scientific knowledge to develop understanding or a practical application for example: a new technology

Internships were essential!

• Bell Labs (Lucent Technologies)
• Procter and Gamble
• Norwich Pharmaceuticals
• Basic Sciences
• Applied Sciences

Toxicology

• Toxicology is the study of the adverse effects of chemical, physical, or biological agents on people, animals, or the environment.
• Toxicologists are scientists trained to investigate, interpret, and communicate the nature of those effects.
“Modes” of Toxicology

Strategic
Pragmatic
Investigative
Mechanism-focused

An Investigative and Mechanistic Strategy

Define the Issue (Opportunity)

ID mechanistic questions relevant to the Problem

Define the Data Plan

• What data are available to characterize the issue?
• What are the consequences?

Analyze
Communicate

• Which answers will be most informative?
• Which questions can be answered definitively?

Available expertise Methods

• What will we do with the data?
• How will data help?

Impact
Audience
Communication Plan

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1-[(3-chlorophenyl)-methyl]-1H-indole-3-carbinol (NSC-743380) induced liver injury and biliary hyperplasia when two doses were given orally to rats, once per day for two consecutive days

The Liver: A Vital Organ

- Main job is to filter the blood coming from the digestive tract-detoxification and metabolism of drugs, get rid of damaged cells, proteins, and old hormones
- With the help of vitamin K, produces proteins that are important in blood clotting.
- Plays a central role in all metabolic processes in the body.
  - Fat metabolism: the liver cells break down fats and produce energy.
  - Bile production: a thick, green-yellow fluid that helps digest food, especially fat
- Storage of glycogen, vitamins, and minerals
- Creating immune system factors to fight against infection
**Biomarkers of Liver Toxicity**

**Hepatocyte Injury**

- Hepatocyte Death
- Neutrophil Activation
- \( T_h \) Lymphocytes
  - Kupffer Cells

**Hepatic Stellate Cell Activation**

- Deposition of Extracellular Matrix
- Fibrotic Pathways
- Myofibroblastic Phenotype

**Local Paracrine Factors**

- TGF-\( \beta \)
- PDGF
- IGF-1

**Liver dysfunction**

- Synthetic Dysfunction
  - Albumin, prothrombin time

**Portal Hypertension**

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**Group Mean Serum Chemistry Values Measured after Two Days of Daily Oral Administration of NSC-743380**

<table>
<thead>
<tr>
<th>Dose Group</th>
<th>Values</th>
<th>Alkaline Phosphatase (units/liter)</th>
<th>Alanine Aminotransferase (units/liter)</th>
<th>Aspartate Aminotransferase (units/liter)</th>
<th>Gamma Glutamyl Transferase (units/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Mean</td>
<td>311</td>
<td>54</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>NSC-743380 100 mg/kg/day</td>
<td>Mean</td>
<td>628*</td>
<td>1498*</td>
<td>1681*</td>
<td>2</td>
</tr>
<tr>
<td>NSC-743380 300 mg/kg/day</td>
<td>Mean</td>
<td>1547*</td>
<td>4215*</td>
<td>4461*</td>
<td>3</td>
</tr>
<tr>
<td>NSC-743380 500 mg/kg/day</td>
<td>Mean</td>
<td>1966*</td>
<td>5186*</td>
<td>7618*</td>
<td>6</td>
</tr>
</tbody>
</table>

*statistically significant difference from control (vehicle) group \( P \leq 0.05 \)
Metabolism of NSC-743380

P: Parent compound
M1: Glucuronide of parent compound
M2: Carboxylic acid of parent compound
M3: Glucuronide of carboxylic acid of parent compound
M4: Glucuronide of carboxylic acid of parent compound
What Would You Do Next?

Investigating the Role of Major Metabolites

[Bar chart showing units/liter for Alkaline Phosphatase, Alanine Aminotransferase, and Aspartate Aminotransferase for different compounds, including Vehicle, NSC-743380 (parent), NSC-741908 (aldehyde), NSC-751172 (carboxylic acid).]

*Statistically significant, P<0.05
## Mean Serum Chemistry Values Measured after Two days of Daily Oral Administration of Parent Compound, Aldehyde Metabolite (NSC-741908), or Carboxylic Acid Metabolite (NSC-751172)

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<tr>
<th>Dose Group</th>
<th>Values</th>
<th>Alkaline Phosphatase (units/liter)</th>
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<th>Aspartate Aminotransferase (units/liter)</th>
<th>Gamma Glutamyl Transferase (units/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Mean</td>
<td>204</td>
<td>68</td>
<td>84</td>
<td>0</td>
</tr>
<tr>
<td>NSC-743380 (parent)</td>
<td>Mean</td>
<td>956*</td>
<td>3942*</td>
<td>4454*</td>
<td>3</td>
</tr>
<tr>
<td>NSC-741908 (aldehyde)</td>
<td>Mean</td>
<td>219</td>
<td>60</td>
<td>89</td>
<td>0</td>
</tr>
<tr>
<td>NSC-751172 (carboxylic acid)</td>
<td>Mean</td>
<td>248</td>
<td>69</td>
<td>84</td>
<td>0</td>
</tr>
</tbody>
</table>

*statistically significant difference from control (vehicle) group ($P \leq 0.05$)

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## What Would You Do Next?

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Defining On- or Off-Target Toxicity for NSC 743380

Comparative analysis of compounds profiled in vitro provided evidence for target-mediated toxicity.
**Hepatotoxicity Appears to be Target Mediated, Not Scaffold**

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<th>Aspartate Aminotransferase (units/liter)</th>
<th>Gamma Glutamyl Transferase (units/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Mean</td>
<td>241</td>
<td>59</td>
<td>113</td>
<td>1.0</td>
</tr>
<tr>
<td>NSC-743380 (active compound)</td>
<td>Mean</td>
<td>377</td>
<td>654*</td>
<td>716*</td>
<td>1.0</td>
</tr>
<tr>
<td>NSC-754516 (same scaffold, inactive)</td>
<td>Mean</td>
<td>220</td>
<td>71</td>
<td>113</td>
<td>1.0</td>
</tr>
<tr>
<td>NSC-763131 (different structure, active)</td>
<td>Mean</td>
<td>322</td>
<td>412*</td>
<td>587*</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*statistically significant difference from control (vehicle) group \(P \leq 0.05\)
What Would You Do Next?

Hepatotoxicity Mitigated by Intravenous Administration

Serum chemistry values from rats dosed once daily with NSC-743380 for two consecutive days

*Statistically significant, P<0.05
Conclusions from IV-Oral Comparison in Rats

- NSC 743380 is hepatotoxic in rats after oral administration > 100 mg/kg (QD dosing), based on clinical chemistry and histopathology noted on Day 2 of dosing.
- IV dosing of 743380 (6.25–25 mg/kg) to rats, QDx5 produced no lethality or clinical chemistry changes.
- There were no microscopic lesions associated with IV administration of 743380 any any tissues examined.
- Plasma levels obtained for IV administration of 743380 and metabolites were higher than what was achieved orally.

Mechanism of Toxicity Not Elucidated

- NSC 743380 was originally identified via a phenotypic screen.
- Targeted proteomics was used to identify direct NSC 743380 binders.
- Characterization revealed that NSC-743380 suppressed the phosphorylation of C-terminal domain of RNA polymerase II.
- Suppression of phosphorylation of the C-terminal domain (CTD) eukaryotic RNA polymerase as was observed in cell lines—could promote cell death.

Toxicol Appl Pharmacol. Author manuscript; available in PMC 2015 Dec 15.
doi: 10.1016/j.taap.2014.10.015
It All Comes Down to a Few Key Words and Phrases

Describe
Hypothesize
Imagine
Design Experiments
Analyze
Explain

Your Passion and Excitement Are the Catalysts for Achievement and Successes during a Research Career

Success Attracts More Opportunities
Advice?

The Journey Is the Fun!
Climbing a ladder was not the focus for my career choices; it is about opportunity. My career is a journey not a destination.

Experience work from a variety of perspectives. Going thoughtfully from one role to another (or one organization to another) can fill expectations of progressing through your career or life.
Setting your sights on a collection of experiences that you wish to have is a measurable way of managing your career.

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