Blood Transcriptomic Findings in Acute Liver Injury

Recent Research Advances in Drug-Induced Liver Injury

2009

FDA/CDER-PhRMA-AASLD Research Conference
National Labor College, Silver Spring, MD
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Richard S. Paules, Ph.D.
National Institute of Environmental Health Sciences
National Institutes of Health, DHHS, USA
Critical Need for Biomarkers

• Clinicians and health policy decision makers need biomarkers of both exposure and adverse effect.

• Genomic technologies offer hope for identifying novel and informative biomarkers and surrogate biomarkers.
Fundamental Premise

• Genomics will provide new insight into the **mechanisms** underlying adverse effects.

• Genomics will provide novel **biomarkers** that will be highly correlative with and predictive of adverse health effects from environmental stresses.
Hypotheses

1. A gene expression signature can be identified that will allow for discrimination between APAP exposures that are **non-toxic** and **toxic** to livers in rats.

   1.1. A gene expression signature can be identified that reveals **incipient liver injury** that manifests as liver injury by traditional indicators only at higher doses and/or at later times.

2. A gene expression signature can be identified in **rat blood** (similar or dissimilar to that in liver) that will allow for discrimination between APAP exposures that are non-toxic and toxic to livers in rats.

3. A gene expression signature can be identified in **human blood** that will be similar to that identified in rat blood that will allow discrimination between no injury and severe injury following APAP intoxication.
Gene Expression Profiling of Rat Livers Reveals Indicators of Potential Adverse Effects

Alexandra N. Heinloth,* Richard D. Irwin,† Gary A. Boorman,† Paul Nettesheim,* Rickie D. Fannin,* Stella O. Sieber,* Michael L. Snell,‡ Charles J. Tucker,* Leping Li,§ Gregory S. Travlos,¶ Gordon Vansant,|| Pamela E. Blackshear,||| Raymond W. Tennant,* Michael L. Cunningham,‡ and Richard S. Paules,*±1

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Mitochondrial Damage after Exposure to 150 mg/kg APAP
Prototype: ACETAMINOPHEN

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Blood gene expression signatures predict exposure levels


*Biostatistics Branch, †Environmental Stress and Cancer Group, ‡Environmental Toxicology Program, §Microarray Group, **Cancer Biology Group, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709; ‖Experimental Pathology Laboratories, Inc., Research Triangle Park, NC 27709; and †Department of Medicine, University of North Carolina, Chapel Hill, NC 27599

Edited by Mark T. Groudine, Fred Hutchinson Cancer Research Center, Seattle, WA, and approved September 21, 2007 (received for review July 25, 2007)
Utilize gene expression acquired from rat peripheral blood cells in a training set to predict sub-toxic or toxic exposure in a blinded test set.

- Compare different bioinformatics approaches for identifying predictor genes.

- Evaluate prediction accuracy of genomic markers vs. traditional toxicologic evaluations.
Study Design – Training Data Set

Sub-Toxic
150 mg/kg

- Blood RNA → Gene Expression
  Individual treated animals hybridized against time-matched control pool
- Liver Tissue sections → Histopathology
- Whole Blood → Hematology
- Serum → Clinical Chemistry

Toxic
1500 mg/kg

Oral Gavage
6 12 24

Time (hours)

Toxic
2500 mg/kg

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Study Design – Test Data Set

Sub-Toxic
150 mg/kg
- Blood RNA → Gene Expression
Individual treated and individual control animals hybridized against time-matched control pool
- Liver Tissue sections → Histopathology
- Whole Blood → Hematology
- Serum → Clinical Chemistry

Toxic
1500 mg/kg
Oral Gavage
3 6 12 24
Time (hours)

Toxic
2000 mg/kg

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### Prediction Accuracy

Blinded Histopathological Evaluation of Test Data Set

<table>
<thead>
<tr>
<th>Pathologist #1</th>
<th>Pathologist #2</th>
<th>Pathologist #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>75%</td>
<td>66.7%</td>
<td>70.8%</td>
</tr>
</tbody>
</table>

24h Data Only: 86% 82% 86%
Prediction Accuracy of Test Data Set

Clinical Chemistry (ALT, SDH): 62.5 %
Only 24h data: 75%

Hematology (Neut/Lymph): 77.8 %
Only 24h data: 71%
Computational Algorithms used to Train Gene Expression Classifiers

- ANOVA models:
  - Dose Main Effect (DME) ANOVA → 152 genes
    • $\kappa$NN → 35 genes
    • Multicategory support vector machine → 20 genes
  - Dose Confounded Effect (DCE)* ANOVA → 264 genes
    • Multicategory support vector machine → 20 genes
- Extracting Patterns and Identifying Genes (EPIG) → 248 genes

* Multiple comparison correction was not performed
Prediction Accuracies for Test Data Set

- **DME - \( \kappa \)NN ANOVA:** 95.8%
  - three toxic dosed samples were predicted as sub/non-toxic
- **DME – SVM ANOVA:** 95.8%
  - two toxic dosed samples were predicted as sub/non-toxic, one non-toxic dosed sample was predicted toxic
- **DCE – SVM ANOVA:** 88.9%
  - eight toxic dosed samples were predicted as sub/non-toxic
- **EPIG:** 91.6%
  - six toxic dosed samples were predicted as sub/non-toxic
Analysis of biological function:

- Analysis of all discriminating gene lists yield similar GO categories

- Top categories for all signature sets point to an activation of an inflammatory response after exposure to toxic doses of acetaminophen, as well as indications of mitochondrial injury
Matrix Approach for Phenotypic Anchori

Building a Compendium of Acute Liver Injury

## Acute Liver Injury Endpoints

<table>
<thead>
<tr>
<th>Primary Expected Adverse Effect</th>
<th>Additional Expected Adverse Effects</th>
<th>Agent</th>
<th>Source of Human Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis, endothelial cell</td>
<td>Necrosis, endothelial cell</td>
<td>Monocrotaline *</td>
<td>Plant alkaloid</td>
</tr>
<tr>
<td>Apoptosis, endothelial cell</td>
<td>Necrosis, endothelial cell &amp; Hemangiosarcomas</td>
<td>Riddelliine</td>
<td>Plant alkaloid</td>
</tr>
<tr>
<td>Cholestasis</td>
<td></td>
<td>17α-Ethinylestradiol</td>
<td>Birth control pills</td>
</tr>
<tr>
<td>Cytomegaly, hepatocyte</td>
<td>Hyperplasia &amp; Bile Duct / Liver Cancers (chronic)</td>
<td>Methyl Eugenol</td>
<td>Flavering agent &amp; Fragrance</td>
</tr>
<tr>
<td>Necrosis</td>
<td>Cirrhosis (chronic)</td>
<td>Thioacetamide *</td>
<td>Fungicide</td>
</tr>
<tr>
<td>Necrosis, centrilobular</td>
<td></td>
<td>Acetaminophen</td>
<td>Analgesic/Antipyretic</td>
</tr>
<tr>
<td>Necrosis, centrilobular</td>
<td>Fibrosis (chronic)</td>
<td>Carbon Tetrachloride</td>
<td>Industrial Solvent</td>
</tr>
<tr>
<td>Necrosis, centrilobular</td>
<td>Oxidative stress</td>
<td>Diquat *</td>
<td>Herbicide</td>
</tr>
<tr>
<td>Necrosis, centrilobular</td>
<td>Apoptosis &amp; Steatosis</td>
<td>Galactosamine *</td>
<td>Aminosugar</td>
</tr>
<tr>
<td>Necrosis, centrilobular</td>
<td>Apoptosis &amp; Liver Cancer (chronic)</td>
<td>N-Nitrosomorpholine *</td>
<td>Industrial corrosion inhibitor</td>
</tr>
<tr>
<td>Necrosis, midzonal</td>
<td></td>
<td>1,2-Dichlorobenzene *</td>
<td>Pesticide</td>
</tr>
<tr>
<td>Necrosis, midzonal</td>
<td></td>
<td>Bromobenzene *</td>
<td>Industrial Solvent</td>
</tr>
<tr>
<td>Necrosis, periportal</td>
<td></td>
<td>Allyl Alcohol</td>
<td>Food additive &amp; Industrial solvent</td>
</tr>
<tr>
<td>Necrosis, periportal</td>
<td>Bile duct hyperplasia &amp; Biliary Cancer (chronic)</td>
<td>Methapyriline</td>
<td>Antihistaminic</td>
</tr>
<tr>
<td>Non-carcinogen</td>
<td>Necrosis, periportal</td>
<td>Eugenol</td>
<td>Flavoring agent</td>
</tr>
<tr>
<td>Non-toxic</td>
<td></td>
<td>1,4-Dichlorobenzene *</td>
<td>Pesticide</td>
</tr>
<tr>
<td>Non-toxic</td>
<td></td>
<td>N-Acetyl-m-Aminophenol</td>
<td></td>
</tr>
<tr>
<td>Non-toxic</td>
<td></td>
<td>Pyrilamine</td>
<td>Antihistaminic</td>
</tr>
<tr>
<td>Oxidative damage</td>
<td></td>
<td>Iron overloading</td>
<td>Diet &amp; Metabolic disorders</td>
</tr>
</tbody>
</table>

* "Standardized" NCT Studies

Collecting Liver, Kidney, Blood/Serum for Transcriptomics, Proteomics and Metabolomics

In-life data including Clin. Chem., Hematology, Pathology

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Gene expression response in target organ and whole blood varies as a function of target organ injury phenotype
Gene expression classifiers generated using the blood data performed better than the liver data at grouping individuals into compound groups in six of the nine dose/time groups.
PCA of 30 Transcripts Identified in 1-Way ANOVA of Blood Genes with Factor “Response to Hepatocellular Injury” Liver Injury Score

No Adverse Effects → Severe Adverse Effects

(* Response to Hepatocellular Injury - glycogen depletion + hypertrophy + fatty change + necrosis)

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Prototype: ACETAMINOPHEN

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# Human APAP Overdose Patients

<table>
<thead>
<tr>
<th>Patient Identifier</th>
<th>Gender</th>
<th>Ethnicity</th>
<th>Age</th>
<th>Peak ALT (U/l)</th>
<th>AST (U/l)</th>
<th>Total Bilirubin</th>
<th>White blood cell counts</th>
<th>INR</th>
<th>Acetaminophen level</th>
<th>Day presented after ingestion</th>
<th>Day blood drawn for study</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>male</td>
<td>caucasian</td>
<td>19</td>
<td>2630</td>
<td>726</td>
<td>0.8</td>
<td>6.8</td>
<td>1.3</td>
<td>&lt;10</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>male</td>
<td>caucasian</td>
<td>25</td>
<td>6446</td>
<td>1804</td>
<td>1.2</td>
<td>10.5</td>
<td>2.1</td>
<td>&lt;10</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>female</td>
<td>caucasian</td>
<td>36</td>
<td>816</td>
<td>1123</td>
<td>0.6</td>
<td>10.6</td>
<td>1.8</td>
<td>22/350</td>
<td>12hrs</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>female</td>
<td>caucasian</td>
<td>40</td>
<td>1582</td>
<td>121</td>
<td>1.6</td>
<td>5.6</td>
<td>1.3</td>
<td>&lt;10</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>female</td>
<td>african american</td>
<td>59</td>
<td>435</td>
<td>552</td>
<td>25.5</td>
<td>17</td>
<td>1.8</td>
<td>&lt;10</td>
<td>18hrs</td>
<td>2</td>
</tr>
</tbody>
</table>

Alexandra Heinloth, NIEHS  
Pierre Bushel, NIEHS  
Paul Watkins, UNC  
Mark Russo, UNC
Human Blood Gene Expression

60 Human Orthologs from Rat Blood Discriminating Gene Set

Human Blood Discriminating Gene Set?

Alexandra Heinloth, NIEHS  Paul Watkins, UNC
Pierre Bushel, NIEHS    Mark Russo, UNC
Human APAP Dosing - Blood Gene Expression

Phase I Pilot Study

Time Points Before and After Single Oral Dose (4 gm; ~50 mg/kg)
-72 - 48 - 24 0 6 18 24 48 72 96

↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓

Time (Hrs)

+ Day 21 Return for Blood Draw

NIEHS / UNC - Paul Watkins & Mark Russo

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