How Our Bodies Adapt to Chemicals

Curtis D. Klaassen, PhD, DABT, ATS
• How Our Bodies Adapt to Chemicals
  or
  Developing Resistance to Toxicity
  or
  Learning to Reprogram the Liver
  or
  Progress Depends on Technology
<table>
<thead>
<tr>
<th>1964</th>
<th>present</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Animal</td>
<td>• Null mice and rats</td>
</tr>
<tr>
<td></td>
<td>• Transgenic mice</td>
</tr>
<tr>
<td>• Analytical methods</td>
<td>• Radioactivity</td>
</tr>
<tr>
<td>• Colorimeter/spectrophotometer</td>
<td>• GC</td>
</tr>
<tr>
<td>• Chromatography</td>
<td>• HPLC-UV</td>
</tr>
<tr>
<td>• Paper</td>
<td>• HPLC-MS/MS</td>
</tr>
<tr>
<td>• TLC</td>
<td></td>
</tr>
<tr>
<td>• Cell fractions (Centrifuge)</td>
<td>• Tissue culture</td>
</tr>
<tr>
<td>• Computer</td>
<td>• Computer</td>
</tr>
<tr>
<td>• One/university</td>
<td>• More than one/person</td>
</tr>
</tbody>
</table>
1964

- Antibodies
  - Radioimmunoassay
  - Western blot

2012

- mRNA
  - Northern blot
  - RT-PCR
  - Bead assays
  - Microarray
  - RNA-Seq

- Transcription factor – DNA binding
  - ChIP-qPCR
  - ChIP-Seq
Drugs and other xenobiotics cross membranes due to their lipid solubility.

Drugs are biotransformed to water-soluble compounds, so they do not pass cell membranes and thus are excreted more readily.

Microsomal enzyme inducers increase cytochrome P-450s and enhance the elimination of some drugs.

There might be two cytochrome p450s.
Examples of Liver-Reprogramming

• P-450 inducers
• Heavy metals
• Plants
• PXR activation
• Development of liver after birth
• Diet
Hypothesis

Therefore, if the purpose of p450 inducers was to enhance the elimination of chemicals, might the inducers also increase the elimination of chemicals that are not biotransformed by enhancing their passage across membranes?
Plasma clearance of DBSP

Klaassen and Plaa, JPET 161:361, 1968

**Plasma DBSP Concentration (mg/100ml)**

- Control
- Phenobarbital

**Time (min)**

Klaassen and Plaa, JPET 161:361, 1968
LIVER CELL AS THE CENTRAL PROCESSING UNIT FOR DRUGS AND NUTRIENTS
A BRICK WALL PROGRESS DEPENDS ON TECHNOLOGY
Effect of PCN on Ouabain uptake in isolated rat hepatocytes

Eaton and Klaassen, JPET 208:381-385, 1979
A brick wall progress depends on technology.
Examples of Liver-Reprogramming

- P-450 inducers
- **Heavy metals**
- Plants
- PXR activation
- Development of liver after birth
- Diet
Rats were pretreated with CdCl$_2$ (2.0 mg Cd/kg, sc) and at indicated time challenged with a lethal dose of CdCl$_2$ (4.0 mg/kg, iv), and mortality was recorded within 48 hrs after Cd challenge.

<table>
<thead>
<tr>
<th>Time after Cd pretreatment</th>
<th>n</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pretreatment</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>2 hr</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>4 hr</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>6 hr</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>8 hr</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>24 hr</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2 day</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>4 day</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>8 day</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>16 day</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>
Cd pretreatment protected against acute Cd hepatotoxicity

Rats were pretreated with CdCl₂ (2.0 mg Cd/kg, sc) and 24 hrs later were challenged with hepatotoxic doses of CdCl₂ (2.0-4.0 mg/kg, iv), and serum SDH was determined 10 hrs later. Data are Mean ± SE of 4-6 rats.

Goering and Klaassen TAAP 74:308, 1984
Cd pretreatment alters subcellular Cd distribution

Rats were pretreated with CdCl₂ (2.0 mg Cd/kg, sc) and 24 hrs later were challenged with hepatotoxic doses of CdCl₂ (3.5 mg Cd/kg, iv), and subcellular Cd distribution was determined 2 hrs later. Data are Mean ± SE of 6 rats.

Goering and Klaassen TAAP 70:195, 1983
Cd pretreatment alters cytosolic Cd distribution

Rats were pretreated with CdCl₂ (2.0 mg Cd/kg, sc) and 24 hrs later were challenged with hepatotoxic doses of CdCl₂ (3.5 mg Cd/kg, iv), and cytosolic Cd distribution was determined 2 hrs later.

Goering and Klaassen TAAP 70:195, 1983
MT-null mice are highly susceptible to chronic Cd-induced nephrotoxicity

Wild-type control and MT-null mice were given CdCl2 (0.05-2.4 mg/kg, sc) daily for 6 weeks, and Blood urea nitrogen (BUN) and renal MT were determined. N =6-8 mice.

The relationship between human exposure to cadmium and renal injury

Klaassen et al., Ann Rev Pharmacol Toxicol 39, 267, 1999
Examples of Liver-Reprogramming

- P-450 inducers
- Heavy metals
- **Plants**
- PXR activation
- Development of liver after birth
- Diet
In 1987, a post-doctoral fellow, Jie Liu, came to my Lab with a “suitcase” of chemicals in Traditional Chinese Medicine that he thought would protect against liver injury.

<table>
<thead>
<tr>
<th>OA</th>
<th>Oleanolic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>UA</td>
<td>Ursolic acid</td>
</tr>
<tr>
<td>UV</td>
<td>Uvaol</td>
</tr>
<tr>
<td>α-H</td>
<td>α-Hederin</td>
</tr>
<tr>
<td>HD</td>
<td>Hederagenin</td>
</tr>
<tr>
<td>GL</td>
<td>Glycyrrhizin</td>
</tr>
<tr>
<td>18β-GA</td>
<td>18 β-Glycyrrhetinic acid</td>
</tr>
<tr>
<td>18 α-GA</td>
<td>18 α-Glycyrrhetinic acid</td>
</tr>
<tr>
<td>HAG</td>
<td>19 α-Hydroxyl asiatic acid 29-0-β-glucoside</td>
</tr>
<tr>
<td>HA</td>
<td>19 α-Hydroxyl asiatic acid</td>
</tr>
</tbody>
</table>

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Oleanolic acid
Oxidative stress and activators

- Nrf2
- Keap1
- CUL3

- Anti-oxidant and inflammation
- Cell survival and proliferation
- Drug metabolism
- Lipid metabolism

A single dose of oleanolic acid (30mg/kg, i.p.) increases Nrf2 translocation into nucleus and induce Nrf2 target genes in wild-type but not Nrf2-null mice

What Genes Might the Keap1-Nrf2 Pathway Alter?
Generation of “Gene dose-response” Model

Constitutively Induced or Suppressed Genes

What Drug Processing Genes are Altered by Nrf2 activation

- Uptake transporters
- Phase-I enzymes
- Phase-II enzymes
- Efflux transporters
Phase-II enzymes: Glutathione Conjugation

Glutathione S-transferase

Gsta2  Gsta3  Gsta4  Gstm1  Gstm2  Gstm3  Gstm4  Gstm6  Gstp1  Gstt3  Mgst3

Fold-induction

Not altered: Gstk1, Gstm5, Gstm7, Gsto1, Gstp1, Gstt1, Gstt2

Wu ... Klaassen. PLoS one 7: e39006, 2012
AhR and Nrf2 interactions

Expression of Which Antioxidant Genes are altered by Nrf2
Antioxidant Genes were Induced with Graded Nrf2 Activation

1) GSH synthesis and regeneration

2) Reduction of hydrogen peroxide

3) Reduction of oxidized protein

4) Reduction of bilirubin and ion sequester
Michael Sporn, “the father of chemoprevention” asked for our Oleanolic Acid so he could make more potent analogues.
• Presently: Take antioxidants

• Future: Have body make antioxidants
Examples of Liver-Reprogramming

- P-450 inducers
- Heavy metals
- Plants
- PXR activation
- Development of liver after birth
- Diet
nuclear receptor structure/function

Dimerization

DBD

LBD

corepressor

ligand

coactivator
### Prototypical Target Genes of Xeno-sensors

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Transcription Factor</th>
<th>Prototypical target genes in liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCDD</td>
<td>AhR</td>
<td>Cyp1a1</td>
</tr>
<tr>
<td>PB</td>
<td>CAR</td>
<td>Cyp2b10</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>PXR</td>
<td>Cyp3a11</td>
</tr>
<tr>
<td>PCN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clofibrate</td>
<td>PPARα</td>
<td>Cyp4a14</td>
</tr>
<tr>
<td>OA</td>
<td>Nrf2</td>
<td>Nqo1</td>
</tr>
</tbody>
</table>
Transporters in Liver

Hepatic induction of rat Oatp1a4 protein expression

Rausch-Derra ...... Klaassen, Hepatology 33:1469, 2001
Guo ...... Klaassen, JPET 300:206, 2002

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Mouse Oatp1a4 induction by PCN is PXR-dependent

Cheng and Klaassen, DMD 34:1863, 2006
## Summary of TF-dependent gene battery using wild-type and TF-null mice

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>TF-dependent gene battery in liver</th>
</tr>
</thead>
</table>
| **AhR**              | Phase I: Cyp1a2, Cyp2b10, Nqo1, Aldh1b1, Aldh7a1  
Phase II: Gstm2, mGst3, Sult5a1, Ugt1a1, Ugt1a6, Ugt1a9, Ugt2b35  
**Transporters:** Mrp4, Mate1, Oatp1a1, Oatp1a4 |
| **CAR**              | Phase I: Cyp1a2, Cyp2b10, Cyp3a11, Nqo1, Aldh1a1, Aldh1a7  
Phase II: Gsta1, Gsta4, Gstm1, Gstm2, Gstm3, Gstm4, Gstt1, Sult1e1, Sult2a2, Sult3a1, Sult5a1, Papss2, Ugt1a1, Ugt1a9, Ugt2a3, Ugt2b1, Ugt2b34, Ugt2b35, Ugt2b36  
**Transporters:** Mrp2, Mrp3, Mrp4, Oatp1a1, Oatp1a4 |
| **PXR**              | Phase I: Cyp2b10, Cyp3a11, Aldh1a1, Aldh1a7  
Phase II: Gsta1, Gstm1, Gstm2, Gstm3, Gstm4, Sult2a2, Sult3a1, Ugt1a1, Ugt1a5, Ugt1a9, Papss2  
**Transporters:** Mrp2, Mrp3, Oatp1a4, Abcg5 |
| **PPARα**            | Phase I: Cyp3a11, Cyp4a14, Nqo1, Aldh1a1, Aldh1a7, Aldh3a2, Aldh9a1  
Phase II: Gsta1, Gstm3, Gstm4  
**Transporters:** Mrp4 |
| **Nrf2**             | Phase I: Cyp1a2, Cyp2b10, Nqo1, Aldh1a1  
Phase II: Gsta1, Gsta4, Gstm1, Gstm2, Gstm3, Gstm4, Gstt2, mGst3, Ugt2b35, Ugt2b36  
**Transporters:** Mrp3, Mrp4, Oatp1a1 |

Aleksunes and Klaassen, DMD 40: 1191, 2012
It is generally recognized that DR3 and ER6 are the prototypical DNA-binding motifs for PXR, based on data obtained from a few genes.

Examples:
- CYP3A4 in humans
  - ER6 (AG(G/T)TCAnnnnnnAG(G/T)TCA) -1kb of TSS

- CYP3A23 in rats
  - DR3 (AGTTCAatgaAGTTCA) 5’-flanking region (-133bp of TSS)

*Is this still true on a genome-wide scale?*

El-Sankary et al., DMD 28, 493, 2000
Schuetz et al., Mol Pharm 54, 1113, 1998
Hunting for specific nucleotides targeted by a TF within the entire genome

If typed 1,500,000 pages
PXR-binding to \( \text{Cyp3a11} \) gene locus in mouse liver

PXR-response element: DR3, DR4, etc.

Cui ... Klaassen, Nucleic Acids Res 38: 7943, 2010
PXR-binding to Oatp1a4 gene locus in mouse liver

PXR-binding fold-enrichment

Corn Oil
PCN

PXR-response element: DR-9 only
Location: 10kb upstream of the transcription site

Cui ... Klaassen, Nucleic Acids Res 38: 7943, 2010
Novel $\text{DR}_{5n+4}$ Periodic DNA-binding Patterns of PXR

Cui ... Klaassen, Nucleic Acids Res 38: 7943, 2010
The $\text{DR}_{5n+4}$ Periodic Pattern for PXR-binding to DNA

1 helical turn, 34 Å, 10 intervals

Watson and Crick (1953 CSH Symp Quant Biol)

$\text{DR}_{5n+4}$ DNA interacts with PXR in the unit of half a helical turn

$n=0$

$n=1$

$n=2$

Cui ... Klaassen, Nucleic Acids Res 38: 7943, 2010
The DR$_{5n+4}$ Periodic Pattern for PXR-binding to DNA

An “Accordion” Model

Cui ... Klaassen, Nucleic Acids Res 38: 7943, 2010

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The $\text{DR}_{5n+4}$ Periodic Pattern for PXR-binding to DNA

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Cui ... Klaassen, Nucleic Acids Res 38: 7943, 2010
The $\text{DR}_{5n+4}$ Periodic Pattern for PXR-binding to DNA

An “Accordion” Model

Cui ... Klaassen, Nucleic Acids Res 38: 7943, 2010
The binding of TFs to DNA does not explain entirely the re-programming of cells

• Why not all genes expressed every tissue?
• Not always a good correlation between activation of TFs and function of liver cells.
The Freedom of Expression is Under Tight Control by Epigenetic Mechanisms

Epigenetics:
Functionally relevant modifications to the genome that do not involve a change in the nucleotide sequence. Epigenetic events control the freedom of gene expression without altering the underlying DNA sequence.

Epigenetic Factors:
- DNA methylation
- Histone modifications
- microRNAs
DNA Methylation as a Suppression Signal for Gene Expression

Gene Activation

Gene Silencing

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Histone Modifications Determine whether the DNA is “Loose” Enough to be Accessed by Transcription Factors

H3K4Me2

GO

H3K27Me3

STOP

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microRNAs usually lead to decreased mRNA stability and suppression of protein synthesis.
Examples of Liver-Reprogramming

- P-450 inducers
- Heavy metals
- Plants
- PXR activation
- Development of liver after birth
- Diet
Comparison of 24hr LD50 of Ouabain in rats of varying age

( Klaassen, JPET, 1972)
Fig. 2. Distribution of ouabain in 7- and 39-day-old rats at various times after 4 mg/kg of ouabain octahydrate (10 ml/kg i.p.). The line that the first letter of each tissue touches is the line for the ouabain concentration in that tissue. Each value represents the mean of four to seven rats.

(Klaassen, JPET, 1972)
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Reprogramming of liver naturally after birth

7825 differentially expressed genes out of a total of 23108 genes in mouse liver
Cui ... Klaassen, 2012
Three Patterns of Liver Transporters during Development

62 critical drug transporters

Cui ... Klaassen, Toxicol Sci, 2012
Examples of Liver-Reprogramming

- P-450 inducers
- Heavy metals
- Plants
- PXR activation
- Development of liver after birth
- Diet
Reprogramming the Liver by Diets

5282 genes had altered mRNAs in at least one diet
Significance and Conclusion

- Liver can be programmed and re-programmed
- This is the way the liver adapts to its environment
- All billion liver cells have the hardware and software to be re-programmed
- It is chemicals that alter the programming
- There are no drugs for liver failure
- If we can learn which chemicals will turn on and off the various programs, we will have drugs for liver diseases
  - Cirrhosis and fatty liver
  - Nutritional functions: obesity and diabetes
  - Excretory functions: jaundice and hyper-bilirubinemia
  - Clotting factors: heart attacks, stroke
  - Cholesterol synthesis and degradation: atherosclerosis
Thank you!

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