The Biochemistry of Detoxification

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Disclosure

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Environmental Toxins

- C.D.C.

“The epidemic of epidemics of **CVD** and **immunological** and **neurological** diseases is likely associated with environmental toxins”

ATSDR/CDC/USPHS monographs on specific toxic metals
Multiple Assailants, Individual Victims

- Knowledge of adverse effects have been based primarily on independent studies of single toxicants. (NIEHS)
- Toxicants (metals, chemicals) can elicit independent, additive or synergistic toxic effects. (C.D.C.)
- Individuals vary considerably in their sensitivity to toxicants and, susceptibility to toxic effects varies with age, gender, pregnancy status, nutritional status, total toxic load and genetics. (C.D.C.)

Single Nucleotide Polymorphisms (SNPs)

Endogenous Detoxification

- Chemical entities (environmental toxicants and endogenously produced toxins, including gut derived)
- Reactive oxygen species (ROS)
- Methionine metabolism - methylation / transsulfuration
- Intertwined adverse, compounding effects of toxic elements
The GI Microbiome and Toxicology

- Toxicologists acknowledge that **toxicokinetic models** need to be **revised** to include the influence of the GI microbiome “pool.”*
- Strong evidence that **toxicant disruption** of the GI microbiome **crosses generations** (e.g. “bad microbiome from grandma”)
- Microbiota that **normally** live in the GI tract perform many useful functions (digestion, train immune system, NT production)
- Promote the expression of hepatic CYPs (*Phase I*)
- Detoxification of arsenic, lead, mercury and chemical entities
- Convert a **phytoestrogen** precursor (hops) to an **anti-inflammatory**, **cardioprotective** compound
- **The metabolic activity of the GI microbiome rivals that of the liver!**


Detoxification of Organic Compounds: Endo- and Xenobiotics

- **Phase I** – oxidative activation
- **Phase II** – conjugation
- **Phase III** – unidirectional excretion via ATP-dependent efflux pumps

Optimal detoxification requires *coordinated* regulation of genes encoding for enzymes in *all* three phases

Phase I: Oxidative Activation

- Cytochrome P450 enzymes (CYPs) add reactive & polar groups to lipophilic substrates → reactive electrophiles (oxidation, hydroxylation, or N-, O-, S-dealkylations)
- Prosthetic heme is absolutely essential for CYP activities.
- Heme biosynthesis (porphyrinogen pathway) requires Fe and Zn.
  Affected by anemias, and inherited enzyme defects
  Inhibited by Pb, Hg, As and chemical entities
  (specific urine porphyrin profiles)

Toxicants Inhibit Phase I Detoxification

Cytochrome P450 isozymes (CYPs)

Hg, Pb, Cd, As, Co, X Cr⁶, glyphosate, O-antigen (K. pneumoniae, P. aeruginosa)

Activated Xenobiotic

**Phase II: Conjugation**

- Conjugation of *activated* toxins to increase hydrophilicity (e.g. GSH, sulfation, acetylation or glucuronidation)
- Catalyzed by broad-specificity transferases (glutathione S-transferases, UDP-glucuronosyl-transferases)
- *Cannot cross* lipid membranes at a **sufficient rate** without specific ATP-dependent **export transporters** (Phase III)

Phase II: Glutathione Conjugation

Activated Xenobiotic

ROS

GSH

Glutathione S-transferases

GS-conjugates

Pb, MeHg, Hg, Cd, As, Cr\textsuperscript{6}, Co

Quenching Reactive Oxygen Species (radical / non-radical)

- **Superoxide Dismutases** (Cu, Zn and Mn)
  \[ \text{SOD} + 2 \text{O}_2^\cdot + 2 \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \]

- **GSH peroxidases** - (selenium)
  \[ 2 \text{H}_2\text{O}_2 + 2 \text{rGSH} \rightarrow \text{GS-SG} + 2 \text{H}_2\text{O} \]
  (Also lipid peroxides \( \rightarrow \) alcohols)

- **GSH reductase** (inhibited by Pb, Hg, Cu, Cd)
  \[ \text{GS-SG} + \text{NADPH} \rightarrow 2 \text{GSH} \]
  \( (Mg \text{ and up to 10\% of total body glucose “disposal”}) \)

References:
- Curr Vascular Pharmacol (2010) 8:259-75
- Biochim Biophys Acta (2009) 1780:869-72
Further Metabolism of GS-conjugates

GS-conjugates

\[ \text{glutamate} \xrightarrow{\gamma-\text{GT (inducible)}} \text{glycine} \xrightarrow{\text{dipeptidase}} \text{Cys S-conjugate} \]

\[ \text{Cys S-conjugate} \xrightarrow{\text{N-acetyltransferase}^*} \text{Mercapturic Acids} \]

\[ \text{Mercapturic Acids} \xrightarrow{\text{Phase III}} \text{Urine} \]

\[ \text{Mercapturic Acids} \xrightarrow{\text{Phase III}} \text{Intestine} \]

\[ \text{Mercapturic Acids} \xrightarrow{\text{Phase III}} \text{Bile} \]
Phase III: Pump it Out!

Membrane-bound efflux pumps - ↓ local cellular toxins (Phase II conjugates and their metabolites)

- **ENERGY DEPENDENT** (B vitamins, Mg, Mn, Fe, CoQ10)
- **MRPs**- multidrug resistance-associated proteins
  
  Differential tissue distribution & substrate specificities

- **OATPs**- organic anion-transport proteins

  Kidney proximal tubule membrane
  Hepatocyte canalicular membrane → bile → intestines


Biofactors (2003) 17:103-14
**Inflammation Compromises Detoxification**

- **Inflammation** of the intestines or liver compromises detoxification (toxicants, end stage metabolites)
- *Intestinal* inflammation *down-regulates* hepatic efflux pumps activity - suppressed basolateral secretion of toxins inhibits Phase II, *increases* oxidative stress
- Keep bowels moving to prevent inhibition of detoxification processes, and *minimize enterohepatic recirculation.*
  
  Ca-D-glucuronate- inhibit microbial β-glucuronidases
  
  Optimize beneficial flora- pre/probiotics (SCFA production), *S. boulardii* (↑sIgA), and *directly* ameliorate inflammation

**Folate**

**Transmethylation “Methionine Cycle”**

- **Methionine**
  - ATP, Mg
  - SAM
  - MTases
  - SAH

- **Homocysteine**
  - P-5-P, Zn, Heme
  - CBS

- **Cystathionine**
  - P-5-P

- **Cysteine**
  - Mg, K

- **Glutathione**

**“Long route”**

- THF
- BHMT (Zn, B-6)
- Betaine
- 5-CH$_3$THF
- MTHFR
- 5,10-methyleneTHF

**“Short route”**

- THF
- BHMT (Zn, B-6)
- Betaine

**Cellular Methylation**

DNA, RNA, protein, neurotransmitters, phospholipids, creatine

**Transsulfuration**

- P-5-P, Zn, Heme
- CBS
High SAM

"short route"

THF

5-CH₃THF

MTHFR

5,10-methyleneTHF

Betaine

BHMT

MS

"long route"

Methionine

SAM

SAH

Homocysteine

Cystathionine

Cysteine

Glutathione

Transsulfuration

CBS

Transmethylation

"Methionine Cycle"

Folate
SNPs- Important Considerations

• Very subtle DNA sequence variations that are **abundant** in the human genome and **frequent** in the general population.

• **SNPs do not cause disease.**

• Some SNPs can affect metabolism and alter disease **risk**, **and ↑ susceptibility to environmental toxicants.**

• Toxicants (metals) can **exacerbate** the effects of SNPs.

• **Multiple** SNPs in a **single gene** increase odds for abnormal **phenotypic expression** (e.g. “sluggish” enzyme).

• SNPs in **multiple genes** may be necessary to affect metabolism / health outcomes (gene-gene interactions).
Formation of THE Methyl Donor-SAM

- Up to 50% of methionine intake catabolized to SAM
- Methionine adenosyltransferase (MAT)
  Requires ATP and Mg
- MAT inactivated by:
  - CCl₄, septic shock, episodes of hypoxia
  - Oxidative stress (NO, ROS) - *reversible* by GSH
  - Bacterial lipopolysaccharides
  - Hepatitis B, HepC-induced cirrhosis

FASEB J(2002)16:15-26
Methionine Synthase (MS) Pathway

- Remethylation- homocysteine to methionine
- Activity Requires:
  - Active form of Folate
  - 5,10 Methylene-THF $\xrightarrow{MTHFR} 5$-L-methyl-THF
  - B-12 (methylated by 5- L-methyl-THF)
- MS pathway inhibited in neuroblastoma cells by: ethanol, Pb, Al, Hg$^{2+}$ and thimerosal

Molec Psychiatry(2004)9:358-70
Consequences of Aberrant Methionine Metabolism

1. ↓ Methionine – from methylation of homocysteine (MS)
2. ↓ Methylation - DNA/RNA, proteins, NTs, PLs (PE→PC)
3. ↓ Transsulfuration - ↓ cysteine, taurine, sulfate and GSH

Potential clinical consequences:
Aberrant neurotransmitter metabolism, developmental delay, psychiatric affects, oxidative stress, ↓ DNA synthesis & repair, immune dysregulation, cancer, poor response to environmental toxins, and ↑ risk for CVD*

Endogenous Arsenic Detoxification

- Entails sequential oxidation, reduction and methylation reactions
- $\text{As}^{+3} \overset{\text{ox}}{\rightarrow} \text{MMA}^{+5} \overset{\text{red}}{\rightarrow} \text{MMA}^{+3} \overset{\text{ox}}{\rightarrow} \text{DMA}^{+5} \text{-GSH}$
- Requires SAM and Arsinite Methyltransferase activity
- Low methionine, folate and cysteine all impede arsenic detoxification

ATSDR Toxicological Profile for Arsenic (2000)
As Detoxification: Folate Supplementation

- **Bangladesh**- DBPC study of 130 adults with *marginal* folate status, 12 weeks +/- folate (400 ug /day)
- Folate vs. placebo:
  - **Plasma**- *total* As; 13.6 % lower, **MMA**; 20 % lower
  - **NO Change** As$_{in}$
  - **Urine**- 10 % ↑ DMA/gm creatinine after 1 week, but no difference between groups after 12 wks. (↑ creatinine)
- Folate-induced methylation of As$_{in}$ increased **detoxification and decorporation** of As despite no change in exposure.

**Glutathione (GSH)**

- $\gamma$-glutamylcysteinylglycine

**Intracellular Functions**

Most abundant intracellular thiol (1-10 mM)

Modulates DNA synthesis & immune function

Regulates nitric oxide homeostasis

Antioxidant defense / Redox control

Facilitates cellular Mg and glucose uptake

**Conjugation of metals and chemicals**

Hypertension(1999)34:1002-6
Low GSH in Patients with:

- Toxic metals/chemicals
- Fe or Cu overload
- Cardiovascular Disease
- COPD/asthma/ARDS
- Diabetes/dysglycemia
- Hypertension
- Chronic stress
- Anxiety
- Aging
- Neurological diseases
- Viral hepatitis/Hep C
- Cirrhosis
- Autism
- Chronic fatigue
- HIV infection
- *Extreme* exercise

Oxidative Stress, GSH and CVD

Cd, As, Pb, Hg, / Fe, Cu, Co

GSH

Hg²⁺

GSH

ROS

↓ Cellular GSH

Ox-LDL (apoB), endothelial dysfunction and, oxidized lipids, proteins and DNA (8-OH-dG)

~ 4 million ·OH/cell/day

GSH, Oxidative Stress and CVD

• Serum rGSH levels are *inversely* correlated with arterial **intimal media thickness** (humans).

• Oxidative stress → Ox-LDL → unregulated uptake of Ox-LDL by macrophages → oxidative damage to macrophages (Macs)

  \[ \text{Macs}_{\text{ox}} \text{ in turn can oxidize normal and small dense LDL} \]

• **Apo-E null rats** - Oral *liposomal* GSH decreased Ox-LDL uptake, Macs$_{\text{ox}}$, Mac cholesteryl ester content, and **aortic lesion area** by *30%* (vs. control liposomes)*

  \[ \text{Am Coll Cardiol}(2006)47:1005-11 \quad \text{Atherosclerosis}(2002)161:307-16 \]

  \[ *\text{Atherosclerosis}(2007)195 :e61-e68 \]
GSH and GSH Peroxidase are Associated with Circulating Lipoproteins

**HDL**

GSHpx

**LDL**

GSHpx

GSH

Paraoxonase-1

Antioxidative

~ 3.5-X > for LDL

~5-X > for LDL

Atherosclerosis (2007) 195:e61-e68
JBC (2002) 277:4301-4308
Atherosclerosis (2005) 181:9-15
**GSH Status and Oxidative Stress**

59 yom, heavy smoker - elevated BP, and blood levels of lead and cadmium

- **RBC GSH**
  - Normal: 1,000 µmoles/L
  - 652 µmoles/L

- **8-OH-dG**
  - Normal: < 8.5 ng/mg cr
  - 26.1 ng/mg cr

- **Oxidized LDL**
  - Normal: < 45 U/L
  - 77 U/L

*(8-hydroxy-2’-deoxyguanosine)*

* First AM urine collection
Increasing GSH Levels

Exogenously

- **NOT** oral encapsulated GSH (intestinal)
- Oral liposomal GSH - ↑ RBC*, brain and heart GSH, ↑ Co excretion, ↓ reperfusion injury (isolated rabbit hearts), neuroprotective (in vitro)
- Nebulized GSH
- Intravenous GSH  $T^{1/2} \sim 14$ min., ↑ RBC GSH & Mg

**Increasing GSH Biosynthesis**

- **Adequate dietary protein (AA)**
  - Methionine, N-AC, undenatured whey protein\(^1\)
- **Antioxidants**: E\(_{\text{MT}}\), C, α-lipoic acid, curcumin\(^2\)
  - Regenerate (reduce GS-SG) and spare rGSH
- **B vitamins**: B-6, riboflavin, niacin (NADPH)
- **Mg, Se** (GSH-Px)

Am J Clin Nutr (2009)\(89\):425  
Am J Clin Nutr (2004)\(80\):1611  
\(^1\) J Appl Physiol (1999)\(87\):1381-5  
\(^2\) J Inorg Biochem (2004)\(98\):266  
\(^2\) Free Rad Biol Med (2008)\(44\):907-17
Upregulate the Rate Limiting Enzyme in GSH Biosynthesis

- **glutamylcysteine synthetase (GCS)**
  - Curcumin and quercetin ↑GSH levels by stimulating the transcription and activity of GCS

- **Onion extract** and quercetin ↑ GSH levels and mRNA for a GCS subunit promoter

- **Oleanolic acid** increases/maintains hepatic GSH

- Induction of gene expression of GCS

References:
**Low Bioavailability of Curcumin**

Systemic bioavailability - increased with piperine that *inhibits* glucuronidation in the gut
Curcumin-phosphatidylcholine complex

Cysteine

↑ Transcription of GCL
Curcumin
Quercitin
Oleanolic acid

Mg, K

γ-glutamylcysteine ligase

Hg²

ADP, Pi

γ-glutamylcysteine

ADP, Pi

ATP, glutamine

ATP, glycine

GSH Synthase

Mg, K

GSH
Metallothioneins - Cytosolic “Toxin Traps”

- ~30% cysteine (-SH)
- Highest concentrations - liver, kidneys, intestines, lungs and testis

Metallothioneins- ROS and Metal Binding

- Excellent free radical scavengers (>rGSH)*
- *Sequester* toxic elements (Hg, Ag, Cd, Pb, Pt) in the cytosol
- Safe *storage/donation* of essential Zn and Cu
- Binding affinities: Bi > Hg > Ag > Cu > Cd > Zn

See Aschner M J, Alzheimers Dis(2005)8:139-45
Zinc and ROS Increase Metallothionein (MT) Gene Expression

• **Displaced Zn** binds to a nuclear metal *transcription factor* that binds to MRE (*promotor region*)

• **ROS** bind to and *activate* the antioxidant response element (ARE)

• **Both mechanisms result in increased MT synthesis**

Common Plant Compounds Induce Hepatic MT

- Oleanolic (OA) and ursolic acids- common triterpenoids that have antioxidant, hepatoprotective, anti-inflammatory and anti-tumor properties
- **Induce hepatic MT** (Nrf2-mediated) that imparts protection from oxidative stress, CCl₄, Cd, acetaminophen and bromobenzene
- OA has long been used in China to treat hepatitis
- Available, synthesized and water soluble derivatives are “in the works” (Rx)

Support for GSH and MT

- **Antioxidants** - vitamin C (TID), mixed tocopherols, lipoic acid, curcumin, etc.
- B vitamins, Mg, Se
- **Inducers**: curcumin, quercitin, oleanolic acid, zinc
- Appropriate intake of high BV protein and energy
- N-AC or methionine (w/ co-factors) - **based on need**

*Excess cysteine* is cytotoxic and neurotoxic, synergistic with glutamate (excitotoxic)*

Excess Cysteine

\[ \text{Cysteine Dioxygenase} \]

Cysteine sulfinate → Taurine

Sulfite

\[ \text{SUOX} \quad \text{Mo} \]

Sulfate

Excess Cysteine

Cysteine Dioxygenase

Cysteine sulfinate

B-6

Sulfite

SUOX

Sulfate

GSH

GCL

O₂

Taurine

B-6

**Essential Ionic Sulfate** \( (SO_4^{2-}) \)

- 80-90% of sulfate is derived from cysteine

- Sulfite oxidase (SUOX)- Mo-dependent, eliminates *sulfite* and produces sulfate

- **Sulfate is important for:**
  - The synthesis of cellular structural components (*mucins*)
  - Regulation/balance of hormones and neurotransmitters
  - Sulfonation of phenols, steroidal hormones, tyramine (cheese), xenobiotics, and some drugs (acetaminophen, prednisone)... *Phase II Detoxification*

Possible Health Implications

- **High sulfite**: sulfite-sensitive asthma, anemia and thiamine deficiency (rats), *maybe* neurotoxicity and breast tumors (animals)

- **Low sulfate** (sulfation) may be associated with:
  - Altered mucosal barriers (GI, lung), intestinal permeability, food sensitivities, IBS, impaired detoxification, chemical sensitivity, migraines, Rheumatoid arthritis, Parkinson’s / Alzheimer's, ASD, systemic autoimmune disease

Take Home Messages

- Endogenous detoxification processes are *inducible* and highly *energy* dependent.
- Phases I-III need to be coordinately up-regulated.
- Normal methionine metabolism (methylation, transsulfuration) is essential for detoxification.
- Toxic elements inhibit Phase I, methylation and Phase II detoxification processes.
- Toxic metals are pro-oxidative and deplete anti-oxidative enzymes, GSH and cysteine.
Take Home Messages

- Metals / chemicals compete for natural processes of detoxification & decorporation (e.g. glutathione).
- GSH and MT are pivotal in protection against toxic elements, chemicals and oxidative stress.
- GSH and MT and can be effectively up-regulated in a sustained manner only if appropriate support is provided.
- Efficient metal decorporation protocols require comprehensive support of endogenous detoxification processes.