RISK ASSESSMENT OF ESSENTIAL ELEMENTS: COPPER: OVERVIEW AND UPDATE

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“Given the importance of copper as an essential mineral for human health, it is conceivable that this and other minerals with health significance should be approached differently from nonessential minerals”

M. Olivares and R. Uauy (1996)
OBJECTIVE

- Overview and update of the Cu data base
- Health issues surrounding copper-induced toxicity and deficiency
- Challenges for developing risk assessment methodologies, strategies and policies that are public health protective
- Health is not only absence of disease but reduction in risk of developing disease
Conceptual Framework for Essential Metals Risk Assessment

- Different from nonessential metal or chemical risk assessment
- Essential: zero intake ≠ optimal
- Homeostatically regulated over a range of intakes
- Basal vs. normative requirements – sufficient nutrient to prevent pathology + provide tissue stores (protective buffer)
- Bioavailability: intake ≠ absorption
A Theoretical U-Shaped Dose-Response Curve

- Risk of Adverse Effects (Deficiency)
- Risk of Adverse Effects (Excess)
- Acceptable Range of Oral Intake (mg/day)
What is Copper?

- Atomic # 29, mass = 63.546
- Member of 3rd transition series (w Zn, Mn, Fe, Ni)
- Isotopes:
  - Two stable $^{63}\text{Cu}$, $^{65}\text{Cu}$
  - Two radioactive $^{64}\text{Cu}$, $^{67}\text{Cu}$
- Three oxidation states: Cu0 (metal), Cu1+ (cuprous), Cu2+ (cupric)
- Redox cycling=essentiality
- Redox cycling=potential toxicity
Some Key Copper Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
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<tbody>
<tr>
<td>Amine oxidases</td>
<td>Oxidation of biogenic amines</td>
</tr>
<tr>
<td>Ceruloplasmin (Ferroxidase I) [Cp]</td>
<td>Plasma transporter of Fe and Cu</td>
</tr>
<tr>
<td>Cytochrome c oxidase [CCO]</td>
<td>Mitochondrial electron transport</td>
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<tr>
<td>Dopamine-β-hydroxylase</td>
<td>Catecholamine metabolism</td>
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<tr>
<td>Haphaestin</td>
<td>Transmucosal Fe transporter</td>
</tr>
<tr>
<td>Lysyl oxidase</td>
<td>Collagen/elastin cross-linking</td>
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<tr>
<td>PAM</td>
<td>Peptide/neuropeptide amidation</td>
</tr>
<tr>
<td>Superoxide dismutase [SOD]</td>
<td>Free-radical scavenging</td>
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</tbody>
</table>
Cu Kinetics

- Fractional g.i. absorption – primary site is duodenum
- Bioavailability – varies inversely with amount ingested, affected by dietary matrix (e.g., Zn, ascorbate, protein, sugars, fat)
- Portal circulation to liver – site of Cu incorporation into Cp for systemic distribution, excess stored in metallothionein (MT) and lysosomes, excess excreted in bile
- Intake ≠ Bioavailability
Cu Transport

- Tightly regulated and coordinated
- Specific Transporters - DMT, ATP7A, Ctr1, ATP7B, others
- Specific Metallochaperones include:
  - Atox1 (HAH1) → to transporter ATPases (A and B)
  - COX17 (yeast) → to mitochondria for incorporation into CCO
  - CCS → to cytosol for incorporation into Cu,Zn SOD
Results??

Homeostatic control:

Precise orchestration of Cu transport to sites of enzyme synthesis/function

Processes ensure that unbound ionic Cu (toxic moiety) does not exist unless homeostatic capacity is exceeded
Inborn Errors of Cu Metabolism

Menkes Disease (MNK): Cu deficiency
• X-linked, freq ≈ 1/300,000, ATP7A defect

Wilson Disease (WD): Cu overload
• Autosomal recessive, freq ≈ 1/30,000, ATP7B defect, severe liver damage
• Treatable with Cu chelation and diet control
Inborn Errors of Cu Metabolism

- WD diagnosis: low Cp, high ratio of non-Cp Cu:Cp Cu, elevated liver Cu, Kayser-Fleischer rings
- WD HZ: WD HZ, freq $\approx 1/90$ are carriers
- Susceptible to Cu overload???
  No evidence
Copper Circulation in Humans

5 mg Cu/day

Intestine

DMT1?

Cu⁺

MT
Atox1

Menkes

Cu⁺

Wilson

MT
Atox1

Liver

Wilson

MT

Menkes

Ctr1

Blood

Cu-histidine

Cu-Albumin

Cu-CP

Cu-Transcuprein

Cu-GSH

Fetus

Milk

Menkes

Brain

BBB

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Other Copper-Related Hereditary Syndromes

- Indian childhood cirrhosis (ICC), Non-Indian childhood cirrhosis (NICC), Idiopathic copper toxicosis (ICT)
- Syndromes similar in etiology and presentation
- Have both genetic component (unidentified) and contribution from elevated intake
- High frequency of parental consanguinity
Deficiency: Effects Evaluation

Humans: Case reports, case series
Depletion-repletion clinical studies
Dose metric: mg/day
Responses: negative Cu balance, alterations in phospholipids, glucose/insulin, immune parameters, cuproenzyme levels, cardiac
Deficiency: Effects Evaluation


Short-term experiments: 1 severely deficient dose, developing young (gestation, lactation and/or postweaning). Dose metric = mg Cu/kg feed (e.g. CuD = 0.6, CuA = 6).

Responses: target-organ specific (blood, heart, brain/behavior, immune), cuproenzyme levels

More recently, studies of marginal Cu def., longer dur.
Deficiency: Effects Evaluation

In both humans and animals, major target organs

- blood and hematopoietic system
- cardiovascular system
- connective tissue and bone,
- nervous system
- immune system
- teratogenicity
Deficiency: Effects Evaluation Summary

- Clinically-evident copper deficiency considered to be very rare except in premature low-birth weight infants, malnourished infants, adults receiving TPN without added Cu.
- Marginal deficiency thought to occur extensively but no available biomarkers of marginal Cu status to confirm.
Deficiency: Effects Evaluation

NEW DATA

Adult onset Cu-deficiency myeloneuropathy

- Resembles subacute combined degeneration of Vit B_{12} def.
- May also present with neuromuscular degeneration similar to ALS
- Clear presentation, diagnostics, progression
- Cu status not assessed in initial diagnostic workup. Often misdiagnosis (e.g., ALS) or no diagnosis (idiopathic neuropathy)
- Myeloneuropathy progressive.
Toxicity: Effects Evaluation

Humans: Case reports
Metabolic-unit studies
Acute exposure studies
Population-based studies (M. Araya)
Dose metric = mg Cu/day, mg Cu/L
Toxicity: Effects Evaluation

Animals:
- Single-dose studies targeting liver and kidney
- One guidelines subchronic toxicity study

NEW DATA
- Two-gen reproductive toxicity study
- Unpublished rabbit dev. tox study
- Infant rhesus monkey study (Araya)

Dose metric: mg Cu/kg feed, mg Cu/L water, mg Cu/day $\Rightarrow$ mg Cu/kg bw/day
Toxicity: Effects Evaluation

- **Acute toxicity:** Gastrointestinal in humans (nausea and vomiting), repeated acute dosing \(\rightarrow\) right-shift in LOAEL and NOAEL
- **Repeated-dose toxicity:** Primary target organ in humans and animals is the liver, other organs include kidney and g.i.t.
Toxicity: Effects Evaluation

- Animal studies (Haywood) show almost complete regression & regeneration in liver and kidney histopathology over dosing period. Appears to be considerable adaptation to copper loading over a range of high doses.
- Liver Cu content remains elevated.
Dose-Response Assessment/Modeling

- Conceptual Approach: Homeostatic model providing an acceptable range of oral intake (AROI) to meet nutritional requirements and avoid toxicity (WHO 2002)
- Common Currency: Risk-risk or risk-benefit comparison – decreasing the risk of one adverse health effect (deficiency) balanced against increasing risk of another adverse health effect (toxicity) (Renwick et al. 2004)
Dose-Response Assessment/Modeling

Key Considerations:

• Quality of studies (toxicity, deficiency)
• Use of biological endpoints of comparable functional significance to define AROI boundaries (or make other adjustments)
• Knowledge of homeostatic mechanisms & of coefficient of variation (CV) of susceptibility within human population
Dose-Response Assessment/Modeling

- Appropriate dose metric (mg/day, mg/kg/d)
- Appropriate response metric (defining acceptable level of response)
- Incorporation of variability
- Incorporation of uncertainty
- CV and/or uncertainty factors (UFs)
- Similar modeling approach for both D and T
Essential Metal Requirements

• “Requirement for what?”
• Related to specified criteria of adequacy
  Prevention of clinical disease?
  Health benefits at intakes > RDA?
  Highest intake level without appreciable risk of adverse effects? (UL)
### Severity Scores for Cu-Associated Outcomes

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Homeostasis</th>
<th>Excess</th>
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<tbody>
<tr>
<td>Gross Deficiency</td>
<td>Metabolic Perturbation</td>
<td>Loss of Cu-dependent Enzyme Activity</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Metabolic Manifestation</td>
<td>Molecular Manifestation</td>
<td>Loss of Cu-Dependent Enzyme Function</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gross Excess</td>
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## Severity Scoring: Deficiency

<table>
<thead>
<tr>
<th>Severity Score</th>
<th>Types of End Points</th>
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<tbody>
<tr>
<td>1D</td>
<td>Cu body burden; MT; urine Cu levels</td>
</tr>
<tr>
<td>2D</td>
<td>Loss of Cu-dependent enzyme function (SOD), changes in blood cell #s or function,</td>
</tr>
<tr>
<td>3D</td>
<td>Severe body wt, changes, organ wt. changes, plasma glucose/insulin, minor histopathology, white blood cell activities/count</td>
</tr>
<tr>
<td>4D</td>
<td>Death, gross histopathology, reproductive/development changes</td>
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## Severity Scoring: Excess

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<tbody>
<tr>
<td>1E</td>
<td>Cu body burden; MT; urine Cu levels</td>
</tr>
<tr>
<td>2E</td>
<td>Changes in cholesterol/triglycerides; lge Cu burdens; nausea, vomiting, diarrhea; enzyme changes without histopathology</td>
</tr>
<tr>
<td>3E</td>
<td>Lge body weight changes; anemia; hemolysis; vitamin/mineral levels; liver enzymes; inflammation; organ weights</td>
</tr>
<tr>
<td>4E</td>
<td>death; gross histopathology; reproductive function changes</td>
</tr>
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</table>
Summary

Rich Cu data base, excellent case study for RA
Areas of research:
Biomarkers – sensitive, specific, dose-responsive, predictive of adverse clinical outcome; few good biomarkers of marginal Cu status
Exposure – Recent multi-route studies (Sadhra et al. 2006, Georgopoulos et al., 2007) show deficiency more of a concern than toxicity