The Scientific Basis for Chelation: Animal Studies and Lead Chelation

Donald Smith, PhD

Microbiology and Environmental Toxicology
University of California
Santa Cruz, CA
Molecular Mechanisms of Chelation

**Action of metal chelators simply described:**

\[ M + L_i \rightarrow ML_i \]

‘Efficiency’ for a chelating agent for mobilizing a toxic metal can be described as:

\[ E = \frac{[ML]}{[M]} \]

\[ \beta = \frac{[ML_i]}{[M] \cdot [L_i]} \]

Modified to reflect major competing cations in body (e.g., Ca\(^{2+}\))

\[ E = \frac{\beta_{ML}}{\beta_{CaL}} \times \frac{[L_i]}{[Ca^{2+}]} \]

**Stability constants don’t tell us everything…**

- Competing metals and ligands
- Kinetics of metal exchange
- Metabolism of chelating agent
- Transport kinetics of the chelator
- Compartmentalization

*In vivo* chelation rxns likely differ extensively from *in vitro* observations

- Chelator ‘efficiency’ best described from animal experiments or clinical observations, not theoretical calc’s
Studies of Aposhian, Dart, Maiorino and colleagues, Graziano, and others have shown:

- **Absorbed DMSA extensively metabolized / biotransformed**
  - All blood / plasma DMSA as altered mixed disulfides
  - >90% as disulfides with plasma proteins, mainly albumin
  - Undergoes enterohepatic circulation and metabolism

- **Peak Pb excretion (urine) ~2 - 4 hrs following single dose**
  - >90% of DMSA excreted in urine as mixed disulfides, primarily w/cysteine
  - Relatively small amt. (~30%) administered DMSA recovered in urine w/in 24hrs

- **Evidence that Pb poisoning status alters metabolism of succimer**
  - Pb poisoned subjects show decreased renal clearance of total DMSA.
Questions Addressed With Animal Studies…

1. What is the efficacy of blood and body Pb reduction?
   – Is brain Pb reduced with chelation?
   – Do changes in blood Pb adequately reflect changes in brain Pb?
   – Are skeletal Pb stores reduced with chelation?

2. Can succimer treatment alleviate neurobehaviroal impacts of Pb?

3. Does succimer treatment, in the absence of Pb, produce negative effects on neurobehavioral measures?
Study Design & Treatment Schedule

Objectives:

1) Reduction of tissue lead, endogenous sources of chelated lead.

2) Efficacy to alleviate lead-induced neurocognitive deficits.
Q: What is the efficacy of blood and body Pb reduction?

Chelation and exposure elimination
Blood Pb Levels By Treatment in Juvenile Monkeys

- Pb Exposure (yrs)
  - 0  1  2
  - Grp 1, Grp 3, Grp 5
  - Grp 2, Grp 4, Grp 6
- N=12/Treatment

- Pb exposure (grp 3 & 4)
- Pb exposure (grp 5 & 6)
- Neurobehavioral assessments
- 2^11Pb tracer
- Succimer chelation

- Pb 1yr + Succimer
- Pb 1yr + Placebo
- Pb 2yr + Succimer
- Pb 2yr + Placebo

- Pb dosing ends for 2yr Pb groups

- 1st Chelation
- 2nd Chelation

- Blood Pb (ug/dL)
- Age (weeks)
Succimer Efficacious, But Rapid Rebound and No Clear Efficacy Post-Treatment in Juvenile Monkeys

Summary

- Succimer significant @ end of treat, AUC
- Rapid rebound
- No effect after day 25

The Use and Misuse of Metal Chelation Therapy
ACMT Conference, Atlanta, 2012

Smith et al., 2000, TAP, 166:230-240
Succimer and Pb Diuresis in Juvenile Monkeys

Substantial inter-animal variability in Pb diuresis with succimer

The Use and Misuse of Metal Chelation Therapy
ACMT Conference, Atlanta, 2012

Smith et al., 2000, TAP, 166:230-240
Succimer Increases Urinary Pb and Decreases Fecal Elimination of Endogenous Pb in Juvenile Monkeys

But…
Whole Body Retention of Lead was High

Cremin et al., 2001, EHP 109:613-620
Q: Is Brain Pb Reduced With Succimer Chelation?
Succimer Chelation in Adult Monkeys

- Adult rhesus monkeys (n = 5-6/trt)
- Pb orally ~5 wks, ending 5 – 8d prior to chelation
- $^{204}$Pb tracer, iv inj. 1/day x 2d

- PFC biopsy -1d of chelation
- Succimer 30 mg/kg/d x 5d + 20 mg/kg/d x 14d


The Use and Misuse of Metal Chelation Therapy
ACMT Conference, Atlanta, 2012
Succimer Does Not Reduce Brain Pb, Adult Monkeys

ANOVA:
Succimer - NS
Time - P<0.05
Brain region - P<0.05

Blood Pb is a Poor Surrogate of Brain Pb, Adult Monkeys

Pre-Succimer, PFC

- Vehicle
- Succimer

\[ r = 0.362, \ p = 0.304 \]
\[ r = 0.693, \ p = 0.026 \]


The Use and Misuse of Metal Chelation Therapy
ACMT Conference, Atlanta, 2012
Reductions in Blood Lead Overestimate Reductions in Brain Lead after Repeated Succimer Regimens in Rodents

- Long-Evans rats, 3 x 3 design (Pb 0, low, high x succimer 0, 1, or 2 regimens)
- Oral Pb PND 1-40 (PND 1-21 via dam, PND 22-40 via water)
- 1 or 2 succimer regimens @ 50 mg/kg/d x 7d + 25 mg/kg/d x 14d (PND 40 and PND 68)

Brain Pb reductions lag behind Blood Pb
(One 21d chelation cycle)

Blood Pb, but not Brain Pb rebounds after chelation
(One 21d chelation cycle)

Two succimer cycles provides added benefit vs one cycle

The Use and Misuse of Metal Chelation Therapy
ACMT Conference, Atlanta, 2012

Stangle et al., 2004, Environmental Health Perspectives 112:3
Q: Can succimer treatment alleviate neurobehavioral impacts of Pb?

- Rodents
- Primates
Rodent Study
3 x 2 Design

- **High Pb**
  - 300 ppm Pb in water
  - High Pb-vehicle, n=20
  - High Pb-succimer

- **Moderate Pb**
  - 300 ppm Pb PN 1-17,
  - 20 ppm Pb PN 18-30
  - Mod Pb-vehicle
  - Mod Pb-succimer

- **No Pb**
  - 300 ppm Na acetate
  - Control-vehicle
  - Control-succimer

Chelation:
- Succimer or apple juice (vehicle)

Succimer dose:
- 50 mg/kg/d x 7d, then
- 25 mg/kg/d 14d,
- Dosing 2x/d via oral gavage

Duration:
PND31 to PND52.

Age (days)
- 0 birth
- 30 weaning
- 51
- 64 → behavioral Testing
  (≈ 8 Months)

Stangle et al., 2007. Environmental Health Perspectives 115: 2

The Use and Misuse of Metal Chelation Therapy
ACMT Conference, Atlanta, 2012
**Rodent study, Tasks administered**

\[ \approx 8 \text{ months to complete} \]

<table>
<thead>
<tr>
<th>Tasks</th>
<th>Cognitive processes tapped</th>
<th>Human task</th>
</tr>
</thead>
</table>
| • Visual discrimination & attention tasks | Associative ability  
Sustained & selective attention inhibitory control | Continuous performance task (CPT) |
| • Olfactory serial reversal learning tasks | Cognitive flexibility  
Inhibition of prepotent responses  
Associative ability | Same task |
| • Extradimensional shift tasks | Cognitive flexibility  
Selective attention  
Inhibition of prepotent responses  
Associative ability | Same task, Wisconsin card sorting task |
| • Olfactory conditional learning task (w/ omission of reward) | Stimulus-response learning, arousal regulation, error monitoring | Similar task |

**Blood and Brain Pb at start of testing:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Pb (μg/dL)</th>
<th>Brain Pb (ng/g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.5 ± 0.1</td>
<td>41 ± 9</td>
</tr>
<tr>
<td>Mod-Pb</td>
<td>12.6 ± 0.8*</td>
<td>1,040 ± 49*</td>
</tr>
<tr>
<td>High-Pb</td>
<td>31.0 ± 0.8**</td>
<td>3,690 ± 260**</td>
</tr>
<tr>
<td>Mod-Pb–succimer</td>
<td>2.8 ± 0.2#</td>
<td>196 ± 14.2#</td>
</tr>
<tr>
<td>High-Pb–succimer</td>
<td>8.5 ± 0.7##</td>
<td>1,370 ± 150##</td>
</tr>
</tbody>
</table>

Stangle et al., 2007. Environmental Health Perspectives 115: 2
Automated testing apparatus

Top view of an automated testing chamber
Succimer treatment of the High-Pb rats was ineffective or only partially effective in alleviating impairments in learning, inhibitory control, and early-session attentional function.

Inhibitory control
Attention task 1
Variable delay, 700 msec cue

Inhibitory control
Sustained attention task
Variable delay, Variable cue

Slopes for High-Pb vs. control; \( p = 0.009 \)

\*\( p \leq 0.07 \), \**\( p < 0.05 \), \#\( p \leq 0.01 \)
Succimer treatment in rodents...

Heightened reactivity to errors of the High-Pb rats was normalized by succimer treatment

*p < 0.01, High-Pb vs. control.
**p < 0.01, High-Pb–succimer vs. High-Pb.

Attention task 1, latency to enter alcove at trial onset

Sustained attention task, % omission errors for trials following an error

Succimer treatment significantly improved learning ability of the Mod-Pb rats

*p = 0.056; **p ≤ 0.03;
#p < 0.01, Mod-Pb vs. control. ###p = 0.03;
†p = 0.006, Mod-Pb–succimer vs. Mod-Pb.

Stangle et al., 2007. Environmental Health Perspectives 115:2
Selective attention is impaired, especially following an error on the prior trial (impaired arousal regulation?)

Stangle et al., 2007. Environmental Health Perspectives 115:2
Succimer Efficacy to Alleviate Pb Neurocognitive Deficits in Primates

Time/ Age
1 Yr  2 Yrs  3 Yrs  4 Yrs

Pb exposure (grp 3 & 4)
Pb exposure (grp 5 & 6)

Neurobehavioral assessments

207Pb tracer
Succimer chelation

Treatments

Pb Exposure (yrs)
0  1  2
Grp 1  Grp 3  Grp 5
Grp 2  Grp 4  Grp 6
N=12/Treatment

Blood Pb (ug/dL)

Pb dosing ends for 2yr Pb groups

Pb 1yr + Succimer
Pb 1yr + Placebo
Pb 2yr + Succimer
Pb 2yr + Placebo

Chelation

1st Chelation
2nd Chelation

0 10 20 30 40 50 60
0 10 20 30 40 50 60
0 10 20 30 40

Week

51 52 53 54 55

Week

Age (weeks)
DRT
5 stages of DRT at age 93, 96, 102, 109, and 115 wks i.e., ~6 mo after the end of chelation

Each monkey tested 5 days/wk, with 30 trials per session.

Stimuli were two identical red squares stimuli.
While the monkey watched, a small food reward was concealed beneath one stimulus.

The monkey was allowed to respond:
**Stage I** - Immediately
**Stage II** - After a brief visual barrier was interposed
**Stage III** - After barrier presented for progressively longer delays of 1, 2, 3, 4, and 5 sec in consecutive sessions
**Stage IV** - Delays of 5, 10, 20, and 30 sec imposed for five consecutive sessions/delay
**Stage V** - Delays of 5, 10, 20, and 30 sec were intermixed randomly within a session for five consecutive sessions.

Testing at each of stages I-III was to a criterion of 90% correct responses/session before advancing to the next stage.
Delayed response task of spatial memory

Stage I
- No barrier
  1. Watch
  2. Reach

Stage II
  1. Watch
  2. Wait < 1 sec
  3. Reach

Stage III
  1. Watch
  2. Wait > 1, 2, 3, 4, or 5 sec
  3. Reach

Stage IV and V
  1. Watch
  2. Wait > 5, 10, 20, or 30 sec
  3. Reach

The Use and Misuse of Metal Chelation Therapy
ACMT Conference, Atlanta, 2012
The Use and Misuse of Metal Chelation Therapy
ACMT Conference, Atlanta, 2012

Preliminary data. Smith, Strupp, Beaudin, Luck, Lasky, Laughlin

Primate DRT:
1 Yr Pb exposure altered post-error performance in stage V and succimer treatment reversed this effect

- The 1-yr-Pb+veh group did not exhibit a decline in correct responses on trials after an error as seen in the controls (p=0.05) and the 1-yr-Pb +succ group (p=0.03)

- The 1-yr-Pb+vehicle monkeys committed significantly more A-not-B errors than controls (p=0.04), whereas succimer fully normalized this effect
Primate DRT: 2 yr Pb exposure delayed the learning rate for stages I and III and succimer treatment alleviated this effect

- The 2-yr-Pb+veh group, but not the 2-yr-Pb+succ group needed more trials than controls to achieve criterion on Stages I and III
- The 2-yr-Pb+veh group committed more repetition errors than the control (p=0.09) or 2-yr-Pb+succ (p=0.05) groups on Stage V trials with a 30 sec delay

Repetition errors are indicative of a failure to adjust behavior in the face of errors.
**Primate DRT:**

**Succimer effects in monkeys **not** exposed to Pb in early and late DRT stages**

Succimer retarded learning rate across stages I-III

Succimer worsened performance disruption on trials after an error with a 10 s delay in stage V

---

* p<0.05 vs control
+ p<0.10 vs control

The Use and Misuse of Metal Chelation Therapy
ACMT Conference, Atlanta, 2012

Preliminary data. Smith, Strupp, Beaudin, Luck, Lasky, Laughlin
Summary: Questions Addressed With Animal Studies…

1. What is the efficacy of blood and body Pb reduction?
   • Eliminating exposure nearly as effective as chelation (if evaluated wks after end of treatment)

2. Is brain Pb reduced with chelation?
   • Possibly; aggressive treatment needed

3. Does blood Pb adequately reflect reduction in brain Pb?
   • NO, blood overestimates brain Pb reduction

4. Are skeletal Pb stores reduced with chelation?
   • NO
Summary (con’t): Questions Addressed With Animal Studies…

5. Can succimer alleviate neurobehavioral impacts of Pb?

Yes, but appears to be selective
  - Improves only some cognitive outcomes
  - Degree of benefit may vary by Pb burden and specific functional deficit

6. Does succimer, in the absence of Pb, cause negative cognitive effects?

Yes, but appears to be selective
  Concerns about use in absence of elevated metals
Acknowledgements

Research Collaborators:
- Barbara Strupp, PhD, Cornell University
- Diane Stangle, PhD, Cornell University
- John Cremin, PhD, University of California Santa Cruz
- Stephane Beaudin, PhD, Cornell University, University of California Santa Cruz
- Nellie Laughlin, PhD, University of Wisconsin, Madison
- Missi Luck, University of Wisconsin, Madison
- Bob Lasky, PhD, University of Wisconsin, Madison, University of Texas Medical School at Houston

Funding support:
- National Institute of Environmental Health Sciences grants #ES07457, ES05950, ES06918, ES05870.
- Centers for Disease Control
- University of California