Therapeutic Implications of Hormesis

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Samueli Institute, Alexandria VA
Dose-Response 2011:
Implications for Toxicology, Medicine and Risk Assessment
Overview of Talk

• Review the window demonstrated by hormesis where therapeutic opportunities exist.

• Explore some top agents and models for clinical application hormesis

• Explore some challenges for development of clinical hormesis

• Discuss conceptual and research needs to advance the clinical utility of hormesis

• Introduce the concept of Rapid Induction of Protective Tolerance (RIPT)
“This suggests that universal organizing principles apply to all networks, from the cell to the World Wide Web.”

General form of Hormetic Dose-Response Relationship

Calabrese and Baldwin 2001c.
Where might hormesis be used therapeutically?

- Radiation Protection
- Carcinogenesis
- Immunological conditions
- Brain Injury and Neurodegenerative Diseases
- Heavy Metal Toxicology
Where might hormesis be used therapeutically?

• Radiation Protection

• Carcinogenesis

• Immunological conditions

• Brain Injury and Neurodegenerative Diseases

• **Heavy Metal Toxicology**
Critical Review and Meta-Analysis of Serial Agitated Dilutions in Experimental Toxicology*

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1 We conducted an overview and quantitative meta-analysis of all experimental literature on the protective effects of serial agitated dilutions (SADs) of toxin preparations.
2 Articles were systematically collected and evaluated for scientific quality using pre-defined methodological criteria and then independently analysed for validity.
3 We found 105 publications exploring the effects of SAD preparations in toxicological systems.
4 The quality of evidence in these studies was low with only 43% achieving one half of the maximum possible quality score and only 31% reported in a fashion that permitted reevaluation of the data.
5 Very few studies were independently replicated using comparable models.
6 Among the high quality studies, positive effects were reported 50% more often than negative effects.
7 Four of 5 outcomes meeting quality and comparability criteria for meta-analysis showed positive effects from SAD preparations.
8 Average percent protection over controls in these preparations was 19.7 (95%CI 6.2–33.2).
9 Further research with special attention to methodological detail and independent replication should be done.

Mean protection index and 95% CI for outcome measures of 26 dilution tests

Cross tolerance protection of low-dose metal exposure is from induced signature HSP and MT

Fig. 6: Relationship between the survival factor and the percentage of similarity. The latter was obtained upon comparison of the heat shock-induced and the stressor treatment-induced protein patterns. Results are the mean of 2 experiments (± standard error).

Jan van Wijk ATHM, 1990.
Biphasic effect of cadmium on cell proliferation in human embryo lung fibroblast cells and its molecular mechanism

![Graph showing the biphasic effect of cadmium on cell proliferation. The graph plots Cd concentration against relative cell proliferation ratio for 12, 24, and 48 hours.](image)

**Fig. 1.** Effects of Cd on HLF cells proliferation. Cells were treated with 0, 0.5, 1.0, 1.5, 2.0, 5.0 or 10.0 μmol/L Cd for 12, 24, and 48 h, respectively. Cell proliferation were tested by MTT assay and expressed as percentages of the value for the control cells, which is set at 100%. Data are presented as mean ± SD of triplicate experiments.

# Long Duration Exposure and Biomarkers

## Cadmium in Prostate Cells

### Cadmium Doses:

<table>
<thead>
<tr>
<th>Control</th>
<th>0 M (Water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Doses</td>
<td>$10^{-6}$ M</td>
</tr>
<tr>
<td></td>
<td>$10^{-7}$ M</td>
</tr>
<tr>
<td>Low Doses</td>
<td>$10^{-18}$ M</td>
</tr>
<tr>
<td></td>
<td>$10^{-21}$ M</td>
</tr>
<tr>
<td>Ultra low Doses</td>
<td>$10^{-32}$ M</td>
</tr>
<tr>
<td></td>
<td>$10^{-36}$ M</td>
</tr>
</tbody>
</table>

**Duration:** 20 weeks

**Cells:** RWPE-1 (Cells) Human Prostate
Effect of Low Level Cadmium Analyses

1. Growth and Toxicity assay
2. MT protein measurements
3. RNA expression analysis by RT-PCR and RPA
4. Cell morphology and transformation
Cadmium Cytotoxicity

MTT values

Cadmium concentration
MT Protein Levels in RWPE-1 Cells (3 weeks)
MT Protein Levels in RWPE-1 Cells (20 weeks)
Reduced Cell Transformation from LD Cd Pretreatment

NL Cells

No Cd pretreatment

Cd $10^{-18}$M pretreatment
Where might hormesis be used therapeutically?

- Radiation Protection
- Carcinogenesis
- Immunological conditions
- **Brain Injury and Neurodegenerative Diseases**
- Heavy Metal Toxicology
NMDA Receptor (Glutamate)

AMPA receptor

KA receptor

Voltage-sensitive Na⁺ channel

Voltage-sensitive Ca²⁺ channel

Voltage-sensitive K⁺ channel (Potassium)

Ca²⁺-dependent Processes

NF-κB inhibitor

Inflammatory cytokines

Arnica (helenalin)

Toxin/Stressor

Cell Death

ATP

Ca²⁺

Na⁺

K⁺

Cl⁻
Subtoxic concentrations of N-methyl-D-aspartate (NMDA) protect neurons against glutamate-mediated excitotoxicity in hippocampal cultures

Fig. 5. Subtoxic concentrations of NMDA protect neurons against glutamate-mediated excitotoxicity in hippocampal cultures. Cultures were incubated with various concentrations of NMDA (10-100 mm) on DIV 7 for 24h, followed by the addition of an excitotoxic concentrations of glutamate (50 μm) and neuronal viability was quantified 24h later. **p < 0.01 versus glutamate (50 μm) alone by ANOVA plus Tukey.

Figure 4

Effect of SAD pre-treatment on glutamate toxicity in cerebellar neurons

- Effects on moderate glutamate toxicity (n > 33)

Middle Cerebral Artery Occlusion Model

Point of Occlusion

Filament (inserted into external carotid and advanced to the middle cerebral artery)

- anterior cerebral artery
- middle cerebral artery
- internal carotid artery
- basilar artery
- occipital artery
- superior thyroid artery
- external carotid artery
- common carotid artery
Middle Cerebral Artery Occlusion

- Intraluminal Filament Method (MCAo)
- Male Sprague-Dawley Rats (275-325 g)
- 2 h Occlusion → 24 h Reperfusion & Recovery

Treatment Protocol:

-- Post-Injury Treatments
-- Variable Dosing Schedule (Potency & Therapeutic Window)
-- I.V. (bolus), 2ml/kg @ 0.5, 2, 4, & 6 hrs post MCAo

Parameters Studied:

-- Infarct Analysis (TTC)
-- Neurological Function (Clinical Neurological Exam)
-- Brain Function (Cortical EEG Analysis)
Temporary MCAo & Reperfusion Model in Rats

bregma

6 h 24 h 72 h

mm from frontal pole
Representative forebrain images following 2 h of MCAo and 24 h recovery

Control

Experimental (LD KCL/Glutamate)

mm from frontal pole

purple = total infarct
green = core infarct
bregma
Neuroprotection from Low-Dose GK30

24 hours

Neurological Scores x 10
Representative forebrain images following 2 h of MCAo and 24 h recovery

Vehicle

ULD Glutamate
Representative forebrain images following 2 h of MCAo and 24 h recovery

Vehicle

ULD KCl

mm from frontal pole

bregma
NMDA Receptor (Glutamate)

AMPA receptor

KA receptor

Toxin/Stressor

voltage-sensitive Ca++ channel

voltage-sensitive Na+ channel

voltage-sensitive K+ channel (Potassium)

Ca++

[Na]^+

[Ca]^{++}

NF-kB inhibitor
Inflammatory cytokines
Arnica (helenalin)

depolarization

Ca++-dependent Processes

CELL DEATH

ATP
Neuroprotection from Low-Dose Arnica

![Bar graph showing Neurological Scores for Core Infarct Volume at 24 hours and 7 days, with Arnica and Control groups compared.](image-url)
Where might hormesis be used therapeutically?

- Radiation Protection
- Carcinogenesis
- Immunological conditions
- Brain Injury and Neurodegenerative Diseases
- Heavy Metal Toxicology
## ANIMAL MODELS FOR THE STUDY OF LOW-DOSE PROTECTIVE EFFECTS

<table>
<thead>
<tr>
<th>EXPERIMENTAL MODEL</th>
<th>TREATMENT</th>
<th>EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic intoxication (Wurmser 1955, Cazin 1987-1991)</td>
<td>Arsenic -7M or -17M</td>
<td>Increase of urinary arsenic excretion</td>
</tr>
<tr>
<td>Liver toxicity (CCl₄) (Bildet 1975-84, Palmerini 1993)</td>
<td>CCl₄ -7M or Phosphorus -7M, -15M, -30M</td>
<td>Protection</td>
</tr>
<tr>
<td>Experimental hepatocarcinoma (De Gerlach e Lans 1991)</td>
<td>Phenobarbital -9M</td>
<td>Decrease of tumor growth</td>
</tr>
<tr>
<td>Skin UV inflammation (Bastide, Poitevin, Bildet 1975-90)</td>
<td>Apis -7M or -9M</td>
<td>Protection</td>
</tr>
<tr>
<td>Adjuvant arthritis (Conforti 1995)</td>
<td>Intraperitoneal adjuvant</td>
<td>Protection</td>
</tr>
<tr>
<td>Bursectomised chicken embryos (Bastide 1993-1994)</td>
<td>Bursin -30-40M in ovo</td>
<td>Recovery of immune function</td>
</tr>
</tbody>
</table>
## MODELS FOR THE STUDY OF LOW-DOSE PROTECTIVE EFFECTS

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<th>EXPERIMENTAL MODEL</th>
<th>TREATMENT</th>
<th>EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cancer</td>
<td>Phenobarbital 1ppm</td>
<td>Reduced foci</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>DDT .01 ppm</td>
<td>Reduced foci</td>
</tr>
<tr>
<td>Multiple tumors</td>
<td>mistletoe lectin</td>
<td>Increased thymidine uptake</td>
</tr>
<tr>
<td></td>
<td>1 ng/ml to 30 pg/ml</td>
<td></td>
</tr>
<tr>
<td>Prostatitis (McLane and McMichael)</td>
<td>chorionic gonadotropin</td>
<td>Improved symptoms</td>
</tr>
<tr>
<td>Brian plasticity/memory (Diamond)</td>
<td>DHEA</td>
<td>Increased</td>
</tr>
<tr>
<td>T-cell function (Sharp)</td>
<td>deltorphin -8M -14M</td>
<td>Biphasic effects</td>
</tr>
<tr>
<td>Aging (Rattan)</td>
<td>Mild heat stress</td>
<td>Anti-aging effects</td>
</tr>
<tr>
<td>Diabetes (Cai)</td>
<td>Zinc MT</td>
<td>Prevention</td>
</tr>
</tbody>
</table>
Challenges in development of clinical hormesis

**Challenges:**
- Conceptual challenges
- Terminology
- Laboratory and toxicology focus
- Variability
- Relevance and utility

**Needs:**
- Need sound mechanistic basis for study of low-dose stimulation
- Need to optimize low-dose effects
- Need to exam the entire dose-response range for various agents
- Need to better communicate hormesis
Challenges to Development of Clinical Hormesis

Terms Used for Hormesis

- Non-linear
- Paradoxical
- Adaptive
- Stimulatory
- Threshold
- Bidirectional
- Biphasic
- BELLE
- Non-monotonic
- U/J-shaped
- Tolerance inducing
- Reparative
- Beneficial
Temporal Sequence of Hormetic Dose-Response Relationship

Calabrese and Baldwin 2001c.
Variability of Protection from Low-dose Toxic Agents

• NMDA ++
• Cyclohexamide +
• PLA₂ -
• Con-G -
• MMP -
• Arnica +
• CN +
• Glutamate +++
• K –
• Silica +
• Mercury/Arsenic ++
• Tularemia +
Very few exposure-protection studies of most agents that matter clinically

<table>
<thead>
<tr>
<th>Language</th>
<th>English</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Country</td>
<td>Finland</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>U.S.</td>
<td>4</td>
</tr>
<tr>
<td>Toxin</td>
<td>Chemical agent - Sarin</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>- Soman (1 combined with Sarin)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biological agent - Tularemia</td>
<td>1</td>
</tr>
<tr>
<td>Model</td>
<td>Animal – rats/mice</td>
<td>4/1</td>
</tr>
<tr>
<td></td>
<td>Central nervous system</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Ex-vivo – spinal cord</td>
<td>1</td>
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<tr>
<td>Outcomes</td>
<td>Physiologic</td>
<td>2</td>
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<tr>
<td></td>
<td>Mortality</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Multiple</td>
<td>2</td>
</tr>
<tr>
<td>Administration</td>
<td>Perfusion</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Injection</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Ingestion</td>
<td>1</td>
</tr>
<tr>
<td>Dilution Ranges</td>
<td>Low dose - 0.003 nM to 1 µM sarin</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>- 1 – 100 µg/kg sarin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 12.5 &amp; 50 µg/kg sarin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 4 &amp; 20 µg/kg soman</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 60 µg/kg soman</td>
<td></td>
</tr>
</tbody>
</table>
"I believe I have a new approach to psychotherapy, but like everything else, it first has to be tested on mice."
RAPID INDUCTION OF PROTECTIVE TOLERANCE (RIPT)

VISION & OBJECTIVES

● A rapid protective countermeasure for CBNR threats?
  ● Reduce CBNR mortality and injury by induction of cellular/whole organism hormetic tolerance.
  ● Target agents are cyanide, botox, phosgene, tularemia, VEE, Anthrax and radiation.
  ● May be effective for emerging infections such as flu and the effects of environmental toxins

CONCEPT

Exposure to sub-toxic doses of toxic compounds can confer protection to or modulation damage from higher doses of same or similar harmful agents.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Current Approach</th>
<th>RIPT Approach</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthrax</td>
<td>Vaccine - 6 doses</td>
<td>1 dose</td>
<td>LD₅₀ increased 3X</td>
</tr>
<tr>
<td>*Tularemia</td>
<td>Antibiotics – 6 weeks</td>
<td>2 X day</td>
<td>LD₅₀ increased 3X</td>
</tr>
<tr>
<td>*Cyanide</td>
<td>Hyperbaric Oxygen</td>
<td>During threat situations</td>
<td>Reduce mortality 30%</td>
</tr>
<tr>
<td>*Brain Trauma</td>
<td>ICU Surgery</td>
<td></td>
<td>40% reduced injury</td>
</tr>
<tr>
<td>Mustard</td>
<td>Barrier Methods</td>
<td></td>
<td>50% reduced damage</td>
</tr>
<tr>
<td>Radiation</td>
<td>Potassium Iodide</td>
<td></td>
<td>60% reduced damage</td>
</tr>
<tr>
<td>*Cadmium</td>
<td>HE-2100</td>
<td></td>
<td></td>
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</tbody>
</table>

A Cellular Hormetic Bioshield?
Publications


