Calcium Dysregulation in Alzheimer’s Disease: Dantrolene for Treatment?

Huafeng Wei, MD, PhD
Associate Professor with Tenure
Department of Anesthesiology and Critical Care
University of Pennsylvania
weih@uphs.upenn.edu

CNTS
March 21, 2014
President Ronald Reagan Suffered from Alzheimer’s Disease (AD) for 10 years
Alzheimer’s Disease (AD)

- Affect 35 million patients worldwide and 6% of population with age ≥65 years old, over 100 millions by 2050

- A devastating neurodegenerative disease, characterized by amyloid and tau pathology in brain and progressive memory loss and cognitive dysfunction

- >182 billions cost in U.S., over 600 billions cost worldwide

- Familial AD (FAD), 5% AD patients, three genes mutation (APP, Presenilin and Tau)

- Sporadic AD, 95% AD patients, similar amyloid and tau pathology in brain, ApoE$_4$ mutation.

- Only symptom relieving but no effective disease modifying drug treatment available at this time
AD Neuropathology Features

Source: http://www.ahaf.org/alz/dis/about/AmyloidPlaques.htm
AD Amyloid Pathology

Mattson MP, Nature, 430, 631-639
AD Tau Pathology

Cell body

Axon

Synapse

Axonal transport

Tau stabilizes microtubules

Tau phosphorylation MT depolymerization

PHF assembly

Source: http://www.mpasmb-hamburg.mpg.de/mandelkow2/taubilder.htm

PHF: Paired Helical Filaments
None of them works in patients!!!
Calcium dysregulation: Alternative hypothesis in neuropathology of AD
Intracellular Calcium Homeostasis

LeFerla FM, Nat Rev Neurosci, 2002, 3: 862-72
Roles of Calcium Dysregulation in Neuronal Apoptosis

Wei H, Anesthesia and Analgesia 2011; 113: 972-4
All three RYR isoforms are expressed in CNS, with RYR3 and RYR2 dominant

Inan S, Wei H, Anesthesia and Analgesia 2010; 111:1400-10

Ryandine receptor calcium channel over activation plays a critical role in AD pathology and memory loss.
Dantrolene

- A ryanodine receptor (RYR) antagonist

- A clinically available drug to treat malignant hyperthermia, muscle spasm, neuroleptic malignant syndrome etc.

- Most common side effects in patients with chronic use is drowsiness, suggesting its passage across blood brain barrier (BBB).

- Dantrolene has been shown to protect neuronal damage induced by multiple insults, including hypoxia, ischemia, trauma, seizure, sepsis etc.

Inan S, Wei H, Anesthesia and Analgesia 2010; 111:1400-10
Hypothesis 1

Early and chronic dantrolene treatment inhibits progress of amyloid, tau pathology, synapse damage, neurodegeneration and memory loss in 3xTG AD mice
3xTG-AD mice

- Transgenic mice with all three genes mutation (APP, Presenilin and Tau) demonstrated in familial Alzheimer’s disease (FAD)

- Calcium dysregulation and synapse dysfunction appears at two months old, long before amyloid and tau pathology, neurodegeneration and memory loss

- An adequate animal model of testing efficacy of drug treatment in Alzheimer’s disease

- Disadvantage of “gene drift”
Schematic time point of experimental design in 3xTG-AD mice

- **0 Months**: Start Alzet pump. ICV Dantrolene 9.24 μg/hour continuous for 3 months.
- **2 Months**: Stop ICV. Start Dantrolene S.C. 5 mg/kg twice a week continuous for 8 months.
- **3 Months**: Morris Water Maze test for memory and learning.
- **4 Months**: Morris Water Maze test for memory and learning.
- **10 Months**: Morris Water Maze rotarod tests.
- **13 Months**: Amyloid, Tau pathology synapse, neurodegeneration in brains.

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
The Morris Water Maze: A sensitive test of rodent memory, learning and behavior.
Dantrolene did not affect motor function or swim speed in 13 month old 3xTG-AD mice

Rotorod Test

Morris Water Maze

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
Effects of dantrolene treatment on reference learning in 3xTG-AD mice at 13 months

Morris Water Maze, Place test, The shorter latency, the better reference learning ability

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
Dantrolene blocks memory loss in 3xTG AD mice at 13 months

MWM, Probe test, The higher percentage, the better reference memory

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
Dantrolene blocks working memory loss in 3xTG AD mice at 13 months old

Morris Water Maze, 21 days working memory test, The shorter latency, the better working memory

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
Dantrolene significantly decreases amyloid plaque level in hippocampus of 3xTG triple transgenic mice

13 month old, hippocampus, red arrows pointing to the plaques

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
Dantrolene significantly decreases amyloid plaque level in hippocampus of 3xTG triple transgenic mice

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
Effects of dantrolene treatment on Tau pathology in 3xTG-AD mice at 13 months

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
Effects of dantrolene treatment on synaptic proteins in 3xTG-AD mice at 13 months old

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
Effects of dantrolene treatment on apoptotic proteins in 3xTG-AD mice at 13 months old

Cerebral cortex, Western Blot Analysis

Peng J et al., Neurosci Lett 2012 April, 516, 274-279
Summary 1

- Early and chronic dantrolene treatment block memory loss in later aged 3xTG AD mice.

- Early and chronic dantrolene treatment reduce amyloid burden in brains of aged 3xTG AD mice.

- Early and chronic dantrolene treatment did not change tau pathology, synapse proteins or apoptotic proteins in brains of aged 3xTG AD mice.
Hypothesis 2

Late treatment of dantrolene still inhibit amyloid pathology and cognitive dysfunction, as a disease-modifying therapeutic agent.
Schematic time point of experimental design in 3xTG-AD mice

0

15

21

22

Month

Start dantrolene: P.O 5 mg/time two times a week continuous for 6 months

Stop dantrolene: Morris Water Maze test for memory and learning roto-rod tests

Morris Water Maze roto-rod tests

MRI Amyloid

Dantrolene Significantly Decreased Amyloid Plaques in 3xTG mice

22 month old, after 7 months dantrolene treatment, hippocampus, Plaques in red color

Dantrolene Significantly Decreased Amyloid Plaques in 3xTG mice

Effect of Dantrolene on Brain Volume in 3xTG-AD mice at 22 month old

MRI: Volume of Hippocampus and Cortex, after 7 months dantrolene treatment

Effect of Dantrolene on Memory and learning in 3xTG-AD at 22 month olds

**MWM, A:** Place trial for reference learning

**B:** Probe test for retention memory

Summary 2

- Late and chronic dantrolene treatment still significantly decreased amyloid burden in brains of aged 3xTG AD mice.

- Extremely old 3xTG in this study did not show significant cognitive dysfunction compared to wild type controls.

Nanocrystal dantrolene or sterile water, ip, 10 mg/kg, daily, for 4 weeks, beginning from 5 months old (starting amyloid pathology and cognitive dysfunction)

Chakroberty et al., PLOS One, 2012, December, 7: e52056
Dantrolene Normalized RyR2 in 3xTG-AD and TASTPM AD Mice

Nanocrystal dantrolene or sterile water, ip, 10 mg/kg, daily, for 4 weeks, beginning from 2 (3xTG) or 5 (TASTPM) months old

Chakroborty et al., PLOS One, 2012, December, 7: e52056
Nanocrystal dantrolene or sterile water, ip, 10 mg/kg, daily, for 4 weeks, beginning from 2 months old

Chakrobority et al., PLOS One, 2012, December, 7: e52056
Dantrolene Significantly Decreased Amyloid Burden in TG2576 Mice Brain

Dantrolene or vehicle PBS, ip, 5 mg/kg, twice a week for 3 months, beginning from 12-15 months

Oules et al, J Neurosci 2012 August, 32, 11820-11834
Dantrolene Inhibited PSD95 loss in TG2576 Mice Brain

Dantrolene or vehicle PBS, ip, 5 mg/kg, twice a week for 3 months, beginning from 12-15 months

Oules et al, J Neurosci 2012 August, 32, 11820-11834
Dantrolene Improved Cognitive Function in TG2576 Mice

Oules et al, J Neurosci 2012 August, 32, 11820-11834

Dantrolene or vehicle PBS, ip, 5 mg/kg, twice a week for 3 months, beginning from 12-15 months
Mechanisms of Dantrolene Therapy in TG2576 Mice

Oules et al, J Neurosci
2012 August, 32, 11820-11834
Acknowledgement

Department of Anesthesiology and Critical Care at PENN
Grace Liang,, Donald Joseph, Saadet Inan, Qing Cheng Meng, Maryellen Eckenhoff, Roderic Eckenhoff, Lee A. Fleisher

Department of Anesthesiology, Second Affiliated Hospital, Zhongshan University, China
Jun Peng, Shuling Peng

Department of Anesthesiology, Tongji Hospital, Huazhong Science and Technology University, China
Zhen Wu, Yuke Tian

Department of Anesthesiology, First City Hospital, Shanghai Jiaotong University, China
Bin Yang, Shitong Li

University of California, Irvine
Frank M. LaFerla

Funding
NIH K08, R01, March of Dimes Birth Defect Foundation, Anesthesia Department at PENN
Thank you !