

Dimebon (Latrepidine) Stimulates Neurite Outgrowth and Protects Against Mitochondrial Depolarization in Cultured Hippocampal Neurons

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BACKGROUND

- Dimebon (latrepirdine) is an orally available, small synthetic molecule that is in Phase 3 clinical development as a potential treatment for Alzheimer's disease (AD) and Huntington disease (HD).
- Impaired mitochondrial function and neurite degeneration are pathological events associated with these neurodegenerative diseases.¹
- Furthermore, mitochondrial depolarization has been proposed as a central step in amyloid beta (A β)-induced neuronal death.^{1,2}

OBJECTIVE

- To evaluate the neurotrophic properties of dimebon, and the effect of dimebon on mitochondrial function, in cultured rat hippocampal neurons.

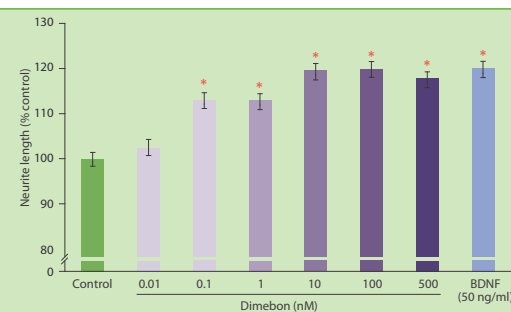
METHODS

- Cultured 3DIV to 7DIV hippocampal neurons transfected with EGFP and immunostained for MAP2 were used for the analysis of neurite development in response to dimebon (0.01–500 nM), brain-derived neurotrophic factor (BDNF) (50–100 ng/mL), or vehicle (distilled water).
- Mitochondrial membrane potential ($\Delta\psi_{mit}$) was investigated in 7DIV hippocampal neurons pretreated with dimebon (10–500 nM) by analysis of the fluorescence intensity of Mitotracker Red-CMXRos loading (50 nM) in microscopy images of fixed cells and by measurements of JC-1 fluorescence ratio (100 nM) in live cells by fluorescence microplate readings.
- Amyloid beta oligomers (A β) were prepared as indicated by Klein (2002)³ and applied to 14DIV to 16DIV hippocampal neurons together with dimebon (100–500 nM) to evaluate A β cytotoxicity (10 μ M, 48 h) by LDH release and the effects of A β (5 μ M, 6 h) on mitochondrial potential by analysis of the fluorescence intensity of Mitotracker Red-CMXRos loading.
- Quantitative analysis of neurite morphology and Mitotracker fluorescence in microscopy images of fixed neurons was made with the ImageJ software.

RESULTS

Neurite Development Analysis

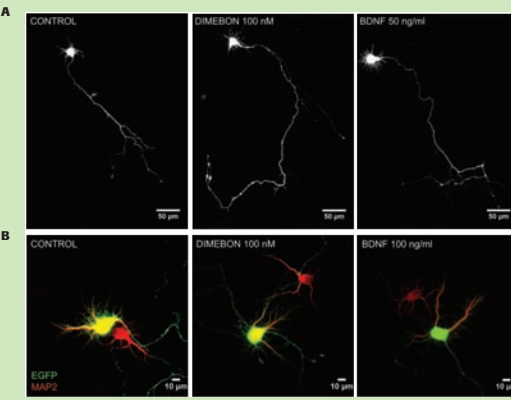
FIGURE 1: Dimebon promotes neurite outgrowth in cultured (3DIV) hippocampal neurons



- Dimebon (0.1–500 nM) was associated with a concentration-dependent significant increase in neurite outgrowth (control: 100.0 ± 2.04%; dimebon 1 nM: 113.0 ± 2.64%; dimebon 100 nM: 119.9 ± 2.45%). **P* < 0.05 vs. control.
- Dimebon's effect was comparable with that achieved with the maximally effective concentrations of BDNF (BDNF 50 ng/mL: 120.0 ± 2.59%).

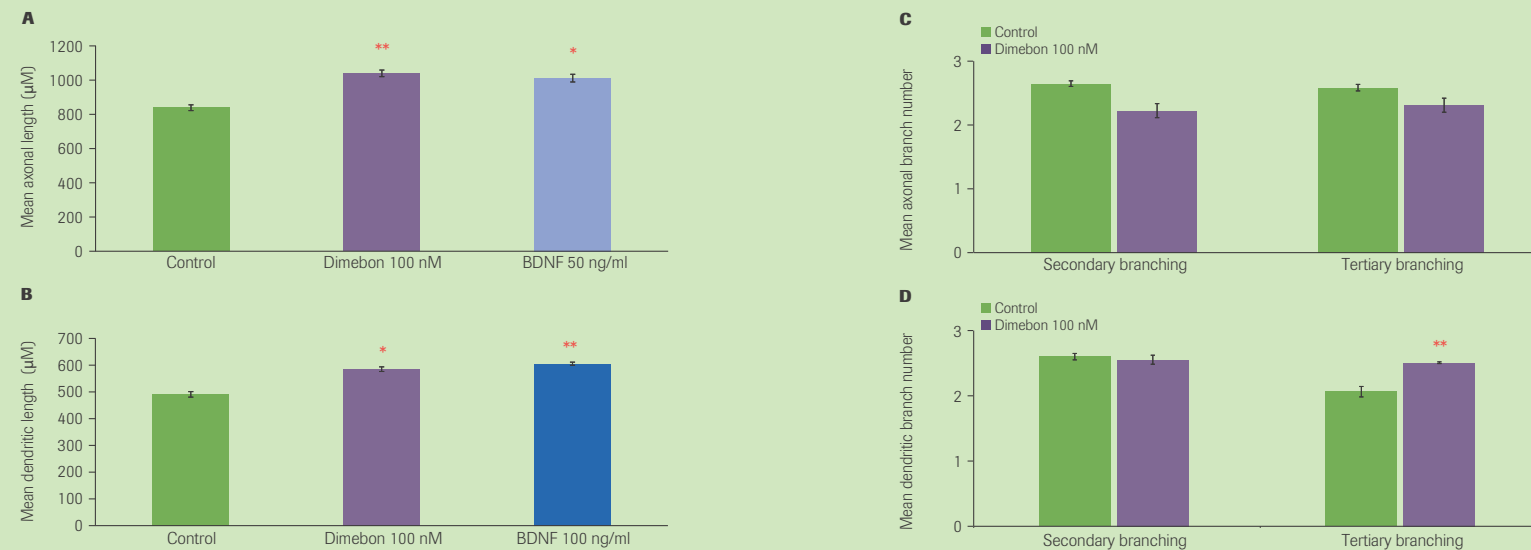
Neurite Development Analysis

FIGURE 2: Dimebon treatment increased axonal length and dendritic length and complexity in cultured hippocampal neurons



- Representative images of (A) axonal (5DIV), and (B) dendritic development (7DIV), of cultured hippocampal neurons expressing EGFP and pre-treated for (A) 48 h and (B) 96 h with vehicle (control), dimebon 100 nM, or BDNF (A: 50 ng/ml; B: 100 ng/ml).

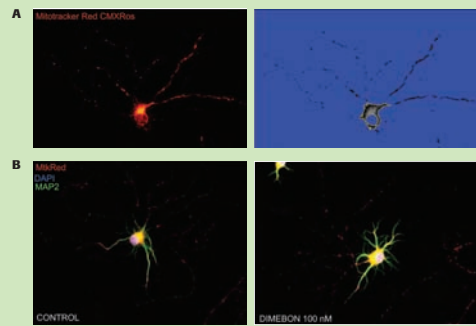
FIGURE 3: Dimebon treatment increased axonal length and dendritic length and complexity in cultured hippocampal neurons



- Dimebon induced a statistically significant increase in (A) mean axon length (control: 838.5 ± 32.7 μ m; dimebon 100 nM: 1040 ± 37.6 μ m), and (B) mean dendritic length (control: 491.7 ± 19.6 μ m; dimebon 100 nM: 585.7 ± 16.3 μ m) of hippocampal neurons. The effect of dimebon (100 nM) was comparable with maximal effective concentrations of BDNF: (A) BDNF (50 ng/ml) on axon growth, and (B) BDNF (100 ng/ml) on dendritic growth. **P* < 0.05; ***P* < 0.01 vs. control.
- Dimebon (100 nM) also increased the complexity of (C) dendritic but not (D) axonal arborization in 7DIV hippocampal neurons. Complexity of dendritic branching was increased through increasing the mean number of tertiary dendritic branches (control: 2.06 ± 0.08; dimebon 100 nM: 2.51 ± 0.01). ***P* < 0.01 vs. control.

Evaluation of Mitochondrial Membrane Potential in Developing Neurons

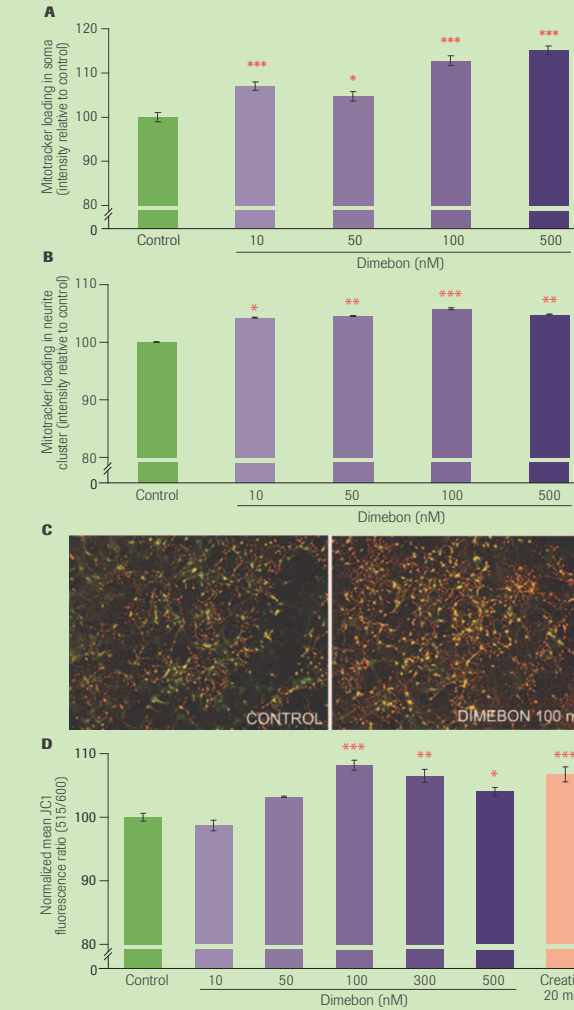
FIGURE 4: Pretreatment of hippocampal neurons with dimebon resulted in mitochondria having more hyperpolarized membrane compared with control



- Intensities of mitotracker (MtkRed) fluorescence were measured in (A) fixed cells separately in soma and neurites after background subtraction and segmentation of the images.
- Developing 7DIV hippocampal neurons pre-treated (B) with dimebon (100 nM; 96 h) had more hyperpolarized mitochondria compared with vehicle-treated neurons (control).

Evaluation of Mitochondrial Membrane Potential in Developing Neurons

FIGURE 5: Pretreatment of hippocampal neurons with dimebon resulted in mitochondria in soma and neurites having more hyperpolarized membranes compared with controls



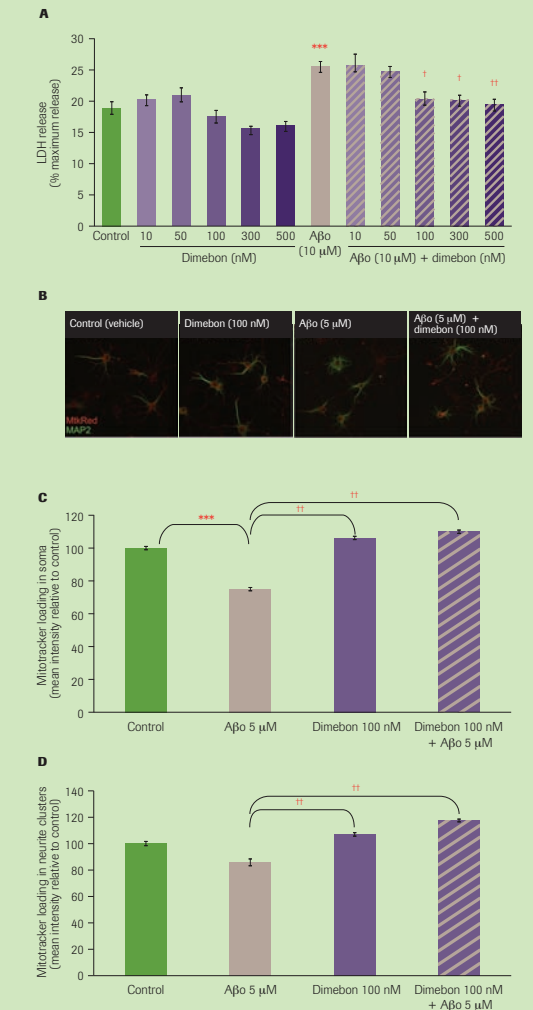
- Dimebon (10–500 nM) induced a dose-dependent and statistically significant increase in the relative fluorescence intensity of Mitotracker loading in (A) somas (% control with dimebon 100 nM: 112.8%), and (B) neurites (% control with dimebon 100 nM: 106.0%).
- Dimebon pretreatment (10–500 nM; 96 h) induced a significant increase in the mitochondrial membrane potential (C, D) shown using normalized JC-1 fluorescence ratio, comparable with energetic substrate, creatine (20 mM, 24 h pretreatment). **P* < 0.05; ***P* < 0.01; ****P* < 0.001 vs. control.
- No increase in mitochondrial biogenesis or mitochondrial cluster density was evident with dimebon pretreatment (100 nM, 96 h) (data not shown).

CONCLUSIONS

- Dimebon stimulated neurite outgrowth of cultured hippocampal neurons, increasing axonal and dendritic length and dendritic complexity.
- Dimebon promoted mitochondrial hyperpolarization and protected against mitochondrial depolarization induced by amyloid beta oligomers in cultured hippocampal neurons.

Evaluating Protection Against Amyloid Beta Oligomer-Induced Mitochondrial Membrane Depolarization

FIGURE 6: Pretreatment of hippocampal neurons with dimebon prevented the decrease in cell viability caused by amyloid-beta oligomers (A β)



- Dimebon pretreatment (100–500 nM; 6 h; A, B) prevented the 9% to 11% decrease in cell viability caused by A β pretreatment (10 μ M; 48h), shown using the LDH release cytotoxicity assay in cultured hippocampal neurons (14DIV–16DIV).
- Dimebon (100 nM) prevented the A β -induced depolarization in (C) somatic (% control: A β 5 μ M: 75.0%; dimebon 100 nM: 106.2%; dimebon 100 nM + A β 5 μ M: 110.0%), and (D) neuritic mitochondria (% control: A β 5 μ M: 85.9%; dimebon 100 nM: 107.0%; dimebon 100 nM + A β 5 μ M: 117.5%). **P* < 0.05; ***P* < 0.01; ****P* < 0.001 vs. control; †*P* < 0.01; ††*P* < 0.001 vs A β .

REFERENCES 1. Kiebertz K, McDermott MP, Voss TS, et al. *Arch Neurol* 2010;67:154–60. 2. Duyckaerts C, Delatour B, Potier MC. *Acta Neuropathol* 2009;118:5–36. 3. Bossy-Wetzell E, Schwarzenbacher R, Lipton SA. *Nat Med* 2004;10 Suppl:S2–9. 5. Klein WL. *Neurochem Int* 2002;41:345–52.

ACKNOWLEDGMENTS This study were sponsored by Medivation, Inc. Medivation Inc., and Pfizer Inc are developing dimebon (latrepirdine) as a potential treatment for AD and HD. Editorial support for the production of this poster was provided by Karen Burrows and Jon Edwards of UBC Scientific Solutions, and funded by Medivation Inc., and Pfizer Inc.

AUTHOR DISCLOSURES Dr Bernales is a full-time consultant to Medivation Inc. Dr Alfaro is a full-time employee of the Fundación Ciencia Para La Vida Chile, which has a research and consulting agreement with Medivation Inc. Dr. Protter is a full-time employee of and holds stock options in Medivation, Inc.