

Cognition Enhancement by Dimebon (Latrepirdine) is Not Mediated by Acetylcholinesterase Inhibition or NMDA Receptor Modulation

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BACKGROUND

- Current treatments for Alzheimer's disease (AD) are limited to agents that inhibit acetylcholinesterase (AChE) activity or those that block the ionotropic *N*-methyl-D-aspartate (NMDA) receptor for glutamate.^{1,2}
- 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).
- Early studies suggested that dimebon inhibited AChE (IC₅₀ = 42 μM) and prevented NMDA-induced seizures (EC₅₀ = 42 mg/kg), although at relatively high concentrations or doses.³
- Subsequent investigations have demonstrated that dimebon (when tested at 10 μM) binds a range of other molecular targets with moderate to high affinity, including various neurotransmitter receptors (eg, histamine, dopamine, norepinephrine, serotonin),⁴ some of which have been implicated in cognition.
- The contribution of these different molecular targets to cognition enhancement properties of dimebon in animals is unknown.

OBJECTIVE

- To assess whether the inhibition of AChE activity or the modulation of NMDA receptor function are involved in dimebon's pro-cognitive effects in rodent models.

METHODS

Novel Object Recognition (NOR) Task

- The NOR task consisted of 2 trials (T1 and T2) separated by a 24-h inter-trial period.
- Male rats were dosed with vehicle or dimebon (0.05, 0.5, or 5 mg/kg p.o.) or with donepezil (1 mg/kg i.p.) 30 min before the acquisition trial (T1).
- During the retention trial (T2) time spent exploring a novel object was compared versus time spent exploring the familiar object.

In vivo Microdialysis

- Acute effects of dimebon (0.05, 0.5, and 5 mg/kg p.o.), donepezil (1 mg/kg i.p.), and galantamine (0.6 mg/kg s.c.) administration on extracellular acetylcholine (ACh) levels were studied in the prefrontal cortex and hippocampus of freely moving rats using *in vivo* microdialysis.

Evaluation of Dimebon Concentrations in Rat Brain and Plasma

- Rats were dosed by oral gavage with 0.05 mg/kg of dimebon in water. Venous blood and brain samples were obtained at 15 min, 30 min, 1 h, 2 h, 4 h, and 6 h and processed.

Effects on AChE Activity

- Dimebon's effect on AChE was assessed using human recombinant AChE from HEK293 cells and AChE from red blood cell fractions as well as rat brain extracts, and compared with donepezil.
- Inhibitory potency was determined by the inhibition of the conversion of the AChE substrate acetylthiocholine-iodide to thiocholine.

In vitro Assays for NMDA Receptor Binding and Activity

- The affinity of dimebon for the NMDA receptor was compared against memantine as follows:
 - Measurement of binding to the phencyclidine site of the NMDA receptor complex.
 - Blockade of NMDA (10 μM) and glycine (10 μM)-induced calcium uptake using the fluorescent calcium indicator dye fluo-3 in cultured rat hippocampal neurons.

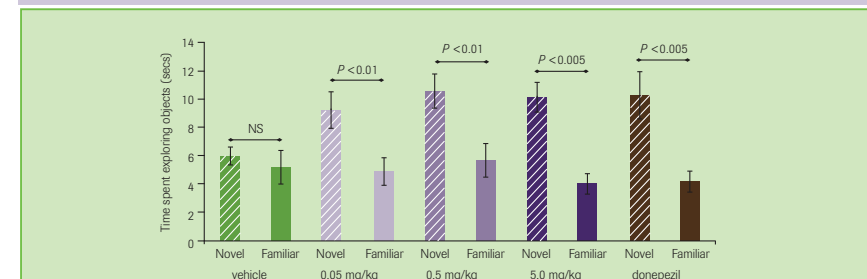
In vitro Assays of Dimebon Interactions with Other Cognition-Related Targets

- Competitive interaction of dimebon with a broad set of neurotransmitter receptors including those implicated with cognition was conducted using high throughput screening.

RESULTS

Novel Object Recognition (NOR) Task

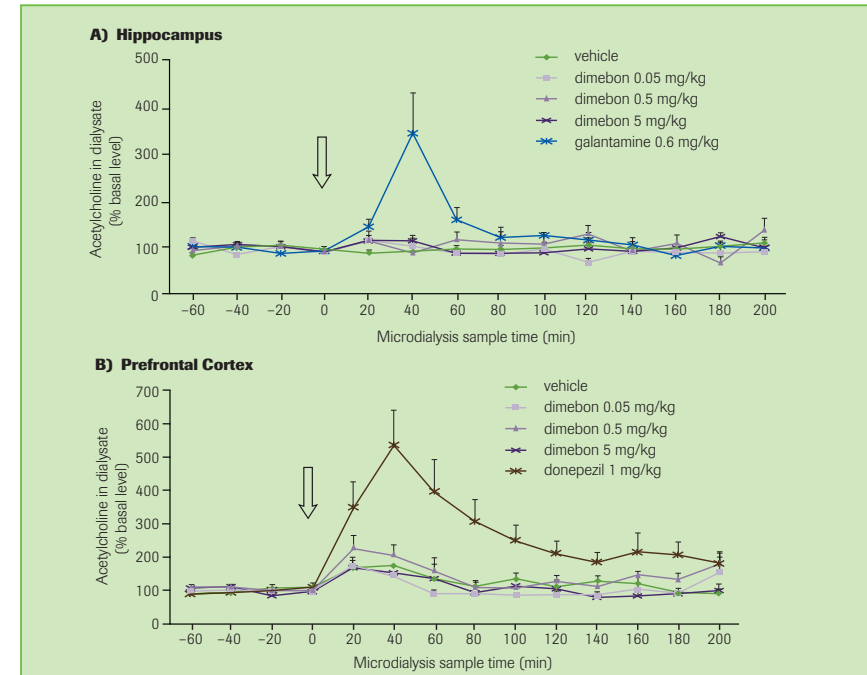
FIGURE 1: Attenuation of natural forgetting by dimebon and donepezil in the NOR task



- Dimebon was associated with a pro-cognitive effect in the NOR model. Time spent exploring the novel (hatched bars) and familiar (solid bars) objects during T2 were compared using a 2-sided Student's *t*-test for paired samples. Data represent mean ± standard error of the mean. NS = Non significant.

In vivo Microdialysis

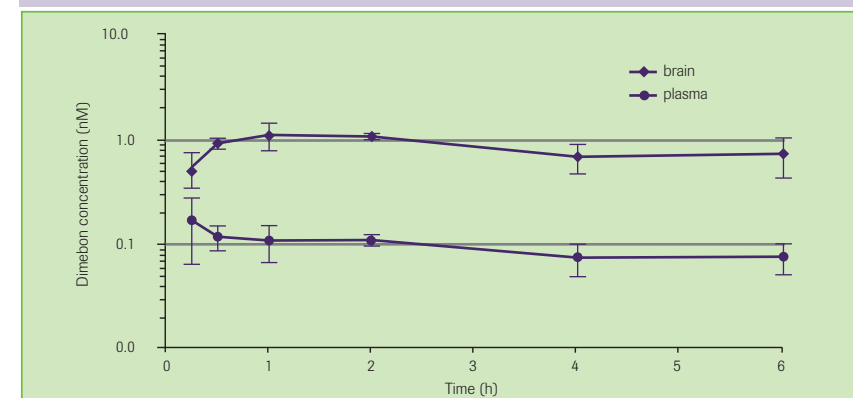
FIGURE 2: Cognition-enhancing doses of dimebon fail to modulate extracellular ACh levels in the (A) hippocampus, and (B) prefrontal cortex of freely moving rats



- Both galantamine and donepezil induced a statistically significant increase in extracellular ACh levels in hippocampus (A, top) and prefrontal cortex (B, bottom), respectively (2-way ANOVA). Arrow indicates the time when the test compound or vehicle were administered. Values are mean ± standard error of the mean, n = 8 per group.

Evaluation of Dimebon Concentrations in Rat Brain and Plasma

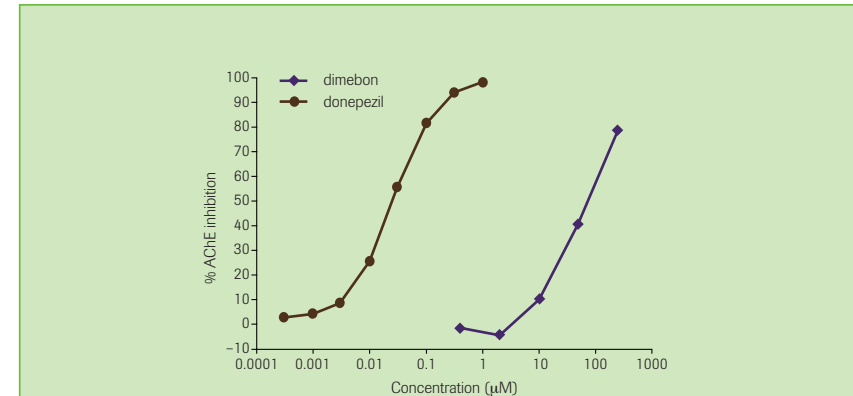
FIGURE 3: Dimebon concentrations in rat plasma and brain over time following oral administration of a cognition-enhancing dose of dimebon (0.05 mg/kg p.o.)



- Brain levels were consistently higher (up to 10-fold) compared with those in the plasma. Values are mean ± standard deviation, n = 3 per time point.

Effects on AChE Activity

FIGURE 4: Inhibitory potency of dimebon and donepezil for recombinant human AChE, human red blood cell (RBC) fraction AChE, and rat brain fraction AChE

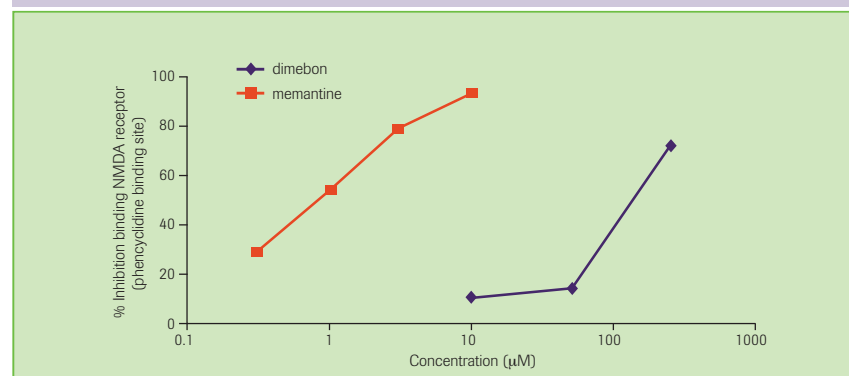


Enzyme source	Dimebon (IC ₅₀ μM)	Donepezil (IC ₅₀ μM)
Recombinant AChE	83 ± 13	0.028 ± 0.005
Human RBC fraction AChE	> 31	0.010 ± 0.003
Rat brain fraction AChE	> 31	0.0119

- The inhibitory potencies (IC₅₀ μM) of dimebon and donepezil for recombinant human AChE were: 83 ± 13 μM and 0.028 ± 0.005 μM, respectively. Percentage inhibition of AChE activity was performed in triplicate.

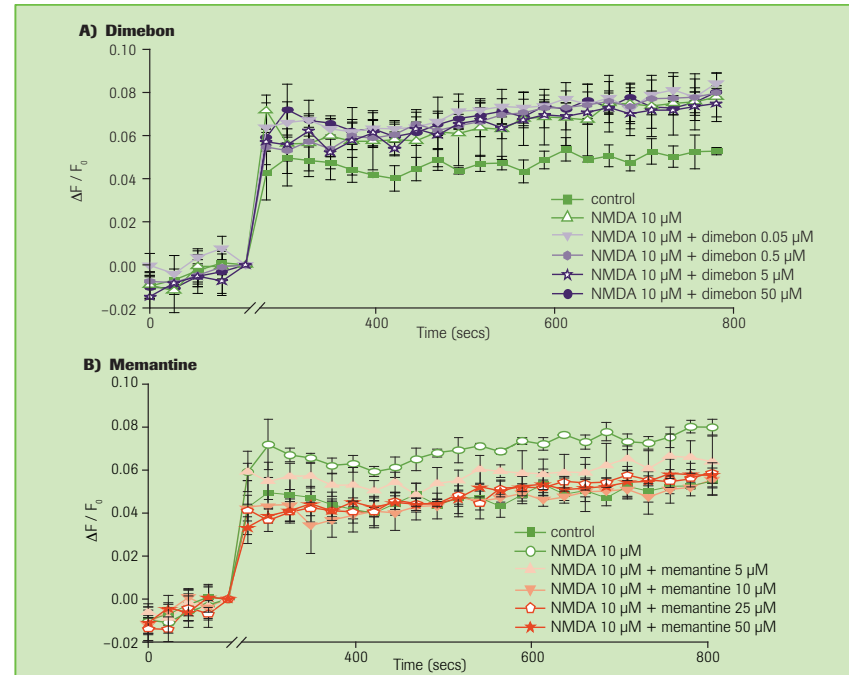
In vitro Assays for NMDA Receptor Binding and Activity

FIGURE 5: Affinity of dimebon and memantine for the NMDA receptor



- Dimebon (K_i = 105 ± 18 μM) was 200-fold weaker than memantine (K_i = 0.54 ± 0.05 μM) for occupying the NMDA receptor. n = 3 per K_i determination.

FIGURE 6: Intracellular NMDA-dependent calcium levels in primary rat neurons following (A) dimebon, and (B) memantine treatment



- Dimebon does not significantly block NMDA-induced calcium influx in cultured rat hippocampal neurons (A, top), unlike memantine (B, bottom). Fluorescence values (F_t) were normalized against initial values (F₀) before NMDA addition (F_t - F₀)/F₀. Traces shown are the mean results of the fluorescence obtained by 4 replicates per treatment 5 min before and 5 min after NMDA addition.

In vitro Assays of Dimebon Interactions with Other Cognition-Related Targets

- Dimebon binds to a number of neurotransmitter receptors; however, brain concentrations associated with cognition enhancement are below the K_i for receptors previously associated with cognitive function. Consistent with its historical use as an antihistamine, dimebon displayed highest affinity for the H1 receptor.

TABLE 1: Receptors to which the affinity (K_i) of dimebon binding was ≤500 nM*

Target	Competing ligand	K _i (nM) mean ± SD
Histamine, H1	pyrilamine	1.3 ± 0.3
Serotonin, 5-HT7	diethylamide	7.0 ± 0.4
Adrenergic, α _{2B}	rauwolscine	17.2 ± 9.9
Adrenergic, α _{1B}	prazosin	21.4 ± 7.0
Adrenergic, α _{1A}	prazosin	38.5 ± 0.0103
Serotonin, 5-HT6	lysergic acid diethylamide	42.2 ± 7.2
Adrenergic, α _{2C}	MK912	44.3 ± 10.1
Adrenergic, α _{1D}	prazosin	51.6 ± 27.6
Serotonin, 5-HT2A	ketanserin	57.4 ± 8.8
Serotonin, 5-HT5A	lysergic acid diethylamide	58 [†]
Serotonin, 5-HT2C	mesulergine	75.3 ± 17.9
Adrenergic, α _{2A}	MK912	92.6 ± 36.6
Imidazoline, I2 (central)	idazoxan	125.0 [†]
Histamine, H2	aminopentidine	201 ± 115
Dopamine, D2S	spiperone	250 [†]
Dopamine, D3	spiperone	290 [†]
Serotonin, 5-HT2B	lysergic acid diethylamide	380 [†]
Dopamine, D1	SCH-23390	380 [†]

*A panel of 75 additional receptor or enzyme targets showed interactions with dimebon that were considerably weaker than 200 nM
[†]All assays performed with n = 3 except where indicated; [†]assay performed with n = 1

CONCLUSIONS

- Dimebon exhibits significant cognition enhancement in a well-validated animal model of short-term memory.
- The results reported here suggest that in rats cognition-enhancing doses of dimebon are not associated with AChE inhibition, NMDA receptor antagonism, or with increases in extracellular ACh levels in the hippocampus or prefrontal cortex.
- Dimebon binds to a number of neurotransmitter receptors, however brain concentrations associated with cognition enhancement are below the K_i for receptors previously associated with cognitive function.
- Further studies are being conducted to determine the pharmacologic basis of the procognitive effects of dimebon in animals.

REFERENCES 1. Birks J. *Cochrane Database Syst Rev* 2006;CD005593. 2. Raina P, Santaguida P, Ismaila A, et al. *Ann Intern Med* 2008;148:379-97. 3. Bachurin S, Bukatina E, Lermontova N, et al. *Ann N Y Acad Sci* 2001;939:425-35. 4. Wu J, et al., *Molecular Neurodegeneration* 2008;3:15.

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AUTHOR DISCLOSURES Drs Giorgetti and Protter are full-time employees of, and hold stock options in, Medivation, Inc. Dr Bernales is a full-time consultant to Medivation, Inc. Dr Alfaro is a full-time employee of the Fundación Ciencia Para La Vida, which has a research and consulting agreement with Medivation, Inc. Dr Drieu La Rochelle is an employee of Biotrial, which has a research and consulting agreement with Medivation, Inc. Dr Cremers is part-owner of Brains On-Line, which has a research and consulting agreement with Medivation, Inc. Dr Altar has a research and consulting agreement with Medivation, Inc. Dr Wronski is a full-time employee of JSW Life Sciences, which has a research and consulting agreement with Medivation, Inc.