MEM 1003 is an L-Type Ca^{2+} Channel Modulator Targeted Towards Alzheimer's Disease Therapy

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INTRODUCTION

Age or disease negatively impacts the ability of neurons to regulate intracellular Ca^{2+} levels. This is of particular importance as abnormal Ca^{2+} signaling has been demonstrated in cells from patients with Alzheimer's as well as Parkinson's disease. An increase in Ca^{2+} entry into the cell alters activity of Ca^{2+} dependent proteins such as ion channels and proteolytic enzymes. This, in turn may result in reduced neuronal excitability and may likely cause neuronal cell death. Excessive Ca^{2+} entry can be exacerbated by an increase in density of L-type Ca^{2+}-channels reported to occur in aging and in Alzheimer's disease brains.

We describe here a novel L-type Ca^{2+} channel blocker, MEM 1003, with selective CNS effects and superior cardiovascular safety profile. MEM 1003 is neuroprotective and should be useful in restoring the excitability of neurons affected by aging and Alzheimer's disease.

METHODS

Receptor binding: The brain membrane preps were generated according to procedure outline in the Current Protocol of Pharmacology. For the binding assay, various concentration of compound and radioligand ([3H]-(+)-PN200-110, ~50pM) were mixed with membrane prep and incubated at room temperature for two hours. The non-specific binding was defined by the amount of binding in the presence of 10 uM final concentration of Nimodipine.

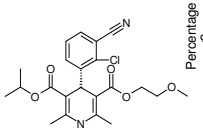
Slow Afterhyperpolarization: Conventional hippocampal slices (400u) were prepared from male Fischer 344 rats and maintained at 30°C in an interface-type holding chamber bathed in oxygenated (95%/5% O/CO2 mix) artificial cerebrospinal fluid (ACSF). Post-burst AHPs were collected in the whole-cell recording configuration using glass microelectrodes (3 MOhm). Cells were held in voltage-clamp at -70 mV for approximately 5 min to facilitate membrane stabilization. Cells were then placed in current-clamp mode and the cell membrane potential was adjusted to -60 mV. CA1 neurons received a 150 ms depolarizing current step (~ 200 to 400 pA) that was sufficient to elicit three or four action potentials. The amplitude of the AHP triggered by this burst was defined as the difference between the voltage immediately before the depolarizing current step and that measured 750 ms after the current step.

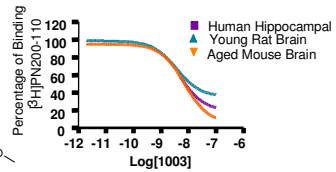
Attentional Set-Shifting:
Subjects: Sprague-Dawley rats (20-29 mo)
Apparatus: Chamber is a black acrylic box (24" x 18" x 18"), divided by a manually lifted divider. Bowls are at the front, separated by a partition. Rats wait in the rear compartment until trials. The bowls are filled with digging media and the rims are scented with oils.
Testing: Following training, rats perform a series of discriminations and reversals within one session- a simple discrimination (SD), a compound discrimination (CD) with a new irrelevant dimension; a compound reversal (CDr) rewarding the previously unrewarded stimulus; an intradimensional shift (IDS) with new stimuli from both dimensions (relevant dimension remains the same); an intradimensional reversal (IDSr); an extradimensional shift (EDS) with new stimuli from both dimensions in which the previously irrelevant dimension is rewarded, and an extradimensional reversal (EDSr). Half the rats are assigned to a scent to media shift and half are assigned to a media to scent shift. Stimuli pairs are maintained for a session. Criterion is six consecutive correct discriminations.
Treatment: MEM 1003 (1.0, 10.0mg/kg) or vehicle is administered ip 30 minutes prior to testing; treatment conditions were randomly assigned and blinded.
Analysis: Data are analyzed using a two-way, repeated measures Analysis of Variance followed by a Bonferroni post-hoc test, *** = p<0.001.

Morris Water Maze
Training: 3 trials/day for 5 days; each trial 120 sec with ITI 40 min, random starting positions. Probe trial given on at end of Day 5. Following acquisition training, rats are classified as either Aged Cognitively impaired (AI) (performance ≥ 3.0 SD from Young Rats), or Aged Cognitively Unimpaired (AU) (performance ≤ 0.5 SD from Young Rats).
Testing: Impaired animals are treated with drug or vehicle for 3 additional days. Probe trial given at end of Day 3. Visible Cued testing followed termination of all drug testing

1. MEM 1003 Binds to Dihydropyridine Binding Sites with High Affinity

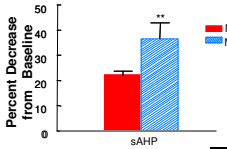
MEM 1003:





	Human Hipp. Membrane	Y. Rat Brain Membrane	Aged Mouse Brain Membrane
MEM 1003	4.8 nM	3.7 nM	7.2 nM
Nimodipine	1.0 nM	1.1 nM	0.7 nM
Nitrendipine	0.3 nM	0.3 nM	0.7 nM

2. MEM 1003 Reduces the Size of the Slow AHP



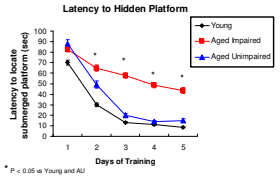
** p<0.1 vs. nimodipine

- sAHP are Ca^{2+}-dependent and become larger with age
- Drugs that reduce the size of the sAHP also improve cognition

Data courtesy of Phil Landfield, Univ. of Kentucky

3. MEM 1003 Improves the Learning and Memory Deficits in Aged Impaired Fischer 344 rats

Acquisition Phase:



* P < 0.05 vs Young and AU

Aged Fischer 344 rats classified as cognitively impaired failed to acquire the water-maze task as efficiently as aged-unimpaired or young rats. Aged-impaired rat platform latencies never fell below 40 sec, whereas aged-unimpaired rat platform latencies were similar to young rats, ≤ 20 sec. All rats readily find the visible cued platform (data not shown)

Probe Phase:

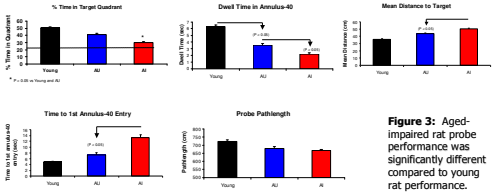


Figure 3: Aged-impaired rat probe performance was significantly different compared to young rat performance.

4. MEM 1003 Significantly Improves AI Deficits on Both Acquisition and on Probe Performance

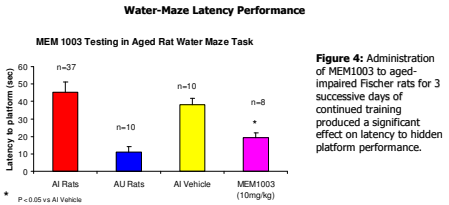


Figure 4: Administration of MEM1003 to aged-impaired Fischer rats for 3 successive days of continued training produced a significant effect on latency to hidden platform performance.

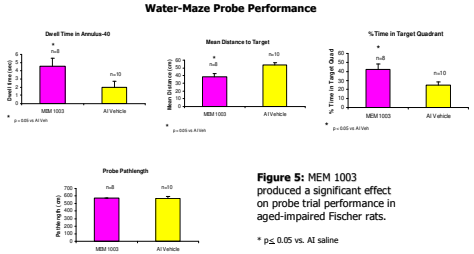
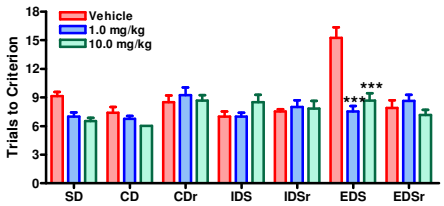


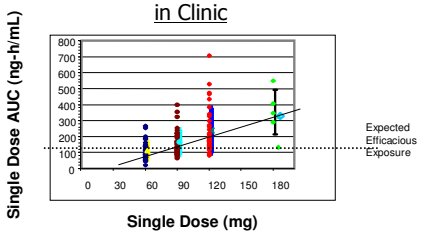
Figure 5: MEM 1003 produced a significant effect on probe trial performance in aged-impaired Fischer rats.

* p≤ 0.05 vs. AI saline

5. MEM 1003 Improves Aged Sprague Dawley Rat Attentional Set-Shifting



6. Preclinical Efficacious Exposures Achievable in Clinic



7. MEM 1003 Preferentially Relaxes Cerebral versus Peripheral Smooth Muscle

Resistance Arteries:

	Relaxation-SMRA EC50 (nM)	Relaxation-MCARA EC50 (nM)
MEM1003	120	43
Nimodipine	15	4.7
Amlodipine	28	N/A
Felodipine	1.6	1.1

HSMRA's contracted with U46619 (a prostanoid receptor agonist)

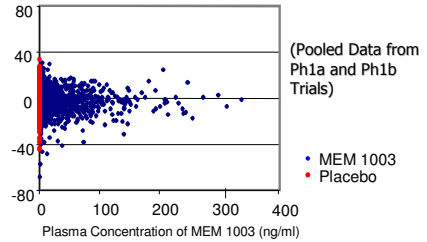
Major Arteries:

	MEM 1003 EC50 (nM)	Nimodipine EC50 (nM)
Coronary Artery	900	70
Middle Cerebral Artery	15	3

Human arteries contracted with 30 mM KCL

MEM 1003 Should improve cerebral blood flow without affecting blood pressure

8. MEM 1003 Does Not Reduce Blood Pressure at Clinical Exposures



CONCLUSIONS

- MEM 1003 binds potently(~ 5nM) and selectively to the brain dihydropyridine binding site
- MEM 1003, possibly by reducing the size of the sAHP and increasing cell excitability, improves spatial memory and attentional performance in aged rats.
- Based on its ability to relax cerebral but not peripheral vascular smooth muscle, MEM 1003 may be able to improve cerebral blood flow without reducing blood pressure..
- MEM 1003 differs from other L-type Ca^{2+} channel blockers because of its selective CNS effects versus peripheral blood pressure lowering effects.
- MEM 1003 is well tolerated in humans at exposure levels predicted to be efficacious from preclinical animal models.
- **MEM 1003 is currently in Phase 2A trials for Alzheimer's disease. Currently there is an unmet need for drugs that address abnormal Ca^{2+} signaling in the CNS**