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RESEARCH PAPER

The acute effects of dimebolin, a potential Alzheimer's disease treatment, on working memory in rhesus monkeys

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BACKGROUND

Dimebolin (latrepirdine), a compound with multiple potential drug targets, is being evaluated in clinical trials for the treatment of Alzheimer's disease (AD) and preliminary results suggest it can slow the disease process. There is also evidence that dimebolin directly improves aspects of cognition. Here we examined the acute effect of dimebolin on components of working memory in non-human primates, young adult (11–17 years old) and aged (20–31 years old) rhesus macaques.

EXPERIMENTAL APPROACH

The effects of dimebolin (3.9–118 μg kg^{-1}) on working memory, as measured by performance on delayed matching-to-sample (DMTS), were examined in the normal young adult monkeys and aged adult monkeys. All the monkeys studied were proficient in the performance of a computer-assisted DMTS task. In a subsequent experiment in the same subjects, dimebolin was administered 15 min before a cognitively-impairing dose (20 μg kg^{-1}) of scopolamine.

KEY RESULTS

In both the young adult and aged monkeys, dimebolin significantly increased the DMTS task accuracies. In young adults, the task improvement was associated with long (retention/retrieval) delay trials, and a protracted enhancement was observed for sessions run 24 h post administration of a single dose. Dimebolin did not significantly attenuate the scopolamine-induced impairment. In the aged monkeys, dimebolin significantly improved the reduced task accuracies associated with long delay intervals.

CONCLUSIONS AND IMPLICATIONS

Here we demonstrated that dimebolin is able to improve components of working memory in monkeys and to induce a protracted response for at least 24 h after administration of a single dose.

Abbreviations

Aβ, amyloid β; AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; AD, Alzheimer's disease; ADAS-cog, Alzheimer's disease assessment scale; DMTS, delayed matching-to-sample

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that results in the deterioration of memory, cognitive function and the ability to care for oneself (Cummings, 2004). Currently, AD affects 26.6 million people worldwide, and this number is expected to quadruple by the year 2050 (Brookmeyer *et al.*, 2007). Current clinical treatments for AD

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are somewhat limited and focus mostly on modulating the symptoms rather than modifying the disease. Cholinesterase inhibitors (donepezil, rivastigmine and galantamine) are the mainstay treatment, yet they provide only limited and transient improvement in AD (Birks, 2006). Donepezil, rivastigmine and galantamine have been shown to reduce cognitive deficits induced by scopolamine, an anti-cholinergic agent in animals (Bejar *et al.*, 1999; van der Staay and Bouger, 2005; de Bruin and Pouzet, 2006; Lindner *et al.*, 2006). The reversal of scopolamine-induced cognitive impairment is a model designed for the evaluation of cognition enhancing compounds relating to AD treatment (Ebert and Kirch, 1998; Buccafusco, 2008). Dimebolin (2,3,4,5-tetrahydro-2,8-dimethyl-5-[2-(6-methyl-3-pyridinyl)ethyl]-1*H*-pyrido[4,3-b]indole, also known as latrepirdine) has shown promise in clinical trials (Doody *et al.*, 2008). The drug has the potential to incorporate disease-modifying properties (neuroprotection) with the relief of certain AD symptoms including memory loss, agitation and cognitive impairment (Doody *et al.*, 2008).

Although dimebolin has been used for its anti-histamine properties in Russia for several years, the compound appears to interact at several neural targets including producing a weak inhibition of cholinesterases (Bachurin, *et al.*, 2001), blockade of glutamate NMDA receptors and L-type Ca^{2+} channels (Bachurin, *et al.*, 2001; Lermontova *et al.*, 2001) and a positive modulation of glutamate AMPA receptors (Grigorev *et al.*, 2003). Each of these properties has the potential to enhance cognition and possibly help in the treatment of AD by increasing levels of ACh (AChE inhibitors) and aiding in long-term potentiation (NMDA and L-type Ca^{2+} channels). Its more recently demonstrated antagonism of brain 5-HT_6 receptors (possibly along with its AMPA action) could also contribute to dimebolin's cognitive-enhancing properties (Schaffhauser *et al.*, 2009). Dimebolin is a neuroprotective agent with the ability to attenuate toxicity induced by amyloid Aβ in rat cortical cultures (Lermontova *et al.*, 2001). Its neuroprotective action has been suggested to be related to its ability to close mitochondrial pores (opened by the neurotoxic insult), thereby preserving mitochondrial function and thus limiting neurotoxicity (Bachurin *et al.*, 2003). Dimebolin's potential for treating AD is suggested from a report of the effects of dimebolin on safety, tolerability and efficacy as assessed in a double-blind, placebo-controlled study encompassing 183 patients with mild to moderate AD (Doody *et al.*, 2008). In this study, dimebolin was administered three times a day for 6 months, while global function, activities of daily living, cognition (ADAS-cog scale) and other non-cognitive symptoms were assessed. At the 6 month time point, dimebolin exhibited significant improvement over placebo in each of the domains assessed. The study was extended an additional 6 months, and at the end of one year, a statistically significant effect of treatment remained (Doody *et al.*, 2008). However, the clinical efficacy of dimebolin was not confirmed in a recent phase III clinical trial (Pfizer Press Release, 2010). Dimebolin has shown some positive effects on cognition in a Huntingdon's disease trial (Kieburtz *et al.*, 2010). In the current study, the effects of dimebolin on working memory, as measured by performance on delayed matching-to-sample (DMTS), were examined under three different conditions in the non-human primates; young adults, aged adults and in the presence of a cognitively impairing dose of scopolamine.

Methods

Subjects

Six Rhesus macaques 11–17 years old served as experimental subjects for the dimebolin studies in young adult monkeys and for the scopolamine reversal studies. Six Rhesus macaques 20–31 years old served as experimental subjects for the dimebolin studies in aged monkeys. Subject information is presented in Table 1. Each subject was individually housed in a stainless steel cage composed of two 127 × 71 × 66 cm units. Six such units, three facing each other, are established in each primate housing room at the Animal Behavior Center of the Medical College of Georgia. To promote psychological well-being, toys and foraging tubes were provided routinely, and monkeys were allowed to observe television programmes each afternoon after testing. DMTS testing was conducted once each weekday. During the test week, monkeys were maintained on a modified feeding schedule such that food (standard monkey chow and other supplements) was withheld beginning at 08:00 h and ending at 17:00 h (when all testing at the facility was completed). During testing, animals obtained 300 mg flavoured reinforcement pellets awarded for correct responses. Standard laboratory monkey chow, fresh fruits and vegetables were provided after 17:00 h during the test week and without modification on weekends. All primates were maintained on a 12 h light/12 h dark lighting schedule, and water was available on an unlimited basis, including during testing. All procedures were reviewed and approved by the Medical College of Georgia Institutional Animal Care and Use Committee and are consistent with Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines. Each subject had previously participated in one or more short-term studies assessing the effects of reversible drugs on DMTS performance, and all were well trained in this task. Prior drug experience had produced no observable untoward effects in the animals. A minimal washout period of 4 weeks occurred before the initiation of the current study.

DMTS procedure

Test panels attached to each animal's home cage presented the task by using a computer-automated system. A 38.1 cm AccuTouch LCD Panelmount TouchMonitor (Elo TouchSystems, Menlo Park, CA) and pellet dispenser unit (Med Associates, St. Albans, VT) were mounted in a light-weight aluminum chassis that was attached to each subject's home cage just prior to testing. The stimuli included red, blue and yellow rectangles on a black background. A trial was initiated by presentation of a sample rectangle composed of one of the three colours. The sample rectangle remained in view until the monkey touched within its borders, at which point the rectangle disappeared and a pre-programmed delay (retention) interval commenced. Following the delay interval, the two choice rectangles were presented below and to the right and left of where the sample had appeared. One of the two choice rectangles was presented with its colour matching the

Table 1

Subject information

ID	Sex	Age (years)	Body weight (kg)	Short delay (s)	Medium delay (s)	Long delay (s)
24	M	17	6.8	20	75	200
987	M	14	10.4	10	45	80
18	M	16	10	40	100	240
573	M	16	12.8	5	25	70
147	M	11	11.2	25	50	100
993	M	14	10.6	30	50	75
11b	M	22	10	10	20	110
h1v	F	25	5.6	7	10	20
7nv	M	31	8.1	15	30	75
979	F	30	9.8	10	25	40
23	M	24	9.4	20	60	200
dp5	F	20	6.6	7	10	20

Study subject information. Short, medium and long delays refer to individually adjusted delay intervals per monkey needed to achieve choice accuracy levels of 75–84%, 65–74 and 55–64% respectively.

stimulus colour, whereas the other (incorrect) choice rectangle was presented as one of the two remaining colours. A correct (matching) choice was reinforced. Non-matching choices were neither reinforced nor punished. The inter-trial interval was 5 s, and each session consisted of 96 trials. If the monkey failed to respond to the sample/choice stimuli within 3 min, the trial was terminated, and the next trial initiated after the 5 s inter-trial interval. Incomplete trials were not repeated and were omitted when calculating task accuracies. The average run time of a 96-session trial was approximately 60–90 min in length. The presentation of stimulus colour, choice colours and choice position (left or right on the screen) were fully counterbalanced according to the method of Gellerman (1933). Five different presentation sequences were rotated through each daily session to prevent the subjects from memorizing the first several trials. Delay intervals were established during numerous non-drug or vehicle sessions prior to initiating the study. Each animal was run at their respective delays an average of 68 sessions and a median 72.5 sessions before receiving a vehicle or pharmacological challenge. Similarly, all animals were required to have at least three stable sessions (task accuracies varying not more than $\pm$7.5% change from baseline) directly preceding the start of vehicle or drug dosing. However, most animals surpassed these criteria (median stable session = 6, average stable session = 5.5) before initiating a vehicle or pharmacological challenge. The duration for each delay interval (Table 1) was adjusted for each subject until three levels of group performance accuracy were approximated: zero delay interval (85–100% of trials answered correctly); short delay interval (75–84% correct); medium delay interval (65–74% correct) and long delay interval (55–64% correct). The assignment of these memory retention intervals based upon an individual's baseline task accuracy is necessary to avoid ceiling effects in the most proficient animals during drug studies, while also serving to insure that each animal begins testing at relatively the same level of task difficulty. In addition to session accuracy, two response latencies also were measured: the 'sample latency', which is the time between presentation of the stimulus rectangle and the animal touching within the sample rectangle; and the 'choice latency', which is the time between presentation of the choice stimuli and the animal touching within one of the choice rectangles.

Drug regimens

This study consisted of three experimental series: a dimebolin dose series and a dimebolin-scopolamine series in young adult animals and a dimebolin dose series in aged animals. Dimebolin was prepared with dihydrochloric acid salt (total molecular weight 392.37), and all drug doses were calculated accordingly. At least two vehicle sessions were administered during each series. Dimebolin (3.9, 11.8, 39.0, 78.5 and 118 μg kg^{-1}) or vehicle (10% dimethylsulphoxide in polyethylene glycol-400) was administered 30 min before initiating DMTS testing. For the dimebolin–scopolamine study, 20 μg kg^{-1} of scopolamine (in normal sterile saline) was administered 15 min before the initiation of testing to produce deficits in DMTS task performance (Buccafusco, 2008). Dimebolin or vehicle (3.9–118.0 μg kg^{-1}) was administered 15 min before the injection of scopolamine to evaluate its ability to attenuate the scopolamine-induced deficits. In each dosing series, doses were given in ascending order to ensure the safety of our monkeys, as this was the first time we had administered dimebolin to our monkeys. Compound solutions were prepared just before use. They were weighed to the nearest 0.1 mg and dissolved in vehicle for an injection volume of about 0.035 mL kg^{-1}. Injections were given in the thigh muscle. There was a minimum 2 week washout between series and a minimum washout of 48 h between acute pharmacological challenges within a series. Similarly, within a series, one session of stable performance within the animal's baseline range was required immediately prior to

any acute pharmacological challenge. Dimebolin was prepared and supplied by Abbott Labs (Abbott Park, IL). Scopolamine hydrobromide was purchased from Sigma-Aldridge (St. Louis, MO). All drug and molecular target nomenclature listed here conforms to the *British Journal of Pharmacology*'s guide to receptors and channels (Alexander *et al.*, 2009).

Statistics

Data for % correct were subdivided according to delay interval for each 24-trial delay component of the session. All statistical analyses were performed on raw data (% trials correct). The first two series in the young adult subjects were performed in duplicate; that is, the same dose–response series was performed in the same subjects on two occasions. One month separated the dimebolin dose series in young adult monkeys with the scopolamine reversal dose series. After 5 months, the series were repeated again with a 1 month separation interval between them. There were no statistically significant differences between the results of the replicate series; therefore, the data from each series were combined for overall statistical analysis and considered repeated measures. The scopolamine trial data were analysed by two-way ANOVA with repeated measures across all factors design (SAS, JMP statistical software, SAS Institute Inc., Carry, NC, USA). The dose series in aged subjects was performed only once. Data from the young adult and aged dose series were analysed by three-way ANOVA with repeated measures across all factors design (SAS, JMP statistical software) to compare immediate effects with effects produced at 24 h. An orthogonal multi-comparison *t*-test was used to compare individual means when there was a significant interaction between dose, delay and time, and a Bonferroni correction for multiple comparisons was used. For each table/figure (below), error values denoted are ± SEM. Differences between means from experimental groups were considered significant at the $P < 0.05$ level (two-sided test). Trends towards significance were considered at the $P < 0.10$ but >0.05. Whenever data were analysed, irrespective of the drug treatment, there was a significant effect of 'delay interval' ($P < 0.001$).

Results

Young adult subjects

The main effect of treatment was not significant. However, there was a significant effect of treatment × delay ($F_{15,235} = 1.9248$, $P = 0.021$) for dimebolin. *Post hoc* analysis revealed that the effects were a long delay with the doses of 3.9, 11.8 μg kg^{-1} (Figure 1A) and 78.5 μg kg^{-1} (Figure 1B), producing a significant effect ($P < 0.001$, $P = 0.002$ and $P = 0.018$, respectively). This effect was independent of time, as the drug treatment × delay × time was not significant.

Scopolamine reversal

In animals pretreated with vehicle, administration of scopolamine (20 μg kg^{-1}) produced a statistically significant ($F_{3,35} = 5.198$, $P = 0.0045$) impairment in task accuracies associated with short ($P < 0.001$), medium ($P < 0.001$) and long ($P = 0.002$) delay trials relative to the mean accuracies obtained after standard DMTS sessions. Scopolamine treatment

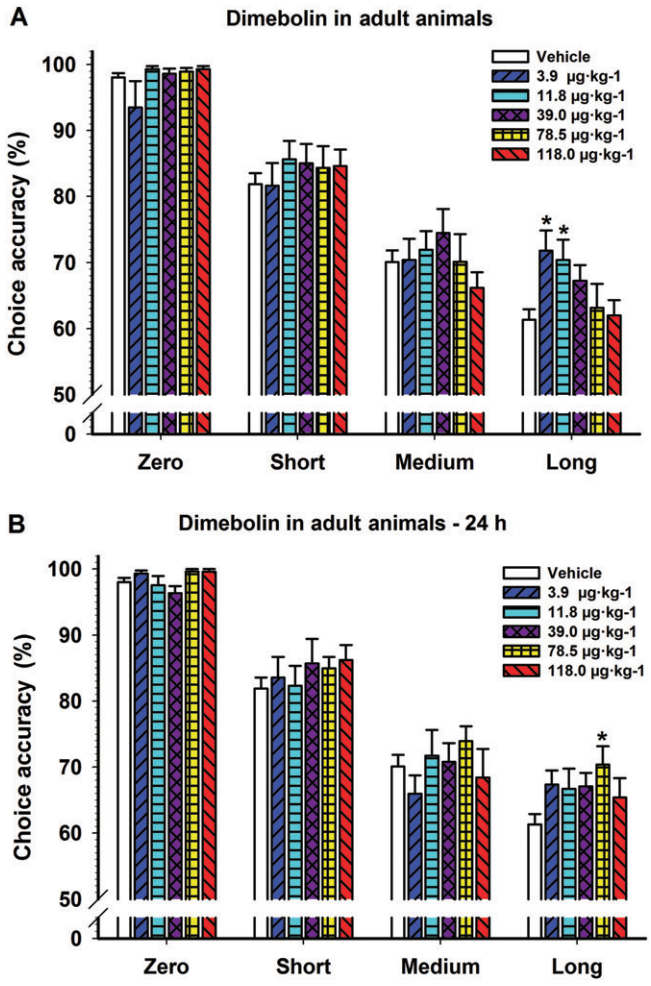

Figure 1

The effect of dimebolin or vehicle on DMTS choice accuracies by six young adult rhesus monkeys. Data were obtained during sessions run 30 min (A) and 24 h (B) after dimebolin or vehicle administration. The 24 h sessions were initiated after no additional administration of the test compounds. Each value indicates the mean ± SEM. *$P < 0.05$, significantly different from respective (0 μg kg^{-1}) vehicle mean.

decreased mean task accuracies associated with short, medium and long delay trials to 57.3 ± 2.4%, 56.3 ± 2.7% and 50.3 ± 2.4% correct, respectively. Scopolamine pretreatment did not significantly impair task accuracies associated with zero delay. Administration of dimebolin 15 min before scopolamine did not significantly attenuate the scopolamine-induced impairment in task accuracies in a manner that was influenced by the drug treatment × delay interval. (Figure 2). In vehicle-treated monkeys, scopolamine administration produced a statistically significant decrease ($P < 0.001$) in the number of trials completed/session relative to standard DMTS baseline. Pretreatment with dimebolin did not attenuate the effects of scopolamine on this variable, and in fact in the presence of scopolamine, all doses of dimebolin except for the highest dose (118 mg·kg^{-1}) were associated with significant reductions in the numbers of trials completed per session (Figure 3).

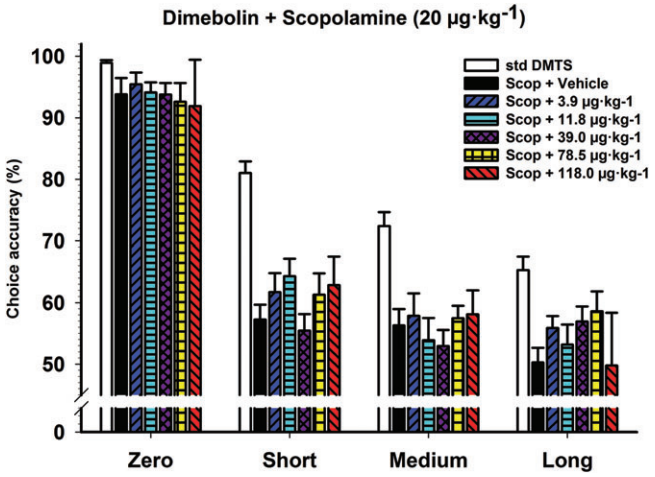

Figure 2

The effect of scopolamine (20 µg kg^{-1}) on DMTS choice accuracies by six young adult rhesus monkeys. Subjects were pretreated either with vehicle or dimebolin 15 min before scopolamine. DMTS sessions began 15 min after scopolamine administration. Each value indicates the mean ± SEM.

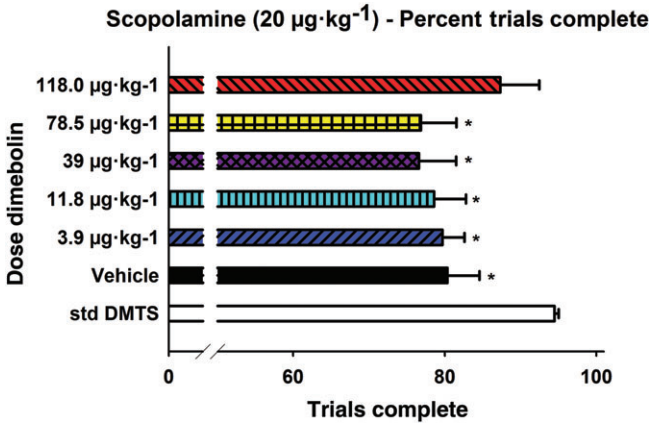

Figure 3

The effect of 20 µg kg^{-1} scopolamine on the number of trials completed per session by six young adult rhesus monkeys in their performance of the DMTS task. Subjects were pretreated either with vehicle or dimebolin 15 min before scopolamine. DMTS sessions began 15 min after scopolamine administration. Data obtained from standard DMTS sessions (only vehicle pre-test administration; Std DMTS) were included for comparison. Each value indicates the mean ± SEM. *P < 0.05, significantly different from respective standard DMTS mean.

Aged subjects

During sessions initiated 30 min (Figure 4A) and 24 h (Figure 4B) after drug treatment, there was a significant improvement ($F_{5,234}$ = 2.6393, P = 0.0241) in task accuracies relative to vehicle. Similarly, there was a significant effect for the drug treatment × delay interval × time interaction ($F_{15,234}$ = 1.7371, P = 0.0451). *Post hoc* analysis revealed a significant (P = 0.009) increase in task accuracies for the dose of

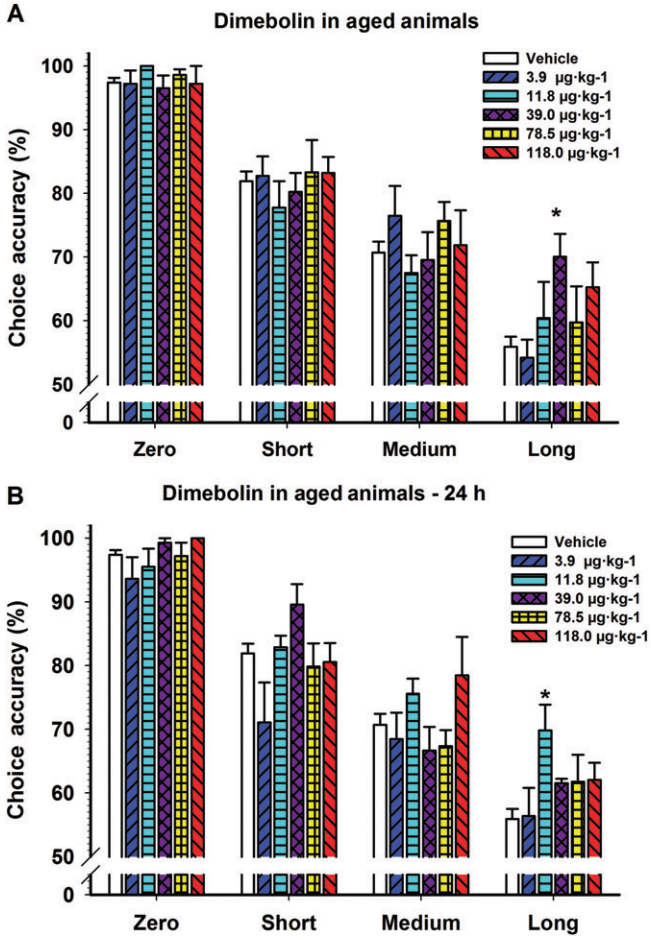

Figure 4

The effect of dimebolin on DMTS choice accuracies by six aged rhesus monkeys. Data were obtained during sessions run 30 min (A) and 24 h (B) after dimebolin or vehicle administration. The 24 h sessions were initiated after no additional administration of the test compounds. Each value indicates the mean ± SEM. *P < 0.05, significantly different from respective (0 µg kg^{-1}) vehicle mean.

39 µg kg^{-1} at the session initiated 30 min after dimebolin administration. A significant effect (P = 0.01) was also seen on sessions run the following day (24 h session) with no other pre-test administration for the dose of 11.8 µg kg^{-1} relative to baseline task accuracies.

Latencies and trials completed

Median sample and choice latencies were not significantly altered by any drug treatment or drug combination relative to vehicle in the study (Table 2). Also, except as noted for the scopolamine series, there were no significant effects of vehicle or dimebolin treatments on the numbers of trials completed per session.

Discussion

The results of this study can be summarized as follows: (i) dimebolin (depending on the dose administered) was associ-

Table 2

Monkey latencies and trials completed by dose

Dose (μg·kg^{-1})	Sample latency Mean (s)	SEM	Choice latency Mean (s)	SEM	% Trials completed Mean	SEM
Dimebolin (young adult)						
0	5.27	1.65	3.14	0.33	98.22	0.69
3.9	5.57	3.82	3.24	0.76	96.88	1.98
11.8	4.30	2.52	3.48	0.98	97.22	2.78
39	3.00	1.23	3.56	0.94	98.61	1.39
78.5	4.10	2.34	2.95	0.45	96.70	2.29
118	4.60	2.95	3.06	0.54	99.48	0.36
Dimebolin + 20 μg kg^{-1} Scopolamine (young adult)						
0	5.87	3.61	3.07	0.6	81.60	6.05
3.9	5.89	3.53	3.52	0.52	78.13	4.80
11.8	4.36	2.3	3.21	0.5	74.13	6.20
39	4.94	2.94	2.91	0.55	71.70	7.71
78.5	3.93	1.28	4	0.77	77.08	6.26
118	2.76	0.64	3.07	0.34	87.85	5.33
Dimebolin (aged)						
0	2.94	0.23	3.25	0.36	99.35	0.62
3.9	2.57	0.44	3.54	0.93	97.94	1.48
11.8	2.77	0.57	3.18	0.89	100.00	0.00
39	2.29	0.32	2.76	0.56	97.79	1.01
78.5	2.67	0.45	2.91	0.62	100.00	0.00
118	2.81	0.44	3.18	0.73	96.88	1.78

DMTS task latencies obtained from three experimental series: (i) dimebolin administration in young adult monkeys; (ii) scopolamine + dimebolin administration in young adult monkeys and (iii) dimebolin administration in aged monkeys. Also, the overall percentage of trials completed following the various dimebolin doses is shown.

ated with modest improvements in the performance of a DMTS task in young adult and aged monkeys; (ii) the positive effects of dimebolin on DMTS performance were observed for up to 24 h after administration; (iii) dimebolin showed a trend towards attenuation of scopolamine-related impairments at long delay and a reversal of scopolamine-related deficits in the number of trials completed in DMTS in young adult monkeys.

The data described here complement a number of recent examples of dimebolin's potential to improve cognition in animal models related to AD and Huntington's disease (Lermontova *et al.*, 2000; Bachurin, *et al.*, 2001; Wu *et al.*, 2008; Grigor'ev *et al.*, 2009; Schaffhauser *et al.*, 2009; Giorgetti *et al.*, 2010) and also suggest that dimebolin may exert lasting effects after a single administration. Pharmacokinetic studies in animals would suggest that this protracted cognitive effect is unlikely to be due to the sustained presence of the drug. While there are no published data available in primates, the half-life of dimebolin is ~1.1 h in the rabbit and ~2 h in the rat (Tishchenkova *et al.*, 1991). Thus, the mechanism of this pharmacokinetic–pharmacodynamic discordance is unknown, but we have observed the phenomenon with drugs from other classes (e.g. the nicotinic acetylcholine receptor agonist nicotine), which have relatively short half lives (Jackson and Buccafusco, 1991; Buccafusco *et al.*, 2009)

In the scopolamine-reversal studies, scopolamine (when administered alone) had no significant effect on zero delays; however, it impaired performance at the short, medium and long delays. This result replicates previous DMTS studies in our laboratory (Buccafusco *et al.*, 2008) and suggests that scopolamine does not impair stimulus discrimination, but that it may impair attention and/or encoding of the stimulus. The latter effect would in turn influence performance at the longer delays, given that the processes of retention and recall rely on attention to (and encoding of) the stimulus (see review, Paule *et al.*, 1998). It should be noted that there was a modest decrease in the number of trials completed in all animals administered scopolamine in this study. This effect could be interpreted as some type of non-mnemonic effect (e.g. impairment of motivation). However, the lack of effect on performance at zero delays (see Dunnett, 1985) and a lack of effect on sample and choice latencies argues against motivational deficits as a source of the scopolamine-related impairments in DMTS performance.

Dimebolin, despite having anti-cholinesterase activity, did not fully reverse scopolamine-related deficits in DMTS

task accuracy, and only the highest dose evaluated (118 μg kg^{-1}) was able to significantly attenuate the scopolamine-induced decrease in the number of trials completed. These results were somewhat surprising given that other cholinesterase inhibitors have been shown to reverse scopolamine-related cognitive deficits in a variety of animal models (Shannon and Peters, 1990; Bejar *et al.*, 1999; van der Staay and Bouger, 2005; de Bruin and Pouzet, 2006; Lindner *et al.*, 2006; Buccafusco, 2008). Dimebolin's lack of effect in our DMTS (scopolamine impairment) studies may be due to its relatively weak potency as an AChE inhibitor. Studies using recombinant human enzyme preparations have shown dimebolin to be roughly 3000-fold less potent at inhibiting cholinesterase activity than donepezil (83 ± 13 μM compared with 0.028 ± 0.005 μM, respectively) (Giorgetti *et al.*, 2010).

In light of the studies described above, it is unlikely that the pro-cognitive effects of dimebolin are due to AChE inhibition alone; it has been suggested that dimebolin exerts its pro-cognitive effects via other receptors/targets. For example, dimebolin was found to bind with high affinity to recombinant 5-HT_6 receptors (K_i = 26.0 nM, human; 119.0 nM, rat) (Schaffhauser *et al.*, 2009). 5-HT_6 receptors are almost exclusively localized in the brain, with high expression in limbic areas and other regions that are known to be critical for normal cognition (Woolley *et al.*, 2004). In a rat social recognition paradigm, dimebolin was found to improve task performance similar to other 5-HT_6 antagonists, although this effect was attained at relatively high doses (10 and 30 mg kg^{-1} i.p.) (Schaffhauser *et al.*, 2009). It is also possible that dimebolin's efficacy in cognition assays and in AD derives from its combined interaction at several drug targets including subtypes of α-adrenoceptors, ionotropic glutamate NMDA receptors and other 5-HT receptor subtypes as well as inhibition of AChE. Interestingly, in diseases such as AD, it has been argued that a multiple drug target approach to therapy may be necessary to address the varied pathological aspects of the disease and its diverse symptoms (reviewed in Youdim and Buccafusco, 2005a,b).

The cognitive enhancement observed after a long delay in both young and aged monkeys in our studies supports the hypothesis that dimebolin is able to improve short-term/working memory. Dimebolin was initially reported to maintain improvement in AD symptoms, including cognitive impairment for at least 6 months in a phase II clinical trial (Doody *et al.*, 2008). The potential disease-modifying action of the drug could partly explain this result, and the protracted mnemonic actions produced by dimebolin in our study would appear to support such an argument. Unfortunately, however, the positive results of our experiments in monkeys and the encouraging early clinical trial results were not confirmed in phase III clinical trials. Notably, in a recent large-scale phase III clinical trial composed of AD patients measuring multiple endpoint of cognition using the ADAS-cog (the Alzheimer's Disease Assessment Scale-cognitive subscale) and the CIBIC+ (Clinician's Interview-Based Impression of Change-Plus Caregiver Input), patients showed no improvement after dimebolin treatment. Dimebolin's lack of effect seen in the phase III trial results is in stark contrast to the earlier finding in the phase II trial. There are notable differences between the two studies that should be discussed. For example, there was a significant placebo effect observed in the later trial that was not present in the first trial. Specifically, in the phase II trial, the placebo-controlled patients showed a cognitive decline in all cognitive measures tested, while in the later phase III trial, none of the placebo controlled patients showed a decline in cognitive function, and, in fact, they showed an improvement in one of the endpoints tested. In addition, there was a discrepancy in age between the two studies. The phase II trial enrolled test subjects in their mid-60s, whereas the average age of the phase III trial was 74.4 years of age. Another difference in the two studies was the heterogeneity of the test populations. The phase II trial encompassed 183 Russian AD patients, while the failed phase III trial test population was made up of 598 patients at 63 sites in North America, Europe and South America. This could explain some of the differences seen in results of the cognitive testing, as there can be language-specific or culturally specific differences that confound the cognitive test results. Unfortunately, the data from the failed phase III dimebolin trial are yet to be published. Thus, more rigorous comparisons are impossible at this time.

Notwithstanding the differences in the study designs in the clinical trials described above, the age of the subjects, placebo effects, etc., the contrast of the positive results with dimebolin in our non-human primate studies and the negative results with dimebolin in phase III clinical trials appears to question the predictive validity of monkey DMTS testing for the evaluation of pro-cognitive agents (specifically for AD). Similar discrepancies between preclinical results in AD-related animal models and clinical evaluations of novel AD-related compounds have been observed on a number of previous occasions and raised as a source of concern (Carlsson, 2008; Simon, 2008; Lowenstein and Castro, 2009). However, it is important to note that it is relatively uncommon for the full results of failed clinical trials to be published in peer-reviewed journals, thus allowing human and animal data to be rigorously compared. The selective reporting of clinical trials (particularly in abstract form or as summary data in reviews or lay publications) may provide a misleading impression of the true efficacy of potential new drugs (see review, McArthur *et al.*, 2010). In the case of DMTS testing in non-human primates, compounds ranging over many diverse structural classes, such as the nicotinic acetylcholine receptor agonists (nicotine, GTS-21, and SIB- 1553A), the muscarinic acetylcholine receptor agonists (WAY-132983), the cholinesterase inhibitors (physostigmine, velnacrine, and donepezil), the α_2-adrenoceptor agonists (clonidine and methylphenidate), when tested with the DMTS method used in this study generally show similar effects as when tested in humans (Buccafusco, 2008).

It is also important to note that there are important similarities between the results described here and those of a previous drug study where modest, pro-cognitive effects were observed in our monkeys, but a lack of pro-cognitive effects were observed in subsequent AD clinical trials. Some years ago, we observed (Terry *et al.*, 2002) that the M1-preferring muscarinic agonist talsaclidine improved DMTS performance in monkeys but only at a single dose (i.e. 0.6 mg·kg^{-1}). In addition, similar to the current dimebolin study, positive effects on DMTS performance occurred at time points after administration that would not necessarily correlate with maximum plasma levels. Collectively, the results of these two

studies indicate that even when positive effects on cognition are observed in non-human primate studies, poor dose–effect relationships and/or pharmacokinetic correlations may confound the ability to predict clinical efficacy.

In summary, the results of the current study indicate that dimebolin improves working memory/short-term memory in both young adult and aged monkeys, and that the compound is capable of enhancing cognition for up to 24 h after a single administration. Collectively, these data indicate that dimebolin may have the potential to improve cognition in age-related cognitive disorders (e.g. AD) as well as conditions not necessarily associated with advanced age (e.g. Huntington's disease).

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Conflicts of interest

None.

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