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ORIGINAL ARTICLE

Spatial memory deficits in mice induced by chemotherapeutic agents are prevented by acetylcholinesterase inhibitors

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Abstract

Purpose These studies determined whether the acetylcholinesterase inhibitors, donepezil and galantamine, both of which are approved for the treatment of cognitive deficits in Alzheimer's disease, can prevent or reverse spatial memory deficits in mice induced by cyclophosphamide and doxorubicin, cytotoxic agents commonly used to treat breast cancer.

Methods Female BALB/C mice were trained in the Morris water maze to identify the location of a submerged platform, and, following baseline assessment of spatial memory, received injections of cyclophosphamide and doxorubicin once per week for 4 weeks to impair spatial memory. Saline or acetylcholinesterase inhibitors were administered daily either concurrent with the chemotherapy injections (prevention) or beginning 1 week following the final chemotherapy injections (reversal), and spatial memory was assessed weekly.

Results Spatial memory declined during and following weekly injections of cyclophosphamide and doxorubicin, and was unaltered when the acetylcholinesterase inhibitors were administered following the manifestation of chemotherapy-induced deficits. In contrast, spatial memory of mice receiving the acetylcholinesterase inhibitors concurrent with chemotherapy did not differ from that at baseline.

Conclusions Results indicate that chemotherapy-induced spatial memory deficits in mice can be prevented, but not reversed by the use of acetylcholinesterase inhibitors concomitant with chemotherapy, suggesting that these agents should be investigated further for the prevention of chemobrain.

Keywords Chemobrain · Cyclophosphamide · Donepezil · Doxorubicin · Galantamine

Introduction

Chemotherapy-related cognitive deficits (CRCDs) are reported by cancer patients receiving chemotherapeutic agents, with a frequency ranging from 15 to 75% [1–3]. The majority of patients who report CRCDs are women [4, 5], with most research conducted in breast cancer patients, many of whom experience declines in at least one neuropsychological test during standard-dose chemotherapy [2, 6]. For some, cognition improves after treatment, but, for more than 50% of those who experience a cognitive deficit, impairment in one or more cognitive domains persists indefinitely [2, 6]. These deficits of attention, learning, memory, and information processing speed [8] are sufficient to impair day-to-day functioning and adversely affect quality of life [7, 8].

The acetylcholinesterase (AChE) inhibitors donepezil and galantamine improve information acquisition, memory, and attention in patients with Alzheimer's disease [9], and improve information processing and memory in schizophrenic patients [10]. These agents prevent the hydrolysis of the neurotransmitter acetylcholine (ACh), whose role in cognitive processes is well established [11]. Thus, AChE inhibitors may be effective for the treatment of CRCDs. Indeed, a recent clinical study reported that the administration of donepezil for 24 weeks to breast cancer patients who received chemotherapy in the prior 1–5 years significantly alleviated verbal memory impairments; no alterations in other cognitive variables were observed [12].

Preclinical studies have demonstrated that donepezil can attenuate learning and memory deficits and impaired executive function exhibited by female BALB/C mice exposed to the combination methotrexate and 5-fluorouracil when

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administered both during and following chemotherapy [13]. However, whether the beneficial effects of donepezil extend to deficits induced by the administration of cyclophosphamide and doxorubicin, a combination that produces cognitive deficits in rats [14, 15] and mice [16, 17] and is commonly used for the treatment of breast cancer, is unknown. It is also not known whether galantamine, an AChE inhibitor and positive allosteric modulator of α7-containing neuronal nicotinic acetylcholine receptors (nAChRs) [18] that improves cognitive performance in several animal models [19], has beneficial effects similar to donepezil. Thus, the objective of these studies was to determine whether donepezil prevents or reverses spatial memory deficits that ensue as a consequence of cyclophosphamide and doxorubicin administration, and whether similar effects could be achieved with galantamine. Because multiple preclinical studies have demonstrated that cyclophosphamide and doxorubicin impair hippocampal neurogenesis and disrupt hippocampal-mediated cognitive function [14, 20–22], and methotrexate and 5-fluorouracil produce similar effects [23, 24], the present study assessed hippocampal-dependent spatial memory in the Morris Water Maze (MWM), a classic behavioral test sensitive to hippocampal damage [25].

Materials and methods

Animals

Adult female BALB/C mice ($N = 120$; Envigo, Indianapolis, USA), 8 weeks of age upon arrival, were housed 2–4 per cage in a temperature and humidity-controlled vivarium on a 12–12 h light–dark cycle (6 am to 6 pm) with food and water available ad libitum. For enrichment, all cages contained a yellow or orange translucent plastic dome with which mice interacted, explored and in which they often rested. Animals were handled daily and acclimated for 1 week prior to study. All behavioral assessments took place between 9 am and 3 pm.

Behavioral training and spatial memory assessment

Morris water maze (MWM) training and assessment of spatial memory were conducted in a circular pool (130 cm diameter, 30 cm high) filled with opaque water maintained at 24 °C. A clear Plexiglas tiered platform (Bottom tier: 15 cm diameter; Top tier: 9.5 cm diameter) was present in the water on all non-probe trials. The pool was enclosed with a white polyvinyl curtain (632 cm × 182 cm) to prevent orientation on the experimenter or other extraneous distal visual cues. Distinct visual stimuli were placed on the interior lining of the curtain to provide static visual cues for spatial orientation. A video camera, mounted above the pool, was used to record all behaviors.

One week following arrival, on day 8, mice received orientation training consisting of five trials/day for a period of 2 days, as described [16]. Spatial memory training began on day 10 and consisted of five trials/day on days 10–14. During each trial, the platform was placed a few cm below the surface of the water in a consistent location for each animal. Mice were placed in the water, facing the wall, in a randomized location different from the location of the platform. Randomization was conducted without replacement, so that each mouse began one trial in each of five possible non-platform zones each day. Each trial continued until the mouse mounted the platform with all four paws, or until 60 s elapsed. If a mouse failed to locate the platform within 60 s, the animal was gently guided to the platform. All mice were left on the platform for a minimum of 15 s before being removed from the water.

On day 15, trials 1 and 2 occurred as described, with trial 3 serving as a probe trial to assess spatial memory. During the probe trial, the platform was removed and mice were allowed to swim for a total of 60 s before being removed from the apparatus. Following the probe trial, two additional platform trials were performed (trials 4 and 5) to prevent possible extinction of spatial memory. Performance was measured in the MWM every 6 days throughout the chemotherapeutic regimen, and weekly for 5 weeks thereafter (Fig. 1). Each assessment day included four platform trials (trials 1, 2, 4, and 5) with the probe trial (trial 3) used to assess spatial memory. Repeated probe trials across 9 weeks of behavioral assessment do not impair memory of the platform location in control BALB/C mice [16], likely due to the presence of two platform trials prior to and following each probe.

Noldus Ethovision tracking software was used for the analysis of behavior in the pool and in each of six equal-sized "virtual" zones that were created using this software. For each trial, the total distance moved and the number of zone entries were determined, and the percent distance moved in and percent entries into the correct zone were calculated. In addition, the mean distance from the pool edge was determined to assess the tendency to maintain contact with, or remain near, the physical barrier of the open field, an indicator of increased anxiety and stress [26, 27]. A significant reduction in distance to the pool edge, relative to baseline performance, was considered an indication of increased anxiety.

Pharmacological agents and administration

Donepezil HCl (Tocris Bioscience, Bristol, UK) and galantamine hydrobromide (Tocris Bioscience) were dissolved [1.5 mg freebase/ml] in 0.9% phosphate-buffered saline

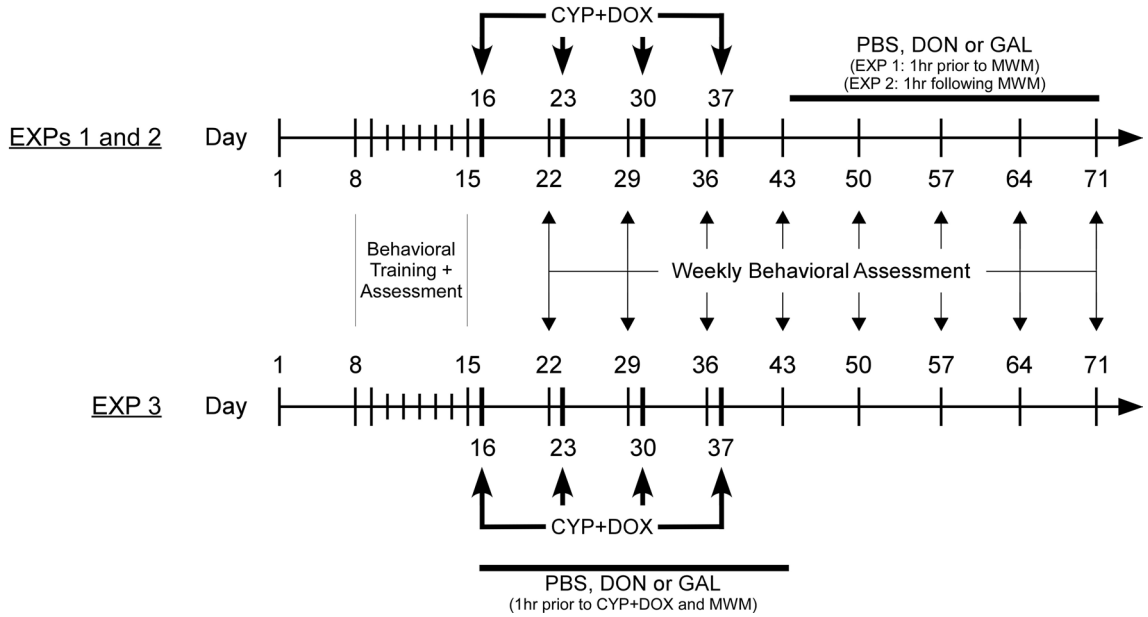

Fig. 1 Experimental timeline. Mice were trained in the Morris water maze from days 8 to 15, and probe trials were performed weekly for 9 weeks beginning on day 15. Activity and spatial memory were assessed by measures of total swim distance, total zone entries, % of the total distance moved in the correct zone, and % entries into the correct zone. Cyclophosphamide (CYP; 25 mg/kg) and doxorubicin (DOX; 2.5 mg/kg) were administered once per week for 4 weeks as indicated, and PBS, donepezil (DON), or galantamine (GAL) was injected daily as shown either 1 h prior to behavioral assessments for experiments 1 and 3 or 1 h following behavioral assessments for experiment 2

(PBS), resulting in a pH of 7.0–7.4, and stored at 4 °C for no longer than 1 week. Cyclophosphamide (Tocris Bioscience) was dissolved [5 mg/ml] in 0.9% PBS, resulting in a pH of 7.4, and stored at − 20 °C for no longer than 1 month. Doxorubicin [2 mg/ml] was purchased as a solution (pH 2.5–4.5) from Teva Parenteral Medicines, Inc (Irvine, CA) and stored at 4 °C.

On day 16, mice began receiving tail vein injections of 2.5 mg/kg doxorubicin followed by 25 mg/kg cyclophosphamide (CYP+DOX) once per week for 4 weeks (days 16, 23, 30 and 37). Because the goal of the present study was to determine whether AChE inhibitors could prevent or reverse the prolonged impairment of spatial memory in female BALB/C mice induced by this dose combination of CYP+DOX, which we have demonstrated [16], and to use the least number of experimental animals as per the ARRIVE guidelines [28], all mice were exposed to CYP+DOX and were either treated with donepezil or galantamine or administered saline to serve as controls.

Donepezil (3 mg/kg, s.c.), galantamine (3 mg/kg, s.c.), or equivalent volumes PBS (s.c.) were administered daily either following cessation of or concurrently with the chemotherapeutic regimen. For experiment 1, the AChE inhibitors or PBS was administered from days 44–71 at 1 h prior to behavioral testing, whereas, for experiment 2, drugs were administered 1 h following behavioral testing. For experiment 3, PBS, donepezil, or galantamine was administered daily during the 4 weeks of chemotherapy, from days 16–43 at 1 h prior to chemotherapy injections or behavioral assessments. The timelines for injections and behavioral measures for the three experiments are shown in Fig. 1. Neither the chemotherapeutic agents nor the AChE inhibitors had a sustained effect on the weight of the mice. One week after the administration of CYP+DOX, weight decreased by 2% in some animals, but all mice exhibited a weight gain by day 50, irrespective of treatment group. The average weight of the mice on day 15 ranged from 18.8 to 20.1 g and final weights on day 71 ranged from 19.3 to 21.0 g, a 3–9% increase with no significant differences among groups.

Statistical analysis

Analyses were conducted using Prism (GraphPad Software; San Diego, CA). To verify an established spatial memory, single-sample *t* tests were performed comparing the % of distance moved in the correct zone and % of correct zone entries to chance performance (1/6 zones = 16.67% for % distance moved in and % entries into the correct zone) on the baseline (day 15) probe trial. For experiments 1 and 2, data from probe trials on days 15–36 were analyzed using a repeated-measure ANOVA with subsequent multiple comparisons (Fishers LSD) to assess changes from baseline performance (day 15) due to cyclophosphamide and doxorubicin administration. Probe trial data from days 43–71 were

analyzed using a multiple 3 × 5 mixed factor ANOVA with subsequent multiple comparisons (Fishers LSD) to assess changes from post-chemotherapy performance (day 43) and to determine differences between treatment groups. For experiment 3, data from probe trials on days 15–71 were assessed using a multiple 3 × 9 ANOVA with subsequent multiple comparisons (Fishers LSD) to assess changes from baseline performance (day 15) and to determine differences between treatment groups. A value of 0.05 was considered significant for each analysis and $\eta^2_{\text{partial}}\left(\frac{SS_{\text{BG}}}{SS_{\text{BG}}+SS_{\text{err}}}\right)$ used to demonstrate the size of effects.

Results

Effects of donepezil and galantamine on chemotherapy-induced spatial memory deficits

Because a recent clinical study reported that donepezil reversed chemotherapy-induced memory deficits [12], the first experiment determined whether donepezil or galantamine could reverse chemotherapy-induced deficits in spatial memory. Of the 40 mice that began this experiment, 17 engaged in floating behavior during training and testing and failed to meet baseline criteria for spatial memory (> 20% of distance moved in the correct zone during the baseline probe trial) and three developed necrosis at the injection site and could not complete the study; these mice were not included in the analyses.

Baseline performance on day 15 (Fig. 2) indicated that mice swam a mean total distance of 693 ± 36 cm with 41 ± 2.6% in the correct zone, significantly greater than

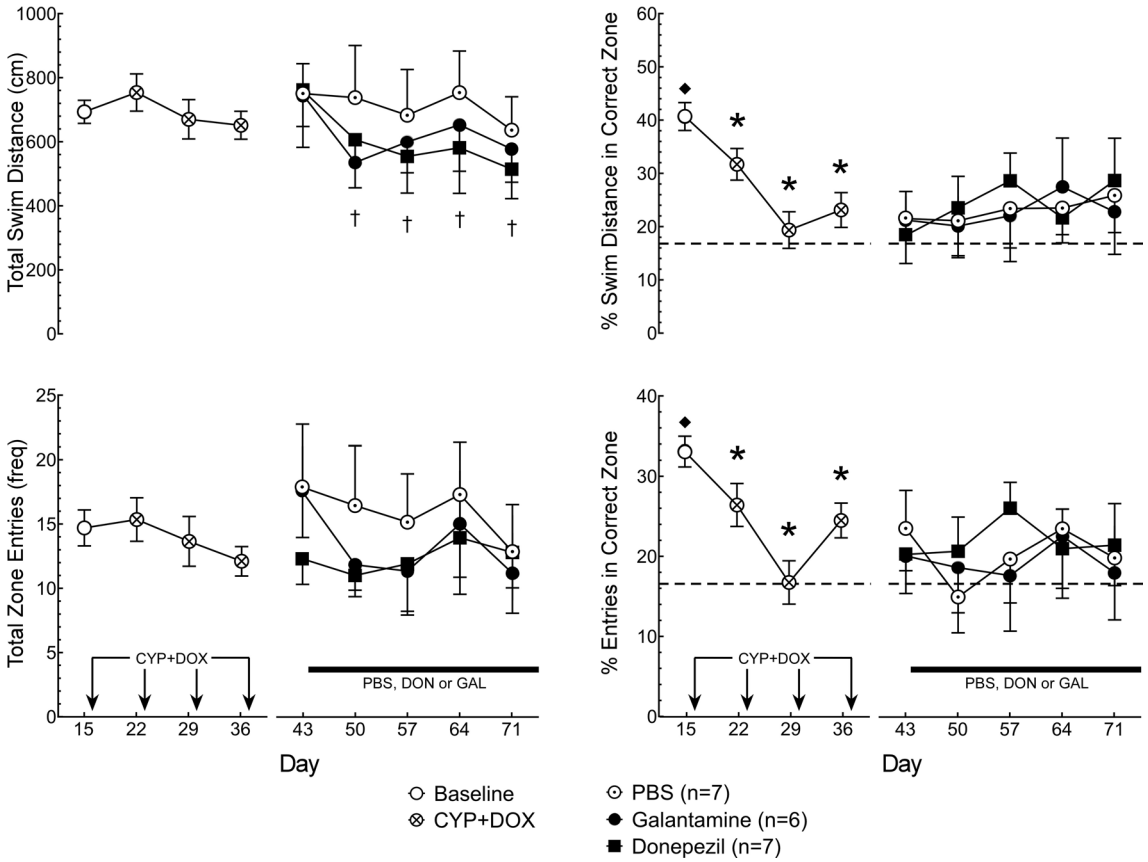

Fig. 2 The effect of chemotherapy on spatial memory is not reversed by the administration of AChEIs prior to behavioral testing. Following training and baseline determinations, mice ($n = 20$) received weekly injections of cyclophosphamide (CYP; 25 mg/kg) and doxorubicin (DOX; 2.5 mg/kg) followed by daily injections of PBS, donepezil, or galantamine as shown in Fig. 1 for experiment 1, and activity and spatial memory assessed as described. The symbols denote significance at $p < 0.05$: ◆, significantly different from chance; *, significantly different from day 15; and †, significantly different from day 43

chance performance (16.67%), indicating spatial memory of the platform location [$t(19) = 9.18$, Cohen's $d = 2.05$]. The mean total number of zone entries at baseline was 14.7 ± 1.4, with 33 ± 1.9% into the correct zone, significantly greater than chance performance (16.67%), indicating spatial memory of the platform location [$t(19) = 8.62$, $d = 1.93$]. Although the absolute values for mean total swim distance and the mean total number of zone entries did not change significantly throughout chemotherapy administration, both the % distance and % entries into the correct zone decreased, the former by 9–21% relative to baseline on days 22–36 [$F(3,57) = 15.63$, $\eta^2_{\text{partial}} = 0.45$], and the latter by 7–16% on days 22–36 [$F(3,57) = 14.19$, $\eta^2_{\text{partial}} = 0.43$]. These effects did not appear to be mediated increased anxiety in the MWM as the distance to the pool edge did not change significantly from baseline (18.57 ± 1.24 cm), ranging from 18.33 ± 1.40 to 19.73 ± 1.33 cm, across days 22–36. Although the present study did not include a control group receiving saline instead of CYP+DOX, we have demonstrated that unexposed control mice do not exhibit a decrement in MWM performance and spatial memory across weeks when using this paradigm [16]. Thus, the reduction in % distance and % entries into the correct zone following CYP+DOX administration is indicative of impaired spatial memory.

On day 43, mice were assigned to treatment groups in a pseudorandom fashion, using the % distance moved in the correct zone to counterbalance spatial memory across groups. The mean total swim distance on day 43 ranged from 748 to 761 cm and did not differ significantly among groups. Furthermore, the mean % distance moved in the correct zone ranged from 18 to 22% and was not significantly different from chance performance (16.67%), indicating impaired spatial memory. Similarly, the average total zone entries ranged from 12 to 18 and did not differ significantly among groups, and the mean % entries into the correct zone ranged from 20 to 23% and did not differ from chance performance (16.67%), indicating impaired spatial memory.

PBS, donepezil, or galantamine was injected daily (days 44–71) approximately 1 h prior to beginning behavioral testing. The mean distance to the pool edge did not decrease significantly on days 50–71 relative to day 43 (18.85 ± 1.72 cm), with group means ranging from 18.39 ± 2.46 to 21.78 ± 3.40 cm, indicating that drug treatment did not alter anxiety during the probe trial. PBS did not affect either the total swim distance, percent distance in the correct zone, the number of total zone entries or the percent of zone entries into the correct zone on days 50–71 relative to day 43. In contrast, the AChE inhibitors decreased total swim distance with a significant effect on days 57–71 for donepezil-injected mice, and a significant reduction on day 50 for galantamine-injected mice [$F(4,68) = 4.09$, $\eta^2_{\text{partial}} = 0.19$]. However, there were no significant differences in the percent distance in the correct zone ($\eta^2_{\text{partial}} = 0.06$), the number of total zone entries ($\eta^2_{\text{partial}} = 0.18$), or the percent of zone entries into the correct zone ($\eta^2_{\text{partial}} = 0.05$) on days 50–71 relative to day 43. Thus, the daily administration of the AChE inhibitors for 4 weeks did not reverse chemotherapy-induced impairments in spatial memory. However, mice who received the AChE inhibitors exhibited reduced swim distance during behavioral assessment, suggesting that the acute effects of an AChE inhibitor administered immediately prior to behavioral testing might have interfered with performance and the assessment of spatial memory.

To investigate this possibility, mice were trained, exposed to CYP+DOX, assigned to treatment groups as in experiment 1, but injections of the AChE inhibitors were at 1 h following behavioral testing to minimize any possible effects of these drugs on behavior. Of the 40 mice that began training for experiment 2, 19 engaged in floating behavior during training and testing and did not meet a minimum criterion for spatial memory (> 20% of distance moved in the correct zone during the baseline probe trial) and three mice presented necrosis at the injection site and could not complete the study; these mice were not included in the analyses.

Baseline performance on day 15 (Fig. 3) indicated that mice swam a total distance of 784 ± 56 cm with 53 ± 3.4% of swim distance in the correct zone, significantly greater than chance performance (16.67%), indicating spatial memory of the platform location [$t(17) = 10.68$, $d = 2.52$]. The total number of zone entries was 16.4 ± 2.0 with 34 ± 1.8% into the correct zone, significantly greater than chance performance (16.67%), indicating spatial memory of the platform location [$t(17) = 9.43$, $d = 2.22$]. As in experiment 1, the absolute values for total swim distance and the number of total zone entries did not change significantly throughout chemotherapy administration, but both the % distance and % entries into the correct zone decreased, the former by 11–21% compared to baseline [$F(3,51) = 6.73$, $\eta^2_{\text{partial}} = 0.28$], and the latter by 10–12% compared to baseline [$F(3,51) = 5.61$, $\eta^2_{\text{partial}} = 0.25$], indicating impairment of spatial memory. These effects did not appear to be mediated by anxiety in the MWM as the distance to the pool edge did not decrease significantly from baseline (18.00 ± 1.21 cm), with group means ranging from 18.74 ± 1.28 to 19.87 ± 1.26 cm, across days 22–36.

On day 43, the mean total swim distance ranged from 706 to 828 cm and the mean total zone entries ranged from 11 to 16; neither measure differed significantly among groups. The mean % distance moved in the correct zone ranged from 15 to 24% was not significantly different among groups, and did not differ from chance performance (16.67%), indicating impaired spatial memory. The mean % of entries into the correct zone on day 43 ranged from 19 to 31%, and although group means did not differ significantly, the mean % entries

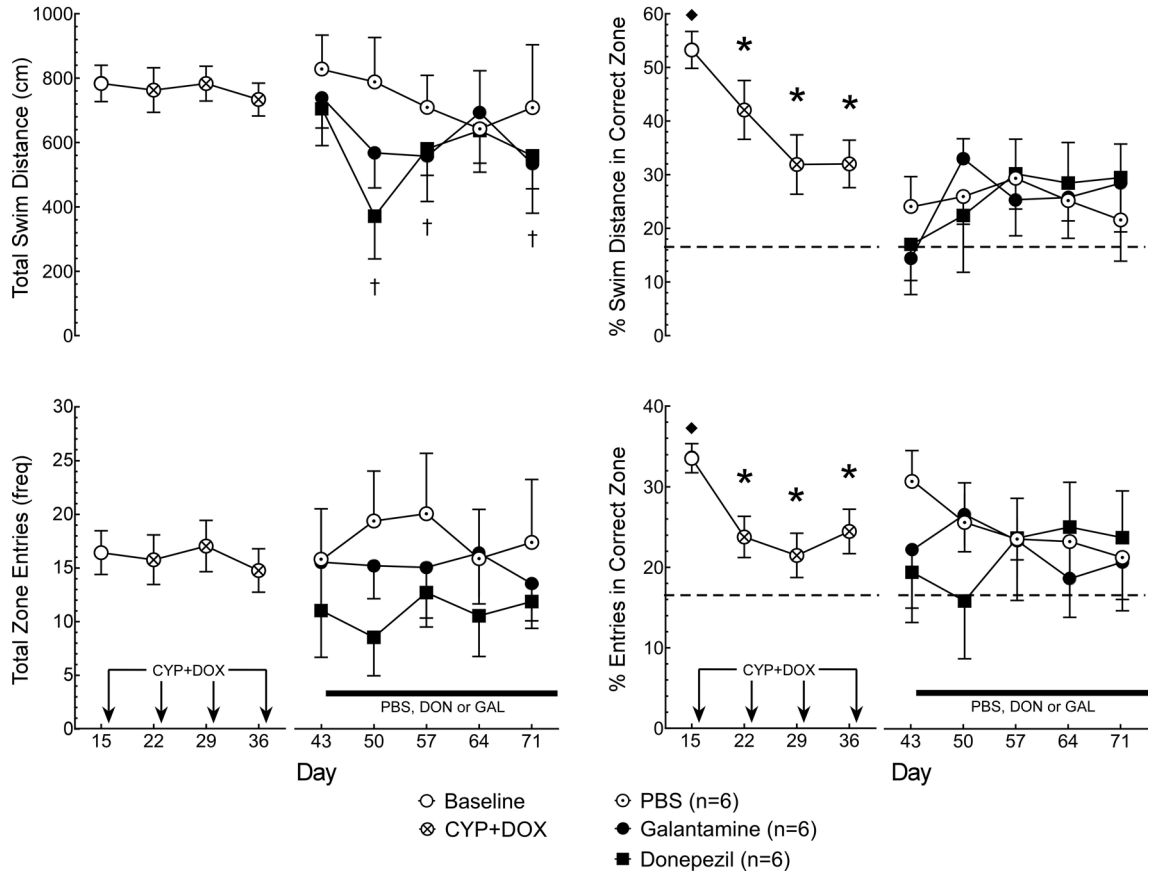

Fig. 3 The effect of chemotherapy on spatial memory is not reversed by the administration of AChEIs following behavioral testing. Following training and baseline determinations, mice ($n=20$) received weekly injections of cyclophosphamide (CYP; 25 mg/kg) and doxorubicin (DOX; 2.5 mg/kg) followed by daily injections of PBS, donepezil, or galantamine, as shown in Fig. 1 for experiment 2, and activity and spatial memory assessed as described. The symbols denote significance at $p<0.05$: ◆, significantly different from chance; *, significantly different from day 15; †, significantly different from day 43

into the correct zone for mice assigned to receive PBS were significantly greater than chance (16.67%) performance [$t(5)=3.87$, $d=2.26$], while the values for mice assigned to receive donepezil and galantamine did not differ from chance, suggesting a smaller impairment in PBS animals for this measure.

The mean distance to the pool edge ranged from 19.36 ± 2.89 to 23.17 ± 3.27 cm on days 50–71 and did not change significantly relative to day 43 (20.21 ± 1.81 cm) for any group, indicating that drug treatment did not alter anxiety during the probe trial. Similar to results from experiment 1, PBS injections had no effect on any subsequent measure when administered following behavioral testing on days 50–71, whereas the administration of donepezil led to a significant decrease in total swim distance on day 50, and galantamine led to significant reductions in total swim distance on days 50, 57, and 71 [$F(4,60)=4.49$, $\eta^2_{\text{partial}}=0.23$]. No significant alterations were determined for measures in the correct zone ($\eta^2_{\text{partial}}=0.07$ for the mean percent distance moved in the correct zone; $\eta^2_{\text{partial}}=0.09$ for the mean percent of entries to the correct zone). Thus, the absence of improved spatial memory during treatment with AChE inhibitors is not a consequence of the enzyme inhibitors interfering with performance during behavioral testing, indicating that these compounds cannot reverse chemotherapy-induced spatial memory deficits.

Effects of donepezil and galantamine on the induction of spatial memory deficits by chemotherapy

Although the AChE inhibitors could not reverse chemotherapy-induced spatial memory deficits, it was possible that these agents could prevent induction of the deficit. This idea is supported by evidence that donepezil administration to mice both during and following exposure to methotrexate and 5-fluorouracil prevented several chemotherapy-induced memory deficits [13]. Thus, experiment 3 determined

whether the AChE inhibitors were effective when administered concurrent with the chemotherapeutic agents.

Mice were trained in the MWM on days 8–14, baseline performance assessed on day 15, and animals were assigned to receive PBS, donepezil or galantamine in a pseudorandom fashion, using the % distance moved in the correct zone to counterbalance spatial memory across groups. Mice received PBS, donepezil or galantamine daily from days 16–43, and injections of CYP+DOX were administered weekly for 4 weeks on days 16, 23, 30 and 37 (Fig. 1). On these days, PBS, donepezil or galantamine was administered 1 h prior to CYP+DOX.

Of the 40 mice that began experiment 3, 16 mice engaged in floating behavior during training and testing and did not meet the minimum criteria for spatial memory (> 20% of distance moved in the correct zone during the baseline probe trial) and two mice presented necrosis at the injection site and could not complete the study; these mice were not included in the analyses.

Total swim distance ranged from 806 to 902 cm and did not differ significantly among groups on baseline day 15 (Fig. 4). Similarly, the mean % distance in the correct zone ranged from 48 to 50%, and did not differ among groups, verifying effective randomization. Furthermore, the overall mean % distance swam in the correct zone (49.4 ± 0.4%) differed significantly from chance (16.67%) performance [$t(21) = 15.39$, $d = 3.28$], indicating spatial memory of the platform location. Pseudorandom assignment based on % swim distance in the correct zone did not result in groups balanced for total number of zone entries. Mice assigned to the PBS group exhibited 36% fewer zone entries than mice assigned to the donepezil group [$F(16,152) = 3.97$, $\eta^2_{\text{partial}} = 0.29$], while the total zone entries of mice assigned to the galantamine group did not differ from other groups. Despite the group differences in total zone entries, the % entries into the correct zone did not differ among groups and the overall mean (33.2 ± 0.7%) was significantly greater than chance (16.67%) performance [$t(21) = 13.31$, $d = 2.88$],

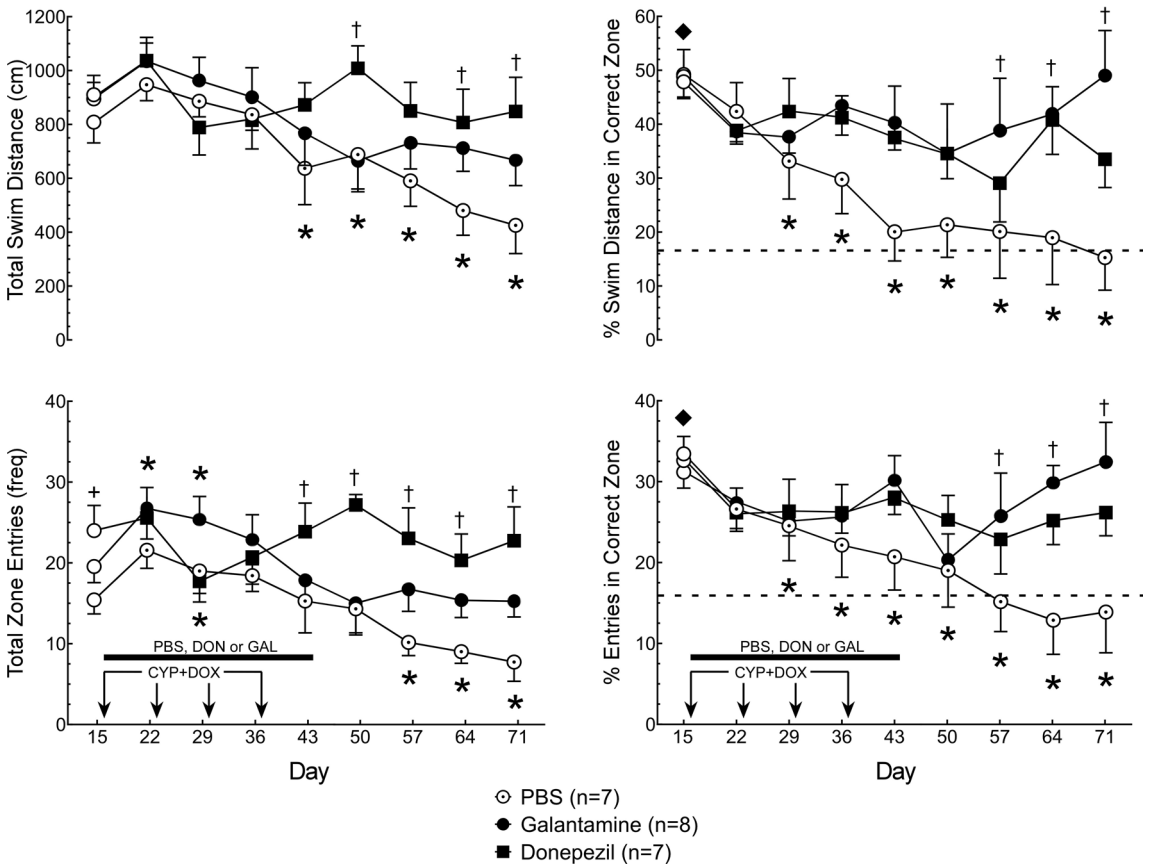

Fig. 4 The administration of AChEIs concurrent with chemotherapy attenuates induced deficits in activity and spatial memory. Following training and baseline determinations, mice ($n = 20$) received daily injections of PBS, donepezil, or galantamine, and weekly injections of cyclophosphamide (CYP; 25 mg/kg) and doxorubicin (DOX; 2.5 mg/kg) 1 h later, as shown in Fig. 1 for experiment 3; activity and spatial memory were assessed as described. The symbols denote significance at $p < 0.05$: ◆, significantly different from chance; *, significantly different from day 15; †, significantly different among groups

again indicating spatial memory of the platform location. The group means for distance to the pool edge at baseline ranged from 19.81 ± 2.17 to 23.66 ± 0.95 cm and did not differ significantly from each other, suggesting similar anxiety in the MWM between groups at baseline.

There was a significant interaction between drug administration and day of assessment for the mean distance to the pool edge [$F(16,152) = 2.12$, $\eta^2_{\text{partial}} = 0.18$]. For mice who received PBS or galantamine, the distance to the pool edge did not differ significantly from baseline across days of assessment. However, mice that received donepezil swam closer to the pool edge on day 71 (21.40 ± 1.24 cm) than at baseline (23.66 ± 0.95 cm), suggesting an increase in anxiety on the final day of assessment for this group.

The total swim distance of mice who received PBS and the chemotherapeutic agents decreased significantly by 15–47% from days 43–71, indicating reduced activity following 4 weeks of chemotherapy. The total swim distance of donepezil-injected mice was unchanged and was significantly greater than that of PBS-injected mice on days 50, 64, and 71, while the total swim distance of galantamine-injected mice decreased by 15–26% from days 50–71 and did not differ from that of PBS-injected mice [$F(16,152) = 3.40$, $\eta^2_{\text{partial}} = 0.26$]. The % distance moved in the correct zone by mice who received PBS and were exposed to CYP+DOX decreased by 34–69% during days 29–71, indicating spatial memory impairment. Both donepezil- and galantamine-injected mice exhibited a smaller reduction (14–21%) in % distance moved in the correct zone, with the former performing significantly better than PBS-injected mice on day 64, and the latter performing significantly better than PBS-injected mice on days 57–71 [$F(16,152) = 2.29$, $\eta^2_{\text{partial}} = 0.19$]. Furthermore, the % distance moved in the correct zone by mice receiving the AChE inhibitors did not differ significantly from baseline day 15.

The number of total zone entries by PBS-injected mice declined on days 57–71, paralleling the decrease in total swim distance by this group. In contrast, donepezil-injected mice exhibited a 29% reduction in total zone entries on day 29, but significantly more zone entries than PBS-injected mice on days 43–71, while galantamine-injected mice exhibited a 27–28% increase in zone entries on days 22 and 29, but did not differ from PBS-injected mice at any assessment time point [$F(16,152) = 3.97$, $\eta^2_{\text{partial}} = 0.29$].

The % entries into the correct zone by PBS-injected mice exposed to CYP+DOX deceased significantly by 28–62% from days 29–71, indicating impaired performance. Donepezil- and galantamine-injected mice exhibited a smaller reduction (25–36%) in the % of entries into the correct zone, and donepezil-injected mice performed significantly better than PBS-injected mice on days 64 and 71, while galantamine-injected mice performed significantly better than PBS-injected mice on days 57–71 [$F(16,152) = 1.81$, $\eta^2_{\text{partial}} = 0.16$]. Furthermore, the % entries in the correct zone by mice receiving the AChE inhibitors did not differ significantly from baseline day 15. Thus, results indicate that when AChE inhibitors are administered concurrently with chemotherapy, spatial memory is preserved, obviating chemotherapy-induced deficits.

Discussion

Results indicate that when donepezil or galantamine is administered during chemotherapy with CYP+DOX, the induction of spatial memory deficits is attenuated. In contrast, neither AChE inhibitor could reverse an established deficit in spatial memory when administered either immediately prior to or following behavioral assessment. Thus, enhancing cholinergic function by administering AChE inhibitors during chemotherapy has a prophylactic effect on cognitive function, but cannot reverse a deficit induced by CYP+DOX administration.

Results extend the findings of Winocur et al. [13] and suggest that the occurrence of spatial memory deficits induced by several chemotherapeutic combinations can be attenuated by AChE inhibitors. The present study, and the study by Winocur et al., utilized healthy mice to examine the use of AChE inhibitors for the prevention or treatment of spatial memory deficits that manifest following the administration of cyclophosphamide and doxorubicin or methotrexate and 5-fluorouracil. One limitation of this approach is that cancer itself may contribute to cognitive decline and chemotherapeutic agents may have more complex effects on cognitive function when administered to individuals with tumors [29, 30]. Thus, it is possible that the beneficial effects of AChE inhibitors observed in the present study and reported by Winocur et al. would not be observed in individuals suffering from cancer. Alternatively, the presence of tumors and/or the interaction of tumors with chemotherapy might produce unique adverse effects on cognitive function for which AChE inhibitors provide some therapeutic benefit.

To this latter point, Lawrence et al. [12] reported that the administration of donepezil to breast cancer patients who had received chemotherapy resulted in significant improvements in two out of five components of the Hopkins Verbal Learning Test (Revised), without improvement on several other tests of cognitive function, suggesting that donepezil may lead to improvements in some cognitive domains of cancer patients receiving chemotherapy, but not others. In this clinical trial, donepezil treatment was initiated 1–5 years following cessation of chemotherapy with the drug administered for 24 weeks prior to assessment. For the present study, donepezil did not reverse chemotherapy-induced spatial

memory deficits in otherwise healthy mice when administered for 4 weeks beginning 7 days following the final exposure to the chemotherapeutic agents. The present findings suggest that: (1) treatment with donepezil may not reverse chemotherapy-induced deficits in spatial memory; (2) there may be a window immediately following chemotherapy during which time the administration of AChE inhibitors cannot improve cognitive function; (3) that a longer duration of drug administration may be necessary to observe a reversal of the cognitive impairment; and/or (4) that chemotherapy alone may have adverse effects on cognitive function that differ from the cognitive-impairing effects of cancer and chemotherapy combined, and that the latter can benefit from treatment with AChE inhibitors. Further studies are warranted to investigate these possibilities.

It has been suggested that cognitive deficits observed in cancer patients are a consequence of, or exacerbated by, depression and increased anxiety precipitated by cancer diagnosis and treatment. In the present study, 43% of mice were removed from the analysis due to poor acquisition of task and/or floating behavior during training and testing, the latter an indicator of anxiety. Similar rates of performance difficulties have been reported for BALB/C mice in the MWM; however, findings indicate that a subset of BALB/C mice perform MWM spatial memory tasks very well [31, 32]. Because immobility, a gold standard indicator of depressive-like behavior in rodents [33] was used as a criterion to remove mice from the study, it is unlikely that depressive-like behavior mediated the impairment of spatial memory demonstrated in the present study. Furthermore, it is unlikely that increased anxiety mediated the observed impairment of spatial memory, since the mean distance to the pool edge among mice that remained in the study indicated little change in anxiety across groups and days of spatial memory testing. Therefore, the preventative effects reported here likely represents the effect of AChE inhibitors on cognitive function rather than an anti-depressant or anxiolytic effect. It should be noted that because this study contained few, if any, mice exhibiting depression-like behavior or increased anxiety during or following chemotherapy, the possibility that AChE inhibitors can prevent or reverse cognitive deficits mediated by depressive-like or anxious behavior remains to be explored.

Because cyclophosphamide, doxorubicin, methotrexate, and 5-fluorouracil all initiate cell death, it is possible that the AChE inhibitors prevent chemotherapy-induced apoptosis via activation of mitochondrial nAChRs and the resulting attenuation of cytochrome c release [34]. However, because these chemotherapeutic agents do not appear to cross the blood–brain barrier in sufficient quantities to induce cell death when administered peripherally at therapeutic doses [35, 36], damage to the central nervous system (CNS) is likely to be a consequence of a peripheral action.

Many chemotherapeutic agents, including cyclophosphamide, doxorubicin, and 5-fluorouracil, increase circulating pro-inflammatory cytokines [37, 38], which may cross the blood–brain barrier or may stimulate central cytokine expression through the vagus nerve [39, 40], leading to neuronal injury [37]. In the periphery, increasing cholinergic activity by inhibiting ACh hydrolysis may attenuate toxicity or damage to cells [41, 42] by suppressing cytokine synthesis via stimulation of α7 nAChRs expressed by macrophages and other cytokine-producing cells, thereby inhibiting inflammation [43]. In the CNS, increasing cholinergic activity can stimulate α7 nAChRs on microglia and attenuate pro-inflammatory responses [44]. Thus, the anti-inflammatory actions of ACh may underlie the ability of AChE inhibitors to attenuate cognitive impairment resulting from chemotherapy, and this possibility should be examined in future studies.

The beneficial effects of galantamine may be attributed to its antioxidant activity, as it has been suggested that CRCDs may result from increased oxidative stress. Konat et al. [15] demonstrated that the concurrent administration of an antioxidant with CYP+DOX can prevent impaired passive avoidance performance in rats. Galantamine, which exerts significant antioxidant activity [45], may prevent an induced memory deficit when administered concurrent with chemotherapy by acting as an antioxidant. A dose–response characterization of the effects of donepezil and galantamine on spatial memory is necessary to determine whether positive allosteric modulation of α7 nAChRs by galantamine provides benefits over donepezil and whether galantamine exerts significant antioxidant effects at prophylactic doses. In addition, whether the prophylactic effects of galantamine are exclusive to spatial memory deficits associated with CYP+DOX administration or apply to other cognitive domains (e.g., working memory, procedural memory), and other chemotherapeutic regimens, remains to be examined.

Conclusions

As cancer survival rates and patient longevity continue to increase, it is important to ensure quality of life of those who suffer from CRCDs. The development of approaches that protect against CRCD development in cancer patients would be beneficial. Further research is necessary to determine how AChE inhibitors interact with chemotherapeutic agents and whether they interfere with the effectiveness of chemotherapy. However, the results of the present studies are promising, and suggest that enhancing cholinergic function during chemotherapy may be a viable approach for the prevention CRCDs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The care and use of animals were approved in accordance with guidelines set by the University of South Florida Institutional Animal Care and Use Committee, the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines of the National Centre for the Replacement, Refinement and Reduction of Animals in Research.

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