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Donepezil reduces cognitive impairment associated with anti-cancer drugs in a mouse model

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ABSTRACT

Adjuvant cancer chemotherapy often causes cognitive impairment that can be long-lasting and adversely affect quality of life. The present study sought to determine if the cognitive enhancing drug, donepezil, can reduce cognitive impairment induced by a combination of methotrexate +5-fluorouracil, two drugs commonly used in cancer chemotherapy. Four groups of mice: (1) chemotherapy-only; (2) chemotherapy + donepezil; (3) saline-only; (4) saline + donepezil, were administered the following learning and memory tests: (1) standard spatial memory (SM); (2) non-spatial cued memory (CM); (3) non-matching-to-sample (NMTS) rule-learning; (4) delayed-NMTS (DNMTS). The chemotherapy-only group was impaired on the SM, NMTS, and DNMTS tasks. Chemotherapy-induced cognitive deficits were significantly reduced in the chemotherapy + donepezil group whose performance on some measures was very similar to that of the saline-only group. There was no evidence that donepezil improved the performance of saline-treated mice. The results confirm the adverse effects of chemotherapy on cognitive function and demonstrate that they can be ameliorated by donepezil, which is widely used to treat cognitive impairment in other clinical populations (e.g., Alzheimer's disease).

1. Introduction

Cancer patients, particularly breast cancer patients, who have undergone chemotherapy often complain of a disruption of mental function, commonly referred to as 'chemobrain' or 'chemofog'. Neuropsychological investigations have confirmed a range of cognitive deficits in such patients, with hippocampus-dependent memory and executive functions (e.g., working memory, directed and sustained attention, task planning) associated with the frontal lobes the most commonly affected (see reviews by Ahles and Saykin, 2007; Dietrich et al., 2008; Vardy et al., 2007; and meta-analyses by Anderson-Hanley et al., 2003; Falleti et al., 2005; Jansen et al., 2005). Consistent with these reports, neuroimaging studies of patients receiving chemotherapy have indicated structural damage in the brain that is most pronounced in the hippocampus and frontal lobes (Inagaki et al., 2007; Schneiderman, 2004). As well, the effects of anti-cancer drugs on cognitive performance have been studied in animal models and the consistent finding is impairment on tests of learning and memory that are sensitive to dysfunction in hippocampal and frontal lobe brain regions (e.g., Fardell et al., 2010; Foley et al., 2008; Lyons et al., 2011; Seigers et al., 2008; Winocur et al., 2006a; see review by Seigers and Fardell, 2011).

While considerable research has been directed at characterizing the nature and extent of cognitive impairment associated with chemotherapy, less attention has been paid to remediation strategies. In a preliminary study of 29 breast cancer survivors who had received anti-cancer drugs, Ferguson et al. (2007) observed enhanced quality of life and improved cognitive performance following a four-week cognitive training course. Pharmacotherapy has been assessed in a few studies with mixed results. For example, the stimulant, methylphenidate (Mar Fan et al., 2008), and the glycoprotein, erythropoietin (O'Shaughnessy, 2002), were found to be ineffective as cognitive enhancers in cancer patients. In rats, the SSRI anti-depressant, fluoxetine, reduced memory deficits caused

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by the anti-cancer drugs, 5-fluorouracil (ElBeltagy et al., 2010) and methotrexate (Lyons et al., 2011). Konat et al. (2008) reported that the anti-oxidant, N-acetyl cysteine, prevented chemotherapy-induced impairment on a test of passive avoidance conditioning.

Given that hippocampus-related memory loss is a common feature of chemotherapy-induced cognitive deficits, it is reasonable to ask whether centrally-acting cholinesterase inhibitors, such as donepezil, could have beneficial effects. Donepezil is one of a few cognitive enhancing drugs that have been approved for treatment of Alzheimer's disease in its early stages. There is also evidence that it can improve cognitive function in patients with traumatic brain injury (Zhang et al., 2004), as well as patients who received radiation therapy for brain tumours (Shaw et al., 2006). Donepezil has been investigated extensively in animals and there are several reports of improved memory performance in donepezil-treated rodents whose hippocampus was compromised as a result of lesions (Meunier et al., 2006), pharmacological agents (Kendall et al., 2011), or normal aging (Hernandez et al., 2006).

In the present study, we asked if donepezil could reduce cognitive impairment induced by the combination of methotrexate (MTX) + 5-fluorouracil (5-FU) in a mouse model. Groups of donepezil-treated and control mice were administered the following learning and memory tests in a water maze: (1) a standard spatial memory test (Morris et al., 1982), highly sensitive to hippocampal dysfunction, in which the mouse uses distal environmental cues to find a submerged platform; (2) a non-spatial test of cued memory that depends on the caudate nucleus and related structures (McDonald et al., 1999), in which a discrete cue signals the location of the platform; (3) tests of non-matching-to-sample (NMTS) rule-learning, and delayed-NMTS (DNMTS). The NMTS and DNMTS tests consist of a series of paired sample and test trials. In the sample trials, a distinctive stimulus signals the location of the platform. In the subsequent test trial, the same stimulus is presented along with a different stimulus and the rat must swim to the new stimulus to find the platform. NMTS and related rule-learning tasks incorporate conditional and working memory components and are highly sensitive to frontal-lobe dysfunction (Moscovitch and Winocur, 2002), but are not typically affected by hippocampal impairment (Aggleton et al., 1986; Winocur, 1992; Winocur et al., 2006b). However, by increasing the interval between sample and test trials in the DNMTS task, increased demands are placed on memory processes and animals with impaired hippocampal function are impaired at longer intervals (Winocur, 1992; Winocur et al., 2006b). Thus, the NMTS and DNMTS tasks, presented in this way, yield dissociable learning and memory functions related respectively to the frontal lobes and hippocampus.

In a previous study, we showed that mice treated with MTX + 5-FU are impaired on the spatial memory, NMTS, and DNMTS tests, but not the cued memory test (Winocur et al., 2006a). In addition to asking if donepezil can reduce chemotherapy-induced cognitive impairment, the present research investigates whether donepezil effects are general or, as suggested by much of the donepezil literature, selective with respect to hippocampal function (e.g., Abe et al., 2003; Hernandez et al., 2006; Kendall et al., 2011; Meunier et al., 2006; Murai et al., 2007; Xu et al., 2002). If the effects are specific to hippocampus-dependent memory, chemotherapy-treated mice should exhibit improvements only on the spatial memory test and at the longer intervals of the DNMTS task. However, if benefits extend to frontal lobe-controlled functions, then improved performance would also be expected in NMTS learning, and at all intervals of the DNMTS task. Based on our previous results (Winocur et al., 2006a), no effect of the anti-cancer drugs is expected on cued memory performance and, therefore, no effect of donepezil is predicted on this task.

2. Materials and methods

2.1. Subjects

Thirty-eight, 3-month old, female BALB/C mice, obtained from the Charles River Laboratories (Saint-Constant, Québec), served as subjects. The mice were housed in plastic shoebox cages (25 × 15 × 10-cm) in groups of 3–5, with free access to standard lab chow and water. They were maintained on a reversed 12-h light/dark cycle (lights on at 1800 h and off at 0600 h). The mice were examined regularly by a veterinarian and their weights recorded every 3 days.

The experimental protocol and all handling procedures were approved by the Trent University Animal Care Committee and conformed to requirements of the Canadian Council on Animal Care.

2.2. Drug treatment

Five weeks before behavioral testing, mice were assigned randomly to one of four groups: chemotherapy (CHEMO; $N = 9$), chemotherapy + donepezil (CHEMO + DON; $N = 10$), saline (SAL; $N = 10$), and saline + donepezil (SAL + DON; $N = 9$).

Each week for four consecutive weeks, mice received an intraperitoneal injection of MTX (50 mg/kg) + 5-FU (75 mg/kg) dissolved in saline, or an equal volume of physiological saline. MTX was obtained from Wyeth Canada, Thornhill, Ontario, 5-FU was obtained from Mayne Pharma, Kirkland, Québec, and donepezil was obtained from Cayman Chemicals, Ann Arbor, Michigan. The doses for both drugs were selected on the basis of preliminary dose–response tests, which showed that they were well tolerated; higher doses caused weight loss or death. Beginning at the same time, mice in the donepezil groups received a daily dose of donepezil (3 mg/kg) dissolved in distilled water every morning throughout the experiment. This dose was found to be effective in improving memory performance in cognitively-impaired adult mice (Murai et al., 2007). Donepezil was administered orally, through a rubber tube attached to a syringe. Mice in the non-donepezil groups received equal volumes of distilled water in the same way. During behavioral testing, the latter treatments were administered one hour before the beginning of testing.

2.3. Behavioural testing

Fig. 1 provides a schematic representation of the experimental design.

All behavioral tests (see Winocur et al., 2006a) were administered in a circular pool (130 cm diameter and approximately 30 cm high), located in the centre of a standard testing room. The pool was filled with opaque water and maintained at room temperature (21 °C). An inverted flower pot, situated a few cm below the surface, served as a platform on which the mice could climb to escape the water. Throughout testing, the water was cleaned after each trial and changed every 2–3 days.

The pool was divided into six zones of approximately equal size. Swimming patterns were monitored by an overhead video camera connected to a recorder and data processing system. The system enabled computation of the time required to mount the platform and the time spent in the platform zone. Records were kept of the animals' swimming routes that were used to count errors and are available on request.

2.3.1. Spatial memory

One week after the final injection of MTX + 5-FU or saline, the mice received two days of orientation training (5 trials/day) in which they learned to swim to the platform, which was visible and in a different location on each trial.

Spatial memory testing began the following day. The platform was now positioned a few cm below the surface and always located in the centre of the north-east zone of the pool. For each trial, the mouse was placed in the water at the edge of the pool, facing the wall, at a different location. The mice were never placed in the north-east zone, where the platform was located. Each trial continued until the mouse mounted the platform with all four paws, or until 60 s. elapsed. If it failed to find the platform in the allotted time, it was guided to the platform. After 20 s. on the platform, the mouse was placed under a heat lamp to await the next trial. Each mouse received 5 such trials/day for 5 consecutive days. On the sixth day of testing, trials 1 and 2 and 4 and 5 were conducted in the usual manner. On the third trial, which served as a probe trial, the platform was removed and the mice were allowed to swim for 60 s.

Two response measures were recorded for each trial of Days 1 to 5 – latency and errors. The latency was the time required to reach and mount the platform. An error was counted each time the mouse entered a zone not containing the platform. If the mouse failed to find the platform within 60 s, it was given an error score of 15 and a latency score of 60 for that trial. On the probe trial of Day 6, the time spent in the zone that normally contained the platform was the only measure.

2.3.2. Cued memory

The cued memory task began 2 days after completion of the spatial memory test. For this task, on each trial, the submerged platform was in a different location, which was signaled by a grey cylinder (30 cm long × 3 cm in diameter), suspended 5 cm

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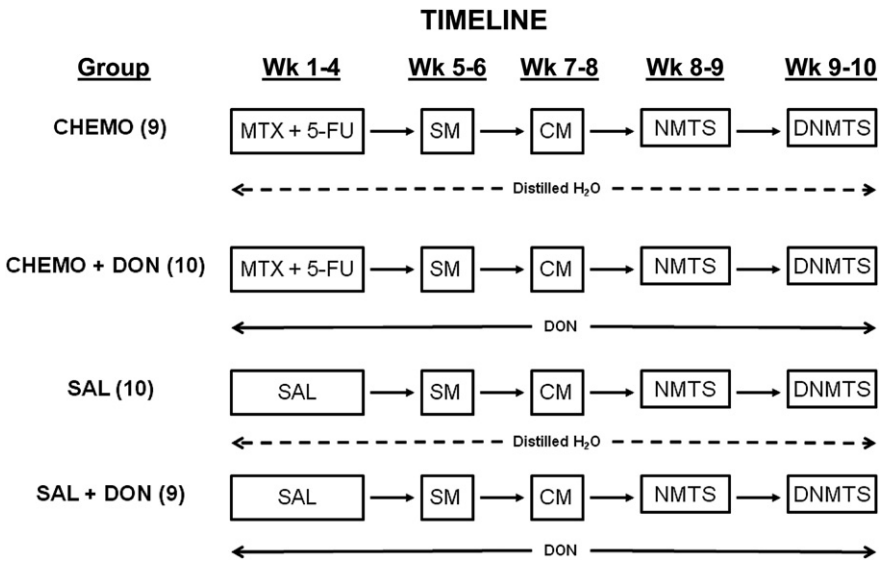

Fig. 1. Schematic representation of timelines for experimental procedures. Note: From the beginning of Week 1 and throughout the experiment, mice in the donepezil groups received daily oral administrations of donepezil dissolved in distilled water, and mice in the non-donepezil groups received daily oral administrations of distilled water. Abbreviations: CHEMO – chemotherapy; DON – donepezil; SAL – saline; MTX – methotrexate; 5-FU – 5-fluorouracil; SM – spatial memory; CM – cued memory; NMTS – Non-matching-to-sample; DNMTS – delayed-NMTS.

above the submerged platform. In all other respects, including a probe trial on Day 6, testing procedures and scoring were identical to those of the spatial memory test.

2.3.3. NMTS

The stimuli for the sample and test trials were black and white cylinders (30 cm long × 3 cm in diameter). For the sample trials, the submerged platform was in a different location and signaled by the black or white cylinder, which was suspended 5 cm above the platform. In the subsequent test trial, the platform was re-located and both cylinders were present, but the cylinder that was not present during the preceding sample trial cued the platform's location.

NMTS testing began two days after the cued-memory test. For each sample trial, the mouse was placed in the south-east zone of the pool and allowed to swim to the submerged platform under the sample cylinder. The mouse remained on the platform for 20 s. and then placed under the heat lamp while the platform was moved and the cylinders put in position for the test trial. The organization of the cylinders and platform took about 10 s. The mouse was then placed in the pool and allowed to swim to the submerged platform or until 60 s. had elapsed. If the mouse failed to find the platform within 60 s, it was guided to the platform and given an error score of 15 and a latency score of 60 for that trial. After 20 s on the platform the mouse was placed under the heat lamp, to await the next pair of trials. There was an interval of 4–5 min between each pair of trials. Ten daily sessions, each consisting of 5 pairs of sample and test trials, were administered.

2.3.4. DNMTS

The day after the completion of NMTS testing, the mice received 5 additional daily sessions. Each session consisted of four paired trials, with delays of 0,[1] 60, 120, or 240 s. between the sample and test trials. The order varied each day according to a random schedule. During DNMTS testing, the interval between successive pairs of trials varied, ranging from approximately 2 min when the sample- test trial delay was 0 s, to 20–25 min when the delay was 240 s. In all other respects, the testing procedure and scoring for the DNMTS test were identical to that followed in NMTS testing.

2.4. Statistical analysis

For the spatial memory, cued memory, NMTS, and DNMTS test trials, we recorded the time required to find the platform (latency) and the number of errors committed in the process. The measures analyzed were the average latency and the average number of errors across all trials on each testing day. To simplify presentation, only error scores will be presented here since the latency and error scores yielded the same pattern of results for all tests. Latency data and their analyses are available on request.

[1] The shortest interval between sample and test trials was the same as in NMTS testing. While designated as 0 s, in fact, the interval was about 10 s, the time required to prepare for the test trial.

For the probe trials on the spatial and cued memory tests, the amount of time spent in the zone where the platform had been located during the learning trials was recorded. The average amount of time was analyzed for these probe trials.

Analysis of variance (ANOVA) was used to test differences between groups on the weight records and behavioral measures. The models for all ANOVAs contained two between-subject factors, chemotherapy (MTX + 5-FU or saline), donepezil (donepezil or distilled water); a within-subject, test-day factor (days); and the interactions between these three factors. The chemotherapy × donepezil interaction directly examined the hypothesis that chemotherapy produces cognitive impairment that is reduced by donepezil. The probe trial measures did not include a day effect or interaction in the model. Significant interactions were followed by *a priori* simple main effect analyses using the appropriate pooled error term as described in Kirk (1968). Analysis of DNMTS scores included an additional within-subject interval factor (0, 60, 120, and 240 s delays). Significant interactions were followed by analysis of simple main effects of treatment group at each interval. All hypothesis tests were performed at an alpha level of 5% and statistics were calculated using PASW Statistics version 18.0.0.

3. Results

3.1. Weight records

The average weights for mice in the various groups at the start and end of the experiment are presented in Table 1. ANOVA confirmed a significant effect of time, $F_{1,35} = 25.85$, $p < .0001$, that represented an overall weight gain in the mice over the course of the study. Neither chemotherapy, $F_{1,35} < 1$, nor donepezil, $F_{1,35} = 1.55$, $p > .20$, significantly affected changes in body weight over time.

The mice were monitored for possible side effects related to drug treatment (e.g., motor impairment, apathy) but, except for a small amount of hair loss in about half of the mice, none was detected.

3.2. Spatial memory

All mice quickly learned to find the visible platform during orientation training and, after two days, were reaching the platform within a few seconds. There was no difference between groups in learning to find the platform.

Over the five days of spatial memory testing, mice receiving MTX + 5-FU only (CHEMO group) made more errors (Fig. 2A) in finding the hidden platform than groups injected with saline ($F_{1,34} = 32.84$, $p < .0001$). ANOVA also indicated that mice treated with

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Table 1
Mean weights.

	SAL		CHEMO		CHEMO + DON		SAL + DON	
	Start	End	Start	End	Start	End	Start	End
Mean	20.70	21.40	20.22	21.00	20.00	21.10	20.44	21.78
S.D.	1.16	0.97	1.09	0.87	0.47	0.38	0.88	0.67

Mean weights in grams for mice in the four groups at the start and end of the experiment (S.D. = standard deviation).

donepezil made fewer errors than mice that were not ($F_{1,34} = 9.55$, $p < .01$). A significant interaction of chemotherapy × donepezil ($F_{1,34} = 7.89$, $p < .01$) indicated that the effect of donepezil was greater in mice that were administered MTX + 5-FU than in mice injected with saline. The main effect of days was significant ($F_{4,136} = 22.47$, $p < .0001$), but there were no significant interactions involving days ($p > .37$ for all interactions).

During the probe trial, mice that received chemotherapy spent less time in the platform zone than saline-injected mice ($F_{1,34} = 18.93$, $p < .0001$; Fig. 2B). The main effect of donepezil was not statistically significant ($F_{1,34} = 1.95$, $p > .10$), although the chemotherapy × donepezil interaction ($F_{1,34} = 3.44$, $p < .08$) was of borderline significance. An examination of simple main effects revealed that, during the probe trial, the CHEMO + DON group spent significantly more time than the CHEMO group in the zone that had contained the platform during the five days of testing ($F_{1,34} = 46.94$, $p < .0001$), reinforcing the impression that chemotherapy-treated mice benefitted from the effects of donepezil. There was no difference between the SAL + DON and SAL groups on this measure ($F < 1$).

3.3. Cued memory

The results for the cued memory test are presented in Fig. 3. As in the spatial memory test, the error rates decreased over the 5 days of testing ($F_{4,136} = 31.25$, $p < .0001$), but there were no significant interactions ($p > .18$ for all comparisons), and no significant main effect of chemotherapy ($F_{1,34} = 1.18$, $p > .25$), or donepezil ($F < 1$) (Fig. 3A). Similarly, there were no group differences in time spent in the zone in which the submerged platform had been located during the probe trial of Day 6 ($p > .05$ for all comparisons; Fig. 3B).

3.4. NMTS

As can be seen in Fig. 4, between Days 1 and 5, there was little difference between groups in learning the NMTS rule. Beginning on Day 6, there was deterioration in the overall performance of the CHEMO group and the variability between scores also increased. ANOVA applied to the data showed a significant effect of chemotherapy ($F_{1,34} = 4.55$, $p < .05$). Although the CHEMO + DON group performed nominally better than the CHEMO group on Days 6–10 and did not appear to differ from the saline groups, the main effect of donepezil failed to yield statistical significance ($F_{1,34} = 2.98$, $p > .05$), nor did we find a significant donepezil × chemotherapy interaction ($F_{1,34} = 1.49$, $p > .20$).

3.5. DNMTS

The average number of daily errors made in finding the platform during the test trials is presented for each interval in blocks of two days in Fig. 5. As can be seen, there was a tendency for all groups to make more errors as the interval increased, with the effect being most pronounced in the CHEMO group. This was confirmed by a significant chemotherapy × interval interaction ($F_{3,102} = 4.33$, $p < .01$), as well as a strong main effect of interval ($F_{3,102} = 68.12$, $p < .0001$). Importantly, a significant chemotherapy × donepezil × interval interaction ($F_{3,136} = 3.10$, $p < .05$) indicated that the effects of donepezil on mice receiving chemotherapy varied with the length of the sample-test trial interval. This was confirmed by significant chemotherapy × donepezil interactions at each interval with the largest occurring at the 240-s interval ($F_{1,136} = 40.50$, $p < .0001$) and the smallest at the 0-s interval ($F_{1,136} = 10.49$, $p < .005$). An examination of simple main effects at each interval confirmed that donepezil consistently resulted in improved performance in mice receiving chemotherapy (CHEMO + DON vs CHEMO groups: $p < .001$ at each interval), while having no effect on saline-treated mice (DON + SAL vs DON: $p > .45$ at each interval).

4. Discussion

The present research confirms that the commonly used anti-cancer drugs, MTX + 5-FU, produce significant cognitive impairment in mice. Deficits observed on the spatial memory test and at the long sample-test trial intervals of the DNMTS test are consistent

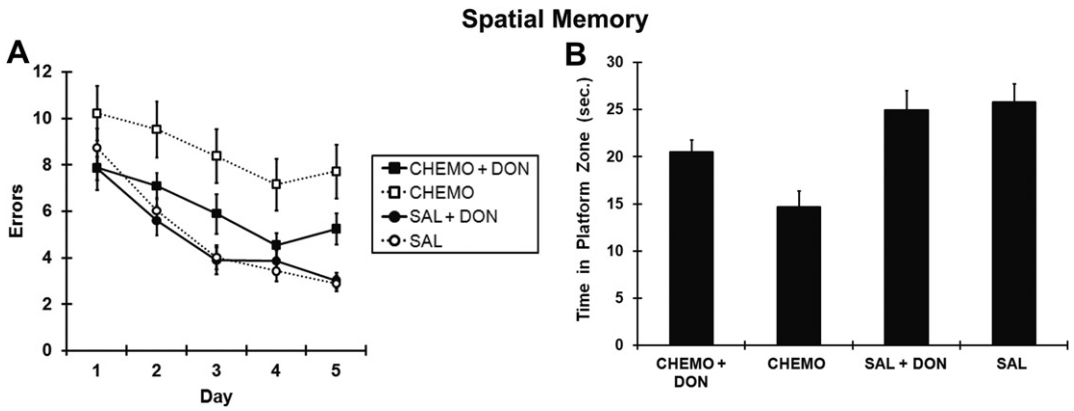

Fig. 2. Spatial Memory. Mean number of errors/trial/day for all groups during learning on the spatial memory test (2A). Mean time (s) spent by all groups in the zone that contained the platform during the spatial memory probe trial (2B). Error bars denote ± SEM.

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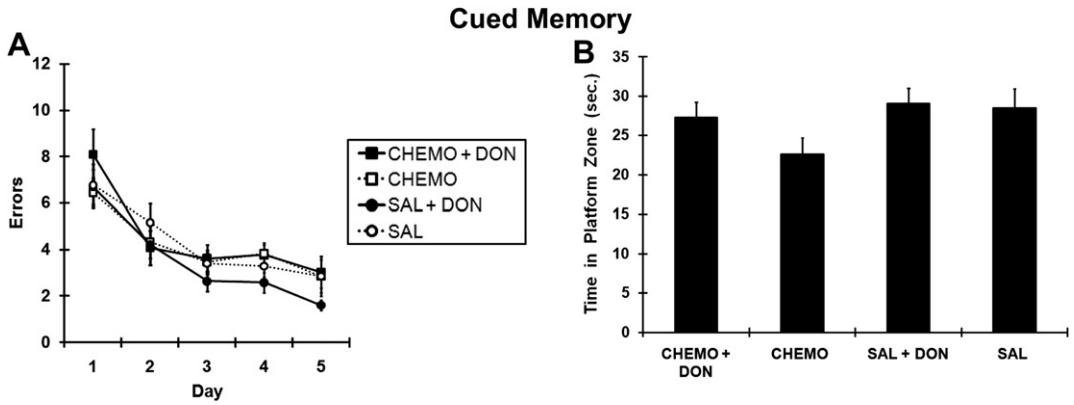

Fig. 3. Cued Memory. Mean number of errors/trial/day for all groups during learning on the cued memory test (3A). Mean time (s) spent by all groups in the zone that contained the platform during the cued memory probe trial (3B). Error bars denote ± SEM.

with previous reports that performance on hippocampus-sensitive tests of memory is particularly vulnerable to the effects of chemotherapy (Fardell et al., 2010; Foley et al., 2008; Lyons et al., 2011; Seigers et al., 2008; Seigers and Fardell, 2011; Winocur et al., 2006a). In addition, in the present study, the CHEMO group was impaired in learning the NMTS rule, a task that is dependent on frontal-lobe function. MTX + 5-FU treatment did not affect performance on the cued memory test, which is thought to be mediated by striatal structures within the basal ganglia.

The pattern of diminished and spared function observed in this research is similar to that reported by Winocur et al. (2006a) and observed in neuropsychological investigations of chemotherapy-treated cancer survivors (Ahles and Saykin, 2007; Dietrich et al., 2008; Vardy and Tannock, 2007; Vardy et al., 2007). In general, the deficits in the CHEMO group were greater than those observed on the same tests in our previous study. Two important procedural variations may explain these differences. For the present study, the dosage of MTX was increased from 37.5 mg/kg to 50 mg/kg and there was an increase, from three to four, in the number of weekly treatments administered. In the clinical neuropsychological literature, chemotherapy-related deficits are usually characterized as moderate but, to our knowledge, there has not been a systematic examination of the relationship between treatment parameters and the nature and extent of cognitive impairment.

In a new finding, learning and memory deficits resulting from the anti-cancer drugs were significantly reduced when mice, receiving MTX + 5-FU, were also administered donepezil. On some measures (e.g., the 120- and 240-s intervals in the DNMTS test), the donepezil effect was so strong that the CHEMO + DON group's performance was very similar to that of the saline groups. On two measures, the spatial memory probe test and NMTS rule-learning, where donepezil treatment appeared to improve the performance of chemotherapy-injected mice (see Figs. 2 and 4), the chemotherapy × donepezil interaction, as assessed by ANOVA, was not statistically significant. However, in both cases, individual comparisons between the CHEMO and CHEMO + DON groups revealed differences that favored the donepezil-treated group. Taken together the results demonstrate that a cognitive enhancing drug that is widely used to treat cognitive impairment in other clinical populations (e.g., Alzheimer's disease) can ameliorate the adverse effects of chemotherapy on cognition in mice.

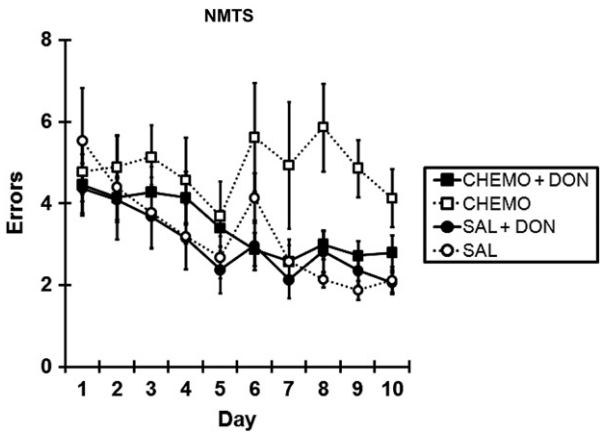

Fig. 4. NMTS rule-learning. Mean number of errors/trial/day on the test trials for all groups during learning on the NMTS test. Error bars denote ± SEM.

Donepezil acts directly on the cholinergic system and increases the synaptic supply of acetylcholine, a neurotransmitter that plays a central role in mediating cognitive processes, especially those that involve the hippocampus. The effects of donepezil on hippocampus-sensitive tasks observed in the present study might therefore be expected. However, the improvements in learning the NMTS rule and at the 0- and 60-s intervals of the DNMTS test are somewhat surprising since successful performance on these tasks is more dependent on the frontal lobes. While there is a large concentration of cholinergic neurons in the hippocampus, a branch of the cholinergic system that originates in the nucleus basalis of the forebrain, projects to the frontal lobes (Whitehouse et al., 1982). As well, clinical evidence suggests that the benefits of donepezil treatment can extend beyond hippocampus-based memory to attentional processes and related cognitive functions (Zhang et al., 2004) under frontal-lobe control. Further research is required, but physiological and neuropsychological evidence points to the possibility that donepezil may prove to be a useful treatment for both the mnemonic and executive deficits associated with chemotherapy.

While the results of the present study point to donepezil as a possible treatment for cognitive deficits resulting from anti-cancer drugs, they also raise a number of questions. For example, how long-lasting are the benefits of donepezil? What is the range of dose effectiveness? Is donepezil generally effective in treating cognitive deficits related to hippocampal or frontal-lobe impairment, or is there some selectivity? In the present study, donepezil was started at the same time as chemotherapy. As a result, it was not possible to separate its effects as a treatment (which would require its administration after inducing cognitive changes) from its ability to prevent cognitive changes from occurring. An important priority for future research is to investigate possible

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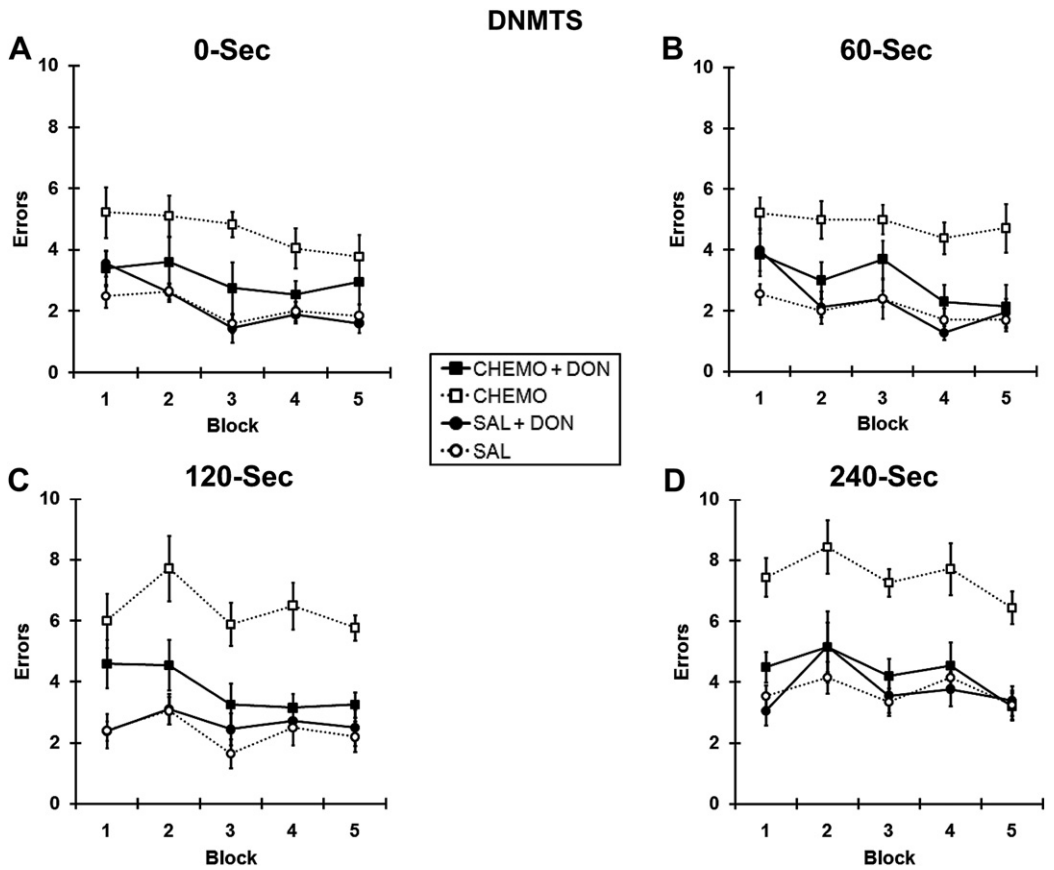

Fig. 5. DNMTS. Mean number of errors/trial/day on the test trials for all groups at all sample-test trial intervals of the DNMTS test. Data are presented over 5 blocks of 2 days each. Error bars denote ± SEM.

relationships between cholinergic impairment and other neurobiological processes (e.g., adult neurogenesis – Kotani et al., 2006; neuroinflammation/cytokine release – Pusztai et al., 2004; oxidative stress – Rajamani et al., 2006) that have been implicated as mediators of cognitive impairment associated with chemotherapy. These issues are central to understanding the mechanisms and boundaries of donepezil treatment of chemotherapy-related cognitive changes and are important topics for future enquiry.

Finally, it is useful to consider the potential benefits of other, non-biological, types of intervention. Lifestyle-related factors (e.g., diet, physical activity, environmental enrichment) are known to protect against cognitive decline (Cotman and Berchtold, 2002; Fratiglioni et al., 2004; Kramer et al., 2004). As well, there is growing evidence that cognitive rehabilitation programs that emphasize training of strategic processes can improve cognitive performance associated with traumatic brain injury (Levine et al., 2000), as well as normal (Stuss et al., 2007) and pathological (Woods and Clare, 2006) aging. These programs typically target losses in memory and executive function that are similar to those seen following chemotherapy. It is reasonable to expect, therefore, that such programs, on their own, or in combination with pharmacotherapy, would be effective in relieving chemotherapy-induced cognitive deficits in cancer patients.

5. Conclusions

The present results confirm that the drug combination of methotrexate + 5-fluorouracil, used in adjuvant cancer chemotherapy causes significant cognitive impairment in healthy mice. The deficit was characterized primarily by a loss of hippocampus-dependent memory and executive function under frontal-lobe control. Both types of deficit were reduced by administration of the cholinesterase inhibitor, donepezil, a cognitive-enhancing drug that is used to treat cognitive impairment in other clinical populations, including Alzheimer's disease.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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