JOURNAL OF NEUROTRAUMA 29:2457–2464 (October 10, 2012)

DOI: 10.1089/neu.2012.2465

Acetylcholinesterase Inhibition Interacts with Training To Reverse Spatial Learning Deficits after Cortical Impact Injury

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Abstract

Cholinergic mechanisms are known to play a key role in cognitive functions that are profoundly altered in traumatic brain injury (TBI). The present investigation was designed to test the ability of continuous administration, starting at the time of injury, of physostigmine (PHY), an acetylcholinesterase (AChE) inhibitor that crosses the blood–brain barrier (BBB), to ameliorate the alterations of learning and memory induced by cerebral cortex impact injury in rats under isoflurane anesthesia. Learning and memory were assessed with the Morris water maze implemented during days 7–11 (WM1), and days 21–25 post-TBI (WM2), with four trials per day for 3 days, followed by target reversal and 2 additional days of training. These groups of Sprague-Dawley male rats were used: TBI treated with PHY at 3.2 μmol/kg/day (TBI-PHY3.2), or 6.4 μmol/kg/day (TBI-PHY6.4), by subcutaneous osmotic pumps, or TBI and no injury (Sham) treated with saline. AChE activity was measured in brain tissue samples of non-traumatized animals that received PHY at the doses used in the TBI animals. In WM1 tests, PHY3.2 improved learning within sessions, but not between sessions, in the recall of the target position, while PHY6.4 had no significant effects. In WM2 tests, PHY improved within- and between-sessions performance at both dose levels. We found that continuous AChE inhibition interacted with repeated training on the water maze task to completely reverse the deficits seen in learning and memory induced by TBI. The PHY treatment also reduced the amount of brain tissue loss as measured using cresyl violet staining.

Key words: acetylcholinesterase; cholinergic; neurorehabilitation; traumatic brain injury; spatial learning.

Introduction

Deficits in memory and learning that can last a lifetime are among the many functional impairments that survivors of traumatic brain injury (TBI) must endure.[1–8]

Central cholinergic mechanisms are known to be essential to learning and memory, among other higher nervous system functions.[9–12] Consequently, interest in the exploration of the role of acetylcholine (ACh) in the pathophysiology and management of TBI has been growing, starting with an early report on the combined use of lecithin and physostigmine,[13] followed by animal experimentation and other clinical therapeutic trials,[1,14–26] but many questions remain about the basic mechanisms of action, and the design of effective treatment regimens with agents that enhance cholinergic function. Inhibition of enzymatic degradation of ACh by inhibition of acetylcholinesterase (AChE) appears to be a straightforward approach. However, the peripheral and central distribution of this enzyme at cholinergically-innervated sites are so wide, and the functions of ACh so distributed, that untoward effects are common and pose a challenge to the efficient use of agents that modulate the activity of AChE in the management of the cognitive deficits of TBI.

This work was undertaken to determine (1) if continuous inhibition of AChE post-TBI, at levels devoid of cholinergic toxicity, could improve learning and memory deficits, and (2) if repeated training could interact with drug treatment to improve cognitive outcomes.

Our previous work[27] demonstrated that AChE inhibition with physostigmine at a continuous infusion rate of 3.2 μmol/kg/day for 28 days improved performance of a complex motor task in TBI-lesioned rats. Greater dosages did not improve performance or induced progressive deterioration of function. Thus in the present investigation we have used physostigmine at 3.2 and 6.4 μmol/kg/day only. The possible effect of repeated training on water maze

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performance was addressed by exposing animals to two 1-week training sessions, separated by a 2-week interval.

Methods

Animals

Sprague-Dawley male rats (250–300 g body mass; Harlan Sprague Dawley, Placentia, CA) divided into four experimental groups were used: TBI treated with saline (TBI-Sal, $n=9$), TBI treated with physostigmine (PHY) at 3.2 μmol/kg/day, (TBI-PHY3.2, $n=9$), or 6.4 μmol/kg/day (TBI-PHY6.4, $n=8$), and sham animals treated with saline (Sham-Sal, $n=10$). Physostigmine hemisulfate or saline were administered continuously with subcutaneous osmotic pumps at the same volumetric flow in all cases. The research environment and protocol for animal experimentation were approved by the Institutional Animal Care and Use Committee of the Veterans Affairs Greater Los Angeles Healthcare System. The animal facility at this Institution is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. The animals were group housed for 1-week to acclimatize them to the vivarium prior to experimentation.

Cerebral cortex impact injury

The rats were anesthetized with isoflurane (induction 3.5%, maintenance 2.0% in 30% oxygen, balance nitrogen). The scalp was shaved and cleansed with povidone-iodine and the animal was mounted in a stereotaxic apparatus in the skull-horizontal position. Following sterile technique, a right parasagittal skin incision was performed, the galea was incised and reflected, and a craniotomy was fashioned with a 6.2-mm trephine, centered at 1.2 mm anterior and 3.5 mm lateral (right side) to the bregma. Severe trauma to the right cerebral cortex was produced as previously reported.[28] The apparatus consisted of a vented stainless steel guide tube mounted on a micromanipulator through which a 20-g weight was dropped from a height of 35 cm to strike a stainless steel circular footplate (4.5 mm diameter) resting on the exposed dura mater of the right motor-sensory cortex, with its center 1.2 mm anterior and 3.5 mm lateral to the bregma. This site was selected to coincide with the maximum activation of the primary motor cortex observed in rats during treadmill walking.[29] The indentation produced in the cortex by the footplate was limited to 3.0 mm. After trauma, the galea was sutured to cover the craniotomy and the skin was closed with 2-0 polypropylene stitches. Sham intervention animals were anesthetized as described above, and the skin and galea incisions were made and then sutured, but no craniotomy and cerebral cortical impact injury were produced. The animals were kept warm to prevent post-operative hypothermia. Antibiotic (cefazolin, 100 mg/kg IM) and analgesic therapy (buprenorphine, 0.05 mg/kg SC) were given twice daily for 3 days after TBI or Sham interventions.

Administration of physostigmine

Physostigmine hemisulfate and ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA; both from Sigma-Aldrich, St. Louis, MO) were dissolved at equimolar concentrations in double-distilled boiled water, and 2 mL of the solution was used to fill each osmotic pump (Alzet model 2ML4; Durect Corp. Cupertino, CA). The addition of EDTA was found to be necessary to prevent degradation of physostigmine in solution. The pumps are cylindrical in shape, are 3 cm long and 0.7 cm in diameter, and weigh 1.1 g empty. They delivered 2.5 μL/h continuously for 28 days. They were inserted into a subcutaneous space created by blunt dissection of the interscapular region of the animals immediately after cerebral cortex impact injury while the animals were still under isoflurane anesthesia. The skin was closed with 2-0 polypropylene sutures. The molar infusion rates of physostigmine hemisulfate were 3.2 and 6.4 μmol/kg/day.

Water maze testing

Spatial learning and memory were evaluated with a modified water maze paradigm,[30] by requiring the rats to swim in a pool 1.7 meter in diameter, with the water kept at 20°C, to find a 12-cm-diameter circular platform submerged 2 cm beneath the surface of the water, which was opacified by the addition of black non-toxic tempera paint. The platform was in a constant position during training, as there were a number of visual cues in the testing room. A video-tracking system (Ethovision; Noldus, Inc. Wageningen, The Netherlands) was used for data acquisition. Initially the animals received four trials per day, with an intertrial interval of 30 min, for 3 consecutive days (Monday, Tuesday, and Wednesday). A trial was initiated by placing the animal in the water with the nose facing the wall of the pool, alternating between quadrants without the target in a random sequence that changed each day. The rat was allowed to swim until it located the submerged escape platform and climbed onto it, or for a maximum of 120 sec, after which it was manually placed on the platform. In either case, the animal was left on the platform for 10 sec before being removed from the water, dried with a towel, and placed in a cage under a warming light. On day 4, the location of the submerged platform was switched to the opposite quadrant. Four trials were implemented on that day, followed by four trials on day 5 (Fig. 1).

Regional brain activity of AChE

Control animals (no TBI) treated with continuous physostigmine hemisulfate infusions as described above were euthanized by decapitation 3 days before the end of infusion while under deep isoflurane anesthesia (3.5% in 30% oxygen balanced with nitrogen). The brain was rapidly removed and flash frozen over dry ice. One coronal brain slice was obtained between coordinates 1 mm rostral and 4 mm caudal to the bregma. Two cortical blocks were dissected on each hemisphere, including the primary motor (M1) and primary somatosensory (S1) areas. One additional block was obtained to include the striatum. These tissue samples were homogenized, and aliquots of these homogenates were used to determine tissue AChE activity with the kinetic method.[31] In order to preserve brain integrity for morphological evaluation, AChE was not measured in brain tissue of TBI animals. However, since there is no indication that drug systemic kinetics could be altered by this model of brain injury, it is thought that these measurements estimate the levels of AChE inhibition obtained in the reported experiments.

Delineation of the cortical infarct area

After euthanasia, the brains were removed, flash frozen on powdered dry ice, and mounted on the stage of a cryostat. Serial coronal frozen sections 20 μm thick were collected on glass slides, heat-dried, fixed in 10% formaldehyde, stained with cresyl violet, dehydrated in an alcohol and acetone series, and sealed with coverslips using mounting medium. The sections were digitized with a Micro-Mac CCD video camera (McBain Systems, Simi Valley, CA), Dazzle video digitizer (Pinnacle Systems, Mountain View, CA), and Adobe Photoshop software (Adobe Systems, San Jose, CA). Contours of the area of tissue loss and of the ipsilateral and contralateral hemispheres were drawn, and their respective areas were computed on slices at 0.8-mm intervals with Image J software (National Institutes of Health [NIH]). The total volume of the lesion for each animal was computed by multiplying the average areas (mm^2) of every two consecutive slices sampled every 0.8 mm, by the interslice distance.

Statistical analysis

For the water maze data, the dependent variables were latency and distance swam to reach the platform and the average speed for each trial. Analysis of variance (ANOVA) was performed with the

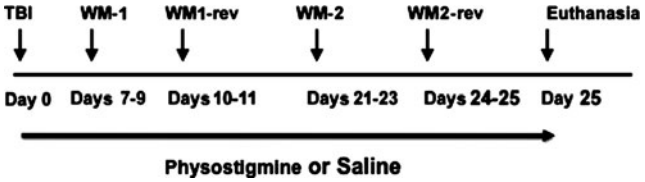

FIG. 1. Timeline showing traumatic brain injury (TBI) or sham-injury (Sham), continuous physostigmine or saline infusion, the first and second training sessions in the water maze (WM1 and WM2), and their reversal (WM1-rev and WM2-rev), as well as animal euthanasia.

factors ''trial (1 to 4),'' ''day (1 to 5),'' and ''treatment'' (Sham saline, TBI saline, TBI 3.2 μmol/kg/day physostigmine, and TBI 6.4 μmol/kg/day physostigmine). Within-daily-session learning was estimated from the performance on the last trial of the day, and between-sessions recall by the performance on the first trial of the day, with the exclusion of days 1 and 4, when the animals were exposed to a novel situation. If the F-ratio of the ANOVA was statistically significant ($p < 0.05$), differences between treatments were assessed by Dunnett's multiple comparison testing, with significance set at $p < 0.05$.

Results

Lesion volumes

At 25 days after cortical injury, the infarcted area under and immediately surrounding the impact site had been replaced by a cyst. The size of this missing tissue area was quantified on coronal slices as described in the methods section and the results are shown in Figure 2. TBI animals treated with physostigmine showed significantly less tissue loss than TBI animals that received saline, but no significant difference existed between physostigmine doses. The cerebral hemisphere on the injured side had a lower cross-sectional area, measured at the same levels as the lesions, than the contralateral hemisphere in all three groups of TBI animals. Expressed as a percentage of the contralateral hemisphere, the value for the three pooled groups was 86.9 ± 0.9%. There were no significant differences among groups (TBI-saline 85.1 ± 2.3%; TBI-3.2 μmol/kg/day physostigmine 86.6 ± 2.4 %; TBI-6.4 μmol/kg/day physostigmine 91.2 ± 2.4%).

Activity of AChE in the brain

AChE activity was decreased in the three regions sampled and at the two infusion rates of physostigmine used (Table 1). Tremors and muscle fasciculations were observed in animals that received

Table 1. Acetylcholinesterase (AChE) Activity in Brain Regions of Non-traumatized Animals Measured with the Kinetic Method of Ellman

Region	*Physostigmine dose µmol/kg/day*	*AChE activity (%)*	*Standard error*
Motor cortex	3.2	77.5	5.7
	6.4	54.0	3.3
Sensory cortex	3.2	82.2	7.2
	6.4	57.2	3.8
Striatum	3.2	67.9	4.6
	6.4	63.8	6.1

Values are expressed as percentages of animals that did not receive physostigmine.

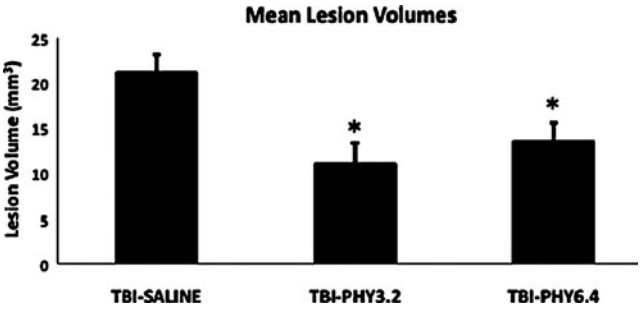

FIG. 2. Mean (bars) and SE (brackets) of lesion volumes (*$p < 0.05$ significantly different by analysis of variance and Dunnett's test comparisons from control TBI-SALINE animals; TBI-PHY3.2, TBI and physostigmine 3.2 μmol/kg/day; TBI-PHY6.4, TBI and physostigmine 6.4 μmol/kg/day; TBI, traumatic brain injury).

physostigmine at 6.4 μmol/kg/day upon awakening from anesthesia, but they ceased within the next 2–3 h and were not observed after that time. This was most likely due to an excessive amount of the drug being released due to manipulation of the pumps during the implantation procedure. No toxic signs were observed in animals that received physostigmine at 3.2 μmol/kg/day.

Water maze learning

The animals were exposed to water maze training twice, the first on days 7–11 post-TBI, (WM1), and the second on days 21–25 post-TBI (WM2), as outlined in Figure 1. ANOVA of the entire data set indicated significance at $p < 0.0001$ for the factors treatment, day, trial, and training session, as well as the interactions of training session with treatment ($p = 0.005$), day ($p < 0.0001$), and trial ($p = 0.03$). Averages of performance throughout the 5 days of the test during training sessions WM1 (left panels) and WM2 (right panels) are shown in Figure 3. Animals with TBI that received saline showed significantly longer trajectories and time delays to reach the submerged platform than sham controls. The difference was evident during both exposures to the water maze. During the first exposure to the water maze, animals with TBI that received 3.2 μmol/kg/day of physostigmine, but not those that received 6.4 μmol/kg/day, showed significantly shorter times (Fig. 3, top panel) and paths (Fig. 3, bottom panel) to the target than TBI animals that received saline. During the second exposure to the water maze, the animals in both physostigmine dose groups achieved similar performance, with significantly lower times and shorter paths to the target than TBI animals that received saline. Regression analyses of the times and paths to the target and the magnitude of tissue loss of every animal with a TBI was performed for both exposures to the water maze to determine if lesion size was correlated with performance. The results of those analyses lacked significance, with R^2 of 0.08 and 0.03 for WM1 and WM2, respectively, in the case of path length to target, and 0.09 for WM2 in the case of latency to target. Only in latency to target for WM1 was a significant correlation found, with R^2 of 0.20, and a significant regression coefficient of latency on lesion volume ($p = 0.027$).

Since some degree of motor impairment is to be expected in TBI, it was considered important to record the average swimming speed of the animals to ensure that the differences observed were due to a cognitive and not a pure motor deficit. The results of this analysis are shown in Figure 4. Contrary to expectations, the swimming speed of the TBI animals that received saline was higher than any

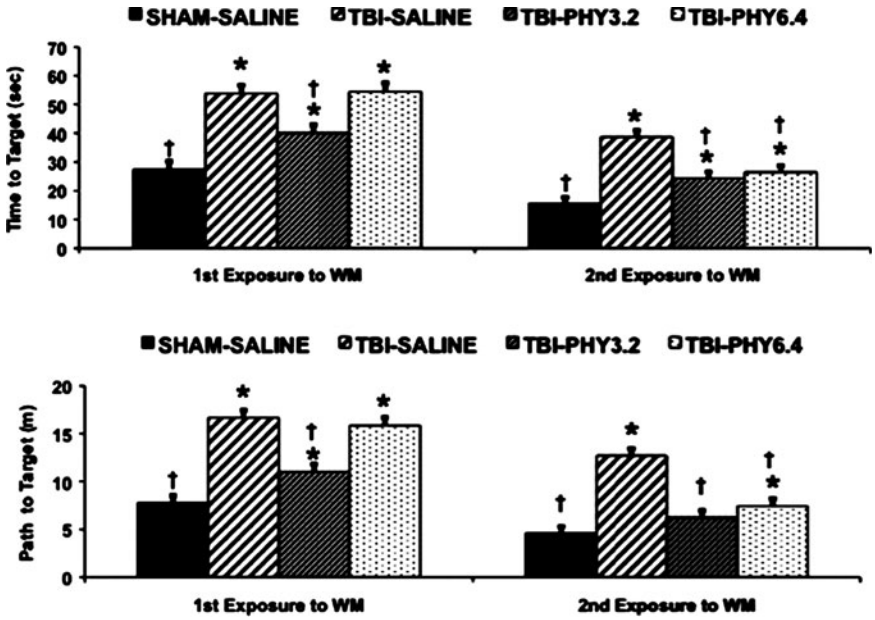

FIG. 3. Mean (bars) and standard error (brackets) of time to reach the target (top panels, seconds), and length of the path followed from the release point to the target (bottom panels, meters), for the first (7–11 days post-TBI, left panels), and second (21–25 days post-TBI, right panels) exposure to the water maze test (WM). Values are averages over trials and days for the four experimental groups. Analysis of variance was performed for every variable and exposure to WM, and if the F-ratio was significant ($p < 0.05$), Dunnett's test was used to assess significant differences ($p < 0.05$) with the TBI-saline (TBI-SALINE, †) or Sham-saline (SHAM-SALINE, *) means (TBI-PHY3.2, TBI and physostigmine 3.2 μmol/kg/day; TBI-PHY6.4, TBI and physostigmine 6.4 μmol/kg/day; TBI, traumatic brain injury).

other group on the first exposure to the test. Observation of animal behavior while searching for the submerged platform explained this phenomenon. Animals that failed to find the platform at an early time point, as was true of the TBI-saline group, tended to start swimming fast in circles close to the wall, while after a few trials, Sham animals or TBI animals treated with physostigmine tended to slow down while in the target quadrant, and make slow side-to-side motions while searching for the target.

In an effort to determine the ability to remember the target position from one day to another, the performance on the first trial of the day (except for days 1 and 4) was computed. This is depicted on the abscissa of the plots in Figure 5. It is apparent that all groups lagged behind the Sham intervention controls during the first WM exposure (Fig. 5, top panel). However, repeated training improved the performance of the animals that received physostigmine, to the level of Sham intervention animals, but did not improve the recall process of those treated with saline (Fig. 5, bottom panel).

The ability to learn within a daily session was tested by comparing the performance among groups on the last trial of the day, depicted on the ordinate of the plots in Figure 5. During the first water maze exposure TBI animals that received physostigmine at 3.2 μmol/kg/day showed a significantly better performance than those on saline, but TBI animals on 6.4 μmol/kg/day physostigmine did not reach that level. Repeated training (second water maze exposure) brought the performance of both groups of TBI animals on physostigmine to levels similar to those of Sham intervention animals.

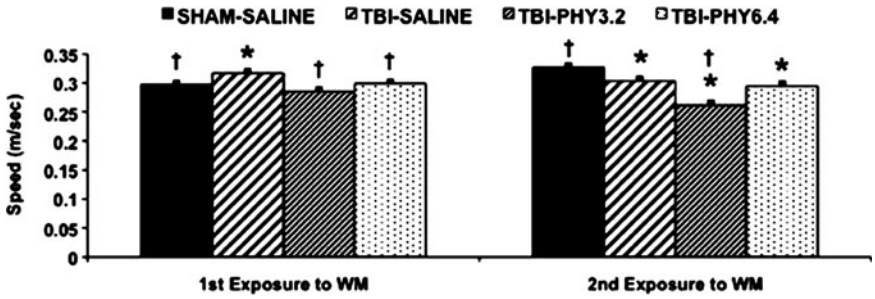

FIG. 4. Mean (bars) and standard error (brackets) of swimming speed during the water maze (WM) test, averaged over trials and days for the four experimental groups (left panel, 7–11 days post-TBI; right panel, 21–25 days post-TBI). Analysis of variance was performed for both exposure times to the WM, and if the F-ratio was significant ($p < 0.05$), Dunnett's test was used to assess significance ($p < 0.05$) versus TBI-saline (TBI-SALINE, †) or Sham-saline (SHAM-SALINE, *) means (TBI-PHY3.2, TBI and physostigmine 3.2 μmol/kg/day; TBI-PHY6.4, TBI and physostigmine 6.4 μmol/kg/day; TBI, traumatic brain injury).

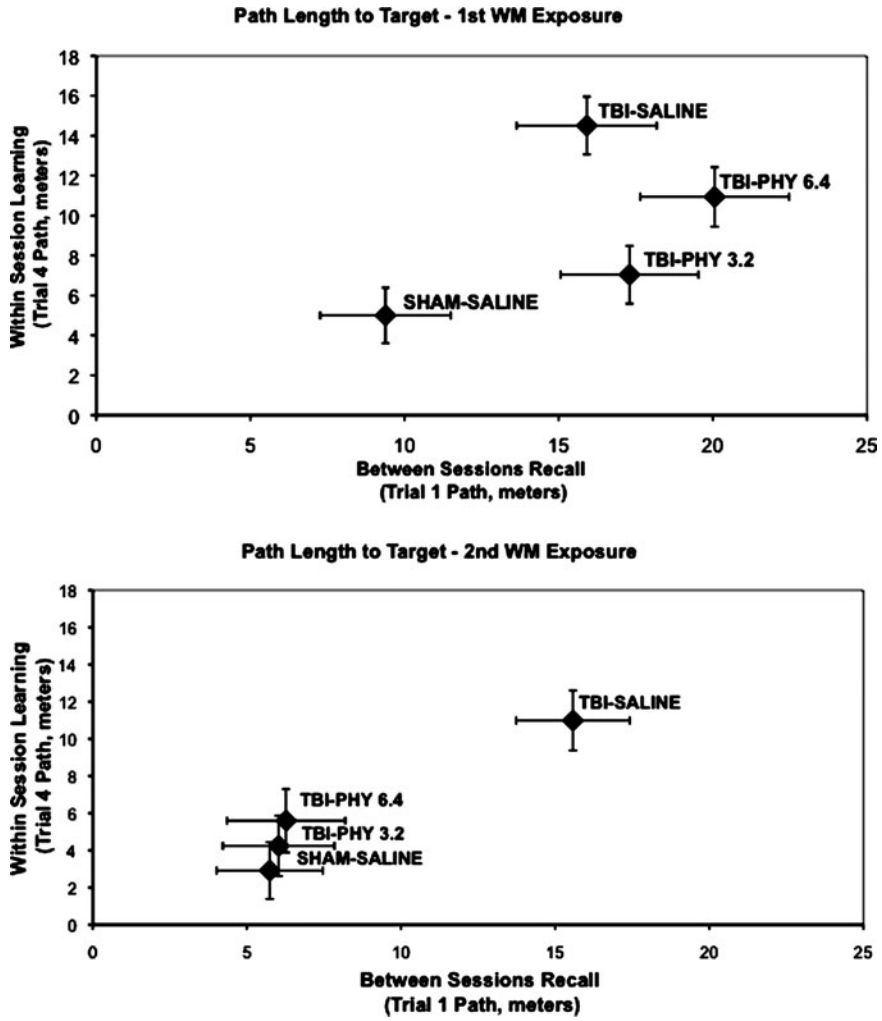

FIG. 5. Means (solid diamonds) and standard error (brackets) of within-session learning (ordinate), and between-sessions recall (abscissa), for all experimental groups during the first (top panel) and second (bottom panel) exposures to the water maze (WM). Analysis of variance was performed for each exposure time and if the F-ratio was significant ($p < 0.05$), Dunnett's test was used to assess significance ($p < 0.05$) versus the TBI-saline (TBI-SALINE) or Sham-saline (SHAM-SALINE) means. For between-sessions recall (abscissa), during the first WM exposure (top panel) all three TBI groups were significantly different from the Sham-saline group, but were no different from one another. During the second WM exposure (bottom panel) both TBI-physostigmine groups and the Sham-saline group were significantly different from the TBI-saline group, but were no different from one another. For within-session learning (ordinate), during the first exposure (top panel) the TBI-saline and TBI-PHY6.4 groups were significantly different from the Sham-saline group, but the TBI-PHY3.2 group was not. The Sham-saline and TBI-PHY3.2 groups were significantly different from the TBI-saline group, but the TBI-PHY6.4 was not. During the second exposure (bottom panel) both TBI-PHY groups and the Sham-saline group were significantly different from the TBI-saline group, but were no different among themselves (TBI-PHY3.2, TBI and physostigmine 3.2 μmol/kg/day; TBI-PHY6.4, TBI and physostigmine 6.4 μmol/kg/day; TBI, traumatic brain injury).

Comparisons of the performance of the experimental groups between both WM exposures indicated that TBI animals that received saline did not improve their path length to target with repeated training (between-sessions recall: WM1 15.9 ± 2.8, WM2 15.6 ± 2.9, $p = 0.93$; within-sessions learning: WM1 14.4 ± 2.6, WM2 10.9 ± 2.5, $p = 0.32$). In contrast, TBI animals that received physostigmine did improve significantly in both variables with repeated training at 3.2 μmol/kg/day (between-sessions recall: WM1 17.4 ± 2.0, WM2 6.0 ± 2.0, $p < 0.001$; within-sessions learning: WM1 7.1 ± 0.8, WM2 4.2 ± 0.8, $p = 0.01$), and 6.4 μmol/kg/day (between-sessions recall: WM1 20.1 ± 2.0, WM2 6.3 ± 2.0, $p < 0.001$; within-sessions learning: WM1 10.9 ± 1.4, WM2 5.6 ± 1.4, $p < 0.01$). Sham animals that received saline improved significantly on within-sessions learning with repeated training (WM1 5.1 ± 0.7, WM2 2.9 ± 0.7, $p = 0.04$), but not on between-sessions recall (WM1 9.3 ± 1.3, WM2 5.7 ± 1.4, $p = 0.07$).

Discussion

Cerebral cortex impact injury has been extensively studied in experimental animals. With this technique, the cortex subjacent to the striking plate undergoes infarction with later development of a cyst and a variable decrease in the volume of the affected cerebral hemisphere.[32–36] This trauma-induced tissue loss was also present in our experiments and physostigmine treatment brought about a decrease of its magnitude. Thus, it was of interest to determine if this morphological damage reduction was the direct cause of the functional improvements observed in the water maze tests. The

lack of correlation between the distance swam to the target and lesion size for both training sessions, and latency to the target for the second training session, disproved this hypothesis. It appears that the levels of cortical impact that interfere with function are lower than those inducing gross anatomical damage. This is in accord with reports in which impairment of water maze behavior has been observed after cortical contusion in the absence of tissue loss.[37]

Increasing the physostigmine infusion rate from 3.2 to 6.4 μmol/kg/day did not improve performance. On the contrary, on the first exposure to the water maze the dose of 6.4 μmol/kg/day was ineffective, and only on the second exposure did it approach the effectiveness of the lower dose. This is in accord with a similar phenomenon seen for the effects of TBI motor deficits in a study in which we explored a wide range of infusion rates that changed cerebral AChE activity from 95% to 40% of control levels.[27]

The conclusion that AChE inhibition combined with repeated training induces further improvement in the water maze is based on two facts. First, during the first training session there was no improvement in between-sessions recall at any dose of physostigmine, and only at the dose of 3.2 μmol/kg/day for within-session learning. The animals in both dose groups showed improvement to levels similar to those of Sham-saline animals in the second training session. Thus, the beneficial effect of physostigmine is only fully achieved with repeated training. The repeated training did not improve the performance of the TBI animals that received saline, which were still significantly different than all the other groups on recall and within-session learning, thus training alone does not have a beneficial effect for TBI animals. Second, ANOVA indicated significance for the interaction between the factors ''training'' and ''treatment.''

A decrement in performance in the water maze of animals with cortical impact injury has been demonstrated by many authors.[38–44] Although we did not find gross morphological changes in the hippocampus, an alteration of neural circuits involving this region after a cerebral cortex impact injury cannot be overlooked. In fact, many authors have demonstrated molecular and fine morphological alterations in the hippocampus following a primary cortical impact injury.[45–48,58]

Selective manipulation of the central cholinergic system induces a profound deficit in water maze performance,[49] making this methodology suitable for the assessment of cholinergic dysfunction.

The brain's cholinergic system is profoundly altered by cerebral cortex impact injury. Decreases in the expression of several muscarinic and nicotinic receptors,[16,50–52] vesicular ACh transporters,[53,54] and acetylcholine turnover[55,56] and release,[57] have been described. Remediation of these deficits is an excellent therapeutic goal for the management of TBI. AChE inhibition is particularly attractive as a treatment for TBI, because it theoretically acts only at sites that are innervated by cholinergic terminals, thus promoting enhancement of cholinergic function where deficits have been pinpointed by pre-clinical research, and sparing activation of receptors with potential toxicity and no functional correlates, as would happen with direct agonists. Moreover, animal experimentation has shown that although cholinergic innervation is lost at the core of the lesion where severe neuronal loss has occurred, it appears intact in the surrounding and contralateral areas, as evidenced by normal levels of choline acetyltransferase (ChAT) and preserved ACh turnover.[55] A number of interventions aimed at improving cholinergic transmission with AChE inhibitors in experimental animals with TBI have been successful, but translation to the clinical arena has met with limited success for a number of reasons. First, there is a lack of large prospective, randomized studies. Most of the evidence that exists, although encouraging, is based on case reports, small open-label series, and only one double-blinded study, all of which had short periods of administration of the AChE inhibitors. In addition, these clinical studies were begun between 18 and 180 weeks post-injury.[26,58] The full benefit of AChE inhibition, as seen in the present study, may be achieved in the acute and subacute periods, when tissue remodeling is still underway. AChE inhibition reverses the cerebral ischemia present in acute TBI, which may be a key factor in the reduction of lesion size, and it enhances perfusion, an index of activation, on the contralateral symmetrical side, which may indicate a beneficial effect on functional compensation.[28,34] Given the dynamic nature of ACh turnover, continuous administration of AChE inhibitors is needed at appropriate rates to avoid toxic effects, while obtaining maximal therapeutic benefits. Thus, careful attention should be paid to the pharmacokinetics of the agents used with individual titration of dosages.

An important element uncovered in the present work is the effect of repeated training on the improvement of cognitive function induced by AChE inhibition. The results show that although ineffective for enhancing recall between days on the first exposure to the water maze, continuous physostigmine infusion combined with training (our animals underwent 40 trials in the water maze before achieving the final outcome) induces improvement in this variable, to levels seen in uninjured animals. Thus, pharmacological treatment alone, in the absence of other rehabilitation modalities, may not be enough to attain full therapeutic benefits.

In summary, AChE inhibition with continuous administration of physostigmine instituted at the time of injury, combined with training, was able to reverse the deficits in memory and learning of animals with cortical injuries. In addition, a reduction in the magnitude of tissue loss was associated with this treatment.

Acknowledgments

This work was supported by Department of Veterans Affairs Rehabilitation Research & Development Merit Review (VA RR&D) #B5089R, and a VA RR&D Research Career Scientist award (to O.U.S.).

Author Disclosure Statement

No competing financial interests exist.

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