Delayed tranexamic acid after traumatic brain injury impedes learning and memory: Early tranexamic acid is favorable but not in sham animals

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BACKGROUND: Early but not late tranexamic acid (TXA) after TBI preserves blood-brain-barrier integrity, but it is unclear if and how dose timing affects cognitive recovery beyond hours postinjury. We hypothesized that early (1 hour post-TBI) but not late (24 hours post-TBI) TXA administration improves cognitive recovery for 14 days.

METHODS: CD1 male mice (n = 25) were randomized to severe TBI (injury [I], by controlled cortical impact) or sham craniotomy (S) followed by intravenous saline at 1 hour (placebo [P1]) or 30 mg/kg TXA at 1 hour (TXA1) or 24 hours (TXA24). Daily body weights, Garcia Neurological Test scores, brain/lung water content, and Morris water maze exercises quantifying swimming traffic in the platform quadrant (zone [Z] 1) and platform area (Z5) were recorded for up to 14 days.

RESULTS: Among injured groups, I-TXA1 demonstrated fastest weight gain for 14 days and only I-TXA1 showed rapid (day 1) normalization of Garcia Neurological Test ($p = 0.01$ vs. I-P1, I-TXA24). In cumulative spatial trials, compared with I-TXA1, I-TXA24 hindered learning (distance to Z5 and % time in Z1, $p < 0.05$). Compared with I-TXA1, I-TXA24 showed poorer memory with less Z5 time (0.51 vs. 0.16 seconds, $p < 0.01$) and Z5 crossing frequency. Unexpectedly, TXA in uninjured animals (S-TXA1) displayed faster weight gain but inferior learning and memory.

CONCLUSION: Early TXA appears beneficial for cognitive and behavioral outcomes following TBI, although administration 24 hours postinjury consistently impairs cognitive recovery. Tranexamic acid in sham animals may lead to adverse effects on cognition. (*J Trauma Acute Care Surg.* 2024;96: 26–34. Copyright © 2023 American Association for the Surgery of Trauma.)

KEY WORDS: Tranexamic acid; traumatic brain injury; water maze; learning; memory.

Traumatic brain injury (TBI) is a major cause of death and disability worldwide, impacting more than 60 million people each year, with incidence rising in the last decade.[1–3] When TBI occurs, pathophysiologic effects are typically divided into a brief, initial and a secondary, elongated phase—the first, occurring within minutes of the head impact, and the latter, manifesting days to weeks after impact.[4,5] Traumatic brain injury survivors will often experience debilitating cognitive dysfunction including deficits in memory, attention, processing speed, and executive functions.[6–8] It is in the first secondary phase hours after TBI that interventions can be applied to mitigate progression of cerebral inflammation and swelling and potentially improve outcomes.[9,10] Presently, few proposed therapies have been successful in improving outcomes, and thus, none has been broadly implemented in the care of TBI patients.[7] A promising post-TBI therapy that could offer such potential is tranexamic acid (TXA).

Tranexamic acid is an antifibrinolytic agent commonly used to reduce blood loss in patients experiencing critical hemorrhage postinjury.[11,12] A synthetic lysine analog, TXA, inhibits fibrinolysis by blocking lysine binding sites on plasminogen and arresting the conversion of plasminogen into plasmin.[12] Independently, TXA may also modulate plasmin's activation of complement, leukocytes, and other host immune cells, thereby inhibiting the postinjury immune response and decreasing neurovascular inflammation and blood brain barrier (BBB) permeability.[13,14] Post-TBI TXA administration decreases mortality and may enhance neurological recovery, especially when administered early after injury.[13,15,16] Beneficial TXA effects may thus be time dependent, leading us to investigate whether the timing of post-TBI TXA administration differentially influences cognitive recovery. We hypothesized that early, but not late, TXA administration after TBI would result in improved murine learning and memory as well as neuroclinical recovery for 2 weeks after injury.

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MATERIALS AND METHODS

Experimental Design and Study Groups

Experimental procedures were approved by the Institutional Animal Care and Use Committee. The Animal Research: Reporting of In Vivo Experiments Checklist was followed, completed, and submitted as Supplemental Digital Content (Supplementary Data 1, http://links.lww.com/TA/D292). CD1 adult male mice (27–32 g) (Charles River Laboratories, Wilmington, MA) were acclimatized in standard housing facilities using dark and light cycles with water and chow ad libitum. On day 0, all mice underwent either sham craniotomy (S) or TBI by controlled cortical impact (CCI; injury [I]) (Fig. 1).

At 1 or 24 hours after CCI, injured animals received a single intravenous (lateral tail vein) injection of either normal saline (NS; placebo [P1]) (Baxter Healthcare Corporation, Deerfield, IL) or TXA (30 mg/kg) (AuroMedics Pharma LLC, E. Windsor, NJ). Uninjured sham animals received either NS or TXA at only 1 hour after sham craniotomy. Tranexamic acid dosing was derived from the standard bodyweight dosage for a 70-kg human receiving a 2-g (28 mg/kg) bolus.[17]

Twenty-five mice were randomly assigned to one of five groups (n = 5 for each): (1) sham craniotomy and NS 1 hour postcraniotomy (S-P1), (2) sham craniotomy and TXA 1 hour postcraniotomy (S-TXA1), (3) CCI and placebo 1 hour post-CCI (I-P1), (4) CCI and TXA 1 hour post-CCI (I-TXA1), and (5) CCI and TXA 24 hours post-CCI (I-TXA24).

Convenience sampling was used to establish animal group numbers, supported by previous reports from our laboratory using identical Morris water maze (MWM) investigations and data analyses. Animal Research: Reporting of In Vivo Experiments guidelines were fulfilled in their entirety (Supplemental Digital Content, Supplementary Data 1, http://links.lww.com/TA/D292).

Severe TBI Murine Model

Controlled cortical impact is a validated murine injury model that replicates severe TBI.[18] On day 0, mice were anesthetized intraperitoneally with ketamine (Mylan Institutional, Rockford, IL), xylazine (Akorn Inc., Lake Forest, IL), and acepromazine (Boehringer Ingelheim, Duluth, GA) (100, 10, and 1 mg/kg, respectively). This was followed by subcutaneous injection of bupivacaine (Fresenius Kabi, Lake Zurich, IL; 0.5 mg/mL) and buprenorphine-SR (1 mg/kg; ZooPharm, Laramie, WY) for extended analgesia. Mice were then placed prone in a stereotactic device, and a left-sided, 4-mm circular craniotomy outline was marked between the bregma and lambda sutures with a 4-mm trephine. A craniotomy was then created on this periosteal outline using a dental drill (Henry Schein, Melville, NY). All animals underwent craniotomy without durotomy. In TBI animals, after skull flap removal, a controlled cortical impactor (AMS201; AmScien Instruments, Richmond, VA) was used to injure the exposed left parietotemporal cortex, using standardized parameters known to replicate severe TBI (impactor tip diameter, 3 mm; impact velocity, 6 m/s; cortical deformation depth, 1 mm). The scalp was sutured closed in all animals once hemostasis was achieved.

Body Weight Loss and Neurological Recovery

Animal body weights were recorded before craniotomy (W0) and daily for the subsequent 14 days, with the weight loss versus W0h expressed as a percentage: ([W0 − Wx]/W0 × 100%, where x = day after CCI/sham).

Animal neurological function was scored using the validated Garcia Neurological Test (GNT), which assesses rodent motor, sensory, reflex, and balancing ability (maximum score, 18).[19] All animals underwent daily GNT scoring for 14 days after CCI or sham craniotomy. Optimal recovery was indicated by a near-maximal or maximal score.[17,18] All animals had a precraniotomy GNT score of 18.

Brain and Lung Water Content

After day 14, water maze trials were completed, animals were sacrificed, and their brain and lungs were removed. The brain was separated into injured (ipsilateral) and uninjured (contralateral) hemispheres, and wet weight (WW) was determined immediately after organ procurement. Dry weight was obtained 72 hours after dehydration at 70°C. Percent tissue water content was calculated using a wet-to-dry ratio (% water content = 100 × [(WW – dry weight)/WW]).

Morris Water Maze

On day 6 post-CCI/sham craniotomy, mice were introduced to daily exercise trials in an MWM (Fig. 1) by one of the authors (M.C.) who had no knowledge of treatment or group allocation. The MWM is a circular tub with a diameter of 100 cm, filled with 22°C water (Supplemental Digital Content, Supplementary Fig. 1, http://links.lww.com/TA/D291). The maze is sectioned into four equal quadrants, with a 10-cm-diameter platform

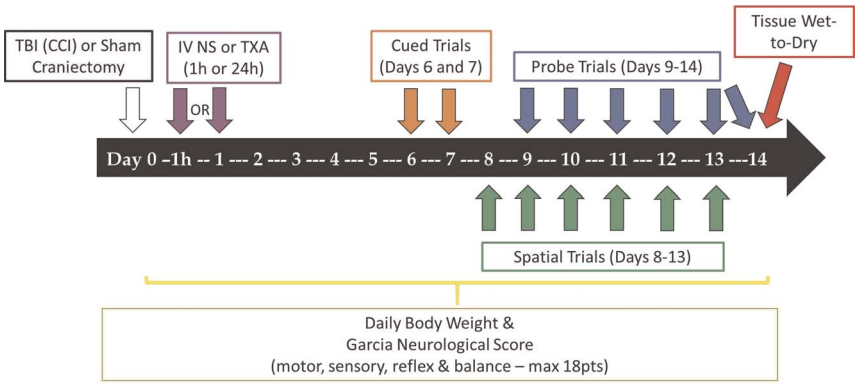

Figure 1. Experimental timeline.

centrally located in the north quadrant (zone [Z] 1). Zone 5 represents the area of the pool where the platform is placed, regardless of whether the platform is actually present. Morris water maze exercises involved cued, spatial, and probe trials, which were conducted and scored by an operator blinded to the animals' experimental groups.

Cued Learning Trials

On days 6 and 7, mice underwent four cued trials per day (totaling eight trials) to familiarize them with the maze and establish platform arrival as the goal. In these trials, the platform was randomly placed in a quadrant above the water level and indicated by a red flag atop the platform center (Supplemental Digital Content, Supplementary Fig. 1, http://links.lww.com/TA/D291). The platform was set 1 cm above water level, thereby making it visible to the swimming mouse; no other visual cues were used. Mice were randomly placed facing the walls of the maze, in different starting locations (north, east, south, west) and given 60 seconds to reach the platform. Mice that did not reach the platform were guided to it by the operator and allowed to stay on the platform for 15 seconds before being removed from the maze and dried. Mice were then placed under a heat lamp, and given 10 minutes to rest and warm between trials.

Spatial Learning Trials

On days 8 to 13 (totaling 20 trials), mice underwent spatial learning trials where the platform was placed solely in the north quadrant, 1 cm below the waterline and without a platform flag. Colorful and distinctive spatial cues were placed on the edge of the pool wall at each cardinal point (north, east, south, west), assisting the animals in localizing the hidden platform (Supplemental Digital Content, Supplementary Fig. 1, http://links.lww.com/TA/D291). The purpose of spatial learning trials was to gauge the animals' ability to use visual cues to navigate to the goal, the platform. Mice underwent four trials per day, with each trial starting with the mouse facing outward at various points relative to the platform to prevent them from readily identifying the platform before entering the maze. Mice were given 60 seconds to reach the platform in each trial. If they failed to reach the platform in this timeframe, they were guided onto it and allowed 15 seconds rest before being removed from the maze. After each trial, the mice were dried and then placed under a heat lamp for 10 minutes to warm between trials.

Probe Memory Trials

The final exercises were probe memory trials conducted on days 9 to 14, during which the platform was removed, but the pool wall cues remained as they were for spatial trials. The aim was to assess animals' long-term memory of platform location using visual cues. Animals were released from various positions along the perimeter of the pool but only given 30 seconds of swim time, after which they were removed from the water. A daily probe trial was conducted after spatial trials on days 9 to 13 to assess memory from the previous day's learning trials. On day 14, four probe trials were performed without any preceding spatial trial, totaling nine probe trials. After each trial, mice were dried and then placed under a heat lamp for 10 minutes to warm and rest between trials.

All MWM exercises were recorded by a camera mounted above the maze. Video recording and analyses were conducted with commercial video tracking software (Ethovision; Noldus, Leesburg, VA). Computerized analyses yielded exercise parameters aimed to quantify animal learning and memory of the platform's location, presence, and absence. Analyzed parameters in each set of trials included the following: delay (latency) to arrive to platform/Z1/Z5 (in seconds), distanced swum to reach platform/Z1/Z5 (in centimeter), duration in Z5 (in seconds, probe trials only), percent duration spent in Z1/Z5 (spatial and cued trials only), and frequency of mouse crossings into Z5 (probe trials only). Swimming velocity was measured in centimeter per second (cm/s). For simplicity, all results for a given parameter were reported and compared as a mean summation across all days tested unless otherwise stated.

Statistical Analysis

All data analyses are presented as mean ± SEM, and graphs were created in Prism (GraphPad Software, San Diego, CA; 2022). A sample size of five animals per group was used, as multiple previous studies[20] using the same MWM exercises demonstrated that four to six animals per group were sufficient in this model to elicit significant differences in both swimming distance to Z5 and probe trial Z1 latency. For all outcomes measured, five post hoc pairwise comparisons were conducted using analysis of variance with Bonferroni's correction to determine significance between group means (corrected $\alpha = 0.008$). p Values of <0.05 were considered statistically significant.

RESULTS

Body Weight Loss and Neurological Recovery

In animals subjected to TBI, extent of animal weight loss (initially), extent of subsequent body weight recovery, and rapidity of this recovery over days is a surrogate of neurological (and systemic) recovery from injury. The particularly high rate of body weight loss recovery after days 5 to 7 in I-TXA1 animals was distinct to that of all other injured animal groups (Fig. 2). Weight loss in the initial 24 hours following CCI/sham craniotomy was ubiquitous with the greatest loss in I-TXA24 (−10.3 ± 1.1%) and I-P1 (−7.2 ± 2.2%, $p > 0.05$). Initially, all groups demonstrated similar rates of weight gain but, by day 7, S-TXA1 and I-TXA1 demonstrated accelerated weight gain (Figs. 2*A* and *B*). On days 12 and 13, S-TXA1 (18.5 ± 3.8%, 18.6 ± 3.1%) had increased weight gain compared with both S-P1 (5.4 ± 2.3%, $p = 0.04$; 5.4 ± 1.7%, $p = 0.02$) and I-TXA24 (5.7 ± 2.6%, $p = 0.049$; 6.6 ± 2.8%, $p = 0.04$) but not I-TXA1 (14.8 ± 2.7%, $p = 0.99$; 14.5 ± 1.2%, $p = 0.99$). Two weeks post-CCI/sham craniotomy, the only difference was between S-TXA1 (19.1 ± 3.5%) and S-P1 (6.1 ± 2.4%, $p = 0.049$).

On day 1 of GNT testing, TXA administered at 1 hour (I-TXA1, 17.6 ± 0.3) significantly improved GNT scores over those of the I-P1 animals (16.4 ± 0.4, $p = 0.01$), but when TXA was administered at 24 hours (I-TXA24, 15.8 ± 0.2, $p = 0.8$ vs. I-P1), GNT remained at I-P1 levels (Fig. 3). I-TXA1 GNT was also significantly better than that of I-TXA24 ($p < 0.001$). Mean GNT differences were no longer significant after day 1 post-CCI/sham craniotomy, and all animals consistently reached maximal scores on days 7 to 14.

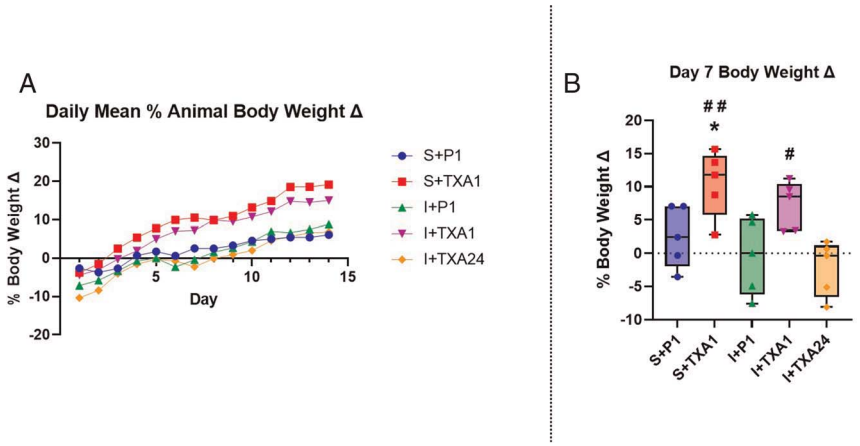

Figure 2. (*A*) Daily mean % animal body weight change for 14 days following sham craniotomy or CCI. (*B*) Specific depiction of panel A on day 7 after CCI/sham craniotomy when group differences begin to amplify where animal bodyweight change is compared with preinjury weight. *$p < 0.05$ versus I-P1; #$p < 0.05$ versus I-TXA24; ##$p < 0.01$ versus I-TXA24.

Brain and Lung Water Content

When comparing water content in all organs, no differences were noted across injured groups regardless of treatment timing (Figs. 4*A–C*). However, uninjured animals treated with TXA (S-TXA1) demonstrated significant less tissue water in sham craniotomy cerebral hemispheres (ipsilateral, 73.5 ± 0.6%, $p < 0.02$ vs. all other groups), uninjured cerebral hemispheres (contralateral, 73.2 ± 0.6%, $p < 0.03$ vs. all other groups), and lungs (69.3 ± 1.8%, $p < 0.01$ vs. S-P1, I-P1, and I-TXA1, but $p = 0.06$ vs. I-TXA24).

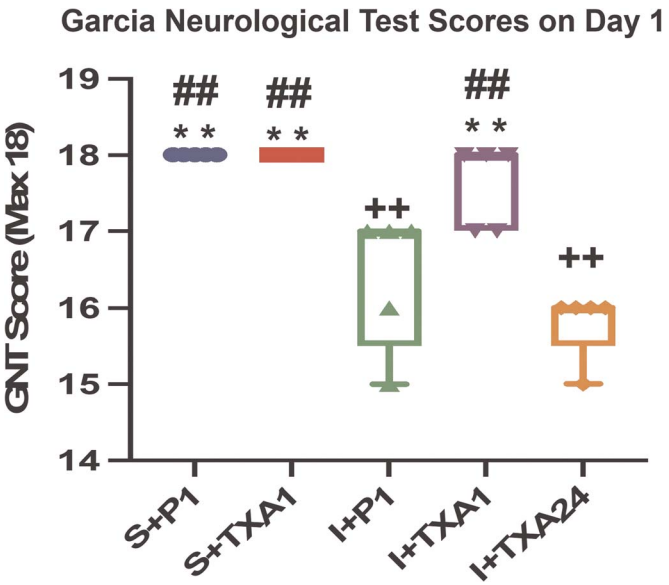

Figure 3. Animal functional neurological recovery 1 day after sham/injury, as calculated by the GNT (max score, 18). Nonstatistically significant trends continued in subsequent days until day 7; after 7 days and beyond, all groups exhibited maximum GNT scores. *$p < 0.05$ versus I-P1; **$p < 0.01$ versus I-P1; ##$p < 0.01$ versus I-TXA24; ++$p < 0.01$ versus S-P1.

Morris Water Maze

In cued learning trials, there were no observable differences in the aforementioned parameters across any group. Unexpectedly, I-TXA1 animals displayed the slowest cued trial swimming velocity (18.9 ± 0.8 cm/s), significantly slower than I-P1 (27.8 ± 2.6 cm/s, $p < 0.01$), S-TXA1 (29.4 ± 1.8 cm/s, $p < 0.01$), and S-P1 (27.6 ± 1.7 cm/s, $p < 0.01$).

During spatial learning trials, no differences were found among groups in platform latency or platform duration, but the I-TXA24 group consistently underperformed the I-TXA1 group in mean total distance traveled to platform (32.4 ± 0.5 vs. 27.0 ± 0.9 cm, $p = <0.001$), zone 1% duration (0.21 ± 0.01% vs. 0.31 ± 0.02%, $p < 0.0001$), and Z1 latency (8.9 ± 0.8 vs. 6.1 ± 0.7 seconds, $p = 0.01$) (Figs. 5*A–C*). I-TXA1 animals also traveled a lower mean distance to reach the platform than I-P (27.0 ± 0.9 vs. 30.5 ± 0.7 cm, $p < 0.01$) (Fig. 5*A*). I-TXA24 mice tended to perform worse than I-P1, although not significantly ($p = 0.6$). The S-TXA1 group performed consistently worse than S-P1 in all spatial parameters mentioned previously and tended to perform worse than injured groups (Fig. 5). The only significant disparity in swimming velocity across all spatial trials was noted in the S-TXA1 group where mice swam significantly faster than in all other groups on day 13 ($p < 0.01$ vs. S-P1, $p < 0.01$ vs. I-P1, $p < 0.0001$ vs. I-TXA1, $p < 0.001$ vs. I-TXA24).

In the memory probe trials (platform removed) conducted on days 9 to 14 postinjury, I-TXA24 (0.16 ± 0.03 seconds) spent the least time in the platform zone (Z5) compared with I-TXA1 (0.51 ± 0.08 seconds, $p < 0.01$), which performed similarly to I-P1 (0.37 ± 0.05 seconds, $p = 1.0$) (Fig. 6*A*). I-TXA24 mice (0.58 ± 0.12 crossings) crossed into the Z5 with the lowest frequency among all groups ($p = 0.02$ vs. I-TXA1, $p = 0.09$ vs. I-P1, $p < 0.0001$ vs. S-P1, $p = 0.03$ vs. S-TXA1) (Fig. 6*B*). No differences were noted across any group in probe latency or distance to Z1 or Z5.

DISCUSSION

In the current study, we explored cognitive and functional neurological recovery using a validated in vivo, murine, severe

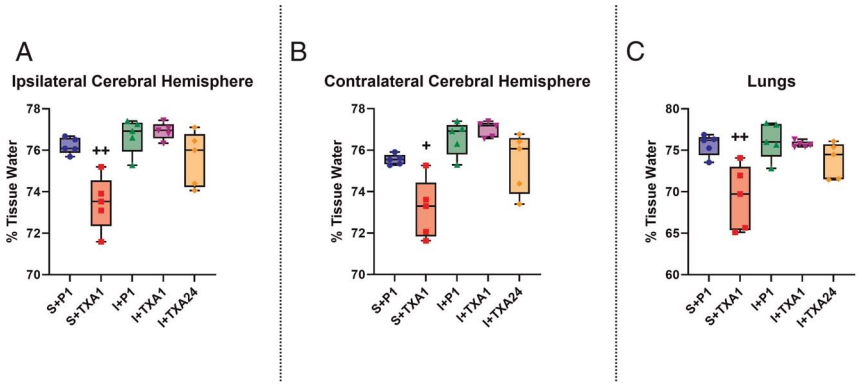

Figure 4. Organ tissue water content: (*A*) ipsilateral (injured) cerebral hemispheric, (*B*) contralateral (uninjured) cerebral hemispheric, and (*C*) pulmonary tissue water measured by wet-to-dry ratios (↑% tissue water = ↑ edema). ++$p < 0.05$ versus S-P1, I-P1, I-TXA1, and I-TXA24; +$p < 0.01$ versus S-P1, I-P1, and I-TXA1, but $p = 0.06$ versus I-TXA24.

TBI model. Our findings suggest that mice receiving TXA 24 hours postinjury performed worse in terms of learning (spatial trials) and memory (probe trials) compared with those treated with TXA 1 hour postinjury, which had similar outcomes to sham animals. Early TXA treatment also facilitated quicker neurological recovery, with improvements in functional neurological scores and body weight recovery. Finally, unexpectedly, in uninjured animals, early TXA resulted in decreased tissue edema, greater weight gain, and earlier improvement of GNT scores but consistently demonstrated hindered learning and memory MWM parameters.

Following TBI, external mechanical forces deform cerebral tissue and initiate a cascade of pathophysiologic events resulting in BBB dysfunction and a rapidly escalating local and systemic proinflammatory host immune response.[21–23] In particular, activated leukocyte and endothelial cells interact closely in the penumbral neurovasculature, promoting release of cytokines and chemokines that further recruit other inflammatory cells advancing the release of reactive oxygen species and cytotoxic proteases. This, in turn, results in further damage to cerebral tissue, fostering neuronal cell death, axon degeneration, oxidative stress, and cerebral edema, which may ultimately lead to brain herniation if left untreated.[21,24–26]

This self-promoting cascade of tissue level injury in TBI patients invariably manifests in downstream cognitive sequelae that often lead to acute, subacute, and chronic impairment and disability. Indeed, TBI is the most important injury responsible for cognitive impairment.[27] Executive cognitive function and memory are the most vulnerable of cognitive abilities affected by TBI, with memory impairment in particular being the main source of long-term disability.[8,27] Such impairments have a profound and pervasive negative effect on activities of daily living as memory and cognitive executive functions control learning, attention, planning, decision making, and social behavior, all of which are required to complete routine tasks.[8] Both focal and diffuse severe TBI can influence these cognitive functions, through alterations of cerebral circuitry in the prefrontal cortex and temporal lobe. Traumatic brain injury also directly influences hippocampal and thalamic function causing histopathological changes, observed first during the secondary response to injury.[27,28] In animal CCI models, the MWM has long been used to test and observe such cognitive deficits, providing understanding of functional recovery after TBI but also aiding in the exploration of therapeutic options for brain injury.[20,29] Similarly, but using a weight-drop TBI model, Schwarzbold et al.[30] showed that mild TBI caused anxiety and depressive behaviors,

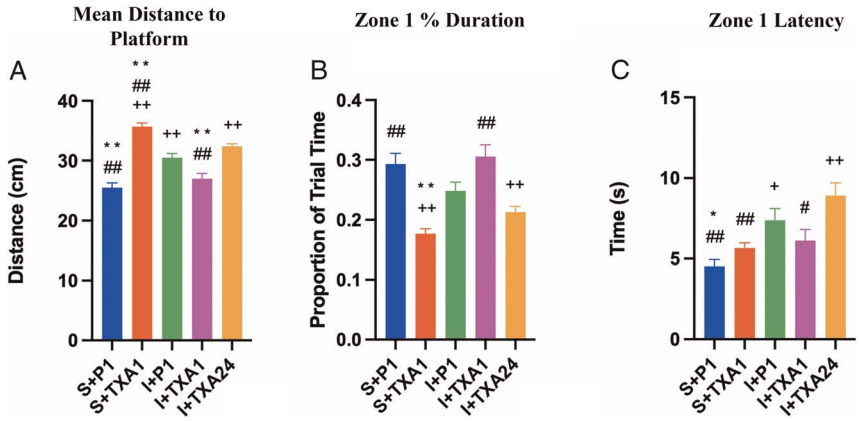

Figure 5. Spatial trials: (*A*) The mean distance traveled to reach the platform. (*B*) Summative time spent in Z1, the quadrant containing the platform, taken as a percentage of total trial time. (*C*) Summative time to reach Z1. *$p < 0.05$ vs. I-P1; **$p < 0.01$ versus I-P1; #$p < 0.05$ versus I-TXA24; ##$p < 0.01$ versus I-TXA24; +$p < 0.05$ versus S-P1; ++$p < 0.01$ versus S-P1.

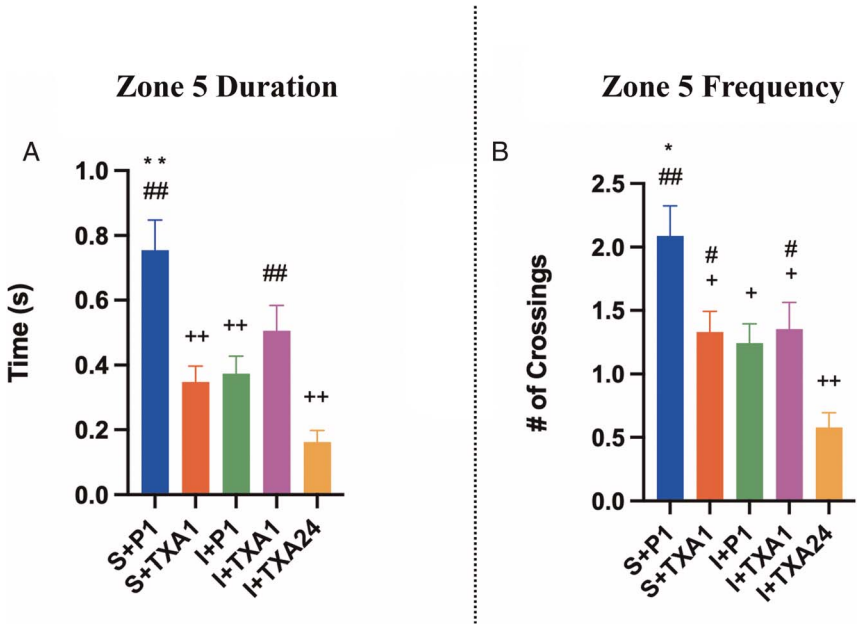

Figure 6. Probe trials: (*A*) Mean amount of time spent swimming in the platform area (Z5). (*B*) Mean number of crossings into the platform area (platform removed). *$p < 0.05$ versus I-P1; **$p < 0.01$ versus I-P1; #$p < 0.05$ versus I-TXA24; ##$p < 0.01$ versus I-TXA24; +$p < 0.05$ versus S-P1; ++$p < 0.01$ versus S-P1.

while severe TBI resulted in significant memory deficits. The same group additionally confirmed histologically that severe TBI resulted in extensive tissue damage to the frontal and parietal cortex, with architectural and neuronal losses in both the cortex and hippocampus.[30] Given the pervasive and significant debilitating burden of severe TBI, identifying effective therapeutics to mitigate cognitive deficits and help improve the long term quality of life of TBI patients is imperative.

Tranexamic acid is one such potential therapeutic that has recently emerged as possessing attributes able to modulate the host immune post-TBI response through inhibition of the plasminogen activation pathway. Plasminogen is transformed into plasmin by two main activators: tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), which are both inhibited by antifibrinolytics such as TXA.[31,32] Tranexamic acid is a synthetic lysine analog that competitively binds to high and medium affinity Kringle domains on plasminogen lysine binding sites, preventing plasminogen from binding to fibrin clots and being cleaved into plasmin.[32,33] The accumulation of plasmin and other byproducts of plasminogen breakdown has been implicated in sustained tissue inflammation, which is reversed by TXA. The time dependence of these effects has been demonstrated in a host of animal and human studies and may be related to the fact that tPA and uPA achieve their peak concentrations at different times following injury.[33] Tissue plasminogen activator peaks in the immediate period after initial injury, while uPA peaks several hours later. In a murine study, Hijazi et al.[34] used an intracerebral hemorrhage TBI model and found that tPA in cerebrospinal fluid peaked immediately after injury and then fell to 50% of maximal concentration 4 hours later, whereas uPA cerebrospinal fluid concentrations did not peak until 8 hours postinjury. This has led some investigators to posit that TXA behaves in two opposing, time-dependent manners: (1) as an antifibrinolytic when tPA-driven plasminogen activation peaks and (2) as a profibrinolytic when uPA-driven activation peaks. Similar to its time-dependent effects on fibrinolysis, TXA appears to have parallel effects on inflammation as well, acting as an early anti-inflammatory and, later on, as a pro-inflammatory driver. This was corroborated in a study where TXA administration reduced C5a generation during early tPA-driven fibrinolysis (anti-inflammatory response) but increased C5a generation during later uPA-driven fibrinolysis (pro-inflammatory response).[33] Such a dichotomous pro-inflammatory and anti-inflammatory TXA effect by timing has also been observed in injury models, which have noted that early TXA administration reduces interleukin 6 and tumor necrosis factor α plasma concentrations, as well as intestinal neutrophil extracellular traps after injury compared with late administration; early administration more effectively reduces tissue edema and microvascular permeability.[13,35,36]

This apparent pathophysiologic time-dependent contradiction may explain the clinical observations several investigators have made in studying survival outcomes in multitrauma and TBI patients receiving TXA. Notably, the Clinical Randomisation of an Antifibrinolytic in Significant Saemorrhage-2 study demonstrated a significant survival advantage in multitrauma patients receiving TXA before, but not after, 3 hours from injury.[37] The same initiative subsequently published the Clinical Randomisation of an Antifibrinolytic in Significant Haemorrhage-3 trial, specifically evaluating brain-injured patients and again noted a reduced risk of head injury–related mortality when TXA was administered within 3 hours of injury in patients with mild-to-moderate TBI.[15] While survival advantages are consistently found with early post-TBI TXA, both animal and human studies have also investigated differential functional neurological recovery with TXA after injury. Using a CCI model, Daglas et al.[38] found that, in male mice, TXA reduced BBB permeability while simultaneously

exhibiting better motor function with improved mouse step angle and gait symmetry up to baseline levels. In a similar setting, our group previously reported how early TXA administration (1 hour post-TBI) resulted in improved GNT scores at 24 and 48 hours concurrent to greater 48-hour body weight loss recovery — results that were reproduced in the current study. In addition, that study also reported how in vivo penumbral BBB permeability significantly increased at 48 hours after CCI and that early, but not late, TXA administration restored BBB integrity to normal.[13] In the current study, we expanded to a more robust neuroclinical evaluation over an extended period of time and found early TXA-related body weight loss recovery to persist beyond a week after injury, significantly greater in I-TXA1 compared with I-TXA24, while I-TXA24 behaved similarly to untreated injured animals (I-P1).

In human studies, effects of early TXA on neurological recovery are mixed. In a cohort of combat TBI patients, Morte et al.[16] again found that early TXA patients suffered lower mortality but also reported that they manifested improved neurological outcomes at discharge measured by change in GCS. Similarly, in a separate military study, patients with traumatic intracranial hemorrhage receiving TXA manifested greater neurological improvement with superior GCS recovery before discharge.[39] In contradistinction, however, the recently published prehospital antifibrinolytics for traumatic coagulopathy and haemorrhage-Trauma trial did not note improved neuroclinical recovery as measured by the Glasgow Outcome Scale—Extended at 6 months after injury in patients receiving prehospital TXA.[40] However, this population was characterized by multiorgan injury (not isolated TBI), and as the trial was not designed to enroll TBI patients, only a small percentage of enrolled patients ultimately were found to have TBI retrospectively through less reliable methodology involving Abbreviated Injury Scale head/neck or GCS scores; no information was provided regarding intracranial content, imaging, neurological examination, neurosurgical intervention(s), or intracranial pressure monitoring. In a similar North American study, the Prehospital TXA for TBI Trial, which was a randomized, double-blind, multicenter phase II trial, also found no improvement in 6-month Glasgow Outcome Scale—Extended in the combined TXA regimen (all bolus/infusion regimens). However, the group that received a 2-g bolus alone demonstrated an improved Disability Rating Scale Score at 6 months.[17] This is particularly relevant, as, in our study, the TXA regimen involved the equivalent of a 2-g bolus in a 70-kg human and no infusions. In our study, the rate of animal body weight loss recovery showed a significantly greater positive inflection after days 4 and 5 but only in TBI animals receiving TXA at 1 hour, further supporting the more elaborate neurological recovery markers elicited in the MWM exercises (spatial learning and memory).

The finding that animals receiving sham craniotomy (without CCI) manifest differences when exposed to 1-hour TXA was surprising. At first, we attributed this to surgical technique or lack of randomization but noted that none of this was the case with all animals randomized to CCI or sham craniotomy and then again randomized to TXA or placebo, indicating no difference in how groups were treated, managed, or analyzed. Nonetheless, S-TXA1 animals demonstrated improved weight loss recovery and reduced cerebral and lung water, but worsened learning and memory across multiple MWM parameters. These contradictory results are perplexing, and further investigation in TXA administration without TBI is needed to determine if this is a true signal or if a beneficial TXA mechanism of action exists in this less or noninjured populations.

Our study investigated a time-dependent effect of TXA administration on cognitive and neurological recovery following severe TBI in mice. However, the presented murine study must be evaluated within the context of certain limitations. First, our model solely used male CD1 mice, as this has been the only mouse sex and strain that our laboratory has used for the past decade. However, other groups have found sex-dependent differences in physiological responses to TBI and even to TXA exposure after TBI, indicating that current results may not be extrapolatable to females.[38,41,42] No doubt significant bias exists in animal research excluding female subjects in studies fearing excessive variability; however, in the current study, the decision to be single sex specific was purposeful because of known sex-related differences in TBI. Second, this was a small animal study, so the results cannot be directly extrapolated to human TBI physiological responses and may not necessarily translate to how TXA functions in other animal species. Third, although TXA dosage used in our study was based on human bodyweight dosing, the most common form of human administration is an initial bolus followed by a multihour infusion. Because of animal husbandry constraints, only a single TXA bolus regimen was used, meaning that the response to standard bolus-plus-infusion TXA in humans could be different. Fourth, with respect to TXA effects in sham craniotomy, we only investigated TXA administration at 1 hour after craniotomy. An additional sham craniotomy group receiving TXA at 24 hours would have helped elucidate a possible delayed effect of TXA (harmful or beneficial) when brain injury was minimal or nonexistent. Also, adding a TBI-placebo group at 24 hours would have better confirmed that administering placebo at 24 hours had no effects. Fifth, the time delay for the late TXA in TBI group was intentionally chosen to be very long at 24 hours. Human TBI literature[37] only shows 3 hours or more as being a worse time to administer TXA, but it remains unknown if the harm/benefit relationship with dose timing is linear, multipeaked, or follows any other relation. Additional trials will have to study this and establish when exactly is the worse timing for TXA administration in trauma and what, if any, the time/benefit relationship is. In this proof-of-concept preliminary study, we wanted to exaggeratedly delay timing to ensure that any signal of worse outcome with greater delay was captured. Finally, some of the comparisons in spatial and probe memory trials demonstrated trends that approached significance (i.e., $p = 0.09$ for probe latency to Z1). Greater sample sizes despite the power calculation may have elicited these to become significant differences.

CONCLUSION

Early but not late TXA administration after severe blunt TBI improves markers of learning and memory in a murine model for up to 14 days after injury. Surrogates of neuroclinical recovery after TBI show that early TXA administration also accelerates neuroclinical and overall recovery when compared with late TXA or no treatment. It remains to be determined if and why early TXA administration in sham animals alters some but not all markers of neurological recovery. This work adds to

an increasing body of evidence indicating that early TXA administration appears to improve postinjury outcomes.

AUTHORSHIP

M.C.C. contributed in the literature search, study design, data interpretation, writing, and critical revision. M.C. contributed in the experimental procedures, data collection, data analysis, data interpretation, writing, and critical revision. P.B. contributed in the literature search, study design, data interpretation, and critical revision. A.T. contributed in the experimental procedures, data collection, data interpretation, and critical revision. A.P.G. contributed in the experimental procedures, data collection, data interpretation, and critical revision. E.A. contributed in the experimental procedures, data collection, data interpretation, and critical revision. K.D.B. contributed in the experimental procedures, data collection, data interpretation, and critical revision. C.J. contributed in the experimental procedures, data analysis, data interpretation, and critical revision. P.S. contributed in the study design, experimental procedures, data analysis, data interpretation, writing, and critical revision. L.J.K. contributed in the data interpretation and critical revision. D.F.M. contributed in the data interpretation and critical revision. D.H.S. contributed in the data interpretation and critical revision. J.L.P. contributed in the literature search, study design, data collection, data analysis, data interpretation, writing, and critical revision.

DISCLOSURE

Conflicts of Interest: Author Disclosure forms have been supplied and are provided as Supplemental Digital Content (http://links.lww.com/TA/D293).

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