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***Paclitaxel* improves outcome from traumatic brain injury**

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Abstract

Pharmacologic interventions for traumatic brain injury (TBI) hold promise to improve outcome. The purpose of this study was to determine if the microtubule stabilizing therapeutic *paclitaxel* used for more than 20 years in chemotherapy would improve outcome after TBI. We assessed neurological outcome in mice that received direct application of *paclitaxel* to brain injury from controlled cortical impact (CCI). Magnetic resonance imaging was used to assess injury-related morphological changes. Catwalk Gait analysis showed significant improvement in the *paclitaxel* group on a variety of parameters compared to the saline group. MRI analysis revealed that *paclitaxel* treatment resulted in significantly reduced edema volume at site-of-injury (11.92 ± 3.0 and 8.86 ± 2.2 mm³ for saline vs. *paclitaxel* respectively, as determined by T2-weighted analysis; $p = 0.05$), and significantly increased myelin tissue preservation (9.45 ± 0.4 vs. 8.95 ± 0.3 , $p = 0.05$). Our findings indicate that *paclitaxel* treatment resulted in improvement of neurological outcome and MR imaging biomarkers of injury. These results could have a significant impact on therapeutic developments to treat traumatic brain injury.

Keywords

Traumatic brain injury; MR imaging; Microtubule stabilization; Neurological function

1. Introduction

Conservative estimates indicate that approximately 1.7 million traumatic brain injuries (TBIs) occur per year in the United States and this injury is the leading cause of death for

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Conflicts of interest

There are no conflicts of interest to report.

ages 1–45 years. In addition, widespread use of improvised explosive devices (IEDs) against US military has resulted in approximately 17% of veterans reporting persistent cognitive deficits and post-concussive symptoms years after blast-TBI (Sources: *Centers for Disease Control and Prevention* and *The Defense and Veterans Brain Injury Center*). In addition to an increasing concern for the acute effects of traumatic brain injury as a significant healthcare issue, TBI is a known environmental risk factor for the development of neurodegenerative diseases such as chronic traumatic encephalopathy (CTE) and Alzheimer's disease (AD) (Fleminger et al., 2003; Gavett et al., 2011). Repetitive mild TBI (mTBI) in boxers can lead to a dementia-like syndrome that includes motor impairment and cognitive symptoms such as bradyphrenia (slowed thinking), confusion, and memory deficits (Corsellis et al., 1973; Critchley, 1957; Martland, 1928; McKee et al., 2009b; Roberts, 1969). It is becoming increasingly evident that chronic mTBI experienced by football players is associated with CTE in mid-life and is evidenced by diffuse neurofibrillary tangles and occasionally, amyloid plaques (Erlanger et al., 1999; McKee et al., 2009b; Omalu et al., 2005). In addition, both moderate and severe head injuries significantly increase the risk of developing AD (Fleminger et al., 2003; Plassman et al., 2000).

Pharmacologic interventions for traumatic brain injury hold promise to improve patient outcome, however no therapeutic has proven clinically effective to date. In this preliminary study, we tested a hypothesis if stabilizing microtubules might provide therapeutic benefits. In TBI, shear and rotational forces generated by impact cause microtubule destabilization and axonal transport disruption, which may progress to axonal swelling and disconnection (Giza and Hovda, 2001; Johnson et al., 2013; Mac Donald et al., 2007; Smith et al., 2003; Tang-Schomer et al., 2010; Tang-Schomer et al., 2012). Neuronal microtubules are part of the cytoskeletal structure and provide a framework for axonal transport processes. Tau protein, which helps stabilize microtubule structure under normal conditions (Brunden et al., 2011), accumulates in the brains of patients with CTE and AD (McKee et al., 2009a; Petrie et al., 2009). These findings led to our hypothesis that preventing microtubule destabilization may improve recovery from TBI. *Paclitaxel* has been administered for more than 20 years as a common treatment for ovarian, breast, lung, bladder, esophageal, and other types of solid tumor cancers (Yusuf et al., 2003), and is well characterized for its role in binding of microtubules and providing structural stabilization (Amos and Lowe, 1999; Arnal and Wade, 1995). The drug inhibits cellular mitosis by stabilizing the GDP-bound tubulin in microtubules thereby preventing depolymerization, and consequently, tumor cell division (Alberts et al., 1994; Amos and Lowe, 1999; Díaz et al., 2003). Although higher doses can be neurotoxic (Gornstein and Schwarz, 2014), limited research has suggested specific neuroprotective and neurotherapeutic effects of low dose *paclitaxel* (Adlard et al., 2000; Hellal et al., 2011). Microtubule stabilizers have been suggested as potential therapeutics for neurodegenerative disease based on effects on the cytoskeleton and, in particular, tau protein, which is the main component of the pathology of CTE (Brunden et al., 2011; Michaelis et al., 2002). A recent investigation into the effects of dynamic stretch injury on micropatterned neuronal cell cultures revealed that *paclitaxel* application prior to injury greatly reduced axonal degeneration, and resulted in greater axon preservation compared to nontreated cultures (Tang-Schomer et al., 2010). However, the application of

paclitaxel to brain injury *in vivo* has not been fully explored. This investigation is to provide evidence of feasibility for this therapeutic strategy through a direct, topical application of the drug to the injury site. As a P-glycoprotein (P-gp) substrate, *paclitaxel* does not readily cross the blood–brain barrier (BBB) and we wished to assess therapeutic efficacy without confounding results by issues related to delivery. Future investigations to overcome this limitation for clinical translation are presented in the discussion.

Here, we present findings that topical application of *paclitaxel* after controlled cortical impact (CCI) improve functional outcome in mice and we assessed the basis for this improvement with *in vivo* MR imaging. Our primary outcome measure was functional improvement in gait, which was associated with improvements in MR imaging biomarkers of injury volume, injury-associated edema, and relative preservation of myelin surrounding the injury.

2. Results

2.1. Functional improvement after application of microtubule-stabilizing drug

At 7 days post-CCI surgery, C57BL/6J mice ($n = 15$, male, 10 wks) were tested in for gait abnormalities using the Cat-Walk automated gait analysis system (Noldus Information Technology, Wageningen, The Netherlands). *Paclitaxel* treated mice ($n = 6$) showed significant gait improvement over saline group ($n = 6$) in several indices that have been shown to be impaired with CCI in a previous study (Neumann et al., 2009). Spatial parameters related to individual limbs were improved as follows (Fig. 1A–F). Mean intensity, which is a measure of paw pressure on the floor was improved for all paws (22%: 78.20 ± 14.5 vs. 64.15 ± 4.1 , 19%: 81.58 ± 14.5 vs. 68.42 ± 5.1 , 19%: 74.17 ± 12.5 vs. 62.23 ± 3.5 , and 19%: 83.86 ± 13.5 vs. 70.73 ± 4.7 for right front (RF), right hind (RH), left front (LF) and left hind (LH) respectively, arbitrary units). Maximum area is the total floor area of the paw contact in cm^2 and was also improved for all paws (RF, 22%: 0.38 ± 0.1 vs. 0.31 ± 0.1 , RH, 52%: 0.40 ± 0.1 vs. 0.26 ± 0.1 , LF, 27%: 0.36 ± 0.1 vs. 0.28 ± 0.1 and LH, 33%: 0.39 ± 0.1 vs. 0.29 ± 0.1). Print area, assessing the complete print that comprises the stance in cm^2 was also improved significantly for all limbs (RF, 21%: 0.44 ± 0.1 vs. 0.36 ± 0.1 , RH, 45%: 0.47 ± 0.1 vs. 0.33 ± 0.1 , LF, 20%: 0.42 ± 0.1 vs. 0.35 ± 0.1 and LH, 25%: 0.45 ± 0.1 vs. 0.36 ± 0.1). The parameter of print width, expressed in cm was significantly improved for RF, RH and LF only (RF, 5%: 0.89 ± 0.1 vs. 0.84 ± 0.1 , RH, 17%: 0.93 ± 0.1 vs. 0.79 ± 0.1 , and LF, 5%: 0.81 ± 0.03 vs. 0.77 ± 0.04). Print length expressed in cm was improved by *paclitaxel* treatment for RH only (12%: 1.02 ± 0.1 vs. 0.91 ± 0.1). The final parameter that showed significant improvement with *paclitaxel* was swing, which is expressed in seconds and measures the time interval between two consecutive paw placements of the same paw. Improvement in this parameter is a decrease in the time interval, which was seen in the RF and LF (RF, –12%: 0.12 ± 0.01 vs. 0.13 ± 0.02). A threshold of $p = 0.05$ was used to determine significant improvement. No significant changes were found in the other parameters of stance, cadence, and walk speed.

2.2. MR imaging biomarkers indicate improvement

At 4 days postinjury, mice were anesthetized with isoflurane and scanned over the entire brain using an ultra hi-res 14 T MRI (Avance III, vertical bore, Bruker BioSpin Corp, Billerica, MA). Several MR acquisitions were used to discriminate different physiological features of the brain injury response to *paclitaxel* treatment. The CCI injury resulted in a reproducible and definable injury on T1-weighted images that we could use to evaluate differences in injury volume in our *paclitaxel* treated mice compared to those treated with saline. Analysis of injury on T1-weighted images indicated a 20% reduction in volume (9.96 ± 2.3 and $7.94 \pm 1.5 \text{ mm}^3$ for saline ($n = 4$) vs. *paclitaxel* ($n = 6$) treatment, respectively $p = 0.05$). Two saline subjects had artifacts that obscured the injury on T1-weighted images and were excluded from the analysis. Sham subjects ($n = 3$) did not have an injury to quantitate, so were not used for this analysis.

Quantitative T2 mapping can be used to assess the size and extent of injury associated edema and hemorrhage in TBI (Kharatishvili et al., 2009). Using thresholding to delineate the voxels with edema, we found that *paclitaxel* treatment reduced the volume of injury-associated edema by 26% (11.92 ± 3.0 and $8.86 \pm 2.2 \text{ mm}^3$ for saline ($n = 4$) vs. *paclitaxel* ($n = 6$) treatment, respectively $p = 0.05$). The two saline subjects that were excluded from the previous T1-weighted analysis did not have a detectable artifact on the T2 maps, so were therefore included in this analysis. As might be expected, the edema volume assessed on the T2 maps was larger than the volume of actual injured tissue measured on T1-weighted images (11.92 ± 3.0 edema volume vs. 9.96 ± 2.3 lesion volume for saline and 8.86 ± 2.2 vs. 7.94 ± 1.5 for *paclitaxel* treated animals (Fig. 2A–C).

We also examined the effect of *paclitaxel* treatment on non-neuronal cells associated with the brain injury. Macromolecular proton fraction imaging (MPF) of myelin content/degradation utilizes a recently published fast single-point method to quantitatively measure the magnetization transfer between protons bound to water and protons bound to macromolecules (Underhill et al., 2009; Underhill et al., 2011; Yarnykh, 2012). We used MPF imaging to investigate if *paclitaxel* treatment had a beneficial effect on the preservation of non-neuronal brain cells following traumatic brain injury. MPF bound % was significantly increased by 6.6% in *paclitaxel* treated subjects (9.45 ± 0.4 vs. 8.95 ± 0.3 , $p = 0.05$), Fig. 3A and B).

Diffusion tensor imaging (DTI) evaluates the directional diffusion of water molecules in the brain. In white matter tracts, water molecules diffuse more readily along the direction of axonal fibers and movement is more restricted in the radial directions. Fractional anisotropy (FA) derived from DTI is a measure of the relative diffusion along fiber tracts, where decreased values reflect a loss of tract integrity including disruption of axonal structure and/or myelin loss, and have been used to evaluate injury from CCI in rodents (Mac Donald et al., 2007). In this study we hypothesized that *paclitaxel* treated subjects would have increased FA values in the white matter tract directly underlying the injury site (external capsule), due to improved microtubule stabilization after injury. However, contrary to our hypothesis no significant improvement was found in the underlying white matter integrity following CCI with administration of *paclitaxel*. CCI surgery decreased FA in the external

capsule, however saline treated subjects were slightly above the statistical threshold due to a higher standard deviation (saline: 0.33 ± 0.02 , $p = 0.057$, *paclitaxel*: 0.33 ± 0.01 , $p = 0.01$ vs. sham: 0.35 ± 0.01 , Fig. 4A and B).

3. Discussion

In this study, we showed that direct application of *paclitaxel* to the site of controlled cortical impact injury in the brain resulted in neurological improvement at 7 days post injury and that injury-related parameters assessed by MR imaging supported this improved outcome. The findings from imaging biomarkers suggested that the neurological improvement could be mediated by reduced overall injury volume, reduced injury-associated edema, and preservation of myelin in tissue surrounding the injury.

Improvement of functional outcome by *paclitaxel* administration after traumatic brain injury has not been reported previously. We assessed outcome following CCI using a Noldus Catwalk automated gait analysis system, which permits simultaneous, observer-independent analysis of both temporal and spatial aspects of interlimb coordination. This system has been used in previous studies to assess the degree of functional impairment after brain injury in rodents (Mountney et al., 2013; Neumann et al., 2009; Wang et al., 2011). One of these previous investigations used the Catwalk system to assess injury in a mouse model with similar CCI parameters to those used in our study and found deficits related to the injury that overlapped with most of the parameters that were improved by *paclitaxel* treatment in our study, including intensity, maximum area, print area, print width, and swing (Neumann et al., 2009). The unilateral injury model to the fronto-parietal cortex that was employed both by our study and this previous investigation caused deficits in spatial parameters involved with interlimb coordination and also affected sensorimotor function, as evidenced by alterations in paw print measurements. These neurological functions were significantly improved with *paclitaxel* administration.

Most of the literature regarding the effect of *paclitaxel* on neuronal populations is devoted to assessing the mechanisms by which the drug causes peripheral neuropathy during chemotherapy (Gornstein and Schwarz, 2014). In fact, these potential mechanisms are still being debated and symptoms are avoided primarily by lowering acute doses and decreasing the dose frequency should symptoms appear during treatment (Carlson and Ocean, 2011). However, prior to our study there were two reports indicating a neurotherapeutic effect of lower dose *paclitaxel* on central nervous system (CNS) injury. Hellal et al. (2011), administered *paclitaxel* directly to the site of spinal cord injury in a rat model. The findings from that study indicated that *paclitaxel* reduced fibrotic scarring by altering the dynamics of microtubule-based cargo transport. This study also found that *paclitaxel* promoted axonal regeneration in the spinal cord injury and improved functional recovery as assessed by the grid test. Another study by Adlard et al. (2000) applied *paclitaxel* to the brain directly following a needle stick injury. Although this study did not assess functional or neurological outcomes, the results from immunohistochemistry indicated a significant reduction in the density of abnormal neurites and a relative preservation of MAP2 labeling of dendrites at the injury site.

Our study used MR imaging to evaluate the effect of *paclitaxel* on the morphological features related to the injury. Use of imaging as a surrogate biomarker for traumatic brain injuries is still under development and the association of imaging findings with clinical symptoms, both acute and chronic, is the goal of numerous research investigations. Imaging modalities such as T2-mapping and DTI as used in this study, as well as positron emission tomography with ^{18}F -fluorodeoxyglucose (FDG-PET) have been proposed to evaluate TBI in clinical settings (Hunter et al., 2012). The imaging modalities selected for this study, in particular the use of macromolecular proton fraction imaging, were in part based on findings from our study of blast-induced mild TBI in veterans (Peskind et al., 2011; Petrie et al., 2014). The caveat being that blast-induced repetitive TBI causes a diffusely distributed injury and our controlled cortical impact injury model produces a focally well-defined lesion-type injury. Therefore, we preferentially chose imaging (T2-mapping and T1-weighted structural imaging) to assess the morphological changes that might be detected by these modalities in response to *paclitaxel* treatment. The reduction of injury volume from T1-weighted images indicates that *paclitaxel* may have affected apoptotic processes in a manner that reduced cell death triggered by the CCI. On T2-maps, the volume of injury edema was decreased by *paclitaxel* application. This finding may indicate that the drug affected the neuroinflammatory response to the injury, possibly through a modification of glial proliferation and motility. Our study in blast-exposed veterans indicated that MPF imaging specifically was a sensitive indicator of blast injury, and other studies have indicated myelin damage as a consequence of TBI (Goldstein et al., 2012; Johnson et al., 2013). Using MPF imaging, our results indicated that *paclitaxel* helped to preserve the myelin density on the injury margins. This finding was not predicted by our hypothesis that *paclitaxel* would stabilize microtubules in neurons. However, we speculate that oligodendrocytes with their highly ordered microtubule arrays might also benefit from stabilization, as do neuronal populations (Lunn et al., 1997). The cellular substrates for the improved imaging outcomes in this study require further investigation.

Other neurotherapeutic applications of microtubule stabilizing drugs have been proposed, which include treatment of AD and other tauopathies (Brunden et al., 2011; Lou et al., 2014). The rationale for this therapeutic strategy is that hyperphosphorylated tau in AD disengages from MTs and this loss of normal stabilization leads to a perturbation of neuronal functions including decreased axonal transport and overall loss of cytoskeletal integrity. One such drug, *Epothilone D*, which has good blood-brain barrier (BBB) penetration, was shown to improve cognitive performance and reduce associated tau pathology in transgenic mouse models (Barten et al., 2012; Brunden et al., 2011). The findings from these studies further support the use of *paclitaxel* and other microtubule stabilizers for neurotherapeutic applications and suggest that this therapeutic strategy may benefit the underlying association between TBI and future development of neurodegenerative disorders.

One obstacle that must be overcome for the development of *paclitaxel* as a therapeutic option for CNS disorders is that, as a P-gp substrate, the drug has limited BBB permeability. Our study used direct application to the injury because our CCI injury model employs a craniotomy. However, this is not often the case for clinically presented TBI, and direct

cortical drug administration may not be feasible in a clinical setting. One potential delivery option under development by our group involves findings that the blood-brain-barrier is transiently disrupted after TBI even in mild cases (Alves, 2014; Si et al., 2014). Experiments to define the parameters of *paclitaxel* uptake to the brain after TBI in relation to BBB disruption are underway by our group. Future studies will focus on more conventional methods of drug delivery as well as an evaluation of drug pharmacokinetics to define brain uptake in relation to BBB disruption and therapeutic time window of drug delivery after injury.

Contrary to our hypothesis, no improvement in the underlying cytoskeletal injury was observed on the fractional anisotropy maps from our DTI acquisitions. There may be several reasons that explain this finding. First, our DTI imaging protocol may not be sensitive enough and the signal-to-noise ratio may be too low to detect subtle improvements from the *paclitaxel* administration. We are working to improve the DTI acquisition protocol by modifying parameters, lengthening the acquisition time and employing stronger head stabilization measures as small movements from respiration and cardiac output can cause motion artifacts at 14 T. Another possibility for this finding is that with direct application of *paclitaxel* to the injury surface, the drug did not penetrate deep enough into the tissue to reach the underlying white matter tracts. It is also possible that the timing of imaging compared to hypothesized therapeutic effect may be not optimized for this biomarker. We performed DTI imaging at 4 days post-injury and it's possible that the therapeutic effects of *paclitaxel* on the white matter tracts could take longer than 4 days to manifest. Alternatively, the acute neuroprotective/neurotherapeutic effects of *paclitaxel* for TBI could be mediated by processes other than the maintenance of cytoskeletal integrity. These different possibilities are under investigation by our group to assess the contributing factors to this unexpected result.

Through preliminary, these results support our hypothesis that direct cortical application of *paclitaxel* may improve recovery from TBI. Future directions for this research will investigate more clinically relevant administration routes and will assess the effect of dose response for therapeutic efficacy. The current study applied the *paclitaxel* directly after CCI injury, however the therapeutic time window for efficacy of administration with respective time of injury is not known. This will also be a focus of further investigation.

4. Conclusions

Pharmacologic interventions for traumatic brain injury hold promise to improve outcome, but no therapeutic has proven clinically effective to date. The goal of this study was to determine if the microtubule stabilizing therapeutic *paclitaxel*, a unique approach recently tested in neurological conditions such as AD and spinal cord injury (Brunden et al., 2011; Hellal et al., 2011), would improve outcome after TBI. Our findings indicate that *paclitaxel* treatment resulted in improvement of neurological outcome and MR imaging biomarkers of injury suggested that the improvement could be mediated by reduced overall injury volume, reduced injury-associated edema, and preservation of myelin in tissue surrounding the injury. These results could have a significant impact on therapeutic developments to treat traumatic brain injury.

5. Experimental procedure

5.1. Subjects

All procedures were conducted in accordance with the animal care guidelines issued by the National Institutes of Health and by the Institutional Animal Care and Use Committee. Adult male C57BL/6 J mice, 10 weeks of age, were purchased from the Jackson Laboratory, Bar Harbor, ME. Animals were kept on a 12-h light/12-h dark cycle with ad libitum access to water and food before and during experimental procedures, and were randomly assigned to three groups (*paclitaxel*, $n = 6$; saline control, $n = 6$; and sham surgery control, $n = 3$).

5.2. Surgery and drug administration

Animals were anesthetized with isoflurane (1.5–2.5% in flowing O₂) during surgery and their core temperature was maintained at 37 °C with a heating pad. After securing the animal in a stereotaxic frame, the scalp was shaved and the skin scrubbed with Betadine. Lidocaine, Bupivacaine (1 mg/kg) and Buprenorphine (0.5 mg/kg) were injected subcutaneously into the scalp and an incision at the midline was made. Fascia and skin were retracted, and a 5 mm craniotomy was performed with a high-speed surgical drill (Dremel, Racine, WI) over the right frontoparietal cortex center point at 2.5 mm behind bregma and 2.5 mm off the midline. Through this opening, the animal was subjected to a controlled cortical impact using a pneumatic impactor (AmScien Instruments, Richmond, VA, USA) with a 3 mm convex tip. The impact injury was generated using the following parameters: 6 m/s strike velocity, 1 mm depth of penetration, and 150 ms contact time. Immediately following impact, 5 µl of sterile isotonic saline or 10 µl of 1.8 mg/ml of *paclitaxel* (Hospira, Inc, Lake Forest, IL) was applied to the cortex at the injury site at a flow rate of 2 µl/min. The scalp was then closed with sutures and the animal was given an interscapular subcutaneous injection of 1.0 ml isotonic saline to prevent dehydration. Sham controls received a craniotomy, but no impact injury.

5.3. Functional assessment

Catwalk gait analysis has been used previously to evaluate impairment following CCI in rodents (Mountney et al., 2013; Neumann et al., 2009; Wang et al., 2011). CCI injury is unilateral and with our mild impact, we required a sensitive test of motor/neurological function. The advantage of the gait analysis system is that it is automated and assessment of gait parameters is observer independent (results are checked only for non “paw” marks such as fecal droppings to be excluded from analyses). Animals underwent gait assessment at 7 days after surgery using the CatWalk automated gait analysis system (Noldus Information Technology, Wageningen, The Netherlands). The testing device included a 1.3-m-long glass plate with dim fluorescent light beamed into the glass from the side. Under dim overhead lighting, the light was reflected downward and the images of the footprints were recorded by the camera under a walkway as the mouse's paws came in contact with the glass surface. Mice performed 3 consecutive runs through the apparatus. Images from each trial were converted into digital signals and processed with a threshold of 179 arbitrary units (au). Following the identification and labeling of each footprint, gait data were generated for spatial parameters related to individual paws (mean intensity, maximum contact area, print area, print width, and print length) and temporal parameters (swing, stance, cadence, and

walk speed). *Paclitaxel* vs. saline controls were compared using a *t*-test and $p = 0.05$ threshold for significance. One sham control died prior to catwalk testing (did not recover from imaging anesthesia) and could not be used in the analysis and that left only 2 shams, which were not enough for statistical comparison to TBI groups.

5.4. Imaging and image processing

At 4 days post injury, mice were anesthetized with isoflurane and scanned over the entire brain using an ultra hi-res 14 T MRI (Avance III, vertical bore, Bruker BioSpin Corp, Billerica, MA). Image acquisitions included T1-weighted structural imaging using a 3D Modified Driven Equilibrium Fourier Transform (MDEFT) sequence with a voxel size of $0.14 \times 0.14 \times 0.25 \text{ mm}^3$, 64 slices, flip angle/repetition time/echo time (FA/TR/TE): $12^\circ/5000 \text{ ms}/1.9 \text{ ms}$ that was used to assess total injury volume and T2 quantitative mapping (T2) with a voxel size of $0.12 \times 0.12 \times 1.0 \text{ mm}^3$, 15 slices, TR = 2000 ms, 16 echoes, spacing: 6.7 ms, TE Effective 1: 6.7 ms, TE Effective 2: 13.4 ms, which was used to measure the volume of the edema directly associated with the injury site.

To evaluate axonal integrity in the white matter, diffusion tensor imaging (DTI) was acquired over the entire brain using a 4-shot echo-planar imaging (EPI) sequence applied along 30 non-collinear diffusion-gradient directions with a voxel size of $0.195 \times 0.195 \times 0.5 \text{ mm}^3$, 35 slices, FA/TR/TE: $90^\circ/8750 \text{ ms}/17.8 \text{ ms}$. DTI processing included the application of a custom algorithm, which allows elimination of image frames contaminated by motion-derived artifacts, followed by eddy current and B0 corrections and confers several advantages. The resultant diffusivity maps exhibit little spatial distortion, artifacts of subject motion or inhomogeneities of magnetic (B0) or RF fields, and show a high degree of white/gray matter contrast. Fractional anisotropy (FA) maps were constructed from DTI by FSL software (Analysis Group, FMRIB, Oxford, UK).

Macromolecular proton fraction imaging (MPF) of myelin content/degradation utilizes a recently published fast single-point method to quantitatively measure the magnetization transfer between protons bound to water and protons bound to macromolecules (Underhill et al., 2009; Underhill et al., 2011; Yarnykh, 2012). They showed that the bound pool fraction was strongly associated with myelin density in both white matter and gray matter. In brief, this modality uses a single magnetization transfer (MT) image with off-resonance saturation, a reference image, and a T1 map to compute an MPF map based on the pulsed MT model with appropriate constraints for other model parameters. Source data included three spoiled gradient-echo (GRE) images (TR/TE = 20/3.2 ms, excitation flip angles [FA] $\alpha = 3, 10$, and 20°) for variable flip angle (VFA) T1 mapping, an MT GRE image (TR/TE = 40/3.2 ms, $\alpha = 10^\circ$) with off-resonance Gaussian saturation pulse (offset = 4 kHz, effective FA 950° , duration 19 ms), and a reference GRE image with the same parameters and without off-resonance saturation. All images were acquired in 3D mode with a $144 \times 144 \times 500 \mu\text{m}^3$ voxel size (FOV $23 \times 23 \times 16 \text{ mm}^3$, matrix $160 \times 160 \times 32$).

5.5. Image analysis

After processing of MR images to generate FA and MPF quantitative maps, all images were thresholded to isolate the feature of interest or to exclude pixels with extremely high values

due to image noise (examples of image thresholding can be seen in subsequent figures for each image result). Manual region-of-interest (ROI) analysis was performed using ImageJ software. For T1-weighted images, the identifiable injury area (hypointensity compared to surrounding uninjured cortex) was manually traced on each slice and the total number of voxels was multiplied by voxel size to estimate injury volume in mm³. On the T2 quantitative maps, threshold bounds were set from 36 to 200, which included regions of edema and ventricles, but excluded normal uninjured cortex and white matter. Manually drawn ROIs on images displayed by Image J software included the volume of voxels within the threshold boundary and total area of injury-associated edema was calculated by multiplying with voxel size in mm³. On MPF maps we wished to assess the preservation of the myelin density associated with the boundary of the injury site. For this purpose we again used image thresholding to exclude hypointense voxels representing the actual injury and hyperintense voxels that represent noise on the brain/skull boundary and drew a large circular ROI that exceeded the injury size on 5 slices that centered on the injury site. For this analysis, lower-bound thresholding was used to exclude hypointense voxels of the injury and include only the voxels that bordered the injury within an ROI of a specified diameter (1.5 mm). The mean intensity of the voxels within this thresholded region was calculated to assess preserved myelin density on the injury boundary. For DTI, the white matter integrity of the external capsule directly below the injury was assessed on FA maps using the mean intensity values over a manually drawn ROI. All ROI analyses were performed by an investigator blinded to treatment group. For all ROI values group-wise mean and standard deviation were compared using unpaired *t*-tests with *p* = 0.05 statistical significance.

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Abbreviations

TBI	traumatic brain injury
IED	improvised explosive device
CTE	chronic traumatic encephalopathy
AD	Alzheimer's disease
mTBI	mild traumatic brain injury
CCI	controlled cortical impact
DTI	diffusion tensor imaging
FA	fractional anisotropy
MPF	macromolecular proton fraction imaging
ROI	region-of-interest
CNS	central nervous system

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Fig. 1.

Gait improvement in paclitaxel treated subjects. (A) Individual paw pressure on the walkway, (B–E) spatial parameters related to paw print and, (F) the temporal parameter of swing were significantly improved in mice receiving paclitaxel after TBI, indicating improved sensorimotor coordination. (* $p < 0.05$) RF: right front, RH: right hind, LF: left front, LH left hind.

Fig. 2.

Volume of injury and Injury-associated edema reduced with *paclitaxel*. (A) Representative coronal T2-quantitative maps from saline vs. paclitaxel subjects that have been thresholded to delineate pixels with edema. Green (saline) and blue (*paclitaxel*) arrows indicate injury edema thru several sections. Total number of injury-associated edema pixels were quantified and converted to mm³. (B) Difference in injury volume from T1-weighted images indicated significant reduction with paclitaxel. (C) Area of edema was larger for both saline and paclitaxel treated subjects than the volume of injury and also reduced significantly by treatment ($p < 0.05$).

Fig. 3.

Paclitaxel preserves myelin density around injury after CCI. Macromolecular proton fraction imaging measures the magnetization transfer between protons bound to water and protons bound to macromolecules. Yarnykh et al. showed that the bound pool fraction was strongly associated with myelin density (Underhill et al., 2011). (A) an example of a raw MPF map of a coronal section from a mouse brain after CCI, which is then thresholded to exclude injury pixels (hypointense regions). Mean myelin density was calculated for a ROI (yellow circle) that was larger than the actual injury (1.5 mm). (B) shows the bound pool

fraction (associated strongly with myelin density) is more preserved with paclitaxel treatment ($*p = 0.05$).

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Fig. 4. Fractional Anisotropy in the white matter underlying injury not improved by *paclitaxel*. (A) indicates representative coronal brain images from saline, paclitaxel and sham subjects with ROI indicated (yellow outline). (B) indicates that subjects undergoing CCI procedure had decreased FA values as compared to sham controls. ($*p = 0.01$, however $\#p = 0.057$, due to high standard deviation in saline group). However, no significant difference was seen between treatment groups.