Pharmacology, Biochemistry and Behavior 94 (2009) 56–62

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Neuroprotective profile of dextromethorphan in an experimental model of penetrating ballistic-like brain injury

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ARTICLE INFO

Article history:
Received 29 May 2009
Received in revised form 8 July 2009
Accepted 13 July 2009
Available online 17 July 2009

Keywords:
Penetrating ballistic-like brain injury
PBBI
Traumatic brain injury
TBI
Dextromethorphan
DM
Motor
Cognitive
Novel object recognition
NOR

ABSTRACT

Dextromethorphan (DM) has been well-characterized as a neuroprotective agent in experimental models of CNS injury. The goal of this study was to determine the neuroprotective profile of DM in a military-relevant model of penetrating ballistic-like brain injury (PBBI). In an acute (3 day) dose–response study, anesthetized male Sprague–Dawley rats were exposed to a unilateral frontal PBBI with DM (0.156–10 mg/kg) or vehicle delivered as an i.v. bolus from 30 min to 48 h post-injury. In a follow-up (7 day) experiment, the 10-mg/kg bolus injections of DM were administered in conjunction with a 6-h infusion (5 mg/kg/h). DM bolus injections alone produced a dose-dependent improvement in motor recovery on a balance beam task at 3 days post-injury. However, more rapid recovery (24 h) was observed on this task when the bolus injections were combined with the 6-h infusion. Moreover, the DM bolus/infusion treatment regimen resulted in a significant (76%) improvement in cognitive performance in a novel object recognition (NOR) task at 7 days post-injury. Although post-injury administration of DM (all doses) failed to reduce core lesion size, the maximum dose of DM (10 mg/kg) was effective in reducing silver-stained axonal fiber degeneration in the cortical regions adjacent to the injury.

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1. Introduction

Traumatic brain injury (TBI) represents one of the most serious injuries occurring on the battlefield, the majority of which are caused by blast and/or penetrating brain injuries incurred by explosive devices or firearms. The most recent epidemiological data, from a retrospective analysis of all inpatient admissions over a 5-year span at the National Naval and Walter Reed Army Medical Centers, reported 56% of severe military TBI casualties were penetrating injuries resulting from either embedded fragments driven into the brain by an explosive blast event (72%) or bullets from assault weapons (23%) (Bell et al., 2009). These data call attention to an increased demand for experimental drug-development research, particularly on military-relevant animal models of TBI, aimed at generating safe and effective clinical therapies.

Dextromethorphan (DM; 3-methoxy-17-methylmorphinan) is a non-narcotic antitussive drug that initially attracted attention in the 1980s due to its anticonvulsant and neuroprotective properties (Choi, 1987; Tortella et al., 1989). Since then, mounting evidence has demonstrated that DM has a high safety profile in humans and is neuroprotective in a variety of experimental injury models including epilepsy, cerebral ischemia, traumatic brain injury (TBI), and neurodegenerative disorders (Werling et al., 2007). DM's neuroprotective properties have been well-characterized and appear to be functionally related to its inhibitory effects on glutamate-induced neurotoxicity via its NMDA receptor antagonist (Tortella et al., 1989), sigma-1 receptor agonist (Klette et al., 1995; Klette et al., 1997), and voltage-gated calcium channel antagonist mechanisms of action (Carpenter et al., 1988).

The aim of the present study was to determine the neuroprotective profile of DM in a military-relevant model of penetrating ballistic-like brain injury (PBBI) (Williams et al., 2005; Williams et al., 2006a; Williams et al., 2006b; Williams et al., 2007). In an acute, dose–response experiment, DM (0.156–10 mg/kg) was delivered via intravenous (i.v.) bolus injections and neurobehavioral (motor) assessments were carried out to 3 days post-PBBI. Based on those results, the maximum efficacious dose of DM (10 mg/kg) was repeated in a follow-up experiment that added a 6-h i.v. infusion (5 mg/kg/h) to the treatment regimen and assessed both motor and cognitive recovery out to 7 days post-PBBI.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats (250–300 g; Charles River Labs, Raleigh, VA) were used in all studies. All procedures were approved by the Walter Reed Army Institute of Research Animal Care and Use

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0091-3057/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.pbb.2009.07.006

Committee. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition. Animals were housed individually under a 12 h light/dark cycle (lights on at 0600) in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. For all experiments, drug treatment was randomized and the investigators performing behavioral and neuropathological assessments were blinded to the treatment group.

2.2. General surgical procedures

All surgical procedures were performed on anesthetized animals. Anesthesia was induced with isoflurane (2–5%) delivered in oxygen. Body temperature was maintained normothermic (37 ± 1 °C) throughout all surgical procedures by means of a homeothermic heating system (Harvard Apparatus, South Natick, MA). Following surgery, animals were placed in a warm chamber maintained by a circulating water-bath heating system (Gaymar Industries, Orchard Park, NY) until recovery from anesthesia. Food and water were provided ad libitum post-operatively. Rectal temperatures were monitored throughout the course of the recovery period, which remained normothermic (37.0–39.0 °C) for all treatment groups.

2.3. Penetrating ballistic brain injury model

The Dragonfly Model # HPD-1700 Variable Pressure Waveform Generator and PBBI probe (Dragonfly Inc., WV) were used to simulate a right frontal ballistic injury of a 7.62 mm NATO round to the rat brain (Williams et al., 2005). Rats were anesthetized (isoflurane; 5% induction, 2% maintenance) and positioned in a stereotaxic frame (Kopf, Tujunga, CA) for probe insertion. The scalp was incised along midline and a 4 mm diameter burr hole was created to expose the right frontal pole of the brain (+4.5 mm AP, +2 mm ML to bregma). The PBBI probe was mounted to a stereotaxic arm and fixed at an angle of 50° from vertical and 25° counter-clockwise from the anterior–posterior axis. The probe was then slowly advanced through the cranial window, along the axis described above, penetrating into the right frontal hemisphere to a distance of 1.2 cm (from dura). A 10% severity level of injury was induced by delivery of a pressure pulse to rapidly inflate/deflate the PBBI balloon (diameter = 0.633 cm, duration = 35 ms), calibrated to equal 10% of the total rat brain volume. Following PBBI, the probe was manually removed, the cranial window sealed with sterile bone wax, and the incision closed with 3-0 nylon suture. Sham animals were not subjected to probe insertion but otherwise received all surgical manipulations including craniotomy.

2.4. Drug treatment

2.4.1. Experiment 1 (3-day dose–response)

Vehicle (saline) or DM (0.156, 0.626, 2.5, or 10 mg/kg, Sigma-Aldrich) was delivered as i.v. bolus injections at 30 min, 2, 4, 6, 24, and 48 h post-injury ($n = 6$ per group). Motor abilities were assessed on a balance beam task (1 and 3 days post-PBBI) and histological endpoints (3 days post-PBBI) consisted of lesion volume (TTC and H&E stained sections) and axonal fiber degeneration (silver staining).

2.4.2. Experiment 2 (7-day maximum dose)

Vehicle or DM was delivered as a 10 mg/kg bolus at 30 min post-PBBI followed by a 6-h infusion (5 mg/kg/h) with additional bolus injections (10 mg/kg) at 24 and 48 h post-injury to the following groups: (1) SHAM (non-injured, $n = 6$), (2) PBBI + VEH ($n = 7$), and (3) PBBI + DM ($n = 7$). Motor abilities were assessed on the balance beam 1 day prior to PBBI (baseline measures) and at 1, 3, and 7 days post-PBBI. In addition, neuroscore assessments were recorded at 30 min (prior to initiation of drug treatment), 6 h, 24 h, 48 h, and 7 days post-injury and cognitive abilities were assessed in the novel object recognition (NOR) task at 7 days post-PBBI.

2.5. Balance beam task

2.5.1. Apparatus

A tapered balance beam (Dragonfly, Inc. Ridgeley, WV) was used to detect non-compensatory footfault deficits following PBBI (Williams et al., 2005). Animals were trained to walk on a flat beam surface, which tapers from 5.5 cm at the startpoint to 1.5 cm at the endpoint (increasing balance difficulty) at a height of 120 cm above the floor. A 2-cm wide ledge is positioned 2 cm below the beam surface along each side of the beam, which serves as a 'crutch' for impaired rats to walk on. Each ledge is divided into three equal longitudinal segments (S1: 5.5 cm–4.1 cm; S2: 4.1 cm–2.8 cm; and S3: 2.8–1.5 segments) and each segment is equipped with a mechanical sensor to record foot faults (steps on the ledge) for each side of the balance beam. Left and right foot faults are recorded separately. A black "goal" box is positioned at the endpoint of the beam.

2.5.2. Testing

Rats were trained prior to the PBBI surgery until each animal was able to successfully ambulate along the beam without footfaults. Animals were allowed to remain in the safety of a dark 'goal' box for 60 s upon successful completion of each trial. On testing days, animals were first placed in the goal box for a 10 min reinforcement period and then given 3 trials with a 60 s intertrial interval (ITI). Footfaults were recorded (and summed) for each side (left/right) for each trial.

2.6. Neuroscore

Neurological deficits were evaluated at 0.5 h, 6 h, 24 h, 72 h, and 7 days after PBBI using a battery of tests similar to that defined previously (Bederson et al., 1986). Briefly, neurological scores (NS) were based on a 10-point sliding scale ranging from 0 (normal) to 10 (severely impaired) comprised from the following 4 neurological tests (weighted in order of testing sequence): (1) contralateral forelimb flexion during tail suspension (maximum score = 1), (2) shoulder adduction (body upward curling behavior) during tail suspension (maximum score = 2), (3) open-field circling behavior (maximum score = 3), and (4) impaired resistance to lateral push (maximum score = 4) (Tortella et al., 1999).

2.7. Novel object recognition (NOR)

The NOR task is used to measure the rat's ability to discriminate between familiar and novel objects. At 6 days post-PBBI, rats were first habituated to an empty open-field arena ($45.5 \times 45.5 \times 61$ cm) for 10 min and returned to their home cage. One hour later, rats were placed in the open-field arena and exposed to two identical objects (A_1 and A_2), for 5 min. Following a 24-h delay, the rats were placed back into the open field (5 min) with one familiar object (A_1) and one novel object (B_2). The time (seconds) that the animals spend exploring each object (sniffing and crawling on it) was recorded. The discrimination index (DI) was calculated using the following formula: $DI = (B_2 - A_1 / B_2 + A_1)$, which corresponds to the difference between the amount of time spent exploring the novel (B_2) and familiar (A_1) objects, corrected for the total time spent exploring both objects. Higher DI scores indicate more time spent exploring the novel object and thus denote better memory retention of the familiar object.

2.8. Histopathology

At the indicated post-injury endpoint, animals were fully anesthetized with ketamine/xylazine (70 and 6 mg/kg, i.p., respectively).

Either fresh brain tissue was immediately collected for analysis of lesion volume using the TTC method (Williams et al., 2000) or animals were transcardially perfused with (4% paraformaldehyde) for evaluation of fiber degeneration using silver staining (FD Neurotechnologies, Baltimore, MD). Quantitative volumetric analysis of lesion size was performed as previously reported (Williams et al., 2000) using a computer-based image analysis system (Loats Assoc., Westminster, MD).

Coronal brain sections (40 mm thick) were cut through the whole cerebrum from +4.0 to −7.0 mm AP to bregma and serial sections collected at 1 mm intervals for H&E and silver staining. A gradient severity score (SS) was assessed on silver-stained sections from vehicle-injected PBBI rats ($n=6$) and PBBI rats treated with the maximum dose of DM (10 mg/kg; $n=6$) animals. The SS was based on the degree of silver reactivity as indicated by the presence of dark black silver-positive granules (0 = no reactivity, 1 = <50% reactivity, 2 = >50% reactivity, 3 = complete reactivity) across 9 distinct brain regions: cortex (cingulate, parietal, temporal, and piriform), thalamus, hippocampus, internal capsule, external capsule, and cerebral peduncle. A mean SS was derived from the average of all slides evaluated.

2.9. Statistical analysis

Histopathological and behavioral endpoints were compared by analysis of variance (ANOVA) followed by a Dunnett's or Fisher PLSD post hoc analysis as appropriate or by using paired *t*-tests between matched groups. Data is presented as the mean ± standard error of the mean (S.E.M), p values <0.05 were considered significant.

3. Results

3.1. Experiment 1 (3-day dose–response)

3.1.1. Balance beam results

Motor abilities were assessed on the tapered balance beam task 1 day prior to PBBI surgery (baseline measures) and at 1 and 3 days post-PBBI. Statistical analysis of footfault deficits was based on the total number of foot faults made over 3 consecutive trials for each animal. Prior to PBBI, all animals had achieved a stable baseline level of performance and were able to successfully cross the beam without footfaults. At 24 h post-PBBI, all injured groups showed significant motor deficits on the beam task (average number of footfaults ranging from 14 ± 4 to 26 ± 5; $p<.05$ between groups; Fig. 1A). At 3 days post-PBBI, animals treated with the 0.626–10 mg/kg dose of DM made significantly fewer footfaults compared to 24 h post-PBBI (Fig. 1A; $p<.05$, paired *t*-test) indicating an improved motor recovery on this task as compared to vehicle-treated PBBI rats (Fig. 1B, $p<.05$ as compared to vehicle, Dunnett's post hoc analysis).

3.1.2. Histopathology

At 72 h post-injury, PBBI produced a consistent pattern of necrotic, hemorrhagic lesions in the injured (right) brain hemisphere that permeated the frontal cortex and progressed through the dorsal–lateral striatum towards the lateral amygdala (Fig. 2A). Quantification of lesion volume indicated that post-PBBI administration of DM (maximum dose) was not effective in reducing necrotic cavity formation as determined from either TTC ($n=6$/group) or H&E ($n=7$–8/group) staining ($p>.05$; xFig. 2B). Furthermore, results of a mixed-factorial (5 groups × 7 coronal level) ANOVA on TTC stained tissues across all doses of DM ($n=6$/group) further revealed that post-injury administration of DM failed to reduce in brain lesion area at each respective brain level evaluated ($p>.05$; data not shown).

However, the high dose of DM (10 mg/kg) was effective in protecting against PBBI-induced axonal degeneration in brain regions distal from the lesion core. Statistical analysis of severity scores (SS), based on the degree of silver reactivity quantified by dark, black, silver-positive granule density at 72 h post-injury ($n=7$–8/group; Table 1), showed that post-injury administration of DM significantly reduced the degree of silver-stained axonal fiber degeneration in the cingulate cortex (66%) external capsule (57%) and thalamus (46%) (Fig. 3; $p<.05$).

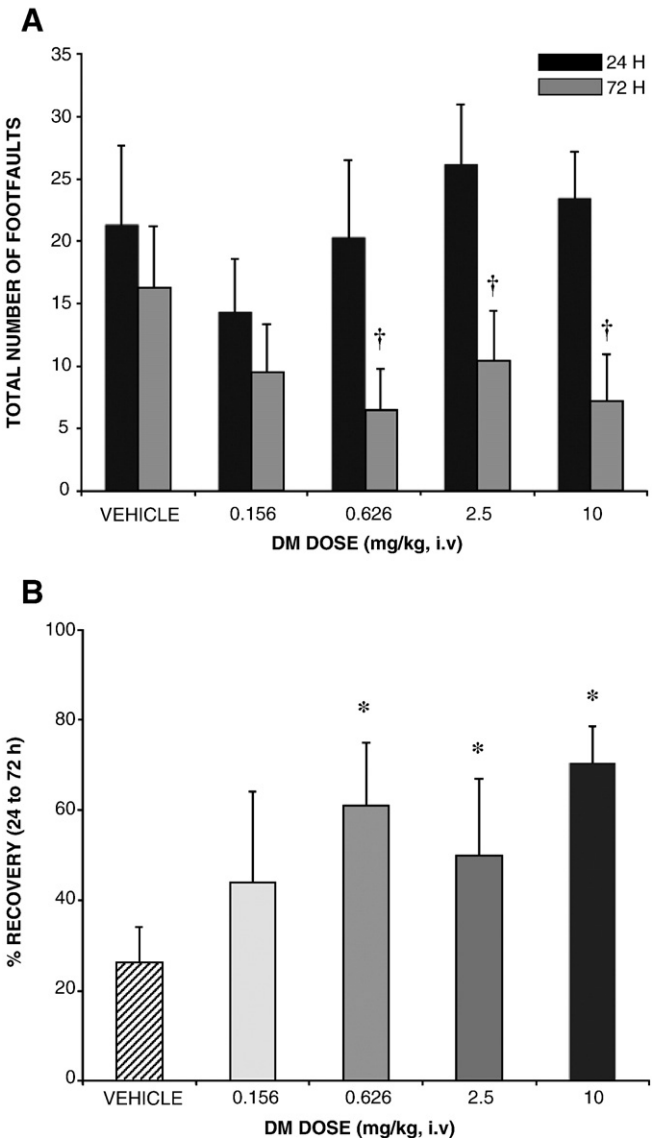

Fig. 1. Dose–response effect of DM on functional recovery (balance beam task, $n=6$/group). A: Total number of footfaults at 24 and 72 h post-injury. B: Percent recovery of footfault deficits (from 24 to 72 h post-PBBI). Columns = group means, error bars = S.E.M., †$p<.05$ as compared to values at 24 h, *$p<.05$ as compared to vehicle.

3.2. Experiment 2 (7-day; maximum dose + infusion)

3.2.1. Balance beam

The balance beam task was repeated on animals that received the maximum bolus dose followed by infusion of DM (10 mg/kg + 5 mg/kg/h × 6-h infusion).

A mixed-factorial (3 groups × 3 time points) ANOVA revealed a significant overall difference between groups ($F(2,17)=10.09$, $p<.05$), which showed DM-treated PBBI animals made significantly fewer footfaults than PBBI + vehicle rats. Further post hoc analysis at each time point revealed that DM-treated rats made significantly fewer footfaults than the vehicle-treated PBBI animals at 24 h (57%) and 72 h (59%), but not at 7 days post-injury (43%; $p>.05$). Notably, PBBI rats that received the combined maximum bolus dose followed

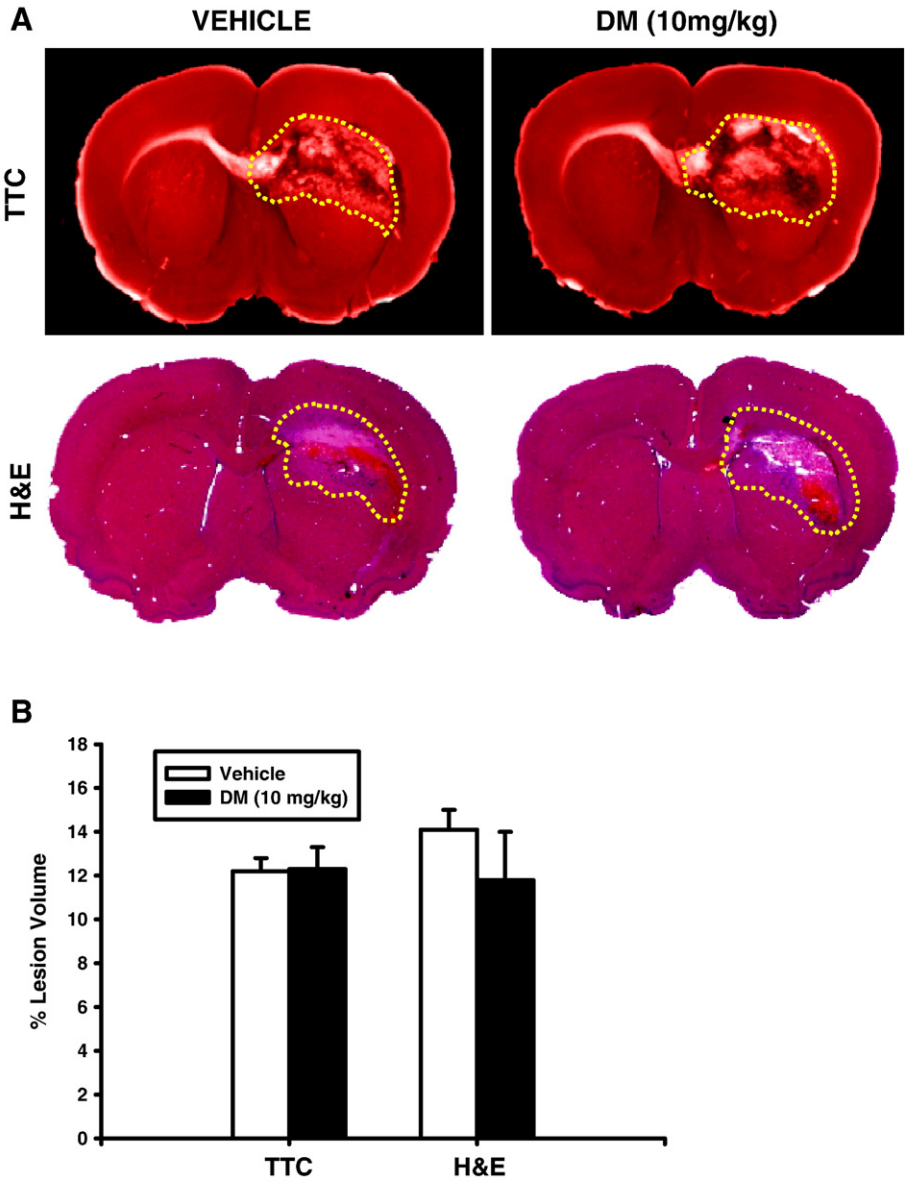

Fig. 2. Representative TTC and H&E stained sections from VEHICLE and DM-treated animals at 72 h post-PBBI with lesion outlined in yellow (A). Quantitative analysis (B) indicated that the highest dose of DM tested (10 mg/kg) was not effective in reducing lesion volume as assessed by either TTC ($n=6$/group) or H&E ($n=7$–8/group) staining ($p>.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

by 6-h infusion of DM made significantly (76%) fewer footfaults at 24 h post-PBBI than animals that received only bolus injections of DM in Experiment 1 (Fig. 4B) suggesting that the prolonged DM infusion further facilitated recovery in this task.

3.2.2. Neuroscore and cognitive results

In addition to balance beam testing, both neuroscore and NOR measures were recorded out to 7 days post-injury. A mixed-factorial (3 groups × 4 post-injury time points) ANOVA revealed a significant difference in neuroscores between treatment groups ($F(2,17)=8.46$, $p<.05$) and a significant group × time interaction ($F(6,51)=2.33$, $p<.05$). Fisher PLSD post hoc analysis further revealed that the PBBI + vehicle animals displayed significant neurological deficits out to 72 h post-injury but had recovered by 7 days post-PBBI (Fig. 5A). On the other hand, the DM-treated rats showed an intermediate level of recovery as early as 24 post-injury, where they did not differ statistically from non-injured animals, suggesting that post-injury administration of DM enhanced recovery on this parameter (Fig. 5A).

ANOVA results of NOR testing at 7 days post-PBBI revealed a significant between-groups effect ($F(2,17)=4.84$, $p<.05$) which showed that SHAM animals exhibited a preference for the novel object, resulting in a higher discrimination index ($DI=0.23\pm0.05$) compared to the PBBI group which explored the familiar and novel objects almost equally ($DI=0.04\pm0.03$). Similarly, DM-treated PBBI animals showed a significantly greater preference for the novel object ($DI=0.17\pm0.05$) compared to vehicle-treated rats. Moreover, the DI of the DM-treated rats was not statistically different from SHAM animals (xFig. 5B). Notably, there were no between-group differences in exploratory activity in the NOR task ($F(2,17)=1.30$, $p>.05$), indicating that the observed deficits were primarily cognitive in nature and not due to motor abnormalities or differences in motivation or exploratory behavior.

4. Discussion

The primary goal of this study was to determine the neuroprotective profile of DM, a well-established neuroprotective agent with

Table 1
Quantification of the degree of fiber degeneration at 72 h post-PBBI.

Brain region	VEH SS	DM SS	% reduction
Cingulate cortex	1.02 ± 0.40	0.41 ± 0.24	66%*
Parietal cortex	1.48 ± 0.44	0.86 ± 0.29	42%
Temporal cortex	1.54 ± 0.42	0.92 ± 0.35	40%
Piriform cortex	0.55 ± 0.18	0.29 ± 0.06	48%
External capsule	1.61 ± 0.27	0.69 ± 0.18	57%*
Internal capsule	1.69 ± 0.38	1.43 ± 0.33	15%
Cerebral peduncle	2.00 ± 0.38	1.71 ± 0.44	14%
Hippocampus	0.16 ± 0.12	0.11 ± 0.07	31%
Thalamus	1.00 ± 0.24	0.54 ± 0.16	46%*

A severity score (SS) was assigned to each region (0 = no silver reactivity, 3 = maximal silver reactivity). Data are presented as mean ± S.E.M.
* $p<.05$ between treatment groups.

clearly defined anti-excitotoxic mechanisms of action, in a military-relevant model of TBI. Overall, results show that post-injury administration of DM provides significant protection against PBBI-induced neuropathology (in areas distal to the lesion core) and improves motor and cognitive outcome. These data, combined with recent pharmacological evidence that neuroprotection via anti-inflammatory mechanisms can improve function in this model (Lu et al., 2009), suggest that the PBBI model is responsive to multiple therapeutic approaches that target different injury mechanisms.

Similar to previous studies, PBBI consistently caused extensive damage at 3 days post-injury to brain regions both proximal (necrotic cavity) and distal (axonal fiber degeneration) to the lesion core (Williams et al., 2005, 2006a,b). Although DM (all doses) failed to reduce lesion size, the maximum dose (10 mg/kg) of DM did provide significant protection against PBBI-induced axonal damage in brain regions remote from the primary lesion.

An essential feature of any potential therapy for TBI is the improvement of functional outcomes following injury. Importantly, both DM and vehicle-treated PBBI animals displayed similar baseline neurologic profiles at 30 min post-injury (prior to DM administration), demonstrating a consistent injury effect across groups. However, by 24 h post-injury, the DM-treated rats showed an intermediate level of improvement, alluding to the neuroprotective potential of DM.

More importantly, the present results demonstrate that post-injury administration of DM improved motor function following PBBI. On the balance beam task, PBBI rats displayed a lack of coordination that was primarily restricted to the contralateral fore- and hindlimbs. Similar to what was previously reported, some degree of spontaneous motor recovery was observed in all PBBI rats by 7 days post-injury (Williams et al., 2005), by which time the motor performance of both vehicle and DM-treated PBBI rats had improved to equal levels. However, in the acute (3-day) study, animals that received the higher concentrations of DM performed significantly better on this task, making fewer footfaults and showing a greater percentage of recovery by 3 days post-PBBI. Moreover, in the chronic (7-day) study, rats that received 6 h DM infusions in addition to the maximum-dose series of bolus injections showed more rapid recovery at 24 h post-PBBI than those that received bolus injections alone. This may be, in part, because the addition of an extended DM infusion resulted in more stable serum levels of DM reaching the CNS.

Previous work suggests that the neuroprotective effects of DM are dependent upon adequate serum levels reaching the brain (Steinberg et al., 1996) but the rapid metabolism of oral administration of DM does not allow sufficient serum concentrations to reach the CNS.

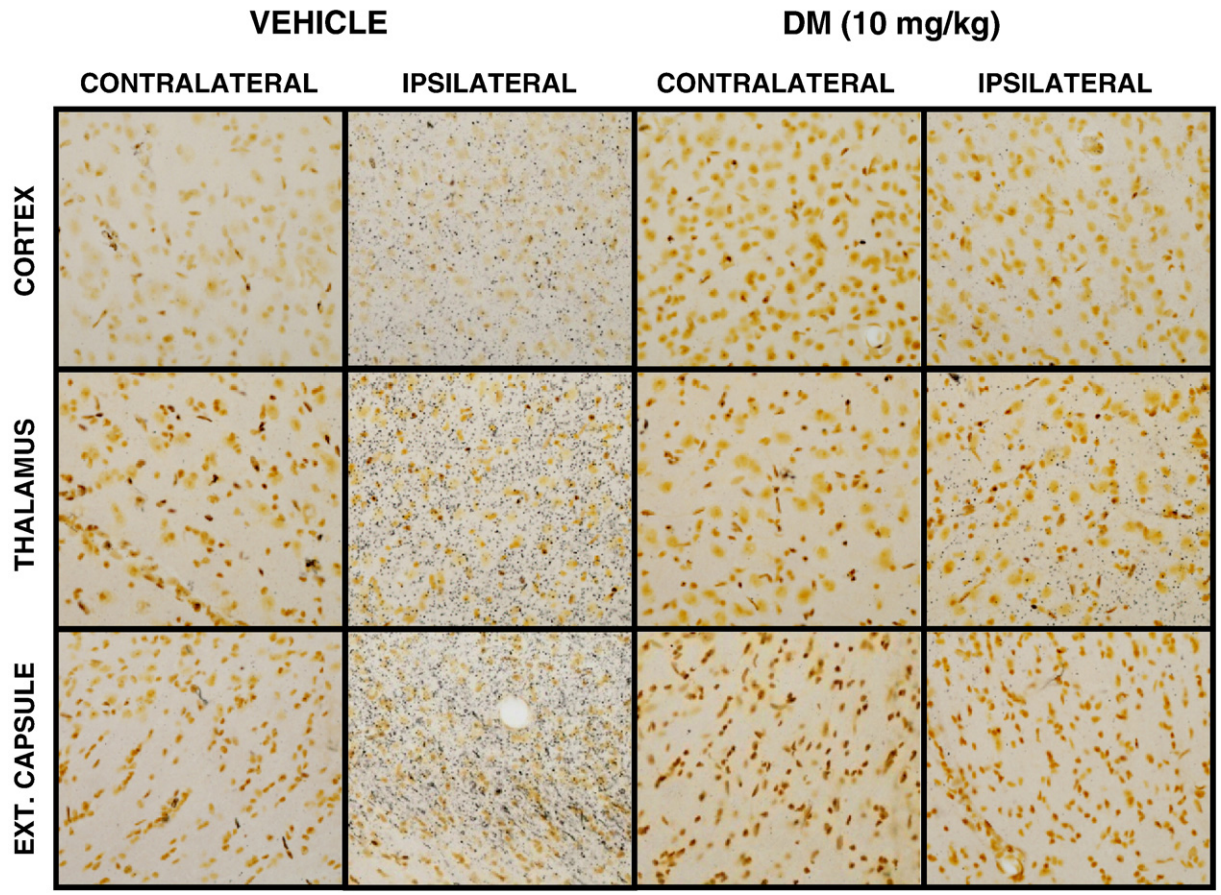

Fig. 3. DM treatment (10 mg/kg, i.v.) reduced axonal fiber degeneration in the cingulate cortex, thalamus, and external capsule as evidenced by a reduction in silver-stained tissue at 72 h post-PBBI (Table 1).

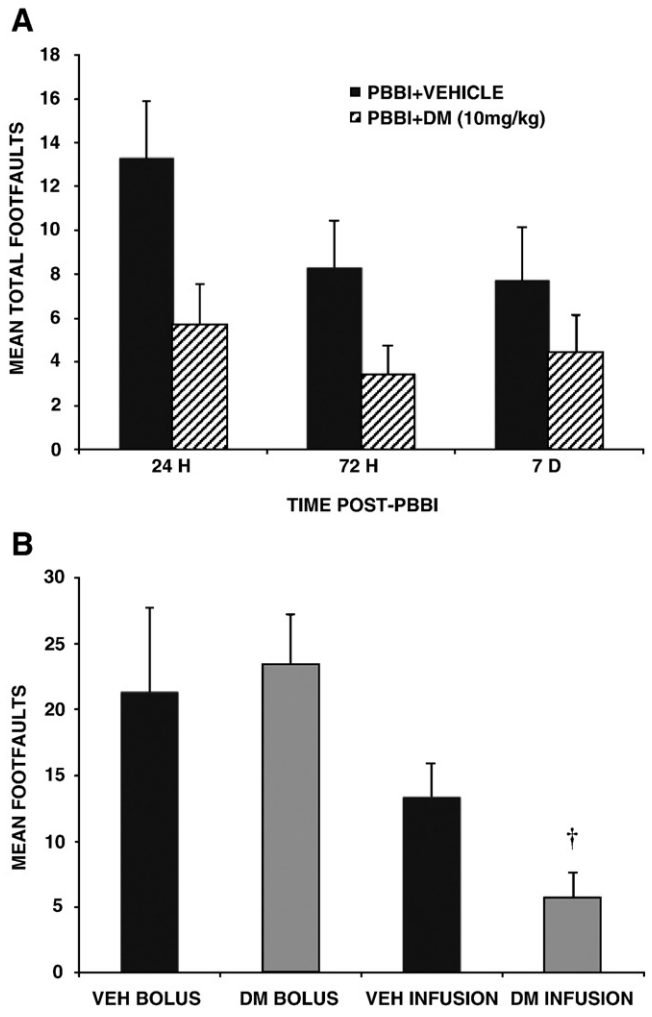

Fig. 4. PBBI rats that were given 6-h DM infusions (5 mg/kg/h) in addition to 10 mg/kg bolus injections, made significantly fewer footfaults than the PBBI + vehicle animals at 24 h and 72 h post-injury (A, *$p<.05$ as compared to PBBI + vehicle). Moreover, at 24 h post-injury, PBBI rats that received 6-h DM infusions made significantly (76%) fewer footfaults than animals that received only bolus injections of DM (B, †$p<.05$ as compared to DM bolus) suggesting that the prolonged DM infusion further facilitated recovery in this task. However, this improvement may be partially due to the duration of IV administration (i.e. bolus injection vs. infusion) as the rats that received 6-h vehicle infusions also tended to perform better, albeit not significantly, than those that received only bolus injections of either vehicle or DM.

When administered orally, DM is primarily metabolized to dextrorphan (DX) by cytochrome *P450* 2D6, which is rapidly glucuronidated and cleared via urine excretion (Schadel et al., 1995). This rapid, first-pass metabolism has been shown to occur to such an extent that even doses of DM that were up to 8 times higher than the normal antitussive dose (60–120 mg/day, oral) were not reliably detectable in the serum levels of Huntington's patients (Walker and Hunt, 1989). To circumvent this limitation, the current study utilized an intravenous administration route (for both bolus injections and 6-h infusion), which facilitates greater serum concentrations of DM reaching the brain. Accordingly, prolonged, continuous infusion may result in more sustained serum levels of DM and presumably potentiate the neuroprotective effects of the parent compound. Alternatively, some neuroprotective actions may be attributable to DM's primary metabolite, DX, which is a potent non-competitive NMDA antagonist in its own right (Carpenter et al., 1988; Choi, 1987; Goldberg et al., 1987).

The present study also provides the first evidence that PBBI produces significant cognitive impairment that is responsive to a therapeutic intervention. The NOR task taps into the rat's innate propensity to explore novel objects in its environment, a critical component to successful foraging activities. Notably, overall exploratory activity was similar across groups, which indicates the observed deficits were primarily cognitive in nature and not the result of attentional, motivational, or motor deficits. In addition, the observation that the motor performance of PBBI animals had improved to the level of DM-treated rats by the time NOR testing was conducted (7 days post-PBBI) provides further evidence that motor dysfunction did not play a significant role in the outcome of this task.

The observation that significant cognitive deficits can be detected in the unilateral frontal PBBI model is not surprising as there is substantial clinical literature demonstrating that severe trauma to the frontal cortex results in impairment of the central executive system (e.g., decision making and working memory) (D'Esposito and Postle, 1999; D'Esposito et al., 2000; Daffner et al., 2000a,b; Godefroy and Rousseaux, 1996, 1997). However, at 7 days post-injury DM treatment resulted in a significant (76%) improvement in cognitive performance in the NOR task, which may be related to a preservation of higher-order brain function as indicated by the amelioration of fiber

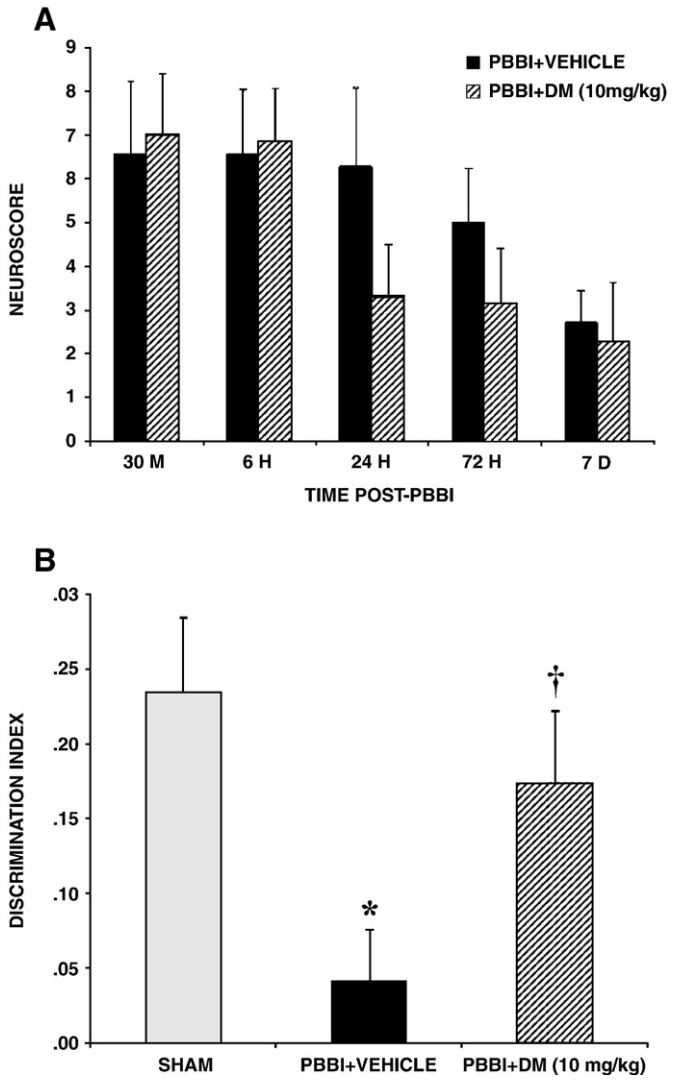

Fig. 5. Effect of DM (10 mg/kg bolus + 6-h infusion) on neuroscore (A) and NOR measures (B) out to 7 days post-injury ($n=6/7$ per group). Initially, both treatment groups exhibited a similar degree of neurological dysfunction (30 min–6 h) although DM-treated animals appeared to exhibit a faster (albeit non-significant) recovery at later time points. Moreover, DM promoted significant recovery on a memory retention task using the NOR paradigm at 7 days post-PBBI (B). Note: higher DI scores indicate better memory retention, *$p<.05$ as compared to SHAM †$p<.05$ as compared to PBBI + vehicle.

degeneration in brain regions remote from the primary injury site. Overall, the finding that DM can improve cognitive performance following PBBI represents an important contribution regarding the sensitivity and usefulness of this experimental model for screening potential therapeutic agents.

The ameliorating effect of DM on PBBI-induced cognitive dysfunction may be most directly related to its role as a high-affinity sigma-1 receptor agonist (DeCoster et al., 1995; Maurice et al., 2001; Walker et al., 1990; Zhou and Musacchio, 1991). Sigma receptors are widely distributed in the mammalian brain and have been implicated in learning and memory processes (Senda et al., 1996) and movement disorders (Matsumoto et al., 1990). Further evidence from in vitro studies suggests that the neuroprotective properties of sigma ligands, such as DM, may partly depend on their ability to suppress excess calcium influx associated with glutamate-receptor mediated excitotoxicity (Katnik et al., 2006; Klette et al., 1995, 1997; Zhang and Cuevas, 2002) and this may be related to the preservation of neuron axonal processes observed in the present study.

Collectively, the results of this study provide evidence that PBBI is associated with acute neurological impairments along with long-term cognitive deficits that are sensitive to neuroprotective intervention. Importantly, it should be noted that DM was ineffective at ameliorating the maturation of core lesion size but rather the therapeutic efficacy of DM to improve functional outcome was associated with significant attenuation of axonal fiber degeneration in regions remote to the primary lesion. Further research is warranted to investigate potential modes of action for DM's neuroprotective effects (i.e. reduced inflammation) and explore the therapeutic sensitivity of remote brain injury as a potential target related to functional recovery following PBBI.

Acknowledgements

We would like to thank Charisma Bautista for her excellent technical skills in support of this study. This material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

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