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Caffeine prevents acute mortality after TBI in rats without increased morbidity

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Abstract

Severe traumatic brain injury (TBI) is associated with a high incidence of acute mortality followed by chronic alteration of homeostatic network activity that includes the emergence of posttraumatic seizures. We hypothesized that acute and chronic outcome after severe TBI critically depends on disrupted bioenergetic network homeostasis, which is governed by the availability of the brain's endogenous neuroprotectant adenosine. We used a rat lateral fluid percussion injury (FPI) model of severe TBI with an acute mortality rate of 46.7%. A subset of rats was treated with 25 mg/kg caffeine intraperitoneally within 1 minute of the injury. We assessed neuromotor function at 24 hours and 4 weeks, and video-EEG activity and histology at 4 weeks following injury. We first demonstrate that acute mortality is related to prolonged apnea and that a single acute injection of the adenosine receptor antagonist caffeine can completely prevent TBI-induced mortality when given immediately following the TBI. Second, we demonstrate that neuromotor function is not affected by caffeine treatment at either 24 hours or 4 weeks following injury. Third, we demonstrate development of epileptiform EEG bursts as early as 4 weeks post-injury that are significantly reduced in duration in the rats that received caffeine. Our data demonstrate that acute treatment with caffeine can prevent lethal apnea following fluid percussion injury, with no negative influence on motor function or histological outcome. Further, we show epileptiform bursting is reduced after caffeine treatment, suggesting a potential role in the modulation of epilepsy development after severe injury.

Keywords

FPI; caffeine; traumatic brain injury; apnea; EEG; neuroprotection

INTRODUCTION

Severe traumatic brain injury (TBI) constitutes a major cause of mortality and morbidity, and the incidence of TBI is on the rise. Both acute mortality and chronic consequences of TBI such as posttraumatic epilepsy (PTE) constitute major unmet medical problems. One of the first acute consequences of TBI is a surge of the neuromodulator adenosine (Clark, et al., 1997). Apnea, caused by central respiratory depression, and resulting hypoxia is a major

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cause of acute mortality after TBI; indeed, prolonged apnea after TBI in humans is associated with a mortality rate of 50% (The Brain Trauma Foundation and The American Association of Neurological Surgeons, 2000).

The rostral end of the brain stem, the medulla oblongata, plays an important role in respiration control (Roth and Roehrs, 2000). Within this respiratory center, the inhibitory neuromodulator adenosine acts at adenosine receptors to regulate respiratory functions (Runold, et al., 1989). It has been demonstrated that overstimulation of both adenosine A₁ and A_{2A} receptors can cause the suppression of vital respiratory and cardiovascular functions (Barraco, et al., 1990, McCarley, 2007, Tseng, et al., 1988). Under physiological conditions, adenosine concentrations are kept within the nanomolar affinity range for its receptors (Fredholm, et al., 2005). However, under conditions of extreme metabolic stress, as occurs during TBI, a surge of micromolar levels of adenosine results (Clark, et al., 1997). In rodents, this increase has been demonstrated as early as 10 minutes after injury (Bell, et al., 1998, Headrick, et al., 1994, Nilsson, et al., 1990). It was further demonstrated that a seizure-induced surge in adenosine coupled to a pharmacologically induced deficiency in metabolic adenosine clearance triggered lethal apnea in mice (Shen, et al., 2010). Likewise, deficient adenosine clearance induced by genetic disruption of adenosine kinase in mice led to intermittent periods of apnea and perinatal mortality (Boison, et al., 2002). These findings suggest that increased brain levels of adenosine can be a likely cause for lethal apnea. In non-toxic concentrations, the methylxanthines caffeine and theophylline are non-selective antagonists of the adenosine receptors (Fredholm, 2007, Fredholm, et al., 1999). Clinically, apnea of prematurity can be treated effectively with caffeine (Schmidt, et al., 2007); likewise, inhibition of adenosine receptors with theophylline was shown to restore spontaneous respiration after spinal cord injury (Nantwi, 2009). Thus, methylxanthines can be used experimentally and therapeutically to influence adenosine-based modulation of respiratory function.

In addition to the risk of acute mortality, severe TBI is a risk factor for the development of PTE; the complexity of TBI and the long latency to clinically evident spontaneous seizures has made the PTE etiology difficult to assess. Gene profiling studies suggest that changes in neuronal plasticity, cell death, proliferation, and inflammatory or immune responses all contribute to the development of PTE (Pitkanen and Lukasiuk, 2009, Pitkanen and Lukasiuk, 2011). Further, changes in homeostatic functions exerted by astrocytes (Stewart, et al., 2010) contribute to the development of PTE, and brief focal, recurrent and spontaneous epileptiform electrocorticography events constitute an early event in the pathogenesis of PTE (D'Ambrosio, et al., 2009). As a homeostatic bioenergetic network regulator, adenosine modulates immune functions including inflammatory processes and cytokine release in the brain (Hasko, et al., 2005), in addition to the regulation of homeostatic functions of astrocytes (Boison, 2008). Clinical studies demonstrate elevated CSF adenosine with micromolar spikes for up to 18 hours following TBI (Bell, et al., 2001), raising the possibility that the post-injury adenosine surge persists long enough to trigger network alterations that may contribute to the development of a posttraumatic epileptic phenotype.

We therefore hypothesized that both acute mortality and posttraumatic chronic consequences might be influenced by an acute surge in adenosine. If true, blockade of adenosine by the non-selective adenosine receptor antagonist caffeine should protect against both acute as well as chronic consequences of TBI. We modeled severe TBI in the rat by lateral fluid percussion injury, which has been shown to cause acute lethal apnea and to trigger PTE (Kharatishvili, et al., 2006). We demonstrate that treatment with caffeine prevents lethal apnea and reduces epileptiform EEG activity without negative impact on neuromuscular behavior or histological outcome.

MATERIALS AND METHODS

Animals

Procedures were conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care according to protocols approved by the Legacy Institutional Animal Care and Use Committee, the USAMRMC Animal Care & Use Review Office, and guidelines from the National Institute of Health.

Lateral Fluid Percussion Injury (FPI)

Male Sprague-Dawley rats (352.2 ± 2.7 g at FPI, $n = 42$, Charles River, Wilmington, MA) were anesthetized with isoflurane (2% isoflurane at 2ml/min in 2:1 N₂O:O₂), then affixed into a stereotactic frame. A 5mm trephine hole was drilled centered at bregma – 4.5mm anterior-posterior and + 2.8mm medial-lateral (Kharatishvili, et al., 2006). TBI was produced by a fluid-percussion device (Custom Design and Fabrication, Richmond, VA). A 21-23 ms fluid pulse with peak pressure of 1.97 ± 0.02 ATM was applied to the exposed dura, measured by an external pressure transducer, digitized by a PowerLab A/D converter (ADInstruments, Colorado Springs, CO), then recorded using Scope (ADInstruments). There were no significant differences in weight and pressure distributions for the severe injury group by post-injury caffeine and mortality, as presented in Table 1. Following FPI, rats were placed in dorsal recumbency for continuous observation of respiratory activity. The duration of apnea was measured from the time from FPI until the first acute inspiratory effort, or “gasp.” Monitoring continued until regular spontaneous ventilation was apparent, or until the complete cessation of inspiratory effort for at least 5 minutes. Sham-injured controls were generated using identical manipulations without impact.

Caffeine

The caffeine dose (Sigma-Aldrich, St. Louis, MO, 25mg/kg, i.p.) was selected based on its pharmacokinetic profile in rats compared with humans. Considering metabolic body weight [body weight raised to the 0.75 power] this is equivalent to about 700mg of caffeine in an 85kg adult human, well below the lethal dose of approximately 10g in humans (Fredholm, et al., 1999).

Neuroscore

Motor function was assessed prior to and 24 hours after FPI by an individual blinded to the experimental condition. Rats were scored from 0 – 4 for their left and right side performance on contraflexion (forelimb reaching), hindlimb flexion, and lateral pulsion. Scores range from 0-4, indicating no response to normal performance respectively, for a total of 24 possible points (McIntosh, et al., 1989).

Open Field Testing

Locomotor activity was assessed in the open field (Yee, et al., 2007). Tests were performed 24 hours and 4 weeks after FPI in squads of 4 animals. Each rat was placed in the center of a 50 × 50cm open field and videotaped for 50 minutes. Total distance traveled was measured using EthoVisionXT (Noldus Information Technology, Wageningen, The Netherlands). Distance was assessed for each animal in 5 minute bins for analysis.

Electrode Implantation

Three weeks after FPI, rats were anesthetized with isoflurane (as for FPI), then affixed into a stereotactic frame. Twisted-pair electrodes (PlasticsOne, Roanoke, VA, USA) were inserted into the brain with respect to bregma: –5.0mm anterior-posterior, +4.0mm medial-lateral, and –7.5mm dorso-ventral. Stainless steel reference screw electrodes (PlasticsOne) were

implanted over the cerebellum. Electrodes were inserted into a multi-channel electrode pedestal (PlasticsOne), and secured using dental cement (CO-Oral-Ite Dental Mft Co, Diamond Springs, CA). An additional 10 naïve adult male Sprague Dawley rats (Charles River) were included in the video-EEG analysis.

Video-EEG Recording

Four weeks after FPI, rats were connected to an amplifier (Grass Technologies, West Warwick, RI) for EEG monitoring. EEG signals were digitized (PowerLab, AD Instruments, Colorado Springs, CO) and recorded to a personal computer (Dell, Round Rock, TX). Video was acquired using an IR sensitive video camera (Noldus). Animals were subjected to 24 hours of continuous video-EEG monitoring. Epileptiform EEG-bursts were defined as high amplitude, periodic spike waveforms with greater than 5 seconds duration (Figure 3). EEG activities associated with grooming, eating, or drinking were excluded from analysis.

Histology

Following video-EEG, rats were deeply anesthetized with isoflurane, then transcardially perfused with 0.9% saline and 4% paraformaldehyde. Brains were post fixed in 4% paraformaldehyde, sunk in sucrose, frozen, and sectioned by cryostat. Nissl staining at Bregma – 3–4mm was used to calculate cortical thinning and lateral ventricular enlargement. Sections were scanned (HP 8350, Hewlett Packard), then quantified using ImageJ (NIH, Bethesda, MD).

Statistics

All statistics were performed using StatView (SAS Institute, Cary, NC). The distributions of weight, injury pressure, and apnea for each group were compared by t-test, and were not different among groups (Table 1). Correlation between caffeine treatment and mortality was assessed using logistic regression followed by a chi-squared test. Effects of caffeine and injury on Neuroscore were assessed by two-way ANOVA. Effects of caffeine and injury on Open Field performance were assessed by repeated measures ANOVA. Incidence of seizure activity was analyzed by logistic regression and the effect of caffeine treatment on burst duration by t-test. Results are presented as mean $\pm$ SEM.

RESULTS

Acute mortality following severe TBI can be prevented by a single acute dose of caffeine

Clinically, acute and prolonged lethal apnea is a pathological hallmark of severe TBI (The Brain Trauma Foundation and The American Association of Neurological Surgeons, 2000). To assess whether the duration of apnea correlates with lethal outcome, we induced severe TBI in rats by lateral fluid percussion injury (FPI). 15 rats received severe FPI, resulting in prolonged apnea and a mortality rate of 47%. A total of 8 rats survived the procedure, whereas 7 animals died within 10 minutes. Apnea was significantly longer in animals that died after severe FPI (Fig. 1A) compared to the apnea duration in survivors. Body weight and FPI pressure distribution were consistent between groups (Table 1). Thus, prolonged apnea correlates with lethal outcome.

To investigate whether lethal apnea is due to an adenosine-related mechanism and thereby preventable, we treated a second group of 8 rats within 1 minute after FPI with a single acute dose of caffeine (25 mg/kg, intraperitoneal). This treatment completely prevented the extended apnea seen in untreated non-survivors, resulting in apnea durations comparable to untreated survivors. Most importantly, caffeine completely prevented apnea-related acute mortality (Fig 1B). There were no statistical differences in the weight and FPI pressure

distributions in any of the injury groups (Table 1). Thus, lethal apnea can be prevented by caffeine treatment after injury.

Caffeine rescue does not impact neuromuscular performance after severe FPI

To rule out the possibility that caffeine rescue aggravates neurological deficits, we performed tests of basic neurological and motor functions 24 hours and 4 weeks after FPI. Neuroscore assessment of motor skills showed significant impairment in severely injured survivors in both groups compared to sham-injured animals, with a trend towards improvement in the rats receiving caffeine (Fig. 2A). Spontaneous locomotor activity in the open field showed a significant reduction in distance traveled by the severely injured rats (Fig. 2B), but no significant effect of caffeine, and no interaction between injury and caffeine. At 4 weeks after FPI, there was caffeine-independent recovery of open field activity in the severe injury group (Fig. 2C). Thus, a single acute dose of caffeine given after the FPI did not negatively impact neuromotor behavior when assessed either 24 hours or 4 weeks after brain injury.

Epileptiform EEG-bursts constitute an early consequence of severe TBI and can be reduced in duration by post-injury caffeine

Severe TBI is associated with the development of posttraumatic epilepsy (PTE) (Frey, 2003, Lowenstein, 2009). Here we used an established model of PTE, which induces spontaneous hippocampal seizures after a latency of 2-12 months (Kharatishvili, et al., 2006). To understand the early consequences of FPI for neuronal excitability in the hippocampus, we acquired simultaneous video and EEG recordings for 24 hours in each animal at four weeks after FPI. Video analysis revealed no evidence of clinical seizures. However, evaluation of the EEGs revealed frequent epileptiform bursting in 7 of the 16 severely injured animals, 1/10 sham injured animals, and 0/10 naïve animals. Bursting was distinguished by amplitude, periodicity, spike waveform, and duration greater than 5 seconds (Fig 3A), and increased EEG-power (Fig. 3C) as compared to quiescent periods (Fig. 3B). EEG activity associated with grooming, eating, or drinking were excluded from the analysis. While the number of bursts was not significantly affected by the single acute caffeine treatment after the injury, the burst duration was significantly reduced in the caffeine-treated rats as compared to the non-caffeinated rats (Fig. 4A). In addition, the total time spent bursting was significantly reduced in the acute caffeine group (Fig. 4B). These data demonstrate (i) that epileptiform EEG-bursts constitute an early pathological consequence of TBI, and (ii) that a single acute bolus of caffeine administered immediately after the injury can reduce the duration of those bursts, suggesting that the acute injury-associated adenosine surge is mechanistically linked to the development of PTE.

A caffeine bolus after severe FPI does not worsen histological damage

As lethal apnea is associated with severe injury, we were concerned that caffeine rescue might be associated with more severe brain damage that was not evident in the behavioral tests presented here. We assessed thinning in the injured cortex with respect to the contralateral cortex, and found a significant effect of severe injury on cortical thickness ($p < 0.05$), but no effect of (or interaction with) caffeine (Fig. 5A). We also assessed lateral ventricle enlargement at the same location, again comparing the area of the ventricle in the injured hemisphere to that of the contralateral hemisphere. Again, we found a significant effect of severe FPI that was independent of caffeine (Fig. 5B). Sample sections used for quantification are shown for each experimental condition (Fig. 5C-F). These results provide additional evidence that caffeine rescue after severe FPI does not result in a more severe injury phenotype.

DISCUSSION

Here we addressed an adenosine-based mechanism for mortality and morbidity following severe TBI by testing the hypotheses that an acute trauma-induced surge in adenosine (Clark, et al., 1997) influences acute and chronic outcomes after TBI, and that transient blockade of adenosine with the non-selective adenosine receptor antagonist caffeine would ameliorate acute and chronic consequences of severe TBI. We provide evidence that in severe TBI induced by a lateral fluid percussion, (i) lethal outcome correlates with apnea duration, (ii) lethal apnea can be prevented by antagonizing the effects of adenosine with a single acute dose of caffeine, (iii) caffeine rescue does not worsen neurological outcome, (iv) EEG bursts occur within 4 weeks after severe TBI, and (v) EEG-bursts can be ameliorated by a single dose of caffeine, suggesting a role of adenosine in their pathogenesis. These findings are of direct therapeutic significance and suggest a novel and unexpected therapeutic potential for caffeine.

The acute administration of caffeine before an injury in caffeine-naïve subjects is generally believed to promote injury and to have pro-convulsant effects (Boison, 2010); pretreatment of rats with caffeine caused significant, dose-dependent mortality after a cortical contusion injury (Al Moutaery, et al., 2003). While acute adenosine receptor blockade prior to TBI can promote injury, post-injury treatment with caffeine, as demonstrated here for the first time, can completely prevent trauma-associated lethal apnea, however without aggravating behavioral outcome parameters. These findings (i) support our hypothesis that lethal apnea following a severe TBI is causally linked to a surge of adenosine triggered by the injury and (ii) demonstrate beneficial effects of post-injury caffeine on morbidity.

Injury to the brain in general, such as TBI, stroke, or excessive seizures, are known to result in a surge of adenosine (Clark, et al., 1997, During and Spencer, 1992, Gouder, et al., 2004, Pignataro, et al., 2008). In further support of our hypothesis, lethal apnea and post-ictal brain shutdown in sudden, unexplained death in epilepsy (SUDEP) (Hirsch, 2010) has been attributed to excessive levels of adenosine due to deficiencies in the metabolic clearance of adenosine (Shen, et al., 2010). In those studies, an acute dose of 40mg/kg caffeine was shown to extend the life-span of mice following excessive seizures, suggesting a mechanistic relationship between SUDEP and lethal post-ictal apnea. To make therapeutic use of post-injury caffeine, it is important to rule out that caffeine, while preventing mortality, might negatively impact neurological outcome. In studies of gross neurological and motor functions we show that the animals that received caffeine show a slight but non-significant improvement compared to non-caffeinated controls, suggesting that there are no overt deleterious effects of the acute caffeine treatment.

Severe TBI is associated with an increased risk of PTE (Lowenstein, 2009). Studying the mechanisms of PTE is complicated by its long latency, often months or years after the traumatic event. Early electrophysiological studies using acute hippocampal slices demonstrate increased excitability at 1 week (Santhakumar, et al., 2000) and as late as 15 weeks (Golarai, et al., 2001) after brain injury. More recently, the progression to spontaneous seizures has been demonstrated in vivo after FPI (D'Ambrosio, et al., 2004), with 92% of rats demonstrating electrographic seizure activity at 8 weeks, progressing to generalized seizures in 50% of the survivors by 1 year after injury (Kharatishvili, et al., 2006). The incidence, frequency, and latency of these seizures demonstrate the face validity of the FPI model for the study of PTE, yet the logistics of the model make it difficult to efficiently propose and test hypotheses. We examined hippocampal electrographic activity at 4 weeks following FPI, and have found epileptiform bursts in the severe injury group, consistent with other studies (D'Ambrosio, et al., 2009). Remarkably, a single acute dose of caffeine given immediately after the FPI was linked to a significant reduction in burst

duration 4 weeks after the injury. This finding indicates potential disease-modifying consequences of early intervention with adenosine signaling at an early time point following FPI. More work is certainly needed to investigate the mechanistic relationship between an early surge of adenosine (blocked here at least partly with caffeine) and subsequent epileptogenesis. While it is not clear from our studies whether these bursts would develop into spontaneous generalized seizures, they provide evidence of early epileptiform activity and constitute a rational early target for evaluating interventions to modify the long-term outcome after TBI.

Caffeine, a non-selective antagonist of adenosine receptors at doses normally reached during human caffeine consumption, is the most widely used psychoactive substance, with a well-understood pharmacodynamic and pharmacokinetic profile (Fredholm, et al., 1999). By antagonizing the function of adenosine, which acts as an endogenous anticonvulsant and neuroprotectant of the brain (Dragunow, 1986, Dragunow and Faull, 1988, Dunwiddie, 1980, Ribeiro, 2005, Ribeiro, et al., 2003), the acute use of caffeine is generally thought to aggravate neuronal injury and to promote epileptic seizures (Boison, 2010). Using a model of closed head injury in female rats, the high mortality associated with pre-injury caffeine was delayed beyond the acute period evaluated in our study (Al Moutaery, et al., 2003). It is important to point out that in the present study we specifically assessed the immediate acute apnea-related mortality that has not been assessed in previous studies. This distinction might be model-dependent, since not all models of TBI recreate the immediate apnea-related mortality studied here.

In contrast, the chronic use of caffeine is generally thought to be neuroprotective, at least in part by effect inversion and adenosine receptor desensitization, at least under certain dosages (Fredholm, 1997, Jacobson, et al., 1996). In line with the neuroprotective effects of chronic caffeine, 3 weeks of caffeine administration to mice provided profound neuroprotection following a cortical contusion injury, whereas an acute dose of caffeine in the same model was without effect (Li, et al., 2008). The detrimental effects of acute caffeine are likely based on the blockade of A₁Rs. This notion is supported by findings that TBI in A₁R knockout mice led to lethal status epilepticus (SE) (Kochanek, et al., 2006). Likewise, A₁R knockout mice subjected to an excitotoxin succumbed to lethal SE (Fedele, et al., 2006). In human populations the use of an A₁R antagonist for the treatment of acute heart failure with renal impairment was associated with seizures as one of the observed side effects (Cotter, et al., 2008). Our current findings demonstrate for the first time that a single acute dose of caffeine, when given immediately *after* the injury prevents lethal outcome and are in apparent contrast to previous data discussed above.

The time-point of acute caffeine administration may contribute to the observed differences in pre- and post-injury caffeine administration. *In vitro*, stretch injury of neurons limits the effect of caffeine on calcium-induced calcium release (Weber, et al., 2002), suggesting that pre-injury caffeine in a caffeine-naïve neuron is additive with injury, but post-injury caffeine is not. *In vivo* studies examining the effect of a single bolus of caffeine on outcome after TBI considered a single time point, 30 minutes, prior to injury, sufficient for the caffeine to be well distributed throughout the brain, yet the relatively short half-life of caffeine (0.8 hours) suggests that some clearance has occurred prior to injury (Bonati, et al., 1984). In addition, caffeine when given prior to the injury is likely to affect the entire brain, whereas caffeine perfusion into the injured brain might be compromised in the most severely affected brain areas. This could be a likely explanation why the acute dose of caffeine after the injury did not worsen morbidity. However, full penetration of caffeine into brainstem is likely, since this region is not directly affected by the lateral fluid percussion injury. In our experiments, we found that post-injury caffeine treatment rapidly restored spontaneous breathing; the reduced hypoxia as a result of limiting apnea may surpass any consequent

negative effects of continued A₁R blockade. The relatively low affinity and rapid clearance of caffeine also may serve to limit the detrimental effects of A₁R blockade demonstrated in A₁R knockout mice (Kochanek, et al., 2006). Based solely on its actions as an adenosine receptor antagonist, we would predict that caffeine delivered to caffeine-naïve subjects immediately prior to TBI might act similarly to post-injury administration to prevent lethal apnea. Of more significant clinical importance, the influence of chronic caffeine consumption on A₁R and A_{2A}R expression (Svenningsson, et al., 1999) indicates that we must consider the protective effects of a single bolus of caffeine in both caffeine naïve and chronically caffeinated subjects.

The fact that caffeine is rapidly absorbed in the gut, readily crosses the blood-brain barrier, and is a relatively weak, non-selective antagonist may partly account for its post-injury efficacy without negative consequences. Adenosine has evolved to maintain homeostasis across organ systems and within the brain across neurotransmitters (Boison, 2008, Boison, et al., 2011, Fredholm, 2007); to maintain stability, there are likely many as yet unrecognized compensatory mechanisms activated in response to typical environmental challenges. The most successful therapeutics may be those that blunt the acute effects of a traumatic event, and then allow endogenous compensatory mechanisms to fully function. Caffeine is a widely consumed psychoactive substance (Barone and Roberts, 1996), and chronic consumption is known to affect adenosine receptor expression (Svenningsson, et al., 1999). Sleep disruption, another common fact of modern life and likely contributor to risk of TBI, may further alter adenosine receptor expression (Basheer, et al., 2004, Elmenhorst, et al., 2009). While chronic caffeine consumption is associated with favorable outcome after TBI (Sachse, et al., 2008), further studies to examine the combined effects of chronic pre-injury caffeine treatment with a protective post-injury caffeine treatment are essential to support the translation of the current findings to broader therapeutic use.

Our results are the first to demonstrate that caffeine limits apnea duration and prevents mortality when administered rapidly following severe TBI, without negative consequences. As apnea is a major cause of pre-hospital mortality, this finding presents a major therapeutic opportunity for first responders in both civilian and military environments. We also demonstrate that a single post-injury dose of caffeine can have long-lasting effects on electrographic burst durations, indicating that caffeine might beneficially influence processes involved in posttraumatic epileptogenesis, an interesting observation that warrants further experimentation. In conclusion, our studies show that, at a safe dose and without adverse neurological outcome, caffeine has the potential to prevent lethal apnea following TBI, and may reduce brain excitability long-term. Since caffeine is a well characterized and widely consumed drug, our findings present a translatable strategy to reduce acute lethal outcome after severe TBI.

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HIGHLIGHTS

- Apnea after severe TBI is correlated with lethal outcome.
- Lethal apnea after severe TBI can be prevented by a single dose of caffeine.
- Caffeine rescue does not worsen neurological outcome.
- EEG bursts occur within 4 weeks after severe TBI.
- EEG bursts are reduced by a single dose of caffeine after injury.

Figure 1. Caffeine treatment prevents acute mortality after severe lateral fluid percussion injury. (A) Acute mortality is associated with longer apnea duration (188 ± 40 s) after severe FPI than for survivors (61 ± 11 s). (B) Treatment with a single IP bolus of caffeine within 1 minute after FPI reduces apnea duration to 53 ± 9 s and prevents acute mortality. * $p < 0.01$.

Figure 2.

Caffeine rescue does not impair behavioral outcome parameters. (A) 24 hours after FPI, gross motor function was assessed by composite neuroscore on a scale of 0 (no function) to 24 (normal function; see methods). Neuroscore is significantly reduced in severely injured rats (* $p < 0.001$), with a trend toward improved function in the caffeine treated rats (# $p < 0.055$). (B) Severe injury results in reduced locomotion in the open field when compared with sham injury ($p < 0.01$), with no influence of caffeine treatment. (C) Four weeks after injury, there is no effect of FPI or caffeine treatment on open field performance.

Figure 3. Epileptiform bursting measured in the injured hippocampus. (A) Bursts associated with eating, drinking, or grooming, or shorter than 5 seconds were excluded from analysis. Bursts were characterized by increased amplitude (A) and power (B, C) as compared to quiescent periods.

Figure 4.

Caffeine treatment reduces the incidence of epileptiform bursting after severe FPI. (A) Burst duration was significantly reduced from 13.35 ± 1.4 seconds in the non-caffeinated rats to 6.94 ± 0.5 seconds in the rats that received an acute caffeine bolus. (B) The total time spent bursting over a 24 hour period was significantly reduced from 68.53 ± 12.78 seconds in the non-caffeinated rats to 11.88 ± 3.0 seconds in the acute caffeine group. (* $p < 0.05$)

Figure 5.

Severe FPI causes (A) cortical thinning and (B) ventricular enlargement, though neither measure is influenced by caffeine treatment (* $p < 0.05$). Sample Nissl stained sections from Bregma - 3.3 mm: (C) Sham, (D) Sham + 25 mg/kg caffeine, (E) Severe TBI, and (F) Severe TBI + 25 mg/kg caffeine used for quantification. The first image contains the whole image used for quantification, with the uninjured hemisphere on the left, and the injured hemisphere on the right. The next column is magnified to illustrate ventricular enlargement. The third and fourth images in each row depict the uninjured and injured cortices. Scale bars at the top of each column 1 mm.

FPI Parameters

Table 1

FPI	Caffeine (mg/kg)	Survival	Weight (g)	FPI Pressure (atm)
Severe	0	Died (n=7)	352 ± 9	1.96 ± 0.04
Severe	0	Survived (n=8)	345 ± 4	1.98 ± 0.04
Severe	25	Died (n=0)	NA *	NA *
Severe	25	Survived (n=8)	356 ± 4	1.96 ± 0.03
Sham	0	Survived (n=9)	358 ± 5	0
Sham	25	Survived (n=10)	349 ± 7	0

* None of the severe injury group died after receiving caffeine.