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Chronic caffeine exposure attenuates blast-induced memory deficit in mice

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ABSTRACT

Objective: To investigate the effects of three different ways of chronic caffeine administration on blast-induced memory dysfunction and to explore the underlying mechanisms.
Methods: Adult male C57BL/6 mice were used and randomly divided into five groups: control: without blast exposure, con-water: administrated with water continuously before and after blast-induced traumatic brain injury (bTBI), con-caffeine: administrated with caffeine continuously for 1 month before and after bTBI, pre-caffeine: chronically administrated with caffeine for 1 month before bTBI and withdrawal after bTBI, post-caffeine: chronically administrated with caffeine after bTBI. After being subjected to moderate intensity of blast injury, mice were recorded for learning and memory performance using Morris water maze (MWM) paradigms at 1, 4, and 8 weeks post-blast injury. Neurological deficit scoring, glutamate concentration, proinflammatory cytokines production, and neuropathological changes at 24 h, 1, 4, and 8 weeks post-bTBI were examined to evaluate the brain injury in early and prolonged stages. Adenosine A1 receptor expression was detected using qPCR.
Results: All of the three ways of chronic caffeine exposure ameliorated blast-induced memory deficit, which is correlated with the neuroprotective effects against excitotoxicity, inflammation, astrogliosis and neuronal loss at different stages of injury. Continuous caffeine treatment played positive roles in both early and prolonged stages of bTBI; pre-bTBI and post-bTBI treatment of caffeine tended to exert neuroprotective effects at early and prolonged stages of bTBI respectively. Up-regulation of adenosine A1 receptor expression might contribute to the favorable effects of chronic caffeine consumption.
Conclusion: Since caffeinated beverages are widely consumed in both civilian and military personnel and are convenient to get, the results may provide a promising prophylactic strategy for blast-induced neurotrauma and the consequent cognitive impairment.

1. Introduction

With the extensive use of explosive blasts as tactical weapons, explosive blast injury is becoming increasingly prevalent and is the major cause of mortality and morbidity in recent military conflicts and during terrorist attacks.[1–3] With the development of body armor, blast-induced traumatic brain injury (bTBI) has attracted increased concerns. A mild to moderate blast injury does not cause serious immediate visible neurological deficits but leads to long-lasting neuropsychiatric abnormalities and severely affects quality of life for victims and their families. It has been described as the "signature injury" of current military operations.[1,4–6] Cognitive dysfunction is one of the most frequent neuropsychiatric disorders after blast exposure. Although there is growing consensus that such mental problems of blast victims have a biological basis,[7] little is known about the mechanisms and prevention or cures for this kind of neurotrauma.

Caffeinated beverages such as coffee, tea, soft drinks, chocolate and many energy drinks are consumed world wide not only in

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civilian but also in military personnel. In the United States and Canada, for instance, the average consumption of caffeine for adults is about 2.4 mg/kg per day.[8] In some heavy coffee drinkers, the value may exceed 15 mg/kg per day. Caffeine is a xanthine and non-selective adenosine receptor antagonist with wide range and complex pharmacological actions.[9,10] In the central nervous system, caffeine administration has been reported to play beneficial roles against several cerebral insults including acute brain damage such as TBI and chronic neurodegenerative diseases such as Parkinson's disease, Alzheimer disease, etc. in both experimental animals[11–13] and clinical patients.[14,15]

According to the regulatory activity in the release of several neurotransmitters, adenosine receptors are considered to affect various behavioral functions including cognition.[16] Previously we had demonstrated that genetic or pharmacological inactivation of adenosine A2A receptor (A2AR) exerted neuroprotective effects against TBI- or bTBI- induced cognitive impairment. As a beverage that is consumed daily and because it is an adenosine receptor antagonist, whether or not caffeine exerts such positive effects on bTBI and the consequent cognitive function is worth exploring. Since caffeine is usually consumed chronically, here we investigated the attributions of three different ways of chronic caffeine exposure to the outcomes in moderate bTBI and the possible mechanisms involved. The findings will help us to determine the potential therapeutic value of caffeine in treating bTBI. Furthermore, the results may provide a rationale for both military personnel and civilian.

2. Methods

2.1. Animals and grouping

Pathogen-free, male C57BL/6 mice of 2–3 months old (weighing 20–24 g) were used. The mice were group housed in a temperature- and humidity-controlled room under a 12-h light–dark cycle in the Experimental Center of Medical Animals of the Daping Hospital/Research Institute of Surgery, the Third Military Medical University (Chongqing, China), with ad libitum access to food and water. All animal procedures used in this study were approved by the Administration of Affairs Concerning Experimental Animals Guidelines of Third Military Medical University and were performed under the supervision of the facility veterinary staff.

All mice were randomly divided into the following five groups: (1) control: without blast exposure, (2) con-water: administrated with water continuously before and after bTBI, (3) con-caffeine: administrated with caffeine continuously for 1 month before and after bTBI, (4) pre-caffeine: chronically administrated with caffeine for 1 month before bTBI and withdrawal after bTBI, (5) post-caffeine: chronically administrated with caffeine after bTBI. Caffeine was purchased from Sigma Chemical Company (Steinheim, Germany). Mice received caffeine administration via drinking water with a dosage of 0.25 g/L, which is a moderate amount equivalent to approximately two to three 8 oz. cups of coffee intake by a person.[17]

2.2. Blast injury model

The compression-driven bio-shock tube (BST-I) apparatus was used to produce blast as described previously.[18] Each conscious mouse was placed into an individual cage. To prevent quaternary blast injury, the cages were fixed on a metal shelf to restrict movement of mice's body during a rapid blast impact. All the cages were positioned at the same vertical plane inside the shock tube to ensure equal pressure exposure (Fig. 1A). The pressure the animals endured was measured by gauge PCB (Printed Circuit Board Piezoelectronics, www.pcb.com) positioned at the metal shelf. The peak overpressure (POB) was (321.2 ± 4.01) kPa and duration time was (50.29 ± 1.86) ms (Fig. 1B).

2.3. Learning and memory tests

Morris Water Maze (MWM) paradigms were used to evaluate learning and memory function at 1, 4, and 8 weeks after blast injury as described previously[19,20] with minor modification. The test consisted of a two-day visible cue task, a five-day reference memory task and a three-day working memory task.

At the beginning of each test period, a two-day visible cue task was performed to pre-train mice to escape from the water by locating the 'visible' platform,[20] which was made 'visible' by a local cue placed directly above the escape platform and was varied between the two days. This served to make the mice familiar with the swimming apparatus and the test procedure, and to screen out those having trouble in swimming or escape behavior.

The spatial reference memory tests consisted of an acquisition phase (day 1–4) and a probe/retention phase (day 5) as previously described[19,21]: Spatial working memory was evaluated from day 6 to day 8 using an adaptation of a four-trial 'repeated acquisition protocol'.[19] The platform was moved to a randomly chosen new position each day, but was kept in the same position for all trials on the same day. The mice were placed at the same starting point in all four consecutive trials of one day and were permitted to swim for 60 s or until they located at the platform. Working memory was measured by escape latency index ((trial1−trial2)/trial1), i.e. the reduction of escape latency from trial 1 to trial 2. The interval between trials was 20 s.

2.4. Neurological deficit scoring

Neurological deficit score was evaluated at 24 h, 1, 4 and 8 weeks post-blast exposure as described previously[22,23]: 0, no observable neurological deficits (normal); 1, failure to extend one of the forepaws (mild); 2, circling to one side of the body (moderate); and 3, loss of walking or righting reflex (severe).

2.5. Measurement of glutamate levels in CSF

At 24 h post-blast exposure, cerebrospinal fluid (CSF) of mice was collected from cisterna magna by puncture for detection of glutamate levels by high performance liquid chromatography (HPLC) using ortho-phthaldialdehyde pre-column derivatisation and fluorescence detection as previously reported.[17,24]

2.6. Quantitative PCR

Quantitative PCR was used to analyze the mRNA expression of inflammatory cytokines TNF-α and IL-1β in lateral cortex and hippocampus of both sides of the brain at 24 h, 1 and 4 weeks post-blast injury.[17,23] The mRNA level of adenosine A1 receptor (A1R) in lateral cortex was determined using the same procedure. The relative abundance of the target gene was normalized to GAPDH and the data were expressed as ratios relative to the control.

2.7. Histopathological evaluation

At 24 h, 1, 4, and 8 weeks after blast injury, anesthetized mice were killed by transcardial injection of saline followed by 4% paraformaldehyde. For immunohistochemistry, coronal paraffin-embedded brain sections of 4 μm thickness were incubated with anti-glial fibrillary acidic protein and anti-neuronal nuclei diluted in PBS (1:1500) for detection of GFAP and NeuN respectively.