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Administration of low dose methamphetamine 12 h after a severe traumatic brain injury prevents neurological dysfunction and cognitive impairment in rats ☆

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ABSTRACT

We recently published data that showed low dose of methamphetamine is neuroprotective when delivered 3 h after a severe traumatic brain injury (TBI). In the current study, we further characterized the neuroprotective potential of methamphetamine by determining the lowest effective dose, maximum therapeutic window, pharmacokinetic profile and gene expression changes associated with treatment. Graded doses of methamphetamine were administered to rats beginning 8 h after severe TBI. We assessed neuroprotection based on neurological severity scores, foot fault assessments, cognitive performance in the Morris water maze, and histopathology. We defined 0.250 mg/kg/h as the lowest effective dose and treatment at 12 h as the therapeutic window following severe TBI. We examined gene expression changes following TBI and methamphetamine treatment to further define the potential molecular mechanisms of neuroprotection and determined that methamphetamine significantly reduced the expression of key pro-inflammatory signals. Pharmacokinetic analysis revealed that a 24-hour intravenous infusion of methamphetamine at a dose of 0.500 mg/kg/h produced a plasma C_{max} value of 25.9 ng/ml and a total exposure of 544 ng/ml over a 32 hour time frame. This represents almost half the 24-hour total exposure predicted for a daily oral dose of 25 mg in a 70 kg adult human. Thus, we have demonstrated that methamphetamine is neuroprotective when delivered up to 12 h after injury at doses that are compatible with current FDA approved levels.

Introduction

Currently there are no approved therapeutic interventions available to prevent cognitive and behavioral deficits following traumatic brain injury (TBI) (Beauchamp et al., 2008). The development of viable treatments has been hindered by the fact that TBI represents a heterogeneous injury that activates multiple neuropathological pathways (Beauchamp et al., 2008; Dolan et al., 2012; Elder et al., 2010; Martin et al., 2008; Okie, 2005). During the primary injury phase, there is a rapid, uncontrolled release of glutamate that leads to calcium dysregulation, the production of reactive oxygen species (ROS), reactive nitrogen species (RNS), lipid peroxidation products, the release of prostaglandins, and the generation of nitric oxide (NO) (Pitkanen et al., 2009; Schober et al., 2012; Shultz et al., 2012; Stoica and Faden, 2010; Zhang et al., 2005). Consequently, these molecules induce microglial activation (Blaylock and Maroon, 2011; Block et al., 2007; Brown and Neher, 2010). Microglia are key mediators of both inflammatory responses and glutamate release (Brown and Neher, 2010). When activated in the presence of excessive glutamate, microglia exhibit a neuro-destructive phenotype and secrete pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-alpha), and interleukin-1 (IL-1) (Block et al., 2007; Brown and Neher, 2010; Stoica and Faden, 2010). The release of these cytokines induces a cascade of neuro-inflammation that perpetuates glutamate excitotoxicity and microglial activation eventually leading to neuronal loss and permanent neurological dysfunction (Blaylock and Maroon, 2011; Stoica and Faden, 2010).

Thus, survivors of severe TBI are likely to experience a degree of lasting neurological impairment, a permanent reduction in cognitive abilities, and psychological disturbances (Daneshvar et al., 2011; Dempsey et al., 2009). In the United States, 1.7 million individuals suffer from a TBI every year (Prevention C.f.D.C.a, 2006; Reeves and Panguluri, 2011; Saulle and Greenwald, 2012). Clearly, there is a crucial unmet need to develop novel, effective therapies that can be administered

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within a clinically relevant therapeutic window following TBI. We have recently published data demonstrating that administration of low dose methamphetamine following severe TBI resulted in improved functional and cognitive outcomes (Rau et al., 2012). In this previous study, we administered methamphetamine beginning 3 h after a severe TBI. While promising, a 3-hour delay to treatment does not represent a clinically relevant time frame. Therefore, in the present study we examined the therapeutic potential of methamphetamine at later time points. In addition, we more thoroughly characterized the pharmacology of methamphetamine by performing a dose response study and pharmacokinetic analysis. Finally, we examined critical gene expression changes in an effort to understand the potential molecular mechanisms of methamphetamine-mediated neuroprotection.

Methods

The Institutional Animal Care and Use Committee at The University of Montana approved all procedures in these studies. Adult, male, Wistar rats (350–500 g) were obtained from Charles River Laboratories (Wilmington, MA) and housed in filtered isolator boxes with a 12-hour light/dark schedule and ad libitum access to food and water. The lateral fluid percussion injury procedure was performed as we have previously published (Rau et al., 2012). Briefly, animals were deeply anesthetized using 2–4% isoflourane. A 5 mm trephination was made over the right hemisphere equidistant from the lambda and the bregma as we previously described (Rau et al., 2012). Animals were given a 20 ms fluid pulse to the dura at 1.9–2.3 ATM of pressure. Approximately 25% mortality was observed with these pressures. All animals became apneic after injury and were manually ventilated with supplemented O_2 until normal breathing resumed. At 7.5 or 11.5 h post-injury, an Alzet osmotic pump (Alzet corp; 2001D; 8.4 μL per hour) containing a methamphetamine solution designed to deliver 0.500, 0.250, or 0.250 mg/kg/h for 24 h was surgically implanted into the inguinal crease and connected to the femoral vein through a catheter as we previously described (Rau et al., 2012). The catheter (Scientific Commodities; Lake Havasu, AZ) from the pump was pre-loaded with a solution of 50% dextrose/50% heparin to prevent clotting. The length of the catheter and inner diameter were calculated to ensure methamphetamine delivery to the end of the catheter 30 min after insertion into the animal. At 8 or 12 h post injury, the animals were lightly anesthetized and a bolus dose of saline or methamphetamine was injected into the tail vein to coincide with the beginning of the pump delivery into the femoral vein. Bolus dosing for the 0.500 mg/kg/h dose was 0.425 mg/kg, 0.212 mg/kg for the 0.250 mg/kg/h dose, and 0.106 mg/kg for the 0.125 mg/kg/h dose. Saline and sham treated animals underwent the same procedure receiving pre-warmed saline. 72 h after injury the animals were re-anesthetized and the Alzet pumps were removed (Rau et al., 2012).

Neurological severity scoring

Neurological severity scoring (NSS) was performed as previously described (Rau et al., 2011, 2012). NSS and foot fault assessments were conducted on days 1, 7, 14, 21, 30, and day 40. Animals were scored from 0–16 with 16 indicating maximal impairment. Scoring criteria for a severe TBI was 16-10, Animals scoring 9 or less on day 1 were excluded as a moderate/mild injury.

Foot faults assessments

Foot fault assessments were conducted as previously described (Rau et al., 2012). Briefly, rats were set on an elevated grid. With each weight-bearing step, the paw may fall or slip off the wire grid. Each time the left forelimb (affected by damage to the right hemisphere) missed a placement on the wire rack it was recorded as a foot fault. The total number of steps (weight bearing movement of the right forelimb) that the rat used to cross the grid was counted, and the total number of foot faults for each forelimb was recorded.

Assessment of cognitive function

The Morris water maze (MWM) was used to assess the impact of methamphetamine on cognitive function (learning and memory) following TBI. The assessment procedure was performed as previously published (Rau et al., 2012). Pre-acclimation began on day 39 post-injury. The training phase began on day 40 post-injury, and the probe trial was conducted on day 45 post-injury. The water temperature was maintained at a constant 19 °Celsius with the clear plexiglas platform 2 cm below the water level. All data was recorded and analyzed using Anymaze software connected to a Logitech camera. All data sets were analyzed by a blinded researcher. There were no significant differences in swim speeds between any of the animals.

Immunohistochemistry

Forty-six days after TBI, rats were deeply anesthetized and perfused with 4% paraformaldehyde (PFA) fixative. Brains were post-fixed for 24 h at 4 °C in 4% PFA and divided into 2 mm coronal sections using a rat brain matrix. Slices were processed, paraffin embedded and sectioned at 7 μm. A 1 in 10 series from each rat was surveyed to identify location relative to Bregma. Sections were mounted at Bregma −3.3, deparaffinized and rehydrated. Antigen retrieval by treatment in 0.1 M citric acid pH 6 was performed, and sections were stained for a pan-axonal neurofilament marker (NF312 Covance, Princeton NJ) at a dilution of 1:1500 overnight at 4 °C in a humid chamber. Slides were washed with PBS 3 × 5 min and incubated with AlexaFluor 488 Goat anti Mouse IgG (Invitrogen, Life Technologies, Grand Island, NY) at a dilution of 1:300 for 1 h at room temperature in the dark. After rinsing in PBS 3 × 5 min nuclei were counterstained with DAPI (Invitrogen) diluted to 1 μg/ml in PBS for 5 min. Sections were rinsed in PBS, rinsed briefly in ddH_2O and coverslipped using FluorSave mounting media (Calbiochem, Darmstadt, Germany). Adjacent sections at Bregma −3.3 were deparaffinized, rehydrated, and permeabilized in 0.1% TritonX100 in PBS for 20 min. Following a PBS rinse slides were stained for 488 NeuroTrace (Molecular Probes, Life Technologies, Grand Island, NY) at a dilution of 1:400 overnight at 4 °C to label neurons. After NeuroTrace labeling, sections were rinsed in PBS 3 × 5 min and counterstained with DAPI as above. All sections were imaged on an Olympus Fluoview FV1000 confocal microscope (Olympus, USA), capturing the single brightest plane of fluorescence. For NF312 stained slides, images were captured at 40× (1.5 zoom) for the CA3 region of the hippocampus. Integrated Optical Density (IOD, a measure of area multiplied by the average density of staining) and axon length (a measure of total length of all one pixel thick open branches) of NF312 staining was measured on the injured hemisphere of the brain sections using ImagePro 6.2 software (ImagePro, Bethesda MD). The sum of the IOD or sum axon length from five saline, six 8 h and four 12 hour methamphetamine treated rats were measured. Treatment group averages of sum IOD or axon length and standard errors of the mean were calculated. Positive staining levels were identified by setting threshold levels from control slides reacted with secondary but no primary antibody. For the NeuroTrace stained slides images were captured at 20× (1.5zoom) in the CA1 region of hippocampus. Two sections per rat were imaged and dead cells were counted manually using ImagePro software. Live cells were round and contained NeuroTrace labeled (green) cytoplasm and blue (DAPI labeled) nuclei. Dead cells were very bright cells with condensed cytoplasm and nuclei and altered morphology. Average dead cells per rat and an average number of dead cells per treatment group were calculated. Four saline and four 8-hour methamphetamine and five 12 hour methamphetamine rats were counted and group averages and standard errors of the mean calculated.

RNA isolation/gene array protocol

Three biological replicates were used for each experimental group and each replicate was run in triplicate. Total RNA was isolated from the rat ipsilateral cortex utilizing Trizol LS (Invitrogen) according to the manufacturer's protocol. To remove any contaminating chemicals