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Delayed sodium pyruvate treatment improves working memory following experimental traumatic brain injury

Nobuhiro Moro[a,b], Sima S. Ghavim[a,b], David A. Hovda[a,b,c], Richard L. Sutton[a,b,*]

[a] Department of Neurosurgery, David Geffen School of Medicine at UCLA, University of California, Los Angeles, Los Angeles, CA 90095, USA
[b] Brain Injury Research Center, David Geffen School of Medicine at UCLA, University of California, Los Angeles, Los Angeles, CA 90095, USA
[c] Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, University of California, Los Angeles, Los Angeles, CA 90095, USA

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ABSTRACT

Prior work indicates that cerebral glycolysis is impaired following traumatic brain injury (TBI) and that pyruvate treatment acutely after TBI can improve cerebral metabolism and is neuroprotective. Since extracellular levels of glucose decrease during periods of increased cognitive demand and exogenous glucose improves cognitive performance, we hypothesized that pyruvate treatment prior to testing could ameliorate cognitive deficits in rats with TBI. Based on pre-surgical spatial alternation performance in a 4-arm plus-maze, adult male rats were randomized to receive either sham injury or unilateral (left) cortical contusion injury (CCI). On days 4, 9 and 14 after surgery animals received an intraperitoneal injection of either vehicle (Sham-Veh, $n = 6$; CCI-Veh, $n = 7$) or 1000 mg/kg of sodium pyruvate (CCI-SP, $n = 7$). One hour after each injection rats were retested for spatial alternation performance. Animals in the CCI-SP group showed no significant working memory deficits in the spatial alternation task compared to Sham-Veh controls. The percent four/five alternation scores for CCI-Veh rats were significantly decreased from Sham-Veh scores on days 4 and 9 ($p < 0.01$) and from CCI-SP scores on days 4, 9 and 14 ($p < 0.05$). Measures of cortical contusion volume, regional cerebral metabolic rates of glucose and regional cytochrome oxidase activity at day 15 post-injury did not differ between CCI-SP and CCI-Veh groups. These results show that spatial alternation testing can reliably detect temporal deficits and recovery of working memory after TBI and that delayed pyruvate treatment can ameliorate TBI-induced cognitive impairments.

Performance of cognitive tasks increases the demand for metabolic fuel in the brain. For example, brain glucose utilization increases during task performance, extracellular glucose levels fluctuate dynamically and regionally during behavioral tests, and glucose concentrations in the hippocampus decrease rapidly and to an extent dependent on task complexity [5,23,25,31]. In normal young adult rats reductions of extracellular glucose during spatial alternation performance is prevented by systemic administration of exogenous glucose, which also improves cognitive performance [23,25,26]. Pre-trial glucose treatment in aged rats, who demonstrate cognitive impairments and more profound and prolonged decrease in hippocampal glucose compared to young adults, is also reported to elevate extracellular glucose levels and improve performance [24].

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide, with an estimated 2% of the US population suffering from long-term neurological and cognitive impairments as a result of injury [19]. Treatment options, particularly in the chronic phase after TBI, are limited. Following an acute phase of neuronal excitation and anaerobic glycolysis, the chronic phase of TBI is characterized by neuronal depression and reductions in cerebral metabolism [3,4,13,17,20,33,42,46]. Interestingly, it was recently reported that systemic glucose injection prior to cognitive testing 11–15 days after injury improved spatial learning ability in rats with fluid percussion-induced TBI [18].

Since the acute pathophysiology of TBI can interfere with glucose metabolism or shunt glucose to alternative pathways [1,2] we, and others, have begun to examine the potential benefits of exogenous pyruvate treatments after experimental TBI. If administered acutely (<24 h) after TBI, systemic pyruvate treatment elevates the brain's extracellular concentration of both pyruvate and glucose, attenuates cerebral inflammation and neuronal cell loss, improves cerebral cytochrome oxidase (CyO) activity and improves

Abbreviations: ANOVA, analysis of variance; 2DG, [^{14}C]2-deoxy-D-glucose; CCI, cortical contusion injury; CyO, cytochrome oxidase; PBS, phosphate buffered saline; rCMRG, regional cerebral metabolic rates of glucose; SEM, standard error of the mean; SP, sodium pyruvate; TBI, traumatic brain injury; Veh, vehicle.

* Corresponding author at: Department of Neurosurgery, David Geffen School of Medicine at UCLA, Box 957039, Los Angeles, CA 90095-7039, USA.
Tel.: +1 310 825 7227; fax: +1 310 794 2147.
E-mail addresses: nmoro@mednet.ucla.edu (N. Moro), sghavim@mednet.ucla.edu (S.S. Ghavim), dhovda@mednet.ucla.edu (D.A. Hovda), rsutton@mednet.ucla.edu (R.L. Sutton).

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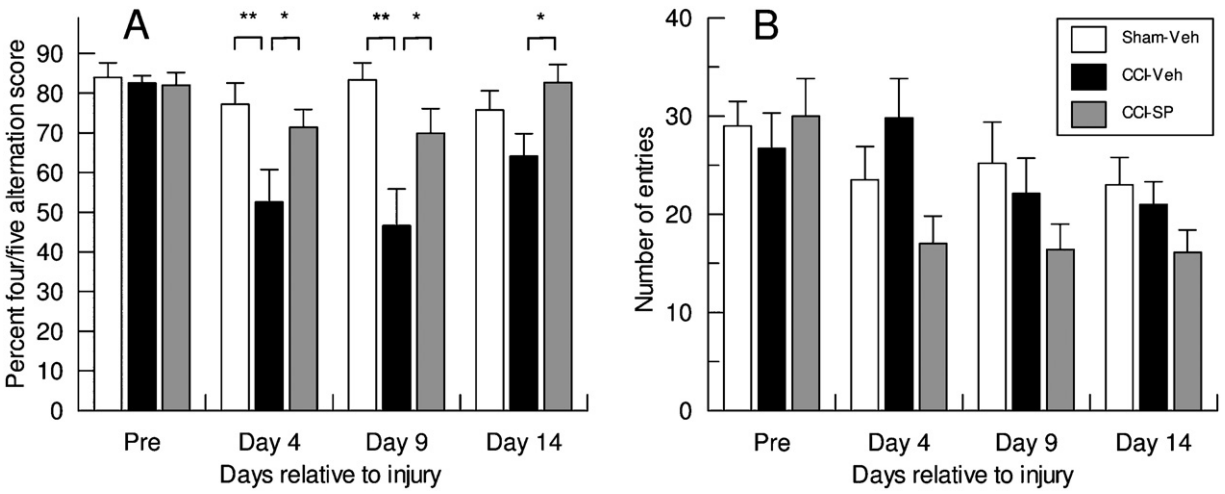

Fig. 1. Mean ± SEM percent four/five spatial alternation score (A) and number of arm entries (B) during plus-maze tests conducted pre- and post-injury in rats with sham or cortical contusion injury (CCI) and treatment with either vehicle (Veh) or sodium pyruvate (SP) 1 h prior to testing on days 4, 9 and 14. CCI significantly worsened the alternation scores on day 4 and day 9 but SP treatment prevented this worsening. Number of arm entries did not differ between groups on any test day. $^{*}p < 0.05$, $^{**}p < 0.01$.

neurological recovery [10,28,48]. However, acute treatments with sodium pyruvate (SP) did not ameliorate working memory impairments in a spontaneous alternation task 1 week after TBI [28].

Based on the recent report showing that delayed glucose treatment improved cognitive outcome [18], the current study was conducted to determine if delayed SP treatments would improve spatial alternation performance in rats with experimental TBI. Potential treatment effects on contusion volume and regional cerebral metabolism were assessed after completion of the behavioral study.

All experimental procedures were reviewed and approved by the UCLA Chancellor's Committee for Animal Research. Efforts were made to minimize pain and discomfort to the animals and the number of subject's used in the study.

Twenty adult male (77 days of age, weighing 336–398 g on the day of surgery) Sprague–Dawley rats (Charles River, Hollister, CA) were pair-housed in plastic cages and maintained in a light (12:12 h light:dark cycle) and temperature controlled (21 ± 1 °C) environment. After one week of acclimatization the rats were tested in a plus-maze to assess pre-surgical spatial alternation ability. After placing the rat in the center of the maze it was allowed to explore freely during a 15 min test and the number and sequence of entries into each arm was recorded. Alternations were counted when each arm was visited within a span of 5 arm entries and this number was divided by total possible alternations (total entries minus four) and multiplied by 100 to calculate the percent four/five alternation score [23,25,28,44]. Chance performance is 45–50%. Based on these alternation scores animals were randomized to 1 of 3 treatment groups. Surgical procedures have been previously reported in detail [10,28]. In brief, isoflurane-anesthetized rats were subjected to either a left hemisphere cortical contusion injury (CCI; using a 5 mm flat-tip impactor at 2.32 m/s velocity, 2 mm tissue compression for 250 ms) or sham injury (no craniotomy).

On days 4, 9 and 14 post-surgery all rats were given an additional 15 min test in the plus-maze to assess working memory performance. One hour prior to each post-surgical test sham injury controls (Sham-Veh: $n = 6$) and one CCI group (CCI-Veh: $n = 7$) were injected (2.5 ml/kg, i.p.) with 0.1 M phosphate buffered saline (PBS, pH 7.6). Another CCI group was injected (i.p.) with 1000 mg/kg of SP (400 mg/ml, dissolved in PBS immediately prior to injection) 1 h prior to each post-surgical test (CCI-SP: $n = 7$). This SP dosage increases cerebral extracellular levels of pyruvate for at least 75 min post-injection [10]. Sham-SP effects were not examined since pyruvate had no effect on alternation performance in young adult rats [35].

Fifteen days after surgery all rats underwent [^{14}C]2-deoxy-D-glucose (2DG) autoradiography procedures to evaluate regional cerebral metabolic rates of glucose (rCMRG) [32,41,42,46]. Briefly, under isoflurane anesthesia the right femoral artery and vein were catheterized and the rats were restrained on a cardboard plank during a 2 h period to allow recovery from anesthesia. 2DG (120 μCi/kg; American Radiolabeled Chemicals Inc., St. Louis, MO) was infused over 30 s via the femoral vein and arterial blood samples were drawn at predetermined intervals over 45 min for plasma ^{14}C and glucose assays. At 45 min rats were given a lethal dose of sodium pentobarbital (100 mg/kg, i.v.), decapitated and their brains were removed and flash frozen (−55 °C, dry ice cooled 2-methylbutane).

Coronal brain sections were cut at 20 μm, saving 3 sequential sections for 2DG autoradiography and 2 sequential sections for CyO histochemistry every 500 μm. Tissue sections and ^{14}C standards were apposed to Kodak Biomax film for 48 h and images were digitally captured with a flat-bed scanner (256 dpi, 8 bit gray scale). A minimum of 5 optical density readings from 8 cortical and 7 subcortical regions of interest (see Fig. 2 for list of regions) were obtained bilaterally using ImageJ software (version 1.42q: National Institutes of Health, Bethesda, MD) and the rCMRG was calculated [41].

CyO histochemistry was performed as previously reported [13,28] to assess cerebral oxidative capacity in tissue sections digitally captured at 600 dpi, obtaining optical density readings in the same regions evaluated using rCMRG. Tissue standards (10, 14, 20 and 25 μm-thick brain sections of naïve rat brain) were included in each batch of CyO reaction medium to establish correlations between section thickness and staining intensity (average Pearson r^2: 99.3 ± 0.002). Slopes of each standard curve were similar (2.002 ± 0.02), so regional optical density readings were normalized (by subtracting the difference between the intercept of the co-stained standards and that of the average for all standards) to control for potential batch staining differences.

The non-injured left and right cortical mantle in CyO stained tissue sections spaced at 1 mm intervals was viewed, traced and quantified (area and volume) using computer-assisted morphometry. The percent tissue loss in the left cortical mantle from +1.2 to −6.8 mm from bregma was calculated using the formula [100 − ((Left/Right) × 100)], as previously described [10,28,44].

All data are reported as the group average ± standard error of the mean (SEM). Plus-maze data were first analyzed by repeated measures analysis of variance (ANOVA), with further analysis of