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Research Report

Pyruvate treatment attenuates cerebral metabolic depression and neuronal loss after experimental traumatic brain injury

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ABSTRACT

Experimental traumatic brain injury (TBI) is known to produce an acute increase in cerebral glucose utilization, followed rapidly by a generalized cerebral metabolic depression. The current studies determined effects of single or multiple treatments with sodium pyruvate (SP; 1000 mg/kg, i.p.) or ethyl pyruvate (EP; 40 mg/kg, i.p.) on cerebral glucose metabolism and neuronal injury in rats with unilateral controlled cortical impact (CCI) injury. In Experiment 1 a single treatment was given immediately after CCI. SP significantly improved glucose metabolism in 3 of 13 brain regions while EP improved metabolism in 7 regions compared to saline-treated controls at 24 h post-injury. Both SP and EP produced equivalent and significant reductions in dead/dying neurons in cortex and hippocampus at 24 h post-CCI. In Experiment 2 SP or EP were administered immediately (time 0) and at 1, 3 and 6 h post-CCI. Multiple SP treatments also significantly attenuated TBI-induced reductions in cerebral glucose metabolism (in 4 brain regions) 24 h post-CCI, as did multiple injections of EP (in 4 regions). The four pyruvate treatments produced significant neuroprotection in cortex and hippocampus 1 day after CCI, similar to that found with a single SP or EP treatment. Thus, early administration of pyruvate compounds enhanced cerebral glucose metabolism and neuronal survival, with 40 mg/kg of EP being as effective as 1000 mg/kg of SP, and multiple treatments within 6 h of injury did not improve upon outcomes seen following a single treatment.

1. Introduction

Traumatic brain injury (TBI) induces an immediate increase in neuronal depolarization and a concomitant increase in energy demand, which can persist for minutes to hours after experimental TBI. These effects are reflected in the elevated cerebral metabolic rates of glucose (CMRGlc) and anaerobic glycolysis (Katayama et al., 1990; Lee et al., 1999; Sutton et al., 1994; Yoshino et al., 1991) and by reduced levels of extracellular glucose and elevated concentrations of extracellular lactate (Chen et al., 2000; Fukushima et al., 2009; Krishnappa et al., 1999). This relatively brief period of hyperglycolysis is followed by a more prolonged (days to weeks) period of reduced CMRGlc (Dunn-Meynell and Levin, 1995; Moro et al., 2011; Prins and Hovda, 2009; Sutton et al., 1994; Yoshino et al., 1991) and increased shunting of glucose to the pentose-phosphate pathway occurs within hours of TBI (Bartnik et al., 2005, 2007). In TBI patients the durations of elevated CMRGlc, low levels of extracellular glucose with elevated lactate, and cerebral metabolic depression are generally more protracted than those observed in experimental TBI models (Bergsneider et al., 1997, 2000; Vespa et al., 2003).

The mismatch between cerebral metabolic or energy demands in the face of decreased levels of glucose, the primary energy source for brain cells, has led several investigators to consider early administration of supplemental fuels to meet the cerebral metabolic demands and/or to avoid “energy crisis” after TBI. Studies have now shown that providing experimental TBI subjects with exogenous lactate (Alessandri et al., 2012; Chen et al., 2000; Holloway et al., 2007; Rice et al., 2002), pyruvate (Fukushima et al.,

Abbreviations: ANOVA, analysis of variance; CCI, controlled cortical impact; CMRGlc, cerebral metabolic rates of glucose; DAPI, 4′,6-diamidino-2-phenylindol dihydrochloride; EP, ethyl pyruvate; FJB, Fluoro-Jade B; SAL, saline (8%); SP, sodium pyruvate; SEM, standard error of the mean; TBI, traumatic brain injury

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2009; Moro and Sutton, 2010; Shi et al., 2015; Su et al., 2011; Zlotnik et al., 2008, 2012) or ketone bodies (Appelberg et al., 2009; Deng-Bryant et al., 2011; Prins and Hovda, 2009; Prins et al., 2004) can improve various measures of cerebral metabolism, neuronal survival and/or neurological outcomes. Our group has also shown that early provision of high doses of glucose in rats can attenuate TBI-induced decreases in CMRGlc and reduce neuronal injury, while providing mild sensorimotor behavioral improvements (Moro et al., 2013; Shijo et al., 2015).

The current studies in rats with unilateral controlled cortical impact (CCI) injury were undertaken to determine the effects of acute administration of pyruvate compounds on CMRGlc and neuronal viability 24 h post-injury. These studies were conducted concomitantly with those to evaluate effects of glucose on these same measures (Moro et al., 2013), and the data for saline-treated controls here is the same as for those studies. Here we compare treatment with sodium pyruvate (SP; 1000 mg/kg, i.p.) with ethyl pyruvate (EP; 40 mg/kg, i.p.), a more lipophilic and electrically neutral derivative of pyruvic acid that is capable of entering cells without use of the monocarboxylate transporters (Kao and Fink, 2010; Tokumaru et al., 2009). We and others have previously shown that EP at 20–40 mg/kg is at least as neuroprotective as SP at doses of 500–1000 mg/kg (Kim et al., 2005; Lee et al., 2001; Moro and Sutton, 2010; Yu et al., 2005). In Experiment 1 we evaluated the effects of a single treatment with SP or EP immediately following injury, to evaluate the hypothesis that supplemental pyruvate could meet the acute energy demands and attenuate neuronal injury after TBI. Experiment 2 was conducted to determine the effects of multiple SP or EP injections, given immediately (time 0) and then at 1, 3 and 6 h post-CCI. The multiple treatment protocol was employed based on evidence of CCI-induced energy demands persisting to at least 2 h post-injury (Lee et al., 1999), three acute SP treatments were needed to reduce contusion volume 2 weeks post-CCI (Fukushima et al., 2009), and post-TBI depolarization or neuronal hyperexcitability may prolong any mismatch between fuel supply and energy demands (Giesemer and Mautes, 2007; Hashemi et al., 2009; Lauritzen et al., 2011; Vespa et al., 2007; Hopwood et al., 2005).

2. Results

2.1. Experiment 1: single saline or pyruvate treatment

2.1.1. Physiological data

As shown in the data of Table 1, there was a significant loss in body weight within 24 h of injury for all CCI groups compared to Sham-SAL controls (p's < 0.05). The largest decline in body weight occurred in the CCI-SP group, where the weight change was significantly greater than that observed for CCI-SAL and CCI-EP groups (p's < 0.05). Also shown in Table 1 are the data for arterial blood gasses and plasma concentrations of glucose and lactate at 24 h post-injury, prior to the [^{14}C]2-deoxy-D-glucose (14C-2DG) injection. All of these physiological measures were within normal ranges and there were no significant differences between the 4 treatment groups.

2.1.2. Cerebral glucose utilization

As detailed in our prior publication (Moro et al., 2013), three shipments of ^{14}C-2DG that differed in their specific activities were used during these experiments, resulting in variable CMRGlc values (μmol/100 g/min) within each group. However, data analysis showed no effect of CCI injury on CMRGlc in right hemisphere structures in subsets of animals injected with the same lot number of 2DG, and treatment effects were similar for ipsilateral (left hemisphere) CMRGlc data and for CMRGlc asymmetry scores

Table 1
Mean (±SEM) change in body weight (in grams) one day post-injury, and the baseline arterial blood pH, gasses, and plasma glucose and lactate concentrations (mmol/L) prior to 2DG injection in Sham and CCI groups given one saline (SAL), sodium pyruvate (SP) or ethyl pyruvate (EP) injection immediately after surgery.

	Sham-SAL	CCI-SAL	CCI-SP	CCI-EP
Sample size	$n=10$	$n=9$	$n=10$	$n=9$
Wt. change	$-$**0.7** ± *1.64*	$-$**8.9** ± *1.65* *,#	$-$**16.5** ± *3.64* ***	$-$**8.9** ± *2.11* *,#
pH	**7.42** ± *0.01*	**7.42** ± *0.01*	**7.42** ± *0.01*	**7.41** ± *0.01*
pCO_2 (mm Hg)	**39.5** ± *1.27*	**38.6** ± *0.58*	**37.9** ± *0.92*	**38.9** ± *0.99*
pO_2 (mm Hg)	**90.8** ± *3.62*	**84.2** ± *0.98*	**85.3** ± *2.39*	**82.6** ± *2.24*
HCO_3s	**26.1** ± *0.48*	**25.4** ± *0.60*	**24.9** ± *0.46*	**24.9** ± *0.24*
tCO_2	**26.9** ± *0.73*	**26.0** ± *0.72*	**25.8** ± *0.55*	**25.6** ± *0.39*
O_2Sat	**96.9** ± *0.35*	**96.4** ± *0.15*	**96.5** ± *0.35*	**96.0** ± *0.33*
Glucose	**9.5** ± *0.29*	**8.9** ± *0.36*	**8.8** ± *0.25*	**8.5** ± *0.27*
Lactate	**0.7** ± *0.08*	**0.8** ± *0.11*	**0.6** ± *0.05*	**0.6** ± *0.05*

* $p < 0.05$ compared to Sham-SAL.
*** $p < 0.001$ compared to Sham-SAL.
# $p < 0.05$ compared to CCI-SP.

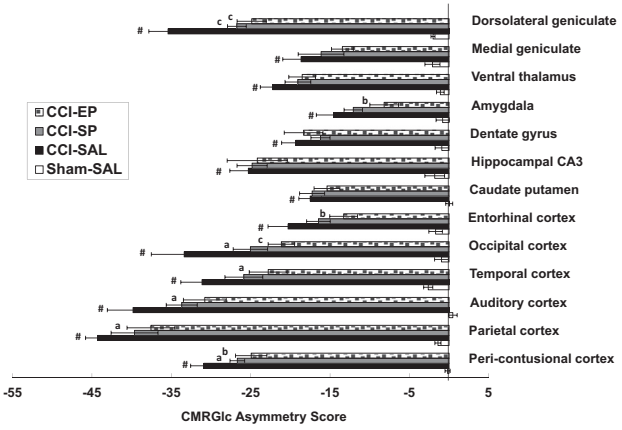

Fig. 1. Mean (bars represent SEM) asymmetry scores [((L-R)/L+R)*100] for CMRGlc in brain regions 24 h after Sham or CCI injury and a single treatment of saline (SAL), sodium pyruvate (SP) or ethyl pyruvate (EP). #$p < 0.001$ compared to Sham injury; [a] $p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ compared to CCI-SAL.

[(left − right/left + right) × 100] (Moro et al., 2013). Therefore, regional cerebral glucose utilization in the current experiments was analyzed based on calculated CMRGlc asymmetry scores. As illustrated in Fig. 1, these asymmetry scores for the Sham-SAL control group ($n=10$) were near zero in all brain regions at 24 h post-surgery, indicating the normally similar metabolism between hemispheres. The reductions in ipsilateral CMRGlc at 24 h after left hemisphere injury produced significantly negative asymmetry values in all brain regions in the three CCI groups compared to Sham-SAL (p's < 0.001). The magnitude of metabolic depression was generally larger in brain regions of CCI-SAL ($n=9$) compared to CCI-SP ($n=10$) or CCI-EP ($n=9$) groups. The single injection of either SP or EP after CCI injury significantly improved CMRGlc asymmetry scores in the midline peri-contusional cortex (p's < 0.05) and occipital cortex (p's < 0.05), as well as for the dorsal lateral geniculate (p's < 0.001). In addition, relative to CCI-SAL controls, improved (i.e., less negative) CMRGlc asymmetry scores by EP treatment after CCI were significant for the parietal ($p < 0.05$), auditory ($p < 0.05$), temporal ($p < 0.05$) and entorhinal cortex ($p < 0.01$), as well as for the amygdala ($p < 0.01$). Although glucose utilization was improved relative to CCI-SAL in more regions after EP treatment, the CCI-SP and CCI-EP asymmetry scores did not differ significantly for any brain region.

2.1.3. Injury severity and neuronal injury in cortex and hippocampus

Ratings of tissue swelling at the contusion site post-injury were