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Pyruvate enhances neurological recovery following cardiopulmonary arrest and resuscitation

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Abstract

Purpose—Cerebral oxidative stress and metabolic dysfunction impede neurological recovery from cardiac arrest-resuscitation. Pyruvate, a potent antioxidant and energy-yielding fuel, has been shown to protect against oxidant- and ischemia-induced neuronal damage. This study tested whether acute pyruvate treatment during cardiopulmonary resuscitation can prevent neurological dysfunction and cerebral injury following cardiac arrest.

Methods—Anesthetized, open-chest mongrel dogs underwent 5 min cardiac arrest, 5 min open chest cardiac compression (OCCC), defibrillation and 3 day recovery. Pyruvate ($n = 9$) or NaCl volume control ($n = 8$) were administered (0.125 mmol/kg/min *iv*) throughout OCCC and the first 55 min recovery. Sham dogs ($n = 6$) underwent surgery and recovery without cardiac arrest-resuscitation.

Results—Neurological deficit score (NDS), evaluated at 2 day recovery, was sharply increased in NaCl-treated dogs (10.3 ± 3.5) vs. shams (1.2 ± 0.4), but pyruvate treatment mitigated neurological deficit (NDS = 3.3 ± 1.2 ; $P < 0.05$ vs. NaCl). Brain samples were taken for histological examination and evaluation of inflammation and cell death at 3 d recovery. Loss of pyramidal neurons in the hippocampal CA1 subregion was greater in the NaCl controls than in pyruvate treated dogs ($11.7 \pm 2.3\%$ vs. $4.3 \pm 1.2\%$; $P < 0.05$). Cardiac arrest increased caspase 3 activity, matrix metalloproteinase activity, and DNA fragmentation in the CA1 subregion; pyruvate prevented caspase-3 activation and DNA fragmentation, and suppressed matrix metalloproteinase activity.

Conclusion—Intravenous pyruvate therapy during cardiopulmonary resuscitation prevents initial oxidative stress and neuronal injury and enhances neurological recovery from cardiac arrest.

Keywords

brain ischemia; cardiac arrest; cardiopulmonary resuscitation (CPR); cerebral blood flow; inflammatory response; metabolism; neurological dysfunction; post-resuscitation period

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6. CONFLICT OF INTEREST

The authors have no professional or personal conflicts of interest which could impact this work.

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1. INTRODUCTION

Neurological damage inflicted by cardiac arrest-resuscitation contributes to high mortality and residual neurological dysfunction after cardioversion.^{1,2} Cardiac arrest-resuscitation imposes global cerebral ischemia-reperfusion. Interruption of cerebral blood flow during arrest depletes high energy metabolites required to maintain neuronal function and integrity. Cerebral oxidative stress during resuscitation and following cardioversion³ can exacerbate structural and functional impairment. Markers of systemic inflammation are elevated after cardiopulmonary resuscitation,^{4,5} and inflammatory mediators, *e.g.* matrix metalloproteinases⁶ are activated in the brain. Oxidative and inflammatory cascades initiated during cerebral ischemia-reperfusion culminate in cell injury and death^{7,8} which can continue for several days following transient ischemic insult.^{9–11} Neuronal death following transient global ischemia involves both necrosis and apoptosis¹² and correlates with clinical impairment of function.¹⁰

Pyruvate, a well documented natural antioxidant and metabolic fuel,¹³ bolsters energy reserves and antioxidant redox state in post-ischemic and oxidant-injured myocardium.¹³ Pyruvate readily traverses the blood brain barrier via monocarboxylate transporters¹⁴ and provides an excellent energy substrate for neurons and astroglia.¹⁵ Pyruvate's antiacidotic,^{16,17} anti-inflammatory¹⁸ and anti-apoptotic¹⁹ properties could minimize neuronal death following cerebral ischemia-reperfusion. Indeed, pyruvate treatment preserves neurons following exposure to oxidants.^{20,21} Pyruvate also improves neuronal survival following hypoglycemic insult²² and minimizes neuronal loss 72 h after transient forebrain ischemia in rats.²³ Intravenous pyruvate stabilized cerebral energy reserves and preserved electroencephalographic activity in pigs subjected to hemorrhagic shock.²⁴ Collectively, these reports suggest that giving pyruvate during cardiopulmonary resuscitation could minimize ischemic injury inflicted on the central nervous system by cardiac arrest.

This study tested whether intravenous administration of sodium pyruvate during cardiopulmonary resuscitation prevented neurological deficit and neuronal injury 2–3 days after cardiac arrest. Open chest dogs underwent 5 min cardiac arrest, 5 min of open chest cardiac compressions and cardioversion. Neurological recovery was evaluated over the next 3 days, then brain tissue was harvested for assessment of inflammation and neuronal injury.

2. METHODS

Instrumentation and protocol surgery

All animal experimentation was conducted according to the *Guide to the Care and Use of Laboratory Animals* (NIH Publication 85-23, revised 1996), and was approved by the Animal Care and Use Committee of the University of North Texas Health Science Center. Twenty-three mongrel dogs weighing 22 ± 4 kg were randomly assigned to sham (3♂, 3♀), pyruvate (4♂, 5♀) or vehicle control NaCl (4♂, 4♀) groups. Pyruvate and NaCl groups underwent an open chest cardiac arrest-CPR-defibrillation protocol¹⁷ under sterile conditions. The experimenters conducting CPR protocol were blinded to the treatments administered. Sham controls underwent the same surgical procedures and 3 days recovery without cardiac arrest-resuscitation. The left saphenous vein was cannulated for infusion of pyruvate or NaCl, and the left antecubital vein was cannulated to administer drugs during resuscitation. Rectal temperature was maintained at 36–37°C using circulating water heating pads. The dogs were sedated with acepromazine ($0.1 \text{ mg kg}^{-1} \text{ i.m.}$), anesthetized with thiopental ($15 \text{ mg kg}^{-1} \text{ iv}$), and then maintained with 1–2% halothane in O_2 (1 L min^{-1}) enriched air. Following tracheal intubation, the dogs were mechanically ventilated to maintain the arterial O_2 saturation, monitored with a pulse oximeter, within physiological limits of 94–96%. Under sterile

conditions the chest cavity was opened via left lateral thoracotomy through the 5th intercostal space, and the pericardium was divided and reflected to expose the left ventricular epicardium. A 17 gauge catheter was implanted in the thoracic aorta to monitor blood pressure and sample arterial blood. This catheter was brought to the surface through the layers of the chest and fixed to the skin. The animals were then allowed to stabilize for at least 15 min before starting the arrest-resuscitation protocol.

Cardiac arrest- resuscitation protocol

To initiate cardiopulmonary arrest, ventricular fibrillation was induced by applying a 9V current to the epicardium, and ventilation was suspended. At 5 min arrest, 1 mg epinephrine (adrenaline) was injected into the left ventricular lumen, intravenous infusion of pyruvate or NaCl was begun, mechanical ventilation was resumed at 12 cycles min⁻¹, and open chest cardiac compressions (OCCC; 60 min⁻¹) were applied to achieve mean aortic pressure ~ 40 mmHg over the next 5 min. Sterile 2M aqueous solutions of pyruvate or NaCl were continuously infused (0.125 mmol·kg⁻¹·min⁻¹) into the left saphenous vein from the beginning of OCCC until 55 min after recovery of spontaneous circulation (ROSC). This rate of pyruvate infusion achieves stable concentrations of ~3.5 mM in systemic arterial plasma,¹⁷ a concentration that stabilized cerebral function in a porcine hemorrhagic shock model.²⁴ After 5 min OCCC the heart was defibrillated with direct current countershocks applied to the epicardium with sterile internal paddles. Up to 8 (3 · 10 J, 3 · 20 J, 2 · 30 J) countershocks were given, with 15 s intervening OCCC, until a spontaneous rhythm was re-established. ROSC was defined as sustained mean aortic pressure of at least 60 mmHg²⁵ at normal sinus rhythm. Sodium bicarbonate (10 mEq, iv) was injected at 30 s ROSC to counter acidosis in the immediate post-arrest period.¹⁷ Lidocaine (1 mg/kg, iv) was injected as needed for treatment of severe ventricular dysrhythmias; 3/8 NaCl and 4/9 pyruvate dogs received lidocaine. Controlled norepinephrine (nor adrenaline) infusion (~1 µg min⁻¹ kg⁻¹) was given as needed to maintain mean aortic pressure between 60–80 mm Hg. After ROSC and 30 min cardiac stabilization, the chest was closed in layers and the dog was allowed to recover.

Plasma 8-isoprostane measurement

Aortic whole blood (10 ml) was collected in heparinized syringes at baseline, 3 min arrest, 3 min OCCC and 5, 15, 25, 60, 90, 120 and 150 min ROSC, and centrifuged to obtain clear plasma. 8-isoprostane, an indicator of lipid peroxidation,³ was measured in flash frozen plasma^{26,27} using competitive enzyme immunoassay (Cayman Chemical, Ann Arbor, MI).

Neurological deficit evaluation

A simplified assessment was used to evaluate neurological recovery on the first post-surgical day. Deficit score was assigned based on six criteria: level of consciousness (0/1/2/3), respiration (0/1), response to oral commands (0/1), walking (0/1), limb movements (0/1), and chewing and drinking (0/1). An aggregate score of zero indicated normal function, and a score of eight, profound impairment.

Neurological function was assessed the day before cardiopulmonary arrest and on recovery day 2 using a standard canine neuro-deficit scoring (NDS) protocol.^{28,29} This procedure evaluates 22 criteria in five categories: level of consciousness, respiratory pattern, cranial nerve function, sensorimotor function, and behavior. A score of zero represents normal neurological function, and 100 represents brain death. Two investigators blinded to the treatments scored each dog and the mean of the two scores was taken as the result.

Tissue sampling for analyses of brain injury

On recovery day 3, dogs were re-anesthetized with acepromazine ($0.1 \text{ mg kg}^{-1} \text{ i.m.}$), and with α -chloralose ($100 \text{ mg kg}^{-1} \text{ i.v.}$), intubated and mechanically ventilated. The brain was removed via craniotomy. The cerebrum was divided by a mid-sagittal cut. The left hemisphere was dissected, and hippocampus and cerebellum were excised and flash-frozen in liquid N_2 for further analyses. The right hippocampus and cerebellum were fixed in 10% neutral buffered formalin, rinsed with 70% ethanol, and then dehydrated in series. Sections ($8 \mu\text{m}$) were processed, paraffin-embedded, and stained with hematoxylin-eosin.

Caspase-3 activity

Activity of caspase-3 was measured in flash-frozen brain tissue according to Wen *et al.*³⁰ Brain tissue was extracted in ice cold lysis buffer for 1 h and homogenized. The extract was diluted in an equal volume of assay buffer containing caspase-3 fluorogenic substrate (Ac-DEVD-AMC; $20 \mu\text{M}$; Pharmingen, San Diego, CA), and incubated for 60 min at 37°C . Fluorescence was measured in a microplate fluorospectrometer (FL600, Boi-tek Instruments, Winooski, VT), at 380 nm excitation/440 nm emission wavelengths. Purified caspase-3 (Pharmingen, San Diego, CA) provided positive control.

Myeloperoxidase activity

Myeloperoxidase activity, an indicator of neutrophil infiltration,³¹ was measured in frozen brain samples as described by Koh *et al.*³² with modifications. 100 mg of frozen brain tissue was pulverized in a liquid N_2 -precooled mortar, homogenized in 1 ml 20 mM K_2HPO_4 buffer, pH 7.4, and then centrifuged (20,000 g, 15 min, 4°C). The pellet was re-extracted 3 times in 150, 100 and 100 μl of 0.5% hexadecyltrimethylammonium bromide/ethylene-diamine-tetraacetate, and the four supernatants were combined. 50 μl of extract was combined with 250 μl of 50 mM K_2HPO_4 /0.017% o-dianisidine hydrochloride/0.0005% H_2O_2 , pH 6.0. Enzyme activity was computed from the rate of absorbance change at 460 nm in a microplate reader (Bio-Tek Instruments, Winooski, VT), by comparison with a horseradish peroxidase standard curve.³³ Myeloperoxidase activity was undetectable in the pellets.

Matrix metalloproteinase (MMP) activity

The aggregate activities of MMPs in the brain samples were assayed by fluorometry.³⁴ Brain tissue was extracted in 20 mM Tris/150 mM NaCl/0.5% Triton-X 100 (pH 7.4). Protein (50 μg) was diluted in assay buffer (50 mM Tris base, 200 mM NaCl, 5 mM CaCl_2 , Brij35 0.02%, pH 7.6) to a final concentration of 1 mg protein/ml. The fluorescence energy transfer peptide substrate TNO211 (Calbiochem) was added to a concentration of $10 \mu\text{M}$.³⁵ The samples were incubated at 37°C for 3 h, and fluorescence was measured at 340 nm excitation and 485 nm emission wavelengths.

Histological examination

Hematoxylin-eosin-stained sections were examined by light microscopy for structural hallmarks of neuronal cell death, including cytoplasmic hypereosinophilia, absence of visible Nissl substance, nuclear condensation (pyknosis) and lack of a discrete nucleolus.^{10,36} Viable and nonviable, hypereosinophilic neurons were counted manually by an investigator blinded to treatments, and percentages of nonviable neurons were computed. Three sections at least $15 \mu\text{m}$ apart were examined for each experiment and mean value was taken. Cerebellar sections were examined for neuronal loss in the same manner.

Paraffin embedded hippocampal sections were terminal deoxynucleotidyltransferase (TdT)-mediated dUTP-biotin nick-end labeling (TUNEL)-stained³⁷ to detect DNA fragmentation due to apoptotic cell death.³⁸ Necrotic and non-neuronal cell death can also give TUNEL positivity,

but such staining is more diffuse than that of apoptotic cells, and characteristic apoptotic bodies are absent.³⁸ For negative controls, TdT enzyme was replaced with double-distilled H₂O.

Brain regional blood flow measurements

In a separate series of 4 sham, 5 pyruvate and 5 NaCl experiments, dogs were surgically prepared as described above, and then underwent the cardiac arrest-resuscitation protocol with 3 hr ROSC. Brain blood flow was measured using radioactive microspheres.³⁹ Briefly, $5 \cdot 10^6$ microspheres (15 μ m diameter) radiolabeled with ⁴⁶Sc, ¹⁰³Ru or ¹⁴¹Ce were injected into the aorta at pre-arrest baseline and at 15 and 180 min ROSC. Duplicate arterial reference samples were collected at a constant rate for 3 min after microsphere injection to confirm adequacy of microsphere mixing in the blood. At the end of the experiment brain was dissected to obtain 1 g sections of hippocampus, cerebellum and frontal, parietal, occipital and temporal cortex. Radioactivities of tissue and arterial reference samples were measured in a gamma counter (Packard Instruments, Meridian, CT). Regional blood flow was expressed as ml·min⁻¹·g⁻¹ of brain tissue.

Statistical analysis

All data are presented as mean $\pm$ SEM. Between-group comparisons of brain blood flow, neurodeficit scores, caspase-3, matrix metalloproteinase and myeloperoxidase activities, and TUNEL analyses were accomplished by one way analysis of variance (ANOVA). Within group differences in plasma 8-isoprostane and cerebral blood flow were analyzed by repeated measures ANOVA. Student-Newman-Keul's multiple comparison test was applied when ANOVA detected significant differences. *P* values <0.05 were taken to indicate statistical significance. Statistical analyses were performed with Sigma Stat software version 3.1. Inter-observer agreement for neurodeficit scoring was evaluated from weighted kappa coefficient,⁴⁰ determined using SAS version 9.1 software.

3. RESULTS

Body temperature and hemodynamics

Each dog's body temperature, pulse and systemic arterial pressure were monitored during pre-arrest baseline, open-chest cardiac compressions and the first 60 min post-arrest recovery. No significant treatment effects on body temperature (Figure 1A), pulse (Figure 1B) or arterial pressure (Figure 1C) were detected, although a trend toward increased arterial pressure in the pyruvate group during the first 30 min recovery was noted.

8-isoprostane

Arterial plasma 8-isoprostane concentration, a global measure of oxidative stress, did not change during arrest, but almost doubled by 5 min ROSC (Figure 2) and remained elevated until 60 min of recovery before subsiding. Pyruvate treatment during OCCC and the first 55 min of ROSC blunted the post-arrest increase in 8-isoprostane.

Brain blood flow

Regional brain blood flow was measured using radioactive microspheres. At 15 min of ROSC blood flow was depressed in frontal cortex (Figure 3C), while the decline in other regions was not appreciable (Figure 3). Pyruvate treatment increased flow in occipital (Figure 3D) and parietal cortex (Figure 3F) and partially prevented the drop in frontal cortical flow (Figure 3C) at 15 min ROSC. However, by 180 min of ROSC all brain regions were underperfused, regardless of treatment, in comparison with baseline or sham control flows.

Post-arrest neurological deficit

A simple evaluation was used to assess neurological function on day 1 post-arrest (Figure 4A). The non-arrested, sham-operated dogs recovered completely from the surgery. All shams were fully conscious, responsive, breathing normally, able to walk, eat and drink; accordingly, each sham dog scored zero. Appreciable neurological impairment was evident in the NaCl group on the day following arrest, but pyruvate treatment during OCCC and the first 55 min ROSC minimized subsequent impairment 24 h later (Figure 4A).

The dogs were evaluated by standard, comprehensive neurological examination [26, 27] before the arrest-resuscitation experiment and on the 2nd day of recovery (Figure 4B). Marked neurological deficit was detected in the NaCl group on day 2 post-arrest. Pyruvate treatment during OCCC and initial recovery mitigated subsequent neurological impairment. Figure 5 presents scores for each of the 22 functions examined on recovery day 2. Modest abnormalities in carinal, muscle stretch and cleaning reflexes were noted in the sham group. Cardiac arrest-resuscitation appreciably impaired the ciliospinal reflex, response to painful stimuli, standing and walking. Pyruvate treatment minimized subsequent impairment of these responses.

Caspase-3

Activity of caspase-3, the effector protease in apoptotic cell death mechanisms, was measured in hippocampus (Figure 6A) and cerebellum (Figure 6B) at 3h and 3 d ROSC. Caspase-3 activity increased in the NaCl group *vs.* sham-arrested controls (Figure 6A) at 3 d recovery. Pyruvate treatment during OCCC and initial ROSC suppressed activation of the cell death effector. Caspase-3 activity did not differ among the sham-arrested, NaCl and pyruvate groups in the cerebellum at 3 h recovery (Figure 6B), nor did pyruvate prevent subsequent activation of caspase 3.

Hippocampal matrix metalloproteinase (MMP) and myeloperoxidase (MPO)

Matrix metalloproteinase and MPO activities were measured in hippocampus at 3 day ROSC. Appreciable MMP activity was evident in the NaCl-treated dogs, but was attenuated when pyruvate was administered during initial resuscitation (Figure 7A). MPO activity, a measure of inflammatory cell infiltration, tended to be higher in NaCl dogs *vs.* shams, which also had detectable MPO activity (Figure 7B) following 3 d post-operative recovery. In pyruvate-treated dogs, hippocampal MPO activity was similar to sham controls, although the decrease *vs.* the NaCl group was not statistically significant.

Hematoxylin-eosin staining

Cardiopulmonary arrest-resuscitation caused appreciable neuronal injury in the pyramidal cell layer of the hippocampal CA1 subregion of NaCl-treated dogs (Figure 8A). Damaged neurons were hypereosinophilic, and lacked normal dendritic structure. Pyruvate treatment during OCCC and early recovery preserved neuronal structure (Figure 8A). By 3 day recovery, nearly 12% of the CA1 pyramidal cells exhibited structural hallmarks of irreversible injury in the dogs treated with NaCl during resuscitation (Figure 8B). Pyruvate treatment substantially improved neuronal survival.

Cerebellar cortical sections were also examined at 3 day recovery. The structure of Purkinje cells remained intact in a non-arrested sham dog (Figure 9A). Degeneration of Purkinje cells was evident in a NaCl-treated dog (Figure 9B) with loss of granular chromatin and indistinct nucleolus and dendritic processes. In contrast, pyruvate treatment during early ROSC minimized neuronal degeneration at 3 day recovery (Figure 9C).

TUNEL assay of DNA fragmentation

DNA fragmentation in the hippocampal CA1 subregion was detected by TUNEL at 3 day recovery. Light microscopic examination of the CA1 region revealed nuclear condensation and evidence of pyknosis. TUNEL-positive nuclei were identified by green fluorescent nuclei with overlapping blue fluorescent DAPI stain (Figure 10A). Percentages of TUNEL-positive nuclei in the three groups are presented in Figure 10B. The NaCl-treated cardiac arrest group displayed considerably higher percentage of TUNEL-positive nuclei than shams. Pyruvate tended to lower the percentage of TUNEL-positive nuclei, although the difference was not statistically significant [$P = 0.056$].

4. DISCUSSION

Cellular energy depletion and oxidant stress activate mechanisms leading to eventual neuronal loss and neurological dysfunction following cardiac arrest-resuscitation. This study demonstrated that intravenous administration of pyruvate, a potent energy-yielding fuel and antioxidant, during cardiac massage and the first 55 min post-arrest ameliorated subsequent neurological deficit, neuronal death and inflammation during the first 3 days of recovery.

Cardiac-arrest resuscitation resulted in significant neurological impairment during the first 2 days of recovery. Intravenous pyruvate during OCCC and the first 55 min of recovery from cardiac arrest attenuated subsequent neurological impairment. During its infusion pyruvate also suppressed systemic arterial oxidative stress. Hippocampal caspase-3 was activated 3 d after arrest; pyruvate treatment suppressed activation of this cell death effector. Pyruvate also suppressed pro-inflammatory myeloperoxidase and matrix metalloproteinase activities in hippocampus. Histological examination at 3 day recovery revealed severe structural damage to hippocampal CA1 pyramidal neurons and TUNEL-detectable DNA fragmentation, both of which were prevented by pyruvate treatment. Although pyruvate moderately increased perfusion at 15 min ROSC in the occipital and frontal cortex, it did not increase perfusion of the hippocampus at any time point. Accordingly, the salutary effects of pyruvate on neurons in this region could not be ascribed to improved perfusion following ROSC. Instead, by bolstering metabolism and antioxidant state in the first 60 min of reperfusion, pyruvate may have prevented inflammatory injury and neuronal loss, and thereby improved neurological recovery.

Pyruvate transport in brain

Within 10–30 s of intravenous administration pyruvate concentration can increase to therapeutically effective levels in the brain.^{14,15} Pyruvate traverses the blood-brain barrier via monocarboxylate transporters (MCTs) which co-transport pyruvate and a proton.⁴¹ This transport is enhanced in acidemic conditions such as post-ischemia, due to increased plasma H^+ concentration. Increased circulating pyruvate and H^+ concentrations also would augment entry of pyruvate into neurons and astrocytes, which are equipped with monocarboxylate transporters MCT-2 and 4.^{20,41} MCT-4 is abundant in ischemia-reperfusion susceptible areas such as the hippocampus.⁴¹

Antioxidant and anti-apoptotic actions of pyruvate during early ROSC

Caspase-3 in brain is activated following transient global ischemia with cardiac arrest.⁴² In this study, caspase-3 activity increased appreciably at 3 d ROSC vs. sham controls, but pyruvate restrained caspase-3 activity to sham levels. Inhibition of caspase-3 prevents delayed neuronal death after transient ischemia⁴³ and improves neurological outcome.⁴² Brain tissue contains high concentrations of polyunsaturated fatty acids that are targets of oxyradical attack. In dogs, 10 min cardiac arrest results in increased cerebrocortical lipid peroxidation products, which remain elevated even after 24 h recovery.⁴⁴ Moreover, arterial 8-isoprostane, an indicator of

cerebral lipid peroxidation, increases following cardiac arrest.³ Pyruvate infusion blunted the increase in plasma 8-isoprostane during OCCC and early reperfusion, indicating decreased post-ischemic oxidative stress. Reactive oxygen species (ROS) can trigger inflammation and apoptosis following ischemia-reperfusion in the brain.⁸ Pyruvate has been shown to dampen ROS formation in brain.^{45,46} Pyruvate and other α -keto carboxylates can directly neutralize oxidants such as H_2O_2 and $ONOO^-$ and thus reduce oxidative stress.^{13,47} Pyruvate also acts as an antioxidant by enhancing NADPH formation which in turn increases glutathione redox state.¹³ Pyruvate concentration-dependently protected cultured human neuroblastoma cells from H_2O_2 toxicity by suppressing H_2O_2 -induced ROS formation and attenuating oxidative stress-induced collapse of the mitochondrial membrane potential.²¹ By dampening oxidative stress at the onset of cerebral reperfusion pyruvate treatment may have prevented activation of inflammatory and cell death pathways triggered by ROS.

Anti-inflammatory effects of pyruvate

Both oxidative stress³ and inflammation¹¹ following cardiac arrest contribute to neuronal death. The CA1 subregion of the hippocampus is particularly susceptible to ischemia and exhibits delayed neuronal death^{11,48} following acute ischemic insult. Matrix metalloproteinases, activated for several days following transient cerebral ischemia,^{49,50} have been implicated in neuronal death in the hippocampal CA1 subregion.^{51,52} ROS^{53,54} and inflammatory mediators activate MMP following cerebral ischemia.⁵⁵ Myeloperoxidase activity increases up to 3 days after cerebral ischemia and correlates with ischemic damage in the brain⁵⁶ and neutrophil infiltration.³¹ Pyruvate suppressed the marked MMP activation evident in the CA1 region at 72 h ROSC. This effect of pyruvate may have helped maintain integrity of the blood brain barrier and thereby minimized neutrophil infiltration, although the trend in the pyruvate group toward lower CA1 myeloperoxidase activity, a neutrophil marker enzyme, didn't achieve statistical significance. The anti-inflammatory effect of pyruvate may have contributed to its ability to prevent neuronal death and neurological dysfunction following cerebral ischemia-reperfusion.

Pyruvate prevents neuronal loss following cardiac arrest-resuscitation

Activated microglia produce NO, oxyradicals and cytokines,^{57,58} and are instrumental in post-ischemic inflammation. Ethyl pyruvate prevented lipopolysaccharide-induced inflammation and nuclear factor- κ B activation in cultured microglia.¹⁸ Intraperitoneal pyruvate, administered at 1 h reperfusion following 12 min ischemia, prevented hippocampal neuronal loss 72 h later.²³ Collectively, pyruvate may have protected neurons during initial cerebral reperfusion following cardiac arrest by minimizing acute energy depletion, oxidative stress and inflammation.

Clinical significance

As early as 1984 pyruvate was infused *iv* to humans without adverse effects.⁵⁹ Pyruvate has proven safe and efficacious as a major component of cardioplegia solutions for cardiopulmonary bypass.⁶⁰ Intravenous pyruvate during resuscitation exerts powerful antioxidant and energetic effects in myocardium that improve immediate cardiac recovery.¹⁷ Thus, pyruvate may be therapeutically effective in ischemia-reperfusion scenarios involving the heart and brain.

Limitations

This study examined neurological injury at 72 h ROSC, a time when hippocampal CA1 neuronal death would be manifest.⁶¹ However, cell death processes following transient ischemic insult can continue for a month.⁴⁸ Indeed, Lee *et al.* demonstrated neuroprotection by pyruvate even at 30 d of recovery from transient forebrain ischemia in rats.²³ Evaluation of

neurodeficit required a high success rate of resuscitation, so cardiac arrest was limited to 5 min with an additional 5 min of OCCC, and an open chest preparation was used to facilitate cardiac massage. Increasing the arrest interval and use of closed-chest OCCC could jeopardize successful ROSC, although it would model clinical scenarios more closely, especially cardiac arrests outside the hospital.

5. CONCLUSIONS

Cardiac arrest-resuscitation results in cerebral inflammation, neurological damage and neuronal loss 3 d later. Acute treatment with intravenous pyruvate prevents oxidative stress during initial ROSC, attenuates inflammatory response, preserves neurological function and prevents neuronal loss. An antioxidant and energy-yielding fuel, pyruvate could provide a safe and effective adjunct to conventional resuscitation to preserve neurological function following cardiopulmonary arrest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ANOVA	analysis of variance
CA	cardiac arrest
BL	pre-arrest baseline
CA1	<i>cornus ammonis</i> region 1 of hippocampus
DAPI	4',6-diamidino-2-phenylindole
MCT	monocarboxylate transporter
MMP	matrix metalloproteinases
MPO	myeloperoxidase
NDS	neurological deficit score

OCCC

open chest cardiac compressions

Q

regional blood flow

RFU

relative fluorescence units

ROS

reactive oxygen species

ROSC

return of spontaneous circulation

TUNEL

terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling

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Figure 1. Body temperature and hemodynamics during cardiopulmonary resuscitation and recovery

Body temperature (panel A), pulse (panel B) and mean arterial pressure (MAP; panel C) were monitored at baseline, during open-chest cardiac compressions (OCCC) and throughout the first 60 min following recovery of spontaneous circulation (ROSC). Values are means $\pm$ SEM from 8 dogs infused with NaCl (circles, solid lines) and 9 infused with pyruvate (triangles, broken lines). No statistically significant treatment effects were detected for these variables.

Figure 2. Plasma 8-isoprostane

8-isoprostane concentration was measured in arterial plasma sampled at pre-arrest baseline (BL), 3 min cardiac arrest (ARR), 3 min OCCC, and at several times during ROSC. Values are means $\pm$ SEM. *P < 0.05 vs. sham; †P < 0.05 vs. NaCl at the same time.

Figure 3. Regional brain blood flows

Blood flows (Q) were measured at baseline, 15 min ROSC and 180 min ROSC in hippocampus (Panel A), cerebellum (Panel B), and frontal (Panel C), occipital (Panel D), temporal (Panel E) and parietal cortex (panel F) of 5 NaCl (hatched bars) and 5 pyruvate (filled bars) experiments. Flows were measured in 4 sham experiments (open bars) at a time corresponding to 180 min ROSC. Values are means $\pm$ SEM. * $P < 0.05$ vs. baseline same treatment; † $P < 0.05$ vs. 15 min ROSC same treatment; ‡ $P < 0.05$ vs. NaCl at the same time.

Figure 4. Neurological deficit after cardiac arrest-resuscitation

Panel A: a simplified neurological evaluation was used to assess recovery on day 1 post-arrest, scored between 0 (normal) to 8 (profound impairment). Data are means $\pm$ SEM, * $P < 0.05$ vs. sham; † $P < 0.05$ vs. NaCl. Panel B: Standard neurodeficit score pre-arrest (hatched bars) and on day 2 following recovery from cardiac arrest-resuscitation (filled bars), expressed as percentages of maximum score. Scores were mean values from 2 treatment-blinded examiners; weighted kappa coefficient = 0.56, indicating good inter-rater agreement.⁶¹ Values are means $\pm$ SEM. * $P < 0.05$ vs day 2 sham; † $P < 0.05$ vs day 2 NaCl.

Figure 5. Individual components of neurodeficit score

Functions were examined at day 2 recovery in sham-arrested controls (open bars) and in dogs subjected to cardiac arrest-resuscitation and treated with NaCl (hatched bars) or pyruvate (solid bars) during OCCC and ROSC. Values are means $\pm$ SEM. * $P < 0.05$ vs sham; † $P < 0.05$ vs day 2 pyruvate.

Figure 6. Activation of cell death effector caspase-3 following cardiac arrest-resuscitation
Caspase-3 activities, expressed as relative fluorescence units (RFU)/mg protein, in hippocampus (Panel A) and cerebellum (Panel B), at 3 h (hatched bars) and 3 day ROSC (solid bars), in sham, NaCl and pyruvate treated experiments. Values are means $\pm$ SEM. * P < 0.05 vs sham; † P < 0.05 vs. NaCl.

Figure 7. Pro-inflammatory enzymes in hippocampus 3 d after cardiac arrest-resuscitation
 Panel A: Hippocampal matrix metalloproteinase (MMP) activities in sham (n=6), NaCl (n=8) and pyruvate (n=8) treated dogs at 3 d ROSC. MMP activity is expressed as relative fluorescence units (RFU) per mg protein. Panel B: Myeloperoxidase (MPO) activity (U/g protein) measured in the same experiments as panel A. Values are means $\pm$ SEM. *P < 0.05 vs. sham; †P < 0.05 vs. NaCl.

Figure 8. Neuronal loss in hippocampal CA1 subregion 3 days after cardiac arrest-resuscitation
 Panel A: Representative hematoxylin-eosin stained sections from sham, NaCl-treated and pyruvate-treated dogs. Panel B: Neuronal loss, expressed as % loss of neuronal structure. Values are means $\pm$ SEM. * $P < 0.05$ vs. sham; † $P < 0.05$ vs. NaCl.

Figure 9. Cerebellar neuronal degeneration 3 days after cardiac arrest-resuscitation

Representative hematoxylin-eosin sections of cerebellar cortex with inner granular, outer molecular and middle Purkinje cell layers. Hypereosinophilia and abnormal architecture of Purkinje cells is visible in NaCl treated dogs (panel B), but not in sham and pyruvate-treated dogs. Insets: enlargements of single cell bodies of Purkinje cells in each condition.

Figure 10. Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling (TUNEL) of DNA fragmentation in the hippocampal CA1 region

Panel A: representative TUNEL and DAPI stained images from sham, NaCl and pyruvate-treated dogs at 3 d ROSC. Green fluorescence: TUNEL-positive nuclei; blue fluorescence: DAPI-staining to detect all nuclei. Panel B: percentages of CA1 cells with TUNEL positive nuclei. Values are means $\pm$ SEM. * $P < 0.05$ vs. sham.