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Fructose-1,6-Bisphosphate Supports Cerebral Energy Metabolism in Pigs after Ischemic Brain Injury Caused by Experimental Particle Embolization

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ABSTRACT

Background. Fructose-1,6-bisphosphate (FDP) is a high-energy intermediate that enhances glycolysis, preserves cellular adenosine triphosphate stores, and prevents the increase of intracellular calcium in ischemic tissue. Since it has been shown to provide metabolic support to the brain during ischemia, we planned this study to evaluate whether FDP is neuroprotective in the setting of combining hypothermic circulatory arrest (HCA) and irreversible embolic brain ischemic injury.

Methods. Twenty pigs were randomly assigned to receive 2 intravenous infusions of either FDP (500 mg/kg) or saline. The first infusion was given just before a 25-minute period of HCA and the second infusion immediately after HCA. Immediately before HCA, the descending aorta was clamped and 200 mg of albumin-coated polystyrene microspheres (250-750 mm in diameter) were injected into the isolated aortic arch in both study groups.

Results. There were no significant differences between the study groups in terms of neurological outcome. Brain lactate/pyruvate ratio was significantly lower (P = .015) and brain pyruvate levels (P = .013) were significantly higher in the FDP group compared with controls. Brain lactate levels were significantly higher 8 hours after HCA (P = .049).

Conclusion. The administration of FDP before and immediately after HCA combined with embolic brain ischemic injury was associated with significantly lower brain lactate/pyruvate ratio and significantly higher levels of brain pyruvate, as well as lower lactate levels 8 hours after HCA. FDP seems to protect the brain by supporting energy metabolism. The neurological outcome was not improved, most likely resulting from the irreversible nature of the microsphere occlusion.

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INTRODUCTION

Cardiac surgery is associated with a significant risk of perioperative stroke, the risk being highest in patients undergoing aortic arch surgery. The cause of postoperative stroke is multifactorial. On one hand, extracorporeal circulation used in these operations may generate microscopic air bubbles and tiny blood clots. The occlusion of the small cerebral arteries caused by these tiny particles may be temporary. On the other hand, the manipulation of a grossly sclerotic heart and the aortic arch may launch macroscopic particles from the vessel walls into the bloodstream. If these particles enter the cerebral vasculature, they may cause permanent ischemia and irreversible ischemic injury, ie, stroke or sudden death in worst cases. [Kawachi 2003].

Hypothermic circulatory arrest (HCA) is often needed to make surgery of the aortic arch possible. HCA itself has a major role in the development of postoperative neurological complications by determining a status of global brain ischemia with metabolic derangements of concern. The length of the arrest and the temperature determine the safety of HCA [McCullough 1999]. We have previously demonstrated that fructose-1,6-bisphosphate (FDP) has marked neuroprotective properties when used during and after a prolonged, 75-minute period of experimental HCA [Romsi 2003; Kaakinen 2005]. FDP is a high-energy intermediate of glycolysis, which increases the activity of key enzymes in the glycolytic chain. FDP has been shown to have benefits in several animal models of brain ischemic injury. FDP has also been shown to decrease the size of cerebral infarction caused by reversible occlusion of the middle cerebral artery [Kuluz 1993] and mitigate ischemic injury due to injection of microspheres into the brain vasculature in rats [Trimarchi 1997].

The aim of this study was to evaluate whether FDP could also protect the brain exposed to HCA of relatively safe duration combined with irreversible embolic ischemic injury in a surviving porcine model, which resembles the actual clinical setting.

MATERIALS AND METHODS

Twenty female juvenile pigs (8-10 weeks old) from a native stock, with a median weight of 26.3 kg (range, 24.3-28.9)

were randomly assigned to receive 2 intravenous infusions of either FDP at 500 mg/kg or equal volume of 0.9% saline solution. The first infusion was given just before a 25-minute period of HCA, and the second infusion was given immediately after the start of reperfusion.

Preoperative Management

All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council (published by the National Academy Press, revised in 1996). The study was approved by the Research Animal Care and Use Committee of the University of Oulu.

Anesthesia and Monitoring

Monitoring of anesthesia, preoperative management, and electrocardiographic, hemodynamic, intracerebral, and systemic metabolic parameters as well as evaluation of behavioral outcome and histopathological analysis were performed as described in detail in our previous study employing the same experimental model of HCA alone or combined with cerebral embolization [Romsi 2003; Dahlbacka 2005].

Cardiopulmonary Bypass

The heart and great vessels were exposed through a right thoracotomy. A membrane oxygenator (Midiflow D 705; Dideco, Mirandola, Italy) was primed with 1 L of Ringer acetate and heparin (5000 IU). After systemic heparinization (500 IU/kg), the ascending aorta was cannulated with a 16 French arterial cannula, and the right atrial appendage was cannulated with a single 24 French atrial cannula. Nonpulsatile cardiopulmonary bypass (CPB) was initiated at a flow rate of 90 to 110 mL/kg per minute, and the flow was adjusted to maintain a perfusion pressure of 50 to 70 mmHg. A 10 French intracardiac sump cannula was positioned into the left ventricle through the apex of the heart. A cooling period of 60 minutes was carried out to attain a brain temperature of 18°C. The $Paco_2$ level was maintained at 5.3 kPa, corrected for temperature, by adding carbon dioxide to the inflowing gas, ie, using pH-stat principles. After 45 minutes of cooling, the first infusion of FDP or placebo was started. When the target temperature was reached, the ascending aorta was cross clamped just distal to the aortic cannula, and cardiac arrest was induced by injecting potassium chloride (40 mmoL) through the aortic cannula. Immediately before initiation of HCA, the descending and the ascending aorta (clamp shifted proximally to the aortic cannula) were cross clamped and the CPB flow rate, designated as preinjection flow, was adjusted to maintain an aortic arch pressure of 50 mmHg. This pressure level was stabilized for 1 minute before injection of 200 mg of polystyrene microspheres (250-750 μm in diameter) into the isolated aortic arch. The embolization protocol herein employed was similar to the one used by Juvonen et al [Juvonen 1998]. After embolization, the postinjection flow rate was adjusted again to maintain a perfusion pressure of 50 mmHg, and it was stabilized for 5 minutes. The 25-minute period of HCA was then initiated. Cardiac cooling with topical ice slush was begun and maintained throughout the HCA period. Similarly, the intracerebral temperatures were controlled and maintained at a level of 18°C with ice packs placed over the head. After the HCA, reperfusion was started and the second infusion of FDP or placebo was started. Five minutes after the start of rewarming, furosemide (40 mg), mannitol (15 g), methylprednisolone (80 mg), lidocaine (40 mg), and calcium bioglyconate (2.25 mmoL Ca^{2+}) were administered. The left ventricular sump cannula was removed after 50 minutes of rewarming, and weaning from CPB occurred about 60 minutes after HCA. No animal needed inotropic drugs postoperatively. During rewarming and after weaning from CPB, a heat-exchanger mattress, heating lamps, and ice packs regulated the temperatures. The animals of both groups were extubated 8 hours after the start of rewarming and were then moved to a recovery room.

Drug Administration and Experimental Protocol

FDP (molecular weight: 336.082 kd, Esafosfina; Biomedica Foscama Industria Chimico-Farmaceutica SpA, Ferentino, Italy) was stored at room temperature in 50-mL injectable powder bottles, 5 g FDP in each bottle. Normal physiologic saline was used as a solvent. The liquid was pale yellow in color and its pH was 5.6. The liquid did not require neutralization with sodium hydroxide as done and described in our previous study [Romsi 2003]. The placebo consisted of a corresponding volume (5 mL/kg) of isotonic sodium chloride with a pH of 6.0 to 6.5. The drugs were administered intravenously in a randomized, double-blinded fashion. The slightly yellow color of FDP was masked by using bright yellow infusion bags in all experiments.

Statistical Analysis

Statistical analysis was performed using SPSS (version 11.5; SPSS, Chicago, IL, USA) and SAS (version 8.02; SAS Institute, Cary, NC, USA) statistical programs. Continuous and ordinal variables are expressed as the median and twenty-fifth and seventy-fifth percentiles. SAS procedure Mixed was used for repeated measurements. Since the measurement intervals were uneven, spatial exponential covariance structure was defined in a repeated statement. Complete independence was assumed across animals (by random statement). Reported P values are as follows: P between groups indicates a level of difference between groups, P time × group indicates behavior between groups over time. Either the Student t test or Mann-Whitney U test were used to assess the distribution of variables between study groups. The Fischer exact test was used to determine the significance of mortality rates between the groups. Significance levels are reported for comparisons with the 2-tailed test ($P \leq .05$).

RESULTS

Comparability of the Study Groups

Median weight of the pigs was 26.7 kg (24.1-30.4 kg) in the FDP group and 26.3 kg (25.0-26.5 kg) in the control group

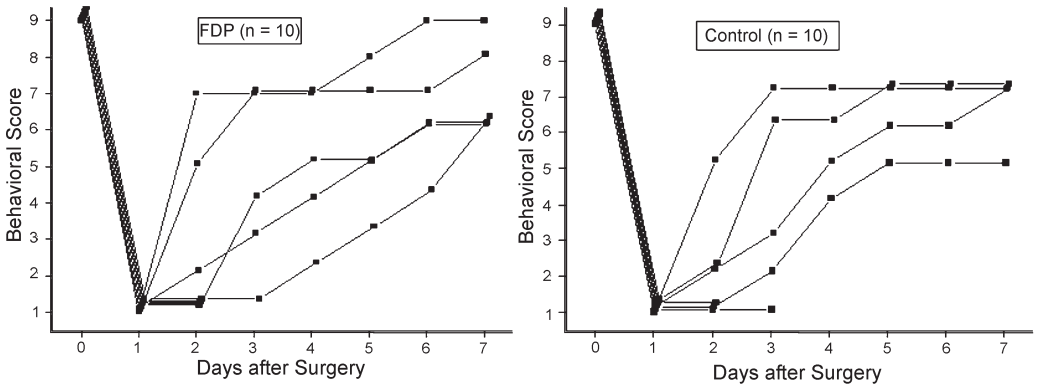

Figure 1. Postoperative behavioral scores in the study groups. The animals were assessed daily for mental status (0-3), appetite (0-3), and motor function (0-3). A total behavioral score of 9 indicates full neurological recovery, lower scores are associated with more severe cerebral injury. Values are shown as medians and twenty-fifth and seventy-fifth percentiles.

(*P* = .56). The median CPB cooling time was 60.0 minutes (60.0-60.0 minutes) in the FDP group and 60.0 minutes (60.0-60.25 minutes) in the control group (*P* = .63). The median CPB rewarming time was 60.0 minutes (60.0-63.0 minutes) in the FDP group and 61.0 minutes (60.0-68.25 minutes) in the control group (*P* = .69). The median total CPB time was 120.0 minutes (120.0-123.0 minutes) in the FDP group and 121.5 minutes (120.0-128.25 minutes) in the control group (*P* = .50). During the whole experimental period, the rectal, blood, epidural, and intracerebral temperatures did not differ between the study groups. Central venous pressure and arterial oxygen partial pressure were significantly higher in the FDP group at baseline, but the overall differences were not significant (*P* = .50 and *P* = .30, respectively).

Mortality and Behavioral Outcome

The 7-day survival rates were 50% (5 of 10 animals) in the FDP group and 40% (4 of 10 animals) in the control group (*P* > .9). The postoperative behavioral scores did not differ between the study groups (Figure 1).

Hemodynamic and Metabolic Data

Hemodynamic and metabolic data are presented in Table 1 and Figure 2. After the administration of FDP, there was a significant increase in serum phosphate levels (*P* < .001) and a significant decrease in serum ionized calcium levels (*P* < .001). In contrast to our previous study, the serum sodium levels were significantly lower in the FDP group (*P* < .001). This was most likely partly due to significantly better fluid balance in the FDP group (*P* = .0059). Increased sodium content led to a significantly higher level of osmolarity in the control group (*P* < .001). Blood glucose and lactate levels did not differ between the study groups.

The administration of FDP caused short-lasting decreases in arterial and venous pH levels, which were statistically significant at the end of cooling (*P* = .05 and *P* = .008, respectively). Also during rewarming, the difference in arterial pH reached statistical significance at the 15-minute interval (7.30 [7.28-7.34] in the FDP group, 7.37 [7.33-7.41] in the control group; *P* = .013). The total differences between groups in terms of pH were, however, not significant (Table 1). The FDP group overcompensated for the lower arterial and venous pH levels within the postoperative hours, as the group behaved differently (*P* = .001 for both) and the pH levels were significantly higher 4 hours after the start of rewarming (*P* = .013 and *P* = .033, respectively) in the FDP group.

The overall $Paco_2$ levels did not differ between the study groups. As in the case of pH, $Paco_2$ levels were significantly higher in the FDP group 15 minutes after the start of rewarming (5.64 [5.46-6.02] in the FDP group, 5.43 [5.09-5.68] in the control group, *P* = .031). This was most likely because of the highly significant difference in $Pvco_2$ at the end of cooling (*P* = .005).

Intracranial Measurements

Intracranial measurements are presented in Figure 3. Brain glucose levels did not differ significantly between the study groups (*P* = .50), although glucose levels tended to be higher in the FDP group in the first postoperative hours. Brain lactate levels did not differ between the study groups as a whole (*P* = .50), but at 8 hours after the start of rewarming lactate levels in the control group were higher (*P* = .049). Brain pyruvate levels were significantly higher in the FDP group (*P* = .013). Brain lactate/pyruvate ratio was significantly lower in the FDP group (*P* = .015). Brain glutamate and glycerol levels did not differ between the study groups (*P* = .40 and *P* > .90, respectively). Intracranial pressure behaved similarly in both study groups (*P* = .90). Brain tissue oxygen partial pressure did not differ between the study groups (*P* = .70).

Histopathologic Data

Histopathologic findings are presented in Table 2. There were no significant differences between groups.

DISCUSSION

FDP has been extensively studied during the last 2 decades. Besides findings on its neuroprotective effects,

Table 1. Experimental Data*

	Baseline	End of Cooling	After the Start of Rewarming				*P* between Groups	*P* Time × Group
			30 min	2 h	4 h	8 h		
Cardiac index, L/min × m^2							.9	.9
FDP	4.78 (4.35-4.93)	3.22 (2.99-3.40)	3.39 (3.09-3.56)	2.70 (2.3-3.34)	3.31 (2.72-3.73)	3.70 (3.38-4.60)		
Control	4.36 (4.18-4.74)	3.30 (2.81-3.48)	3.35 (3.02-3.53)	2.92 (2.62-3.09)	3.15 (2.77-3.39)	3.84 (3.31-5.00)		
Mean arterial pressure, mmHg							.8	.7
FDP	89 (76-99)	48 (45-52)	60 (55-66)	81 (74-87)	71 (67-79)	69 (64-80)		
Control	91 (83-100)	47 (44-51)	59 (51-63)	75 (68-82)	74 (69-80)	71 (66-77)		
Central venous pressure, mmHg							.5	.14
FDP	4 (2-5)†	3 (1-4)	2 (1-2)	4 (2-5)	4 (2-6)	5 (3-7)		
Control	3 (2-4)†	2 (0-4)	3 (0-4)	3 (2-4)	3 (2-4)	5 (3-8)		
Vascular resistance, dyne × sec × cm^{-5}							.8	.8
FDP	1848 (1768-1949)	1430 (1221-1601)	1853 (1480-2198)	2924 (2164-3653)	1962 (1758-3118)	1660 (1287-2170)		
Control	2027 (1844-2411)	1341 (1171-1889)	1672 (1576-1998)	2579 (2275-3092)	2306 (2125-2814)	1542 (1385-2380)		
Fluid balance, mL							.006	.008
FDP	200 (75-500)	1950 (1475-2125)†	1600 (1440-1960)‡	625 (500-1360)	1000 (650-1290)	1250 (760-1580)†		
Control	150 (25-200)	2425 (2050-2600)†	2350 (2140-2425)‡	1275 (875-1910)	1350 (940-1990)	1725 (1475-2010)†		
Cerebral perfusion pressure, mmHg							.8	.4
FDP	79 (70-95)	41 (31-50)	54 (50-60)	72 (67-80)	61 (53-71)	51 (43-66)		
Control	88 (80-96)	45 (40-47)	53 (46-57)	69 (63-75)	63 (58-69)	54 (46-62)		
Hematocrit, %							.3	.4
FDP	25 (21-26)	15 (13-18)	16 (14-19)	21 (18-23)	19 (16-21)	19 (15-22)		
Control	24 (23-25)	15 (14-16)	15 (14-17)	19 (17-22)	19 (17-21)	17 (14-19)		
Arterial pH							.8	<.001
FDP	7.56 (7.51-7.58)	7.05 (7.02-7.07)†	7.34 (7.31-7.36)	7.44 (7.40-7.46)	7.54 (7.49-7.56)†	7.55 (7.53-7.56)		
Control	7.54 (7.52-7.57)	7.07 (7.06-7.10)†	7.35 (7.32-7.42)	7.41 (7.39-7.44)	7.49 (7.45-7.51)†	7.54 (7.52-7.55)		
Venous pH							.8	<.001
FDP	7.48 (7.46-7.51)	6.99 (6.97-7.03)‡	7.27 (7.25-7.30)	7.38 (7.34-7.42)	7.46 (7.41-7.50)†	7.48 (7.46-7.52)		
Control	7.48 (7.45-7.49)	7.08 (7.05-7.11)‡	7.30 (7.26-7.35)	7.36 (7.34-7.38)	7.43 (7.38-7.45)†	7.47 (7.45-7.48)		
$Paco_2$, kPa							.6	.6
FDP	5.04 (4.96-5.21)	13.30 (12.57-13.36)	5.94 (5.54-6.03)	6.05 (5.62-6.15)	5.49 (5.30-5.63)	5.27 (5.11-5.60)		
Control	5.01 (4.86-5.35)	13.05 (12.36-13.72)	5.95 (5.77-6.25)	5.81 (5.41-6.33)	5.71 (5.40-5.78)	5.34 (4.93-5.56)		
$Pvco_2$, kPa							.055	<.001
FDP	6.06 (5.79-6.30)	14.71 (14.28-14.90)‡	7.23 (7.03-7.58)	7.37 (6.93-7.86)	6.77 (6.51-7.02)	6.39 (6.10-6.90)		
Control	6.02 (5.80-6.45)	12.84 (12.26-13.67)‡	7.20 (6.64-7.52)	7.20 (7.00-7.50)	6.98 (6.75-7.47)	6.26 (6.16-6.69)		

*Values are shown as medians and twenty-fifth and seventy-fifth percentiles. Fluid balance represents fluids given intravenously minus cumulative diuresis.

†$P < .05$ between study groups.

‡$P < .01$ between study groups.

reports of the beneficial impact of FDP on ischemic injury have also been reported for the myocardium [Tavazzi 1990], kidney [Li 1994], intestine [Sawchuk 1986], and lung [Markov 2002].

The mechanism of action of FDP is not completely understood, and several different mechanisms have been suggested. FDP stimulates phosphofructokinase and pyruvate kinase, key enzymes in glycolysis, but it may also work as an alternative substrate during glycolysis [Tavazzi 1992]. FDP produces 4 mol of adenosine triphosphate (ATP) instead of 2 mol produced in anaerobic metabolism of glucose, thus preserving ATP stores in cells and re-establishing cellular energy-producing mechanisms after ischemia [Bickler 1996]. FDP has also been shown to inhibit the increase of intracellular calcium during ischemia [Bickler 1992], which is considered to be one of the main mechanisms leading to cell death. Part of the neuroprotective effect of FDP is derived from its ability to diminish the injury to astrocytes, which are closely linked to neurons in terms of brain metabolism [Kelleher 1996]. FDP also

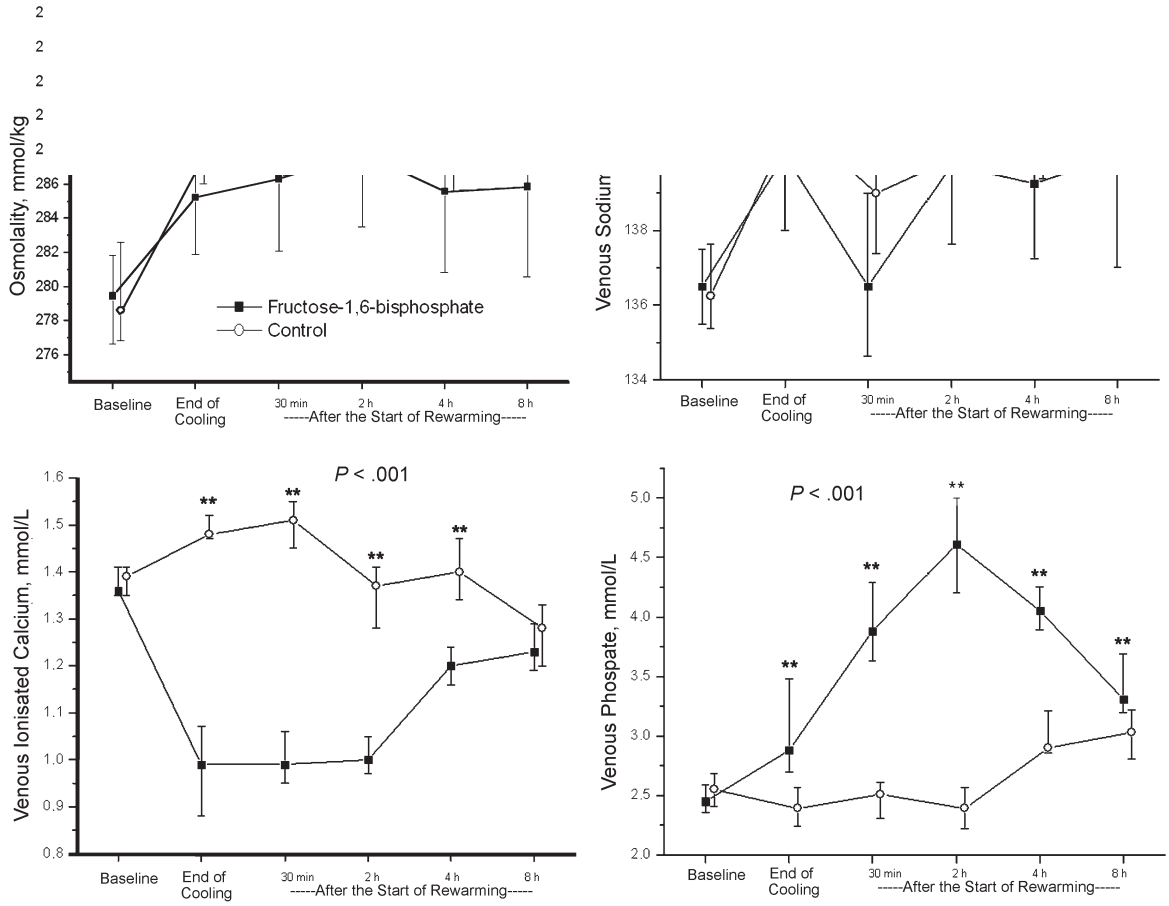

Figure 2. Metabolic parameters. Values are shown as medians and twenty-fifth and seventy-fifth percentiles. $^{**}P < .01$ between groups at any study interval.

inhibits the release of oxygen-free radicals by activated neutrophils and reduces glutamate release and nitric oxide synthase in vitro [Bickler 1996; Cardenas 2000; Sola 2003]. FDP has also been shown to protect cerebrovascular endothelial cells from ischemia, possibly leading to better blood flow during reperfusion [Gobbel 1994].

In the present study, the brain lactate/pyruvate ratio and pyruvate levels as well as brain lactate improved in the FDP group, suggesting cerebral metabolic recovery to some extent. The use of FDP did not show any relevant benefit in terms of 7-day outcome, in contrast to our earlier results of reversible global ischemic injury to the brain [Romsi 2003]. Indeed, the latter study did not include in the experimental protocol an embolic load to the brain, which resulted in a markedly different experimental setting. Assuming that cerebral complications are the main determinants of postoperative outcome after HCA, based on the data reported herein it seems that FDP cannot do much in the territory of the brain beyond an irreversible arterial occlusion as caused by the embolic load herein employed. The microspheres generate a permanent block in the vessels they occlude, making it impossible to deliver therapeutic substances as well as blood to the tissues downstream, ie, the ischemic core, after embolization. Thus, the ischemic brain injury in this study can be considered far more severe than the reversible global ischemia of isolated HCA inflicted on the animals in our previous study with FDP.

In the embolization study by Trimarchi, the authors report a positive result [Trimarchi 1997]. However, the rats operated on were subjected only to a unilateral brain injury, as the small (48.4 μm) particles were injected into the right common carotid artery. Therefore the injured areas of the brain most likely have received blood and FDP from collateral circulation via circulus Willisii. In our study, the embolization was performed bilaterally by injecting the relatively large embolic particles through the aortic cannula. Thus the embolization affected both sides of the brain and its vasculature, making the chance of collateral blood flow to the injured area quite small.

These findings do not negate a neuroprotective efficacy of FDP, as the endpoints in our experimental model of neuroprotection are just as imprecise as those within the clinical setting. As suggested by microdialysis findings, metabolic support to the brain is rather clear. This can beneficially

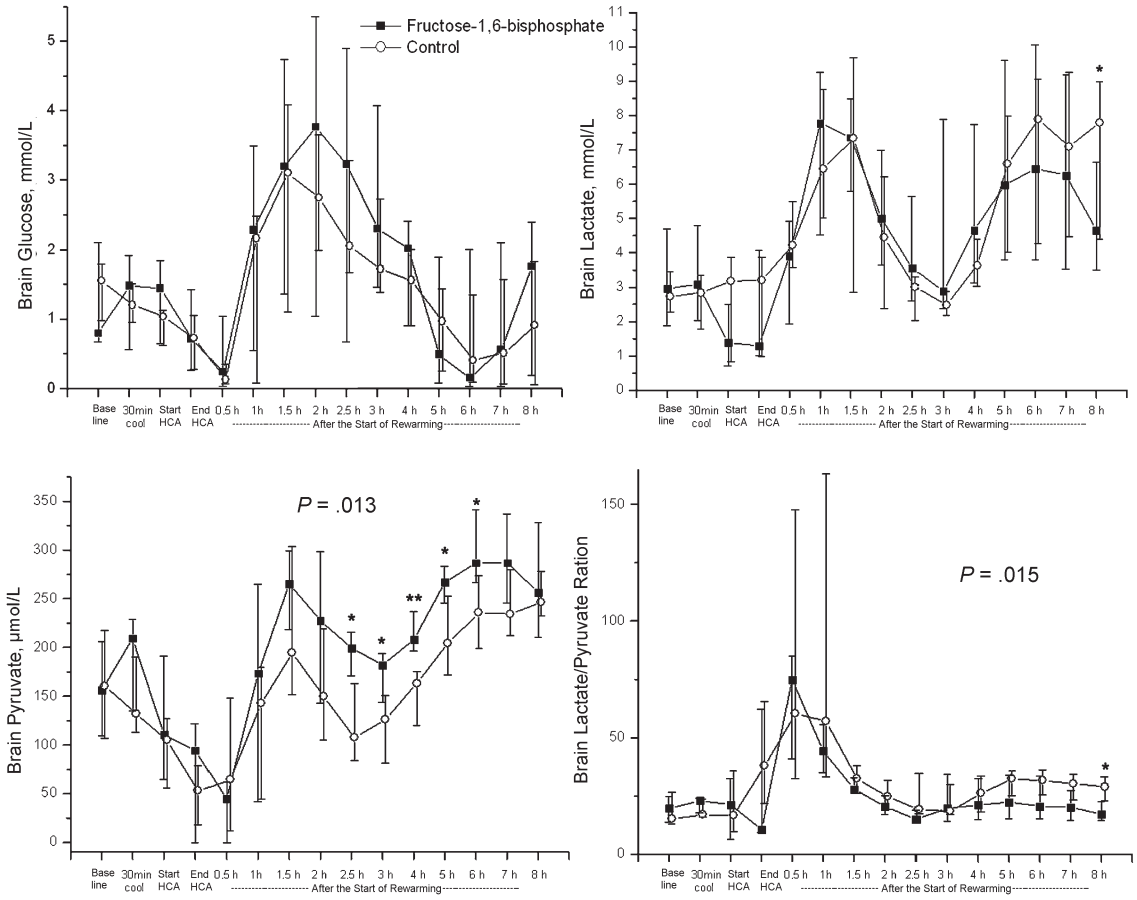

Figure 3. Intracranial parameters. *P < .05 between groups. **P < .01 between study groups at any study interval. Values are shown as medians and twenty-fifth and seventy-fifth percentiles.

affect the recovery of brain areas that have undergone global ischemia that is, within due limits, temporary and not irreversible as in the case of particle embolism. This supporting effect of FDP on brain metabolism was clear in our previous study with reversible global ischemia, generating significantly favorable results also in terms of neurological outcome as well as histopathologic findings, also measured by electron microscopy [Romsi 2003; Kaakinen 2005]. Furthermore, a neuroprotective effect could be expected on the ischemic penumbra areas near infarction, where the blood flow has not stopped completely. Although speculative, FDP may prevent postoperative neurocognitive derangements and other markers of milder cerebral injury that cannot be recognized in a rough experimental model involving animals.

Our findings support the theory that the main mechanism of action of FDP is due to the supportive role on the carbohydrate metabolism of brain tissue recovering from hypoxic injury, as reflected by the lower lactate/pyruvate ratio and higher pyruvate levels. Increased lactate/pyruvate ratio is a good marker of cerebral ischemia [Hillered 1990], and high pyruvate levels indicate adequate tissue reperfusion [Persson 1992].

We noticed a dramatic drop in venous ionized calcium levels due to a highly increased phosphate load. This FDP-dependent phenomenon has also been described previously [Hassinen 1991; Romsi 2003]. No differences were detected between the groups in terms of cardiac index and mean arterial pressure, as we have also demonstrated previously, indicating that hardly any negative inotropic effect on myocardium actually occurred [Romsi 2003].

The differences in pH, $Paco_2$, and $Pvco_2$ were clearly linked to the administration of FDP. Low pH of the drug led to increased levels of CO_2. Although high $Paco_2$ dilates cerebral vessels [Duebener 2002] and itself has protective properties to the brain [Dahlbacka 2005], the difference between groups is hardly significant from a clinical point of view and the effect was short-lasting. The lower levels of sodium and osmolarity observed in the FDP group most likely are due to better fluid balance.

In conclusion, 2 intravenous infusions of FDP at 500 mg/kg before and after irreversible embolic injury on the brain were associated with significantly higher brain pyruvate levels and lower lactate/pyruvate ratios, but failed to provide positive impact on outcome. These findings do support

Table 2. Histopathologic Brain Scores*

Protocol	Pig #	Survival, d	Cortex Score	Thalamus Score	Hippocampus Score	Posterior Brainstem Score	Cerebellum Score	Total Score	Number of Brain Infarctions
FDP	1	7	1	4	2	4	3	14	1
	2	1	4	4	2	2	3	15	0
	3	7	1	1	1	3	3	9	1
	4	1	4	3	3	1	3	14	0
	5	7	2	2	1	1	3	9	1
	6	7	3	2	2	1	3	11	0
	7	2	3	2	4	3	3	15	3
	8	2	2	5	2	2	4	15	0
	9	2	4	3	2	3	0	12	1
	10	7	3	3	1	1	3	11	4
Mean		4.3	2.7	2.9	2.0	2.1	2.8	12.5	1.1
Control	1	1	3	4	2	3	3	15	0
	2	1	6	2	4	4	1	17	0
	3	4	2	2	3	1	3	11	1
	4	1	3	1	1	3	3	11	1
	5	7	2	1	2	3	3	11	2
	6	7	1	1	2	2	3	9	1
	7	7	1	1	1	3	0	6	0
	8	2	3	4	1	3	4	15	1
	9	1	3	3	1	2	3	12	1
	10	7	1	3	1	1	1	7	2
Mean		3.8	2.5	2.2	1.8	2.5	2.4	11.4	0.9
P		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Signs of brain ischemic damage were scored as follows: 1, dark or eosinophilic neurons or cerebellar Purkinje cells, edema; 2, moderate edema, hemorrhages; 3, severe edema, infarct foci. The total score is the sum scores of each specific brain area. N.S. indicates not significant.

previous reports that FDP has supporting effects on brain metabolism after ischemia. Based on these and our previous results, FDP does provide metabolic support to the ischemic brain after embolic injury, but it seems that reperfusion of the injured tissue is needed to improve the neurological outcome.

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