Relative Higher Hematocrit Attenuates the Cerebral Excitatory Amino Acid Elevation Induced by Deep Hypothermic Circulatory Arrest in Rats

Meijuan Zhu, Yanhua Zhao, Yongjun Zheng, Diansan Su, and Xiangrui Wang

Hemodilution is a commonly used technique in cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA). Our previous study showed that lower hematocrit aggravated the brain injury after DHCA. Because the excitatory amino acids are critical pathways of ischemic neuronal damage, the purpose of the present study was to investigate the effects of different degrees of hemodilution on the excitatory amino acid content in different brain areas after DHCA. Adult Sprague-Dawley rats were randomly divided into four groups: group I hematocrit (Hct) 10% (H1), group II Hct 20% (H2), group III Hct 30% (H3), and control group (C). All animals except those in the control group underwent DHCA at 18°C for 90 minutes. Different degrees of hemodilution were accomplished by changing the composition and volume of the priming solution used in CPB. High-performance liquid chromatography was used to determine the concentration of glutamate (Glu), aspartate (Asp), glycine (Gly), gamma-aminobutyric acid (GABA), and taurine (Tau) in the cerebral cortex, hippocampus, and thalamus. We found that the concentration of these five amino acids in the hippocampus and cortex were all increased after DHCA. Glu, Asp, and Gly in the hippocampus and cortex were significantly lower in the Hct 30% group than in the other two groups ($p < 0.05$). There was no significant difference in the GABA and Tau concentrations among the three groups. In summary, excitatory amino acids increased significantly after DHCA, and relative higher hematocrit attenuates this response.

Introduction

DEEP HYPOTHERMIC CIRCULATORY ARREST (DHCA) is widely used in complex surgical procedures targeting congenital heart disease and large cerebral aneurysms (Elmstekawy and Rubens, 2011). DHCA is a strategy of total circulation arrest when the body temperature is less than 18°C induced by cardiopulmonary bypass (CPB). The approach provides a bloodless field for surgery and without miscellaneous cannula (Gega *et al.*, 2007). DHCA is still an essential technique used during many complex cardiac surgeries.

Hemodilution is a common method applied in the CPB or DHCA to counteract the deleterious rheologic consequences of hypothermia, such as increased viscosity and red cell rigidity (Dian-San *et al.*, 2006). However, Sakamoto *et al.*, (2004) demonstrated that severe hemodilution results in decreased perfusion pressure and an increased cerebral metabolic rate of oxygen during DHCA of a piglet. Our previous study showed that low hematocrit worsens brain injury induced by DHCA in rats (Dian-San *et al.*, 2006). However, the exact mechanisms underlying this detrimental effect are not clear. Cerebral excitatory amino acids play an important role in the pathogenesis of ischemic brain injury. The basic pathology of brain

injury induced by DHCA is hypoxia and ischemia during circulation arrest. In the present study, we document the changes of amino acids after DHCA in rats with varying degrees of hemodilution.

Materials and Methods

This study was approved by the School of Medicine, Shanghai Jiaotong University Animal Care and Use Committee. All procedures were performed in accordance with the guidelines of the National Institutes of Health (NIH) for animal care (guide for the care and use of laboratory animals, Health and Human Services, NIH Publication No. 86-23, revised 1985).

Animals and groups

Twenty-four male adult Sprague-Dawley rats weighing 400–450 g were randomly divided into four groups ($n=6$ each): group I Hct 10%(H1), group II Hct 20%(H2), group III Hct 30%(H3), and control group (C). The composition of the priming fluid was varied to obtain the different hematocrit levels. In the Hct 30% group, the CPB prime consisted of

10 mL heparinized donor blood. Blood was used from donor rats that were anesthetized with pentobarbital (40 mg/kg), and blood was withdrawn through the femoral artery. The remainder of the prime consisted of Ringer's lactate 4 mL, mannitol (20%) 1 mL, heparin (100 UI) 1 mL, and 6% hydroxyethyl starch 4 mL (HAES-sterile®; Fresenius Kabi, Bad Homburg, Germany). In the Hct 20% group, the CPB prime consisted of Ringer's lactate 10 mL, 20% mannitol 1 mL, heparin (100 UI) 1 mL, and hydroxyethyl starch 4 mL. In the Hct 10% group, the CPB prime solution was identical to that of the Hct 20% group, with the exception that before CPB, 10 mL of blood was withdrawn and replaced with Ringer's lactate 5 mL and 6% HAES 5 mL administered intravenously.

Experimental protocol

CPB was conducted as previously described (Su *et al.*, 2005; Dian-San *et al.*, 2006). The CPB circuit included a roller pump (Masterflex®; Cole-Parmer Instrument Co., Vernon Hills, IL) with sterile silicone tubing (1.6 mm), connected with a 10-mL syringe as reservoir and a custom designed membrane oxygenator for rats with a surface area of 0.1 m² and a heat exchanger fixed in the oxygenator. The rats were anesthetized with pentobarbital (40 mg/kg) and ventilated with 100% oxygen. Both femoral arteries were cannulated with a 22G catheter, left side for monitoring mean arterial blood pressure, blood gas analysis, and the arterial outflow for the CPB circuit. A multiorificed 14G *iv* catheter was inserted in the right common jugular vein as the venous inflow for the CPB circuit.

Animals in the three target Hct groups were cooled to a rectal temperature of 16°C to 18°C over 30 minutes, at which point, CPB and ventilation were discontinued. Temperature was maintained by surface cooling for 90 minutes. CPB was resumed after 90 minutes of arrest, and the animals were actively warmed over 40 minutes using the heat exchanger to achieve a body temperature of 35°C. Sixty minutes after weaning from CPB, rats were sacrificed. Brain tissue was dissected and prepared for the measurement of amino acids with high-performance liquid chromatography (HPLC).

Amino acid measurement

The cortex, hippocampus, and thalamus were dissected and the amino acids were measured by HPLC as described

previously (Lopez-Meraz *et al.*, 2012). After homogenization in perchloric acid (0.1 M), the homogenates were centrifuged at 10,000 rpm at 4°C for 20 minutes. The supernatant was used for the measurement of amino acids. After derivatization with ophthaldehyde (OPA), the mixture of supernatant and OPA was injected into the HPLC with a reversed-phase 3.9×150-mm column (Nova-Pack, 4 µm, C18, Waters®). The concentrations of glutamate (Glu), aspartate (Asp), glycine (Gly), gamma-aminobutyric acid (GABA), and taurine (Tau) were measured from this protocol.

Statistics

One-way ANOVA was used to analyze the difference among the four groups with SPSS10.0. Statistical significance was assumed when $p < 0.05$.

Results

The hematocrit values had no differences among groups before CPB. During CPB, the hematocrit decreased to the desired levels in the different groups because of the different priming. During the experiment, PO₂ and PCO₂ were at the acceptable range.

Compared with the control group, the concentration of the five amino acids (Glu, Asp, Gly, GABA, and Tau) in the hippocampus, cortex, and thalamus increased significantly after DHCA (Table 1). Compared with the group H2, the concentration of Glu, Asp, and Gly in the hippocampus and cortex was increased significantly in group H1 ($p < 0.05$), while these three amino acids in the hippocampus and cortex decreased significantly in the group H3 ($p < 0.05$, Table 1). There were no significant differences among the three experimental groups for the concentration of GABA and Tau in the three brain areas (Table 1).

Discussion

The major finding of the present study was that cerebral excitatory amino acids increase after deep hypothermia circulation arrest and relative high hematocrit (30%) attenuated the elevation.

Cerebral hypoxia and ischemia underlie the basic pathology after DHCA. The toxic effects of excitatory amino acids

TABLE 1. AMINO ACID CONCENTRATION CHANGES AFTER DEEP HYPOTHERMIC CIRCULATION ARREST (MICROMOLAR CONCENTRATION)

Brain areas	Group	Glu	Asp	gly	GABA	Tau
Cortex	C	1.31±0.18	0.33±0.1	0.31±0.11	0.72±0.13	1.62±0.16
	H1	2.11±0.17 ^{a,b}	1.12±0.2 ^{a,b}	1.11±0.14 ^{a,b}	1.14±0.29 ^a	2.41±0.18 ^a
	H2	1.86±0.14 ^a	0.83±0.16 ^a	0.9±0.12 ^a	1.07±0.14 ^a	2.23±0.24 ^a
	H3	1.59±0.07 ^{a,b}	0.56±0.18 ^{a,b}	0.58±0.14 ^{a,b}	1.06±0.26 ^a	2.47±0.28 ^a
Hippocampus	C	0.84±0.12	0.25±0.1	0.41±0.14	0.8±0.24	1.44±0.23
	H1	2.24±0.32 ^{a,b}	0.85±0.15 ^{a,b}	1.45±0.21 ^{a,b}	1.54±0.14 ^a	2.23±0.27 ^a
	H2	1.47±0.19 ^a	0.68±0.14 ^a	1.11±0.17 ^a	1.49±0.16 ^a	2.24±0.23 ^a
	H3	1.14±0.15 ^{a,b}	0.47±0.08 ^{a,b}	0.79±0.12 ^{a,b}	1.51±0.21 ^a	2.13±0.26 ^a
Thalamus	C	0.48±0.06	0.33±0.11	0.71±0.09	1.11±0.18	1.22±0.20
	H1	0.74±0.13 ^{a,b}	0.54±0.10 ^{a,b}	0.97±0.14 ^{a,b}	1.84±0.24 ^a	2.12±0.25 ^a
	H2	0.71±0.20 ^a	0.59±0.11 ^a	1.01±0.11 ^a	2.08±0.33 ^a	1.95±0.23 ^a
	H3	0.75±0.16 ^{a,b}	0.53±0.16 ^{a,b}	0.97±0.13 ^{a,b}	1.82±0.48 ^a	2.08±0.4 ^a

^a $p < 0.05$ compared with the control group.

^b $p < 0.05$ compared with the H2 group.

are known to participate in ischemic neuronal damage (Lipton and Rosenberg 1994). Glutamate is the major neurotransmitter in the brain that plays many neurophysiological functions, including cognition, memory, movement, and sensation (Ferraro *et al.*, 2009). Glu and Asp all interact with the glutamate receptors to produce its physiological role. There are two kinds of glutamate receptors, the metabolic and the ionic type. The former combines with G protein-coupled receptors, while the latter combines directly with the ionic channel. The ionotropic glutamate receptors are NMDA receptors and AMPA receptors. The cerebral concentration of Glu and Asp can increase too high, which can overactivate the NMDA receptor that can cause various consequences, including the over-activation of protein kinases, endonucleases, phosphoglycerate kinase, and nitric oxide synthase, and the formation of peroxide, thus leading to the degradation of DNA and death of neurons (Taoufik and Probert, 2008). In the present study, we found that the excitatory amino acids Glu, Asp, and Gly increased significantly after DHCA, which was consistent with previous studies (Tseng *et al.*, 1999). In a canine DHCA model, Tseng *et al.*, (1999) reported that DHCA significantly increased brain Glu, Asp, and Gly.

There are basically two reasons to apply hemodilution in CPB and DHCA. One reason is that hemodilution ameliorated the microcirculatory disturbances induced by DHCA because of the rheologic changes (Skaryak *et al.*, 1995; Jonas *et al.*, 2003; Sakamoto *et al.*, 2004). Another reason is that the increased cardiac output with blood hemodilution may complement the oxygen carrier capacity decreased because of the hemodilution. Although experience with cardiovascular surgery in adults suggests that very low Hct levels can be tolerated (Cooper, 1990), our previous studies demonstrated the neurological damage after DHCA especially in the low hematocrit group (Dian-San *et al.*, 2006). The number of injured neurons in the hippocampus CA1 and parietal cortex increased significantly with the lower hematocrit (Dian-San *et al.*, 2006). Jonas *et al.*, (2003) found that lower hematocrit was associated with adverse perioperative and developmental outcomes at one year in infants. There are some limitations associated with this study, including only measuring whole tissue amino acid levels, whereas extracellular assessment may show more robust changes as well as only a single time point was assessed. Nevertheless, in the present study, we found that the higher hematocrit attenuated the elevation of excitatory amino acids induced by DHCA, which indicated that the increased cerebral blood flow during hemodilution, while in a fixed cardiac output by CPB (Sakamoto *et al.*, 2004), cannot balance the reduced oxygen carrying capacity. In summary, excitatory amino acids increased significantly after DHCA, and a relative higher hematocrit attenuated this increase.

Disclosure Statement

No competing financial interests exist.

References

- Cooper JR, Jr. Perioperative considerations in Jehovah's Witnesses. *Int Anesthesiol Clin* 1990;28:210–215.
- Dian-San S, Xiang-Rui W, Yongjun Z, Yan-Hua Z. Low hematocrit worsens cerebral injury after prolonged hypothermic circulatory arrest in rats. *Can J Anaesthesiol* 2006;53:1220–1229.
- Elmestekawy EM, Rubens FD. Deep hypothermic circulatory arrest: alternative strategies for cerebral perfusion. A review article. *Perfusion* 2011;26 Suppl 1:27–34.
- Ferraro L, Tomasini MC, Beggiato S, Guerrini R, Salvadori S, Fuxe K, *et al.* Emerging evidence for neurotensin receptor 1 antagonists as novel pharmaceuticals in neurodegenerative disorders. *Mini Rev Med Chem* 2009;9:1429–1438.
- Gega A, Rizzo JA, Johnson MH, Tranquilli M, Farkas EA, Eleftheriades JA. Straight deep hypothermic arrest: experience in 394 patients supports its effectiveness as a sole means of brain preservation. *Ann Thorac Surg* 2007;84:759–766; discussion 766–757.
- Jonas RA, Wypij D, Roth SJ, Bellinger DC, Visconti KJ, du Plessis AJ, *et al.* The influence of hemodilution on outcome after hypothermic cardiopulmonary bypass: results of a randomized trial in infants. *J Thorac Cardiovasc Surg* 2003;126:1765–1774.
- Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* 1994;330:613–622.
- Lopez-Meraz ML, Rocha LL, Miquel M, Ortega JC, Perez-Estudillo CA, Garcia LI, *et al.* Amino acid tissue levels and GABAA receptor binding in the developing rat cerebellum following status epilepticus. *Brain Res* 2012;1439:82–87.
- Sakamoto T, Nollert GD, Zurakowski D, Soul J, Duebener LF, Sperling J, *et al.* Hemodilution elevates cerebral blood flow and oxygen metabolism during cardiopulmonary bypass in piglets. *Ann Thorac Surg* 2004;77:1656–1663; discussion 1663.
- Skaryak LA, Kirshbom PM, DiBernardo LR, Kern FH, Greeley WJ, Ungerleider RM, *et al.* Modified ultrafiltration improves cerebral metabolic recovery after circulatory arrest. *J Thorac Cardiovasc Surg* 1995;109:744–751; discussion 751–742.
- Su DS, Wang XR, Zheng YJ, Zhao YH, Zhang TJ. Retrograde cerebral perfusion of oxygenated, compacted red blood cells attenuates brain damage after hypothermia circulation arrest of rat. *Acta Anaesthesiol Scand* 2005;49:1172–1181.
- Taoufik E, Probert L. Ischemic neuronal damage. *Curr Pharm Des* 2008;14:3565–3573.
- Tseng EE, Brock MV, Kwon CC, Annanata M, Lange MS, Troncoso JC, *et al.* Increased intracerebral excitatory amino acids and nitric oxide after hypothermic circulatory arrest. *Ann Thorac Surg* 1999;67:371–376.

Address correspondence to:
Xiangrui Wang, MD, PhD
Department of Anesthesiology
Renji Hospital
School of Medicine
Shanghai Jiaotong University
1630 Dong Fang Road
Shanghai 200127
China

E-mail: wangxr2010@yahoo.com