Inhibition of microRNA-29c protects the brain in a rat model of prolonged hypothermic circulatory arrest

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ABSTRACT

Objective: We sought to investigate the cerebroprotection of a novel microRNA mechanism by targeting peroxisome proliferator-activated receptor gamma coactivator 1-alpha in a rat model of prolonged deep hypothermia circulatory arrest.

Methods: The right carotid artery and jugular vein of male Sprague–Dawley rats were cannulated for cardiopulmonary bypass. Circulatory arrest was conducted for 60 minutes when the pericranial temperature was cooled to 18°C. The sham group received the surgical procedure without cardiopulmonary bypass and deep hypothermia circulatory arrest; the deep hypothermia circulatory arrest group received cardiopulmonary bypass and deep hypothermia circulatory arrest; lentivirus control vector or lentiviral vector containing antagomiR-29c was given to the deep hypothermia circulatory arrest + vector group or the deep hypothermia circulatory arrest + antagomiR-29c group by intracerebroventricular administration 5 days before cardiopulmonary bypass (n = 8, for each of the 4 groups). Neurologic function was evaluated by the modified hole board test and beam balance task during 14 postoperative days. Expressions of caspase-3, peroxisome proliferator-activated receptor gamma coactivator 1-alpha, and miR-29c in the hippocampus were measured by Western blot and quantitative reverse transcription polymerase chain reaction. Malondialdehyde was measured using the Malondialdehyde Assay Kit (Beyotime, Jiangsu, China).

Results: Pretreatment with antagomiR-29c significantly decreased the expression of microRNA-29c and increased the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha in the hippocampus ($P < .05$ vs deep hypothermia circulatory arrest group). The level of malondialdehyde in the hippocampus was lower in the deep hypothermia circulatory arrest + antagomiR-29c group ($P < .05$ vs deep hypothermia circulatory arrest group). The neurologic functions were markedly protected in rats pretreated with antagomiR-29c as evidenced by improvement of vestibulomotor and cognitive performance during the early postoperative period. In the deep hypothermia circulatory arrest + antagomiR-29c group, histologic scores of the hippocampus were improved and the level of caspase-3 in the hippocampus was lower ($P < .05$ vs deep hypothermia circulatory arrest group).

Conclusions: Inhibition of miR-29c attenuates neurologic injuries induced by prolonged deep hypothermia circulatory arrest through a peroxisome proliferator-activated receptor gamma coactivator 1-alpha pathway. (J Thorac Cardiovasc Surg 2015;150:675-84)

Inhibition of miR-29c attenuates neurologic dysfunction after DHCA.

Central Message

Inhibition of miR-29c by intracerebroventricular administration of lentivirus vectors containing antagomiR-29c attenuates neurologic injuries induced by prolonged DHCA through a PGC-1 α pathway.

Perspective

The current study highlights the potential role of miRNAs in neuroprotection after DHCA, a special procedure of brain ischemia. The results indicate that inhibition of miR-29c conducts neuroprotective effects against injuries induced by DHCA through a PGC-1 α -dependent pathway. To our best knowledge, this is the first report that miRNAs play a critical role in neurologic injuries of DHCA. Regulation of miRNAs may become a novel therapeutic strategy for attenuation of neurologic injuries after DHCA.

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Abbreviations and Acronyms

CPB	= cardiopulmonary bypass
DHCA	= deep hypothermia circulatory arrest
MDA	= malondialdehyde
miR-29c	= microRNA-29c
miRNA	= microRNA
PGC-1 α	= peroxisome proliferator-activated receptor gamma coactivator 1-alpha
ROS	= reactive oxygen species

Supplemental material is available online.

Cardiopulmonary bypass (CPB) and deep hypothermia circulatory arrest (DHCA) are required for the repair of complex pediatric and adult congenital cardiac and aortic arch lesions. Although the overall mortality and morbidity after surgery with DHCA have been substantially improved, neurologic deficit induced by DHCA remains a major complication. New onset of cerebrovascular events was seen in 5.4% of patients after noncomplex hemiarch surgery with the aid of DHCA.¹ Moreover, it was also reported that approximately 25% patients had a temporary neurologic dysfunction after DHCA,² and that the incidence of neuropsychologic deficit within 12 weeks after the operation with DHCA was as high as 55%.³

Ischemia–reperfusion injury caused by circulation arrest contributes mainly to the neurologic injury after DHCA. It has been reported that one of the principal roots for ischemia–reperfusion injury is the oxidative stress triggered by the excessive production of free radicals.⁴ Overproduction of reactive oxygen species (ROS) as by-products generated from mitochondria has been implicated in acute brain injuries, such as stroke from cerebral ischemia.⁵ Mitochondria activate signaling pathways through ROS production and regulate mitochondria-dependent apoptosis pathways after cerebral ischemia.⁶ Oxidative stress and apoptotic neuronal death signaling pathways also have been involved in the brain in patients undergoing CPB and DHCA.⁷ Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) is a potent stimulator of mitochondrial biogenesis and respiration, and powerfully regulates oxidative and mitochondrial metabolism in a number of tissues, including the central nerve system.⁸ However, it is still not known whether PGC-1 α is expressed in the brain after DHCA and functions as a regulator of oxidative stress and apoptotic neuronal death.

As negative regulators of gene expression,⁹ microRNAs (miRNAs) have been identified to play critical roles in brain ischemia and injury, neuroprotection, and

neurodegeneration.¹⁰ Transient focal ischemia induces extensive temporal changes in rat cerebral microRNAome.¹¹ Collective evidence shows that miR-29c is enriched in the brain¹² and plays dramatic roles in the brain development¹³ and neurite outgrowth.¹⁴ With the help of bioinformatics-based databases^{15,16} and the published report,¹⁷ miR-29c is indicated to be an endogenous regulator of PGC-1 α . Therefore, we tried to explore the possible neuroprotection of using microRNA-29c (miRNA-29c) as a regulator of PGC-1 α in the brain after DHCA in the current study.

MATERIALS AND METHODS**Animal Care**

Male Sprague–Dawley rats weighing approximately 350 g were used in the current study. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of China Medical University (Shenyang, China) and was in accordance with the Guide for the Use and Care of Laboratory Animals (National Institutes of Health, Bethesda, Md).

Cardiopulmonary Bypass and Deep Hypothermia Circulatory Arrest

A rat model of CPB and DHCA was conducted according to the method reported previously.^{18,19} Briefly, animals were anesthetized with chloral hydrate and isoflurane in 60% oxygen, and the anesthesia was maintained with 2.0% to 2.5% isoflurane and fentanyl (25 mg/kg, intravenous, as a bolus injection). After orotracheal intubation with a 14G cannula, the animals were mechanically ventilated with a tidal volume of 10 mL·kg⁻¹ and respiratory rate of 60 to 65 beats·min⁻¹. After a systemic heparinization (150 IU), the right carotid artery was cannulated for aortic inflow. A multistaged venous return cannula was placed near the junction of the inferior vena cava and right atrium through the right external jugular vein, and the tail artery was cannulated for blood pressure monitoring and blood sampling. The CPB circuit consisted of a venous reservoir, a peristaltic pump, and a custom-designed small-volume oxygenator. The entire circuit was primed with 10 mL of 6% hetastarch. Physiologic data, including mean artery pressure, temperature, and blood gas analysis, were collected. CPB was conducted at a flow rate of 160 to 180 mL·kg⁻¹·min⁻¹ and was consecutively decreased by half during the cooling period. Rats were cooled to a pericranial temperature of 18°C over 30 minutes (pH stat cooling was used) by using a heat exchanger and topical cooling with ice bags and a cooling blanket. Then CPB was stopped, and venous blood was drained to the reservoir carefully. DHCA was confirmed by the absence of systole and measurable blood pressure. After 60 minutes of DHCA, CPB was re-instituted and the flow rate was gradually increased to 160 to 180 mL·kg⁻¹·min⁻¹. The rats were rewarmed to a pericranial temperature of 34°C over 30 minutes and then CPB was weaned. After decannulation, rats were maintained anesthetized with 0.5% to 1.2% isoflurane, intubated, and ventilated for 2 hours while warming the rats to a pericranial temperature of 36°C to 37°C. After spontaneous ventilation had resumed, animals were extubated and allowed to recover in an oxygen-enriched environment for 12 hours. Animals demonstrating signs of severe neurologic dysfunction, such as immediate signs of cerebral death, were excluded from the protocol.

Lentivirus Vectors for AntagomiR-29c

Chemical modified antisense oligonucleotides of rat miR-29c (antagomiR-29c) lentivirus gene transfer vector were constructed by Genechem (Shanghai, China). The recombinant lentivirus vector of antagomiR-29c

FIGURE 1. Experimental groups and protocol. CPB, Cardiopulmonary bypass; DHCA, deep hypothermic circulatory arrest; TU, transfection unit.

and the control lentivirus vector without antagomiR-29c were prepared and titered to 1×10^9 transfection unit $\cdot \text{mL}^{-1}$.

Intracerebroventricular Injection

After anesthesia, a puncture needle was implanted into the lateral ventricle (bregma; 0.8 mm posterior, -4.8 mm dorsoventral, -1.5 mm lateral).²⁰ Proper placement of the needle in the lateral ventricle was indicated by the presence of cerebral spinal fluid in the needle hub.¹¹ Once correct needle placement was confirmed, the solutions were injected over 1 minute. The animals were included in the study only if they had a normal neurologic function 3 days after the intracerebroventricular injection.

Experimental Protocol

Rats were randomly assigned to 4 groups, as shown in Figure 1. The sham group received only the surgical procedure without CPB and DHCA. The other 3 groups were subjected to CPB and a 60-minute DHCA. The DHCA + antagomiR-29c group and the DHCA + vector group received intracerebroventricular injection of approximately 1×10^7 transfection unit lentivirus vectors of antagomiR-29c or the control lentivirus vectors in a total fluid volume of 10 µL 5 days before the surgical procedure, whereas the vehicle alone of the same volume was given to the sham group and DHCA group.

In a parallel series of experiments, rat brains were collected 2 hours after the surgical procedure from the 4 groups ($n = 4$ per group) for evaluating the expressions of miR-29c, PGC-1α, and caspase-3 and measuring malondialdehyde (MDA) levels.

Neurologic Assessment

Cognitive functions were assessed by the modified hole board test as reported previously.¹⁸ Briefly, rats were housed in an area divided into a home cage (80 × 60 × 50 cm) and a test arena (40 × 60 × 50 cm) with a centrally placed hole board (20 × 40 cm). Fifteen holes covered by lids were staggered on the board. After being opened, coil springs forced the lids back to their original position. Three holes baited with puffed rice were marked with white tape. All holes were flavored with the aroma of black currants

to cover the odor and smell of the puffed rice. The scores for the modified hole board test with a range from 0 (normal) to 24 (severe cognitive dysfunction) are provided in Table E1. Vestibulomotor function was assessed using a beam balance task where the rat was placed on a 1.5 cm wide beam. The duration it remained on the beam was recorded (maximum = 60 seconds).²¹

Malondialdehyde Measurement

As a marker of oxidative stress and free oxygen radical-mediated damage, MDA levels of the hippocampus were measured using the MDA Assay Kit (Beyotime, Jiangsu, China) according to the procedure recommended by the manufacturer. MDA concentration was calculated in nanomoles per gram of protein.

Histologic Study

After evaluation of the vestibulomotor and cognitive function during a 14-day postoperative period, animals were killed for histologic evaluation. Brain tissues were sectioned (5 µm) and stained with hematoxylin-eosin. The neuronal damage of the hippocampus CA1 region was evaluated qualitatively by a pathologist blinded to the grouping. Neurons showing features such as shrunken cell bodies, triangulated, pyknotic nuclei, and eosinophilic cytoplasm were identified as a dead neuron. Normal neurons and death neurons were counted using a defined rectangular field area with magnification of 200×. A pathologic score was used to evaluate the neurologic damage: 0 = no damage; 1 = 0% to 12.5% damage; 2 = 12.5% to 25% damage; 3 = 25% to 50% damage; and 4 = more than 50% damage.⁷

microRNA Extraction and Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA was isolated by using Trizol reagent (Invitrogen, Carlsbad, Calif) according to the manufacturer's instruction. The first-strand cDNA was generated using the Reverse Transcription System Kit (Invitrogen). Real-time polymerase chain reaction was performed according to a standard protocol using a StepOne Plus system (Applied Biosystems, Foster

TABLE 1. Physiologic data

	Group	Baseline	CPB		Post-CPB		F	P value
			Cooling 20 min	Warming 20 min	15 min	120 min		
Weight (g)	Sham	351 ± 14					F = 0.172	.914
	DHCA	350 ± 10						
	DHCA + vector	353 ± 12						
	DHCA + antagomiR-29c	354 ± 8						
MAP (mm Hg)	Sham	74 ± 4					F(group) = 1.665 F(time) = 290.459	.194 .000
	DHCA	75 ± 5	45 ± 4*	49 ± 3*	75 ± 4	76 ± 6		
	DHCA + vector	76 ± 7	46 ± 3*	49 ± 4*	74 ± 4	75 ± 4		
	DHCA + antagomiR-29c	79 ± 7	46 ± 4*	50 ± 3*	77 ± 5	76 ± 6		
Heart rate	Sham	317 ± 11					F(group) = 0.077 F(time) = 1983.430	.926 .000
	DHCA	321 ± 10	86 ± 5*	303 ± 12*	315 ± 5	321 ± 10		
	DHCA + vector	313 ± 15	91 ± 9*	301 ± 12*	318 ± 10	320 ± 8		
	DHCA + antagomiR-29c	318 ± 17	88 ± 8*	310 ± 13*	316 ± 14	318 ± 10		
Pericranial temperature (°C)	Sham	33.6 ± 0.2					F(group) = 0.199 F(time) = 3082.746	.820 .000
	DHCA	33.7 ± 0.2	21.6 ± 0.5*	28.3 ± 0.7*	35.1 ± 0.2*	35.7 ± 0.4*		
	DHCA + vector	33.8 ± 0.2	21.8 ± 0.6*	27.9 ± 1.0*	35.2 ± 0.4*	35.8 ± 0.3*		
	DHCA + antagomiR-29c	33.8 ± 0.2	21.2 ± 0.5*	28.1 ± 1.1*	35.2 ± 0.3*	35.8 ± 0.2*		
pH	Sham	7.4 ± 0.02					F(group) = 1.898 F(time) = 59.717	.155 .000
	DHCA	7.41 ± 0.02	7.33 ± 0.03*	7.45 ± 0.02*	7.4 ± 0.03	7.44 ± 0.03*		
	DHCA + vector	7.4 ± 0.02	7.32 ± 0.03*	7.46 ± 0.02*	7.38 ± 0.05	7.43 ± 0.03*		
	DHCA + antagomiR-29c	7.42 ± 0.03	7.35 ± 0.04*	7.46 ± 0.02*	7.39 ± 0.03	7.45 ± 0.02*		
PaO ₂ (mm Hg)	Sham	262 ± 51					F(group) = 2.660 F(time) = 73.082	.074 .000
	DHCA	258 ± 57	417 ± 60*	317 ± 61*	128 ± 18*	306 ± 48*		
	DHCA + vector	271 ± 80	429 ± 77*	323 ± 79*	138 ± 27*	360 ± 61*		
	DHCA + antagomiR-29c	274 ± 73	457 ± 85*	342 ± 78*	142 ± 12*	373 ± 88*		
PACO ₂ (mm Hg)	Sham	42 ± 2					F(group) = 0.127 F(time) = 18.964	.881 .000
	DHCA	42 ± 3	36 ± 2*	38 ± 3*	44 ± 3*	41 ± 4		
	DHCA + vector	40 ± 5	37 ± 4*	39 ± 4*	45 ± 2*	41 ± 5		
	DHCA + antagomiR-29c	40 ± 4	37 ± 3*	38 ± 3*	44 ± 3*	41 ± 2		

CPB, Cardiopulmonary bypass; DHCA, deep hypothermia circulatory arrest; MAP, mean arterial pressure; PACO₂, alveolar carbon dioxide tension; PaO₂, arterial oxygen tension.

**P* < .05 versus baseline of the same group. n = 8 per group.

City, Calif). The U6 served as an internal control. Changes in expression were determined by the $2^{-\Delta\Delta CT}$ method.

Western Blot

Caspase-3 and PGC-1 α were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotted with anti-caspase-3 and anti-PGC-1 α antibody (Abcam, Cambridge, Mass), respectively. The caspase-3 and PGC-1 α expressions in each brain tissue sample were analyzed with National Institutes of Health Image and quantified as a relative fold to the sham group after normalization with β -actin.

Statistical Analysis

Parametric values are reported as mean $\pm$ standard deviation. The Kruskal-Wallis test was used for nonparametric values, and the Mann-Whitney *U* test was used as a posttest to identify the specific differences among the groups. Parametric values were analyzed by 1-way or 2-way repeated-measures (time and group) analysis of variance followed by the Bonferroni post hoc test. Statistical analyses were performed using SPSS version 19.0 (SPSS Inc, Chicago, Ill).

RESULTS

Mortality and Exclusion

A total of 37 rats were enrolled in the protocol for neurologic assessment. One rat in the DHCA + vector group

was excluded because of cerebral death after the surgical procedure, and 4 rats were excluded because of death during the recovery period (1 in sham group; 1 in DHCA + antagomiR-29c group; 2 in DHCA group). Complete data were obtained in the remaining 32 rats (n = 8, for each of the 4 groups).

Physiologic Parameters

Table 1 indicates the physiologic parameters from the 4 groups during the surgical procedure. No significant differences in body weight and physiologic values of the baseline were observed among the 4 groups (n = 8, for each of the 4 groups). The hemodynamic data and the data of blood gas analysis were similar at any time point during the CPB and DHCA among the 3 groups receiving CPB and DHCA (*P* > .05).

Neurologic Assessment

Vestibulomotor function assessed by the beam balance task is summarized in Figure 2 (n = 8, for each of the 4

FIGURE 2. A-F, Vestibulomotor function assessed by the beam balance task after the surgical procedure. n = 8, for each of the 4 groups. **P* < .01 versus sham group. #*P* < .05 versus DHCA group. DHCA, Deep hypothermic circulatory arrest.

groups). Vestibulomotor functions of animals in the DHCA and DHCA + vector groups were damaged remarkably during the early period after DHCA, as evidenced by the shorter balance beam time compared with the sham group

(*P* < .01 at postoperative days 3, 5, 7, and 9 of the DHCA group and DHCA + vector group), and such functions were recovered in approximately 2 weeks (vs sham group, *P* > .05 at postoperative days 11 and 13 of the DHCA group

FIGURE 3. A-C, Cognitive function assessed by modified hole board test after the surgical procedure. n = 8, for each of the 4 groups. **P* < .05 versus sham group. #*P* < .05 versus DHCA group. DHCA, Deep hypothermic circulatory arrest.

FIGURE 4. MDA contents in the hippocampus. $n = 4$, for each of the 4 groups. * $P < .05$ versus sham group. # $P < .05$ versus DHCA group. DHCA, Deep hypothermic circulatory arrest; MDA, malondialdehyde.

and DHCA + vector group, respectively). Although damaged vestibulomotor functions were also detected in the rats with pretreatment with antagomiR-29c during the early period after DHCA (vs sham group, $P < .01$ at postoperative days 3, 5, 7, and 9), the vestibulomotor function of the DHCA + antagomiR-29c group was still protected to a significant extent when compared with the DHCA group ($P < .05$ at postoperative days 3, 5, 7, and 9).

DHCA also affected the cognitive function of animals as illustrated in Figure 3. On postoperative days 10 and 12, the

hole plate scores of the DHCA, DHCA + vector, and DHCA + antagomiR-29c groups were higher than in the sham group ($P < .05$). However, the plate scores of the DHCA + antagomiR-29c group at postoperative days 10 and 12 were still significantly lower than those in the DHCA group ($P < .05$). On postoperative day 14, no significant differences of the plate scores were found among the 4 groups ($P = .064$).

Malondialdehyde Levels

MDA levels were significantly increased in the DHCA and DHCA + vector groups compared with the sham group ($P < .05$) (Figure 4). Intracerebroventricular injection of antagomiR-29c remarkably reduced the level of MDA in the DHCA + antagomiR-29c group ($P < .05$ vs DHCA group).

Expression of microRNA-29c

The results of quantitative reverse transcription polymerase chain reaction showed that miR-29c was enriched in the hippocampus (Figure 5). DHCA slightly decreased the expression of miR-29c in the DHCA and DHCA + vector groups ($P > .05$ vs sham group). However, intracerebroventricular injection of antagomiR-29c before DHCA significantly decreased the expression of miR-29c ($P < .05$ vs DHCA group).

Western Blot

As illustrated in Figure 6, A and C, PGC-1 α was expressed in the hippocampus of rats. After DHCA, PGC-1 α expressions decreased significantly in the DHCA and DHCA + vector groups ($P < .01$ vs sham group). Transfection with lentivirus vectors of antagomiR-29c markedly increased the expression of PGC-1 α in the DHCA + antagomiR-29c group ($P < .01$ vs DHCA group).

Densitometric analysis of the Western blot images revealed a weak signal of caspase-3 in the sham group (Figure 6, B and D). The expression of caspase-3 increased dramatically after DHCA in the DHCA and DHCA + vector groups ($P < .01$ vs sham group), whereas pretreatment with antagomiR-29c significantly reduced the expression of caspase-3 in the DHCA + antagomiR-29c group ($P < .01$ vs DHCA group).

Histologic Examination

Representative coronal brain sections of the hippocampus and the cerebellar vermis stained with hematoxylin-eosin are shown in Figure 7, A. In sham animals, neurons were intact with a clear shape and abundant cytoplasm. In the DHCA and DHCA + vector groups, many neurons with features of dead neuron (eg, shrunken cell bodies, triangulated, pyknotic nuclei, and eosinophilic cytoplasm) were detected. Pretreatment of antagomiR-29c attenuated the morphologic changes in the DHCA + antagomiR-29c

FIGURE 5. miR-29c expression in the hippocampus. $n = 4$, for each of the 4 groups. * $P < .05$ versus DHCA group. DHCA, Deep hypothermic circulatory arrest; miR-29c, microRNA-29c.

FIGURE 6. Results of Western blot. A and B, Representative Western blot pictures showing PGC-1α or caspase-3 expressions. C and D, Densitometric quantification of PGC-1α or caspase-3 expressions (folds to the sham group). $n = 4$, for each of the 4 groups. * $P < .01$ versus sham group. # $P < .01$ versus DHCA group. DHCA, Deep hypothermic circulatory arrest; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

group, and most neurons were kept intact. The pathologic score of the DHCA + antagomiR-29c group was lower than that of the DHCA or DHCA + vector group ($P < .05$) (Figure 7, B).

DISCUSSION

In the current study, CPB and DHCA were imitated in a long-term recovery model of rats, and the DHCA time was extended to 60 minutes beyond the duration of what is commonly required in the clinical practice. After DHCA, the level of MDA and the expression of caspase-3 in the hippocampus increased significantly, which indicated that ROS and mitochondria-dependent apoptosis pathways contributed to the neurologic deficits induced by DHCA. Antioxidant interventions have become the therapeutic strategy to reduce the cerebral damage related to both ischemia and reperfusion.²² One of the main findings of the current study is that inhibition of miR-29c, a brain-enriched miRNA, reduced the level of MDA and apoptosis of the hippocampus after DHCA.

DHCA is an extreme variant of cerebral hypoperfusion, and the ischemia-reperfusion injury is still the principal root for the neurologic injury after DHCA. Neuron death and apoptosis are the key cellular events in the ischemic central nervous system. It is well established that multiple genes and their target proteins are responsible for such pathologic processes after ischemia-reperfusion. miRNAs

recently have become a novel target for cerebral protection against ischemia. The dramatic roles of miRNAs, such as miR-134, miR-30a, and miR-592, in the pathophysiologic process of cerebral ischemia have been identified in different models.²³⁻²⁵ In the current study, inhibition of miRNA-29c demonstrated significant neuroprotection against neurologic injuries caused by a prolonged DHCA in rats. miR-29c is enriched in the brain and is activated as an endogenous regulator of BACE1 protein.¹² miR-29c is expressed during mice brain development and increases neurite outgrowth by decreasing PTEN expression.¹⁴ Over-expression of miRNA-29a, also a member of miRNA-29 family, has been indicated to attenuate the neurologic injury after forebrain ischemia.²⁶ However, the current data showed that miRNA-29c was a negative mediator of cerebral protection against neurologic injuries of DHCA.

Another major finding of the present study is that the expression of PGC-1α in the hippocampus was markedly enhanced by inhibition of miRNA-29c. Activation of miR-29a-c in mouse livers consistently decreases protein levels of PGC-1α.¹⁷ PGC-1α is highly expressed in tissues with high metabolic demands and is generally thought to be the master regulator of mitochondrial biogenesis and ROS scavenging enzymes, including manganese superoxide dismutase 2 and the uncoupling protein 2.²⁷ PGC-1α gene expression is induced in cultured skeletal myotubes by ischemia-like conditions.²⁸ Hypoxia stimulates PGC-1α

FIGURE 7. Histologic examination. A, Representative coronal brain sections of the hippocampus and cerebellar vermis stained with hematoxylin-eosin; a, b, c, d, magnification of 40 $\times$; e, f, g, h, magnification of 200 $\times$; i, j, k, l, magnification of 400 $\times$; a, e, i, sham group; b, f, j, DHCA group; c, g, k, DHCA + vector group; d, h, i, DHCA + antagomiR-29c group. B, Pathologic scores. $n = 8$, for each of the 4 groups. $*P < .05$ versus DHCA group. DHCA, Deep hypothermic circulatory arrest.

protein expression in the mouse cerebral subcortex.²⁹ PGC-1 α is induced by cerebral ischemia and provides a neuroprotective effect against ischemic brain injury in the hippocampus by ameliorating oxidative stress.³⁰ An increased PGC-1 α expression protects cultured neural cells from oxidative stress-mediated cell death.²⁷ Icaritin protects against brain ischemic injury by increasing SIRT1 and PGC-1 α expression.³¹ As the necessary mediator, PGC-1 α also conducts protective effects against brain ischemia induced by alpha-lipoic acid.³² Collectively, PGC-1 α is vital for neuron survival under the condition of ischemia or hypoxia

through a mitochondrial pathway. Therefore, it is plausible that PGC1- α is the target protein by which inhibition of miR-29c conducts neuroprotection after DHCA in rats.

Study Limitations

Because of the large number of possible targets, the mode of action for miR-29c regulating PGC-1 α is not well established in the current study, which needs to be investigated in further research. Another limitation of the current study is that other target proteins may be implicated in the neuroprotection of miR-29c and should be identified in detail.

CONCLUSIONS

The current study highlights the potential role of miRNAs in neuroprotection after DHCA, a special procedure of brain ischemia. The results indicate that inhibition of miR-29c conducts powerful neuroprotective effects against injuries induced by DHCA through a PGC-1 α -dependent pathway. To the best of our knowledge, this is the first report that miRNAs play a critical role in neurologic injuries of DHCA. Regulation of miRNAs may be a novel therapeutic strategy for attenuation of neurologic injuries after DHCA.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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Key Words: hypothermic circulatory arrest, brain, microRNA

Discussion

Dr J. Lawton (St Louis, Mo). You showed upregulation or more increased expression of PGC-1 α . Are there further experiments you could do to directly correlate that increased expression with the benefits you see? In other words, it would be difficult to inhibit it because it is so important in the mitochondrial oxidative stress.

Dr Gu. As we know, the gene transfer to the brain tissue may be more challenging than other organs because it is difficult for gene transfer vectors to get through the blood-brain barrier. That is why we chose the intracerebroventricular injection of the anti-miR-29c. Further

experiments would be performed to explore the mechanisms of the observed cerebral protections.

Dr Lawton. But are there other ways you could specifically implicate that as the mechanism for the benefit you see? Maybe in future studies. I have a couple concerns regarding translation in the future to human benefit. So the first would be the fact that you gave your anti-miRNA 5 days before the deep hypothermic circulatory arrest. The second one is in your neurologic function you showed that there were no differences after 9 days or 14 days. In other words, the benefit of the inhibition of the miRNA was lost. Do you think these benefits would be more long term if you looked at something else?

Dr Gu. It does need some time to achieve better transfection effects after injection of lentiviral vectors. In general,

what occurs more often is transient neurologic dysfunction after DHCA, such as cognitive dysfunction. We used a rat model to mimic the neurologic dysfunction after DHCA in the current study, and our results showed that anti-miR-29c pretreatment attenuated the transient neurologic dysfunction. There is still a long way before anti-miRNA treatment can be used clinically.

Dr Lawton. Do you have any hypotheses about why other groups have shown that miRNA is beneficial to the brain, whereas you showed that blocking the miRNA was beneficial?

Dr Gu. miRNAs negatively regulate the expressions of the target proteins. Whether the miRNA or blocking the miRNA is beneficial to the brain is decided by the functions of their target proteins.

EDITORIAL COMMENTARY

Cerebral protection during deep hypothermic circulatory arrest: Can a molecular approach via microRNA inhibition improve on a millennia-old strategy?

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See related article on pages 675-84.

In this issue of the *Journal*, Wang and colleagues¹ report on their investigation into cerebral protection during deep hypothermic circulatory arrest (DHCA) with a novel microRNA (miRNA) mechanism. The goal of DHCA is to cool the patient's body, effectively reducing the global cerebral metabolic rate of glucose and oxygen consumption. By decreasing the brain's metabolism, DHCA prevents cellular damage by two interrelated mechanisms: (1) it

lowers the demand for glucose to prevent adenosine triphosphate (ATP) depletion with concurrent anaerobic waste product accumulation that leads to necrosis, and (2) it reduces the effects of hypoxia, including activation of intracellular proteases and mitochondrial dysfunction that lead to generation of free radicals and apoptosis.² The cerebral protection offered by DHCA is essential for the success of aortic arch surgery and complex pediatric and adult congenital cardiac procedures.

Hypothermia slows cellular metabolism by approximately 7% for every 1°C by which the body is cooled.³ The resulting protective effect of hypothermia has been known since antiquity; Hippocrates often advocated packing the wounds of soldiers with snow and ice.⁴ Hypothermia was introduced into surgical practice in the mid-20th century,² and the earliest successful open heart surgeries were implemented under hypothermic conditions.⁵ After Griep and coworkers⁶ reported the initial experience of using hypothermia for cerebral protection during aortic arch repair, this technique was implemented worldwide in aortic surgery.

Despite its protective effects, hypothermia remains a *macro* level approach to limit neurologic damage during aortic surgery. Many adjunctive therapies have been

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TABLE E1. Cognitive function assessed by modified hole board test

	Level of impairment							Score	Normal
	0	1	2	3	4	5	6		
Declarative memory									
Wrong choice of 3 trials	0	1	2	3	4	5	>5	0-6	0-1
Omission errors of 3 trials	0	1	2	3	4	5	>5	0-6	0
Working memory									
Repeated choice of 3 trials	0	1	2	3	4	5	>5	0-6	0-1
Cognitive performance									
Time needed to complete 3 trials	<200 s	200-399 s	400-599 s	600-799 s	800-999 s	1000-1199 s	>2000 s	0-6	0-1