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Administration of 5-methoxyindole-2-carboxylic acid that potentially targets mitochondrial dihydrolipoamide dehydrogenase confers cerebral preconditioning against ischemic stroke injury

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ABSTRACT

The objective of this study was to investigate a possible role of mitochondrial dihydrolipoamide dehydrogenase (DLDH) as a chemical preconditioning target for neuroprotection against ischemic injury. We used 5-methoxyindole-2-carboxylic acid (MICA), a reportedly reversible DLDH inhibitor, as the preconditioning agent and administered MICA to rats mainly via dietary intake. Upon completion of 4 week's MICA treatment, rats underwent 1 h transient ischemia and 24 h reperfusion followed by tissue collection. Our results show that MICA protected the brain against ischemic stroke injury as the infarction volume of the brain from the MICA-treated group was significantly smaller than that from the control group. Data were then collected without or with stroke surgery following MICA feeding. It was found that in the absence of stroke following MICA feeding, DLDH activity was lower in the MICA treated group than in the control group, and this decreased activity could be partly due to DLDH protein sulfenation. Moreover, DLDH inhibition by MICA was also found to upregulate the expression of NAD(P)H-ubiquinone oxidoreductase 1(NQO1) via the Nrf2 signaling pathway. In the presence of stroke following MICA feeding, decreased DLDH activity and increased Nrf2 signaling were also observed along with increased NQO1 activity, decreased oxidative stress, decreased cell death, and increased mitochondrial ATP output. We also found that MICA had a delayed preconditioning effect four weeks post MICA treatment. Our study indicates that administration of MICA confers chemical preconditioning and neuroprotection against ischemic stroke injury.

1. Introduction

Stroke is a leading cause of disability and death in the United States. Fortunately, the brain can be induced to tolerate stroke injury. One promising strategy to achieve this tolerance is called preconditioning [1–3], wherein exposure to non-injurious stimuli affords protection against subsequent injurious ischemic challenges. Preconditioning in the brain can be triggered not only by brief episodes of ischemia-reperfusion [4,5] but also by administration of certain chemicals or drugs [6–10]. As ethical considerations do not allow for the use of brief repeated occlusion of the cerebral arteries to elicit stroke tolerance in humans, animal models of chemical preconditioning have been actively investigated in hopes to develop a clinically-useful approach to stroke preconditioning in humans. Moreover, chemical preconditioning may also be useful as a prophylactic approach to neuroprotection [11,12]. The reason for this is that while the onset of stroke is often sudden and unpredictable, individuals with a higher than normal risk of stroke could benefit from prior measures that enhance the brain's tolerance to potential ischemic injury. Such is the case for patients who are scheduled to undergo cardiovascular procedures during which the brain needs to be prophylactically protected against possible stroke injury.

Mitochondria are a known target for preconditioning against stroke injury [13–15]. When oxygen and nutrients supply to the affected area of tissues come to a halt upon ischemia, mitochondrial ATP production is severely decreased. The decrease in ATP content triggers functional impairment of ATP-dependent calcium channels, leading to overload of

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cellular and mitochondrial calcium [16,17] that in turn can trigger glutamate excitotoxicity [18,19]. On the other hand, as cells have to undergo anaerobic respiration in the absence of oxygen, lactate formed from pyruvate via lactate dehydrogenase accumulates, which results in a decrease in cellular pH and closure of mitochondrial permeability transition pore (MPTP) [20]. Upon reperfusion, a sudden resumption of blood flow can over energize mitochondrial respiration, leading to a spike in mitochondrial generation of reactive oxygen species (ROS) and opening of MPTP [21–24]. This opening can release cytochrome c that then activates cell death pathways and causes tissue infarction [25,26]. Moreover, ROS production could further accentuate cellular apoptosis as ROS can induce oxidative stress and impair protein functions [27–35]. Therefore, based on the key roles of mitochondria in cell death and ischemic reperfusion injury, numerous mitochondrial proteins have been assessed or suggested as targets for preconditioning against ischemic stroke injury [14,36].

In this paper, we describe our findings that mitochondrial dihydrolipoamide dehydrogenase (DLDH) could be a target for chemical preconditioning against ischemic stroke injury. DLDH is a family member of flavin-dependent, pyridine dinucleotide oxidoreductases [37]. It is the third component of α-ketoglutarate dehydrogenase complex, pyruvate dehydrogenase complex, and branched chain amino acid dehydrogenase complexes. DLDH is also involved in the glycine cleavage system [38,39]. Each of these complexes or pathways is fundamental for mitochondrial bioenergetics and cell survival. Yet, mouse with a loss of 50% DLDH protein content is viable and fertile [40], suggesting that DLDH function could be inhibited to an extent to which no harm ensues. Our main findings indicate that dietary inhibition of DLDH for 4 weeks using chow supplemented with 5-methoxyindole-2-carboxylic acid (MICA), a specific inhibitor of DLDH function [41–44], significantly decreased infarct volume after transient middle cerebral artery occlusion (tMCAO), while no detrimental effects on food intake, body weight gain, blood glucose concentrations, and mitochondrial electron transport chain activities were detected in the absence of stroke. The preconditioning mechanism in the absence of stroke appeared to involve decreased DLDH activity and increased NAD(P)H: ubiquinone oxidoreductase-1 (NQO1) expression via activation of the Nrf2 signaling pathway. This mechanism was also found to operate upon stroke after MICA feeding. Our data indicate that decreased oxidative stress and apoptosis and increased mitochondrial ATP output are involved in stroke neuroprotection induced by MICA/DLDH preconditioning.

2. Materials and methods

2.1. Animals

Young male Sprague-Dawley rats (approximately 3 months old) were used in this study. The use of animals was approved by Institutional Care and Use Committee of University of North Texas Health Science Center and the protocol was in accordance with NIH Guidelines for the Care and Use of Laboratory Animals. Rats were randomly grouped for MICA groups and control groups.

2.2. Chemicals and reagents

5-methoxyindole-2-carboxylic acid (MICA) was purchased from Fisher Scientific (Hanover Park, IL). Lipoamide, BSA, nitro-blue tetrazolium (NBT) tablets, NADH, NAD^+, succinate, ATP, antimycin A, cytochrome c, 2,3,5-triphenyltetrazolium chloride (TTC), and pyruvate were obtained from Sigma (St. Louis, MO). Dihydrolipoamide was synthesized from lipoamide using sodium borohydride as we previously reported [45]. Amino caproic acid was purchased from MP Biochemicals. Acrylamide, bis-acrylamide, Coomassie brilliant blue, Bradford protein assay solution, and streptavidin-HRP were purchased from Bio-Rad. DLDH antibodies and HRP conjugated secondary antibodies were from US Biological (Swampscott, MA) and Invitrogen (San Diego, CA), respectively. Mito-ID extracellular O_2 sensor kit for the measurement of mitochondrial respiration was purchased from Enzo Life Sciences (Farmingdale, NY). Immunoblot membranes and ECL immunochemical detection solution were from GE Healthcare (Piscataway, NJ). Protein sulfenic acid probe DCP-Bio1 was purchased from Karafast (Boston, MA). Other antibodies were obtained from Abcam (Cambridge, UK).

2.3. Administration of MICA

MICA-containing chow was made by TestDiet (Richmond, IN). For feeding studies, rats were fed with diet supplemented with 0.33% MICA for 4 weeks. Rats were randomly assigned to either control or MICA-treated groups. Control rats were fed a standard rodent diet (Prolab RMH 1800, 5LL2), and the MICA-treated rats consumed a 5LL2 diet containing 0.33% of MICA. All rats had ad libitum access to food and water. Food consumption and body weight were monitored. The average dose of MICA derived from 0.33% diet supplement was equivalent to 200 mg/kg per rat per day, a dosage adopted from a previous study [42]. Dietary feeding of MICA was followed by stroke surgery or decapitation for tissue collection. No death caused by MICA feeding was observed. For MICA injection studies, rats were injected with MICA (200 mg/kg body weight) 24 h before ischemic surgery.

2.4. Transitional middle cerebral artery occlusion (tMCAO)

For tMCAO surgery, an intraluminal filament model was adapted as previously described [46,47]. Rats were anesthetized by 1–3% isoflurane in 30% oxygen using an anesthetic vaporizer and flowmeter. The left MCA was occluded by a 4-0 monofilament suture (coated with silicon to a diameter of 0.30 – 0.33 mm) introduced via internal carotid artery. After a 60 min occlusion, the suture was withdrawn for reperfusion. Subsequently, rats were maintained in the University of North Texas Health Science Center Vivarium. For sham surgery, anesthesia and surgery were performed as described for tMCAO except that no suture was introduced.

2.5. Measurement of infarct size

Brain ischemic injury was assessed by measuring the infarct volume using 2,3,5-triphenyltetrazolium chloride (TTC) staining [46,47]. Briefly, brain slice was incubated for 30 min in a 2% solution of TTC in physiological saline at 37 °C, and then fixed in 10% formalin. The stained slice was then digitally scanned and subsequently measured for the ischemic lesion size (AlphaEaseFC) [48]. The percentage of infarction volume over total brain volume was calculated as previously described [49].

2.6. Preparation of brain mitochondria

Mitochondria isolation from whole brain was carried out using Percoll gradient centrifugation as previously reported [50] with slight modifications [45,51]. Brains were removed rapidly and homogenized in 15 ml of ice-cold mitochondrial isolation buffer containing 0.32 M sucrose, 1 mM EDTA and 10 mM Tris-HCl, pH 7.1. The homogenate was centrifuged at 1330*g* for 10 min and the supernatant was saved. The pellet was resuspended in half volume (7.5 ml) of the original isolation buffer and centrifuged again under the same conditions. The two supernatants were combined and centrifuged further at 21,200*g* for 10 min. The resulting pellet was resuspended in 12% Percoll solution prepared in mitochondrial isolation buffer followed by centrifugation at 6900*g* for 10 min. The obtained soft pellet was resuspended in 10 ml of the mitochondrial isolation buffer and centrifuged again at 6900*g* for 10 min. All of the mitochondrial pellets obtained after centrifugation were either used immediately or frozen at −80 °C until analysis. Protein concentrations were determined by Bradford assay [52].