*Neuroscience* 231 (2013) 345–352

# MITOCHONDRIAL BIOENERGETIC DEFICITS IN THE HIPPOCAMPI OF RATS WITH CHRONIC ISCHEMIA-INDUCED VASCULAR DEMENTIA

**J. DU,[a] M. MA,[b] Q. ZHAO,[b]* L. FANG,[c] J. CHANG,[b] Y. WANG,[d] R. FEI[d] AND X. SONG[e]**

[a] *Department of Vascular Surgery, China–Japan Union Hospital, No. 829 Xinmin Street, ChaoYang District, Jilin University, Changchun 130021, Jilin Province, People's Republic of China*

[b] *Department of Neurology, China–Japan Union Hospital, No.126 Xiantai Street, Erdao District, Jilin University, Changchun 130033, Jilin Province, People's Republic of China*

[c] *Department of Neurology, The Second Hospital of ChangChun, No. 1239 Xiangyun Street, LvYuan District, Changchun 130062, Jilin Province, People's Republic of China*

[d] *Department of Cell Biology, School of Basic Medical Sciences, No. 126 Xinmin Street, ChaoYang District, Jilin University, Changchun 130021, Jilin Province, People's Republic of China*

[e] *School of Public Health, No. 1163 Xinmin Street, ChaoYang District, Jilin University, Changchun 130021, Jilin Province, People's Republic of China*

**Abstract—Vascular dementia (VD), defined as a loss of memory and cognitive function resulting from vascular lesions in the brain, is the second-most-common cause of dementia in the elderly, after Alzheimer's disease. In recent years, research has focused on the pathogenesis of VD, and mitochondrial bioenergetic deficits have been suggested to contribute to VD onset. To further investigate the role of mitochondria in VD, we used a rat model of VD, which involved permanent bilateral occlusion of the common carotid arteries (with a 1-week interval between artery occlusion to avoid an abrupt reduction in cerebral blood flow) leading to chronic cerebral hypoperfusion. Prior to occlusion, male Wistar rats underwent 7 days of Morris water maze training. Only animals that could swim and passed the Morris water maze test were chosen for the study. After 5 days of Morris water maze training, mitochondria from the hippocampi of rats, which were randomly selected from animals that could complete the Morris water maze test, were isolated for functional assessment. Mitochondria isolated from the hippocampi of rats from the ischemia group had decreased pyruvate dehydrogenase protein levels, and increased oxidative stress, as manifested by increased hydrogen peroxide production. The ischemia group mitochondria also exhibited decreased respiration coupled to decreased expression and activity of the electron transport chain complex IV (cytochrome *c* oxidase). These results indicate that the mitochondrial oxidative metabolism is inhibited in the hippocampi of rats following chronic ischemia-induced VD. As the mitochondrial oxidative metabolism deficits, namely mitochondrial bioenergetic deficits directly affect the functions of neurons, it may contribute to VD onset.**


**Key words: vascular dementia, mitochondria, bioenergetic deficit.**

## INTRODUCTION

Vascular dementia (VD) involves the impairment of memory and cognitive function following cerebrovascular disease. VD is considered to be the second most common cause of cognitive impairment after Alzheimer's disease in the elderly (Lee, 2011). Data from developing countries suggest that VD accounts for ~30% of the age-adjusted dementia prevalence (Kalaria et al., 2008). The prevalence rate of VD is approximately 1–4% of people of 65 years of age in Western developed nations (McVeigh and Passmore, 2006). Recently, in mainland China, a nationwide investigation found the prevalence of VD to be approximately 0.8% of population aged 60 years and older (Dong et al., 2007), with another study revealing the total annual cost (direct, illness related, and cost arising from informal care) of dementia in developing countries being at least USD 73 billion (Kalaria et al., 2008). Since 1995, the total healthcare cost for VD patients has become the highest among all other forms of dementia (include Alzheimer's disease) (Hill et al., 2005). With the exponential increase for VD in people over the age of 65 years, VD is becoming an increasingly significant public health problem in the 21st century (Moretti et al., 2007).

In recent years, research has focused on the pathogenesis of VD. Although there are two main theories that have been proposed to explain the pathogenesis (1) hypoperfusion and (2) cerebral microembolism, the pathogenesis of VD remains unclear currently (Román, 2002). Therefore, treatments are based on symptoms and are unable to reverse the process. Current findings suggest a potential causal role of mitochondrial dysfunction in Alzheimer's disease pathogenesis and in Parkinson's disease pathogenesis

*Corresponding author. Tel: +86-139-44835259; fax: +86-431-84641026.
E-mail addresses: djs3043@126.com (J. Du), mamingcc2009@163.com (M. Ma), 1292860277@qq.com (Q. Zhao), stargazer8554@163.com (L. Fang), jlspspc@126.com (J. Chang), 584659752@qq.com.cn (Y. Wang), fayrui64@yahoo.com.cn (R. Fei), songxiangfu2008@yahoo.cn (X. Song).

*Abbreviations:* ANOVA, analysis of variance; CoA, coenzyme A; COX, cytochrome *c* oxidase; EDTA, ethylenediaminetetraacetic acid; ETC, electron transport chain; $H_2O_2$, hydrogen peroxide; HRP, horse-radish peroxidase; NADH, reduced nicotinamide adenine dinucleotide; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase; ROS, reactive oxygen species; TCA, tricarboxylic acid; VD, vascular dementia.

0306-4522/12 $36.00 
http://dx.doi.org/10.1016/j.neuroscience.2012.11.062

(Sas et al., 2007; Dodson and Guo, 2007). Some studies have revealed that there were decreases in hippocampal mitochondrial cytochrome *c* oxidase (COX) activity and gene expression of VD models (Zhao et al., 2011). However, there is little information about the changes in mitochondrial protein levels and the relationship between mitochondrial dysfunction and VD pathogenesis. Mitochondria, organelles in eukaryotic cells, are the site of oxidative phosphorylation (OXPHOS). These double-membrane organelles provide a highly efficient route for eukaryotic cells to generate ATP from energy-rich molecules (Chan, 2006). In addition, mitochondria help maintain cellular $Ca^{2+}$ homeostasis, regulate apoptotic cell death via the mitochondrial permeability transition pore, and produce reactive oxygen species (ROS) as a byproduct of OXPHOS (Chan, 2006; Trushina and McMurray, 2007; Jing et al., 2011). Under normal conditions, pyruvate (derived from glucose catabolism) and other sources of energy that contain fatty acids are ultimately metabolized to acetyl coenzyme A (CoA), which enters the tricarboxylic acid cycle (TCA-cycle). The reducing equivalents that are generated from TCA-cycle activity are used by the mitochondrial electron transport chain (ETC)/respiratory chain to power the synthesis of ATP. The process of mitochondrial generated energy is a multi-enzymatic process. Therefore, if any stage in the pathway is inhibited, mitochondria will be unable to utilize oxygen efficiently and the production of ROS, reactive nitrogen species including the superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals (HO), nitric oxide (NO) and peroxynitrite ($ONOO^-$) will increase, which is highly toxic to cells. In this study, we aimed to identify how mitochondrial dysfunction contributes to VD onset using a rat model of chronic ischemia-induced VD (Horecky et al., 2009).

## EXPERIMENTAL PROCEDURES

### Animals

A total of 132 male Wistar rats weighing 300–350 g, which passed the 7-day Morris water maze test training session, were used in this study. Rats were housed in a cage (six per cage) under controlled conditions of temperature (20–25 °C) and light (14 h light/10 h dark), with water and food available *ad libitum*. All procedures were performed in accordance with the institutional guidelines for animal care and use of the Jilin University. Animals were randomly assigned to different experimental groups: 2-month ischemia group ($N = 30$); 3-month ischemia group ($N = 30$); 4-month ischemia group ($N = 30$); 2-month pseudo-operation group ($N = 14$); 3-month pseudo-operation group ($N = 14$); 4-month pseudo-operation group ($N = 14$).

### Surgery

Surgery was performed as previously described (Cechetti et al., 2010). Rats were anesthetized with chloral hydrate (350 mg/kg, intraperitoneally); a neck ventral midline incision was made, and the common carotid arteries were then exposed and gently separated from the vagus nerve. In the ischemia group (2-, 3-, and 4-month; $N = 90$), the rat common carotids were occluded with a 1-week interval between interventions, the right common carotid being the first to be assessed and the left one being occluded 1 week later. In the pseudo-operation group (2-, 3-, and 4-month $N = 42$), rats received the same surgical procedures without carotid artery ligation.

### Morris water maze

Two months (2-month ischemia group + 2-month pseudo-operation group), 3 months (3-month ischemia group + 3-month pseudo-operation group), and 4 months (4-month ischemia group + 4-month pseudo-operation group) after surgery, the rats were submitted to behavioral testing for spatial learning and reference memory using the Morris water maze (Kumar et al., 2009; Han et al., 2011; Parle and Bansal, 2011). The maze was made of a circular tub (height 50 cm, diameter 130 cm) filled to a depth of 30 cm with water maintained at 26 ± 1 °C. Water was made opaque by the addition of skimmed milk.

The pool was divided into four equal quadrants: northeast (NE), northwest (NW), southeast (SE), and southwest (SW). A circular platform (diameter 11 cm), painted in white, was placed 1 cm below the surface of the water in the middle of the NE quadrant of the pool. The position of the platform was kept unaltered throughout the training session. Visual cues were placed on the walls surrounding the pool and the positions of the cues were kept unchanged throughout the experimental period. The rats were trained in the water maze in 10 sessions on 5 consecutive days, two sessions on each day. During each training session, the rats were placed in the water so that they faced the sidewall of the pool. Each session consisted of four trials. The trials were always initiated from different positions in the tank. For each rat, the quadrant in which the platform was located remained constant, but the point of immersion into the pool varied between N, E, S, and W so that the rat was not able to predict the platform location from the location at which it was placed into the pool. The rat was then given 180 s to search for the platform. Once the rat located the platform, it was permitted to remain on it for 10 s. In each training session, the latency time to escape onto the hidden platform was recorded. If the rat was unable to find the platform within 3 min, it was guided to the platform and allowed to remain on it for 10 s; the latency time was recorded as 180 s. After each trial the rats were dried, placed back into their home cages, an allowed to rest for 3–5 min before the next trial.

### Isolation of mitochondria

Mitochondria from the hippocampi were randomly isolated from rats in each group as previously described (Irwin et al., 2008; Pecinová et al., 2011). Rats were decapitated, and the whole brain, minus the cerebellum, was rapidly removed. The hippocampi were separated, minced, and homogenized at 4 °C in mitochondrial isolation buffer (320 mmol/L sucrose, 1 mmol/L EDTA, 10 mmol/L Tris–HCl, and Protease Inhibitor Cocktail Set I (pH 7.4, Calbiochem, Darmstadt, Germany). The hippocampal homogenates were centrifuged at 1500*g* for 5 min. The supernatant was centrifuged for 10 min at 21,000*g*, and the mitochondrial pellet was resuspended in mitochondrial isolation buffer. Mitochondria were washed twice, centrifuged at 9000*g* for 10 min, and resuspended with mitochondrial isolation buffer. Mitochondrial protein concentrations were quantified using the BCA protein assay kit (Beyotime, Shanghai, China). The resulting mitochondrial samples in which each mitochondrial sample corresponds to one rat were used immediately for respiratory measurements and $H_2O_2$ production or stored at −70 °C for later protein and enzymatic assays.