

Lipid Peroxidation in Chronic Cerebral Hypoperfusion-Induced Neurodegeneration in Rats

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ABSTRACT

Introduction: Reduced cerebral blood flow is associated with neurodegenerative disorders and dementia, in particular. Experimental evidence has demonstrated the initiating role of chronic cerebral hypoperfusion in neuronal damage to the hippocampus, the cerebral cortex, the white matter areas and the visual system. Permanent, bilateral occlusion of the common carotid arteries of rats (two vessel occlusion - 2VO) has been introduced for the reproduction of chronic cerebral hypoperfusion as it occurs in Alzheimer's disease and human aging. Increased generation of free radicals through lipid peroxidation can damage neuronal cell membrane. Markers of lipid peroxidation have been found to be elevated in brain tissues and body fluids in neurodegenerative diseases, including Alzheimer's disease, Parkinson disease and amyotrophic lateral sclerosis. **Materials and Methods:** Malondialdehyde (MDA), final product of lipid peroxidation, was estimated by thiobarbituric acid-reactive substances (TBARS) assay kit at eight weeks after induction of 2VO in the rats and control group. **Results:** Our study revealed a highly significant ($p < 0.001$) increase in the mean MDA concentration ($12.296 \pm 1.113 \mu\text{M}$) in 2VO rats as compared to the control group ($5.286 \pm 0.363 \mu\text{M}$) rats. **Conclusion:** Therapeutic strategies to modulate lipid peroxidation early throughout the course of the disease may be promising in slowing or possibly preventing neurodegenerative disorders.

KEYWORDS: Neurodegenerative diseases, chronic brain ischaemia, lipid peroxidation, malondialdehyde.

INTRODUCTION

Neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease are characterized by progressive and irreversible loss of neurons from specific regions of the brain.¹ These disorders are not so uncommon and present a substantial medical and social problem.¹ Aging is a major risk factor for neurodegenerative disorders,² and reduced cerebral blood flow (CBF) is associated with aging and dementia.³ A chronic reduction in CBF is believed to induce the neurodegenerative processes. Chronic cerebral hypoperfusion as it occurs in human aging and Alzheimer's disease can be induced by permanent, bilateral occlusion of the common carotid arteries (two vessel occlusion, 2VO) of rats.⁴ Experimental evidence has shown that chronic cerebral hypoperfusion can initiate the neural damage to the hippocampus, the cerebral cortex, the white matter areas and the visual system.^{5,6,7,8}

However, the CBF pattern in 2VO rats is not perfectly identical to that as it occurs in aging or demented

humans. Following induction of occlusion in 2VO rats, the CBF drops sharply and then starts normalizing after about three months due to compensatory and adaptive mechanisms. However, there is a relatively long period of time (eight weeks to 12 weeks), the chronic oligemic phase, during which the cerebral hypoperfusion in 2VO rats is comparable to that in humans.⁹

Cerebral hypoperfusion-induced neuronal cell death can be visualized with hematoxylin-eosin dye. With conventional staining procedures no obvious loss of neurons was seen during the first week after 2VO induction.¹⁰ At two weeks, six to 29% of the animals exhibited hippocampal injury in the CA1 area.¹¹ At four weeks, this had increased to 55%¹⁰, while at eight to 13 weeks, total hippocampal damage was seen in 67% of the 2VO rats.¹² This demonstrates that the neurodegeneration in the hippocampus gradually increased with time and that the most extensive neurodegeneration takes place during the chronic phase (eight to 13 weeks) of 2VO-induced cerebral hypoperfusion.

Oxidative stress is widely recognized nowadays as an important feature of many neurological disorders because brain is highly susceptible to lipid peroxidation due to its high oxygen utilization, low levels of antioxidants, and high levels of polyunsaturated fatty acids (the substrate for lipid peroxidation). Peroxidation of membrane lipids can result in numerous effects, including increased membrane rigidity, decreased activity of membrane bound enzymes, altered activity of membrane receptors and altered permeability.^{13,14} There are suggestions that oxidative injury plays a role in Alzheimer's disease, Parkinson's disease, multiple scler-

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rosis, schizophrenia, mitochondrial encephalopathies and brain injury due to ischemia and reperfusion.¹⁵ However, not much is known about the possible role of lipid peroxidation-induced oxidative stress in the pathogenesis of chronic cerebral hypoperfusion -induced neurodegeneration in 2VO rats.

The present study was, therefore, designed to evaluate the lipid peroxidation status during chronic phase of cerebral hypoperfusion in 2VO rats by estimating the malondialdehyde (MDA) concentrations in the brain tissue.

MATERIALS AND METHODS

The present study was conducted on Wistar rats of either sex, weighing 200-250 g. The rats were obtained from Universiti Sains Malaysia and were kept in standard plastic cages. They were maintained on ad libitum food and water and were treated in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institute of the Health. Every effort was made to minimize suffering to the rats.

After one week of acclimatization, rats were randomly divided into two groups. Group A (n=7) rats served as a control and they were not subjected to 2VO. Group B (n=7) rats were subjected to permanent, bilateral common carotid arteries occlusion. They were anesthetized with intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). After making a small midline cervical incision, both common carotid arteries were gently isolated and separated from the carotid sheath and vagal nerve. They were then permanently ligated with sterilized silk suture and the incision was stitched and the wound treated with betadine solution. The body temperature was maintained throughout the surgical procedure and recovery period by heat lamps.

Group A and Group B (after 8th week of 2VO) rats were sacrificed by euthanization with ether. The brain was quickly dissected out and rinsed with phosphate buffer saline (pH 7.4) to remove any red blood cells and clots. It was weighed and sonicated with 1 ml/100mg of cold RIPA buffer containing 1 mM EDTA. Samples were centrifuged at 1600 revolutions per minute for 10 min at 4°C for thiobarbituric acid-reactive substances (TBARS). Supernatant was collected and stored at -80°C.

Determination of Lipid peroxidation

TBARS measure the concentration of MDA, an end product of the oxidation and decomposition of polyunsaturated fatty acids containing three or more double bonds. Although this method has been criticized for its lack of specificity, sensitivity, and reproducibility, it is still one of the easiest and most frequently used methods.

MDA levels were estimated by TBARS Assay Kit (Cayman Chemicals, USA) strictly according to the instructions laid down in the catalogue. The absorbance was read at 530-540 nm using a multi detection microplate reader Infinite M200. The average absorbance of each standard and sample was calculated. The absorbance value of the standard was subtracted from its value and from both standard and sample values of absorbance. The corrected absorbance values of each standard were plotted as a function of MDA concentration. The values of MDA for each sample were calculated from the standard curve.

$$\text{MDA } (\mu\text{M}) = \{\text{Corrected absorbance} - y \text{ intercept}\} / \text{slope}$$

Statistical Analysis

The data were analyzed using Predictive Analytics Software (PASW version 18.0) and are presented here as mean \pm SEM values. Comparison between the groups was done by independent t-test (Gaussian). Differences were designated as significant when $p < 0.05$ at 95% confidence interval.

RESULTS

The mean MDA level in the 2VO group ($12.296 \pm 1.113 \mu\text{M}$) was found to be highly significant ($p < 0.001$) as compared to the control group ($5.286 \pm 0.363 \mu\text{M}$) as shown in Fig 1.

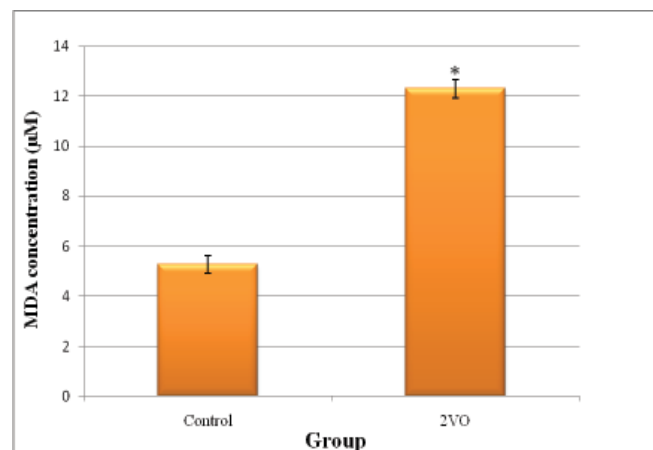


Figure 1. Mean MDA concentrations in the 2VO and control groups.* $p < 0.001$

DISCUSSION AND CONCLUSION

Oxidative stress has long been linked to the neuronal cell death that is associated with neurodegenerative disorders. Numerous studies have reported increased lipid peroxidation in the brain of patients with Alzheimer's disease compared with age-matched controls.^{16,17,18} As lipid peroxidation can be measured, not only in brain tissue but also in body fluids, it might be used as a useful marker of disease progression and as a monitor of therapeutic efficacy.

death in chronic cerebral hypoperfusion begins with neuronal ATP depletion due to reduced CBF and consequent hypoxia and hypoglycemia. In ischemic brain injury or stroke, the loss of ATP is followed by generation of reactive oxygen species (ROS) lethal to neurons at high concentration. The ROS in turn initiate lipid peroxidation; generating lipid peroxides that are degraded to reactive aldehyde products such as MDA.¹⁹ However, increased lipid peroxidation and a decreased capacity of the antioxidant systems have primarily been associated with reperfusion after ischemia, which is very gradual in permanent, chronic 2VO due to cerebral blood flow compensation.²⁰

The concentration of MDA, indicative of lipid peroxidation, was significantly increased ($p < 0.001$) after eight weeks of 2VO in our study, as is typically seen in ischemic brain injury. Similarly, increase in MDA level was reported by Aytac et al. after 10 days of 2VO in rats.²¹ However, Institoris et al. could not find any significant change in the levels of superoxide dismutase suggesting that the degree of oxidative stress is not remarkable in this model or other antioxidant mechanisms are induced against oxidative agents.²²

Our preliminary data require confirmation, but it is currently considered that 2VO produces a permanent ischemic/oligemic condition sufficient enough to initiate and maintain oxidative stress, which could be the reason for the persistent and progressive neuronal damage.

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