The Three-Vessel Occlusion as a Model of Vascular Dementia – Oxidative Stress and Mitochondrial Failure as an Indicator of Brain Hypoperfusion

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Abstract

Brain energy disorders and oxidative stress due to chronic hypoperfusion are considered to be major risk factors in the pathogenesis of dementia. The aim of our study was to evaluate changes of brain creatine kinase (BB-CK) reaction and mitochondrial respiratory chain function in male Wistar rats exposed to chronic cerebral hypoperfusion. Three-vessel occlusion (3-VO) was accomplished without thoracotomy using a minimally invasive surgical approach for the occlusion of the brachiocephalic trunk and the left common carotid artery (CCA). The forward rate constant of creatine kinase (kfor) was measured in vivo by saturation transfer of ^{31}P magnetic resonance spectroscopy (MRS) at 2 and 10 weeks of permanent 3-VO. The function of the mitochondrial respiratory chain in vitro was assessed polarographically at 10 weeks after 3-VO. As compared to the controls, the significant 42 % reduction of kfor at 2 respiration. 10 weeks indicated disorders in brain energy metabolism, which is in agreement with the 12 % decrease of the oxidative phosphorylation coefficient (ADP:O) and with the 14 % decrease of the oxidative phosphorylation rate (OPR) measured in isolated mitochondria obtained from the hippocampal tissue. Oxidative modification of the creatine kinase system (inactivation of enzymes) and metabolic disorders due to chronic 3-VO, thus, may participate in vascular cognitive impairment and neuronal degeneration.

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Keywords

^{31}P NMR • Brain 3-VO hypoperfusion • Creatine kinase • Mitochondria • Oxidative phosphorylation

Introduction

Various rodent models have been proposed for studying the pathophysiology of vascular dementia, including Alzheimer's disease (AD). Three-vessel occlusion (3-VO), i.e., both common carotid and one vertebral artery, was accepted as a reliable model (de la Torre and Fortin 1994; de la Torre 2002). To avoid surgical stress, we attempted to use a new surgical approach for 3-VO without thoracotomy and without artificial ventilation. The surgical procedure and the results of our investigations are reported in this study. We assume that chronic cerebrovascular insufficiency results in less oxygen and glucose availability to the CNS which reduces mitochondrial oxidative phosphorylation and ATP production. Cytosolic brain-type creatine kinase, BB-CK which is coexpressed with ubiquitous mitochondrial creatine kinase (uMtCK), is significantly inactivated by oxidation in Alzheimer's disease (AD) patients (Aliev et al. 2003). Creatine kinase has been shown to play fundamental role in cellular energetics of the brain, disturbance of this enzyme may impair the AD process. If the brain CK reaction is inhibited in the model of AD and the ATP turnover correlates with the flux through the CK reaction, then we should be able to monitor cerebral effect of the simulated AD in rats by ^{31}P-MRS saturation transfer experiments in vivo.

The technique of phosphorus magnetic resonance spectroscopy (MRS) is suitable for studying energy metabolism in living tissues including the brain. Furthermore, the magnetization transfer using the ^{31}P-MRS technique allows for in vivo measurements of material fluxes caused by key enzymes of the energy metabolism (Kašparová et al. 2005a, b). ATP can be replenished with PCr via the creatine kinase (CK) reaction (89.1). Creatine is phosphorylated by CK to produce phosphocreatine (PCr):

$$\mathrm{MgATP^{2-} + Cr} \quad \rightleftarrows \quad \mathrm{PCr^{2-} + MgADP^{-} + H^{+}} \tag{89.1}$$

In this work we studied kinetics of the above reaction in aged rat brains under severe chronic hypoperfusion conditions. With respect to reduced cerebral blood flow, it should be noted that prolonged brain ischemia could produce the amyloid-β peptide precursor (AβPP) found at increased levels in the hippocampus of AD brains and which has been implicated in formation of senile plaques (de la Torre 2004).

The aim of this study was to demonstrate that the pseudo first-order rate constant kfor of the CK forward reaction is a sensitive indicator of changes in the *early state* of dementia rat models (Aliev et al. 2004; Horecký et al. 2009; Vančová et al. 2010; Horecky et al. 2011; Aliev et al. 2011; Palacios et al. 2011). Mutations in amyloid precursor protein (AβPP) are associated with early onset AD and result in abnormal processing of AβPP and accumulation of the Aβ peptide, the main constituent of

amyloid plaques in AD brain. Recent data on a direct interaction between AβPP and the precursor of uMtCK support an emerging relationship between AD, cellular energy levels, and mitochondrial function (Aliev et al. 2004).

Therefore, after ^{31}P-MRS measurements, the rat brains were subjected to biochemical analysis focused on mitochondrial oxidative phosphorylation parameters because common features of VD and AD include the marked decrease of cerebral glucose metabolism caused by disturbed acetyl-CoA synthesis and critically lowered oxidative phosphorylation. AD is an age and brain ischemia-related, progressive neurodegenerative disorders characterized by loss of memory, low energy metabolism, oxidative stress, and impairment of multiple cognitive functions. Mitochondrial dysfunction in the brain of neurodegenerative disorders associated with aging may contribute to the pathogenesis of AD.

Materials

Aged (16 months old) male Wistar rats supplied by Velaz (Czech Republic) were housed at 22 ± 2 °C, 45 % relative humidity, 12 h light/dark photoperiodicity in air-conditioned rooms with free access to standard commercial laboratory pellets ST1 (Top Dovo, Slovak Republic) and water *ad libitum*. Prior to the operation, the animals were fasted overnight with free access to water. All animals received human care in compliance with Institutional Animal Ethic Committee and with the Guidelines of European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes.

Methods

Minimally Invasive Surgery

The rats were anesthetized by i.p. injection of ketamine (50 mg/kg b.w.) and xylazine (4 mg/kg b.w.), placed in a supine position on the operating table, and left to respire (breathe) spontaneously. The steps of the procedure are as follows:

1. Ventromedial neck skin and manubrium incision
2. Occlusion of brachiocephalic trunk (BCT) to eliminate blood flow through the right vertebral artery (VA) and through the right common carotid artery (CCA)
3. Occlusion of the left common carotid artery
4. Reconstruction of the manubrium and skin with 3–0 silk sutures.

In Vivo ^{31}P MRS

Phosphorus saturation transfer experiments were performed on a 4.7 T 200 MHz SISCO imaging spectrometer, equipped with a horizontal magnet, at 81 MHz.

A tree-turn 1.6 cm diameter surface coil was positioned over the skull of an animal anesthetized by 0.8–1.0 % of halothane. First, the static magnetic field was shimmed using proton signal, which showed typical line width of 20–35 Hz. Then, the phosphorus flip angle was adjusted to minimize the broad signal coming from the bone. The time of irradiation of γ-ATP resonance was varied from 0.3 to 1.6 s which resulted in an exponential decay of PCr signal to a steady-state value. The saturation was accomplished by an on-resonance series of 10 μs DANTE pulses with interpulse delays of 400 μs (Morris and Freeman 2011; Clark et al. 1991). Either 96 or 128 transients were accumulated in the interleave mode with the repetition time of 5 s. The dependence of the longitudinal magnetization of the PCr signal, $Mt(\mathrm{PCr})$, on the time t of the γ − ATP signal saturation is given by the equation (Forsen and Hoffman 1963; Bittl et al. 1987):

$$Mt(\mathrm{PCr}) = M0(\mathrm{PCr})\{1 - kfor \text{ x } T1\mathrm{sat}(\mathrm{PCr})\ [1 - \exp(-\mathrm{t}/T1\mathrm{sat}\ (\mathrm{PCr}))]\} \tag{89.2}$$

where $M0(\mathrm{PCr})$ is the magnetization of PCr in the absence of γ-ATP, *kfor* is the forward creatine kinase reaction rate constant, T1sat(PCr) is the apparent longitudinal relaxation time in the presence of γ-ATP saturation, and t is the irradiation time. The $T1\mathrm{sat}(\mathrm{PCr})$ value was calculated as a slope of the semi-logarithmic regression of $Mt(\mathrm{PCr}) - \mathrm{M}\infty\ (\mathrm{PCr})$ against t where $M\infty\ (\mathrm{PCr})$ is the steady-state magnetization of PCr after a long-term irradiation of the γ-ATP signal. The pseudo first-rate constant *kfor* was calculated according to the equation:

$$kfor = [1 - M\infty(\mathrm{PCr})/M0(\mathrm{PCr}]/T1\mathrm{sat}(\mathrm{PCr}) \tag{89.3}$$

Instead of the magnetization values, corresponding PCr signal intensities were used in these calculations. $M\infty(\mathrm{PCr})$ was read from the spectrum with 10 s irradiation of the γ-ATP resonance, and $M0\ (\mathrm{PCr})$ was obtained from the reference spectrum measured with the irradiation offset in the mirror position relative to the PCr resonance and with the irradiation time of 1 s. As a check of validity of the results, the $T1(\mathrm{PCr})$ values were calculated using the following equation:

$$T1\ (\mathrm{PCr}) = T1\mathrm{sat}(\mathrm{PCr})[M0(\mathrm{PCr})/M\infty(\mathrm{PCr})]. \tag{89.4}$$

In Vitro Mitochondrial Respiration

The rats were euthanized with i.p. injection of thiopental (Spofa, Czech Republic, 150 mg/kg b.w.). The brain tissue was subsequently removed and placed in an ice-cold isolation solution containing (in mmol L^{-1}) 225 mannitol, 75 sucrose, and 0.2 EDTA (pH 7.4). The tissue sample was minced and homogenized in the isolation solution using a glass–teflon homogenizer. Brain mitochondria were isolated

at 4 °C by differential centrifugation (Sarma et al. 1976). Mitochondrial protein concentration was estimated by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Respiratory chain function was measured in a respiratory buffer containing (in mmol L^{-1}) 100 HEPES, 5 KH2PO4, 120 KCl, 0.5 EDTA, and 2 % dextran (ph 7.2 at 30 °C), by means of Oxygraph Gilson 5/6H (USA) using a Clark-type polarographic oxygen electrode. Sodium glutamate (5 mmol) was used as a NAD substrate for complex I. To initiate state 3 respiratory activity, 500 nmol of ADP was added to the cuvette. When all the ADP was converted to ATP, state 4 respirations were measured. Parameters of oxidative phosphorylation RCR [S3/S4] – respiratory control ratio, ADP:O – coefficient of oxidative phosphorylation, and OPR [nmoL ATP mg prot min^{-1}] (oxidative phosphorylation rate) were determined.

Statistics

The results were evaluated using ANOVA and Student's *t*-test for unpaired data; $p < 0.05$ was considered as statically significant.

Results

Using a new minimally invasive surgical approach for brachiocephalic trunk and left common carotid artery occlusion (Fig. 89.1), the time of surgery decreased from 45 to 7 min and perioperative survival increased from 95 % to 100 %, as compared to de la Torre and Fortin's (de la Torre and Fortin 1994) approach for intrathoracic occlusion of the left subclavian artery (Fig. 89.2).

^{31}P Magnetic Resonance Spectroscopy

Comparing with control aged group, the significant 42 % reduction in the forward rate constant of creatine kinase (k*for*) 10 weeks after 3-VO was found (Graph 89.1). These findings indicated disorders in brain energy metabolism.

Oxidative Phosphorylation

In agreement with ^{31}P magnetic resonance spectroscopy, the rate of ATP production in complex I showed a significant decrease in brain mitochondria 10 weeks after 3-VO. There was a significant decrease in the mitochondrial oxidative phosphorylation rate (OPR) by 14 %. 3-VO induced a significant decrease of brain mitochondrial energy production due to acute oxidative stress. There was also a significant decrease in coefficient of oxidative phosphorylation ADP:O by 12 % and in the respiration control index (RCI) by 6 % (Graph 89.1).

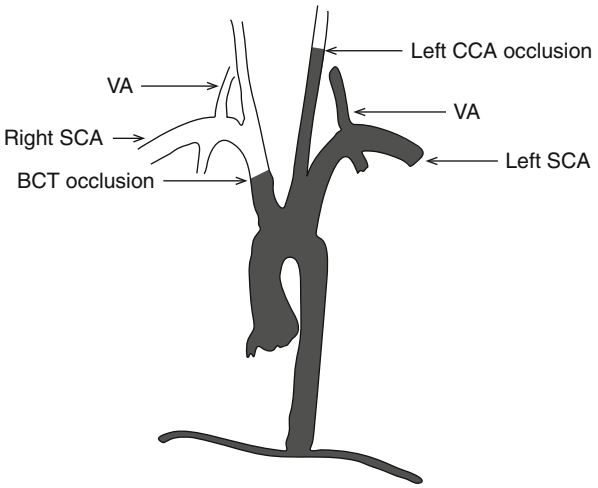


Fig. 89.1 New minimally invasive transmanubrial approach for 3-VO. Extrathoracic occlusion of brachiocephalic trunk (BCT) and left common carotid artery (CCA). VA, vertebral artery; SCA, subclavian artery (Reprinted with permission of *Journal of the Neurological Sciences*: Horecký, J., Baciak, L., Kašparová, S., Pacheco, G., Aliev, G. and Vancová, O. Minimally invasive surgical approach for three-vessel occlusion as a model of vascular dementia in the rat-brain bioenergetics assay. J Neurol Sci. 2009;283(1–2):178–181. doi: 10.1016/j.jns.2009.02.348. Epub 2009 Mar 9)

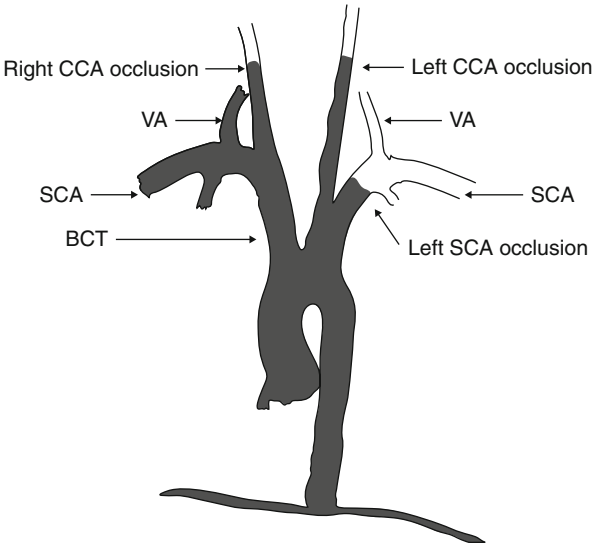
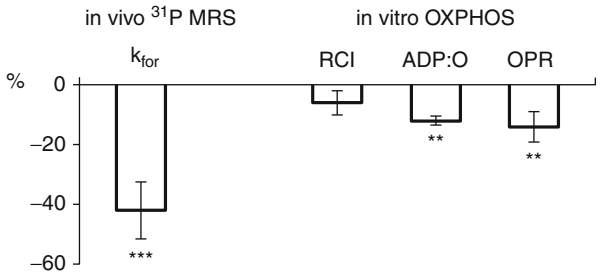


Fig. 89.2 Original de la Torre and Fortin model of 3-VO. Intrathoracic occlusion of left subclavian artery (SCA) and extrathoracic occlusion of both common carotid arteries (CCA). *VA* vertebral artery, *BCT* brachiocephalic trunk (Reprinted with permission *Journal of the Neurological Sciences*: Horecký, J., Baciak, L., Kašparová, S., Pacheco, G., Aliev, G. and Vancová, O. Minimally invasive surgical approach for three-vessel occlusion as a model of vascular dementia in the rat-brain bioenergetics assay. J Neurol Sci. 2009;283(1–2):178–181. doi: 10.1016/j.jns.2009.02.348. Epub 2009 Mar 9)



Graph 89.1 Percentual changes of brain bioenergetics measured in vivo by ^{31}P MRS and in vitro by mitochondrial OXPHOS before (0) and 10 weeks after 3-VO in aged rats. *MRS* magnetic resonance spectroscopy, *kfor* forward rate constant of creatine kinase, *OXPHOS* oxidative phosphorylation, *RCI* respiration control index, *ADP:O* coefficient of oxidative phosphorylation, *OPR* oxidative phosphorylation rate, $^{***}p < 0.001$, $^{**}p < 0.01$ (Reprinted with permission *Journal of the Neurological Sciences*: Horecký, J., Baciak, L., Kašparová, S., Pacheco, G., Aliev, G. and Vancová, O. Minimally invasive surgical approach for three-vessel occlusion as a model of vascular dementia in the rat-brain bioenergetics assay. J Neurol Sci. 2009;283(1–2):178–181. doi: 10.1016/j.jns.2009.02.348. Epub 2009 Mar 9)

Discussion

The original de la Torre and Fortin rodent model of three-vessel occlusion (de la Torre and Fortin 1994) is characterized by the intrathoracic occlusion of left subclavian artery to eliminate blood flow through the left vertebral artery. However, such a surgical procedure is time consuming (45 min) and stresses the animal (endotracheal intubation, artificial ventilation, thoracotomy), resulting in 5 % perioperative mortality. Therefore, we developed a novel, minimally invasive, transmanubrial approach for the occlusion of the brachiocephalic trunk to eliminate blood flow through the right vertebral artery and parallel right common carotid artery. In comparison with the de la Torre and Fortin method of 3-VO, our minimally invasive transmanubrial osteomuscular sparing surgical approach without thoracotomy reduced the time of surgery from 45 to 7 min and increased perioperative survival from 95 % to 100 %. The efficiency of our model was also verified in our previous studies on chronic or transient cerebral hypoperfusion (Kašparová et al. 2005a, b; Horecký et al. 2005, 2006). Brain energy disorders and oxidative stress due to chronic hypoperfusion are considered to be major risk factors in the pathogenesis of dementia (Zhu et al. 2004a, b).

One of the most important hallmarks in the pathogenesis of senile or vascular dementia is the marked decrease of cerebral glucose metabolism (Schubert 2005) caused by disturbed acetyl-CoA synthesis and critically lowered oxidative phosphorylation (Meier-Ruge et al. 1994). Our 3-VO model proved that it is very sensitive to oxidative modification of CK system (inactivation of enzymes) or oxidative phosphorylation in neuronal cells that

cause cognitive impairment (Horecký et al. 2009). Thus, we suppose that the above rat model of vascular dementia can be very useful for various preventive and therapeutic strategies in vivo (Vančová et al. 2010; Horecky et al. 2011; Aliev et al. 2008, 2011; Palacios et al. 2011).

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Conflict of Interest
Authors declared no conflict of interest.

Disclosure
Part of information included in this chapter has been previously published (Horecký, J., Baciak, L., Kašparová, S., Pacheco, G., Aliev, G. and Vancová, O. Minimally invasive surgical approach for three-vessel occlusion as a model of vascular dementia in the rat-brain bioenergetics assay. J Neurol Sci. 2009; 283(1–2):178–181. doi: 10.1016/j.jns.2009.02.348. Epub 2009 Mar 9). Reprinted with permission *Journal of the Neurological Sciences*: Horecký, J., Baciak, L., Kašparová, S., Pacheco, G., Aliev, G. and Vancová, O. Minimally invasive surgical approach for three-vessel occlusion as a model of vascular dementia in the rat-brain. This article represents an update of current knowledge regarding the implication of oxidative stress and mitochondrial failure as an indicator of brain hypoperfusion in the context of the three-vessel occlusion as a model for the vascular dementia.

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