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Research Report

Pathophysiological rat model of vascular dementia: Magnetic resonance spectroscopy, microimaging and behavioral study

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ABSTRACT

Chronic cerebral hypoperfusion and aging can be related to vascular dementia manifested by the decline in cognitive abilities and memory impairment. The identification of specific biomarkers of vascular disorder in early stages is important for the development of neuroprotective agents. In the present study, a three-vessel occlusion (3-VO) rat model of vascular dementia in the middle-aged rat brain was used to investigate the effect of global cerebral hypoperfusion. A multimodal study was performed using magnetic resonance spectroscopy, MR-microimaging, histology and behavioral tests. Our measurements showed a signal alteration in T2-weighted MR images, the elevation of T2 relaxation times and histologically proven neural cell death in the hippocampal area, as well as mild changes in concentration of proton and phosphorus metabolites. These changes were accompanied by mild behavioral alterations in the open field and slightly decreased habituation. The analysis of the effects of vascular pathology on cognitive functions and neurodegeneration can contribute to the development of new treatment strategies for early stages of neurodegeneration.

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1. Introduction

Vascular dementia (VaD) is a degenerative illness caused by a variety of vascular lesions resulting in restricting blood supply to different brain regions. It is the second most-common cause of dementia after Alzheimer's disease (AD), both referring to a progressive decline in cognitive abilities and memory impairment (Nagata et al., 2007; Querfurth and LaFerla, 2010). Persistent reduction in cerebral blood flow induces hypoxia/ischemia of the brain tissue, deprivation of oxygen and nutrients and can lead to cell death (Bennett et al., 2009). Global cerebral hypoperfusion compromises memory processes and together with advancing age can contribute to neurodegeneration (De la Torre, 2005). Furthermore, cerebrovascular lesions and infarctions appear to play a major role in the pathogenesis and clinical expression of non-genetic sporadic AD (De la Torre, 2005; Querfurth and LaFerla, 2010; Román and Kalaria, 2006). Decreased cerebral blood flow is probably associated with AD (Bürklen et al., 2006; de la Torre, 2005; Farkas et al., 2007) and the degree of cerebral hypoperfusion has been suggested as a predictive marker for progression of AD (Borroni et al., 2006). Hypoxia/ischemia was found to affect mostly hippocampal, striatal and cortical areas of the brain (Block, 1999) and degenerated neurons in the CA1 layer of hippocampus were found in many models of chronic cerebral hypoperfusion (Bennett et al., 2009; Ferreira et al., 2011; Liu et al., 2006; Torre and Fortin, 1994; Yang et al., 2011). In humans, vascular dementia is often unrecognized or misdiagnosed at its early stages and its recognition might offer substantial benefits and could support the development of neuroprotective strategies.

A number of various rodent models mimicking cerebrovascular diseases (e.g. VaD) have been introduced. Post-stroke models (Kimura et al., 2000) and occlusion models (Ferreira et al., 2011; Kitamura et al., 2011; Plaschke et al., 1999) and also impaired myelinization model (Assaf et al., 2003) are used for reconstruction of the status of VaD. These models are useful for studying the inherent development and underlying mechanisms of pathology, reflect important aspects of the clinical manifestation of the disease and are crucial for the evaluation of therapeutic efficacy of new disease-modifying agents. Induction of chronic hypoperfused condition by permanent ligation or occlusion of cerebral vessels is used as an accepted approach for the simulation of a VaD condition. In previous studies we have developed and used the three-vessel occlusion (3-VO) model, based on the occlusion of both common carotids and one vertebral artery (Horecký et al., 2009).

Magnetic resonance (MR) provides the unique information on both alterations of the brain with regard to metabolite concentration and morphologic changes under physiological and pathological conditions. MR is capable to monitor the progression of the disease and consequences of therapeutic treatment. Due to its multimodal capability, MR has become the most versatile method for the diagnosis of a variety of disorders, including dementias. Recent advances in MR technology with high magnetic field strengths, specialized RF coils and the fast-switching of strong gradients allowed an improvement in spatial resolution from microimaging (μMRI) with pixel size of about 100–300 μm e.g. for DWI (J. Zhang et al., 2002) to MR-microscopy (pixel size < 100 μm) scale for T_2- and diffusivity mapping *ex vivo* on microscopy inserts to human scanners (Berg et al., 2003). On special animal high field (B = 14.1 T) scanners the animal rat brain could be investigated at pixel size of 33 μm in SWI imaging (e.g. Marques et al., 2009). MR-microscopy permits to distinguish different structures at microscopic level and to assess neuronal damage (Pirttilä et al., 2001). The quantitative assessment of pathology by MR can be accomplished by measurement of tissue MR relaxation times (T_1, T_2). There are contradictory results regarding alterations of T_2 times in AD/VaD cases; several authors reported increase of T_2 times due to higher water content in the brain tissue (Dawe et al., 2014; Kirsch et al., 1992; Laakso et al., 1996; Wang et al., 2004), some of them found no significant alterations of relaxation times (House et al., 2006) and some authors reported also a decrease of T_2 times due to iron accumulation (Laakso et al., 1996). Prolongation of T_2 times in white matter was found to be associated with memory impairment (Dawe et al., 2014). Moreover, in recent years, research based on μMRI has been focused on the detection of amyloid plaques, the dominant pathologic feature of dementia (Benveniste et al., 1999; Braakman et al., 2006; Dhenain et al., 2002; Vanhoutte et al., 2005; Wadghiri et al., 2003; Wengenack et al., 2008).Using in vivo localized ^{1}H magnetic resonance spectroscopy (MRS), several metabolite concentrations in the brain tissues can be detected – the most important in cerebral metabolism being N-acetyl-aspartate (NAA), choline-containing compounds (Cho), creatine (Cr), phosphocreatine (PCr), taurine (Tau), myo-Inositol (mIns), glutamine/glutamate (Gln/Glu), γ-aminobutyric acid (GABA), glutathione (GSH) and lactate (Lac) (Michaelis et al., 2009). Additionally, ^{31}P MRS permits the monitoring of biochemical processes in the brain, including high-energy phosphate metabolism and the assessment of intracellular pH in vivo by measuring the concentration of ATP, PCr and inorganic phosphate (Pi). It is also possible to derive dynamic metabolite parameters as the forward rate constant of creatine kinase (Kašparová et al., 2005).

In this work, we investigate the mid-term effect (4 weeks) of an animal 3-vessel occlusion (3-VO) pathology applied to middle-aged rats (11-months old) by means of in vivo phosphorus ^{31}P, proton ^{1}H MR-spectroscopy and behavioral testing. The analysis was completed by *ex vivo* μMRI, and histology. By using ^{31}P and ^{1}H MRS we aimed to assess cerebral neurochemical profile. In addition, we examined a possible correlation between pathologic findings in the rat brain and behavioral tests on the 3-VO rats in early states of brain neurodegeneration. Pathology related changes in MRS and MRI-data may be useful as specific early diagnostic markers. These findings could improve patient management, safety and treatment efficacy.

2. Results

2.1. MRS

An evaluation of relative concentrations of the metabolites from proton spectra showed significant decrease in the concentration of myo-Inositol. An illustrative proton spectrum with results of the analysis is shown in Fig. 1a and the

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