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Research report

Neurochemical changes in unilateral cerebral hemisphere during the subacute stage of focal cerebral ischemia-reperfusion in rats: An ex vivo ^{1}H magnetic resonance spectroscopy study

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ABSTRACT

Understanding the subacute may shed light on the mechanism of cerebral ischemia. The present study aimed to explore metabolic features underlying subacute stage of ischemia-reperfusion injury and developing effective treatments. Rats were divided into three groups: the permanent middle cerebral artery occlusion (pMCAO), transient cerebral focal ischemia (tMCAO) and sham group. Evaluation of animal models was performed by the neurological deficit, MR images and pathological morphological abnormality. To elucidate metabolic changes, we conducted a comparative analysis of metabolic composition of unilateral brain tissue using ^{1}H nuclear magnetic resonance spectroscopy. The successful model was observed low signal on T1WI and high signal on T2WI lesions in the left cerebral. Histopathological results confirmed the formation of apparent lesions in the left striatum, hippocampus CA1 and cortex tissues of subacute cerebral ischemia rats and showed that rats with focal cerebral ischemia-reperfusion could alleviate the extent of pathological damage degree. In pMCAO rats 7 days after surgery, decreased levels of N-acetyl aspartate (NAA), γ-aminobutyric acid (GABA), glutamate (Glu) and succinate (Suc) concomitantly with increased levels of glutamine (Gln), myo-inositol (m-Ins) and lactate (Lac) were observed compared to the control. Whereas, increased level of Lac with decreased levels of NAA, GABA, Glu, Suc, creatine (Cre) were observed in the tMCAO rats. This demonstrated that experimental subacute ischemic stroke in rats caused extensive perturbation in energy metabolism, the tricarboxylic acid cycle and GABA shunt, which provided essential information for understanding the pathogenesis of subacute cerebral ischemia-reperfusion and provided guidance in choosing the suitable therapeutic schedule.

1. Introduction

Stroke, as a serious cerebrovascular disease, is a leading cause of long-term disability and death seriously affecting the life quality of over 16 million people every year in the world (Strong et al., 2007). Approximately 80% of all strokes are ischemic type events in both developed and developing countries (Palm et al., 2010). Thus, stroke causes a considerable burden on both the concerned families and community. For example, more than 40 billion U.S. dollars were lost due to stroke in 2007 (Roger et al., 2012). Therefore, investigation of pathogenesis underlying ischemic stroke is of great importance to advance the development of its treatment. The mechanism for ischemic injury is complex and mainly includes neuronal excitotoxicity, energy metabolism disequilibrium, oxidative stress, inflammation and cell apoptosis (Chan, 2001; Liesz et al., 2009). In addition, ischemic stroke has been also associated with metabolic disorder. Previous in vivo studies showed that cerebral ischemia led to metabolic alterations in lactate (Lac), creatine (Cre), N-acetyl aspartate (NAA) and choline (Cho) by using magnetic resonance spectroscopy (MRS) (Graham et al., 1992; van der Toorn et al., 1996). Most literature focus on metabolites for the pathological mechanism of acute phase, such as lactate, which is related to anaerobic metabolism onset and NAA, which is related to neuron viability (Juranek and Bačiak, 2009). Yan, Dai et al. showed that increases in lactate, glutamate (Glu), taurine (Tau) and decreases in NAA were already detectable at 1 h post-stroke in a permanent middle cerebral artery occlusion (pMCAO) rat model (Yan et al., 2015). However, the studies of metabolic changes in unilateral cerebral hemisphere during the subacute stage of focal cerebral ischemia-reperfusion are rarely reported.

Metabolomics attempts to develop new low-molecular-weight metabolites in biological samples using advanced analytical

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techniques and has been applied to explore metabolic mechanisms underlying diseases and determine the effectiveness, safety and doses of therapeutic interventions (Yuan et al., 2012; Zhang et al., 2012). Nuclear magnetic resonance (NMR) has been used as an attractive tool in metabolomics research due to its advantages, such as simple sample preparation, rapid analysis and non-destructive analysis (Wang et al., 2014a). High-resolution nuclear magnetic resonance (NMR) based metabolomic has proved to be very useful for studying ischemic stroke injury (Jiang et al., 2011; Jung et al., 2011). Metabolic disturbance serves as a potential mechanism of ischemia, including elevation of acetate and decreases of Glu and aspartate (Asp) in the ischemic tissue 6 h after occlusion (Håberg et al., 2009; Nonaka et al., 1998). Wang et al. observed 13 metabolites changed significantly in the serum of ischemic stroke injury, malonic acid and glycine (Gly) are the most noticeable variable metabolites (Wang et al., 2014c).

As a transition from acute to chronic, the subacute condition according to the temporal classification by Pitkonen et al. (2012), might clarify the mechanism of the development of cerebral ischemic and might provide a new target for clinical diagnosis and treatment. Unfortunately, less attention has been paid to metabolism and related pathogenic processes during the subacute ischemic period.

In this work, a focal cerebral ischemia rat model was established with the suture-occluded method to imitate human ischemic stroke. Reperfusion was accomplished by softly pulling out the filament, 2 h later. Then we analyzed metabolic changes in the brain of rats with a 7-day ischemic injury following cerebral ischemia-reperfusion using an NMR-based metabolomic approach. The aim of this study is to explore metabolic features underlying ischemia-reperfusion injury and thereby further understand its potential metabolic mechanisms.

2. Results

2.1. Evaluation of animal models

All experimental rats had no behavioral abnormalities before surgery with Longa method score was 0 point. Twenty-four hours after surgery, the rats showed apathetic, sluggish, piloerection, irritability, and did not fully stretch the right forepaw. The neurological damage was evaluated by the magnetic resonance (Fig. 1). In the sham operation rats, there was no abnormal signal in both T1WI and T2WI. MR study in pMCAO rats demonstrated hypointense signal on T1WI and obvious hyperintense signal lesions on T2WI in the left cerebral cortex and basal ganglia. Low signal on T1WI and high signal on T2WI were only observed in the basal ganglia of tMCAO rats.

2.2. Histopathological examination of brain tissues

Microscopic examination showed the representative HE-stained sections of brain from sham, pMCAO and tMCAO group (Fig. 2). The cortex, hippocampus CA1 and striatum regions of the left cerebral hemisphere from sham rats showed normal histology and arranged in neat rows without inflammatory reaction. Whereas, rats with experimental stroke showed histological abnormalities including sparse organization structures, interstitial edema, pyknotic nuclei, cell vacuolation degeneration, capillary proliferation etc. In the left cortex and striatum region of pMCAO rats, HE staining results showed more serious inflammatory cell infiltrate, nerve cell necrosis as well as vascular and gliocyte proliferation compared with tMCAO rats. More seriously, liquefactive necrosis was found with most neurons and glial cells disappeared in the pMCAO striatum. It is also clear that the pyramidal cells of hippocampus CA1 loss, thinning of the cell layer, nucleus shallow, loose matrix and vacuolization were observed in tMCAO rats, but less seriously than that in pMCAO rats. These histopathological results confirmed the formation of apparent lesions and functional changes in the left striatum, hippocampus CA1 and cortex tissues of MCAO rats and it found that rats with focal cerebral ischemia-reperfusion could alleviate the extent of pathological damage degree.

2.3. ^{1}H NMR spectra and PLS-DA analysis

Representative ^{1}H NMR spectra of the left cerebrum extracts obtained from the tMCAO group(A), pMCAO group (B) and sham group(C) 7 days after the operation, are shown in Fig. 3A–C.

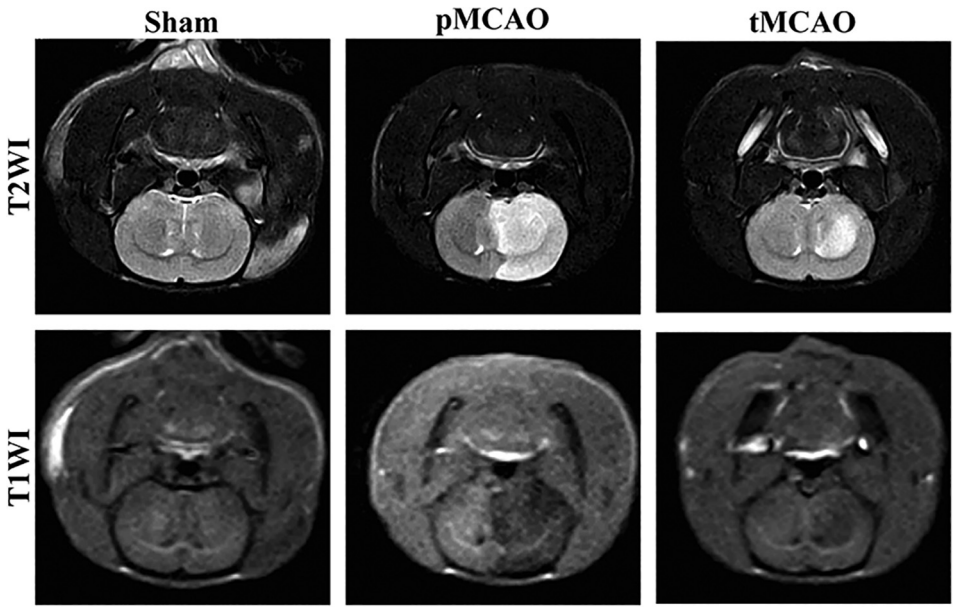

Fig. 1. Representative magnetic resonance (MR) images following surgery on 24 h. The sham operated rats have no abnormal signal; the pMCAO rats observed low signal on T1WI and high signal on T2WI lesions in the left cortex and basal ganglia; the lesions of tMCAO rats only detected in the basal ganglia demonstrating high signal on T2WI, low signal on T1WI.