MR evaluation of cerebral oxygen metabolism and blood flow in stroke-like episodes of MELAS

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A R T I C L E I N F O

Article history:
Received 23 June 2012
Received in revised form 5 September 2012
Accepted 10 September 2012
Available online 11 October 2012

Keywords:
Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
Magnetic resonance imaging
Oxygen extraction fraction
Cerebral blood flow

A B S T R A C T

Metabolic information is essential in the investigation of the pathophysiology of stroke-like episodes in patients with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). Here, we used magnetic resonance imaging to evaluate the dynamic metabolic changes before and after a stroke-like episode in two patients with MELAS caused by the mitochondrial DNA mutation A3243G. We performed functional magnetic resonance imaging, including arterial spin labeling and oxygen extraction fraction imaging, and generated cerebral blood flow and oxygen extraction fraction maps. We recruited eight healthy volunteers to define the normal range of the oxygen extraction fraction. We detected a heterogeneous reduction in the oxygen extraction fraction in the brain in the interictal period as well as at the onset of a stroke-like attack. However, the oxygen extraction fraction in the stroke-like lesions normalized in the acute stage. The stroke-like lesions showed consistent hyperperfusion in the acute phase but hypoperfusion in the chronic phase. We have demonstrated the utility of using new magnetic resonance imaging techniques in the evaluation of the pathophysiology of stroke-like lesions. The increased utilization of oxygen in an acute lesion is a novel finding in our study, which might play a role in the oxidative stress.

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1. Introduction

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is a maternally inherited encephalomyopathy, characterized by seizures, headaches, lactic acidosis, vomiting, and recurrent stroke-like episodes [1]. It is mainly associated with mitochondrial DNA mutations, mostly A3243G, which cause a failure of mitochondrial protein synthesis resulting in impaired ATP production. In contrast to the rapid progress in understanding the molecular pathophysiology of MELAS, the precise pathogenesis of the stroke-like episodes remains controversial. Possible mechanisms include angiopathy leading to ischemic change [2], energy failure caused by defects in the oxidative metabolic pathways of energy production [3] and [4], or increased energy demand because of neuronal hyperexcitability [4].

To clarify the mechanisms of the stroke-like episodes in MELAS, investigators have assessed various pathophysiological parameters of the cerebral lesions in vivo by neuroimaging. There have been many studies concerning changes in cerebral blood flow in patients with MELAS, which have revealed hyperperfusion in the acute stage (within 1 month) and hypoperfusion in the chronic stage (several months later) [4–9,10]. There have been a few studies of the metabolic condition in the stroke-like episodes, including oxygen uptake, glucose metabolism, and oxidative stress [10–12]. Using single-photon emission computed tomography or PET, increased glucose metabolism [10–11], decreased cerebral oxygen metabolism [11–12], and mild increased oxidative stress have been observed in the acute stage of stroke-like lesions [10]. A drastic decrease in cerebral oxygen metabolism and cerebral glucose uptake was found globally in the brains of interictal MELAS patients [13–15].

Most of these studies were cross-sectional, but serial studies could provide more valuable information for the longitudinal evaluation of the pathophysiology of stroke-like lesions. However, PET is not ideal for serial studies because of its high cost and necessary radiation exposure. With the recent development of new MRI techniques, patients can be followed-up frequently and noninvasively. Recently, Tsujikawa et al. used continuous arterial spin labeling (ASL) to assess cerebral blood flow in MELAS patients [16]. Oxygen extraction fraction (OEF) imaging is a newly developed technique that enables quantitative assessment of the OEF in brain tissue using MRI. This technique has been tested in
volunteers and was able to show a decrease in OEF caused by hypercapnia [17]. It has also been used to detect misery perfusion, which is characterized by elevated oxygenation in the ischemic region [18]. It is possible that OEF imaging could be used to assess oxygenation in the stroke-like episodes of MELAS patients.

The purpose of our study was to assess the dynamic metabolic changes during the development of stroke-like lesions in patients with MELAS. Metabolic information was obtained by functional MRI, including measurements of the ADC, CBF, and OEF.

2. Subjects and methods

2.1. Subjects

2.1.1. Case 1

A 14-year-old girl developed normally until the age of 11 years when she had an acute episode of headache, vomiting, blurred vision, and left hemiparesis. Cranial MRI showed high T2 signal in the right parieto–occipito-temporal lobes. She was diagnosed as having viral encephalitis and given corticosteroids. Six months later, she had more headaches that were followed by hemiparesis and myoclonus in her right arm. Plasma lactate was raised to 5.2 mmol/L. She was diagnosed as having MELAS during this second episode by a genetic test that confirmed an A3243G mutation in her blood. Although she improved slightly after treatment with B vitamins, coenzyme Q10, L-carnitine, and L-arginine, she had three more episodes at the ages of 13 and 14 years. The patient has been followed-up since her fourth episode, and she experienced a fifth episode during this period. During her fourth episode, she suffered dysarthria and right hemiparesis which resolved after treatment with L-arginine and L-carnitine. About 50 days after the fourth episode, she experienced a fifth episode, when she developed simple partial status epilepticus involving the eyes, face and neck. There was no apparent impairment of language, sensory or movement function during her fifth episode. She underwent MRI four times: on the 30th day before the fifth episode, and on the 2nd (44 h after the onset of simple partial status epilepticus), 10th days, and 3 months after the onset of the fifth episode.

2.1.2. Case 2

A 12-year-old girl was apparently normal until the age of 8, when she noticed headaches as well as focal motor seizures affecting her left arm. A right occipital high-signal area was seen on T2-weighted MRI. Although oxcarbazepine was administrated, seizures were still present during sleep. When she was ten years old, she suffered a second episode with fever, headache, and mild weakness in her left limb. Cranial CT revealed hypodense in right parieto-occipital lobe. She recovered from this attack. However, three months later, she had hemiparesis in her right arm. MRI showed a left occipito–parieto-temporal lesion. Her symptoms resolved after this third stroke-like episode with treatment of anticonvulsants and coenzyme Q10. Mitochondrial DNA analysis detected mtDNA A3243G mutation in her white blood cells. She had a fourth episode at the age of 12, when she suddenly had headache and focal motor seizures affecting her left arm and leg. Different from her previous attacks, repetitive headaches and focal seizures lasted three months in her fourth episode. Multiple lesions involving the right occipital, parietal and temporal lobes were identified on DWI by MRI. This patient underwent three MRI examinations in our hospital: on the day 2 (34 h after the onset of headache and focal seizures), day 20 and 2 months after the onset of the fourth episode.

Eight healthy volunteers, age-matched to the patients, were recruited to form a control group. Informed written consent was obtained from the volunteers, and the patient’s parents. All the protocols in this study were approved by the ethics committee of Peking University First Hospital.

2.2. MRI examinations

MRI studies were performed using a 3.0T MRI scanner (GE Healthcare, Milwaukee, WI) and an eight-channel head coil. Initially, routine clinical pulse sequences were used, including axial T1-weighted FLAIR, axial T2-weighted FLAIR, and DWI sequences. Then, ASL and GESSE sequences were performed to obtain the hemodynamic information. ASL can be used to measure CBF by using intravascular water as the endogenous contrast agent. The parameters for ASL were: TR = 800 ms, TE = 22.8 ms, EC = 250 kHz, matrix = 128×96, NEX = 1, slice thickness = 6.0 mm, and space between the slices = 1.5 mm. GESSE is a multi-echo gradient and spin echo MRI sequence [18]. The parameters for GESSE were: TR = 1.5 s, TE = 56 ms, EC = 62.5 kHz, matrix 128×128, FOV = 240×240 mm, slice thickness = 7.5 mm, NEX = 4, and scanning time = 12 min 55 s. Thirty-two echoes with an echo spacing of 1.5 ms were acquired and 32 images were obtained as raw data for calculation of the OEF. Only one axial slice just above the corpus callosum was acquired in this study so that the potential crosstalk, signal interference between two adjacent slices, and bone-gas interface artifact could be minimized.

A theoretical signal model applied using in-house software, which describes the signal dephasing phenomena in the presence of deoxyhemoglobin, was used to post-process the acquired images and obtain a quantitative measurement of the OEF [19]. After the OEF map was generated, six ROIs were placed in the anterior, middle, and posterior of the bilateral hemispheres. To minimize the effects of large inhomogeneities in the background magnetic field, which would result in geometric distortion in the spin echo images and severe signal loss in the gradient echo images, voxels with a low signal-to-noise ratio that resulted from artifacts were excluded automatically. To better match the ROI and lesion region, the location and size of some ROIs were adjusted by a radiologist. The size of the ROIs ranged from 180 to 250 mm². The OEF values of normal controls were obtained from eight healthy volunteers. The 95% confidence level of the controls’ OEF was defined as the normal OEF range. CBF maps were also generated using in-house software. The perfusion status of the lesion was determined by an experienced radiologist.

3. Results

The normal range of OEF measured in the controls was 0.276–0.373. For Case 1, the first MRI examination was performed on day 20 after the fourth episode, which was also 30 days before the fifth episode. The left cingulate cortex was affected in the fourth episode, which could be seen in the T2-weighted image (Fig. 1A). A decreased OEF was detected in the right hemisphere and the left frontal lobe (Fig. 1E). The CBF map was unremarkable except for a small region of hyperperfusion in the left frontal lobe (Fig. 1D). On day 2 after the onset of the fifth episode, T2-weighted images showed new lesions in the bilateral frontal lobes (Fig. 1F); meanwhile, a marked reduction in OEF could still be seen in these areas (Fig. 1J). CBF increased drastically in these regions (Fig. 1I). The swelling of the lesion resolved on day 10 after the onset of the episode (Fig. 1K), and OEF increased to normal levels (Fig. 1O). Remarkable hyperperfusion in CBF remained in the right lesion, while it decreased to some extent in the left lesion (Fig. 1N). Three months later, the perfusion in the right frontal lobe had reversed to hypoperfusion (Fig. 1S) and a re-duction in the OEF was again detected in some brain regions (Fig. 1T). The OEF values in the brain in the selected slices are presented in Table 1.

For Case 2, MR images were obtained on day 2 after the onset of fourth episode. A new hyperintense lesion involving the right parietal lobe was observed on FLAIR and DWI images (Fig. 2B, C). Hyperperfusion in the right parietal lobe on the CBF map (Fig. 2D) and extensive reduction on the OEF map (Fig. 2E) could be seen at this time-point. Twenty days after the onset, the lesion still showed hyperperfusion on the CBF map (Fig. 2I) while the OEF of the parietal lobe slightly increased (Fig. 2J). The high signal of the lesion became faint on DWI images (Fig. 2H).
Two months after the episode, although the high signal on DWI almost disappeared (Fig. 2M), the CBF maintained increased in the lesion region on CBF maps (Fig. 2N). The OEF map showed a mosaic pattern since there was extensive OEF reduction in the left parietal lobe while the OEF in the lesion region increased to normal (Fig. 2O). The OEF values of the brain in the selected slice are presented in Table 2.

Fig. 3 shows the changes in OEF values in the new lesions in the two patients throughout their episodes. The trend towards an increase in OEF after the acute stage can be seen clearly on this scatter plot.

ADC values remained normal in both cases, except for a slightly decreased ADC in the new lesion in Case 1, which returned to normal a week later.

4. Discussion

Among all the MELAS patients we studied, for the two patients presented here we were able to provide full imaging data throughout the whole clinical course of a stroke-like episode. This enabled us to gain insight into the pathophysiological conditions associated with lesion development.

Many studies have shown almost consistent results regarding the perfusion status around the time of a stroke: i.e., hyperperfusion in the acute stage followed by hypoperfusion in the subacute and chronic stages [9,11,16–21]. Our study revealed similar lesion-associated chronological changes in CBF. The perfusion change was mostly a passive response, caused by acidosis in the early stage and down-regulated metabolic needs in the late stage. It is noteworthy that, the acute stage may be prolonged with focal hyperperfusion in the lesion persisting for a long time, just as in Case 2. The persistence of focal hyperperfusion in the lesion was consistent with the long-lasting manifestation of symptoms in this episode. After the early stage, CBF was markedly decreased in Case 1. The decrease in CBF in chronic lesions may result from a low metabolic level. This may be due to substantial neural apoptosis in lesions after an episode, which would contribute to the hyporemia after the acute stage [16].

As far as we know, this is the first study reporting OEF changes during a stroke-like episode. One month before the episode, Case 1 exhibited decreased OEF in particular brain regions. Lindroos et al. performed a PET study in 14 patients with mitochondrial 3243 mutations, which showed the cerebral metabolic rate for oxygen was decreased by 26% in the gray matter as well as in the white matter. Decreased OEF was present globally in these patients, suggesting defective mitochondrial respiratory chain function irrespective of central nervous system symptoms [15]. In our study, the two cases both showed extensive OEF reduction in some brain regions in the acute stage, including the lesion

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Table 1

<table>
<thead>
<tr>
<th></th>
<th>Day 30 before the fifth episode</th>
<th>Day 2 after the onset</th>
<th>Day 10 after the onset</th>
<th>3 months after the onset</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left frontal (lesion)</td>
<td>0.267</td>
<td>0.339</td>
<td>0.302</td>
<td>0.277</td>
<td>0.315±0.024</td>
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<tr>
<td>Left parietal</td>
<td>0.336</td>
<td>0.317</td>
<td>0.336</td>
<td>0.291</td>
<td>0.329±0.036</td>
</tr>
<tr>
<td>Right frontal (lesion)</td>
<td>0.257</td>
<td>0.265</td>
<td>0.301</td>
<td>0.293</td>
<td>0.319±0.027</td>
</tr>
<tr>
<td>Right parietal</td>
<td>0.272</td>
<td>0.297</td>
<td>0.289</td>
<td>0.322</td>
<td>0.321±0.022</td>
</tr>
</tbody>
</table>

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Fig. 1. Serial magnetic resonance images of a 14-year-old girl with MELAS. The images were taken 30 days before (A–E) and on day 2 (F–J), day 10 (K–O), and 3 months (P–T) after the onset of the fifth episode.
The manifestation of OEF reduction in the acute stage reliably indicated the pathophysiological conditions in the two patients in our study. However, in our study, the decrease in OEF showed heterogeneity to some extent. For example, in the left frontal lobe of Case 1, there was a small zone with a higher OEF than that of the area around it (Fig. 1E, arrow), reflecting a heterogeneous degree of defective oxidative phosphorylation in the brain. Interestingly, the zone with a higher OEF before the episode was spared in the subsequent attack (Fig. 1F, arrow). Another example was the increased OEF in the left cingulum during the interictal period in Case 1 (Fig. 1E). Based on the T2W image (Fig. 1A), we predicted that the lesion in the left cingulum might be in the subacute stage. So the heterogeneity of OEF in the brains of the patients came from the various pathophysiological conditions. Our results indicated that OEF imaging by MRI had better resolution than PET, and it may be more useful in the monitoring of metabolic changes in the brain tissue.

Another important finding in our study was that the OEF increased rather than decreased as time passed by after the episode onset, reaching to the level of normal controls (See Fig. 3). The increase of OEF in the lesion could have two explanations. One explanation was that some unknown mechanism upgraded the oxygen metabolism and made a more efficient utilization of oxygen. Another possibility was that only neurons with lower ratio of mitochondrial mutations survived the episode and revealed a higher OEF. Clearly, increased oxygen use in defective mitochondria is a double-edged sword for these cells, because increased ATP production is accompanied by increased generation of reactive oxygen species. Because CBF and glucose metabolism also increased in the acute stage and markedly enhanced oxidative stress in the subacute stage [9], we speculate that increased oxygen use in the mitochondria after the acute episode might exacerbate oxidative stress in MELAS.

The mechanism that initiates an episode in MELAS patients remains an enigma. The persistent presence of decreased OEF during the interictal period indicates that oxidative phosphorylation can normally meet the demands of neural survival. It is considered that events such as trauma, fever, or seizures will cause stroke-like episodes by increasing the energy demand. It is likely that the degree of OEF reduction is not the crucial factor for the generation of a stroke-like episode, because the brain regions with the lowest OEF in the two cases did not develop lesions as we expected. Therefore, the triggering factor for a stroke-like lesion is still to be elucidated.

Our study provided a useful method to trace metabolic changes throughout a stroke-like episode. Although PET is the optimal technique to evaluate metabolism, it is not suitable for longitudinal studies because of its high cost and necessary exposure to radiation. ASL without

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**Table 2**

|                | Day 2 after the onset | Day 20 after the onset | 2 months after the onset | Control  
|----------------|-----------------------|------------------------|--------------------------|---------
| Left parietal  | 0.258                 | 0.225                  | 0.173                    | 0.329 ± 0.036 |
| Right frontal  | 0.224                 | 0.254                  | 0.297                    | 0.319 ± 0.027 |
| Right parietal (lesion) | 0.258          | 0.292                  | 0.314                    | 0.321 ± 0.022 |
contrast media is more appropriate to provide hemodynamic information for serial studies [16]. OEF imaging offers a promising and convenient way to observe oxygen metabolism in vivo. We successfully applied this technique in the evaluation of MELAS to demonstrate abnormalities in oxygen metabolism. One limitation is that unexpected paramagnetic sources, like microbleeding, could cause artifacts. Moreover, the accuracy of this technique should be tested and compared to that of PET.

In conclusion, using magnetic resonance OEF imaging, we showed that the OEF in the brain was decreased in the interictal period and in the acute stage in patients with MELAS, but increased after the onset of stroke-like attack until the subacute stage. An increase in oxygen use, CBF inflow, and glucose intake may exacerbate the oxidative stress and enhance cytopathy in the brain tissue. The increase of CBF in the acute stage is a sensitive indicator of a stroke-like lesion. The precise factors that trigger an episode remain to be elucidated. However, using new MRI techniques, it is now possible to obtain brain metabolic information throughout a stroke-like episode; this will be of great value in dissecting the pathophysiology of metabolic abnormalities.

5. Funding

This work was supported in part by the National Natural Science Foundation of China [No. 30870864 to Z Wang and No. 81201154 to Xie Sheng].

Conflict of interest

None.

References