Real-time imaging of infarction deterioration after ischemic stroke in rats using electrical impedance tomography

Lu Cao1, Haoting Li1, Danchen Fu, Xuechao Liu, Hang Ma, Canhua Xu, Xiuzhen Dong, Bin Yang2 and Feng Fu2

Department of Biomedical Engineering, Air Force Medical University (Fourth Military Medical University), Xi’an 710032, People’s Republic of China

1 Lu Cao and Haoting Li contributed equally to this work.
2 Author to whom any correspondence should be addressed.

E-mail: binyang@fmmu.edu.cn or fengfu@fmmu.edu.cn

Keywords: electrical impedance tomography, ischemic stroke, rat model

Supplementary material for this article is available online

Abstract

Objective: This study investigated the feasibility of electrical impedance tomography (EIT) for monitoring the deterioration of ischemic lesion after the onset of stroke. Approach: Fifteen rats were randomly distributed into two groups: rats operated to establish a right middle cerebral artery occlusion (MCAO) (n = 10), and sham-operated rats (n = 5). Then, the operated rats were kept 2 h under anesthesia for EIT monitoring. Subsequently, descriptive statistical analysis was performed on whole-brain resistivity changes, and repeated-measures analysis of variance (ANOVA) on the average resistivity variation index. Additionally, pathological examinations were performed after 6 h of infarction. Main results: The results obtained showed that ischemic damage developed in the right corpus striatum of the rats with MCAO, whereas the brains of the sham group showed no anomalies. The descriptive statistical analysis revealed that the whole-brain resistivity changes after 30, 60, 90, and 120 min of infarction were 0.063 ± 0.038, 0.097 ± 0.046, 0.141 ± 0.062, and 0.204 ± 0.092 for the rats with MCAO and 0.029 ± 0.021, 0.002 ± 0.002, 0.017 ± 0.011, and −0.001 ± 0.011 for the sham-operated rats, respectively. The repeated-measures ANOVA revealed that the right MCAO model resulted in a significant impedance increase in the right hemisphere, which continued to increase over time after infarction. Significance: The overall study results indicate that EIT facilitates monitoring of local impedance variations caused by MCAO and may be a solution for real-time monitoring of intracranial pathological changes in ischemic stroke patients.

1. Introduction

Ischemic stroke is a serious cerebrovascular disease originated by an occluded blood vessel in the neck or brain (Randolph S A 2016) that accounts for 87% of all stroke cases (Bamford et al 1991). The occlusion may be caused by thrombus, embolus, or severe stenosis (Randolph S A 2016). Given its high incidence, ischemic stroke is the leading cause of death in China, with 2 million new cases each year and 25% of survivors suffering from different degrees of disability (Zhou et al 2016). Digital subtraction angiography is a common examination for patients who have suffered from acute stroke syndrome that provides clinicians with detailed anatomic information. Consequently, it is the current gold standard (Caldwell et al 2017). Further, other imaging modalities—such as computed tomography, magnetic resonance imaging, and diffusion weighted imaging—are also recommended by stroke teams (Caldwell et al 2017). It is obvious that for patients who already present with an acute stroke syndrome these imaging methods are enough for the clinician to make a diagnosis and continuous real-time monitoring will be unnecessary. Conversely, for patients who have no symptoms but with high risk of ischemic stroke, such as unconscious patients being transported in ambulance or patients who may suffer from recurrence of cerebral vascular occlusion or stenosis after thrombolysis treatment, real-time monitoring would be a useful complement to the traditional imaging methods because it can provide stroke physicians with early detection of
intracranial pathological changes, based on which they can perform further inspection or administer protective medicine to improve the prognosis of the patient. However, none of the above methods is suitable for continuous real-time monitoring because they either require the use of radiation and contrast agent or costly and bulky equipment.

Electrical impedance tomography (EIT) provides a safe, cost-efficient, portable, and continuous real-time imaging solution, in which electrical stimulation is applied to electrodes on the body surface, and the impedance distribution or variation inside the human body is reconstructed using an inverse problem algorithm (Adler and Boyle 2017). Owing to its high temporal resolution, nonradiative property and sensitivity to the conductivity changes of biological tissues, EIT has been successfully applied to monitor mechanically ventilated patients (Prérichs et al 2017). Recently, cerebral EIT has become another research hotspot after lung EIT. Several studies have shown that pathological and normal brain tissues have markedly different impedance characteristics ($\Omega_{\text{blood}} < \Omega_{\text{brain}} < \Omega_{\text{ischemia}}$, where $\Omega$ represents impedance) (Yang et al 2017, Cao et al 2019). Thus, EIT is promising for monitoring the deterioration of ischemic lesion and providing early stroke warning.

Extensive research has been conducted on the application of EIT for monitoring stroke in animals. In regards to hemorrhagic stroke, Dai et al and Xu et al successfully monitored subarachnoid hemorrhage and intracranial hemorrhage in piglet models using 16 cortical electrodes (Dai et al 2010, Xu et al 2010). Manwaring et al obtained the impedance changes resulting from cerebral hemorrhage in piglets by adding an intraparenchymal sensor electrode to an existing eight-electrode configuration (Manwaring et al 2013). Further, a research team at Loughborough University used an eight-electrode EIT system to monitor the cerebral impedance variation in vitro from skinless ovine models. They simulated intracranial hematomas by injecting gel samples with the same conductivity as blood into different locations and found that EIT can be used to accurately localize the simulated hematomas (Ayati et al 2015). These studies demonstrate the feasibility of using EIT to monitor hemorrhagic stroke.

There are fewer studies on the application of EIT monitoring of ischemic stroke in animals, mainly because the ischemic stroke model is more sophisticated, and many models are not suitable for synchronous EIT data collection. For instance, the craniotomy–electrocoagulation method can damage the skull, preventing the placement of the EIT electrodes, and the microsphere-induced microembolization method is sophisticated and results in slow lesion development (Walberer and Rueger 2015). Thus, early EIT studies mainly verified its feasibility for monitoring cerebral ischemia by using global instead of local ischemia models. For example, Holder simulated global cerebral ischemia in rats by permanent diathermy of vertebral arteries and reversible occlusion of the common carotid arteries, and performed EIT using cortical and scalp electrodes, respectively. He observed an impedance increase of 50%–200% using the cortical electrodes and a 10% increase using the scalp electrodes. He also observed an impedance drop after rectifying occlusion of the common carotid arteries (Holder 1992). More recently, Yang et al applied EIT for monitoring local infarction through a rabbit model. In this model, focal cerebral infarction was obtained by injecting a photosensitizer through the ear vein and directing a cold light into a hole drilled into the skull. They performed EIT using a ring of 16 cortical electrodes and found that EIT can detect impedance changes after 10 min of lighting and localize the infarction area after 20 min of lighting, confirming that EIT is sensitive to impedance variation in the cortex resulting from infarction (Yang et al 2014). However, ischemic strokes are mainly located in middle cerebral arteries (MCAs). Thus, the feasibility of EIT for detecting deep focal infarction warrants further research.

Among experimental ischemic stroke models, the intraluminal suture MCA occlusion (MCAO) in rats and mice is the most frequently used model, as it is less invasive and easy to implement both permanent and transient ischemia in a controlled manner (Durukan A and Tatlisumak T 2007). It involves inserting a filament into the internal carotid artery and advancing until it blocks the initial segment of the MCA. This model provides reproducible MCA territory infarctions (involving both the frontoparietal cortex and lateral caudoputamen) (Durukan A and Tatlisumak T 2007), allows reperfusion by retracting the suture and has been widely adopted to study ischemic stroke (Fluri et al 2015). Dowrick et al were the first to introduce the MCAO model into the EIT field and performed 3D EIT monitoring using 40 spring-loaded cranial electrodes on seven experimental rats (Dowrick et al 2016). Their results showed markedly increased voltage measurements but severe artifacts in the reconstructed images, thus failing to identify the infarcted cores in all cases. Therefore, there is no valuable EIT monitoring image of MCAO. By comparing the abovementioned experimental animal studies on cerebral EIT, it can be concluded that this failure may be caused by the shielding effect of the skull (Dowrick et al 2016). Moreover, to achieve 3D imaging, Dowrick et al applied 40 spring-loaded electrodes with limited contact area, which increases the contact impedance and instability and reduces the upper limit of the safety current, posing a challenge for reconstruction. Thus, in this study, EIT monitoring of the MCAO in rats was conducted using fewer cortical electrodes. Further, baseline data were collected after onset of MCAO and measurement was not intermittent but continuous, which are all different from previous studies and highlights that this study aimed to confirm the feasibility of EIT for monitoring the deterioration of the ischemic lesion rather than the blood fluctuations caused by filament insertion. In addition, the sham group was defined as parallel control and repeated-
measures ANOVA was adopted to analyze the time effect. EIT data were thoroughly evaluated from two aspects: voltage measurement and reconstructed images. The study may provide a reliable measurement setup and analysis method for EIT monitoring of MCAO.

2. Materials and methods

2.1. Animal preparation
This study was approved by the Animal Research Ethics Committee of the Air Force Medical University and conducted following its guidelines on animal experiments. Fifteen healthy male Sprague Dawley rats (240–270 g) were obtained from the animal experiment center. The rats were randomly distributed into two groups: 10 in the right MCAO group (rats 1–10) and five in the sham-operated group (rats 11–15). The rats were anesthetized using Isoflurane (induction 5% and maintenance 2%). A temperature sensor was placed inside the rectum for body temperature monitoring which was controlled at 37°C ± 0.5°C using an electrical heating pad (Beijing Sinotech, Beijing, People’s Republic of China) (Crupi et al 2018). The hair on the top of the head was shaved, and the skin and periosteum were removed using a scalpel. An elliptical window of 2.5 cm in the coronal direction (width) and 3 cm in the sagittal direction (length) was formed for placing eight sterilized copper electrodes of 0.93 mm in diameter (dental nails, Hangzhou Westlake Biomaterial, Zhejiang Province, People’s Republic of China). The electrodes were nailed into the skull uniformly along the edge of the elliptical window (figure 1) but did not penetrate through the endocranium, with the depth being controlled below 1 mm.

2.2. MCAO model
The MCAO model was established using the Koizumi method (Morris et al 2016). After conventional skin preparation and sterilization, an incision of 2–3 cm in length was made in the middle of the neck. The right common carotid artery (CCA) was then exposed through blunt dissection. The internal and external carotid arteries (ICA, ECA) were then separated along the forward direction of the CCA. The proximal ends of the CCA and ECA were then ligatured. The ICA was occluded using an artery clip. A thread was knotted and attached to the proximal end of the clip without tightening. A micro-cut was made 1 mm under the bifurcation of the CCA. A filament with the head coated with silicon of 0.34 ± 0.02 mm in diameter (A5-243420, Beijing Sinotech) was inserted into the ICA through the cut. With the filament through the thread, the thread was tightened to ensure no bleeding from the cut and allowing the filament to pass through. The artery clip was then removed and the filament was introduced along the ICA up to 18.5 ± 0.5 mm (Fluri et al 2015, Crupi et al 2018). After the start of the MCA was occluded, the thread was tightened to fix the filament. Finally, the neck skin was sutured, and the rat was ready for subsequent EIT measurements. Figure 2 illustrates the MCAO operation. The rats in the sham group were operated in the same manner, excluding the filament insertion.

2.3. EIT monitoring
Data were collected using our in-house FMMU-EIT5 system, which can operate within a frequency range of 1–190 kHz and output currents in the range 10 μA–1.25 mA. A previous study using the system on a physical human head phantom demonstrated its ability to detect small disturbances of 0.35% in volume (1.99% in the cross-sectional area) and 17% in resistivity (Shi et al 2018). Data acquisition speed is up to 3 fps, satisfying the requirements for cerebral EIT monitoring. Copper electrodes were connected to the FMMU-EIT5 system using leads with a hooked frontal end. A sinusoid current of 250 μA and 50 kHz was applied between pairs of opposite electrodes; the resulting potential differences were measured by the remaining adjacent electrode pairs at a rate of 1 fps.
Data collection in this study can be divided into two steps. (1) After placement of the electrodes, data were collected for 10 min to observe the cerebral impedance variations, aiming to ensure stable electrode contact and no intracranial hemorrhage (rats with apparent intracranial hemorrhage would be excluded from experiment, but no such case occurred). (2) After the MCAO operation, data were collected for 2 h to obtain the cerebral impedance variations in rats of the MCAO and sham groups.

Image reconstruction was performed using a circular finite-element mesh and the damped least-squares algorithm, which can be expressed as equation (1) (Xu et al 2011):

\[
\Delta\rho = (J^T J + \lambda R)^{-1} J^T \Delta V
\]  

(1)

where \(\Delta\rho\) is the impedance change vector between two different time points, \(J\) is the Jacobian matrix (Yorkey et al 1987), \(\lambda\) is a regularization parameter (here \(\lambda = 0.1\)), \(R\) is a regularization matrix (using the Standard Tikhonov method, \(R = \text{diag}(J^T J)\)) and \(\Delta V\) is the boundary voltage change vector.

2.4. Data analysis

To thoroughly analyze the ability of EIT for detecting intracranial impedance changes resulting from the right MCAO, we conducted statistical analyses on raw voltage measurements and the reconstructed images, respectively. For voltage measurements, as the amplitude of the exciting current was fixed, whole-brain resistivity (WBR) was calculated using equation (2) to reflect the variation of voltage measurements (Dowrick et al 2016):

\[
WBR = \frac{\sum_{i=1}^{8} \sum_{j=1}^{8} V_{ij}}{T}
\]  

(2)

where \(V_{ij}\) is the measured voltage of electrode pair \(j\) under excitation of electrode pair \(i\), and \(I\) is the amplitude of the exciting current. As the contact impedance of the exciting electrodes was unknown, their measured voltage was disregarded. Considering individual differences between animals, the mean WBR of the first 20 frames after data acquisition was calculated for each animal to obtain \(WBR_{\text{reference}}\) and then determine the WBRCs for the remaining period of monitoring using equation (3):

\[
WBR_{t} = \frac{WBR_{t} - WBR_{\text{reference}}}{WBR_{\text{reference}}} \times 100\%
\]  

(3)

where \(WBR_{t}\) is the WBR at time point \(t\) and \(WBR_{t}\) is the change percentage in WBR at time point \(t\) in relation to the reference time.

Next, five given times (\(T0 = 0, T1 = 30, T2 = 60, T3 = 90\), and \(T4 = 120\) min) were considered from the 2 h monitoring session. At each given time, the average WBCROV 10 frames was calculated, and a descriptive statistical analysis was performed to comparatively analyze the WBRCs from the two groups.
For reconstructed EIT images, the right and left hemispheres were defined as two separate regions of interest because, anatomically, the unilateral MCA mainly supplies blood ipsilaterally. Then, the ARVI for both regions was computed using equation (4) (Sadleir et al. 2008):

\[
ARVI = \frac{\sum_{i=1}^{N} \Delta \rho_i}{N}
\]

where \(\Delta \rho_i\) is the reconstructed impedance change per element and \(N\) is the number of elements. The average ARVI of the two regions, \(ARVI_{\text{right}}\) and \(ARVI_{\text{left}}\), were calculated using the reconstructed images. A repeated-measures ANOVA was then applied to \(ARVI_{\text{right}}\) and \(ARVI_{\text{left}}\) with the sham group defined as parallel control of the MCAO group and \(p < 0.05\) being considered as significant. The above analyses were implemented on the SPSS20 software (IBM Corporation, Armonk, NY, USA).

2.5. Pathological validation

The animals were humanely euthanized 6 h after the operation, and their brains extracted and sliced into five 2 mm coronal sections. The sections were incubated in 2% 2,3,5-triphenyltetrazolium chloride (TTC) buffered solution for 20 min at 37 °C. After 10 min of incubation, the sections were flipped to ensure even staining on both sides. Then, samples were washed once in 1 × phosphate buffered saline solution and subsequently fixed in 4% paraformaldehyde solution at 4 °C until photographed. Additionally, we conducted hematoxylin–eosin and elastic Van Gieson (EVG) staining on two rats from each group for further microscopic examination of the micromorphology of neurons and the structure of the right MCAs. Specifically, after the animal was deeply anesthetized, a midline incision was made, the ascending aorta was cannulated, and the descending aorta was occluded. The animal was perfused serially with isotonic saline (150 ml) and 4% (250 ml) paraformaldehyde in a phosphate buffer solution (0.1 M, pH 7.2). Then, the brain was removed, fixed in 4% paraformaldehyde for 7 d, and embedded in paraffin. One brain was sectioned coronally into 4 µm slices, deparaffinized and stained with hematoxylin and eosin solution to observe the micromorphology of neurons. Another brain was sampled at the right MCA, and the sample sectioned into 4 µm slices, deparaffinized, stained with Verhoeff’s solution, and counterstained with Van Gieson’s solution to examine the structure of right MCAs (Sakaguchi et al. 1988, Chan 2014).

3. Results

EIT data were successfully collected from all 15 rats, which showed stable vital signs after operation. After recovering from anesthesia, the sham-operated rats were able to move normally, whereas the rats with MCAO exhibited different degrees of left foreleg dysfunction.

3.1. Pathological results

Figure 3 shows typical TTC results from the two groups. The healthy cerebral tissue is dark red, while the right corpus striatum and cortex in the MCAO brains are from white to light pink, indicating the presence of ischemic damage.

The microstructure of brain tissue under a light microscope with 20 × magnification is shown in figure 4. After 6 h of operation, the sham-operated rat brains were uniformly stained and most of the neurons show normal structure and clear nucleoplasm, while both hemispheres exhibited several dark neurons caused by inappropriate sample preparation. However, the right hemisphere tissues of the rats with MCAO exhibited pale white and a tendency to swell. Moreover, the microscopic examination showed reduced number of cells, swollen neuron cells, shrunken and hyper-staining nuclei, and neuron necroses, whereas no apparent anomalies were in the left hemisphere—similar to the sham-operated rats.

In addition to observing the changes in neuron morphology by HE staining, EVG staining was also performed to show vascular wall morphology, as shown in figure 5, where significant vessel wall thickening and corrugations in the right MCA appeared in the MCAO group. This is consistent with the presence of cerebral vasospasm (Van Citters et al. 1962, Ohtake et al. 2004, Park et al. 2008, Nishino et al. 2017).

3.2. EIT raw voltage

The WBRC values of the 15 rats at different given times were calculated using the equations in section Materials and Methods, section 2.4, and plotted over time. The two groups of rats showed markedly different trends in WBRC, as shown in figure 6. First, the rats with MCAO showed an overall trend of continuous increase except for rat 1, which exhibited less than 10% WBRC increase within 2 h, whereas five rats exhibited increase between 10% and 20% (rats 2, 4, 5, 8, and 10), and four rats exhibited increase above 20% (rats 3, 6, 7, and 9). Hence, 90% of the rats exhibited a marked WBRC increase above 10% after right MCAO. In addition, except for rats 3 and 6, the WBRC curves experienced a rapid increase followed by decreasing trends over time, with peaks.
of different magnitudes. In contrast, the sham-operated rats showed no marked WBRC evolution. The WBRC increase within 2 h remained below 5% in all the sham-operated rats, being notably smaller than that in the rats with MCAO.

A descriptive statistical analysis was performed on the WBRC values at five given times (T0–T4); its results are listed in table 1. During the 2 h monitoring, the average WBRC of the rats with MCAO increased and peaked at 20.4% at the end, whereas that of the sham-operated rats showed no apparent variation pattern, with the maximum remaining below 3%.

The WBRC results show that the EIT voltage measurements can reflect cerebral impedance increase tendency caused by deep focal infarction. However, the voltage measurements do not provide location information, and thus cannot be used to localize the infarcted core and explain the peaks over time in the rats with MCAO. Hence, image reconstruction becomes necessary.

3.3. EIT image reconstruction

Figure 7 shows the reconstructed EIT images for the two groups of rats at T1–T4. The background was the average of the first 20 frames after data collection onset. Additionally, appropriate temporal adjustment of the foreground
frame was made to avoid the peak of the curve, but in no case longer than 5 min. Moreover, 10 EIT frames were averaged before reconstruction to improve the EIT signal-to-noise ratio. Two color bars with different scales are used to present the reconstructed images because the 2 h WBRC increase for rats 6, 7, and 9 was above 20%, which is far more than that of other rats.

The images of the sham-operated rats showed no apparent variations in the 2 h monitoring session. In contrast, the images of the rats with MCAO showed different degrees of impedance increase in the right hemisphere, which became more pronounced over time, identifying the infarction core. Rats 3, 4, and 6 showed apparent impedance decrease in some areas after 90 min. This was possibly caused by subarachnoid hemorrhage (Walberer and Rueger 2015). However, this did not affect the localization of infarction in the right hemisphere.

Image reconstruction was also performed using a shorter time interval for rat 5 (with MCAO) to further reveal its impedance variation over time. With the color bar scale fixed at $\pm 0.6$, figure 8 shows that after 1 h of MCAO, an area of increased impedance appeared, and its location was roughly consistent with the infarction core. Additionally, the area expanded and the impedance further increased after 1.5 h. Hence, EIT images reveal not only abnormal cerebral impedance variations at the early stage of MCAO like raw voltage measurements do but also the rough location and deterioration of infarcted cores.

The ARVI_{right} and ARVI_{left} values at T0–T4 were obtained from the reconstructed images for each rat. Repeated-measures ANOVA was performed on these two parameters, with the sham-operated rats being the parallel control of the rats with MCAO. First, Mauchly’s test of sphericity was applied to the two groups of data, yielding $p$-values below 0.05. Thus, the null hypothesis of sphericity was rejected. $F$ critical value estimation was then performed using the Greenhouse–Geisser method, yielding correction coefficients of 0.328 and 0.399, respectively.
The additional supplementary table S1 (stacks.iop.org/PM/41/015004/mmedia) lists the test results for ARVIright, where the intergroup difference, time effect, and the group–time interaction are statistically significant ($p < 0.05$). Hence, the right MCAO resulted in significant ipsilateral cerebral impedance increase compared to normal brain tissues, and the increase varied with time, being more notable as the ischemic damages progressed, thus confirming the suitability of EIT for monitoring local cerebral impedance increases caused by ischemic lesion deterioration. Additionally, the time effect test showed that the measured impedance in both the MCAO and sham groups drifted up over time.

The additional supplementary table S2 lists the test results for ARVIleft, where only the time effect was significant ($p < 0.05$), indicating that the measured impedance drifted up over time, being consistent with the results of ARVIright.

The comprehensive multiple comparison would include 40 paired $t$-tests ($4 \times 10$), but many of the comparisons are unnecessary. Thus, the following four pairs of time points were selected based on the ex-ante test principle: $T_0$–$T_1$, $T_0$–$T_2$, $T_0$–$T_3$, and $T_0$–$T_4$, and the mul-

---

**Table 1. Descriptive statistic results of WBRC.**

| Occlusion time [min] | MCAO ($n = 10$) | Sham ($n = 5$) |
|----------------------|-----------------|----------------|
|                      | Mean [%]        | SD [%]         | Mean [%] | SD [%] |
| 0                    | 0.2             | 0.1            | 1.4      | 0.9    |
| 30                   | 6.3             | 0.4            | 2.9      | 2.1    |
| 60                   | 9.7             | 4.6            | 0.2      | 0.2    |
| 90                   | 14.1            | 6.2            | 1.7      | 1.1    |
| 120                  | 20.4            | 9.2            | −0.1     | 1.1    |

---

**Figure 7.** Reconstructed EIT images at $T_1$–$T_4$ for the two groups. Image orientation is indicated in the bottom-left diagram, where A, L, P, and R represent the anterior, left, posterior, and right parts of the brain, respectively. The color bars represent the possible range of colors from diminished resistivity (red) to normal baseline intensity (green) and increased resistivity (blue). Scales of $\pm 0.6$ and $\pm 1.2$ correspond to the upper and lower limits of the resistivity values. Two color bars with different scales are used because the 2 h WBRC increase for rats 6, 7, and 9 was far higher then others.
multiple comparison was performed using the Bonferroni method, with $\alpha = 0.05$ and nominal level of significance $\alpha' = 0.05/4 = 0.0125$. The additional supplementary table S3 lists the results of the multiple comparison for G1 to G4. For G1, ARVI_{right} was significantly higher at T1–T4 compared to the baseline at T0 ($p < \alpha'$) and peaked at T4, with confidence interval [16.3, 32.9]% at the 95% level. For G2–G4, ARVI_{right} at T1–T4 showed no significant variations compared to T0.

Figure 6 shows that the WBRC for the rats with MCAO exhibited not only an overall increasing trend but also different magnitudes of fluctuation at interspersed moments (except for rats 3 and 6). Therefore, to further investigate the cerebral impedance variations at the vicinity of such fluctuations, EIT image reconstruction was performed for every fluctuation using the data obtained from rats 5 and 8 at shorter intervals of 1 min (background frame defined as the average of the initial 20 data frames). As shown in figure 10, both rats present impulse-like peaks with roughly equal duration of approximately 6–7 min. Additionally, the right side of the reconstructed images exhibit similar trends to the fluctuations. Specifically, an area with impedance increases appeared and then rapidly expanded with darker color. The impedance peaked and then dropped to a level higher than that at the onset of the fluctuation. In contrast, the left side of the reconstructed images showed no apparent changes.
Hence, besides an overall trend of continuous ipsilateral impedance increase, the right MCAO can also result in several rapid ipsilateral impedance fluctuations. This is possibly caused by vasospasm induced by the contact between the head of the filament and the artery inner wall. The EVG staining results (figure 5) support this hypothesis, and along with the results shown in figure 10, suggest the ability of EIT for detecting both ischemic stroke and cerebral blood flow variations.

4. Discussion

This study investigated the feasibility of using EIT to assess the early deterioration of deep cerebral ischemia by monitoring the right MCAO model in rats. We successfully established a right MCAO model in 10 rats and five other sham-operated rats for which filament insertion was not performed. The 15 rats showed stable vital signs after operation and thus were appropriate for EIT monitoring. The MCAO model was first reported in 1986 and has undergone several modifications and improvements afterwards. Research has shown that the location and size of infarction is affected by many factors, such as diameter, material, and operating depth of the filament in the MCAO model (Durukan A and Tatlisumak T 2007). Shimamura et al suggested that coating the filament head with silicon produces more stable and larger infarction damages (Shimamura et al 2006). Therefore, we used silicon-coated filaments in this study. Additionally, the following parameters were strictly controlled to yield consistent infarction among the rats with MCAO: rat weight of 240–270 g, filament diameter of 0.34 ± 0.02 mm, and insertion depth of filament of 18.5 ± 0.5 mm. TTC and hematoxylin–eosin staining showed that the rats with MCAO exhibited apparent ischemic damages in the unilateral corpus striatum after operation, laying the foundation for EIT measurements.

EIT data were successfully collected on all 15 rats for 2 h using eight T-shaped copper electrodes nailed into the rat skull. The insertion depth of the electrodes was controlled under 1 mm to ensure the integrity of the endocranium and prevent other cerebral injuries. Additionally, during monitoring, the rats were kept anesthetized to avoid noise introduced by body motion. The EIT hardware system used in the experiment, FMMU-EIT5, was developed by our research team. A test of the system using a physical head phantom confirmed that the system satisfies the requirements for cerebral monitoring (Shi et al 2018). In contrast to previous animal studies that usually set the data before the stroke onset as the baseline frame, our study collected baseline data after onset of MCAO. We adopted this monitoring protocol to avoid the influence caused by blood flow variations during filament insertion. Furthermore, it is more consistent with clinical applications, because data before stroke onset tend to be unavailable. This kind of monitoring protocol also highlights that this study aimed to confirm the feasibility of EIT for monitoring the deterioration of the ischemic lesion after the onset of stroke, which has not previously been validated.

Raw voltage measurements showed that the mean WBRC of the rats with MCAO increased and peaked at 20.4% at the end of the 2 h monitoring session, whereas that of the sham-operated rats showed no apparent variation patterns, with maximum WBRC below 3% (table 1). This WBRC trend is physiologically reasonable and consistent with findings from other studies (Yang et al 2016, Yang et al 2017, Song et al 2018), confirming the ability of EIT for detecting cerebral impedance increase due to deep cerebral infarction. Moreover, when setting color bar static at ±0.6, the reconstructed EIT images retrieved an alarming area with impedance increases after 1 h of the stroke, and its location was consistent with the right MCA territory. The area further expanded and darkened after 1.5 h of infarction (figures 7 and 8). It should be noted that when using dynamic color bars, the warning will be more timely. Repeated-measures ANOVA confirmed a significant impedance increase of the right MCAO ipsilaterally (p < 0.05), and the increase became more significant as the occlusion duration was prolonged (table 2). Thus, EIT reflects not only abnormal cerebral impedance variations at the early stage of stroke through voltage measurements but also the rough location and deterioration trend of ischemic damages through the reconstructed images. The relation between MCAO and ipsilateral impedance increase may be due to the following mechanisms. First, at the initial stage of MCAO, there exists a gap between the head of the filament and the artery inner wall. As thrombus gradually forms, the blood flow decreases and the impedance increases. Second, at an early stage of ischemic stroke, brain cells experience cytotoxic edema and volume expansion by the ischemia itself and hypoxia, decreasing both the intercellular space and the electrical current density through tissue under the same excitation, thus increasing the impedance of affected tissue (Song et al 2018). However, studies have revealed that after MCAO is successfully established, the ensuing decrease of blood flow in MCA over a long period is very limited (Sim et al 2016). Thus, it is clear that the overall impedance increase trend in the 2 h session was mainly attributed to the second mechanism. Three rats (rats 3, 4, and 6) showed apparent impedance decreases in some areas after 1.5 h of MCAO (figure 7), possibly caused by subarachnoid hemorrhage, a compilation of the MCAO model (Walberer and Rueger 2015). In MCAO operation, subarachnoid hemorrhage can occur when a filament is inserted too deep or is not fixed securely and moves forward to penetrate the bifurcation of the MCA and anterior communicating artery. A visual examination of the removed rat brain confirmed light hemorrhagic damage near the right MCA in rats 3 and 6, whose location was consistent with the area of decreased impedance in the EIT images.
Unexpectedly, the WBRC curves showed that the right MCAO resulted in not only ipsilateral cerebral impedance increases but also fluctuations. The reconstructed EIT images showed that the fluctuations persisted for short periods (approximately 6–7 min) and had magnitudes of 2%–7% (figure 10). This phenomenon is very likely due to vasospasm induced by the contact between the filament head and artery inner wall (Nishino et al 2017, Ogami et al 2017), as the vasospasm aggravates local ischemia, resulting in impedance increase. Then, the blood re-dilates and the blood supply resumes, thus decreasing the impedance. To verify this hypothesis, we performed EVG staining of the right MCA, finding that the right MCA of the rats with occlusion was apparently constricted compared to that of the sham-operated rats, which was probably caused by the recurrent vasospasm (Van Citters et al 1962, Ohtake et al 2004, Park et al 2008, Nishino et al 2017). This result suggests that EIT may also be suitable to detect cerebral blood flow variations.

This study confirmed the feasibility of EIT to evaluate the early deterioration of deep cerebral ischemia and has laid a foundation for diagnosing ischemic stroke using EIT by performing animal experiments. EIT voltage measurements can reflect impedance increase caused by deep cerebral infarction, and the reconstructed images enables rough localization of the ischemia core and reflection of the local blood flow decrease caused by vasospasm. Clinically, EIT can complement traditional imaging modalities such as digital subtraction angiography, computed tomography, and magnetic resonance imaging. Portable EIT can be used for real-time monitoring of patients with high risks of ischemic stroke, such as unconscious patients being transported in ambulance or anesthetized post-operative patients with high incidence of relapse of infarction and occurrence of vasospasm. EIT images show apparent regional changes within a very short time of stroke onset, and is therefore a feasible warning system for medical personnel to perform further inspection of patients and administer protective medicine.

Figure 10. Reconstructed EIT images at WBRC peaks for rats 5 and 8 at intervals of 1 min. The WBRC curves with peak indicator are presented in the right-hand insets. There are three and four peaks in WBRC curves of rats 5 and 8, respectively. Image orientation is indicated in the top-right diagram, where A, L, P, and R represent the anterior, left, posterior, and right parts of the brain, respectively. Color bars represent the possible range of colors from diminished resistivity (red) to normal baseline intensity (green) and increased resistivity (blue). The scales correspond to the upper and lower limits of the resistivity values.
Although EIT is a prospective portable and safe solution for monitoring and early warning of ischemic stroke, various challenges remain to be solved before it is clinically applied. First, comparing our results to those obtained by Dowrick et al, it can be concluded that various electrodes have no effect on voltage variations but affect the quality of the reconstructed images (Dowrick et al 2016). Dowrick et al used 40 spring-loaded scalp electrodes positioned directly on the skull, whereas we used only eight electrodes nailed into the skull without penetrating the endocranium, thus greatly improving the stability of contact and reducing measurement noise. The results of this study cannot confirm whether EIT is ready for the clinical diagnosis of stroke and can replace traditional imaging methods because this study lacked the application of noninvasive scalp electrodes. Currently, noninvasive EIT electrode systems used for brain imaging are still a hot research topic among different groups worldwide. However, it is also necessary to carry out applied research on animals synchronously, which lays a foundation for further study. Next, we aim to carry out further experiments using different scalp electrode systems to explore the possibility of non-invasive animal experiments.

Second, our study simplified the head model as a homogeneous circle, introducing modeling errors during image reconstruction. Although the modeling error in this study does not affect the identification of the ischemic core, it can cause a significant misreading during reconstruction of human images. Thus, an anatomically accurate human head model with realistic distribution of skull resistivity should be used in further research.

In this study, the rats were anesthetized to ensure stable electrode–skull contact. However, in clinical applications, electrodes may be loosened or displaced by the patient’s motion or operation by the physician, resulting in image artifacts. Moreover, repeated-measures ANOVA showed that, even without the electrodes being loosened or displaced, the impedance measurement slowly drifts over time, which can be attributed to changes in the contact status of the electrodes. Clinically, this is likely caused by perspiration from the patient or evaporation of the conductive gel. Thus, a data processing method to identify and compensate for changes in the contact of electrodes needs to be developed. Nevertheless, repeated-measures ANOVA also showed that the drift did not influence the assessment of intergroup effects.

Finally, although histology is useful for assessing image accuracy, it only provides information on the state of the brain after the rats are euthanized, it does not provide any information on the physiological changes at the time of EIT data acquisition. This is especially true for the vasospasm, because blood flow varies instantaneously, and the final pathological results can only show a trace of vasoconstriction. Therefore, when the WBRC curve changes, blood flow distribution needs more accurate monitoring through techniques such as transcranial Doppler. For ischemic lesion progression, as impedance changes are unpredictable, simultaneous magnetic resonance imaging data would be ideally collected along with EIT data as reference for comparisons.

5. Conclusion

In this study, we verified that EIT is sensitive to impedance variations caused by MCAO and vasospasm in rats, providing insights into its feasibility for monitoring ischemic stroke deterioration. However, further developments are required, including the design of an electrode system for the human head and the development of data preprocessing and reconstruction methods, before application of EIT to this end in clinical practice.

Data availability

The EIT data used to support the findings of this study are included within the article.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Funding statement

This work was supported by the National Nature Science Foundation of China (Grant Nos. 61771475, 51837011 and 31771073), and the Natural Science Foundation of Shaanxi Province (Grant No. 2014JM2-6092).

ORCID iDs

Lu Cao https://orcid.org/0000-0002-5159-7615
Haoting Li https://orcid.org/0000-0002-0496-6705
Bin Yang https://orcid.org/0000-0001-9687-5968
