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Molecular and Structural Changes in Induced-Brain Stroke Tissue Using FTIR Imaging Spectroscopy, Scanning Electron and Atomic Force Microscopy

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1. Background

Stroke, i.e. loss of brain function(s) due to disturbance in the blood supply to the brain, is the main cause of adult disability (e.g. paralysis) in the world, leaving more than half of the patients dependent on daily assistance. In Qatar, stroke is a major health problem with an estimated incidence of 238/100,000 per year for the population over 45 years old [1]. Stroke patients are often hospitalized and/or subjected to intensive rehabilitation programs for long periods of time, and their quality of life is severely affected socially and economically. Around 10% of the hospital beds in Qatar are occupied by stroke patients [1]. Thus, without major advances to improve prevention, treatment and rehabilitation of stroke, the social and economic costs of this disease will increase dramatically.

There are pathological and physiological changes on the cellular and molecular levels associated with stroke. The objective of this work is to determine the molecular and structural changes occurring in the tissue of rat's brain. Vibrational spectroscopy, i.e. Fourier transform infrared (FTIR) imaging spectroscopy, was used as rapid and objective diagnostic platform to investigate the pathological and pathological changes in the rat's brain sections three weeks after stroke. FTIR spectroscopy was also used to differentiate between the biochemical makeup of the white and grey matters of a healthy control brain samples. Also, in the current study, scanning electron (SEM), energy dispersive X-ray spectroscopy (EDX), and atomic force microscopic (AFM) techniques were assessed to study the structural changes in the rat's brain tissues after experiencing an induced stroke.

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2. Experimental

2.1. Sample preparation

Rats were anesthetized using 2–3% isoflurane. Experimental stroke was induced in rats by 90-min occlusion of the right middle cerebral artery with an intraluminal filament. Rats were euthanized with a lethal dose of sodium pentobarbital and transcardially perfused with 4% paraformaldehyde. Rat's brains were extracted, embedded in paraffin and then serially sliced, using semi-automated rotary microtome, into 5 μm thickness sections for the FTIR imaging and AFM analysis and 35 μm thickness for the SEM and EDX analysis. The brain sections were mounted on MirrIR CFR, Low-e microscope slides for the FTIR imaging analysis, and on aluminum metal for the SEM analysis and EDX analysis. The paraffin was removed from the samples by using xylene and isopropanol.

2.2. Instrumentation

2.2.1. FTIR Imaging Measurements

The FTIR images were obtained using FTIR spectrometer (Agilent Technology) at a reflection mode within the range of 4000–700 cm^{-1}. Spectral images were analyzed using Metlab software (The Mathworks Inc.). Origin 2015 software was used for graph drawing. Principal component analysis (PCA) was performed to study the spectral data variations between the FTIR spectra and images.

2.2.2. Scanning Electron Microscopy (SEM)

Rat's brain sections of 35 μm thickness were mounted on aluminum slides for SEM analysis. All the samples were viewed with a FEI Quanta 200, USA scanning electron microscope at 10 kV. SEM micrographs of the brain stroke and healthy rat's sections were compared. Elemental distribution in both healthy and induced stroke brain sections were investigated by using energy dispersive X-ray spectroscopy (EDX) equipped with SEM. The spectra provided a semi-quantitative view of the elemental composition of both weight and atomic percent.

2.2.3. Atomic Force Microscopy (AFM)

Bruker atomic force microscopy (AFM) was used for imaging and quantitatively determining the local elastic properties of healthy and induced stroke rat's brain sections. A controllable and constant force was applied at each data point and using the resulting force-distant curve for the formation the AFM images. Brain sections were scanned at 10 μm by 10 μm. About 100 force-distance curve were collected for each healthy and induced stroke brain sections and two random scan lines of force-distance curves was recorded.

3. Results and Discussions

The FTIR spectroscopy results indicated that the white matter is richer in lipid content than the grey matter as shown in Figures 1 and 2. The infrared spectrum images showed a decrease in the lipid content of the white matter associated with the induced stroke brain sections. FTIR bands assigned to the bio-chemical makeup such as proteins, lipids and ester varied in positions, line-shape, and intensity between control and induced stroke brain samples. The spectral images showed that there is a configuration changes is associated with the lipid bands in the rat's brain white matter that experienced stroke.

The FTIR spectral images of the white matter in the induced stroke brain sections indicated that amide I and ester bands experienced a bio-chemical changes as shown in Figure 3 and 4. Figure 5 shows the second derivative of the collected FTIR spectra from induced stroke brain sections. In Figure 5, there are spectral differences that assigned to ester and protein regions. Figure 6a represents the loading spectra of the first three principal component analysis (PC1, PC2 and PC3). The variations principally were located in the regions of amide I band at (~1695–1637 cm^{-1}) and small variation in the amide II band at (1543 cm^{-1}). Figure 6b represents the loading spectra of the PC4, PC5 and PC6. The variations principally were located in the protein region, mainly amide I band at (~1695–1637 cm^{-1}) and ester band at about 1730 cm^{-1}. The use of FTIR imaging and chemometric analyses such as principal component analysis (PCA) of spectral data allows to investigate and differentiate spectral images pattern collected from control and stroke rat's brain samples.

The scanning electron microscope results showed that lesion region in the induced stroke brain sections are enriched by the selected elements such as Fe and Ca as shown in Figure 7 (a & b). Scanning electron microscope (SEM) micrographs indicated that there is structure change in the induced stroke brain section. The structure of stroked brain sample in the nanometer scale appeared to be significantly rough compared to the control brain sample (Figure 8 a & b).

Atomic force microscope (AFM) images showed that the stroke brain section is swollen compared to healthy brain sections. The AFM images of the induced stroke brain sections appeared more stretched when compared to the control brain section image as shown in Figure 9 (a & b). AFM results also showed that the force-distance curves in Figure 10, recorded using control (healthy) brain sections (blue) and induced stroke brain sections (red). The force-distance showed that the AFM cantilelver deflection of the healthy brain samples is higher than the induced stroke brain section. This indicate that the healthy brain section are softer and elastic than the induced stroke brain sections.

4. Conclusion

FTIR imaging spectroscopy, scanning electron and atomic force microscopy techniques were able to analyze and differentiate between the healthy and induced stroke rat's brain sections on the molecular, structural and global levels making them valuable tools to investigate, diagnose and study the structural plasticity of the stroke induced brain. FTIR imaging spectroscopy in combination with multivariate analysis such as principal component analysis (PCA) is a non-destructive technique that proves to be rapid, accurate and straightforward to be performed. It constitutes a powerful approach to be used as a medical diagnosis tool to investigate the pathological changes associated with stroke in the brain tissues.

Keywords

Fourier Transform Infrared (FTIR) imaging spectroscopy, Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Brain tissue, Stroke, Chemometric Analysis

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Reference

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