G54 Biochemical Characteristics of Diffuse Axonal Injury by Fourier Transform Infrared Micro-Spectroscopy

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After attending this presentation, attendees will learn a new method of Fourier transform infrared (FTIR) Micro-spectroscopy to study the diffuse axonal injury.

This presentation will impact the forensic science community by providing participants with a better understanding of the biochemical and histopathological characteristics of diffuse axonal injury.

Fourier transform infrared (FTIR) imaging and microspectroscopy have been extensively applied in the identification and investigation of both healthy and diseased tissues. FTIR imaging can be used to determine the biodistribution of several molecules of interest (carbohydrates, lipids, proteins) for tissue analysis, without the need for prior staining of these tissues. Molecular structure data, such as protein secondary structure and collagen triple helix exhibits, can also be obtained from the same analysis. Thus, several histopathological lesions, for example diffuse axonal injury, can be identified from FTIR analyzed tissue images, the latter which can allow for more accurate discrimination between healthy tissues and pathological lesions.

The goal of the study was to assess whether Fourier transform infrared spectrometry (FTIR) micro-spectroscopy could produce distinct spectral information on biochemical characteristics of diffuse axonal injury (DAI) and to set them as molecular markers to diagnose atypical DAI.

Method: DAI was produced by rotational acceleration in rats and rabbits. Paraffin-embedded brain tissues from rats with head trauma were studied by hematoxylin and eosin staining, immunohistochemistry (please specify the staining), silver staining, and FTIR micro-spectroscopy. The characteristics of DAI were analyzed morphologically and molecularly. Biochemical parameters of DAI in rabbits' brain tissues were also conducted by using a chemical FTIR mapping.

Results: The most relevant bands identified were the amide A, B, I, and, II showing crucial spectral differences between apparent normal region and DAI region, including the peak position blue shift and the increased intensity of DAI. Comparing to single spectral band, the I1650/I1550 ratio was increased and rationally used as a molecular marker for diagnosing DAI. These novel preliminary findings supported further exploration of FTIR molecular profiling in clinical or forensic study, and were in accordance with histopathology.

In rabbits study, the N–H stretch (3290 cm^{-1}) of amide A, the CH_3 symmetric stretch of mainly proteins (2873cm^{-1}), fibrocollagenous tissues (1638 cm^{-1}), nucleic acids and phospholipids C-O stretch represented by PO_2^- symmetric stretch (1081 cm^{-1})were tested. All of the above represent changes of proteins, collagens, nucleic acids and phosphonolipid. Red, green, and their gradient color of the Infrared spectra (FTIR-mapping) represented the IR absorptive intensity of the tested microstructure. Red indicated strong absorption and thus means a greater quantity of material is present at this peak; Green means a weak absorption, indicating of little quantity of material at the peak. The infrared spectra of the above four vibration peaks were different even if the infrared spectrum was consistent with the result of HE staining. Hence, each vibration peak represented a different chemical structure. With the help of different peaks of the infrared spectra, the extent a certain chemical substance that is involved in the pathological change can be explored, to reveal the pathophysiological process. The results of this study showed that N-H stretching (3290 cm^{-1}) of amide A, CH_3 symmetric stretch of mainly proteins (2873 cm^{-1}), and nucleic acids and phospholipids C-O stretch represented by PO_2^- symmetric stretch (1081 cm^{-1}), all widely participated in the diffuse axonal injury. The red region is mostly consistent with diffuse axonal injury lesion. The approaches described in this manuscript significantly enhance the rate and quality of spectroscopic analyses of tissue specimens, allowing realization of the statistical sampling and further numerical analysis to explore associations between molecular chemical changes and pathologic information. This imaging method also provides accurate information about the exact distribution of each component in the composite material, which is crucial for understanding its performance when in contact with pathologic processes.

In summary, it is proposed that FTIR be used as a new tool integrating both molecular and histopathological assessments to investigate the degree of biochemical and pathological characteristics of DAI diffuse axonal injury.

Fourier Transform Infrared (FTIR) Micro-Spectrosco, Histopathology, Diffuse Axonal Injury

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