Differences in Raman Spectra of Aluminium Treated Brain Tissue Sample

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Citation

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Abstract

Raman microscopy was used to measure the effect of aluminium exposure on paraffin-embedded brain tissue sections. Despite the complexity of the system, we are able to show a differential Raman spectral characteristic between the Al-treated and untreated brain tissue samples. These are attributed to changes in the packing and conformation of protein material. Much work remains to be done to quantify the effect and to optimise experimental conditions, nevertheless, the results reported here demonstrate the benefits of these Raman microspectroscopic studies in such complex biosamples.

INTRODUCTION

Raman spectroscopy is a laser technique that provides vibrational spectra from different molecular species due to their different molecular bonds. But the technique only become viable as a medical tool in the mid 1980's with introduction of the Fourier Transform Raman instrument ([1]). The inherent weakness of the Raman effects is the intense Rayleigh scatter coupled with sample auto-fluorescence. Hence visible-light Raman spectrometers are poor spectroscopic tools for biological samples. However, the longer wavelength used in Fourier Transform Raman systems reduced the effects of tissue fluorescence ([2]). Besides reduced fluorescence intensity, less photolytic degradation occurs due to the longer wavelength laser, allowing higher laser powers to be used. The high throughput and multiplexing capability of the instruments also partially compensates for the low scattering efficiency and poor detector sensitivity in the near infrared. Raman spectra of tissue have been used to differentiate between normal and diseased tissues as well as different chemical state of organs and cells. The chemical structural change of cells and tissues is a good indicator of disease and is often apparent in Raman spectroscopy before it can be detected by the usual conventional methods. Raman spectrum can permits the studies of protein dynamics, drug interactions and single cells, bacteria, and viruses. Raman effect has been shown to adequately quantify species in vivo, such as arterial plaque and silicone molecules from ruptured breast implant. Dapple et al. ([3]) have isolated globules of fat surrounded by connective tissue, adding an element of spatial selectivity to the analysis. Brain tissue can also be studied, although analysis of cerebral tissue is difficult because the biochemistry and pharmacology of brain tissue is complex and heterogeneous ([4]). But using Raman spectroscopy, white and grey matter can easily be differentiated with micrometer resolution ([5]). Several methods have been developed to investigate the biochemistry associated with brain injury and activity ([6], [7]). Some of the techniques are slower with broader species sensitivity while others are fast and have species limited sensitivity. An ultraviolet resonance Raman system, using UV resonance Raman spectra of some small-molecules, peptides and lipids (free fatty acids) was used to measure the effect of aluminium exposure on paraffin-embedded brain tissue.

So far we have not came across any data showing the effect of aluminium exposure on brain tissue sample using Raman spectroscopy, but there are information on the use of Raman spectroscopy and other similar techniques to detect other chemical compounds in the brain tissue ([8]).

MATERIAL AND METHODS

Male Wistar rats weighing 170-180g (age three weeks) were divided into four groups with n=5 in each group; (a) aluminium treated group, (b) aluminium treated and a dietary supplement of 20 mg/g of vitamin E, (c) 20 mg/g vitamin E supplemented diet alone and (d) control group received only normal chow. Aluminium was administered i.p. as aluminium lactate (10 mg Al/Kg body weight) 5 times a week for 4 weeks, and the animals were not injected on

Saturday and Sunday. Following treatment the animals were killed 24hrs after the last administration and brain tissue embedded in paraffin wax and stored at –80 C. Frozen embedded paraffin brain tissue samples were mounted on a cryostat chuck with Histoprep (Fisher Diagnostics). Thin transverse tissue sections (5-30 µm) for Raman micro-spectroscopy were cut using a cryostate/microtome. It should be noted that several materials (ranging from glass to steel) were used to mount the tissue in order to obtain a method suitable for the analysis. No cover slip was used for spectroscopic measurements. Similarly, brain tissue obtained from male Wistar rats cut into sections treated as follows; (i) incubated with PBS, (ii) incubated with aluminium (10 mg Al/Kg), and (iii) incubated with iron (10 mg Fe/Kg), for 1 hour at 37 ° C. After the incubation brain samples were paraffin-embedded and thin sections (30 µm) prepared as described above.

ANALYSIS

Raman spectroscopic measurements were carried out using two systems. One was a Perkin Elmer System 2000 FT-NIR Raman spectrometer, using a Nd: YAG laser to provide excitation at 1064 nm. This deposited up to 500 mW of energy at the sample into a spot-size of c.a. 100 µm, and enabled a Raman spectrum spanning the range 4000 to 200 cm^{-1} . Micro-measurements were made using a Rensihaw System 2000 micro-Raman. Examination of the sample was performed with a 50x infinity corrected objective, with integration times of 128 second in most cases. Near-infrared (782 nm) laser light was generated in a GaAlAs laser diode system, and focused onto the sample through the objective operating in backscatter mode, using adjustable mirrors and dichroic beamsplitters, with a laser power on the sample of 1-13 mW. Light emitted from the tissue sample was collected by the same objective, passed through the beamsplitter and notch-filtered to reject Rayleigh scattered light. The Raman signal was focused with a chromatic lens, via a 50 micron slit, onto a grating, which dispersed the Raman signal onto a deep-depletion CCD detector cooled to –70 °C. The whole system was PC-controlled using Renishaw's WIRE software, which performed data processing and storage. At least three Raman spectra over a range of 150-1800 cm^{-1} (5 cm^{-1} resolution) were obtained from each site selected. Spectra were normalised to the phenylalanine band near 1004 cm^{-1} .

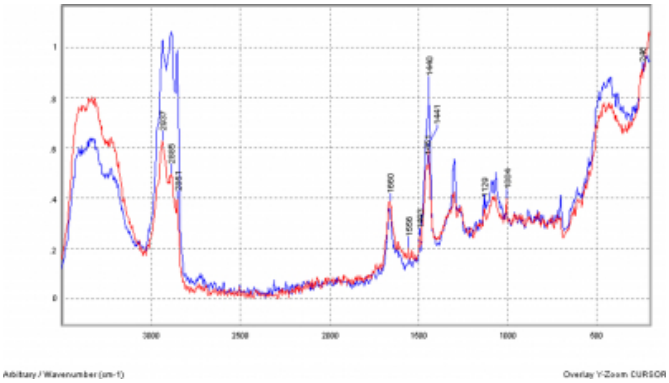
RESULTS

BRAIN RAMAN SPECTROSCOPY

Figure 1 - 3 revealed the spectral analysis of aluminium treated samples and aluminium untreated samples the spectra are normalised to the 1004 cm^{-1} band of phenylalanine. The results showed consistent shape changes in the methyl and methylene CH deformations at 1460 cm^{-1} . Note the relative differences in the intensity of these C-H deformation vibrations, that the amide I band is similar, and the shape and intensity changes in the C-H stretching region near 2900 cm^{-1} , and in the CH_2 twist and wag motions near 1300 cm^{-1} . These are attributable to the paraffin wax. Although there is no clear evidence of the C=O bond, the main CH bands are characteristic of lipids.

Figure 1

Figure 1: FT-Raman (1064 nm excitation) of control (blue) and aluminium (red).



The changes observed in both the relative intensity and the shape of the C-H bands, show differences in the packing and conformation of the long lipid molecules. The blue spectrum shows good protein bands, with amide 1 (1663 cm^{-1}), CH bending (1455 cm^{-1}), and phenylalanine (1004 cm^{-1}) being particularly clear. The red spectrum additionally shows a complex structure, including carbonyl (1730 cm^{-1}), with a very weak phenylalanine peak, but yet there is no evidence for unsaturation, though the signal-to-noise ratio is admittedly very low. The green spectrum shows features from both. Having formulated a method of mounting the sample, brain tissue materials were deposited on a scalpel blade and the results revealed clearer differences in terms of the different treatment. Figure 2 and 3 shows the spectra of brain tissue sample treated with/or without aluminium and with or without vitamin E supplementation. The data show nearly all the features observed from previously (Figure 1), with major differences around 1300 cm^{-1} , bands attributed to the CH_2 twist and wag motions of lipids, and to the amide III protein band.

Figure 2

Figure 2: Raman spectra of control (blue), aluminium treated (red) and vitamin E supplemented group alone (green).

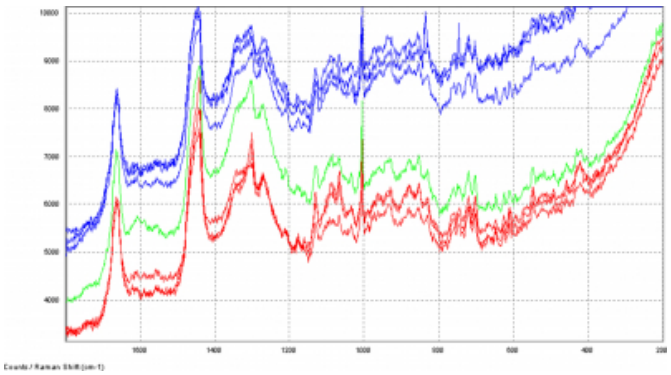
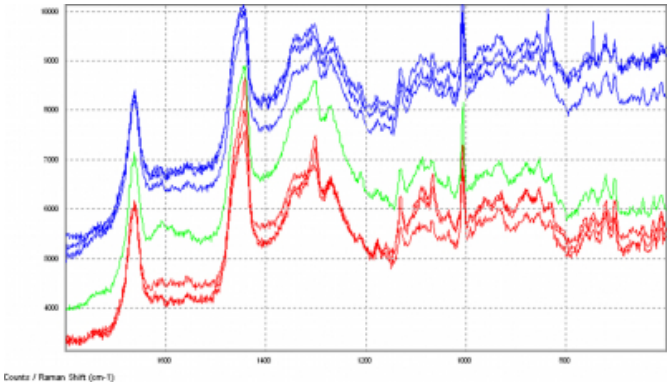


Figure 3

Figure 3: Raman spectra identical to the above figure 2, control (blue), aluminium treated (red) and vitamin E supplemented group alone (green) but for only 1800 cm – 600 cm with a differences around 1300 cm .



ALUMINIUM AND IRON INCUBATION

Figures 4 - 6 represent Raman spectra from in vitro studies of brain tissue sample - the patterns are similar to those observed in the in vivo studies. Figure 4 presents spectra of control brain tissue sample showing repeatability from three fresh points of analysis. In figure 5, the spectra show the results from aluminium-treated material, and indicate the variable ratios between the amide I band, at 1640 cm^{-1} , and the aromatic/carboxylic features near 1600 cm^{-1} . However, the areas where previous changes were noticed remain the same, particularly in the 1300 cm^{-1} region, which seem quite similar. Repeatable sets of spectra were observed in brain tissue incubated with iron alone (Figure 6). The reproducibility from the different areas is excellent, and looks quite similar to the control material.

Figure 4

Figure 4: Raman spectra of control brain tissue sample incubated with PBS for 1 hour at 37 C.

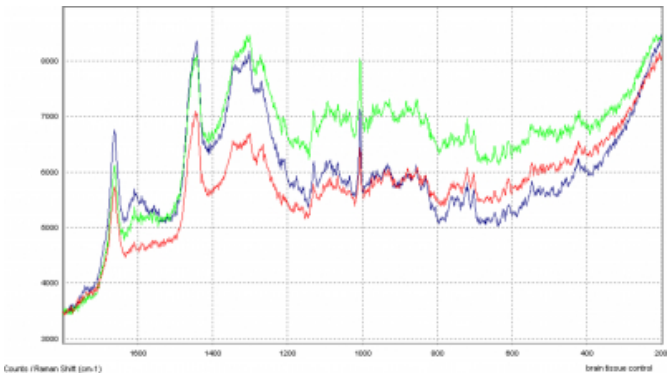


Figure 5

Figure 5: Raman spectra of brain tissue incubated with aluminium (10 mg Al/Kg) for 1 hour at 37 C.

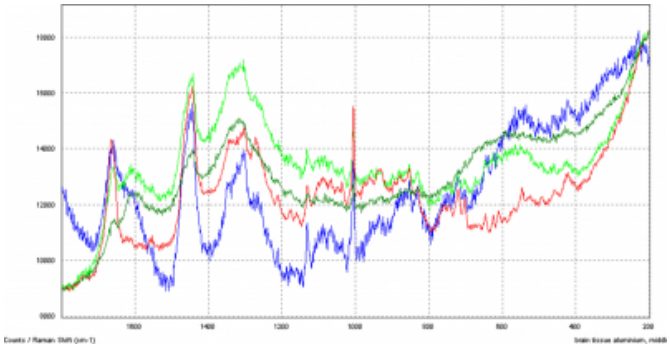
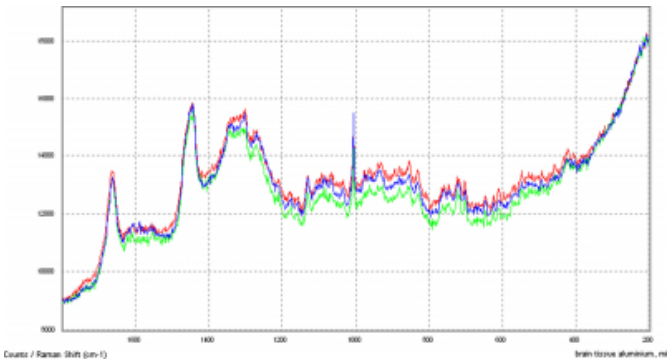


Figure 6

Figure 6: Raman spectra of brain tissue incubated with 1ron (10 mg Fe/Kg) for 1 hour at 37 C.



COMPARATIVE STUDIES OF ALUMINIUM AND IRON

Comparison of control, aluminium treated and iron treated is presented in figure 7. The studies vindicate what was observed in the in vivo studies, although the comparative studies of the in vivo and the in vitro control (Figure 8), and aluminium treated (Figure 9) highlight some spatial

differences between the studies, but these do not nullify what previously observed in the individual study.

Figure 7

Figure 7: Comparison of control (blue), aluminium (red) and iron (green) samples.

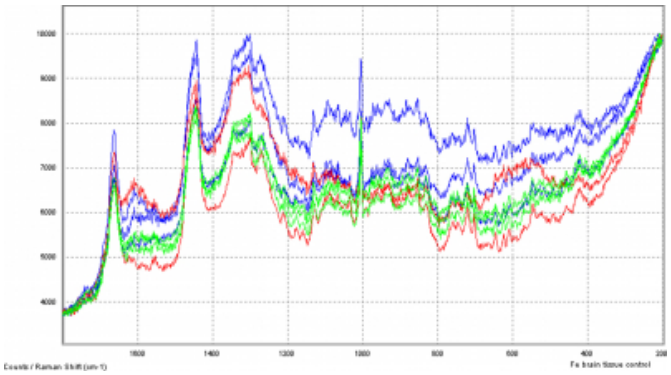


Figure 8

Figure 8: comparison between controls (dark blue) and controls (light blue).

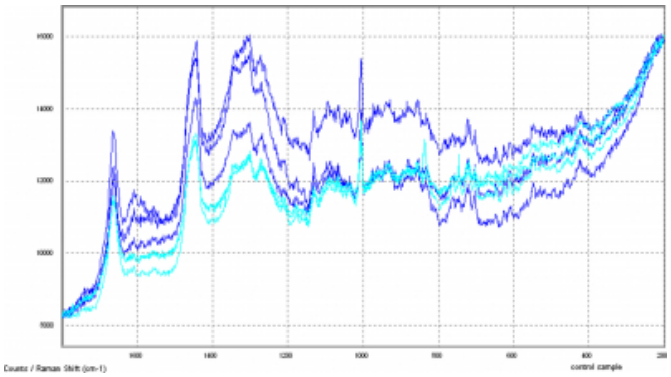
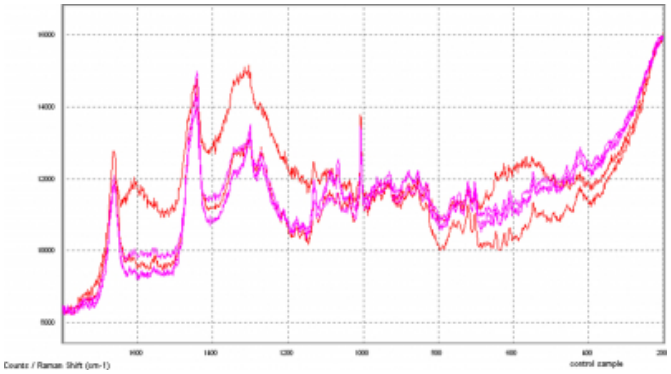


Figure 9

Figure 9: comparison between (red) and (purple) aluminium treatment.



DISCUSSION

Judging from the result of the spectra the results are in the order of magnitude of the noise and showed consistent pattern from spectrum to spectrum. The Raman spectrum of each morphologic structure is well described by the observed changes in packing and conformation. Despite the limitation of the weakness of Raman signals, we are able to clearly show a differential Raman spectral characteristic between the Al-treated and untreated brain tissue samples. The bands at 1663 and 1455 cm $^{-1}$ are attributable to the amide I and III vibration of a structural protein such as elastin ($_{9}$). The intense band at 1440 cm $^{-1}$ can be assigned to the CH2 and CH3 bending mode of proteins ($_{10}$) and the 1100 cm $^{-1}$ are attributable to desmosine/isodesmosine which are specific for elastin ($_{10}$). These results indicates that the main spectral features in the smooth muscle cells spectra are similar to those observed in the elastic laminae and collagen and are usually dominated by protein bands at 1660 and 1270 cm $^{-1}$ i.e. the amide I and III vibration respectively. The region of 1455, 1334, 1030, 935 and 850 cm $^{-1}$ are C-C or C-H bending. The obvious differences between the protein dominating in the smooth muscle cells, elastic laminae and collagen fibre are actually the intensity of vibrations in the phenylalanine (1004 cm $^{-1}$), desmosine/isodesmosine (1334 and 1105 cm $^{-1}$) and amide III (1265 cm $^{-1}$) bands ($_{9}$, $_{10}$). The changes brought by the aluminium-exposed tissues are easily distinguished from the control (Figure 2). The Raman band of elastin and collagen observed here might be due to the presence of -amyloid proteins, which have been reported to have a band of 1628 cm $^{-1}$. These proteins are highly linked with the onset of Alzheimer's disease as previously stated. Although the 1730 cm $^{-1}$ carbonyl bands was not observed in every instance and coupled to the observation of the 1660 and 1455 cm $^{-1}$ bands this strongly indicates triglycerides ($_{9}$). Thus we shown here the ability of Raman spectroscopy to detect changes between aluminium exposed sample and control samples in both in vivo and in vitro preparations. Much work remains to be done to quantify the effect and to optimise experimental conditions, nevertheless, the results reported here demonstrate the benefits of these Raman microspectroscopic studies in such complex biosamples. This approach could be of great interest for the study of endothelial and cerebral tissue in research related to various heart disease and brain disorders respectively. For example, the studies related to Alzheimer's disease, a neurodegenarative disease, would benefit in this case.

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