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In-vitro analysis of normal and aneurismal human ascending aortic tissues using FT-IR microspectroscopy

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Abstract

FTIR microspectroscopy has shown to be a proven tool in the investigation of many tissue types. We have used this spectroscopic approach to analyse structural differences between normal and aneurismal aortic tissues and also aortas from patients with congenital anomalies like aortic bicuspid valves. Spectral analysis showed important variations in amide I and II regions, related to changes in alpha-helix and beta-sheet secondary structure of proteins that seem to be correlated to structural modifications of collagen and elastin. These proteins are the major constituents of the aortic wall associated to smooth muscular cells. The amide regions have thus been identified as a marker of structural modifications related to these proteins whose modifications can be associated to a given aortic pathological situation. Both univariate (total absorbance image and band ratio) and multivariate (principal components analysis) analyses of the spectral information contained in the infrared images have been performed. Differences between tissues have been identified by these two approaches and allowed to separate each group of aortic tissues. However, with univariate band ratio analysis, the pathological group was found to be composed of samples from aneurismal aortas associated or not with an aortic bicuspid valve. In contrast, PCA was able to separate these two types of aortic pathologies. For other groups, PCA and band ratio analysis can differentiate between normal, aneurismal, and none dilated aortas from patients with a bicuspid aortic valve.

Keywords: FTIR microspectroscopy; Aortic tissue; Aneurysm; Aortic bicuspid valve; PCA analysis

1. Introduction

Human aneurism is a common pathology encountered daily in the cardio-vascular surgery unit. Different pathologies of the aortic wall, such as aortic ectasia, stenosis or aneurysm are known [1]. The most important corresponds to dilations with or without atheroma (accumulation of lipid, calcium, and fibrosis). The exact physiopathology of this aortic dilatation is unknown but implication of Matrix Metalloproteinases or presence of congenital valve malformation is clearly associated with pathology. Macroscopic examination confirms modifications of physical characteristics with dilation, decrease of elastance and aortic wall resistance. The development of aneurysm is multifactorial [2–6]; the final outcome of the pathology being a rupture of aortic wall, causing the death of patient in the following minutes.

At present, the main dilemma for the surgeon is to take a decision, during a heart-surgical act, whether it is necessary to replace the ascending part of aortas at the same time. To date, there is no tool available that can help the surgeon to take the proper decision. For the patient, a second surgery and replacement of the aorta many years later, increases the risk of complications by a factor of 10 [7,8]. Therefore, development of new methods that can assist the surgeon could be very helpful and beneficial to patients.

Characterisation of the different tissue types is an important issue in view of evaluating the stage of the aortic disease and it could be in the future an important parameter of the surgical decision. Application of spectroscopic methods offers the

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possibility to analyse the structural information contained in a larger area of the tissue sample and to combine morphological and chemical information.

In this study, we make a preliminary attempt to use Fourier-transform infrared (FT-IR) microspectroscopy as an in vitro analytical tool for discriminating between normal and pathological human aortic tissues on the one hand, and to differentiate between the different pathological tissue types, encountered during surgery, on the other hand.

2. Material and methods

2.1. Aortic samples

Biopsies of aortic tissues obtained after surgeries were classified following the 4 different groups: normal tissues, tissues from patients with bicuspid valves, aneurismal tissues from patients without bicuspid valves, and aneurismal tissues from patient with bicuspid valves. Bicuspid aortic valve is a congenital disease with only 2 aortic leaflets instead of three normal leaflets.

Bicuspid aortic valves are often associated with moderate dilations of the ascending aorta which are more difficult to assess.

Normal tissues were obtained on heart explants after a cerebral death. The different pathological samples were conserved after surgical replacement acts of pathological aortas.

All samples were first conserved in sterile physiological serum just after surgery. Only tissue stripes of 20 mm × 8 mm size were snap-frozen in liquid nitrogen and conserved at −80 °C. Infrared imaging was performed on 10 μm thick cryosections placed on infrared transparent ZnSe windows.

2.2. Infrared imaging of aortic tissue sections

Spectral images were acquired using the infrared imaging system Spotlight 300 (Perkin Elmer Life Sciences, France). Acquisition areas were disposed in the thickness of aortas, across the three layers of the aortic wall as this is schematised in the orcein stained section which reveals the lamellar structures (Fig. 1A). IR images were acquired with a liquid nitrogen cooled mercury cadmium telluride (MCT-A) line detector composed of 16 pixel elements which can be operated either at 6.25 or 25 μm/pixel resolution. In our study we used the highest spatial resolution. Spectral resolution was set to 4 cm^{-1}. Each absorbance spectrum composing the IR images, and resulting of 32 scans, was recorded for each pixel in the transmission mode using the Spotlight software (Perkin-Elmer). An example of an IR image based on the total absorbance and an individual pixel spectrum is shown in Fig. 1B. In these conditions, a typical area of about

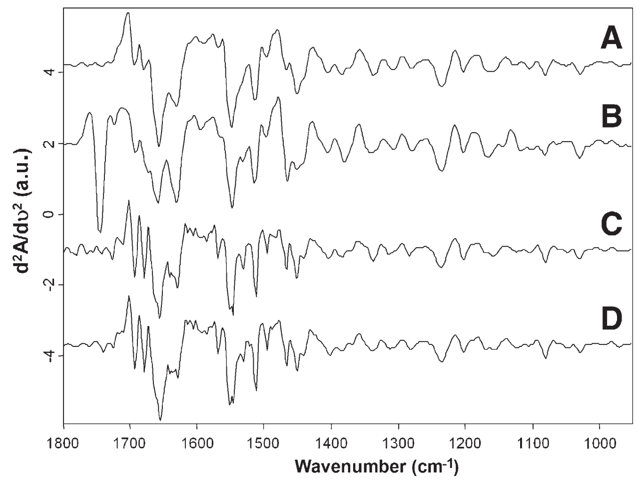

Fig. 2. Normalised second derivative of mean FT-IR spectra of aorta of four different groups of tissues. A, healthy tissue; B, tissue from patient with aortic bicuspid valve; C, aneurismal tissue from patient with normal (tricuspid) valve; and D, aneurismal tissue from patient with aortic bicuspid valve. All other conditions are as in Fig. 1.

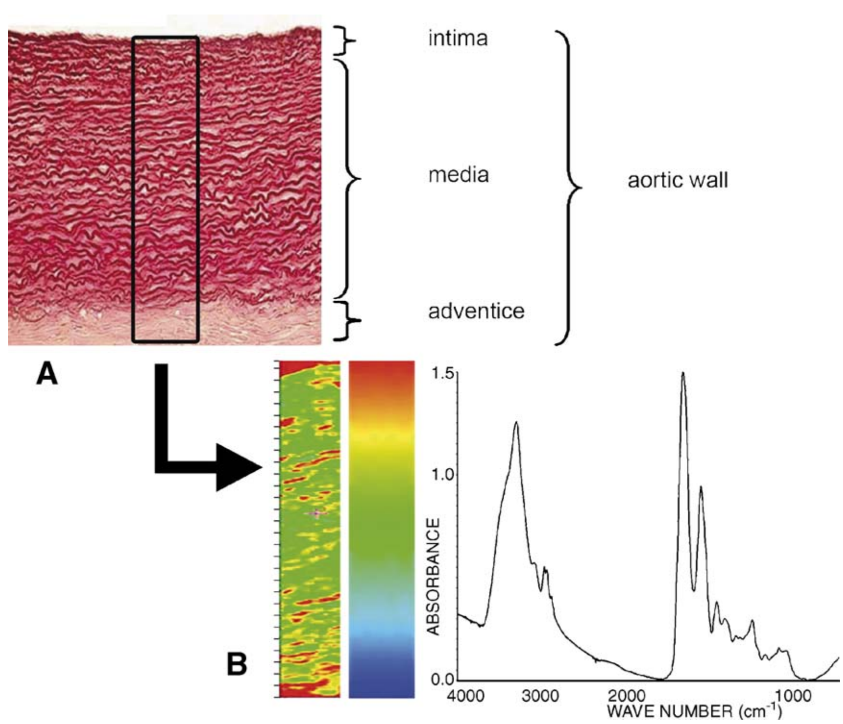

Fig. 1. (A) Histological preparation of aortic tissues. Section of orcein stained tissue showing presence of the three layers (intima, media, and adventice) of the aortic wall. The black striates present in tissue correspond to elastic fibres. (B) Total absorbance FT-IR image obtained after spectral acquisition in the selected area represented by the black triangle. The arbitrary colour scale represents total absorbance of spectra in the frequency range 4000-720 cm^{-1}. Each pixel (size: 6.25 μm) corresponds to one spectrum (32 scans at 4 cm^{-1}) which can be extracted from the image and displayed as shown.