Atherosclerotic plaque characterization using NMR spectroscopy

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INTRODUCTION

Cardiovascular and cerebrovascular diseases, currently the first and third cause of death in developed countries, will soon be a major burden to public health worldwide (1). Several investigations have established that the acute clinical complications of these diseases are associated with atherosclerotic plaque disruption and thrombosis (2). Atherosclerotic plaque composition rather than degree of stenosis determines its instability. Thus, noninvasive *in vivo* techniques that can harvest both chemical and spatial information on the distribution of different plaque components may allow risk stratification in asymptomatic, as well as symptomatic, patients with cardiovascular and cerebrovascular diseases, plaque monitoring, and possibly guide targeted therapy. It is thought that plaque vulnerability is highly dependent on its various constituents, such as lipids, calcification, blood, inflammatory cells, and fibrous tissues. Generally, the presence of necrotic cores, inflammation, hemorrhage, and thinning of the fibrous cap thought to render a plaque vulnerable. A high lesion celullarity, for example, is found to disrupt plaque stability. MRI allows serial monitoring with high-resolution images of multiple vascular territories in the same individual. It does not involve ionizing radiation and provides noninvasive appraisal of the pathophysiology of atherosclerosis. Another important feature of this technique is that it permits *in vivo* nondestructive characterization of the tissue sample's chemical constituents. Whereas special techniques of MRS *in vitro* allows to predict with high precision the chemical constituents of atheromatous plaque in a systematic way, providing a very important method to study the evolution of the pathological process caused by atherosclerosis disease and pave the way for future *in vivo* studies.

The aim of the present study was to investigate the feasibility and efficiency of MRS in detecting different stages of human atherosclerotic plaques. The disease severity, as consequence of lipid alterations, can be detected by variations in ^{1}H and ^{13}C spectral characteristics.

MATERIALS AND METHODS

Atherosclerotic lesions were graded according to the American Heart Association's (AHA's) Committee on Vascular Lesions classification (Table 1) (3).

Table 1: Tissue samples data.

Subject	Age (yrs)	Gender	Race	Atherosclerotic lesion classification	Cause of death	Hypertension	Diabetes
3738	75	Male	Caucasian	VII	Perforated chronic duodenal ulcer	-	-
3739	90	Female	Caucasian	V	Ruptured abdominal aortic aneurysm	+	-
3909	47	Male	Caucasian	V	Pulmonary thromboembolism	+	-
3922	33	Male	Caucasian	I-II	Chronic hepatopathy	-	-
4175	70	Male	Caucasian	V	Pulmonary Tuberculosis	-	-

Human abdominal aortas (n = 5) were obtained *en bloc* during routine autopsies within 8 hours after death. Subsequently, the tissue samples were submitted to a careful dissection. Adjacent specimens were then excised with parallel blades, fixed with 10% buffered formalin, and stored at 4˚C until usage. It has been demonstrated that arterial tissue remains feasible for *post mortem* lipid analysis for up to 4 to 5 days if it is kept at this temperature (4). All proceedings followed a protocol approved by the institution's research ethics committee (CAPPesq/HCFMUSP).

The ^{13}C and ^{1}H High-Resolution NMR experiments were performed using a VARIAN spectrometer operating at 9.4 Tesla. A VARIAN 7-mm MAS double-resonance probe was used and the spinning speed was adjusted at 5 kHz. The ^{13}C and ^{1}H NMR spectra were obtained using a single Direct Polarization sequence (DP). Adamantane (29.2 and 38.3 ppm) and distillated water (4.7 ppm) were used as reference.

RESULTS

The presences of the cholesterol and cholesteryl ester in arterial tissues were confirmed by the comparison of experimental and simulated spectra. Figure 1-a and -b, respectively, show the ^{13}C and ^{1}H High-Resolution spectra in an increasing tissues degradation process as follows: 3922 (healthy sample), 41755, 3909, 3739 and 3738. In figure 1-a the one labeled as "bckgrn zirc&solv", presents the ^{13}C spectra associated with the solvent (buffered formalin, narrow line) used to keep the samples fresh and is present in all spectra. Also there is a broad line associated to the NMR sample holders, which was subtracted from all the spectra presented in figure 1-a. In region of 71 to 10 ppm (aliphatic carbons), the spectra show a complex set of peaks of different intensities, associated with the molecules of cholesterol and cholesteryl ester. Due to the semi-solid state of the samples and the characteristics of the NMR spectrometer, the spectra are rather broad making it difficult to association them precisely to different chemical group of the lipid molecules (cholesterol and cholesteryl ester).

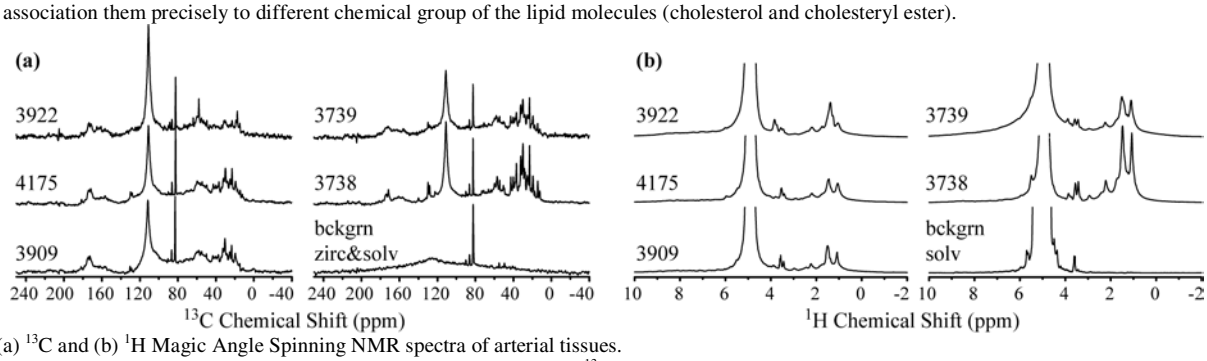

Figure 1: (a) ^{13}C and (b) ^{1}H Magic Angle Spinning NMR spectra of arterial tissues.

The experimental spectra do not contain the peaks presented in the simulated ^{13}C high resolution spectra of triglycerides and phosphatidyl-choline which suggest that either the concentration of these molecules in the atheromatous plaque is very low or the NMR technique used in this experiment is not adequate to observe the more rigid substances. Also, no evidence of collagen, which is one of the components of fibrous cap of atheromatous, was found.

Figure 1-b shows the ^{1}H High-Resolution NMR for the same samples in the same order. Again, the last one, labeled as "bckgrn solv", shows the ^{1}H spectra associated with the solvent (~ 3.6 ppm) and the water (broad line at ~ 5 ppm) which are present in all the samples.

The simulated ^{1}H spectra of cholesterol and cholesteryl ester molecules show a pattern of complex lines (multiplets and singlets due to the *J* coupling) in the range of 0.70 to 5.27 ppm, approximately. As can be seen in figure 1-b, there are peaks in this same range of chemical shift, which could be associated with these molecules. This hypothesis is supported by the fact that the ^{13}C spectra also confirm the presence of this kind of molecules.

The ^{13}C and ^{1}H NMR spectra were used to determine the content of cholesterol, cholesteryl ester and some other substances in atherosclerotic plaque. As can be seen in figure 1-a and -b the set of ^{13}C and ^{1}H spectra show an evident behavior, i.e., the intensity of the peaks associated with the cholesterol and cholesteryl ester molecules for both nuclei increase when the degenerative process of tissues increase.

CONCLUSION

As it is known the atherosclerotic plaque composition rather than degree of stenosis determines its instability. MRS can be used to discriminate if there is a specific compound responsible for the instability of these plaques. In our preliminary study we showed that the predominant substances in these plaques are cholesterol and colesteryl ester while triglycerides and phosphatidyl-choline are rare. We will follow our study using more sophisticated NMR techniques and more specimens hopping that with further study it is possible to discriminate the cause of plaque instability.

REFERENCES

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