LABORATORY INVESTIGATION

ATHEROSCLEROSIS

Time domain echo pattern evaluations from normal and atherosclerotic arterial walls: a study in vitro

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ABSTRACT The aim of this study in vitro of human fresh specimens was to quantitatively evaluate the contribution of the aqueous phase–intima interface (the first 400 msec of the reflected signal) in normal and atherosclerotic arterial walls. Seventy-five samples were studied, 15 normal, 15 fatty, 15 fibrofatty, 15 fibrous, and 15 calcific. A broadband transducer (4 to 14 MHz) was used. The aqueous phase–intima reflection (expressed in dB, mean ± SD) was lowest in the fatty plaques (−35.3 ± 2.5), differing in a highly significant way from that in all other groups: normal (−13.2 ± 8.8), fibrofatty (−20.4 ± 8.3), fibrous (−13.0 ± 9.7), calcific (−5.9 ± 3.4). The echo coming from the intima-media transition was of relatively low amplitude in normal and in fatty samples; typically, strong reflections from the intima-media transitions were present in the other pathologic subsets. In conclusion, the time domain echo pattern of the arterial wall may provide a useful clue to the structure of the plaque. Circulation 77, No. 3, 654–659, 1988.

ULTRASONIC tissue characterization of atherosclerosis has been attempted in several studies in vitro.1–12 The purpose of these studies has been twofold: (1) to test new variables of potential diagnostic use, and (2) to provide basic information for a better definition of limits and applicability of clinical echocardiography.

Findings in vitro show that, in predominantly fatty samples of aortic wall, the values of the internal backscatter tend to overlap with those found in normal walls.9 In this previous study, the value of the specular echoes of aqueous phase–tissue interface — which is supposed to be strongly angle dependent — was purportedly “gated out.” However, the detection of specular reflections is fundamental for the border identification with conventional echocardiographic instruments.13

The aim of this study was to quantitatively evaluate the contribution of the “first interface” intimal echo in various normal and pathologic subsets of arterial wall.

Materials and methods

Experimental procedure. Fresh specimens of arterial wall were taken from human aortas at autopsy and those with one of four distinct kinds of lesions (fatty, fibrofatty, fibrotic, and calcified) and with regions, whenever possible, of relatively normal tissue were chosen for study. The ultrasonic examination was started immediately after excision. Excised samples of aorta were cut down the anterior midline, opened flat, mounted on a sample holder (consisting of a rectangular metallic hollow rim with pins around its periphery), and placed in a distilled water bath at 20° C.

The sample holder is able to stretch the specimen in order to assume normal incidence for the interrogating beam. This device enabled the aortic sample to be stabilized while it was maintained in the focus of the transducer, thus avoiding interference with ultrasonic measurements. Once the ultrasonic study was completed, the tissue was removed from the water bath. Seventy-five aortic specimens (15 for each pathologic subset, see below) were therefore analyzed. In each specimen, four measurements were obtained with micrometer displacement of the transducer, the aim being to minimize phase cancellation effects.14, 15

Ultrasonic and pathologic characterization were separately performed by different individuals in a totally “blinded” fashion.

Ultrasonic technique. A single transducer (Aerotech, model gamma, 15 MHz nominal frequency, 0.5 diameter, 8.5 cm focal distance, 2 cm focal length) acting as both transmitter and receiver was used. Broadband characteristics were achieved by designing this transducer to operate over the frequency range of about 4 to 14 MHz on the left tail of the frequency response of the 15 MHz center frequency quartz plate. The use of a small diameter transducer reduced the phase cancellation artifacts caused by phase-sensitive transducers.14, 15 The ultrasonic beam width (4 mm evaluated at −3 dB) was determined at 10 MHz (continuous-wave signal) by means of a metallic Rayleigh reflector. The transducer was excited with a broadband pulser/receiver (Panametric, model 5052 PR). The received signal was captured with a transient recorder (Tektronik, model 7912 AD, conversion rate of 100 MHz, 9 bits of amplitude resolution).

The transducer was kept at a constant angle of 0 degrees with
respect to the perpendicular axis of the sample holder; a "normal" incidence of the ultrasonic beam over the tissue sample was therefore obtained.

During the acquisition, the gate length was fixed at 5 μsec to allow digitization of the signal from the full thickness of the specimen. A quantitative analysis was also performed on each ultrasonic record, under computer control, from the first 400 μsec of the reflected signals to allow evaluation of the purely "aqueous phase-intima" interface (400 μsec is the time length of the transmitted burst).

The peak-to-peak amplitude represents the greatest half cycle value computed on the radiofrequency signal within the 400 μsec analysis window.

For each specimen, a single value corresponding to the average of the four peak-to-peak values was obtained and assumed to be representative of that specimen.

The ultrasonic backscatter from the aqueous phase-intima interface was obtained with the tissue in a position similar to that of the stainless steel plate when it was inserted in place of the tissue sample.

**Pathologic characterization.** After ultrasonic measurements each aortic region was studied histologically with Weigert-van Gieson stain. As stated above, according to generally accepted criteria, five pathologic subset were identified: normal aortic walls, fatty plaques (characterized by accumulation of lipids in the intima), fibrofatty plaques (usually characterized by a fibrous cap and a lipid core), fibrous plaques (i.e., wall thickened by connective tissue), and calcified plaques (walls in which the atheromata were calcified).

With the use of metallic (highly echogenic) sample holder as a spatial marker, the transducer was oriented towards the center of the specimen. Therefore, only the central part of the specimen was histologically characterized. After histologic characterization, only specimens falling into one of the pathologic subsets described above were considered for analysis. Specimens with significant necrosis and hemorrhage were not included.

**Statistical analysis.** For each subset the mean value and the SD were measured. Differences were tested for significance by analysis of variance, with subgroup analysis by the Newman-Keuls test.

**Results**

Ultrasonic backscatter from the aqueous phase-intima interface in calcific plaques was greater than that in normal or fibrous tissue, and that in normal and fibrosis tissue exceeded that in fatty plaques (table 1).

A typical reflected signal from a representative sample from each of the five groups is shown in figures 1 to 5.

A relatively prominent "first interface" (aqueous phase-intima) echo was apparent in the normal (figure 1) and fibrofatty samples, and it tended to be even more prominent in fibrous (figure 4) and calcific (figure 5) specimens. In particular, in the calcific samples the calcium deposit was often covered by a fibrous layer. In our examples, the first interface echo was present, but the close calcific reflection was much greater (figure 5). The time-domain appearance of the fatty plaque was very peculiar, since only a small intimal reflection was apparent, and it was usually less prominent than the signal coming from the intima-media transition (figure 2).

**Discussion**

The appearance of the radiofrequency signal in the time domain supplies information potentially helpful for a better understanding of some clinical B scan patterns and in studies in vivo of tissue characterization.

A constant feature in the normal specimens is the presence of relatively strong aqueous phase-intima and media-adventitia interfaces that are spaced by the low-amplitude medial reflections.

This is consistent with the "double-line pattern" invariably observed on the typical B mode image of a longitudinally scanned normal artery wall.16 This pattern has been related to the presence of relatively sharp changes in acoustic impedance at the level of the luminal-intimal and media-adventitia transitions.16 The echo-generating properties of the adventitia, as compared with the relatively silent acoustic behavior of the media, may be related to its high collagen content, which has been shown to be largely responsible for the acoustical properties of the tissues.

In the fatty plaque, often no significant "first interface" echo may be detected. The peak-to-peak value in the first 400 μsec is significantly less than the corresponding value in the normal wall.

This first interface echo is particularly important for border identification, since a "leading-edge enhancement" is electronically performed by conventional echocardiographic instruments. The small echo reflected from the intima in the fatty plaque, together with the lower internal backscatter value,9 might explain

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**Table 1**

<table>
<thead>
<tr>
<th>Tissue group</th>
<th>First interface echo value (dB)</th>
<th>Comparison</th>
</tr>
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<tbody>
<tr>
<td>NL</td>
<td>-13.2±8.8</td>
<td>p&lt;.01</td>
</tr>
<tr>
<td>FA</td>
<td>-35.3±2.5</td>
<td>p&lt;.01</td>
</tr>
<tr>
<td>FF</td>
<td>-20.4±8.3</td>
<td>NS</td>
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<tr>
<td>FI</td>
<td>-13.0±9.7</td>
<td>NS</td>
</tr>
<tr>
<td>CL</td>
<td>-5.9±3.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

CL = calcific; FA = fatty; FF = fibrofatty; FI = fibrous; NL = normal.
FIGURE 1. Schematic representation of the ultrasonic and histologic appearance of a normal aortic wall. Left, The ultrasonic record in the time domain is shown; the amplitude value is normalized to the highest value ( = 1) displayed in figure 5. Middle, Schematic representation of the three layers of the aorta. Right, A histologic view of the entire wall: (1) Weigert-Van Gieson stain $\times 32$ (2) higher magnification of the subendothelial layer of the intima, and (3) higher magnification of the elastic membranes of the media (2 and 3, Weigert-Van Gieson stain $\times 80$). The timing marker is also shown.

FIGURE 2. Schematic representation of the typical ultrasonic and histologic appearance of a fatty plaque. Left, Ultrasonic record in the time domain. Middle, A schematic representation of the three layers of the aorta. Right, Histologic view of the plaque (the arrow indicates the intimal thickness). The timing marker is shown.
why soft plaques can be ultrasonically silent in the B scan image.

The possible occurrence of strong reflections from the intima-media transition of atherosclerotic plaques is consistent with the frequent occurrence, on the B scan image of atherosclerotic walls, of high-amplitude echoes between the inner and outer lines. This fact should also be considered as a potential source of error when trying to estimate the arterial wall thickness with B scan imaging,16, 17 and explains the less satisfactory correlation between the echocardiographic and histologic measurements of arterial wall in the presence of complex atherosclerotic plaques, which fail to exhibit the characteristic double-line pattern of the B scan picture.

Implications for characterization studies in vivo. From the presented data one might conclude that the luminal-intimal interface provides some information in addition to the internal backscatter, and it should not be necessarily gated out in studies in vivo of tissue characterization in which the radiofrequency signal is used.

**FIGURE 3.** Schematic representation of the typical ultrasonic and histologic appearance of a fibrofatty plaque. *Left,* Ultrasonic record in the time domain. *Middle,* A schematic representation of the three layers of the aorta. *Right,* Histologic view of the plaque (the arrow indicates the intimal thickness). The timing marker is shown.

**FIGURE 4.** Schematic representation of the typical ultrasonic and histologic appearance of a fibrous plaque. *Left,* Ultrasonic record in the time domain. *Middle,* A schematic representation of the three layers of the aorta. *Right,* Histologic view of the plaque (the arrow indicates the intimal thickness). The timing marker is shown.
There is also a theoretical reason for this, since the pathology of the plaque is always intimal, and therefore the exclusion a priori of the first interface echo implies the loss of some ultrasonic information, at least in some cases.

There is a further reason to "gate in" the intimal echo. The rationale for analysis of the internal backscatter only is its supposed angle independence. However, in arterial walls, even the internal backscatter is strongly angle dependent, and there is no particular reason for exclusion of the first interface echo.

This would also probably make easier the correct acquisition of an adequate sample volume of the arterial wall in vivo. Our findings apply to an experimental situation of aqueous phase–intima interface, but since the impedance of water is similar to that of blood (which replaces water in the interface occurring in vivo), these findings might apply to the situation in vivo.

However, extrapolation of these findings to conditions in vivo is fraught with many technical problems, including the necessity of perfect alignment of the ultrasound beam, attenuation caused by anatomic structures between skin surfaces and artery wall, etc. Furthermore, the primary lipids in atherosclerotic plaques are known to be cholesterol esters, most if not all of which change physicochemical properties above 20°C and below body temperature. The radiofrequency ultrasound signal might depend in part on the physical nature of the lipids present within lesions, and this appears to be temperature dependent, suggesting that the results of these experiments might be different if the temperature were increased to that of body temperature.

In conclusion, the first interface aqueous phase–intima reflection may provide a useful clue for the identification of the structure of the plaque in vitro.

References

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