Contrast-enhanced ultrasound imaging of periadventitial vasa vasorum in human carotid arteries

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Received 5 March 2008; accepted after revision 1 August 2008; online publish-ahead-of-print 29 August 2008

Aims Arterial vasa vasorum (VV) are known to be involved in the atherosclerotic process. The aim of the present study was to explore whether ultrasound imaging with contrast agent is able to visualize adventitial VV in human carotid atherosclerosis.

Methods and results We studied with standard ultrasound 25 patients with carotid stenosis >50% (ATS group) and 15 patients without carotid artery plaques and an intima-media thickness (IMT) <1.0 mm (CTRL group). All patients underwent contrast ultrasound to evaluate periadventitial VV and B-flow imaging (BFI) modality was used to improve and measure periadventitial flow signal. On contrast-enhanced images, a fast microbubble flow and a homogeneous and linear periadventitial contrast signal were detectable in the adventitial area in all patients of both groups. Periadventitial signal thickness by BFI was higher in patients with carotid atherosclerosis than in the control group (mean ± SD: CTRL 0.80 ± 0.06 mm; ATS 1.10 ± 0.11 mm; P < 0.001). Moreover, considering the whole study population, the adventitial signal thickness significantly correlated with IMT values (r = 0.88, r² = 0.77; P < 0.0001).

Conclusion Periadventitial contrast signal was detected in all patients and BFI thickness was higher in patient with carotid atherosclerosis and correlated with IMT.

KEYWORDS Contrast ultrasound; Vasa vasorum; Atherosclerosis; Carotid artery; Imaging

Introduction

Vasa vasorum (VV) are a plexus of microvessels physiologically located in the adventitia of most medium and large arteries, including the aorta and coronary, carotid, and femoral arteries.1 The presence of VV is essentially related to arteries and veins nutritional needs.

The possible involvement of VV in atherosclerosis was originally introduced in 1876 by Köester2 and then in 1938 by Winternitz et al.3 and was revived by Barger et al. in 1984,4 who clearly showed in post-mortem samples that coronary atherosclerotic segments presented a rich vascular network extending from the adventitia to the full thickness of media and intima.

Neangiogenesis originating from the adventitial VV of the artery and extending into the atherosclerotic plaque represents a pathological ectopic neovascularization,5 which may contribute to the progression of the atherosclerotic lesion from an asymptomatic fibrotic plaque to a lesion with a high risk of rupture (‘vulnerable plaque’), by favouring leucocyte recruitment and intraplaque hemorrhages.6–12

By using the three-dimensional micro-computed tomography (CT) technique, Kwon et al.13 have demonstrated in an experimental model of hypercholesterolaemia an increase in the density of adventitial VV even before the development of the atherosclerotic lesion in porcine coronary arteries. Notably, Herrmann et al.14 have demonstrated that such an increase in the density of adventitial VV occurs before the onset of endothelial dysfunction, which is considered one of the first functional alterations in the atherosclerotic process.

Microbubbles are widely used in cardiovascular imaging. Ultrasound imaging with contrast agent has been recently used in the carotid arteries for the assessment of the vessel lumen, improving the visualization of the luminal profile of the plaque and of intima-media thickness (IMT).15,16 Moreover, the possibility of using contrast ultrasound-enhanced imaging to study the neovascularization of the atherosclerotic plaque is very appealing.17
Recently, Vicenzini et al. described the visualization of contrast microbubbles within carotid plaques as a marker of neovascularization, even though no systematic histological validation was performed. Shah et al. reported a good correlation between carotid contrast ultrasound imaging of intraplaque neovascularization and a semiquantitative histological score on surgical specimens.

Furthermore, in a recent study performed by our group, a good correlation between the intraplaque ultrasound contrast enhancement and the quantitative histological neovessel density has been demonstrated. Although previous studies were focused on intraplaque neovessels, the aim of the present study was to assess the possibility of visualizing in vivo adventitial neovascularization associated with atherosclerosis by contrast ultrasound.

Therefore, we evaluated the presence of periadventitial contrast enhancement, both in pulse inversion and in B-flow images (BFI), as a possible marker of the VV network, both in patients with and without carotid atherosclerosis.

**Methods**

**Study population**

Between March 2005 and October 2005, we enrolled in our study 25 patients with carotid atherosclerosis (ATS group), who were admitted to our Cardiovascular and Thoracic Department in order to undergo an ultrasound study of the epiarticular arteries. Only patients with carotid stenosis at least >50% were included in the group. Subjects with allergy to blood derivatives and/or human albumin were excluded from the study due to the potential risk of developing allergy to the ultrasound contrast agent.

Furthermore, a control group was enrolled, including 15 patients (CTRL group) with no carotid stenosis and IMT < 1 mm at the echo-colour Doppler, i.e. subjects without echographic signs of atherosclerosis. The two populations were matched for age, sex, and clinical features.

Age, sex, and body mass index (BMI) of each patient were noted along with any traditional risk factors such as hypercholesterolaemia (defined as the presence of total cholesterol >200 mg/dL), arterial hypertension, diabetes mellitus, active smoking, cardiovascular history, and any ongoing drug therapies.

The protocol was approved by the ethical committee of Vita-Salute San Raffaele University and all patients were informed about the study characteristics and gave their consent to ultrasound imaging with contrast medium.

**Standard and contrast-enhanced carotid ultrasound**

All patients underwent a standard echo-colour Doppler examination of extra-cranial carotid arteries (common carotid artery, internal and external carotid artery) by means of a high-frequency linear probe (Vivid 7–GE Medical Systems, GE Healthcare, Chalfont St. Giles, United Kingdom, with linear probe 7 MHz). The severity of the stenosis was assessed by complying with the guidelines set by the 2003 Consensus Conference of the American Society of Radiology.

Moreover, we measured the IMT of the far wall at the level of the common carotid artery proximal to the bifurcation bilaterally. We used the mean value obtained from five measurements for our analysis.

All patients were also submitted to contrast-enhanced ultrasound imaging. As sonographic contrast agent, we used Optison® (GE-Amersham Health), which is composed of microbubbles of human albumin containing perfluoron. Optison has been approved by the FDA and by EMEA for clinical use in echocardiography. A vial of Optison was diluted with saline up to 10 mL (Optison 3 mL + saline 7 mL) and then a 2 mL bolus was injected and repeated as needed through a peripheral vein. Good images could be obtained for ~2 min after each bolus. No patient received a dose >6 mL of Optison, which is far lower than the maximum recommended dose of 8.7 mL.

Contrast ultrasound studies were performed with the same Vivid 7 device, equipped with a 7 L linear probe, using a dedicated real-time contrast imaging modality, based on the pulse inversion principle, which enhances the signal from sonographic agents compared with tissue signal. In all cases, we used a low mechanical index (0.08–0.10) and the image was optimized by modifying dynamic range, persistence, and filter settings.

The contrast study was completed by using the BFI method for the assessment of periadventitial VV. BFI is a non-Doppler technique based on coded ultrasounds, which enhances signal coming from moving reflectors, such as red blood cells and contrast microbubbles, thus depicting blood flow, with a high frame rate and a high spatial and transverse resolution. This, along with the possibility of automated background subtraction, improved our ability to strictly focus on periadventitial flow signal. After automated background subtraction, the image was then optimized with the lowest gain allowing to obtain a homogeneous flow image in the main lumen and periadventitial area. After the examination, the patients were monitored for 30 min.

The ultrasound examination was performed by one of the researchers (S.C.). All the studies were digitally stored for a subsequent off-line analysis. At first, quality and baseline features of standard and contrast-enhanced images have resulted from a consensus decision by two readers (S.C. and M.M.), particularly the presence of a signal coming from contrast microbubbles in pulse inversion images was carefully evaluated. In a subsequent separated session, in BFI images, the thickness of the rim of homogeneous flow signal close to the adventitia was measured at the level of the far wall of the common carotid artery (BFI thickness), by a single researcher (M.M.) in blind approach with regard to the clinical data. Five measurements were carried out bilaterally and the mean value was then employed for the analysis. This parameter was taken as a possible indicator of the extension of the network of adventitial VV.

The intraobserver reproducibility was determined from repeated measurements of all subjects included in the study. According to the method described by Bland and Altman, the 95% limits of the agreements (corresponding to the ± 2SD of the difference between the first and the second examination) varied between 0.131 and 0.134 mm.

**Statistical analysis**

Data analysis was carried out by a single investigator unaware of the patient features. When variables had normal distribution, the values were expressed as mean ± standard deviation (SD). In order to identify the differences between the groups of parameters, we employed the t-test for non-coupled data according to the relevant applications. Statistical significance was defined as P < 0.05. Linear correlation was used to assess the relationship between variables.

**Results**

The two populations were similar with regards to age, gender, BMI, and traditional risk factors distribution (Table 1).

As expected from control population inclusion criteria, the median values of IMT in the CTRL group were significantly lower compared with the ATS group (mean ± SD: CTRL 0.73 ± 0.13 mm; ATS 1.08 ± 0.14 mm; P < 0.001).

After the administration of the sonographic contrast agent, we obtained an excellent opacification of the main
vascular lumen and an improved definition of the luminal profile, beginning ~30 s after the injection and lasting ~2 min. No side effects after contrast infusion were observed, the exam was well tolerated and good quality images were available in all patients both for standard and for contrast ultrasound.

In all patients of both groups, longitudinal scans during pulse inversion contrast imaging showed a signal coming from microbubble flow near the external part of the carotid wall and parallel to the major axis of the vessel, partially superimposed with the bright echo signal corresponding to the adventitial layer. Only linearly moving echogenic spots were considered as a sign of contrast bubbles flow within VV. The use of BFI modality with background subtraction provided a sufficient intensification of contrast signal with suppression of the adventitial tissue for the quantitative analysis in all patients of both groups (Figure 1). With this modality, a flow signal coming from the main lumen could be observed, along with an adjacent, longitudinal rim of flow signal in the periadventitial area, which was particularly well defined in the far wall region of the vessel, where its thickness was measured. This parameter was taken as a possible indicator of the local extension of the periadventitial network of VV.

As shown in Figure 2, mean values of BFI signal thickness (BFI-T) were significantly higher in the ATS group than in the CTRL group (mean ± SD: CTRL 0.80 ± 0.06 mm; ATS 1.10 ± 0.11 mm; P < 0.001; Figure 2).

In the combined ATS and control groups, we observed a statistically significant correlation between IMT and BFI-T (r = 0.88, r² = 0.77; P < 0.0001; Figure 3).

Discussion

Pathological studies have demonstrated that atherosclerotic plaque neovascularization does not originate from the main arterial lumen, but from the adventitia, where a network of VV physiologically exists.6

Previous studies on contrast ultrasound imaging of VV have mainly focused on intraplaque neovascularization; to our knowledge, this study represents the first attempt to identify the network of periadventitial VV in human carotid arteries with ultrasound imaging by a combined use of sonographic contrast agent and BFI.

Table 1 Baseline characteristics of the study population. Values of age and body mass index are expressed as mean ± standard deviation

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline characteristics of the study population. Values of age and body mass index are expressed as mean ± standard deviation</th>
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<tbody>
<tr>
<td>Patients, n</td>
<td>Control group</td>
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<tr>
<td>Age</td>
<td>64.5 ± 11.7</td>
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<td>Male (%)</td>
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<td>Diabetes (%)</td>
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<td>BMI (kg/m²)</td>
<td>25.5 ± 2.44</td>
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Figure 1 Contrast-enhanced images of the common carotid artery in patient without atherosclerosis (A) and in patient with carotid atherosclerosis (C) and relative contrast-enhanced images combined with B-flow images (BFI) (B and D, respectively). In all panels, the asterisk marked the carotid artery lumen, and the IMT was marked with the open arrow. The solid arrow marked periadventitial tissue contrast enhancement in A and C, and the BFI thickness in BFI images (B and D).
Contrast microbubbles behave as pure intravascular tracers and they have already been used to study tissue perfusion in the myocardium and other organs and to study tumour angiogenesis, thus supporting the concept that contrast signal detected in periadventitial area in our study can result only from microbubbles flowing through vascular structures, i.e. the local VV network.

Interestingly, the correlation observed between BFI-T and IMT is consistent with the dynamic association of adventitial VV hyperplasia with developing atherosclerosis, previously reported in animal models and post-mortem studies. A persistently increased arterial wall thickness; therefore, changes in neovascularization could occur earlier than IMT reductions and contrast ultrasound could be able to monitor this phenomenon in the clinical setting. Indeed, one case of regression of VV after aggressive statin therapy in a diabetic patient has been previously described by Feinstein and the interest in the potential achievement of plaque progression reduction with angiogenesis inhibitors is growing up.

**Conclusions**

By enabling the visualization of periadventitial VV, the study of carotid atherosclerosis by contrast ultrasound and BFI may represent a useful tool for non-invasive characterization of patients with carotid atherosclerosis. Notably, in view of the association of VV proliferation with the development of atherosclerosis, contrast ultrasound imaging may provide and additional surrogate marker beyond IMT to characterize the disease and to assess the response to anti-atherosclerotic therapies.

**Conflict of interest:** S.C. has received consultancy honorarium from GE Healthcare. S.B.F. has received speaking honorarium from KOS Pharmaceutical, Takeda Pharmaceutical and GE Health Care, and a research grant from Takeda Pharmaceutical. The other authors have no commercial associations or conflict of interest in connection with the submitted article.

**References**


Micro-CT has been widely employed to study the anatomy of VV both in physiological and in pathological conditions, but its use is limited to the experimental settings. Magnetic resonance imaging with gadolinium infusion has been employed in humans to evaluate carotid plaque VV, according to a kinetic model of image intensity; a good agreement with histology on surgical specimens was found. A similar approach has been used to derive indexes of the extension of carotid adventitial VV. Imaging of coronary VV appears also to be possible with the combination of intravascular ultrasound and contrast and initial experience in humans has recently been reported.

IMT is currently the most widely used surrogate marker of atherosclerosis, but it represents only an anatomical marker and demonstrates only small changes in atherosclerotic regression trial and does not reflect the phenotypic variability of the atherosclerosis.

Thus, the assessment of the arterial neovascularization by contrast ultrasound might become a novel marker beyond IMT for measuring the result of antiatherosclerotic therapies. Experimental studies have shown that plaque neovascularization can regress and that this can happen, despite a persistently increased arterial wall thickness; therefore, changes in neovascularization could occur earlier than IMT reductions and contrast ultrasound could be able to monitor this phenomenon in the clinical setting. Indeed, one case of regression of VV after aggressive statin therapy in a diabetic patient has been previously described by Feinstein and the interest in the potential achievement of plaque progression reduction with angiogenesis inhibitors is growing up.


