The use of Contrast Enhanced Ultrasound in Carotid Arterial Disease

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Abstract Traditionally, stroke risk stratification has centred on the degree of internal carotid artery stenosis, and the presence of focal neurological symptoms. However, degree of stenosis alone is a relatively poor predictor of future stroke in asymptomatic patients; the Asymptomatic Carotid Surgery Trial highlighting the need to identify a subgroup of asymptomatics that may benefit from intervention. Attempting to define this subgroup has inspired imaging research to identify, in vivo, high-risk plaques. In addition to pre-operative risk stratification of carotid stenosis, contrast enhanced ultrasound (CEUS) may be employed in monitoring response to plaque-stabilising therapies.

Unlike most contrast agents used for computed tomography and magnetic resonance imaging, microbubbles used in CEUS remain within the vascular space and can hence be used to study the vasculature. In addition to improving current carotid structural scans, CEUS has potential to add extra information on plaque characteristics. Furthermore, by targeting microbubbles to specific ligands expressed on vascular endothelium, CEUS may have the ability to probe plaque biology.

This review describes the current carotid ultrasound examination and the need to improve it, rationale for imaging neovascularisation, use of CEUS to image neovascularisation, microbubbles in improving the structural imaging of plaque, potential problems with CEUS, and future directions.

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Introduction

Microbubble-based contrast agents are injected intravenously to enhance ultrasound scans, and are composed of 1–10 μm diameter albumin or lipid shells filled with air or high molecular weight gas. Their small size allows them to recirculate, whereas larger bubbles are retained in the pulmonary circulation.

The discovery that gas filled bubbles can act as ultrasound contrast agents was initially made because the density change from blood to intrabubble gas causes reflection of sound waves, just as the density change from air to cliff-face creates an audible "echo". However, modern ultrasonic methods of distinguishing microbubble from native tissue rely on the fact that ultrasound waves cause microbubbles to compress and expand, whereas tissue is virtually incompressible.1 By fortunate coincidence, the resonance frequency for microbubbles (the frequency at which compression and expansion occurs most readily) is within the range of frequencies used for clinical ultrasound. Sound waves of very low energy, therefore, will return detectable signal from microbubbles, provided the transmitted wave is around the microbubble resonance frequency. Native tissue, however, will not respond to such low energy waves.

Unlike most contrast agents used for computed tomography (CT) and magnetic resonance imaging (MRI), microbubbles remain within the vascular space and are hence well suited to study the vasculature. By improving visualisation of the lumen, contrast enhanced ultrasound (CEUS) can improve current carotid structural scans. Furthermore, CEUS may be able to add extra information on plaque characteristics, such as neovascularisation, deemed to elucidate plaque instability. In addition, manufacturing microbubbles targeted to specific vascular endothelial ligands, CEUS may have the ability to probe plaque biology.

The current carotid ultrasound examination

With the publication of the results of the European Carotid Surgery Trial (ECST)2 and the North American Symptomatic Carotid Endarterectomy Trial (NASCET),3 the importance of accurate assessment of the degree of internal carotid artery diameter reduction has been highlighted. There has been extensive work undertaken in the generation of reliable and reproducible criteria for the calculation of internal carotid artery stenosis using unenhanced duplex ultrasonography. This is based on largely velocity criteria, is standardised and been used in the creation of national and international society consensus documents.4–8 Duplex ultrasonography is likely to remain the first line imaging modality in this context5; as such, much work has been undertaken in its refinement.

The need to improve the current ultrasound assessment

Traditionally, stroke risk stratification has revolved around the degree of internal carotid artery luminal narrowing, and the presence of recent focal neurological symptoms pertaining to the ipsilateral cerebral hemisphere. However, it has become apparent that the degree of stenosis alone is a relatively poor predictor of future stroke in asymptomatic patients. Indeed, the Asymptomatic Carotid Surgery Trial (ACST)9 highlighted the need to identify a subgroup of asymptomatics that would benefit from intervention.

The attempt to define this subgroup has inspired research into imaging modalities which can identify, in vivo, plaques at high risk of causing acute cardiovascular events. Histological and functional techniques have determined the features of such plaques, including: inflammation; extra-cellular matrix degradation; neovascularisation; intra-plaque haemorrhage; and apoptosis.10–12

Imaging techniques to identify these biological features of the plaque in vivo could improve risk stratification, facilitating clinicians’ treatment decisions and monitoring response to plaque-stabilising therapies. To this end, imaging intra-plaque inflammation, neovascularisation, haemorrhage and apoptosis have been attempted with some success using MRI and/or nuclear medicine. However, high prevalence of carotid disease and expense associated with these techniques preclude them from replacing ultrasound as first line investigation.13–17

Using (non-contrast enhanced) ultrasound to identify plaque constituents has been attempted with "Gray-Scale Median" (GSM) evaluation. Studies in which patients were imaged prior to surgical endarterectomy have consistently demonstrated that plaques which are echolucent on ultrasound, with a low GSM, have high lipid, haemorrhage and macrophage on histology. Conversely, echogenic plaques, with a high GSM, have a higher fibrous content.18–22 GSM is also associated with clinical findings: evidence of cerebral infarction on CT is more common in the presence of echolucent plaques rather than echogenic, regardless of symptomatic status.22,23 Echogenicity on ultrasound has also been shown to predict ipsilateral ischaemic stroke; patients with echoluent plaques are at increased risk compared to those with echogenic plaques.24–26 However, hazard ratios for development of stroke that are associated with differing echogenicity scores are not sufficiently great to warrant translation into clinical practice. Furthermore, studies investigating the use of GSM in selection for carotid artery stenting (CAS) have had conflicting results. The Imaging in Carotid Angioplasty and Risk of Stroke (ICAROS) study revealed that high echolucency increases risk of stroke as a complication of CAS.27 Subsequently, Reiter and colleagues showed no such relationship between plaque echolucency and stroke risk with CAS.28

Imaging neovascularisation: the rationale

Imaging intra-plaque neovascularisation may be a means by which ultrasound can identify the high-risk plaque. In healthy large vessels, the vasa vasorum runs through adventitia and outer media,29 penetrating intima only in pathology.30 Recent work has afforded a central role to intra-plaque neovascularisation in initiation, progression and rupture of atherosclerotic plaques.12,31–38 In animal models, progression of disease can be reduced if neovascularisation is inhibited with angiotatin,39 and growth enhanced by administration of Vascular Endothelial Growth Factor (VEGF).40
Associations between human plaque vulnerability and angiogenic activity were originally noted in the late 1980s. Subsequently it was found that plaques bearing hallmarks of vulnerability, including inflammation, haemorrhage, lipid accumulation and thin fibrous caps, are also associated with increasing neovascularisation. Neovascularisation appears to be an early feature of atherosclerosis, predating macrophage infiltration, and as the plaque progresses so too does intimal neovascularisation. The majority of studies show carotid plaques retrieved at endarterectomy have many more microvessels and transcripts known to promote neovascularisation when they originate from symptomatic, compared to asymptomatic patients. Furthermore, symptomatic plaques have been shown to contain abnormal, immature vessels that may precipitate plaque instability through their acting as sites of vascular leakage and so inflammation.

Contrast enhanced ultrasound to image neovascularisation

Limiting the success of MRI in imaging plaque neovascularisation is the ceiling on imaging timepoints and the fact that the contrast agent leaks from the vascular space. CEUS does not suffer from these limitations and allows quantification of vessels measuring less than 100 μm in diameter. Unlike CT or MRI contrast agents, microbubbles remain within the vasculature and act as “surrogate red blood cells”; hence they are true intravascular tracers which can be imaged in real time. Combined with high temporal and spatial resolution of ultrasound, CEUS is well placed to study neovascularisation (Fig. 1).

Hitherto, three groups have correlated CEUS imaging results with histological plaque neovascularisation. Feinstein reported a moderate correlation value of 0.64; Coli reported an increase in neovascularisation in patients demonstrating extensive enhancement; Giannoni et al. reported diffuse contrast uptake in plaques from symptomatic patients, all of whom had increased number of microvessels confirmed on histology. The latter group described the common presence of small vessels within plaque underlying ulcerations. Up until this point, these three groups used subjective visual assessment made by human readers to ascribe binary or discreet scores to the imaging findings—quantification can be improved by generating time-signal intensity curves (Fig. 2) and using automated image analysis to produce a continuous variable. This approach has been demonstrated by Xiong and colleagues who studied 104 carotid stenoses, revealing that plaque enhanced intensity and the intensity normalised against carotid luminal intensity were both significantly greater in symptomatic versus asymptomatic atherosoma.

An alternative approach is to investigate periadventitial, rather than intra-plaque, vasa vasorum. A recent study compared quantified B-flow imaging (BFI) CEUS of peri-adventitial vasa vasorum in patients with atherosclerotic carotid stenosis compared with control carotids, showing a significant difference as well as a correlation of BFI with intima-media thickness (IMT).

Future work will have to address current unanswered questions about how best to quantify microbubble signal, particularly how normalisation can account for administered contrast dose and pharmacokinetic factors. Acquisition techniques and methods of image analysis need to be standardised, including in motion correction. Work with flow phantoms may help with these issues. Validation of the technique against a standardised clinical score, such as the modified Rankin, Barthel or Frenchay, is required. Alternatively, comparison with a standardised plaque histological score can be undertaken, such as that produced by the American Heart Association in 1995 or a modification.

Microbubbles to improve structural imaging of plaque

Further to potentially providing information on neovascularisation as a biological feature of the atherosclerotic plaque, CEUS has been shown to increase accuracy, sensitivity and specificity of the structural disease assessment. The concept of “Doppler-rescue” has emerged, whereby contrast is introduced during an US examination at the point of failure to obtain diagnostic images with unenhanced scanning. It should be stated that in experienced hands, “Doppler-rescue” is rarely required and this, generally, would indicate the need for alternative imaging. This should also be considered against the recent advances in imaging technology. Furthermore, CEUS has been shown to be of use in the depiction of unsuspected wall irregularities, ulceration, and dissection, as well as improve the resolution of IMT.

Potential problems with contrast enhanced ultrasound

A small number of microbubbles will be destroyed by the incoming ultrasound wave, and when this occurs capillary damage may ensue. This in turn recruits vascular endothelial growth factor (VEGF)-producing inflammatory cells, stimulating neovascularisation. This phenomenon may be a deterrent in the use of CEUS in diagnostics due to the
theoretical risk of potentiating plaque instability by promoting angiogenesis. However, at the low energies employed for CEUS, very few microbubbles will be affected and this is therefore a theoretical risk.

The undesirable effects that have been associated with SonoVue\textsuperscript{\textregistered}, the most commonly used microbubble in the UK, were in general, non-serious, transient and resolved spontaneously without residual effects. In clinical trials, the most frequently reported adverse reactions were headache (2.3%), injection site pain (1.4%), and injection site bruising, burning and paraesthesia (1.7%).\textsuperscript{66} Fatal adverse events (AEs) have occurred following the administration of microbubbles. In the case of SonoVue\textsuperscript{\textregistered}, approximately 160,000 doses have been administered and 3 deaths have been temporally related, although causal relationship was uncertain. This fatal AE rate is an order of magnitude higher than for MRI and CT contrast agents, probably reflecting the comorbidities of the recipients (microbubbles are commonly used in high-risk patients for echocardiography).\textsuperscript{67}

It is important to consider the cost related to the use of ultrasound contrast agents. Although no formal health economic assessment has been undertaken for the use of microbubbles in carotid arterial disease, the technique has been described as being cost-effective in the context of gastrointestinal imaging.\textsuperscript{68}

**Future directions for contrast enhanced ultrasound**

Current microbubbles are used as blood pool agents because, for all intents and purposes, they remain in the vascular space. However, over time they have been shown to be passively taken up by macrophages, such as Kupffer cells in the liver, with the rate of phagocytosis being a function of their shell’s constituents.\textsuperscript{69} Conjugation of microbubbles with ligands creates the possibility of actively targeting to specific molecules accessible from the vascular...
space. Kaufmann et al. have quantified vascular inflammatory changes occurring at different stages of murine atherosclerosis utilising microbubbles targeted to vascular cell adhesion molecule-1 (VCAM-1).70 Similarly, microbubbles have been surface conjugated with the disintegrin echistatin which binds to αv- and α5β1-integrins expressed by the endothelium of neovessels.71 These tailored microbubbles have been used to assess endogenous and fibroblast growth factor-2-induced therapeutic neovascularisation in a rodent model of hindlimb ischaemia. Such molecular imaging with targeted microbubbles offers the possibility of early pharmacodynamic readouts for drugs in development, assisting drug discovery in the field of vascular inflammation.

The carotid plaque has been imaged in 3-dimensions (3D) with unenhanced ultrasound.72,73 The development of contrast modes on high frequency 3D transducers offers the opportunity to image carotid plaques in their entirety during a single microbubble acquisition. This potentially addresses two issues: out of plane motion for which there is no solution in 2-dimensional (2D) CEUS bar the exclusion of affected frames; and the false assumption that the single slice imaged by 2D CEUS is representative of the entire inhomogeneous plaque. As it is practically impossible to select the same plaque slice for imaging over two sessions using 2D CEUS, 3D imaging should improve reproducibility and monitoring of therapy.

In addition to imaging, microbubbles may have a future role in enhancing selectivity for delivery of therapeutics. Ultrasound itself can improve uptake of drugs by causing cavitation in the local cell membrane or increasing capillary permeability, but the energy levels required to do so exceed safety limits.74 However, by using ultrasound to destroy microbubbles (ultrasound targeted microbubble destruction (UTMD)) the amount of energy required to create these local pores is reduced to within acceptable limits. Moreover, if microbubbles are loaded with a drug of interest, either on their surface or within the shell, UTMD limits. Moreover, if microbubbles are loaded with a drug of interest, either on their surface or within the shell, UTMD can further improve the selectivity of the drug delivery system.74–76

Conclusion

The paradigm shift in our understanding of features of carotid atherosclerotic plaque instability has changed the focus of carotid imaging from assessing structure (i.e. degree of luminal narrowing) to exploration of the plaque biology. To this end, the advent of 2D CEUS presents the possibility of imaging plaque microvasculature, a proven hallmark of vulnerability.

The application of CEUS for carotid atherosclerosis primarily lies in the pre-operative risk stratification of patients with asymptomatic stenosis, and monitoring the response to plaque-stabilising therapies. However, prior to CEUS entering the clinical arena in this context, it is essential that quantification techniques are refined and internationally standardized, and that prospective studies are performed to elucidate the natural history of the evolution and involution of plaque microvasculature. This will include the establishment of appropriate time intervals for follow-up scanning, and the full health-economic assessment thereof.

Conflict of Interest

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None

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