Chlamydia Pneumoniae and Carotid Plaque Morphology: The Link with Ischemic Stroke

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Background

There has been some anecdotal evidence linking certain infectious pathogens (such as Chlamydia pneumoniae) with stroke. C. pneumoniae infection may be one of several contributing factors for the development of inflammation of blood vessels and atherosclerosis. Furthermore, a number of inflammatory markers (such as plasma CRP, fibrinogen, IL-6, IL-1ra, ESR, WCC) have recently been linked with acute ischemic stroke. However, their value in predicting short and long-term prognosis has been questioned and deemed to be not useful in defining outcomes due to the lack of evidence and cost implications.

Aims

The aims of this study are to (i) investigate the presence of C. pneumoniae in both echogenic and echoluent carotid plaques; and (ii) explore their role in the destabilization of the plaque, leading to embolization and cerebrovascular events.

Methods

(i) Study design: A prospective study involving carotid plaque specimens collected from routine endarterectomy surgical operations. Carotid plaques were removed en bloc during surgery to preserve the entire plaque structure.

Carotid plaque samples: Twenty specimens of carotid atherosclerotic plaques (ten predominantly echolucent, Types I & II and ten predominantly echogenic Types III & IV) were used. The non-invasive ultrasonic appearance (i.e. echogenicity or echolucency) and the quantitative grading of these plaques were determined pre-operatively using duplex scanning aided by computerized measurements according to the criteria adopted by Gray-Weale et al. (1988). (iii) Immunohistochemistry: Levels of C. pneumoniae, Matrix Metalloproteinase-3 (MMP3), Nitric Oxide Synthase (NOS), Superoxide Dismutase (SOD) enzymes in echogenic (fibrous) and echolucent (lipid-laden) atherosclerotic carotid plaques were determined using immuno-histochemical and Western blotting techniques. (iv) Histological methods: Haematoxylin-Eosin stain was performed to stain the slides that will be used for morphological studies by light microscopy. For Laser Scanning Confocal Microscopy (LSCM), slides were stained with saturated aqueous fluorescein for a few minutes and then dehydrated, cleared and mounted in DPX. Carotid plaque specimens were first examined by light microscopy (at magnification 10, 40 times) to characterize the following histological features: necrosis, calcification, fibrous cap, erosion or ulceration, hemorrhage, fibrous tissue and lipid content. Sections from the same specimens were then analyzed using LSCM; slides were viewed with 488 excitation and 515 LP emission. The presence or absence as well as the pattern of distribution of these markers (proteins) were correlated with the histological and preoperative ultrasonic appearances of the plaques.

Results
Ultrasound imaging of carotid plaques revealed 10 with echolucent (lipid-laden/unstable) properties and 10 with echogenic (fibrous/stable) features. Intense immune-reactivity for C. pneumoniae was observed in two thirds of echolucent plaques, clustering near endothelial cells of the intimal region, and in the neo-endothelial regions of the specimens. By contrast, low level and scattered C pneumoniae immune-reactivity was observed in echogenic plaques. MMP-3 (stromelysin) level was higher in echolucent than in echogenic plaques. High levels of this enzyme were observed near regions of ulceration, necrosis and in areas where the fibrous cap was thin or torn. Isoforms of NOS enzymes were seen in all carotid plaques irrespective of intra-plaque features however, levels of inducible NOS (NOS-II) were higher in echolucent than in echogenic plaques. Higher levels of immune-reactive SOD were also observed in plaques with higher degree of stenosis (more than 75%–80% measured by ultrasound scan). No direct relationship was evident between the neurological features experienced by the study group and the immuno-histochemical findings. There was however a relationship between the morphology of the plaque and the immuno-histochemical results. Both bright-field microscopy and Laser Scanning Confocal Microscopy (LSCM) were used to generate 3D images of surgically removed carotid plaques. 3D imaging of carotid plaques, using LSCM showed that most specimens were predominately composed of lipid material, comprising necrotic core of amorphous debris and cholesterol clefts, with varying degrees of fibrous tissue present in all plaques. Regions of actual fibrous cap disruption and some ulceration were also seen. Fraying of the fibrous cap was notable with fibrous cap erosion and exposure of underlying necrotic core to lumen. The extent of breaks in the fibrous cap varied with each plaque. Evidence of carotid plaque vulnerability (to rupture) was demonstrated by reduced fibrous cap thickness, a large lipid-necrotic core, and increased inflammatory cells infiltrate with evidence of cracking.

Discussion
Although the findings of this study are suggestive that C. pneumoniae may be involved in the worsening and destabilization of the carotid atherosclerotic plaque, more evidence is needed to ascertain whether C. pneumoniae infection may be an independent, modifiable risk factor for ischemic stroke. Results of this study also showed that MMP3 or its pro-enzyme may play an important role in digesting fibrous tissue of the plaque, leading to thinning or tearing of the fibrous cap. This might result in subsequent destabilization of the plaque, leading to embolization and cerebrovascular events. Furthermore, increased level of SOD expression in carotid plaques might be linked to the degree of stenosis of the affected carotid artery. Enzymes identified in this study may have been influenced by (i) inflammatory conditions prevailing in these plaques (e.g., C. pneumoniae might be responsible for triggering cascades of inflammatory events leading to activation of these enzymes), or (ii) shear stress which might be responsible for stimulation of these enzymes. The 3D geometry of carotid plaque also showed evidence of shearing stresses, resulting
from fluid flow which might contribute to the degree of instability of the plaque. This is a complex process which might also include the mechanical activation of enzymes such as matrix metalloproteinases, nitric oxide synthases and Superoxide Dismutase. The behavior of these enzymes in response to fluctuations in the velocity field in both echogenic and echolucent plaques is yet to be investigated. Possible activation of these enzymes by fluid dynamical instabilities and the implications of such activation on the destabilization and progression of atherosclerotic plaques require further investigation. Once the role of 3D geometry and microrheology on plaque stability has been investigated and quantified, more opportunities will be available for translating the results into clinical applications.

Conclusion
There is a need for stronger evidence to assist in the diagnosis, treatment, and secondary prevention of stroke in patients in whom an infectious cause for stroke is probable. It has been suggested that mechanically induced or flow-sensitive enzymes contain positive and negative shear stress response elements in their encoding genes and can be stimulated or suppressed according to the nature of blood flow. However, it is still not possible to show any definite link between specific enzyme(s) and cerebrovascular events. Such an association is probably multifactorial and mediated by a combination of the degree of stenosis, plaque morphology, dynamic and biological factors. We are currently investigating the link between certain inflammatory markers and cerebral blood flow and cerebral auto-regulation in patients presenting with Transient Ischemic Attack (TIA or mini stroke) for the first time to explore further the role of inflammation in stroke. This study has just started; it involves 200 patients recruited from Qatar and UK. One of its outcomes will be to correlate impaired cerebral auto-regulation with a range of pro-inflammatory and anti-inflammatory markers in first time TIA patients. In doing so, we will study the profile, rather than just the level, of these inflammatory markers in each patient, taking into account the time lapse between onset of symptoms and the collection of blood specimen (for cytokines analysis) from each patient. “Part of this study was made possible by grant NPRP 6 - 565 - 3 - 141 from the Qatar National Research Fund (a member of Qatar Foundation). The statements made herein are solely the responsibility of the author[s].”