X-Ray Imaging of Intraplaque Hemorrhage in Aortas of ApoE^-/-/LDL^-/- Double Knockout Mice

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Aims:
Pathologic neovascularization is a consistent and well-known feature of plaque development and progression (1-4). In the event of a plaque hemorrhage it is presumed that ectopic neovascularization as well as vasa vasorum might be the source of the hemorrhage and the reason for the accumulation of erythrocytes within the plaque (5). The accumulation of erythrocytes after intraplaque hemorrhage is considered as an important factor in the transition from a stable to an unstable atherosclerotic plaque (6). Damaged erythrocytes generate conglomerations of iron within the atherosclerotic lesion. Due to the different x-ray photon energy-dependant attenuation coefficients of iron, calcium and the surrounding tissue, localization of iron deposits and calcium within an atherosclerotic lesion should generally be possible using high-resolution x-ray imaging. The relation of fibro-calcified lesions, as determined by CT using the Agatston score, and cardio-vascular events has been demonstrated in the past, but imaging of advanced, vulnerable lesions with clinical imaging modalities like CT or MRI has remained difficult (7, 8).

Therefore, this study explores the possibility of 3D Nano-Computed Tomography imaging to directly detect hemorrhage in the arterial wall by virtue of iron accumulation and to differentiate intraplaque hemorrhage from calcified lesions. The present study was designed to demonstrate nano-CT’s technical feasibility for imaging iron deposits as a marker of intraplaque hemorrhage and for imaging calcified lesions as a consequence of intraplaque hemorrhage in the aortas of apoE^-/-/LDL^-/- double knockout mice.

Methods:
Experimental Design
Animal studies were performed according to the „German Animal Protection Law“ (1993). Approval of the institutional animal care and use committee was obtained before the start of this study.
Five apoE^-/-/LDL^-/- double knockout mice (80 weeks) were infused in situ with Microfil® contrast agent. After removing the heart and aorta en bloc from the animals, samples were segmented (n=12) and scanned with nano-CT between 900 nm – 2.5 µm isotropic voxel size.

Nano-Computed Tomography
Samples were scanned using a nano-computed tomograph (Nano-CT_2011), manufactured and developed by SkyScan® (Kontich, Belgium). The microfocus X-ray source is a pumped type source (open type x-ray source) with a LaB6 cathode. The electron beam is focused by two electromagnetic lenses onto the surface of an x-ray target. The x-ray target (Au) contains a thin tungsten film plated on the surface of the beryllium window producing x-ray emission reaching a minimum spot size of < 400nm. At this small spot size, small-angle scattering enhances object details down to 150 nm isotropic voxels size. The X-ray detector consists of a 12-bit digital, water-cooled CCD high-resolution (1280 x1024 pixel) camera with fibre optic 3.7:1 coupling to an X-ray scintillator and digital frame-grabber. In our
experimental setting, samples were positioned on a computer controlled rotation stage and scanned 180° around the vertical axis in rotation steps of 0.25 degrees at 40 kV. Acquisition time for each view was 2.4 seconds. Relative position of the object to the source determines geometric magnification and thus the pixel size defined by the cone-beam geometry of the system. Maximum possible magnification is also limited by the specimen size, which has to be within the cone-beam in its horizontal diameter. Raw data were reconstructed with a modified Feldkamp cone-beam reconstruction modus resulting in two dimensional 8-bit gray-scale images consisting of cubic voxels.

**Histology**

After nano-CT scanning, the entire tissue block was embedded in paraffin wax and sectioned. The 6µm-sections were stained with hematoxylin and eosin. Contiguous serial sections within each sample were prepared to detect iron by staining with Perls’ Prussian blue reaction with 3,3’-diaminobenzidine (DAB) intensification as described previously (9).

**Results:**

Two types of opacities within the atherosclerotic lesions were identified. One type of opacities manifested as clusters of randomly distributed punctuate deposits, being predominantly localized in the descending aorta (Figure 1). Histology confirmed these deposits as being iron deposits after intraplaque hemorrhage (Figure 2). The other opacities detected in nano-CT scans of the aortic samples were confluent accumulations and primarily located in the aortic arch. These opacities were exclusively found as being calcium (Figure 3). Vasa Vasorum neovascularization was present and related to advanced atherosclerotic lesions in the descending aorta.

**Conclusion:**

The advanced technology and high resolution of nano-CT allows localizing iron deposits within advanced atherosclerotic lesions. These findings enable us to detect intraplaque hemorrhage by high-resolution x-ray imaging. Differentiation of iron deposits and calcified lesions is only possible by estimating the size of the opacities. Measuring the density of the two different sized opacities within the atherosclerotic lesions does not allow any statement concerning its substance.
FIGURE 1. A+B Maximum intensity projection of a nano-CT scan (coronal view) demonstrating iron deposits along the descending aorta. C Maximum intensity projection (axial view) of a nano-CT scan. The arrows pointing at accumulations of iron within the atherosclerotic lesion. * marking the lumen of the descending aorta. D Single slice of a nano-CT image. The arrows pointing at iron deposits within the atherosclerotic plaque. * marking the lumen of the descending aorta.

FIGURE 2.
Figure 2. A Histological slice (HE) of a atherosclerotic lesion demonstrating vasa vasorum in the aortic wall (black arrows). B Perls’ Prussian staining. Blue arrows pointing at iron deposits, black arrows hinting at vasa vasorum in the aortic wall.

FIGURE 3

Figure 3. A Maximum intensity projection (coronal view) of a nano-CT scan. Arrows pointing at Calcium within the atherosclerotic lesions of the aorta. IVC describing the inferior vena cava. B Axial nano-CT single slice image showing calcifications along the lumen of the aorta.

References