Use of contrast-enhancement and high-resolution 3D black-blood MR Imaging to identify inflammation in rabbit atherosclerotic plaques

Jin Hur[1], Byoung Wook Choi[1], Young Jin Kim[1], Hye-Jeong Lee[1], Hua Sun Kim[1], Ji Eun Nam[1], Kyu Ok Choe[1]

Dept. of Radiology[1], Yonsei University College of Medicine, Severance Hospital, Seoul, Korea

Abstract

Purpose: We investigated the contributing factors for plaque enhancement and examined the relationships between regional contrast enhancement and the inflammatory activity of atherosclerotic plaques in an experimental rabbit model using contrast-enhanced high-resolution 3D black-blood magnetic resonance imaging (MRI) in comparison with histopathology.

Materials and Methods: Ten atherosclerotic rabbits and three normal control rabbits underwent high-resolution 3D contrast-enhanced black-blood MRI. MR images and the corresponding histopathological sections were divided into four quadrants. Plaque composition was analyzed for each quadrant according to histopathological (percent of lipid-rich, fibrous, macrophage area and microvessel density) and imaging criteria (enhancement ratio (ER), ER=SIpost/SIpre).

Results: A total of 62 non-calcified plaques (n=248, 156 lipid-rich quadrants and 92 fibrous quadrants) were identified based on histopathology. Mean ER values were significantly higher in atherosclerotic vessel walls than in normal vessel walls (2.01 ± 0.29 vs 1.58 ± 0.15, p = 0.022). Mean ER values were significantly higher in macrophage-rich plaques compared to the macrophage-poor plaques (2.21 ± 0.28 vs 1.81± 0.22, p = 0.001). Using multiple regression analysis, macrophage area and microvessel density were independently associated with ER values that reflected plaque enhancement (p <0.001).

Conclusion: Contrast-enhanced high-resolution 3D black-blood MRI may be an efficient method to predict plaque inflammation.

Introduction

❖ Recent reports have shown that association of inflammatory cells and neovessels in atherosclerosis is a histological hallmark of high-risk active lesions.

❖ Contrast-enhanced MRI, which employs gadolinium-based extracellular contrast agents for imaging, provides both morphological as well as functional assessment of atherosclerotic plaque formation .

❖ Gadolinium-based agents passively distribute from the intravascular into the extracellular fluid space. Some previous studies reported that Gd-DTPA is a non-specific contrast agent and was unable to discriminate between normal and atherosclerotic vessel walls.

❖ We developed a new, contrast-enhanced black-blood single-slab 3D turbo / fast SE pulse sequence to quantitatively evaluate the inflammatory activity of atherosclerotic plaques in an experimental rabbit model.

❖ We hypothesized that plaque enhancement would reflect inflammatory activity and therefore the proposed imaging method would be a valuable tool for detecting plaque inflammation.

❖ The purpose of this study was to investigate the contributing factors for plaque enhancement and to investigate the relationship between regional contrast enhancement and inflammatory activity of atherosclerotic plaques in the experimental rabbit model by using contrast-enhanced high-resolution 3D black-blood MRI in comparison with histopathology.

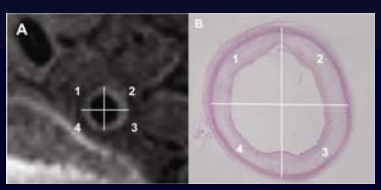

Fig.1 Transverse pre-contrast MR image (A) with the matched histopathologic Hematoxylin & Eosin stained section (B) were divided into four quadrants.

Material & Methods

1. Animal Protocol

❖ Ten male New Zealand white rabbits (weight 3.0 to 3.5 kg) were used for the animal model of atherosclerosis. Three male rabbits (weight 3.0 to 3.5 kg) were used as control animals and maintained on a normal rabbit chow diet for 2 weeks. In the experimental rabbits, aortic atherosclerosis was induced by a combination of a high-cholesterol diet (0.3% cholesterol-enriched diet) for 6 months and two times of aortic balloon denudation injury (at 1 week and 1 month after starting the high-cholesterol diet).

❖ The aortic denudation injury was performed three times from the diaphragm to the iliac bifurcation (abdominal aorta).

2. Magnetic Resonance Imaging

❖ The rabbits were imaged in the craniocaudal direction and in the supine position in a 3.0 Tesla (T) whole-body MRI scanner (MAGNETOM Trio, Siemens Medical Solutions) using an eight-channel body coil. High-resolution images were obtained using the proposed, T1-weighted single-slab 3D black-blood turbo / fast SE pulse sequence with fat suppression before and after the administration of contrast agents.

❖ Imaging parameters were as follows: TR/TE, 630/24 msec; field of view, 192x128 mm^2; matrix, 320x214; echo train length, 17; signal averages, 4; and slab thickness, 0.6 mm. The scan range included the segment of the abdominal aorta immediately below the diaphragm to the iliac bifurcation.

❖ Two minutes after administration of 0.1 mmol/kg of gadolinium-based contrast agent (Omniscan™) via the ear vein, coronal T1-weighted imaging was repeated with the same protocol as the pre-contrast scan, which typically took 15 minutes.

3. Histopathology

❖ Serial sections of the abdominal aorta were cut at 3-mm intervals to match the corresponding MR images.

❖ 5 μm sections were cut and stained with Masson's trichrome and Van Gieson's stain. Additionally, all sections were immunohistochemically stained with RAM-11 antibody which binds to macrophages (Dako, Carpinteria, CA, USA) and a CD-31 antibody which binds to endothelial cells (clone JC70A; Dako, Carpinteria, CA, USA).

4. Image Analysis

❖ 62 histopathology sections for atherosclerotic wall and 28 sections for the normal control were included in this study and histopathology sections and the matched MR images were divided into 4 quadrants (atherosclerotic wall, n=248 and normal wall, n=72, respectively) for analysis (Fig 1).

❖ After matching the MR image slices with the corresponding histopathology slices, each 3 mm sections of MR images were reconstructed into 0.6 mm sections with no overlap. We then compared every first slice of the histopathology slices (5 μm thick section) with the MR image slices (0.6 mm).

❖ On histopathology, percent of lipid-rich, fibrous, macrophage area and microvessel density were analyzed for each quadrant.

❖ To obtain plaque SI values on MRI, ROIs were manually placed inside the matched plaques. Two radiologists independently measured the SI at three randomly selected points for each quadrant. The mean SI values were used for analysis. Enhancement ratio (ER) of the SI was calculated as follows; ER=SIpost/SIpre, where SIpost is the SI of the wall after contrast enhancement and SIpre is SI of the wall before contrast enhancement.

4. Statistical Analysis

❖ Statistical significance of differences in calculated ER of aortic walls between atherosclerotic rabbits and the normal control was assessed using a Student's t test for independent samples. The statistical significance of differences in calculated ER of plaques according to different plaque types determined by histopathology was assessed using a Student's t-test for independent samples. Multiple linear regression analysis was performed to determine independent factors for plaque enhancement.

Results

❖ Mean ER values were significantly higher in atherosclerotic vessel walls than in normal vessel walls (2.01 ± 0.29 vs 1.58 ± 0.15, p=0.022).

❖ The mean ER values for each quadrant are summarized in Table 1. The mean ER values were 2.03 ± 0.32 and 1.83 ± 0.23 for the lipid-rich and fibrous quadrants, respectively. The mean ER values were significantly different between the lipid-rich quadrants and fibrous quadrants (p=0.001).

❖ Mean ER values were significantly higher in macrophage-rich plaques compared to the macrophage-poor plaques (2.21 ± 0.28 vs 1.81± 0.22, p=0.001).

❖Using multiple regression analysis, macrophage area and microvessel density were independently associated with ER values that reflected plaque enhancement (p<0.001) (Table 2, Fig 2).

Table 1	Enhancement Ratio Measurements of Lipid-rich and Fibrous Quadrants		
	Pre SI*	Post SI†	ER‡
Lipid-rich quadrants	238 ± 41 (range, 168-331)	483 ± 125 (range, 285-707)	2.03 ± 0.32 (range, 1.33-2.79)
Fibrous quadrants	231 ± 39 (range, 165-314)	437 ± 97 (range, 267-654)	1.83 ± 0.23 (range, 1.24-2.51)

* Pre SI (signal intensity): SI of the wall before contrast enhancement.

† Post SI (signal intensity): SI of the wall after contrast enhancement

‡ ER (enhancement ratio) of the SI was calculated as follows; ER=SIpost/SIpre.

Table 2	Results of the Multiple Linear Regression Model		
	ER (Adjusted R^2 =0.437)		
Parameters	Unstandardized coefficient	Standard error	P value
Total plaque Area	0.047 (0.111)	0.023	0.156
% of lipid area	-0.001 (-0.06)	0.005	0.522
% of fibrous area	-0.003 (-0.131)	0.005	0.418
% of macrophage area	0.009 (0.599)	0.001	< 0.001
Microvessel density	0.006 (0.436)	0.001	<0.001

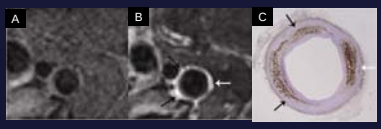

Fig 2. (A) 3D-high resolution pre-T1 weighted MR image and corresponding transverse slice. (B) 3D-high resolution post-T1 weighted MR image and corresponding transverse slice. On post-contrast transverse MR image, the atherosclerotic wall showed marked contrast enhancement (white and black arrows). (C) Corresponding histopathological section (RAM-11 positive staining, Magnification x 12.5) demonstrates abundant macrophage accumulation (white and black arrows) in the area matched with marked contrast enhanced area on MR image.

Conclusion / Summary

❖ Strong evidence suggests that plaques with high macrophage accumulation within the plaque and significant neovascularization are unstable and susceptible to rupture .Therefore, the ability to quantitatively assess the inflammatory status of a plaque using noninvasive imaging could be of tremendous value for studies evaluating the effectiveness of novel therapies intended to inhibit plaque inflammation.

❖ Our results show that the percentage of macrophage area and microvessel density was independently associated with plaque enhancement, reflecting the occurrence of plaque inflammation.

❖ Based on these results, we believe that contrast-enhanced high-resolution 3D black-blood MRI may be an efficient way to predict plaque inflammation.

❖ In addition, the ability to quantitatively assess the inflammatory status of a plaque using high-resolution 3D black-blood MRI will be of tremendous value for monitoring the therapeutic effect intended to inhibit plaque inflammation in the future.