Nuclear Imaging of Vulnerable Atherosclerotic Lesions
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Coronary artery disease is the most important cardiovascular scourge that mankind has faced in the twentieth century. It will continue to be the leading cause of morbidity and death in the next century, both in men and women, and in developing and developed nations alike. The management of coronary artery disease has almost always been based on demonstration of the severity of luminal stenosis. Such an approach does not characterize the plaque morphology that happens to be the major determinant of clinical outcome. Limited success has been achieved by the use of angioscopy and intravascular ultrasonography or more recently with optical coherence tomography. All these techniques are invasive and have limited depth resolution. Appropriate targeting strategies with radionuclide imaging techniques could identify the predominant cellular population in the atherosclerotic plaque and help predict the likelihood of clinical events. For an achievement of high target-to-background ratio in radionuclide imaging, it is important to identify the morphologic characteristics of the lesion that are uniquely expressed during the evolution of atherosclerotic process (hot-spot imaging) or those that are selectively deleted from the affected area (cold-spot imaging).

Evolution of Atherosclerotic Lesions

Development of atherosclerotic lesions is an immunoinflammatory response of the intima to injury and involves a complex interplay of components of blood vessels wall with blood elements. The injury is initiated by oxidatively modified lipids that permeate through the endothelial layer. A concurrent expression of selectins and adhesion molecules on the endothelium leads to recruitment of monocytes and their subendothelial migration. The altered release of vasoactive substances from the endothelium facilities phenotypic alteration of medial smooth muscle cells. At this time, the interaction of endothelial cells, adherent monocytes, and modified lipids leads to release of growth factors that induce proliferation or phenotypically altered smooth muscle cells and eventually their migration to the intima. Monocytes and smooth muscle cells in the subendothelial space ingest modified low-density lipoprotein (LDL) cholesterol and evolve into foam cells. The modified lipid uptake in the monocytes occur through non-classical LDL scavenger receptors and is not inhibited by the intracellular lipid. Foam cells are restricted from moving away from the lipid core. Any one of the three major constituents of the plaque, namely lipids, inflammatory cells, and proliferating muscle cells, can be targeted with radiolabeled agents for noninvasive detection of the lesion.

Strategic Targeting of Vulnerable Atherosclerotic Lesions

Fourteen million people in the United States have coronary artery disease.1 Although recognition of each one of them may constitute the ideal situation, it should be most desirable to identify a subset of patients with likely development of acute coronary event. Of the 14 million, 1 million people have development of acute coronary event, and 400,000 die of that event every year. It is now well recognized that progressive luminal stenosis of the coronary artery is often not associated with an acute event and that the
trombotic occlusion usually occurs as a result of plaque rupture. The plaques that are vulnerable to rupture have large lipid cores, attenuated fibrous cap, and intensified infiltration of macrophages. It has been proposed that the macrophages release metalloproteinases that digest matrix and induce fibrous cap rupture. The plaque rupture exposes thrombogenic lipid core leading to thrombotic luminal obstruction. Therefore for the identification of vulnerable plaques, morphologic techniques such as IVUS, MRI, OCT and CT have capitalized on attenuated fibrous caps and large lipid cores. Nuclear imaging can be best exploited for localization of macrophage infiltration in the atherosclerotic plaque. It is conceivable that only those macrophage antigens should be targeted that are expressed on the resident macrophages and not borne by the circulating monocytes.

**Targeting Macrophage Infiltration in Atherosclerotic Plaques**

The macrophage infiltration in the vessel wall follows a phasic process that includes a reversible adhesion, leukocyte activation and activation dependent binding of leukocytes. The endothelial injury induces expression of E- or P-selectin. Corresponding integrin molecules on the monocytes such as L-selectin facilitates interaction of the monocytes with the endothelium that slows the monocytes rolling along the vessel wall. Such an interaction is a reversible process that only attracts monocytes to the endothelial surface from the blood stream to facilitate more definitive steps of interaction. If endothelium has been sufficiently insulted to express chemotactic peptides (such as MCP-1), then monocytes adhere strongly to the endothelium. If the chemoattractant is not available, the selectins are shed, and the monocytes roll back to the blood stream. The interaction of the endothelial chemoattractants and the monocyte receptors lead to activation of β1- or β2- integrins such as LFA-1, VLA-4, or Mac-1. These integrins bind firmly to endothelial expression of immunoglobulin gene superfamily of adhesion molecules such as ICAM-1 or –2 and VCAM-1. By now the monocytes are committed to permeate through the endothelial cell junctions mediated by coadherins.

Indium 111 oxine-radiolabeled autologous monocytes were first used in the patients with angiographic evidence of peripheral vascular disease. After a transient imaging of the lungs during the initial dynamic acquisition, the major site of the in vivo uptake of radiolabeled monocytes was the spleen. Focal sites of uptake were visible over the carotid or femoral arteries only in 40% of patients. It seems more logical to target the integrins or receptors for chemoattractants on monocytes because they may be exclusively expressed by the infiltrating monocytes. Iodine 131-labeled MCP-1 has been shown to selectively accumulate in lipid-rich, macrophage-rich regions of experimental atherosclerosis model by macroautoradiography. If proven feasible for clinical imaging, it will provide a major breakthrough imaging for atherosclerotic lesions.

Subendothelial monocytes after migration develop scavenger receptors and ingest oxidized LDL more avidly (than native LDL). Scavenger receptors are not down-regulated with an increase in the cholesterol content of the cell increases. At least 4 types of macrophages scavenger receptors for oxidized LDL uptake have been recognized and include scavenger receptor I or II (SRA), CD36, CD68, and FcγRII. Up
to 50% of cholesterol uptake may occur via CD36 and 30% via SRA. These receptors are unique to the recruited macrophages and are not borne by circulating monocytes; the radiolabeled ligands for these receptors may constitute attractive targets for noninvasive imaging. The Fc receptors have been targeted with limited success by radiolabeled nonspecific immunoglobulin G (IgG). Imaging of the peripheral arterial lesions was undertaken in 4 patients with radiolabeled IgG. Although 1 hour after injection, increased focal localization of $^{111}$In-labeled IgG was seen in 9 of 12 angiographically documented lesions, the lesion-to-normal tissue contrast ratio was inadequate for clinical application.

**Association of Macrophages with Plaque Vulnerability**

Although the mechanisms that convert a stable plaque into a vulnerable plaque are not well understood, recent literature supports that cell death in the neointima may contribute to vulnerability of plaque to rupture. In a study of non-ulcerated atheromatous plaques, a high incidence of apoptosis was observed in inflammatory cells, predominantly in macrophages surrounding the lipid core. This finding is corroborated by other studies of human plaques suggesting that macrophage cell death is perhaps responsible for core formation and expansion. Other studies have proposed that the chronic loss of SMC in atherosclerotic plaques in human aorta may lead to fibrous cap thinning and vulnerability. In addition to direct cap thinning, SMC apoptosis may also contribute to plaque vulnerability by recruitment of inflammation in the fibrous cap, by induction of expression of MCP-1 and IL-8.

In addition to plaque vulnerability, apoptosis of macrophages may contribute to the actual process of plaque rupture. In a study in the hearts of victims of sudden death secondary to plaque rupture, there was a strikingly high prevalence of apoptotic nuclei at the rupture sites by in situ end-labeling. Further characterization demonstrated that the apoptotic cells in the culprit lesions suggested exclusive apoptosis of macrophages, these macrophages expressed caspase-1 or ICE. Immunoblot studies of the plaques demonstrated cleaved active band of ICE in the ruptured plaques; whereas only unprocessed ICE was present in stable plaques, both processed and unprocessed ICE bands were not seen in normal vessel wall. On quantitative analysis of the prevalence of cells and incidence of apoptosis in various cell types, apoptotic cells were predominantly observed at the rupture site and only occasionally encountered in the regions of the same plaque remote from the site of rupture; apoptosis was most prevalent in macrophages. Stable plaques demonstrated minimal evidence of apoptotic cells, which was predominantly confined to SMC.

**Noninvasive Detection of Apoptosis of Macrophages in Atherosclerotic Plaques**

Since Annexin-V can identify the cell membrane alterations associated with apoptosis and the process of apoptosis contributes to plaque vulnerability and plaque rupture, we used radiolabeled Annexin-V for the detection of experimental atherosclerotic lesions. Atherosclerotic lesions were induced in NZW rabbits by deendothelialization of the infradiaphragmatic aorta followed by 12 weeks of a high fat, high cholesterol diet. All animals received 0.5-1 mg of Annexin-V labeled with 7-10 mCi of technetium-99m
intravenously for in vivo imaging studies. The left lateral decubitus gamma images showed clear delineation of radiolabel within the abdominal aorta 2 hours after Annexin administration. Ex-vivo images showed a robust uptake of radiotracer in the infradiaphragmatic aorta within the lesion distribution corresponding to the in-vivo images. In contrast, the uptake of radiolabel was absent in areas without grossly visible atherosclerotic lesions; these areas were predominantly localized to the non-denuded descending thoracic aorta. In contrast to vessels with plaques, there was no localization of radiotracer within the presumably normal vessel wall; ex-vivo imaging confirmed the lack of radiotracer uptake. The accumulation of $^{99m}$Tc-Annexin-V in atherosclerotic lesions in the balloon-denuded region of the aorta was approximately 9.3-fold greater than in the corresponding control abdominal aortic region. The mean±SEM percent-injected dose per gram uptake in the specimens with lesions (0.054±0.0095%) was significantly higher than the background activity in the normal specimens (0.0058±0.001, p< 0.000). Histopathologic correlation of the severity of atherosclerotic lesions and the radiotracer uptake demonstrated that Annexin accumulation predominantly occurred in AHA type IV lesions with only minimal uptake in type II and III lesions. A large proportion of the cells stained positively for the presence of apoptosis in type IV lesions, which had shown the maximal radiotracer uptake. Further, there was a modest direct relationship of Annexin-V uptake with total macrophage burden (r=0.47, P= 0.04); no association was observed between SMC burden and radiotracer uptake (r=0.08, P= 0.73).

To further characterize the precise localization of Annexin-V, both radiolabeled and fluorescein-labeled Annexin-V were injected in 50-week-old ApoE deficient mice. Autoradiographically identified regions of $^{99m}$Tc-Annexin-V uptake in ascending aorta and carotid arteries demonstrated localization of fluorescent tracer in macrophage-rich regions of AHA type IV lesions, which were subsequently determined to harbor apoptotic cells.

**Radionuclide Detection of MMP Upregulation in Atherosclerotic Lesions**

Inflammation within the atherosclerotic plaque may further perpetuate plaque instability by production of MMP. When activated by cytokines (TNF-γ, IL-1), macrophages secrete inactive MMP, including interstitial collagenases (MMP1), gelatinase B (MMP9), stromolysins 1-3 (MMP3, 10, 11), and a membrane type. When activated by plasmin or by inactivation of intrinsic inhibitors in tissue, MMP can degrade the connective tissue matrix. Mechanical testing in vitro of cap tissue shows that an increase in the number of macrophages and a reduction in collagen and glycosaminoglycans content reduce the amount of stress needed to fracture the tissue. Sections of plaques laid on a gelatin substrate in vitro show active degeneration of collagen in lipid-rich plaques. Plaque cap rupture can therefore be seen resulting from a destructive process initiated by macrophages that gains ascendancy over the repair process of collagen deposition by SMC.

The MMP production is predominantly seen in the vicinity of macrophage predominance in human coronary atherosclerotic lesions. Since active digestion of fibrous cap by MMP upregulation and activation may be an important event in plaque instability, noninvasive
detection of MMP should allow better prediction of clinical outcomes in CAD patients. To proof the principle a broad-spectrum MMP inhibitor (specificity for MMP-1-3, 7-9, and 13, Ki 1-15 nM; Bristol-Myers Squibb, North Billerica, MA) radiolabeled with indium-111 was used for imaging experimental atherosclerotic lesions in NZW rabbits. Lesions were induced by balloon deendothelialization of the infradiaphragmatic aorta and diets were manipulated by feeding 0.5% high cholesterol either continuously or interrupted with normal chow. Animals were randomized as follows: Group I (control group) animals were unmanipulated rabbits and received normal chow for 4 months. Group IV animals received continuous high cholesterol diet for 4 mo (test group); group III (diet-interrupted group) received high cholesterol diet for 2 mo - normal chow for 1 mo - high cholesterol diet again for 1 mo; and group II (diet withdrawal group) received high cholesterol diet for 2 mo - normal chow for 2 mo. By noninvasive gamma imaging, the abdominal atherosclerotic lesions were visualized best at 3 hours in group IV. The percent injected dose per gram uptake of radiolabeled MMP inhibitor was maximum in group IV lesions (0.033±0.019; lesion-to-nonlesion ratio 11:1), and significantly higher than interrupted diet group III (0.015±0.005, p=0.01). Uptake in group I control animals (0.003±0.001) was minimum and similar to diet withdrawal group II (0.01±0.003, p=ns). Threshold analysis of histologic sections after immunostaining showed a significant increase of MMP in plaque segments demonstrating a high radiolabeled MMP inhibitor uptake (MMP-1= 12.5±2.1, MMP-3= 14.3±1.8, MMP-9= 1.3±0.5 mm²) relative to those with low uptake (MMP-1= 7.7±0.2, MMP-3= 9.1±1.5, MMP-9= 0.2±0.05 mm²; p< 0.03). These preliminary observations suggest that in vivo quantitation of MMP in atherosclerotic plaques is feasible, and correlates with their pathologic distribution in the plaque. The observations have also confirmed the previous belief that withdrawal of the hyperlipidemic diet (and use of statins) abrogates MMP upregulation in the plaque.