Drugs of the Future 2004, 29(7): 705-714

CCC: 0377-8282/2004

Review Article

Detection of atherosclerosis: methods, agents and developments

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Abstract

Nuclear medicine is an important diagnostic tool in the evaluation of patients with a variety of diseases. This modality uses radiopharmaceuticals that target the area of interest due to functional rather than morphological abnormalities, adding important information to other imaging methods such as magnetic resonance imaging and computed tomography. One of the challenges for nuclear medicine is the evaluation of atherosclerosis. Atherosclerosis originates from increased endothelial permeability and enhanced endocytosis of LDL, which carries endogenous serum cholesterol. Plaque formation occurs when the LDL particles accumulate in the vessel wall. Of special interest is the identification of vulnerable plaque, which is weakened by apoptosis and inflammatory reactions. These vulnerable plaques represent a life-threatening danger, as rupture releases thrombogenic gruel with possible downstream occlusive thrombosis. Since vulnerable plaque is characterized by increased apoptosis, it is worthwhile to investigate the possible targeting of radiolabeled annexin V to the lesion. For patient studies, this agent would preferably be labeled with technetium-99m (^{99m}Tc). This radionuclide offers all the characteristics for adequate patient studies. Indeed, under experimental conditions, ^{99m}Tc-annexin targets the plaque lesions prone to rupture.

Introduction

With the discovery of X-rays by Röntgen at the end of the nineteenth century, new avenues were opened for medical diagnosis, allowing the visualization of radio-opaque structures such as bones. Half a century later, the application of radio-opaque contrast media made it possible to image soft tissue body parts such as kidneys, liver and the larger vessels. Although the function of these organs was made visible to a certain extent, the information obtained with X-rays was essentially of a morphological nature, even with later inventions such as (high-resolution) computed tomography (CT). Functional imaging had to wait until the radionuclide tracer principle, first described by Chievitz and Hevesy in 1935 (1), was further developed and became part of medical practice. Nowadays, radiotracers are used for both diagnostic and therapeutic purposes, but the principle of localization is similar for all these agents and relies upon the physiological changes produced by disease. The functional information obtained from nuclear medicine methods adds to the morphological data obtained from radiology and helps us to understand and treat diseases.

One of the diseases that finds its origin in an abnormal biochemical cellular process is atherosclerosis, and radiotracer methods are considered an appropriate and logical way to map the various stages of this serious disorder. In this article we shall review the possibilities for targeting atherosclerosis with radiotracers and summarize early clinical results and which agents are under development. Prior to this, we shall highlight various pathophysiological aspects of atherosclerosis and put the more conventional methods for imaging atherosclerosis into perspective.

Epidemiology

Atherosclerosis is mainly present among the populations of developed countries. In contrast with western countries, atherosclerosis is much less prevalent in central and south America, Africa and Asia. For example, the

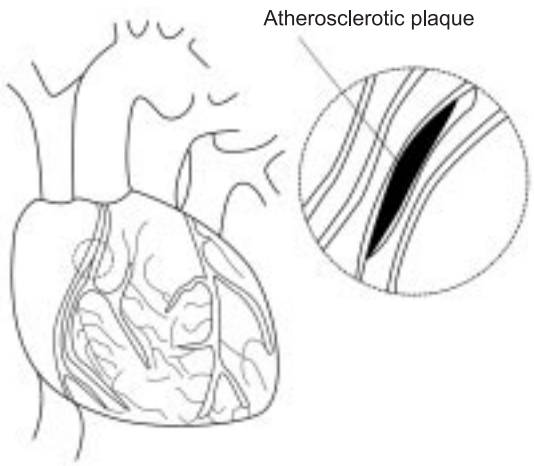

Fig. 1. Atherosclerosis development in a coronary artery.

mortality rate for ischemic heart disease in the United States is among the highest in the world and 6 times higher than in Japan, a difference which is usually attributed to lifestyle and dietary customs. In The Netherlands, the mortality from atherosclerosis increased between 1996 and 2002 by 10%. However, the number of deaths attributable to ischemic heart disease (including myocardial infarction) decreased by 29% between 1996 and 2002.

Etiology, pathogenesis and pathophysiology

It is generally accepted that the common forms of coronary heart disease result from the combination of unhealthy environment, genetic susceptibility and increased life span. Such vascular disorders find their origin in the development of fatty streaks, a collection of lipid-containing macrophages, often already present during childhood. Such lesions tend to occur at bifurcations and ostia of the arteries (2), where there is a turbulent flow with variable shear stress levels. Shear stress is a force that is developed on the vascular wall by the streaming blood. Variations in shear stress on the vascular wall cause endothelial dysfunction (Figs. 1 and 2). These variations in shear stress also give rise to increased endothelial permeability and enhanced endocytosis of low-density lipoprotein (LDL), which is the carrier of endogenous serum cholesterol.

The transport of dietary and endogenously produced lipids occurs by several lipoprotein particles. Chylomicrons provide the primary transport of dietary lipids, while very-low-density lipoproteins (VLDL), LDL and high-density lipoproteins (HDL) function to transport endogenous lipids. Triglyceride-rich VLDL particles contain apolipoprotein B-100 (apo B-100), apolipoprotein E (apo E) and apolipoprotein C (apo C) (3). After triglyceride removal in peripheral tissues, a portion of the remaining VLDL remnants are metabolized to LDL particles by further removal of core triglycerides and dissociation of apolipoproteins other than apo B-100.

In humans, the major part of serum cholesterol (endogenous lipids) is carried by LDL particles, which are taken up by cells via LDL receptors that recognize an *N*-terminal domain of apo B-100 (4). The liver synthesizes apolipoproteins and these components function to transport fatty acids to adipose tissue and muscle. One of the lipoproteins, HDL, has a beneficial effect on atherosclerosis. HDL is believed to absorb cholesterol from LDL, as well as to mobilize cholesterol from developing and existing atheroma and transport it to the liver for excretion in the bile, thereby inhibiting the development of atherosclerosis.

When LDL particles accumulate in the endothelial wall, certain enzymes can oxidize them. Oxidized LDL is easily ingested by macrophages, forming foam cells. LDL urges monocytes to move to the endothelial wall and it induces adhesion of the cells to the endothelium. Furthermore, LDL induces the release of cytokines and

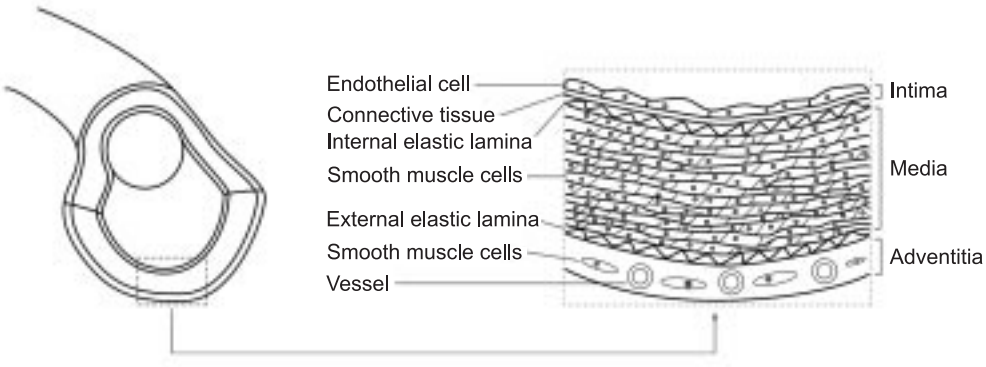

Fig. 2. The normal structure of a large artery.

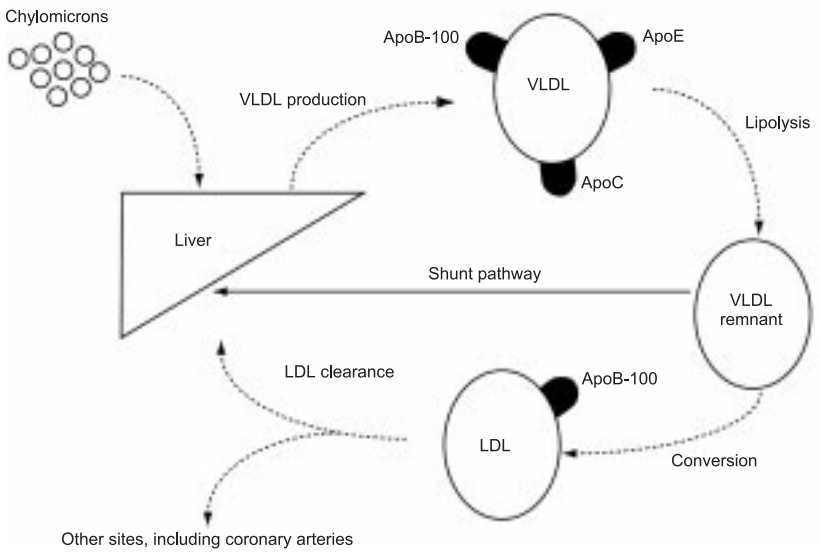

Fig. 3. LDL regulation. Dietary lipids are transported by chylomicrons to the liver. The liver produces very-low-density lipoproteins (VLDL), the core of which contain triglycerides and cholesteryl esters. VLDL triglycerides move to muscle and adipose tissue and the particles then undergo exchange reactions with other lipoproteins. The VLDL remnant will be produced by lipolysis of VLDL that can be taken up by the liver or will be converted to LDL, the core of which contains only cholesteryl esters. Furthermore, it can be cleared by the liver or will be transported to other sites in the circulation.

growth factors, thereby stimulating an inflammatory reaction.

Macrophages play an important role in the initiation of lesion formation, because they have a large amount of cytokines (regulatory proteins) and other secretory products. They stimulate the oxidation of LDL (reaction of LDL with reactive oxygen particles) in the lesions and they also stimulate, via growth factors, the proliferation of smooth muscle cells in the lesions. Smooth muscle cells migrate in the intima, where they proliferate. If the hypercholesterolemia persists, smooth muscle cell proliferation and cell matrix deposition transform the fatty streak into a progressively growing atherosclerotic fibrous lesion. In the lipid core, the foam cells, endothelial cells and smooth muscle cells that have taken up the LDL die by apoptosis (gene-regulated cell death) and there remains lipid debris. A fibrous cap of smooth muscle cells and cellular matrix surrounds this lipid core (Figs. 3 and 4).

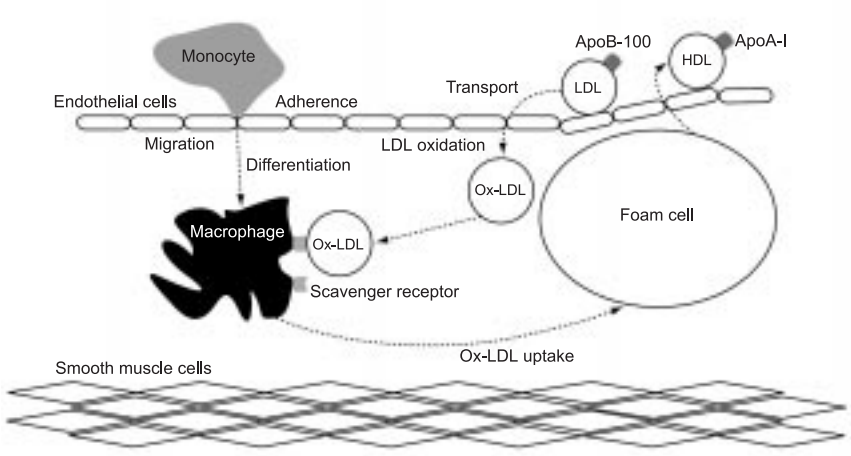

Fig. 4. LDL is subject to oxidative modification in the intima. This oxidized LDL (Ox-LDL) induces the expression of adhesion molecules, which make monocytes attach to endothelial cells. After adherence to endothelial cells, the monocytes will migrate into the subendothelial space and differentiate into macrophages. The uptake of Ox-LDL via scavenger receptors by macrophages leads to foam cell formation, after which Ox-LDL is subject to esterification and storage in lipid droplets. Eventually, the Ox-LDL is converted to more soluble forms and/or exported to extracellular HDL acceptors via cholesterol transporters.

Table I: Major components of well-developed atheromatous plaque.

Fibrous cap	Necrotic core
Smooth muscle cells	Cellular debris
Macrophages	Cholesterol crystals
Foam cells	Foam cells
Lymphocytes	Calcium
Collagen	Lymphocytes
Elastin	Extracellular lipids
Proteoglycans	Oxidized LDL

Atherosclerosis is considered to be a chronic inflammatory disorder (5). Atherosclerotic lesions are like arterial wounds in which smooth muscle cells and inflammatory cells are abundant and in which lipids and fibrous elements accumulate. Vallabhajosula and Fuster (6) have summarized the three principal components of atherosclerotic plaque: 1) cells, basically smooth muscle cells, macrophages and lymphocytes; 2) connective tissue, including collagen and elastic fibers; and 3) intracellular and extracellular lipid deposits. Table I summarizes these components in further detail. The composition of atherosclerotic plaque varies from person to person and may give rise to a variety of lesions, including thrombosis, thromboembolic occlusion and aneurysm. The plaque lesion always involves the intima of the wall of large- and medium-sized arteries, including the aorta and the carotid and coronary arteries (Fig. 5).

In the eighties, it was believed that the danger of atherosclerosis arose from a growing stenotic plaque causing gradual narrowing of the vessel and limiting the downstream perfusion of the cardiac muscle. There is now substantial evidence that the so-called vulnerable plaques represent a life-threatening danger. They are usually not significant on angiographic studies, which casts doubt on the “gold standard” character of such studies (7). However, vulnerable plaques are blamed for the majority of sudden cardiac deaths due to plaque rupture and downstream occlusive thrombosis (Fig. 5). In fact, it seems that many cases of death attributed to myocardial infarction and unstable angina find their origin in plaque erosion (8).

In vitro studies have suggested that the infiltration of macrophages weakens the fibrous cap locally (9). It has been found that acute ischemic events appear to be due to the disruption of plaques with large lipid cores, followed by the exposure of the thrombogenic gruel to the circulation and subsequent thrombus formation (10). Smaller plaques with securely contained lipid cores and thick caps are considered more stable and may remain without clinical signs during a lifetime (11). At present, the challenge is to characterize the plaque by imaging and identifying lesions that are at risk for rupture. For this purpose, several invasive and noninvasive techniques have been under study. Without exception, these are all imaging

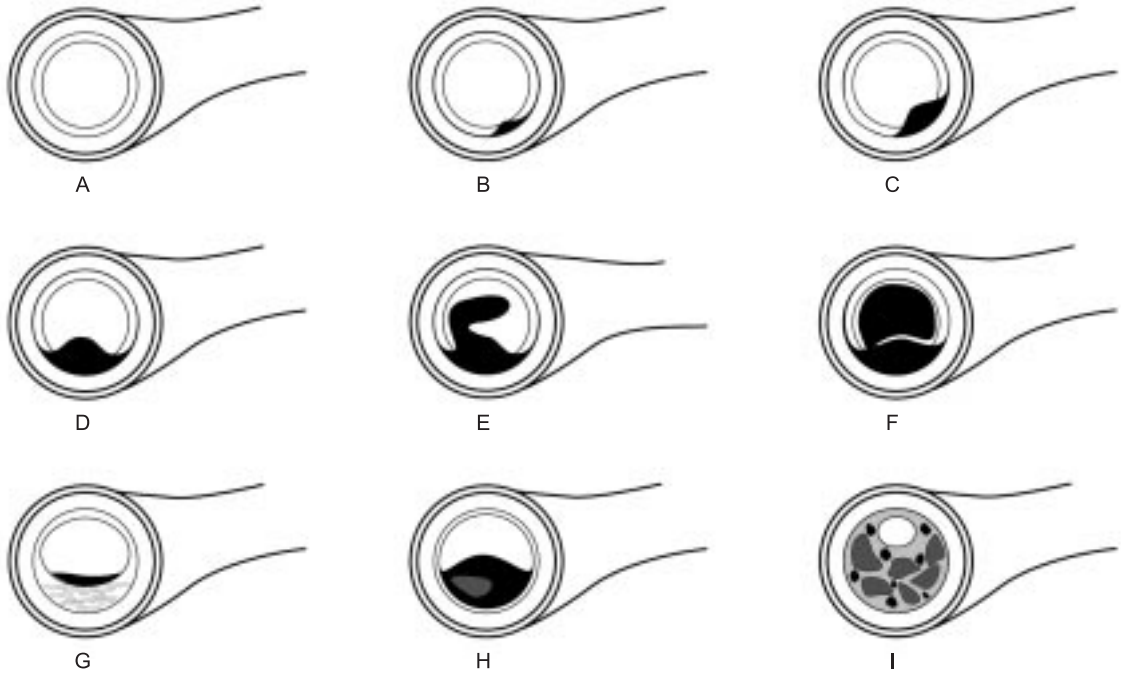

Fig. 5. Development of atherosclerotic plaque. A: Normal structure of artery. B: Fatty streak. C: Atheroma plaque. D: Vulnerable plaque. E: Ruptured vulnerable plaque. F: Occlusive thrombosis. G: Eroded vulnerable plaque with nonocclusive thrombus. H: Vulnerable plaque with calcified nodule. I: Critically stenotic vulnerable plaque with extensive calcification and old thrombi.

techniques that attempt to identify the morphological and functional characterization of the plaque. From the foregoing, it is clear that such a test should preferably be able to identify vulnerable plaque, and moreover, be able to differentiate between stable and unstable plaque. In the following we shall discuss these imaging techniques and the ongoing work in this field of atherosclerosis.

Radiological imaging methods

Intravascular techniques

Angiography provides information on the luminal diameter and measures stenosis. Atherosclerotic disease can only be detected when the stenosis is severe enough to reduce or inhibit blood flow. Early, silent plaques can hardly be detected by angiography. Such plaques, however, may also disrupt and their detection requires another test. For this purpose, angioscopy and intravascular ultrasound are more suited, because these tests permit the direct imaging of the vascular wall and possible plaques. However, due to their invasive nature, these methods remain research tools rather than clinical tools, and it should be emphasized that there is always the danger that the catheterization itself may rupture a plaque, carrying a significant risk. Various in-depth papers on these intravascular methods have recently been published (12, 13) and emphasize the morphological character of this test, imaging only the vessel lumen. It should be realized that atherosclerosis usually develops in the arterial wall and that the lumen caliber may stay virtually unaltered. Nevertheless, ultrasound techniques have provided new insights into plaque growth and composition, and more specifically on the content of the lipid core (14).

In combination with these catheter-based imaging techniques one could use Raman spectroscopy. This method characterizes the chemical composition of tissue by processing the collected light that is scattered by this tissue when it is illuminated with a laser. This technique is hindered by strong background fluorescence and light absorption by blood. These problems are at least partly overcome with near infrared (NIR) spectroscopy in the 750-2500 mm range, providing deep penetration. There is good correlation between NIR spectra and plaque composition and histology (15, 16). Taking into account the invasive nature of these intravascular techniques, it should be emphasized that these modalities presently remain research tools rather than clinical tools.

Tomographic methods

Over the past decade, magnetic resonance imaging (MRI) has proven extremely useful for the detection of cardiovascular disorders. Advantages include the noninvasive nature of the technique, the fact that the patient is not exposed to ionizing radiation and a high spatial resolution of about 10 μm.

In vivo imaging of coronary arteries has been achieved recently but the technique poses considerable technical difficulties due to cardiac and respiratory motion. Nevertheless, various investigators have succeeded in developing MRI techniques for plaque imaging and it has been possible to determine plaque anatomy and composition under *ex vivo* and *in vivo* conditions (17, 18). Image enhancement with contrast media such as gadolinium-DTPA has not been very successful in the identification of vulnerable plaques, although parameters were developed from the detection of inflammation and growing plaque (19-21).

The situation is different with a newly developed agent composed of carbohydrate-coated colloidal iron oxide particles (so-called paramagnetic iron oxide) with a size in the range of 30-100 nm. These particles are taken up by macrophages and remain within these cells. In view of the fact that vulnerable plaque contains many of these actively engulfing inflammatory cells, it has been suggested that these iron oxide particles may accumulate to a significant degree in this type of lesions. This hypothesis has been confirmed by Schmitz *et al.* and Winter *et al.* (22, 23), who found a large number of the particles in subendothelial layers and neovasculature, respectively, of atherosclerotic plaque. Other modalities based on MRI techniques are magnetic resonance angiography (MRA) and magnetic resonance spectroscopy (MRS) (24, 25). Table II summarizes the various radiological methods together with their respective advantages and disadvantages.

Nuclear medicine imaging

A better understanding of the etiology and pathogenesis of atherosclerotic plaque has mobilized the nuclear medicine community to develop radiotracers to depict this disorder and possibility distinguish between stable and unstable plaque. Naghavi *et al.* (26) have pointed out that radionuclide techniques will probably never become the methods of choice for the detection of vulnerable plaque in the general population due to resistance to the application of ionizing radiation and the huge costs involved. However, the present issue is not to develop a screening method but to create the possibility of imaging atherosclerotic plaque and characterizing its status. Moreover, vulnerable plaque is functionally active at the cellular and metabolic levels and, as discussed earlier, this paves the way for noninvasive nuclear medicine imaging, rather than for methods based on altered morphology.

Vallabhajosula and Fuster (6) and Cerqueira (27) have summarized the potential targets and possible radiotracers for the visualization of atherosclerotic plaque. Their data, together with the most recent developments, are listed in Table III.

Table II: Radiological methods for imaging atherosclerosis.

	Advantages	Disadvantages
Angiography	Detailed vascular morphology	Invasive, no information about plaque composition
Angioscopy	Detailed information about vascular wall	Invasive, research tool
IVUS	Detecting vulnerability of whole plaque	Invasive, research tool
Raman spectroscopy	Characterization of the chemical composition of the plaque	Strong background fluorescence, absorbance by blood of the laser light, long acquisition time
NIR	Deep penetration	Invasive, research tool
MRI	Noninvasive, imaging without ionizing radiation, characterizes plaque composition and microanatomy, high-resolution images of the vessel wall and lumen, repeatable	Only imaging of larger vessels, long acquisition time, structural information
MRA	Images of the vessel lumen, including coronary artery, high resolution	Stenosis length is overestimated*
MRS	Characterizes plaque (lipid + calcium)	Requires special techniques

*Stenosis length is overestimated as severity increases because of disturbed patterns of flow with turbulence distal to severe stenoses. IVUS = intravascular ultrasound; NIR = near-infrared; MRI = magnetic resonance imaging; MRA = magnetic resonance angio-graphy; MRS = magnetic resonance spectroscopy.

Table III: Targets and possible radiotracers for the visualization of atherosclerotic plaque.

Target	Possible radiotracer
(Transformed) smooth muscle cells	^{99m}Tc-labeled ET-1, ^{99m}Tc-labeled purines (G-protein signaling pathway), ^{111}Indium-labeled Z2D3 antibody
AMA	^{131}I-labeled anti-AMA monoclonal antibody
Macrophages	^{99m}Tc-labeled "highly oxidized" modified LDL
Apoptotic cells	^{99m}Tc-labeled annexin V
LDL receptor (on macrophages present in atheroma)	Apo B-based peptide labeled with ^{99m}Tc (SP-4, P-199 and P-215)

LDL = low-density lipoprotein; ET-1 = endothelin-1; AMA = aminomalonic acid

According to these and our own investigations, the requirements for the ideal radiotracer are as follows:

1. Ability to detect atherosclerotic disease on the basis of its specificity for the lipid core and/or macrophage density
2. Ability to image lesions in coronary, carotid and ileofemoral arteries
3. Ability to assess progression and regression of disease and monitor the effect of therapy
4. Ability to identify plaques prone to rupture
5. Ability to predict significant clinical events
6. Ability to provide a prognostic indicator
7. Use of a radionuclide that is cheap and available on a wide scale
8. Ability to detect disease within a few hours after application and provide a sufficiently high target-to-nontarget ratio for quantitative evaluation

These requirements can be translated into practice by stating that the radiopharmaceutical should have high avidity for metabolically active plaque and be rapidly cleared by the kidneys. Furthermore, the labeling procedure should be easy and make use of the radionuclide technetium-99m (^{99m}Tc), which is easily available in most parts of the world and provides a limited radiation burden to the patient due to its physical half-life of 6 hours.

Various efforts have been undertaken to develop a radiopharmaceutical with the features mentioned above. This has resulted in different agents which can be grossly divided into two categories*, i.e.*, more specific and less specific agents.

Specific radiopharmaceuticals

Peptides and proteins

The group of specific agents consists of various peptides comprising approximately 20 amino acids. The rationale for the use of peptides for the imaging of atherosclerosis is that cell surfaces express a variety of receptors that bind small peptides with high affinity (28). Two different classes of peptides have been evaluated as possible agents for the imaging of atherosclerotic plaques: peptides that associate with the apo B portion of LDL and those that associate with endothelin analogues.

With regard to the first class of peptides, it should be mentioned that the uptake of LDL by hepatocytes or fibroblasts is mediated by the LDL receptor on those cells that recognize a particular part of apo B. Also, macrophages present in the atherosclerotic plaque contain these LDL receptors and peptides based on the apo B portions of LDL will thus be bound to them. This elegant principle has been successfully tested in animals and

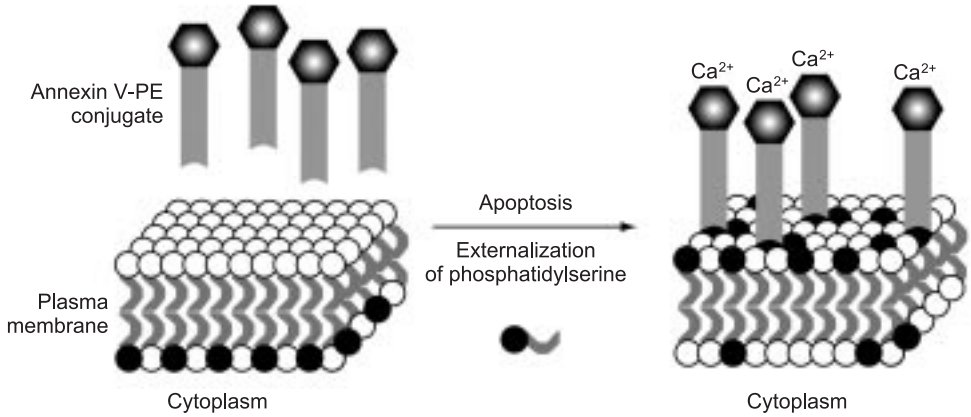

Fig. 6. Schematic representation of Ca^{2+}-dependent binding of annexin V to apoptotic cell membrane in which phosphaptidylserine is expressed on the luminal side (after www.bdbiosciences.com).

patients (29), but has not yet been evaluated in well-controlled clinical trials.

The second class of peptides includes endothelin (ET) analogues, of which ET-1 is the major effector compound (30). This peptide is synthesized by endothelial smooth muscle and inflammatory cells. ET-1 favors vascular cell proliferation and vasoconstriction through activation of specific ET_A and ET_B tyrosine kinase receptors on vascular smooth muscle cells. In transformed smooth muscle cells, such as in areas of atherosclerosis, ET-1 binds selectively to these receptors (30). Unfortunately, this means of targeting did not result in sufficiently high target-to-nontarget ratios for adequate imaging.

Based on the fact that plaque cells demonstrate increased programmed cell death, it might be worthwhile to investigate the possibilities of radiolabeled annexin V for depicting lesions. Annexin V is a Ca^{2+}-dependent 36-kD protein that shows high affinity for phosphatidylserine-containing membranes (Fig. 6). This compound can be labeled with ^{99m}Tc and is under investigation for the imaging of apoptosis in infarcted myocardial tissue (31). Plaque instability may well be caused by apoptosis of smooth muscle cells of the fibrous cap. It is also likely that apoptosis contributes to the accumulation of dead macrophages in the necrotic core, which thereby undergoes an increase in volume, resulting in plaque instability (32). Preliminary studies have shown that ^{99m}Tc-annexin V accumulates in experimental atherosclerosis in the rabbit aorta with a target-to-nontarget ratio of 9.3. In their study, Kolodgie *et al.* (33) demonstrated that radiotracer uptake predominantly occurred in advanced lesions, which were characterized by higher macrophage burden and increased apoptosis compared to less advanced lesions.

Altogether, it seems that ^{99m}Tc-annexin V is a promising agent for identifying vulnerable plaque. In order to distinguish the various degrees of plaque vulnerability, quantitative scintigraphic analysis may be necessary.

Nonspecific radiopharmaceuticals

Immunoglobulins

As mentioned before, atherosclerotic lesions contain so-called foam cells, which are macrophages that have died after taking up oxidized LDL. Such macrophages express Fc receptors to which the Fc part of immunoglobulins can bind. Indeed, the accumulation of radiolabeled immunoglobulin G (IgG) at sites of inflammation/infection has been the subject of various studies with regard to the scintigraphic detection of these lesions. However, this radiotracer did not prove to be successful due to the low ratio between lesion-incorporated radioactivity to circulating activity (34).

A different approach using immunoglobulins could be the development of a monoclonal antibody against antigens isolated from human atherosclerotic lesions. Aminomalonic acid (AMA) has been isolated from human atherosclerotic lesions and has been shown to mediate calcium deposition in the atheroma (35). Moreover, it is important for monocyte recruitment in the intima and for foam cell formation. Indeed, a radioiodinated monoclonal antibody against AMA has been shown to localize in experimental atherosclerotic lesions.

Generally, radiolabeled monoclonal antibodies localize in liver and bone marrow, thereby giving rise to an extensive radiation burden to the patients. This targeting also obscures the accumulation of the radiopharmaceutical in lesions in the large vessels. This disadvantage also holds for antibodies raised against antigenic proteins that appear on the cell surface of transformed smooth muscle cells in atherosclerotic lesions. Although experiments with one of these antibodies, indium-111-labeled Z2D3 (36), proved to be successful in demonstrating carotid lesions, proof that it visualizes aortic lesions is still lacking.

It should be noted, moreover, that monoclonal antibodies have a number of other disadvantages with regard to possible human use. These antibodies are usually generated in mice and it is therefore conceivable that they contain murine viral material and that human anti-mouse

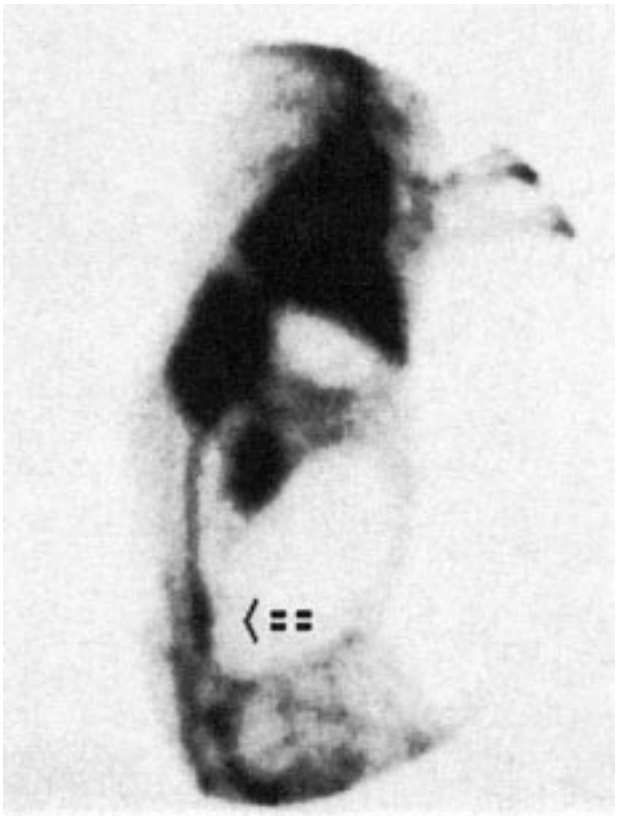

Fig. 7. Scintigram taken after application of ^{99m}Tc-Ox-LDL in rabbits with diet-induced aortic atherosclerosis (see arrow) (modified from Ref. 39).

antibodies (HAMA) may be raised in patients, resulting in allergic reactions (37).

LDL and oxidized LDL

Macromolecules such as LDL are known to accumulate in arterial lesions (38). Radiolabeled LDL may therefore target atherosclerotic lesions and Atsma *et al.* (39) have provided proof of principle in experimental atherosclerotic lesions. These authors also demonstrated that LDL uptake is considerably higher and that higher target-to-background ratios can be reached when "highly oxidized" modified LDL, labeled with ^{99m}Tc, was applied (Fig. 7). This is consistent with the fact that this modified LDL is taken up rapidly by macrophages present in the lesion (2).

Conclusions

The endoluminal approach to visualizing atherosclerotic plaque has been replaced by research efforts to image atherosclerosis by noninvasive methods (Table IV). Two noninvasive imaging methods hold promise for the future, *i.e.*, MRI and nuclear medicine. Both methods have the potential to image plaque and to determine its composition and microanatomy. However, both methods are intrinsically different. Whereas MRI visualizes the morphological characteristics, the challenge for nuclear medicine is to target the abnormal biochemical and cellular processes that result in plaque formation. In this respect, nuclear medicine has the ability to depict the functional aspects, which may add to the morphological data obtained from high-resolution MR images.

Of special clinical interest is the effort to identify vulnerable plaque, as this is important for risk stratification in patients suffering from atherosclerosis. Vulnerable plaque is characterized by apoptosis of cells such as smooth muscle cells and macrophages. Thus, the use of ^{99m}Tc-labeled annexin V, which targets the apoptotic process, has been suggested and successfully tested in experimental atherosclerosis prone to rupture. This agent may therefore hold promise for the identification and follow-up of vulnerable plaque before clinical disease has been allowed to manifest itself.

Table IV: Clinical imaging of atherosclerosis.

	% Stenosis	Wall	Lipid	Fibrous	Calcium
Invasive:					
X-ray angiography	●○⊕				
Angioscopy			●		
IVUS	●	●		●	●
Photonic spectroscopy*			●**		●**
Noninvasive:					
MRI	○⊕	○⊕	○⊕	○⊕	○⊕
MRA	●	●			
MRS			●○⊕	●○⊕	●○⊕
Scintigraphy			○⊕	⊕	

● Coronary artery; ○ Carotid artery; ⊕ Aorta.
*Raman spectroscopy and near-infrared spectroscopy; **In combination with IVUS; IVUS = intravascular ultrasound; MRI = magnetic resonance imaging; MRA = magnetic resonance angiography; MRS = magnetic resonance spectroscopy.

Acknowledgements

The authors are indebted to Mr. Gerrit Kracht for artistic assistance.

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