Short Communication

Dating Components of Human Atherosclerotic Plaques

Isabel Gonçalves, Kristina Stenström, Göran Skog, Sören Mattsson, Mihaela Nitulescu, Jan Nilsson

Rationale: Atherosclerotic plaques that give rise to acute clinical symptoms are typically characterized by degradation of the connective tissue and plaque rupture. Experimental studies have shown that mechanisms to repair vulnerable lesions exist, but the rate of remodeling of human plaque tissue has not been studied.

Objective: In the present study, we determined the biological age of different components of advanced human atherosclerotic plaques by analyzing tissue levels of $^{14}$C released into the atmosphere during the nuclear weapons tests in the late 1950s and early 1960s.

Methods and Results: Atherosclerotic plaques were obtained from 10 patients (age 46 to 80 years) undergoing carotid surgery. Different regions of the plaques were dissected and analyzed for $^{14}$C content using accelerator mass spectrometry. At the time of surgery, the mean biological age of the cap region was 6.4 ± 3.2 years, which was significantly lower than that of the shoulder region (12.9 ± 3.0 years, $P<0.01$), the interface toward the media (12.4 ± 3.3 years, $P<0.01$), and the core (9.8 ± 4.5 years, $P<0.05$). Analysis of proliferative activity and rate of apoptosis showed no signs of increased cellular turnover in the cap, suggesting that the lower $^{14}$C content reflected a more recent time of formation.

Conclusions: These results show that the turnover time of human plaque tissue is very long and may explain why regression of atherosclerotic plaque size rarely is observed in cardiovascular intervention trials. (Circ Res. 2010; 106:1174-1177.)

Key Words: atherosclerosis ■ plaque ■ dating ■ $^{14}$C

Observations from animal models of atherosclerosis and human autopsy studies suggest that plaque development is initiated by accumulation and oxidation of lipoproteins in the arterial wall extracellular matrix, leading to activation of inflammation and intimal fibrosis. Advanced plaques with large lipid necrotic cores covered by thin fibrous caps are more susceptible to rupture and responsible for the development of the majority of clinical events. It has been postulated that the stability of atherosclerotic plaques depends on their ability to produce effective fibrotic repair responses. In humans, plaques develop silently over several decades, and it is not known to what extent they have a capacity to undergo continuous repair and remodeling. In this study, we aimed to clarify this by determining the biological age of individual components of human plaques. We used a method that allows assessment of human tissue turnover based on the incorporation of $^{14}$C released by nuclear weapons tests in the late 1950s and early 1960s, the so-called "bomb-pulse." Atmospheric levels of $^{14}$C increased rapidly after these detonations, but as a result of diffusion of $^{14}$CO$_2$ into other parts of the ecosystem. Through dietary intake of $^{14}$C from plants, as well as animals that live on plants, the $^{14}$C concentration of human cells and tissues is generally expected to closely parallel that in the atmosphere at the time they were formed, with an average lag time of ≈1 year. The potential of using the bomb-pulse to study replacement rates for human tissue was realized already at the beginning of the nuclear weapons test era. Refined measurement methods, using samples containing only a few tens of micrograms of carbon, have recently opened new possibilities and stimulated further applications of the technique. By determining $^{14}$C levels in different tissues, it has been shown that the formation of human cortical neurons, as well as the eye lens, almost entirely takes place around the time of birth and that human gallstones begin to form 10 years before they give rise to symptoms. It has also been shown that 10% of all fat cells and 0.5% to 1% of all of cardiomyocytes are turned over annually in humans.

Original received October 15, 2009; revision received February 3, 2010; accepted February 5, 2010.

From the Departments of Clinical Sciences Malmö (I.G., S.M., M.N., J.N.) and Cardiology (I.G.), Malmö University Hospital; and Physics (K.S.) and GeoBiosphere Science Centre, Quaternary Sciences (G.S.), Lund University, Sweden.

Correspondence to Isabel Gonçalves, CRC Entrance 72:91/12, Malmö University Hospital, SE-20502 Malmö, Sweden. E-mail isabelgoncalves@med.lu.se

© 2010 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.109.211201

1174
**Methods**

We examined 10 atherosclerotic plaques from patients undergoing carotid surgery at the Malmö University Hospital during 2007 to 2009. The mean age of the patients was 65 (range, 46 to 80) years. Five of the patients had symptoms such as stroke (n = 1), transient ischemic attacks (n = 1), and amaurosis fugax (n = 3), whereas 5 were referred to surgery because of a nonsymptomatic carotid stenosis of >70%. Informed consent was given by each patient. The study was approved by the regional ethical committee. After surgical removal, plaques were snap-frozen in liquid nitrogen. A 1-mm-thick transverse section of the plaque was used for histology, and a consecutive 1-mm-thick section was dissected to separate the fibrous cap, shoulders, core, and interface between the core and the underlying media of the plaque. The 14C content of isolated plaque fragments was quantified by accelerator mass spectrometry (AMS) using the 250 keV single-stage AMS facility (Lund University). Before these measurements, the carbon was extracted from the samples and converted into elemental carbon (graphite). This was achieved in a 2-step process involving the conversion of the carbon in the sample to CO2, followed by reduction of the CO2 to graphite. The graphite from each sample was pressed into a sample holder and placed in the ion source of the single-stage AMS facility, together with standard samples of known activity and background samples (14C-free) processed in the same way. The results from the AMS measurements were converted to calendar years using the CaliBomb software and the Levin data set representative for Europe (Figure 1A). Histograms were converted to calendar years using the CaliBomb software and the Levin data set representative for Europe (Figure 1A). Histograms were converted to calendar years using the CaliBomb software and the Levin data set representative for Europe (Figure 1A).

**Results**

The fibrous cap was identified as the most recently formed part of the plaque (Figure 1B). At the time of surgery, the mean biological age of the cap was 6.4 ± 3.2 years, which was significantly lower than that of the shoulder (12.9 ± 3.0 years, P < 0.01), the interface toward the media (12.4 ± 3.3 years, P < 0.01), and the core (9.8 ± 4.5 years, P < 0.05). It should be noted that this is the location where most of the growth of the lesion.

**Discussion**

This study demonstrates that it is possible to determine an approximate biological age of human atherosclerotic plaque components using 14C analysis by AMS. The finding that the fibrous cap is the youngest part of advanced plaques suggests that this is the location where most of the growth of the lesion.
takes place or alternatively that the tissue turnover is higher in the fibrous cap than in the rest of the plaque. The latter possibility is in line with the notion that active proliferation and extracellular matrix synthesis by smooth muscle cells in the cap is required for maintaining plaque stability. However, our observation that proliferative activity and apoptosis were not increased in the cap argues against a higher tissue turnover as a possible explanation to the lower $^{14}$C in the cap in this study. These results demonstrate that the turnover of most of the atherosclerotic plaque tissue occurs at a very low rate. Results from lipid-lowering intervention trials have consistently shown that significant reductions in cardiovascular mortality occur without any or only marginal reduction of atherosclerotic plaque size. The finding that the age of most of the plaque tissue is $\approx$10 years helps to explain these observations and suggests that, at least with existing therapies, reduction of plaque size is an unlikely response to treatment. This is also in line with the notion that statins primarily function by reducing plaque inflammation. The regression of atherosclerosis observed in experimental animal studies generally relates to relatively early lesions, and these findings cannot be directly extrapolated to humans.

There are several limitations of the present study that need to be considered. First, the study is based on relatively few patient samples. However, with the exception of the plaque obtained from a patient who had a major stroke at a young age, the results consistently demonstrate that the rate of tissue turnover in human atherosclerotic plaques is very low. Second, the uncertainty of AMS determination results in an uncertainty of up to $\pm$3 years in the biological age of tissue sample (Figure 1). The uncertainty increases with more recently formed tissues because the difference in atmospheric $^{14}$C levels between years is lower. High-precision bomb-pulse dating may also be limited by small variations of $^{14}$C concentration in different environments and types of diets. Nevertheless, the effect of these variations is likely neither to exceed the uncertainty reported in this study nor to influence the conclusions. Finally, although the proliferation and apoptosis findings argue against an increased cellular turnover in the fibrous cap, they do not exclude the possibility of an increased turnover of noncellular proteins as an explanation for the younger biological age of the cap.

In conclusion, using $^{14}$C determination by AMS, we show that the biological age of human atherosclerotic plaque...
components varies between 5 and 15 years of age, with the cap being the youngest. It should be noted that this does not exclude that the plaque in itself is older and that the original plaque components have been replaced. The very slow turnover of atherosclerotic plaque tissue may help to explain why reduction of atherosclerosis has been difficult to achieve in cardiovascular intervention trials.

Sources of Funding
This study was supported by the Swedish Research Council (2008-8311 and 2007-4163), Swedish Heart and Lung Foundation, Swedish Medical Society, Ernhold Lundström Foundation, Zoëga’s Foundation, Michaelsen Foundation, Tore Nilsson Foundation, Lars Hierta Medical Society, Ernhold Lundström Foundation, and the Knut and Alice Wallenberg Foundation.

Disclosures
None.

References

Novelty and Significance

What Is Known?

- Atherosclerotic plaques develop over many years.
- Tissue remodeling is important for maintaining plaque stability.
- The rate at which remodeling occurs in human cells and tissues has not been studied.

What New Information Does This Article Contribute?

- The turnover of human plaque tissue is very slow, ranging from 5 to 15 years in different regions of the plaque.
- The fibrous cap is the youngest part of the plaque.

The ability to mount effective tissue repair responses is of critical importance for maintaining plaque stability, but the rate at which tissue turnover occurs in human atherosclerotic plaques has not been studied. We have taken advantage of the possibility to determine the time of formation of human tissue by analyzing the content of 14C released into the atmosphere during the nuclear weapons tests in the late 1950s and early 1960s. The 14C content of atherosclerotic lesions removed at carotid surgery was determined by accelerator mass spectrometry. The results demonstrate that most of the plaque tissue is formed 10 years or more before the time of surgery but that fibrous cap is several years younger. The very slow tissue turnover of atherosclerotic lesions may explain the relatively limited effect on plaque size observed in clinical intervention trials.
Short Communication: Dating Components of Human Atherosclerotic Plaques
Isabel Gonçalves, Kristina Stenström, Göran Skog, Sören Mattsson, Mihaela Nitulescu and Jan Nilsson

Circ Res. 2010;106:1174-1177; originally published online February 18, 2010; doi: 10.1161/CIRCRESAHA.109.211201

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/106/6/1174

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/