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JJCC-1295; No. of Pages 9
ARTICLE IN PRESS
Journal of Cardiology xxx (2016) xxx–xxx
Contents lists available at ScienceDirect
Journal of Cardiology
journal homepage: www.elsevier.com/locate/jjcc

Original article

Human pericoronary adipose tissue as storage and possible supply site for oxidized low-density lipoprotein and high-density lipoprotein in coronary artery

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ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form 22 March 2016
Accepted 25 March 2016
Available online xxx

Keywords:
Human coronary plaques
Immunohistochemical staining
Low-density lipoprotein
Pericoronary adipose tissue
Vasa vasorum

ABSTRACT

Background: Thickening of the pericoronary adipose tissue (PCAT) is a proven risk factor for coronary artery disease, but it is poorly understood whether PCAT stores pro-atherogenic substances with oxidized low-density lipoprotein (oxLDL) and low-density lipoprotein (LDL), and an anti-atherogenic substance with high-density lipoprotein (HDL) and supply them to the coronary intima.
Methods: Using immunohistochemical techniques, the localization of oxLDL, LDL and HDL in PCAT and its adjacent coronary segments was examined in 30 epicardial coronary arteries excised from 11 human autopsy cases.
Results: PCAT stored oxLDL and HDL in all, but LDL rarely, in 77 specimens examined, irrespective of the presence or absence of coronary plaques and underlying disease. The percentage (%) incidence of oxLDL, HDL and LDL deposits in intima was, respectively, 28, 10, 35 in 29 normal segments, 80 ($p < 0.05$ vs. normal segments), 12, 75 in 19 white plaques (growth stage), 57, 36, 90 in 15 yellow plaques without necrotic core (NC; mature stage), and 40, 21, 100 ($p < 0.05$ vs. normal segments) in 14 yellow plaques with NC (end-stage of maturation) as classified by angioscopy and histology.

In coronary intima, oxLDL deposited in either a dotted or diffuse pattern whereas HDL and LDL showed diffuse patterns. Dotted oxLDL deposits were contained in CD68(+)-macrophages traversing the border of PCAT and adventitia, external and internal elastic laminae. Diffuse oxLDL and HDL deposits colocalized with intimal vasa vasorum.
Conclusions: The results suggested that, as a hitherto unrecognized supplying route, the human PCAT stores oxLDL and HDL and oxLDL is supplied to coronary intima either by CD68(+)-macrophages or vasa vasorum and HDL by vasa vasorum, and that deposition of oxLDL and HDL in the intima increased with plaque growth but the former decreased while the latter increased further with plaque maturation. Molecular therapy targeting PCAT before plaque maturation could be effective in preventing atherosclerosis.

Introduction

Pericoronary adipose tissue (PCAT) lies on the epicardial coronary artery and is part of the epicardial adipose tissue. PCAT is considered to release, as in the case of other perivascular adipose tissues, a wide range of cytokines that modulate vascular smooth muscle cell contraction, proliferation and migration. In addition, it

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; NC, necrotic core; oxLDL, oxidized low-density lipoprotein; PCAT, pericoronary adipose tissue.

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http://dx.doi.org/10.1016/j.jjcc.2016.03.015

releases pro-inflammatory cytokines such as interleukin-6, interleukin-8 and monocyte chemoattractant protein-1, which contribute to adventitial inflammation and atherosclerosis development [1–9]. Oxidized low-density lipoprotein (oxLDL), derived from LDL is the core substance that induces atherosclerosis and is a clinically proven risk factor for coronary events [10–13]; their counterpart, high-density lipoprotein (HDL) decreases the risk of coronary heart disease by facilitating cholesterol uptake from cholesterol-loaded macrophage-foam cells in plaques for transport back to the liver [14–16]. However, it is poorly understood whether these lipoproteins are stored in human PCAT, and are supplied to the adjacent coronary artery to induce or suppress atherosclerosis.

We examined by immunohistochemical techniques whether or not the human PCAT acts as a storage and supply site of these substances to the adjacent coronary intima, which is the site of atherosclerosis.

Materials and methods

Angioscopic and immunohistochemical studies of excised human PCAT and adjacent coronary artery

Ethics

This ex vivo study was carried out after approval of the ethical committees of the Japan Foundation for Cardiovascular Research, Funabashi-Futawa Hospital and Toho University, and after obtaining written informed consent from the families concerned on the use of excised coronary artery and surrounding adipose tissue for angioscopic and histological studies to clarify the mechanisms of atherosclerosis.

Subjects

The 30 proximal to middle segments of coronary arteries (11 left anterior descending arteries, 9 left circumflex arteries, 10 right coronary arteries) and surrounding adipose tissue were carefully excised from 11 successive patients who had died and were autopsied from April 1, 2010 and May 31, 2015 at Funabashi-Futawa Hospital or Toho University Medical Center Sakura Hospital [61.2 ± 3.4 years (mean $\pm$ SD); 3 females, 8 males; death from acute myocardial infarction (3), diabetic nephropathy (3), cerebral infarction (1), subarachnoid hemorrhage (1), visceral cancer (2), sudden death (1)].

Classification of coronary plaques and normal segments by conventional angioscopy

In the present study, a conventional coronary angioscopy system (details described elsewhere [17]) was used to classify coronary plaques and normal segments.

Plaques and normal segments. By conventional coronary angioscopy, plaque was defined as a nonmobile, protruding or lining mass clearly demarcated from the adjacent normal wall and with a shape, location and color that did not alter after saline flushing. A normal segment was defined as a milky-white and smooth-surfaced portion without any protrusions [18].

Plaque color assessment. Because color definition differs observer to observer, influenced by their experience and visual sense, we developed a more objective method for color definition.

Color is a spectrum of visible light wavelengths. A given visible color band changes successively to the next, and there are no definite border between two color bands. This is the reason why color definition differs observer to observer, influenced by their experience and visual sense. Therefore, we developed a more objective method for color definition. Namely, plaque images obtained by conventional angioscopy were classified as white or yellow by an AquaCosmos image analyzer (C7746, Hamamatsu Photonics, Hamamatsu).

Namely, based on the relationship between color spectrum generated through a prism by a Xenon lamp and its light wavelength, the light wavelength of "yellow" was defined as $575 \leqq 595$-nm. The color band of this area was imaged through an angioscope and it was separated by the image analyzer into three primary colors, namely red, green and blue. The intensity ratio of red: green: blue of yellow color was 1.0:1.38–1.46:0.47–0.60. Therefore, plaque color was defined as "yellow" when its intensity ratio was within this range. Similarly, white light which was generated by the Xenon lamp was separated into three primary colors. The intensity ratio of white color was 1.0:0.9–1.1:0.9–1.1. Therefore, plaque color was defined as "white" when the intensity ratio was within this range [19].

Observation of excised coronary arteries by conventional angioscopy

A Y-connector was introduced into the proximal portion of the respective coronary artery for perfusion with saline solution at a rate of 20 mL/min and then the angioscope was introduced through the connector into the artery to evaluate it for plaques. A total of 29 normal segments, 19 white plaques and 29 yellow plaques, which matched the aforementioned criteria, were confirmed by conventional angioscopy in 30 coronary arteries. Externally, the location of each observed plaque or normal segment could be identified because the light irradiated by the angioscope tip was visible through the arterial wall (Fig. 1).

Selection of plaques and normal segments. The 4–5-mm long section of artery in which the observed plaque was located and its surrounding PCAT were isolated by transecting its proximal and distal ends at the shorter axes. Normal segments were similarly isolated. In total, 29 normal segments, 19 white plaques, 29 yellow plaques, and 29 normal segments, surrounded by PCAT, were classified using images recorded on digital video disks by two independent observers who did not participate in conventional angioscopy. They randomly selected the normal segments and plaques for further examination, and embedded them in O.C.T. Compound (Sakura Fintek USA Inc., Torrance, CA, USA) before being stored at −20 °C.

Immunohistochemistry of OxLDL, CD68(+)-macrophages and CD31 (endothelial cells of vasa vasorum)

Definition of PCAT. Epicardial adipose tissue located within 3 mm (diameter of the proximal to middle segments of a coronary artery) of the external elastic lamina of an epicardial coronary artery was arbitrarily defined as PCAT because it was more likely to directly

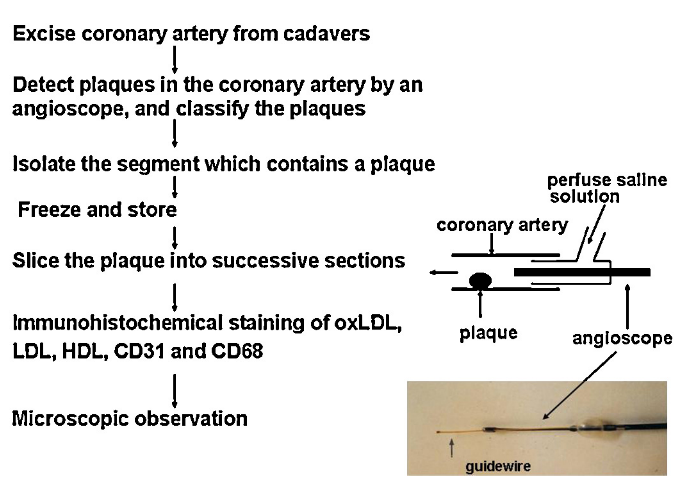

Fig. 1. Procedure of the present study.

Please cite this article in press as: Uchida Y, et al. Human pericoronary adipose tissue as storage and possible supply site for oxidized low-density lipoprotein and high-density lipoprotein in coronary artery. J Cardiol (2016), http://dx.doi.org/10.1016/j.jjcc.2016.03.015