Atherosclerosis 243 (2015) 540–545

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

In vivo evidence for chylomicrons as mediators of postprandial inflammation

Marijke A. de Vries [a], Boudewijn Klop [a], Arash Alipour [a], Gert-Jan M. van de Geijn [b], Lenneke Prinzen [b], Anho H. Liem [c], Pedro Valdivielso [d], José Rioja Villodres [d], José Ramírez-Bollero [d], Manuel Castro Cabezas [a, *]

[a] Department of Internal Medicine, Center for Diabetes and Vascular Medicine, Rotterdam, The Netherlands
[b] Department of Clinical Chemistry, Rotterdam, The Netherlands
[c] Department of Cardiology, Sint Franciscus Gasthuis, Rotterdam, The Netherlands
[d] Internal Medicine Unit, Hospital Virgen de la Victoria and Department of Medicine and Dermatology, IBIMA, University Málaga, Campus de Teatinos s/n, 29010 Málaga, Spain

ARTICLE INFO

Article history:
Received 16 July 2015
Received in revised form
16 October 2015
Accepted 20 October 2015
Available online 23 October 2015

Keywords:
Apolipoproteins
Apolipoprotein B48
Atherosclerosis
Leukocyte activation
Monocytes
Neutrophils
Postprandial lipemia
Triglycerides

ABSTRACT

Background and aims: The postprandial situation is a pro-inflammatory condition most likely linked to the development of atherosclerosis. We evaluated the relationship between apolipoprotein (apo) B48 and fasting and postprandial leukocyte activation markers.
Methods: Leukocyte activation markers and apo B48 were determined in 80 subjects with and without coronary artery disease (CAD). Twelve healthy subjects underwent an oral fat loading test (up to 8 h).
Results: Fasting apo B48 was significantly higher in patients with CAD (n = 47, 8.1 ± 5.2 mg/L) than in subjects without CAD (n = 33, 5.9 ± 3.9 mg/L, p = 0.022). Fasting apo B48 and triglycerides correlated positively with fasting monocyte CD11b and neutrophil CD66b expression. Plasma apo B48 and leukocyte activation markers increased after an oral fat load. No correlations were found between fasting or postprandial triglycerides and postprandial leukocyte activation markers. We observed no correlations between postprandial apo B48 and postprandial neutrophil CD11b or CD66b expression.
Conclusion: This study suggests that chylomicron remnants may be responsible for postprandial leukocyte activation in the circulation. The postprandial chylomicron response may be a stronger mediator of postprandial inflammation than postprandial triglyceridemia.

1. Introduction

The postprandial situation is a pro-inflammatory condition most likely linked to the development of atherosclerosis. Postprandial lipemia activates circulating leukocytes, as has been demonstrated *in vitro* and *in vivo* [1–3]. Activated leukocytes can adhere to the intact endothelium and migrate to the subendothelial space, where the development of atherosclerosis is initiated [4].

Both, increased leukocyte activation and postprandial triglycerides have been linked to the presence of coronary artery disease (CAD) and peripheral artery disease [5–10]. The measurement of postprandial lipemia is time-consuming, requiring postprandial measurements during several hours after an oral fat loading test [11]. It has been suggested that fasting apolipoprotein (apo) B48 may be used as a surrogate marker for postprandial lipemia [11,12].

Diet-ingested lipids are transported to the circulation in chylomicrons. In the circulation, triglycerides present in the chylomicrons are rapidly hydrolyzed by the enzyme lipoprotein lipase, and chylomicron remnants are formed [13]. Each chylomicron carries a single apo B48 molecule on its surface [14]. Since apo B48 is synthesized exclusively in the intestine, the level of apo B48 represents the total amount of circulating chylomicrons and their remnants. Chylomicron remnants can migrate into the arterial wall, where they can induce foam cell formation without the need of prior oxidation or other modifications [15,16]. Although the infiltration rate into arterial tissue is approximately 10 times lower than that of

* Corresponding author. Department of Internal Medicine, Center for Diabetes and Vascular Medicine, Sint Franciscus Gasthuis, Rotterdam Po Box 10900, 3004 BA Rotterdam, The Netherlands.
E-mail address: m.castrocabezas@sfg.nl (M. Castro Cabezas).

http://dx.doi.org/10.1016/j.atherosclerosis.2015.10.025
0021-9150/

low-density lipoprotein (LDL) particles, their efflux rate is also approximately 20 times lower [17]. Thus, chylomicron remnants are more easily retained in the subendothelial space than LDL particles. It has been postulated that chylomicron remnants are mainly involved in the early stages of atherogenesis, and fasting apo B48 levels have been associated with the development of atherosclerosis [18–21]. Carotid intima media thickness, a marker of subclinical atherosclerosis, was positively associated with fasting apo B48 [19]. In addition, fasting apo B48 was higher in patients with peripheral artery disease [20], and predicted the risk of coronary events independent of LDL cholesterol [21].

Chylomicrons may not only be directly involved in the development of atherosclerosis, but also indirectly, by stimulating inflammation following interaction with circulating leukocytes [1,2]. The aim of this study was to evaluate the relationship between apo B48 and leukocyte activation markers in the fasting and the postprandial situation.

2. Materials and methods

2.1. Participants

In order to assess the relationship between fasting apo B48 and markers of leukocyte activation, subjects who were scheduled to undergo a diagnostic coronary angiography were included. The design of this case–control study has been described extensively elsewhere [22]. Exclusion criteria were the presence of inflammatory disorders, plasma C-reactive protein above 10 mg/L, and disorders of kidney, liver and thyroid function. To investigate the role of postprandial apo B48 on leukocyte activation, a second group of healthy volunteers underwent an oral fat loading test. The design of this postprandial study has been described elsewhere [23]. The Institutional Review Board of the Sint Franciscus Gasthuis Rotterdam and the regional independent medical ethics committee of the Maasstad Hospital Rotterdam approved both studies. All participants gave written informed consent.

2.2. Study design

For the case–control study, anthropometric measures, the use of medication and cardiovascular history were recorded on the day of the angiography. Shortly before coronary angiography, fasting venous blood was obtained from a peripheral vein of the forearm. Blood samples were collected in tubes containing EDTA (1 mg/mL) and kept on ice until processed for determination of leukocyte activation markers. Coronary angiography images were scored by an independent cardiologist.

For the postprandial study, volunteers visited the hospital after an overnight fast. A fasting venous blood sample was drawn. After venipuncture, volunteers ingested fresh cream in a concentration of 50 g of fat per square meter body surface. Blood samples were obtained at 2-hourly intervals, for up to 8 h after fat ingestion.

2.3. Analytical methods

Parameters for glucose, C-reactive protein, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were determined using Synchron LX-20 analyzers (Beckman Coulter, Brea, CA, USA) according to standard procedures in our laboratory. LDL cholesterol values were calculated using the Friedewald formula. Apo AI and B were determined by rate nephelometry using an IMMAGE analyzer (Beckman Coulter). Blood cell counts were determined using LH750 analyzers (Beckman Coulter, Miami, FL, USA). The leukocyte differentiation was determined as a five-part differentiation on the same instruments. Apo B48 was measured using a high-sensitivity commercial ELISA (Shibayagi Co Ltd, Ishihara, Japan) [24]. Two plates with 4 replicates per plate of a low value internal quality serum control (mean 2.96 mg/L) were run. The mean intra-assay coefficient of variation (CV) at this low concentration was 8.7%, the total CV for both assays was 10.3%. The inter-assay CV was (Geometric substract) 5.5%.

2.4. Leukocyte activation markers

Blood samples for the measurement of leukocyte activation markers were collected in EDTA and were determined by flow cytometry on the same day. The expression of leukocyte activation markers on the cell surface was determined using fluorescent labeled monoclonal antibodies (Beckman Coulter). Antibodies against CD66b were labeled with fluorescein isothiocynate (FITC). Antibodies against CD11b were labeled with phycoerythrin (PE). Antibodies against CD45 labeled with PE-Texas Red (ECD) were used to differentiate leukocytes from erythrocytes and platelets. Whole blood was added to a combination of CD66b-FITC, CD11b-PE and CD45-ECD antibodies. Cells were incubated for 15 min in the dark at room temperature. In parallel, blood was incubated with FITC- and PE-conjugated mouse IgG1 as isotype control to correct for nonspecific binding. Erythrocytes were lysed by adding ice-cold isotonic erythrocyte lysing solution (NH_4Cl 0.19 M; $KHCO_3$ 0.01 M; $Na_2EDTA \cdot 2H_2O$ 0.12 M, pH 7.2) for 15 min. For the cross-sectional study, a Coulter Epics XL-MCL flow cytometer with a 488 nm Argon ion laser and EXPO 32 software were used for measurement and analysis. For the postprandial study, a Navios flow cytometer (Beckman Coulter) was used for measurement and Kaluza software version 1.2 (Beckman Coulter) was used for analysis. The fluorescence intensity of each cell was expressed as the mean fluorescence intensity, given in arbitrary units (au). Lymphocytes, monocytes and granulocytes were identified based on their side scatter and the level of CD45 on their cell surface.

2.5. Statistical analysis

Data are given as mean ± SD in the text, tables and figures. Baseline differences between the groups were tested with Independent Samples T Test for normally distributed continuous variables and with Mann–Whitney U Test for continuous variables with skewed distributions (apo B48, triglycerides, monocyte CD11b, neutrophil CD11b and CD66b). Differences between fasting and postprandial values were tested with Wilcoxon Signed Rank Test. Correlation analysis was carried out using Spearman correlation statistics. The total area under the curve (AUC) and the AUC corrected for baseline values (dAUC) were calculated by the trapezoidal rule using Graphpad Prism version 5.0 (LA, USA). Statistical analysis was carried out with PASW statistics version 22.0 (IBM SPSS Statistics, New York, United States). P-values <0.05 (2-tailed) were considered statistically significant.

3. Results

3.1. Baseline characteristics

A total of 80 patients participated in the cross-sectional study. Their baseline characteristics are listed in Table 1. The CAD group consisted of older patients and included more males. They had higher body mass index, systolic blood pressure and fasting triglycerides. Plasma apo A1, LDL cholesterol and HDL cholesterol were lower than in subjects without CAD. Fasting apo B48 was significantly higher in patients with CAD (8.1 ± 5.2 mg/L) than in subjects without CAD (5.9 ± 3.9 mg/L, $p = 0.022$). Data suggested a trend for higher expression of leukocyte activation markers in