Relationship between Reactive Oxygen Metabolites and Carotid Intima-Media Thickness in Subjects with Hypercholesterolemia

Kazuhiko Kotani    Harumi Koibuchi    Michiaki Miyamoto    Toshiyuki Yamada    Nobuyuki Taniguchi
Department of Clinical Laboratory Medicine, Jichi Medical University, Tochigi, Japan

Key Words
Oxidative stress · Reactive oxygen species · Diacron reactive oxygen metabolites · Low-density lipoprotein cholesterol · Carotid atherosclerosis

Abstract
Objective: It was the aim of this study to investigate whether there is any relationship between oxidative stress, as assessed by the diacron reactive oxygen metabolite (d-ROM) test, and carotid atherosclerosis among hypercholesterolemic patients. Subjects and Methods: A well-defined group of patients with type II hypercholesterolemia (n = 81, mean age 59 years) was studied to observe the correlation between the levels of serum d-ROMs and carotid artery intima-media thickness (IMT) using B-mode ultrasound, in relation to the traditional atherosclerotic risk factors (age, sex, smoking, body mass index, blood pressure, glucose and lipid panels). Results: The mean level in low-density lipoprotein cholesterol (LDL-C) in this population was 4.45 mmol/l, d-ROMs were 323.2 Carr U, and IMT was 0.91 mm. A multiple regression analysis revealed a positive and significant correlation between IMT and d-ROMs (β = 0.27, p < 0.05), along with age and LDL-C. Conclusion: These results indicate that the increased oxidative stress levels using the d-ROM test, independent of aging and increased LDL-C levels, may be associated with carotid atherosclerosis even in hypercholesterolemic patients.

Introduction
Oxidative stress (OS) is crucial in atherogenesis [1, 2]. Hypercholesterolemia is an atherosclerotic risk factor, and the role of OS in hypercholesterolemia on atherosclerotic formation has so far received a great deal of attention. Among the several clinically applicable biomarkers, the diacron reactive oxygen metabolite (d-ROM) test has been shown to indirectly quantify the free radical production by measuring the hydroperoxidation of organic compounds [3–5]; hence, this test is presently used as an OS marker.

The carotid arterial intima-media thickness (IMT) is a well-recognized index for cardio-/cerebrovascular disease risks [2]. In spite of the clinically widespread usage of OS markers, few studies have examined associations between carotid IMT and OS markers [2, 6]. Positive correlations have been reported between NADPH oxidase activity and IMT in healthy persons [2] as well as between

Received: July 19, 2009
Revised: January 10, 2010

Kazuhiko Kotani, MD, PhD
Department of Clinical Laboratory Medicine, Jichi Medical University
3311-1 Yakushiji, Shimotsuke-City
Tochigi 329-0498 (Japan)
Tel. +81 285 587 386, Fax +81 285 449 947, E-Mail kazukotani@jichi.ac.jp

© 2010 S. Karger AG, Basel
1011–7571/10/0196–0496$26.00/0
Accessible online at: www.karger.com/mpp
reactive oxygen species formation by mononuclear cells and IMT in hypertensives [6]. However, because there is still no currently accepted reference marker which can be used to assess OS, these markers might not necessarily show similar findings. To elucidate the roles of OS on health and diseased status, more studies are required using various markers.

Furthermore, unexpectedly, the independent association of OS markers such as d-ROMs with carotid atherosclerosis in a specifically restricted population of patients with hypercholesterolemia remains to be fully demonstrated in clinical practice. Therefore, a well-defined group of type II hypercholesterolemic patients was studied to evaluate the correlation between circulating d-ROMs and carotid IMT levels in relation to atherosclerotic risks.

**Subjects and Methods**

The study population (81 patients, 52 females, 22 males; average age 59.2 ± 11.0 years) was diagnosed with a diagnosis of type II hypercholesterolemia using the World Health Organization criteria, a relatively common hyperlipidemic type. They were free of clinical overt cardio-/cerebrovascular diseases. No subject receiving medication for blood pressure (BP) and glucose/lipids was included. The study was approved by the Jichi Medical University Ethics Committee, and each patient gave an informed consent.

In addition to the current smoking status by self-report, the following values were determined after an overnight fast: body mass index, seated systolic/diastolic BP (SBP/DBP), blood glucose, triglycerides, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). The d-ROM test values were obtained using a kinetic spectrophotometric assay (FREE system; Diacron, Grosseto, Italy) [3], with the intra- and interassay coefficients of variation being 2.1 and 3.1%, respectively. The IMT of common carotid arteries was ultrasonographically determined with a 10-MHz linear type B-mode probe (Aloka Co., Japan) in the supine position. Three measurements of IMT, bilaterally examined with longitudinal/transverse scans in plaque-free segments, were averaged: one at the thickest site and the other points 1 cm upstream and 1 cm downstream from the thickest site.

For the correlations between IMT and the other variables including d-ROMs, we used Pearson’s rank coefficient test as well as a multiple regression analysis adjusted for all measured atherosclerotic risks such as age, smoking, SBP/DBP, glucose and lipid panels. Because triglycerides had a skewed distribution, the values were logarithm-transformed in analyzing. A p value <0.05 was considered significant.

**Results**

The average levels ± standard deviation of each variable in all patients were as follows; body mass index 24.2 ± 3.4; SBP 137.1 ± 13.9 mm Hg; DBP 79.3 ± 8.8 mm Hg; LDL-C 4.45 ± 0.74 mmol/l; HDL-C 1.63 ± 0.35 mmol/l; glucose 5.37 ± 0.83 mmol/l; d-ROMs 323.2 ± 63.9 Carr U; IMT 0.91 ± 0.24 mm. The median (interquartile range) of triglycerides was 1.4 mmol/l (1.2–2.1). There were 10 current smokers.

In the simple correlation analysis, IMT was significantly correlated with age (r = 0.33, p = 0.003), SBP (r = 0.26, p = 0.018), LDL-C (r = 0.22, p = 0.047) and d-ROMs (r = 0.25, p = 0.024). The multiple regression analysis, adjusted for all the variables, revealed that the correlation of IMT with age, LDL-C and d-ROMs independently remained significant (table 1).

**Discussion**

This study found a significant positive relationship between the d-ROMs and carotid IMT, independent of age and LDL-C, in a population with type II hypercholesterolemia. Our study confirmed previous findings on the significant correlation between age, LDL-C and IMT, even in the present restricted study group.

The detailed mechanisms of the correlation between the ROMs and IMT are not clear; however, OS is a mediator on the initiation-to-progression of atherosclerosis with a vicious cycle [1]. Not only the presence of hypercholesterolemia-induced OS but also OS itself can enhance IMT, which in turn enhances OS. Another possi-

---

**Table 1. Multiple regression analysis for carotid artery IMT with atherosclerotic risks and ROMs**

| Variable         | β coefficient | p value  |
|------------------|---------------|----------|
| Age              | 0.279         | 0.023*   |
| Men              | 0.152         | 0.193    |
| Current smoking  | 0.005         | 0.973    |
| Body mass index  | -0.060        | 0.677    |
| SBP              | 0.211         | 0.126    |
| DBP              | -0.114        | 0.362    |
| LDL-C            | 0.230         | 0.034*   |
| (log-)triglycerides | 0.083                   | 0.469    |
| HDL-C            | -0.155        | 0.181    |
| Glucose          | 0.170         | 0.238    |
| d-ROMs           | 0.265         | 0.021*   |

* Statistically significant.
bility is the relevance of potential lifestyle: diet might affect OS independently of LDL-C, and a lack of exercise could also increase the d-ROM levels [5].

The d-ROM test has been shown to have several advantages: this test is easy to perform, stable, sensitive, inexpensive and fully automated [3, 4]. Additionally, the d-ROM test values have been reported to correlate with the other OS markers (i.e. malondialdehyde), and a number of published studies have acknowledged the clinical implications of the d-ROMs as an OS marker [5]. However, it has been suggested that the d-ROMs may not always reflect the OS itself [7]. Further studies on the features of d-ROMs are thus necessary.

**Conclusion**

Collectively, the increased OS levels as assessed by the d-ROM test, independent of aging and increased LDL-C levels, may be associated with carotid atherosclerosis even in patients with hypercholesterolemia. More studies are needed to elucidate this phenomenon.

**Acknowledgements**

This study was supported in part by a Grant-in-Aid for the Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to K.K.) and the Foundation for the Development of the Community, Japan.

**References**

1. Madamanchi NR, Hakim ZS, Runge MS: Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. J Thromb Haemost 2005; 3: 254–267.
2. Zalba G, Beloqui O, San José G, Moreno MU, Fortuño A, Díez J: NADPH oxidase-dependent superoxide production is associated with carotid intima-media thickness in subjects free of clinical atherosclerotic disease. Arterioscler Thromb Vasc Biol 2005; 25: 1452–1457.
3. Iamele L, Fiocchi R, Vernocchi A: Evaluation of an automated spectrophotometric assay for reactive oxygen metabolites in serum. Clin Chem Lab Med 2002; 40: 673–676.
4. Vassalle C: An easy and reliable automated method to estimate oxidative stress in the clinical setting. Methods Mol Biol 2008; 477: 31–39.
5. Kotani K, Sakane N, Tsuzaki K, Matsuoka Y, Sano Y, Hamada T, Yamada K: Lifestyles and oxidative stress in type 2 diabetic patients. Scand J Clin Lab Invest 2008; 68: 516–518.
6. Watanabe T, Yasunari K, Nakamura M, Maeda K: Carotid artery intima-media thickness and reactive oxygen species formation by monocytes in hypertensive patients. J Hum Hypertens 2006; 20: 336–340.
7. Harma MI, Harma M, Erel O: d-ROMs test detects ceruloplasmin, not oxidative stress. Chest 2006; 130: 1276.