LETTERS TO THE EDITOR

Low Density Lipoprotein Oxidation and Variant Angina: Role of Methodologic Procedures in Assessment of Oxidizability of Low Density Lipoprotein

I read with great interest the results of the study by Miwa et al. (1) dealing with the role of copper-induced peroxidized low density lipoprotein (LDL) in patients with variant angina. The authors conclude from their observations that LDL in these patients was highly susceptible to peroxidative modification in association with less vitamin E content than that in patients with inactive coronary spasm. This conclusion may be questionable, although the results are provocative and may justify further studies. Oxidized LDL, isolated from atherosclerotic arteries, is similar to LDL oxidized in vitro with copper (range 5 to 20 μM) (2). Many groups use copper ions to peroxidize LDL in vitro (2) and probably study reactions involving preformed hydroperoxides. In fact, copper ions will not cause peroxidation in completely peroxide-free LDL (3). This phenomenon highlights the risk of misleading results in interpreting the pathophysiologic significance of copper-peroxidized LDL. Computer simulation revealed that the average concentration of Cu^{2+} was 10^{-18} mol/liter, with a conservative upper limit of 10^{-11} mol/liter, whereas the mean free iron (Fe^{3+}) was 10^{-23} mol/liter (4). Thus, micromolar concentrations of copper are millions-fold higher than physiologic concentrations. In addition, one should not infer from in vitro oxidation by free copper (Cu^{2+}) that such a process occurs in vivo because Cu^{2+} is unlikely to occur in vivo. Copper is a member of the first transition series of elements, and in aqueous solution, ions of these elements form well defined aqua ions. Because these water molecules can be displaced completely by other ligands only with difficulty, one can infer that Cu^{2+} never occurs on aqueous media. Several studies (2,5,6) used different methods to oxidize LDL (cells, polymorphonuclear leukocytes, xanthine oxidase, ceruloplasmin).

Another important issue is the length of the LDL isolation procedure. Miwa et al. (1) isolated LDL with sequential ultracentrifugations, and LDL was then extensively dialyzed (40 to 48 h). Both the long-term ultracentrifugation procedure (2 days) and dialysis and the long-term storage of LDL at 4°C before use may induce a loss of endogenous antioxidants (7) and contribute to generating prooxidant conditions. We prefer to use a single-step, rapid ultracentrifugation with a vertical rotor (1 h) coupled with the Sephacryl S-300 column (2 min) to desalt LDL samples (5,6).

A major determinant of LDL peroxidation is its fatty acid composition (2). Linoleic and arachidonic acids enhance oxidizability with major susceptibility of double bonds to peroxidation. In contrast, the increased content of oleic acid, which contains a single double bond, enhances the resistance to oxidation. Therefore, the fatty acid composition must be determined in studies aimed at identifying differences in LDL peroxidation among different classes of patients.

The malondialdehyde (MDA) content of LDL was assayed by the thiobarbituric acid method (1). Artifactual peroxidation during the procedure could be prevented by adding the chain breaker butylated hydroxytoluene (10 mmol/liter), the iron chelator deferoxamine (100 μmol/liter) and ethanol (0.16%) to the samples before the TBA reagents are added (5,6). Malondialdehyde is a widely used marker of peroxidation; however, as the authors acknowledge, the propagation phase of lipid peroxidation is a more adequate estimation of LDL susceptibility to peroxidation. The following indexes should be determined (6): 1) the initial 234-nm absorbance; 2) the lag time, defined as the interval between the intercept of the linear least square slope of the curve with the initial absorbance axis; 3) the maximal rate of oxidation, calculated from the slope of the absorbance curve during the propagation phase; 4) the maximal amount of dienes produced. Finally, the authors did not provide a pathophysiologic explanation of why patients with variant angina had low levels of vitamin E (1).

In conclusion, although the primary finding of the study (1) is very interesting, further studies are necessary before definitive conclusions addressing the issues raised by Miwa et al. can be made.

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Reply

We thank Napoli for his interest in our report (1). We agree that cupric ion–induced oxidation of low density lipoprotein (LDL) is unlikely to occur in vivo, and the concentrations of cupric ion used in our in vitro experiments were much higher than those in physiologic concentrations. Our study demonstrated that plasma LDL from patients with active variant angina was highly susceptible to cupric ion–induced peroxidative modification in association with a lower vitamin E content than LDL from patients without coronary spasm. Although the precise mechanisms by which oxidative modification of LDL is triggered in vivo are yet to be elucidated, the susceptibility to oxidation demonstrated in our study may be extrapolated or generalized to other oxidation processes, such as free radical–induced oxidation of LDL.

We also appreciate the comments made by Napoli with regard to our methods, including the isolation procedure and the estimation of the oxidative susceptibility to cupric ion used in our study and would like to clarify some points mentioned by him. Actually, we used a sequential rapid ultracentrifugation step with a semivertical rotor (3 h × 2). Indeed, the concentration of vitamin E in LDL may have been reduced after dialysis, and the LDL particles used in our study may not

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