Kinetics of Fatty Acid Oxidation in Low Density Lipoproteins Evaluated by Registration of the Oxidizer Consumption and Reaction Product Yield

V. N. Titov, G. G. Konovalova, D. M. Lisitsyn*, S. D. Razumovskii*, I. B. Nezhdanova, and V. V. Kukharchuk

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 7, pp. 45-47, July, 2005
Original article submitted June 29, 2004

Oxidation of arachidonic acid by ROS *in vitro* can be evaluated by the formation of reaction products (conjugated dienes); this is preceded by a lag period caused by the action of antioxidants (α-tocopherol, β-carotene, and ascorbic acid). In case of ozone titration the oxidizer is consumed even during the lag period, when conjugated dienes are not yet forming. Comparison of the oxidation rate constants for antioxidants, arachidonic and oleic monoenic fatty acids suggests that during the lag period Cu^{2+}-initiated forms of O_2 oxidize primarily oleic acid, whose reaction rate constant is much higher than those of antioxidants. Presumably, the duration of lag period during oxidation of arachidonic acid and formation of conjugated dienes is determined also by the content of triglycerides and oleic fatty acid in low density lipoproteins.

Key Words: *arachidonic acid; oleic fatty acid; ozone; conjugated dienes; antioxidants*

The levels of primary and secondary products of essential polyenic arachidonic fatty acid (AA) oxidation are often used for evaluation of the intensity of peroxidation in clinical practice. The primary and secondary products of AA oxidation are conjugated dienes (CD) and MDA, respectively [3]. Intensive *in vivo* formation of ROS (peroxide) is regarded as a mechanism of activation of peroxidation of, *e.g.* lipids, and usually as a pathological process [1]. By recording the formation of CD alone, it is difficult to explain the existence of lag period (induction period) of the reaction, when the oxidant is consumed, but no CD is forming yet.

The formation of lag period during registration of CD can be explained by the effects of antioxidants (α-tocopherol, β-carotene, ascorbic acid, flavonoids) [9]. However, 10-fold increase of *in vitro* concentrations of α-tocopherol and β-carotene in the medium does not prolong the period of CD induction; antioxidant effects largely depend on the reaction conditions [8]. We showed that oxidation of many substrates and formation of the reaction products can be more reliably evaluated by the consumption of the oxidant (ROS) [7] than by the yield of a reaction product. In order to clear out possible causes of the lag period formation, we recorded the kinetics of FA oxidation in the same LDL samples simultaneously by the formation of reaction products (CD) and by ROS (ozone) consumption.

MATERIALS AND METHODS

LDL fraction was obtained from the sera of 23 patients with familial hypercholesterolemia and less pronounced hypercholesterolemia of unknown origin. LDL were isolated by ultracentrifugation at hydrated density of 1.019-1.062 g/cm^3 and dialyzed. Protein was measured

Laboratory of Clinical Biochemistry of Lipids and Lipoproteins, Institute of Clinical Cardiology, Ministry of Health of the Russian Federation; *Institute of Chemical Physics, Academy of Sciences of the Russian Federation, Moscow. ***Address for correspondence:*** vn_titov@mail.ru. V. N. Titov

0007-4888/05/1401-0038

by the method of Lowry, LDL were diluted to a concentration of 50 μg/ml with buffered 0.15 M NaCl (pH 7.4). LDL oxidation was induced at 37°C by adding 3×10^{-5} M $CuSO_4$ (Cu^{2+} served as ROS initiator). Optical density of the incubation mixture was repeatedly measured at λ=234 nm on a 220A spectrophotometer (Hitachi). CD content was estimated using molar extinction coefficient (2.44×10^4 mol/cm) (Fig. 1).

Lipids were extracted from LDL by the method of Folch, after which the kinetics of O_3 consumption during oxidation of double bonds (DB) in the entire pool of fatty acids (FA) was recorded automatically on a Russian DB analyzer (DBA). The content of DB in LDL FA pool was estimated in millimoles of O_3 used for titration. For the analysis of DB, FA in 10 μl CCl_3 was introduced in DBA reactor. The sensitivity of the method reaches 10^{-9} M; the reproducibility in a series was 4.5%. Stilbene solution in CCl_4 served as the reference sample. Ozone oxidation rate constants for C 18:1 oleic monoenic and C 20:4 AA essential FA were determined on DBA. An example of registration of O_3 consumption kinetics during DB oxidation in FA pool and of antioxidants in LDL is shown in Fig. 2.

RESULTS

The formation of CD during ROS oxidation of AA in LDL starts after 20-110-min induction period (lag period). After this optical density increases due to CD formation and attains a plateau; the longer the lag period, the less CD are formed and the lower is the rate of their oxidation by ROS [12].

The consumption of O_3 for substrate oxidation in the same LDL sample in DBA started from the first seconds, similarly as ketone formation, and presumably, O_3 reacted with the substrates other than AA. We consider that the lag period during evaluation of CD is the time of O_3 reaction with the LDL substrates whose oxidation rate constants are higher than in AA. To verify this hypothesis, we determined by the method of kinetic ozonolysis the reaction rate constant for oxidation of AA (C 20:4) and compared this parameter to that for monoenic oleic FA C 18:1.

The rate constant for oxidation of oleic mono-FA with O_3 was 1×10^6 liter/mol×sec, that for AA 2.4×10^5 liter/mol×sec. Naturally, antioxidants are also oxidized by ROS during the lag period. According to our data, rate constants for oxidation of various antioxidants by ozone were: 1.4×10^3 liter/mol×sec for α-tocopherol, 4.0×10^4 for β-carotene, and 3.3×10^5 for ascorbic acid. The reaction rate constant for oxidation of oxidize various antioxidants by ROS was lower than for oxidation of FA [2].

Oleic FA can be the most real substratum for O_3 oxidation to CD formation. Presumably, oleic FA in

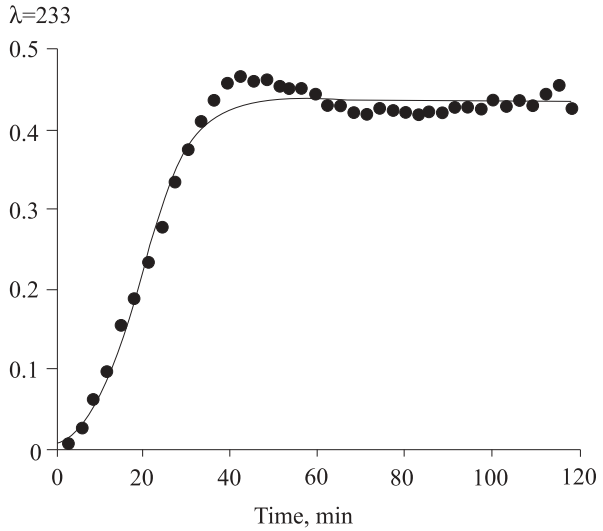

Fig. 1. Kinetics of arachidonic acid oxidation by Cu^{2+}-initiated ROS and conjugated diene formation in LDL. Lag period is not shown.

biology is an ROS trap. The oxidizer disrupts DB in it, forming two short-chain C 8:0 FA, which are rapidly oxidized in mitochondria without formation of radicals. The content of ROS acceptors in LDL from volunteers is presented by the following concentrations (mmol/mmol LDL) [11]: 30-930 for oleic FA, 48-250 for AA, 680-1832 for linoleic FA, 2.9-14.9 for α-tocopherol, 0.03-1.87 for β-carotene, and 0.02-0.06 for ascorbic acid.

The content of all oxidation substrates in LDL from volunteers varied within a range of 4-5-fold values, and only the level of oleic FA changed 30-fold. Hence, the higher serum level of triglycerides, the higher their content in LDL, the longer lag period, the greater percent of Cu^{2+}-initiated ROS oxidize oleic FA, and the lesser percent of them remains for AA oxidation. It was previously shown [14] that rabbit LDL enriched with oleic acid *in vivo* are characterized

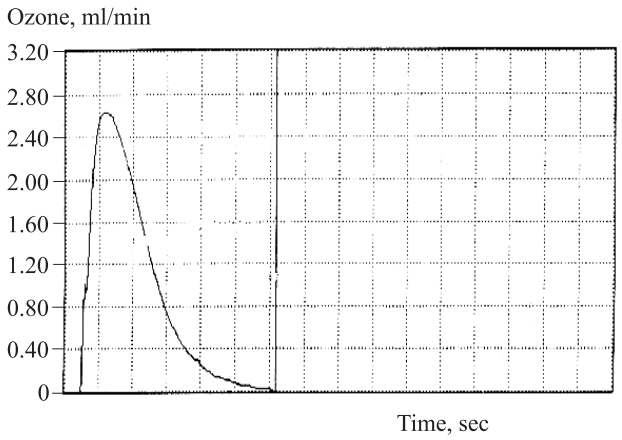

Fig. 2. Kinetics of O_3 consumption during oxidation of fatty acids and antioxidants in LDL sample.

by longer lag period during CD formation in comparison with control animals. Activation of CD formation after dilution of LDL sample can result from lower content of oleic FA in incubation medium [8].

The maximum optical density of CD on the plateau is a result of AA oxidation under conditions of oxidizer deficiency [10]. Therefore, in patients with familial hypercholesterolemia (with minimum content of triglycerides in LDL) LDL are characterized by a short lag period and active AA oxidation. In patients with familial combined hyperlipemia the residual content of triglycerides in LDL and oleic FA is high. LDL are characterized by a long lag period and low AA oxidation. Long lag period is characteristic of oxidation of "large" LDL with low hydrated density, while short lag period is typical of oxidation of "small" LDL with high density. It is therefore hardly possible that CD formation truly reflects the oxidative stress status [10].

Hence, oleic FA ranks first among ROS traps [5] and it is oxidation of oleic FA that forms the lag period during CD formation. Oleic FA can be the main ROS acceptor *in vivo* and the most active antioxidant. Even few essential molecules of α-tocopherol and β-carotene, just transferred by LDL to cells, can activate angiogenesis *in vivo*, for which the OH-group should be retained [6]. The topology and stereospecificity of α-tocopherol distribution in the membrane indicate its specific, but still unclear function [4]. LDL is not the only transporter of tocopherols to cells [15]. We suppose that monotonic oleic FA endogenously synthesized by all primates is an important factor forming the lag period during evaluation of CD formation; of all FA and antioxidants it is oxidized by ROS with the highest reaction rate constant.

REFERENCES

1. Yu. A. Vladimirov, *Biokhimiya*, **69**, No. 1, 5-7 (2004).
2. A. B. Gagarina and N. M. Evteeva, *Khim. Fizika*, **21**, No. 7, 41-49 (2002).
3. N. K. Zenkov, V. Z. Lankin, and E. B. Men'shchikova, *Oxidative Stress* [in Russian], Moscow (2001).
4. P. Kwinn, *Biokhimiya*, **69**, No. 1, 74-84 (2004).
5. G. I. Klebanov, Yu. O. Teselkin, I. V. Babenkova, *et al.*, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 1, 15-22 (2000).
6. T. Miyazova, H. Inokuti, H. Hirokane, *et al.*, *Biokhimiya*, **69**, No. 1, 85-88 (2004).
7. S. D. Razumovskii, S. K. Rakovskii, D. M. Shopov, and G. E. Zaikov, *Ozone and Its Reaction with Organic Compounds* [in Russian], Sofia (1983).
8. A. P. Savchenkova, L. B. Dudnik, N. P. Solov'eva, and O. A. Azizova, *Biomed. Khim.*, **49**, No. 6, 576-587 (2003).
9. A. K. Tikhaze, V. Z. Lankin, G. G. Konovalova, *et al.*, *Byull. Eksp. Biol. Med.*, **128**, No. 8, 186-189 (1999).
10. Y. Dotan, D. Lichtenberg, and I. Pinchuk, *Prog. Lipid Res.*, **43**, 200-227 (2004).
11. H. Esterbauer, G. Jurgens, O. Quehenberger, and E. Koller, *J. Lipid Res.*, **28**, 495-509 (1987).
12. H. Esterbauer, H. Puhl, M. Dieber-Rotheneder, *et al.*, *Ann. Med.*, **23**, 573-581 (1991).
13. J. Goulet, B. Lamarche, G. Nadeau, and S. Lemieux, *Atherosclerosis*, **168**, 115-124 (2003).
14. S. Parthasarathy, J. C. Khoo, E. Miller, *et al.*, *Proc. Natl. Acad. Sci. USA*, **87**, 3894-3898 (1990).
15. N. Weber, E. Klein, and K. D. Mukherjee, *J. Nutr.*, **133**, 435-441 (2003).