A Probucol Analog Protects Lipoproteins Better Than Probucol

L. F. Dmitriev and M. V. Ivanova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 5, pp. 491-493, May, 1995
Original article submitted May 20, 1994

Using low density lipoproteins, the antioxidant activity of probucol and one of its analogs was examined, and the analog was found to be more effective than probucol in protecting these lipoproteins against oxidation. It is pointed out that the choice of particular probucol analogs for clinical treatment should depend on the antioxidant index of low density lipoproteins in the patient concerned.

Key Words: *low density lipoproteins; lipid peroxidation; probucol; α-tocopherol*

Lipid peroxidation (LPO) is most often looked upon as a catch-all process responsible for membrane damage. In our view, the prime consideration in studies designed to elucidate the role of LPO in a disease or pathological condition should be the intensity of this process. In evaluating its intensity in biological structures such as membranes and lipoproteins, three qualitatively distinct cases may be singled out: intensive, moderate, or weak LPO. Moderate peroxidation may be regarded as a normal process that is strictly controlled *in vivo*. However, whereas LPO may be normally just under control in membranes, it must be completely blocked in lipoproteins. Leading roles in either case are played by tocopherol and ubiquinol.

Human low density lipoproteins (LDL) of relative molecular weight 2.5×10^6 contain, on average, 1300 molecules of polyunsaturated fatty acids bound to lipids of different classes. There are approximately 6 tocopherol molecules per LDL particle [6], so that normally (i.e., when the ratio of tocopherol to polyunsaturated fatty acids is 1:200) LDL are well protected from LPO.

Oxidative modification of LDL is held to be an important factor in atherogenesis [15]. If the notion that atherogenesis is associated with LPO activation is accepted, then a study of LDL modification through oxidation and of methods by which it can be controlled presents immediate interest. Such modification is very likely to occur *in vivo* when the balance between pro-oxidant and antioxidant agents is upset.

Although the participation of antioxidant enzymes in LPO regulation is obvious, currently there is no evidence to suggest that atherogenesis can be inhibited by altering the activity of these enzymes in the desired direction. For this reason, the most promising way of exercising control over LPO appears to lie in the use of artificial antioxidants.

In the study described here we initiated LPO in LDL *in vitro* and tested probucol and its analog of relative molecular weight 537 for their antioxidant activity.

MATERIALS AND METHODS

LDL were isolated by a routine method [8]. To incorporate probucol and its analog (both were synthesized at the Center for Elastomers, Kazan) into LDL, we added an estimated amount of the antioxidant to these lipoproteins in ethanol and then subjected the samples to dialysis for 18 h at 4°C. LPO was initiated with ultraviolet light (λ=320-380 nm). As the initiator, we used Azo-

Institute of Clinical Cardiology, Moscow. (Presented by E. I. Chazov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0005-0474$12.50

isobutyronitril (Fluka), which forms the primary radicals R·(RO_2·) under certain conditions, for example in the reaction R-N=N-R → N_2+2R· [12]. In this way the rate of LPO initiation can be controlled. Hydroperoxide concentrations were estimated by the absorption of dienes at λ=232 nm (ε =2.5×10⁴ $M^{-1}cm^{-1}$) [14]. The antiradical activity of probucol and its analog was measured using diphenylpicrylhydrazyl (DPPH) [13]. The tocopherol used was DL-tocopherol (Merck).

RESULTS

LPO is customarily divided into two stages: the formation of lipid hydroperoxides (stage 1) and their breakdown with the formation of secondary oxidation products (stage 2). Stage 1 is controlled by antioxidants, and measurement of lipid hydroperoxides in liposomes or LDL can give a reliable indication of the efficacy with which natural or artificial antioxidants act.

Figure 1 depicts the kinetics of lipid hydroperoxide (LOOH) accumulation in probucol-free LDL (*1A* and *1B*) together with curves describing LOOH formation in LDL containing 25 μM (*2*) and 50 μM (*3*) probucol. We used both native LDL and LDL subjected to aging (for 6 h at 25°C). In the native LDL the oxidation process ended much earlier than in the aged LDL (cf. curves *1A* and *1B*). In our view, the difference between these two curves can be explained by the change in the accessibility of the oxidation substrate for oxygen in LDL during aging. In the aged LDL both phospholipids and cholesterol esters are probably oxidized, but this, as can be seen from Fig. 1, did not affect the duration of the induction period. In all three cases, its duration was solely determined by the level of the antioxidant.

Figures 2 and 3 show, respectively, the kinetics of LOOH formation in LDL and the variation in DPPH absorption with time when LDL were incubated with probucol or its analog of molecular weight 537. Inspection of curves *2* and *3* in Fig. 2 shows an obvious difference in the lengths of the induction period, indicating that the probucol analog was more effective in protecting the LDL from oxidation than was probucol itself. In fact, it was at least four times as effective as probucol. On the other hand, the rates of DPPH reduction by probucol and its analog (Fig. 3) indicate that the two differ only slightly in antiradical activity, and the somewhat higher activity of the analog is possibly due to its greater mobility [2].

The duration of the induction period is a complex and fairly conservative parameter, reflecting both the content of polyunsaturated fatty acids and that of the antioxidant(s). In comparing native LDL, this parameter may be used as an antioxidative index of individual lipoproteins. Thus, if two or three assays of lipoproteins from a particular patient point to a low value of this index, this may be taken as a justification for raising its level by means of artificial antioxidants. The necessary and sufficient condition for checking the result of such a correction is the ability of LDL to resist oxidation *in vitro* under standard conditions of LPO initiation. Currently, probucol is one of the more promising lipophilic antioxidants, possessing as it does several useful properties. LDL incubated with probucol *in vitro* are effectively pro-

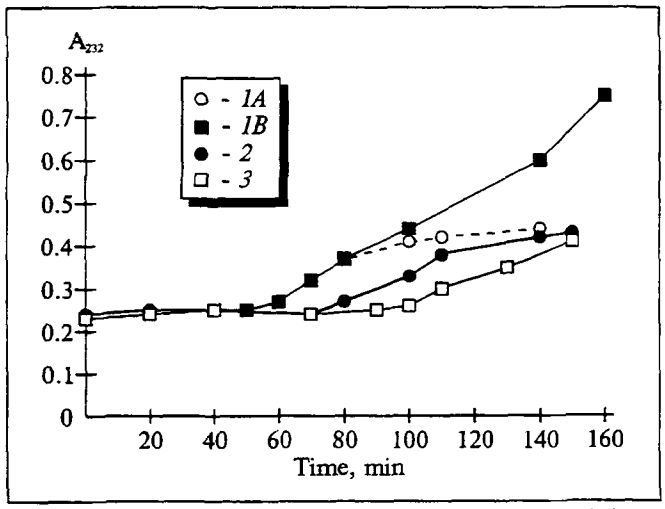

Fig. 1. Kinetic curves of LDL oxidation (formation of diene hydroperoxides) by the azo initiator (AIBN). *1A* and *1B*): LDL without probucol (native and aged, respectively); *2*) probucol 25 μM; *3*) probucol 50 μM. Tris–HCl 25 mM, pH 7.4; AIBN 1 mM. To record dienes, 40 μl of LDL were added to 2 ml of the buffer solution.

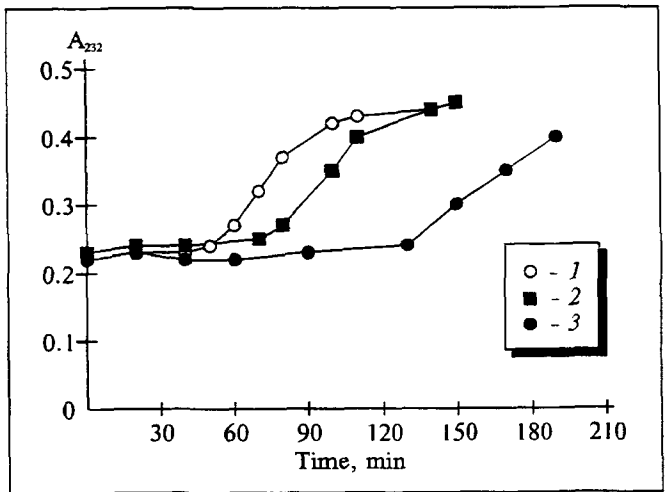

Fig. 2. Effect of probucol and its analog on the kinetics of LDL oxidation. *1*) LDL without probucol; *2*) LDL with probucol, 25 μM; *3*) LDL with the probucol analog, 25 μM. Same incubation conditions as in Fig. 1.

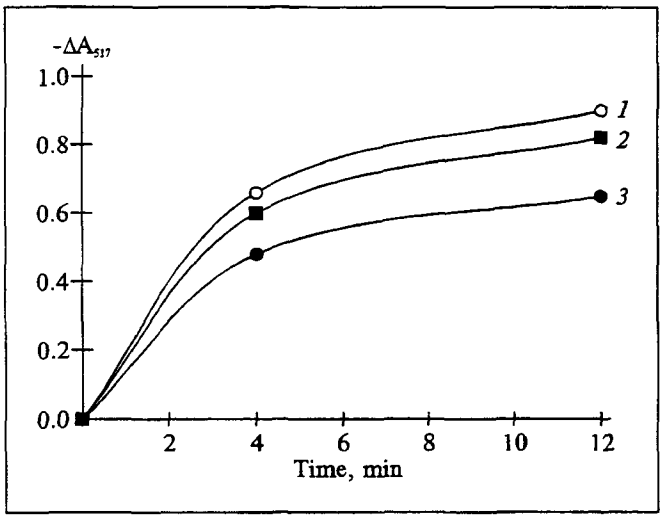

Fig. 3. Kinetic curves of diphenylpicrylhydrazyl (DPPH) reduction [(N·) → (DPPH(NH))] by antioxidants in solution. *1*) probucol analog; *2*) tocopherol; *3*) probucol.

tected from oxidation without showing the negative consequences of oxidative modification such as engulfment by macrophages or cytotoxicity [11]. Probucol also prevents hydroperoxide formation and lowers total blood cholesterol. Probucol is transported together with lipoproteins and exhibits high antioxidative potential [4,5]. The degree of protection afforded to LDL by probucol is comparable to that given by ascorbate, as can be seen by recording the formation of thiobarbituric acid-reactive products and measuring the level of LDL degradation by macrophages [9].

The ultimate goal of using artificial antioxidants is to redress the disturbed balance between pro-oxidant and antioxidant agents and to maintain tocopherol at a sufficiently high level. Tocopherol is a unique antioxidant [2] that enables lipid radicals to be sustained at a particular level without running the risk of hydroperoxide accumulation [1]. Moreover, tocopherol suppresses thrombosis by maintaining the balance between prostacyclin and thromboxane [10]. It follows, then, that probucol and its analogs may well supplement or partly replace tocopherol and thus delay or even totally block the onset of atherosclerosis by reducing the impact of risk factors to a minimum.

Probucol has already found fairly extensive clinical application [3,7]. Its concentrations in the serum and LDL of patients were reported to be 12.8 and 6.7 μg/ml, respectively, after a 12-week period of daily intake in a dose of 0.5 g [7]. As regards probucol analogs, the best prospects are offered by the clinical use of different analogs - with higher and with lower antioxidant activity as compared to probucol. Which analog(s) to use would then depend on the individual antioxidant index of LDL in the patient concerned.

This work was performed with the participation of the Center for Elastomers (Kazan), where probucol and its analog were synthesized.

REFERENCES

1. L. F. Dmitriev and M. V. Ivanova, *Dokl. Ross. Akad. Nauk*, **324**, 459-464 (1992).
2. A. N. Erin, V. I. Skrypin, L. L. Prilipko, and V. E. Kagan, in: *Oxygen Radicals in Chemistry, Biology, and Medicine* [in Russian], Moscow (1988), pp. 109-129.
3. V. Z. Lankin, V. M. Revenko, V. P. Lupanov, *et al.*, *Kardiologiya*, № 9, 41-44 (1993).
4. O. I. Aruoma, P. J. Evans, H. Kaur, *et al.*, *Free Radic. Res. Commun.*, № 10, 143-157 (1990).
5. R. L. Barnhart, S. J. Busch, and R. L. Jackson, *J. Lipid Res.*, **30**, 1703 (1989).
6. H. Esterbauer, H. Puhl, M. Dieber-Rotheneder, *et al.*, *Ann. Med.*, **23**, 573-581 (1991).
7. R. Fellin, A. Gasparotto, *et al.*, *Atherosclerosis*, **59**, 47-52 (1986).
8. R. J. Havel, H. A. Eder, and H. H. Bragdon, *J. Clin. Invest.*, **34**, 1345 (1955).
9. I. Jialal and S. M. Grundy, *J. Clin. Invest.*, **87**, 597-601 (1991).
10. C. W. Karpen, A. J. Merola, R. W. Trowyn, *et al.*, *Prostaglandins*, **22**, 651-661 (1981).
11. A. Negre-Salvayre, Y. Alomar, M. Troly, and R. Salvayre, *Biochim. Biophys. Acta*, **1096**, 291-300 (1991).
12. E. Niki, T. Saito, A. Kawakami, and Y. Kamiya, *J. Biol. Chem.*, **259**, 4177-4182 (1984).
13. A. Tamura, T. Sato, and T. Fuji, *Chem. Pharm. Bull.*, **38**, 255-257 (1990).
14. M. Tien and S. D. Aust, in: *Lipid Peroxides in Biology and Medicine* (K. Yagi, ed.), New York (1982), pp. 23-39.
15. J. L. Witztum and D. Steinberg, *J. Clin. Invest.*, **88**, 1785-1792 (1991).