Biochemical Pharmacology, Vol. 40, No. 9, pp. 1975–1980, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

PHENOTHIAZINES INHIBIT COPPER AND ENDOTHELIAL CELL-INDUCED PEROXIDATION OF LOW DENSITY LIPOPROTEIN

A COMPARATIVE STUDY WITH PROBUCOL, BUTYLATED HYDROXYTOLUENE AND VITAMIN E

C. BREUGNOT,* C. MAZIÈRE,† S. SALMON,†‡ M. AUCLAIR,† R. SANTUS,‡ P. MORLIÈRE,§ A. LENAERS* and J. C. MAZIÈRE†‡‖

* Institut de Recherches Servier, 11 rue des Moulineaux, 92150 Suresnes; † Laboratoire de Biochimie, Faculté de Médecine Saint-Antoine, 27 rue Chaligny 75012 Paris; ‡ Laboratoire de Physico-Chimie de l'Adaptation Biologique, INSERM U312, Muséum National d'Histoire Naturelle, 43 rue Cuvier 75231, Paris Cedex 05; and § Laboratoire de Recherches en Dermatologie, INSERM U312, Hôpital H. Mondor, 94010 Créteil, France

(*Received* 13 *September* 1989; *accepted* 11 *June* 1990)

Abstract—The effect of two phenothiazines, chlorpromazine (CPZ) and trifluoperazine (TFP) on the copper and endothelial cell-induced peroxidation of low density lipoprotein (LDL) has been studied and compared to that of drugs previously shown to protect LDL against peroxidation: probucol (PBC) and butylated hydroxytoluene (BHT). Incubation with CPZ or TFP inhibited in a dose-dependent manner LDL peroxidation induced either by copper ions or by cultured endothelial cells. Both the electrophoretic mobility and the thiobarbituric reactive substance content of LDL returned to almost normal values in the presence of 50 μM CPZ or TFP. The two studied phenothiazines also strongly inhibited the hydrolysis of LDL phosphatidylcholine which accompanies copper or endothelial cell-induced peroxidation of the particle. CPZ and TFP were as effective as PBC and BHT in inhibiting the LDL peroxidation. Whereas copper or endothelial cell-oxidized LDL were recognized and rapidly catabolized by mouse peritoneal macrophages, CPZ- or TFP-, as well as PBC- or BHT-treated LDL were not. Moreover, it was found that, in contrast to vitamin E, neither CPZ nor PBC reacted with model peroxy radicals formed by gamma irradiation of aerated ethanol. The possible mechanisms underlaying this protective effect of phenothiazines against LDL oxidative modification are discussed.

Low density lipoprotein (LDL¶) is the main cholesterol carrier in plasma and is, in most tissues, specifically recognized and catabolized via the apo B/E receptor pathway, which leads to feed-back of cellular cholesterol biosynthesis and up-regulation of cholesteryl ester formation [1]. During the last years, attention has been focused on the importance of low density lipoprotein oxidation in atherogenesis. When oxidized by endothelial [2, 3] or smooth muscle cells [3, 4], LDL is no longer recognized by the apo B/E receptor but avidly taken up by the scavenger receptor of macrophages, inducing cholesteryl ester accumulation. This process is currently believed to play a role in the atherogenic process [5, 6].

LDL incubated with endothelial cells are oxidized, as demonstrated by the increase in thiobarbituric reactive substance (TBARS) content [2, 3]. Its electrophoretic mobility is increased, due to an increase in the negative net charge of the particle [2, 3]. Moreover, about 50% of the LDL phosphatidylcholine is hydrolysed [7]. It is worthy of note that LDL oxidized *in vitro* by incubation with copper ions exhibits very similar modifications [8].

Recently, the protective effect of probucol (PBC), an hypocholesterolemic drug, against endothelial cell or copper-induced LDL oxidative modification has been demonstrated [9]. PBC has also been reported to have a marked anti-atherogenic action in animal models [10, 11]. This anti-atherogenic action is supposed to be related to its so-called "antioxidant" effect rather than to its relatively weak hypocholesterolemic potency [10]. Nevertheless, the actual mechanism of this important phenomenon is still unclear. Indeed, it has never been *directly* demonstrated that PBC could scavenge superoxide anion or oxy radicals which have been postulated to be involved in copper or endothelial cell-induced modification of the LDL particle. Recent studies pointed out that other lipophilic compounds, which are not classically considered as good radical scavengers, such as chlorpromazine, are in certain conditions able to inhibit the peroxidation of microsomal phospholipids [12, 13]. We thus investigated the effect of two phenothiazines, chlorpromazine (CPZ) and trifluoperazine (TFP) on copper and endothelial cell-induced LDL modification. We found that under our experimental conditions, CPZ and TFP were as effective as PBC in protecting LDL against peroxidation.

‖ To whom correspondence should be addressed.

¶ Abbreviations: BHT, butylated hydroxytoluene; CPZ, chlorpromazine; LDL, low density lipoprotein; PBC, probucol; TFP, trifluoperazine.

MATERIALS AND METHODS

Biochemicals. Chlorpromazine, trifluoperazine,

butyl hydroxytoluene (BHT), vitamin E and $CuSO_4$ were from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Probucol was a generous gift of Merrell-Dow Laboratories. Ham F10 and RPMI 1640 mediums, fetal calf serum, penicillin and streptomycin were from Gibco (Grand Island, NY, U.S.A.). The CBA mice were from IFFA-CREDO (l'Arbresle, France). The 1-stearoyl-2-[^{14}C]arachidonoyl phosphatidylcholine (2.1 GBq/mmol) was from Amersham (Buckinghamshire, U.K.). Silica gel plates F1500 were from Schleicher and Schuell (Dassel, F.R.G.).

Cell culture. The rabbit endothelial cell line, initially established by Buonassisi and Ventner [14], was a gift from Prof. D. Steinberg (La Jolla, University of San Diego, CA, U.S.A.). Cells were grown in 35-mm Nunc Petri dishes containing 1 mL Ham F10 medium supplemented with 15% foetal calf serum, penicillin and streptomycin, at 37° in 5% CO_2 humidified atmosphere. Experiments were performed on confluent cells.

LDL preparation. LDL was prepared from fresh normal human blood by sequential ultracentrifugation according to Havel *et al.* [15]. LDL was taken as the 1.024–1.050 fraction. After extensive dialysis against 5×10^{-3} M Tris/5×10^{-2} M NaCl pH 7.4 buffer containing 0.04% EDTA, LDL was filtered through 0.45 μM Millipore and stored at 4°. Protein determination was done according to Lowry *et al.* [16].

Oxidative modification of LDL. For all experiments dealing with LDL oxidation, LDL was previously dialysed against 5×10^{-3} M Tris/5×10^{-2} M NaCl pH 7.4 buffer devoid of EDTA. Auto-oxidation by Cu^{2+} was performed by incubating 0.2 mg LDL with 5×10^{-6} M $CuSO_4$ in 1 mL of serum-free Ham F10 medium, for 24 hr at 37°. Endothelial cell-induced modification of LDL was achieved by incubating 0.2 mg/mL of the LDL solution for 24 hr at 37° in the presence of confluent cells in 1 mL of serum-free Ham F10 medium.

The extent of lipid peroxidation was assessed by measurement of the thiobarbituric acid-reactive products (TBARS) according to Hessler *et al.* [17], calculated as nanomoles of malondialdehyde per milligram of LDL protein and expressed as percentage of control (LDL oxidized in the absence of drugs). In the case of endothelial-cell induced LDL peroxidation, the control was the LDL incubated with cells during 24 hr at 37° in Ham F10 medium in the absence of drugs. The electrophoretic mobility of the LDL was checked by agarose gel electrophoresis using a Ciba-Corning system. Results are expressed as the relative electrophoretic mobility of the particle, i.e. the ratio of the electrophoretic mobility of the oxidized LDL vs the electrophoretic mobility of the control (LDL incubated in the absence of copper or endothelial cells).

In experiments dealing with phosphatidylcholine hydrolysis, [^{14}C]phosphatidylcholine-loaded LDL was prepared according to Steinbrecher [18]. The specific radioactivity of the labeled LDL was about 4.8×10^5 dpm/mg protein. After a 24 hr incubation of 0.2 mg of labeled LDL in the presence of 5×10^{-6} M $CuSO_4$ or confluent endothelial cells, lipid analysis was performed by thin layer chromatography as previously described [19]. Results are expressed as percentage of [^{14}C]phosphatidylcholine hydrolysed.

LDL degradation by mouse peritoneal macrophages. For experiments dealing with LDL degradation by mouse peritoneal macrophages, either native LDL or Cu^{2+}-treated LDL (incubated in the absence or in the presence of 50 μM PBC, CPZ or BHT) were labeled using [^{125}I]Na according to the method of Bilheimer *et al.* [20]. After extensive dialysis against 5×10^{-3} M Tris/5×10^{-2} M NaCl/0.04% EDTA pH 7.4 buffer, LDL fractions were filtered through 0.45 μM Millipore and stored at 4°. The specific radioactivity was about 300 dpm/ng LDL protein, and the free iodine content of each fraction was under 2.5%. Mouse peritoneal macrophages were prepared from CBA female mice (18–22 g) as described by Edelson and Cohen [21]. After a 48-hr culture in RPMI medium supplemented with 10% heat-inactivated fetal calf serum, cells were washed and incubated for 5 hr at 37° in Ham F10 medium supplemented with 0.2% bovine serum albumin, in the presence of either [^{125}I]native LDL (control), or [^{125}I]Cu^{2+}-treated LDL, or [^{125}I]Cu^{2+}-treated LDL incubated with 50 μM PBC or CPZ or BHT. LDL degradation was measured according to Goldstein and Brown [22]. Results are expressed as nanograms of LDL (native or modified)/milligrams of cellular proteins.

Reactivity of PBC, CPZ and vitamin E with free radicals. Peroxy radicals were produced by gamma radiolysis of aerated pure ethanol [23]. The dose delivered was up to 800 Gy. The PBC, CPZ and vitamin E final concentrations were 10^{-4} M.

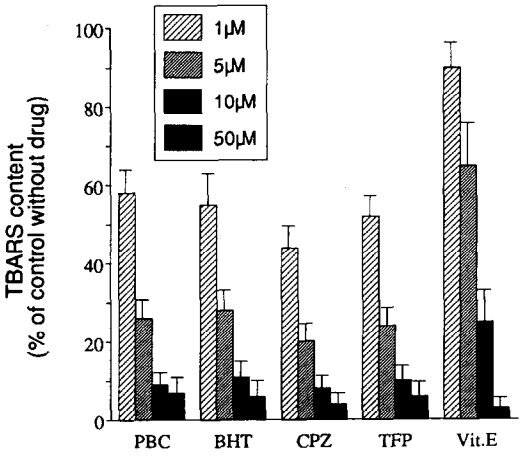

Fig. 1. Effect of probucol (PBC), butylated hydroxytoluene (BHT), chlorpromazine (CPZ), trifluoperazine (TFP) and vitamin E (Vit. E) on the TBARS content of copper-oxidized LDL. The LDL (0.2 mg/mL) was incubated for 24 hr at 37° in Ham F10 medium containing 5×10^{-6} M $CuSO_4$ in the absence or in the presence of the drugs at indicated concentrations. TBARS were determined by the thiobarbituric acid assay (see Materials and Methods). Results are expressed as percentages of control (LDL oxidized in the absence of drugs). Means of six experimental values ± SD.

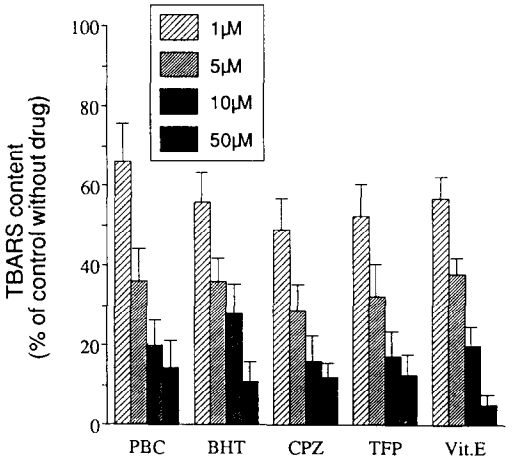

Fig. 2. Effect of probucol (PBC), butylated hydroxytoluene (BHT), chlorpromazine (CPZ), trifluoperazine (TFP) and vitamin E (Vit. E) on the TBARS content of endothelial cell-oxidized LDL. The LDL (0.2 mg/mL) was incubated for 24 hr at 37° with confluent endothelial cells in the absence or in the presence of the drugs at indicated concentrations. TBARS were determined by the thiobarbituric acid assay (see Materials and Methods). Results are expressed as percentages of control (LDL oxidized in the absence of drugs). Means of six experimental values ± SD for PBC, BHT, CPZ and TFP. Means of three experimental values ± SD for vitamin E.

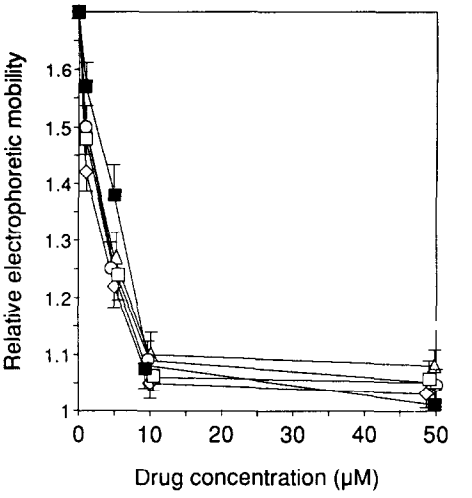

Fig. 3. Effect of probucol (PBC), butylated hydroxytoluene (BHT), chlorpromazine (CPZ), trifluoperazine (TFP) and vitamin E (Vit. E) on the relative electrophoretic mobility of copper-oxidized LDL. The LDL (0.2 mg/mL) was incubated for 24 hr at 37° in Ham F10 medium containing 5×10^{-6} M $CuSO_4$ in the absence or in the presence of the drugs at indicated concentrations. The relative electrophoretic mobility was calculated as the ratio of the electrophoretic mobility of the copper-oxidized LDL vs the electrophoretic mobility of the control (unoxidized) LDL. Means of six experimental values ± SD. (○) PBC; (△) BHT; (◇) CPZ; (■) TFP; (■) vitamin E.

The reactivity of PBC and vitamin E with peroxy radicals was followed by high performance liquid chromatography using a 5 μ Nova Pak C_{18} column (Waters) at a flow rate of 1.5 mL/min, with methanol:ethanol:water 86:10:4 (by vol.) as eluent (spectrophotometric detection at 210 nm). Under these conditions, retention times were 1.45 and 4.35 min for probucol and vitamin E, respectively. The CPZ separation was also achieved by high performance liquid chromatography, but using a 5 μ Porasil column (Waters) at a flow rate of 2 mL/min, with dichlorobutane:ethanol:ammonia (100:10:1 (by vol.) as eluent (spectrophotometric detection at 250 nm).

RESULTS

Figures 1 and 2 display the effects of CPZ, TFP, PBC, BHT and vitamin E on copper or endothelial cell-induced peroxidation of LDL, as assessed by the measurement of their TBARS content. It was observed that CPZ and TFP reduced the TBARS level in a dose-dependent manner, both in copper and endothelial cell-treated LDL. More than 85% reduction of the copper-induced formation of TBARS was observed in the presence of either 10 μM CPZ or TFP (Fig. 1). At the same concentration, these compounds decreased by 75–80% the oxidation of the particle by endothelial cells (Fig. 2). It can also be observed in Figs 1 and 2 that CPZ and TFP were as efficient as PBC or BHT in inhibiting both copper or endothelial cell-induced LDL peroxidation. On the other hand, it is of note that under 10 μM, vitamin E appeared less efficient than the other studied compounds in protecting LDL against copper-induced peroxidation, whereas it strongly reduced the TBARS formation in LDL incubated with endothelial cells (more than 90% reduction in the presence of 50 μM vitamin E).

CPZ or TFP also reduced in a dose-dependent manner the relative electrophoretic mobility of the copper-treated LDL, and these compounds were as effective as PBC or BHT. Figure 3 shows that the relative electrophoretic mobility of the LDL was reduced to about 1.05 for 10 μM CPZ or TFP, and to about 1.1. for PBC and BHT at the same concentration, whereas the mean relative electrophoretic mobility of the copper-treated LDL was 1.7 ± 0.06 as compared to the control (native LDL). As observed for the TBARS content, the protective effect of vitamin E against the copper-induced increase in LDL electrophoretic mobility was less marked, at low concentrations, than that of BHT, PBC or phenothiazines (Fig. 3). Concerning the endothelial-cell induced modification of the LDL, 10 μM of PBC, BHT, CPZ, TFP or vitamin E reduced the relative electrophoretic mobility to 1.05–1.1 vs 1.36 ± 0.03 for the particle incubated with cells in the absence of the drugs (data not shown).

Since LDL modified either by copper ions or by endothelial cells are recognized by the scavenger receptor of macrophages [2, 8], we also investigated the effect of phenothiazines on the recognition of copper-treated LDL by mouse peritoneal macrophages. Table 1 shows that, as expected, native LDL was very weakly catabolized by mouse peritoneal macrophages, whereas copper-oxidized LDL was

Table 1. Effect of probucol (PBC), butylated hydroxytoluene (BHT) and chlorpromazine (CPZ) on the degradation of Cu^{2+}-treated LDL by mouse peritoneal macrophages

Conditions	Degradation by mouse peritoneal macrophages (ng/mg cell protein)
Native LDL	2.0 ± 0.1
Cu^{2+}-oxidized LDL	
without drug	109.5 ± 14.8
+50 μM PBC	11.5 ± 0.5
+50 μM BHT	17.1 ± 0.5
+50 μM CPZ	14.1 ± 0.7

After a 24 hr preincubation with 5 M Cu^{2+} in the absence or in the presence of the drugs, LDL fractions were labeled with [^{125}I]Na and then tested for degradation by mouse peritoneal macrophages as described in Materials and Methods. Results are expressed as ng [^{125}I]LDL degraded/mg of cell protein (on a LDL protein basis). Means of three experimental values ± SD.

Table 2. Effect of probucol (PBC), chlorpromazine (CPZ) and trifluoperazine (TFP) on the LDL phosphatidylcholine hydrolysis induced by copper ions or by endothelial cells

Drug (50 μM)	Phosphatidylcholine hydrolysis (%) induced by Cu^{2+}	Endothelial cells
None	54.2 ± 4.0	35.0 ± 12.2
PBC	7.5 ± 2.0	6.6 ± 3.0
CPZ	3.2 ± 1.8	4.6 ± 1.6
TFP	5.5 ± 2.4	6.2 ± 2.8

The LDL was labeled with [^{14}C]phosphatidylcholine as described in Materials and Methods. The specific radioactivity was about 4.8×10^5 dpm/mg LDL protein. After a 24 hr incubation of 0.2 mg of labeled LDL with 5 μM Cu^{2+} or with confluent endothelial cells in the absence or in the presence of the drugs at 50 μM, lipid analysis was performed by thin layer chromatography. Results are expressed as percentage of [^{14}C]phosphatidylcholine hydrolysis. Means of six experimental values ± SD for copper-induced peroxidation of LDL, and of eight experimental values ± SD for endothelial cell-induced modification.

actively degraded. As previously shown [9], PBC strongly reduced the degradation of copper-treated LDL by macrophages. It can be also observed that CPZ was at least as efficient as PBC, at the same concentration (50 μM), in preventing macrophage degradation of copper-treated LDL.

The LDL peroxidation is also accompanied by the hydrolysis of about half of the phosphatidylcholine of the particle [7]. We thus investigated the effect of phenothiazines on this phenomenon using [^{14}C]arachidonoylphosphatidylcholine-labeled LDL. In the presence of 50 μM CPZ or TFP, the phosphatidylcholine hydrolysis was reduced by more than 85% in LDL incubated either with copper ions or endothelial cells (Table 2). In both cases, PBC also inhibited phosphatidylcholine hydrolysis, with a similar efficiency.

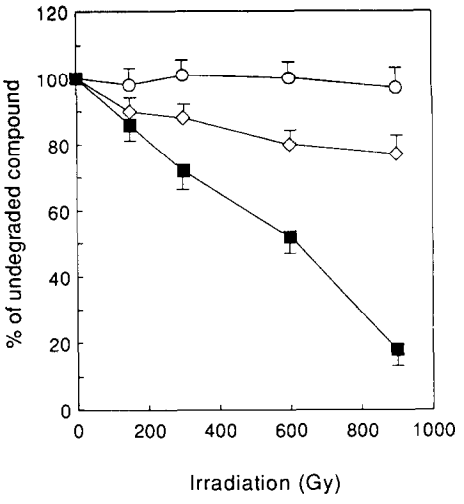

Fig. 4. Comparative reactivity of vitamin E, probucol and chlorpromazine with peroxy radicals formed by gamma radiolysis of aerated pure ethanol. The final concentration of all compounds was 100 μM. Results are expressed as the percentage of unalterated compound, checked by high performance liquid chromatography as described in Materials and Methods. (■) Vitamin E; (○) probucol; (◇) chlorpromazine.

Experiments were also carried out to study the reactivity of PBC and CPZ with peroxy radicals, which are the most important oxygenated radicals encountered in lipid peroxidation [24, 25]. For this purpose, we used model peroxy radicals (RO2·) obtained by gamma radiolysis of aerated ethanol. It can be observed in Fig. 4 that whereas vitamin E was readily destroyed, as expected, by peroxy radicals, PBC did not significantly react with RO2·, up to 800 Gy. The CPZ was also poorly reactive in our experimental system, since only 20–25% of the drug were destroyed following 800 Gy irradiation.

DISCUSSION

Taken all together, our results clearly demonstrate that phenothiazines strongly protect LDL against copper or endothelial cell-induced modification, as assessed by the TBARS content and the electrophoretic mobility of the particle. They also inhibit phosphatidylcholine hydrolysis which accompanies LDL oxidative modification. Moreover, phenothiazine-treated LDL incubated with copper ions or endothelial cells are no longer recognized by the scavenger receptor of mouse peritoneal macrophages. In fact, in the presence of 50 μM CPZ or TFP, all the studied parameters returned to almost normal values. It is also worthy of note that the two studied phenothiazines were as efficient as probucol or as the well known free radical scavenger BHT in preventing LDL oxidative modification. Concerning BHT, Van Hinsberg et al. [26] previously reported the protective effect of this compound against both copper and endothelial cell-induced peroxidation of LDL. The less efficiency of vitamin E at low concentrations (under 5 μM) is also consistent with

observations of Esterbauer *et al.* [27], which found that addition of 1–5 μM vitamin E only poorly increases the lag-phase of LDL oxidation induced by copper ions. By contrast, vitamin E readily protects LDL against cell-induced modification as described for endothelial cells [7, 26], monocytes [28] and macrophages [29].

The mechanisms by which phenothiazines can protect LDL against both copper and endothelial cell-induced modification remain unclear. The question of a possible "free radical scavenging effect" of these drugs has to be discussed. In our experimental system, chlorpromazine reacted very poorly with model peroxy radicals. Janero and Burghardt [13] also failed to demonstrate a significant free radical scavenging effect of chlorpromazine. Katsuoka and Ohnishi [30], studying the protective effect of chlorpromazine against rat heart ischemia, found a little scavenging effect of the drug using 1,1-diphenyl-2-picrylhydrazyl as a free radical generating system, but only at 10^{-4} M. It is worthy of note that at 30 μM, a concentration which in our experiments led to 80–90% inhibition of the LDL peroxidation, these authors did not observe any significant reaction of chlorpromazine with free radicals [30]. In another experimental system, using tetraphenylporphyrin as photosensitizer and arachidonic acid as a lipid radical generating system, chlorpromazine at 10 μM only slightly inhibited diene and triene production (Dr Iliou, personal communication), whereas at this concentration we already observe a marked protective effect of the drug against LDL peroxidation (see Figs 1 and 2).

The effect of phenothiazines on the LDL microviscosity and its eventual influence on the propagation of the oxidative process has also to be considered. Nagatsuka and Nakazawa [31] pointed out a possible role of the physical state of the lipid phase in the susceptibility of lipids to peroxidation: these authors showed that compounds which decrease membrane fluidity such as cholesterol and cepharantin inhibited the radiation-induced peroxidation of lipids in liposomes, although they have no significant radical-trapping ability. It is of note that phenothiazines are known to interact with phospholipids [32], affecting membrane microviscosity [33, 34]. Since phospholipids represent about 25% in mass of the LDL [35], an effect of phenothiazines on the physical state of the LDL could be involved in the inhibition of the copper or endothelial cell-induced peroxidation of the particle. Experiments are now undertaken in our laboratories in order to check the effect of phenothiazines on the LDL physico-chemical properties during the time–course of its oxidative modification by copper ions or endothelial cells.

Concerning the protective effect of probucol against LDL peroxidation induced either by endothelial cells or by copper ions, although it is now admitted that it is due to "antioxidant" properties of the drug, such properties have to our knowledge never been *directly* demonstrated. It is worthy of note that, in our experimental system probucol did not react with model peroxy radicals produced by irradiation of aerated ethanol whereas vitamin E was almost completely destroyed. However, in recent studies, Barnhart *et al.* [36] demonstrated the appearance of the probucol oxidation products spiroquinone and diphenoquinone during the course of LDL auto-oxidation. But even in this case, there is no clear argument allowing to correlate the production of such compounds to the protective effect of the drug against LDL oxidative modification. Thus, the contribution of a "physical" effect of probucol in its protective effect against LDL peroxidation cannot be ruled out. Probucol has been shown to interact with the LDL particle [37, 38], and recently Goldberg and Mendez [39] evoked the marked effect of this drug on lipid structure: at very low concentrations, probucol markedly reduced the phase-transition temperature of phospholipids.

In conclusion, the mechanisms by which drugs such as phenothiazines or even probucol can protect LDL against copper or cell-induced peroxidation are not entirely elucidated. But it clearly appears, in our experimental system, that compounds which are not believed to be classical "anti-oxidants" such as phenothiazines are as efficient as probucol or as free radical scavengers such as BHT or vitamin E in preventing LDL modification. Thus, although further experiments are needed to support this hypothesis, we suggest that LDL peroxidation could be prevented by different mechanisms, i.e. not only by the presence of actual free radical scavengers, but also by insertion of lipophilic compounds such as phenothiazines or probucol, which can alter the LDL structure and render the particle less sensitive to the oxidative attack. It is conceivable that many other lipophilic drugs could have a similar effect. This opens a wide field of research in the investigation of drugs which can slow down the atherogenic process by inhibiting LDL lipid peroxidation.

Acknowledgements—J. C. Mazière thanks la Ligue National Contre le Cancer (Comité de Paris), for financial support. Albert Lenaers (Institut de Recherches Servier) gratefully acknowledges Prof. D. Steinberg (La Jolla, CA, U.S.A.) for the gift of the endothelial cell strain.

REFERENCES

1. Brown MS and Goldstein JL, Receptor-mediated control of cholesterol metabolism. *Science* **191**: 150–154, 1976.
2. Henriksen T, Mahoney EM and Steinberg D, Enhanced macrophage degradation of LDL previously incubated with cultured endothelial cells: recognition by receptors for acetylated LDL. *Proc Natl Acad Sci USA* **78**: 6499–6503, 1981.
3. Morel DW, DiCorletto PE and Chisolm GM, Endothelial and smooth muscle cells alter low density lipoprotein *in vitro* by free radical oxidation. *Arteriosclerosis* **4**: 357–364, 1984.
4. Heinecke JW, Rosen H and Chait A, Iron and copper promote modification of low density lipoprotein by human arterial muscle cells in culture. *J Clin Invest* **74**: 1890–1894, 1984.
5. Steinberg D, Metabolism of lipoproteins and their role in the pathogenesis of atherosclerosis. *Atherosclerosis Rev* **18**: 1–23, 1988.
6. Ylä-Herttuala S, Palinski W, Rosenfeld ME, Parthasaraty S, Carew T, Butler S, Witzum JL and Steinberg D, Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic

lesions of rabbit and man. *J Clin Invest* **84**: 1086–1095, 1989.
7. Steinbrecher UP, Parthasaraty S, Leake DS, Witzum JL and Steinberg D, Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc Natl Acad Sci USA* **81**: 3883–3887, 1984.
8. Steinbrecher UP, Witzum JL, Parthasaraty S and Steinberg D, Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL. Correlation with changes in receptor-mediated catabolism. *Arteriosclerosis* **7**: 135–143, 1987.
9. Parthasaraty S, Young SG, Witzum JL, Pittman RC and Steinberg D, Probucol inhibits oxidative modification of low density lipoprotein. *Proc Natl Acad Sci USA* **77**: 641–644, 1986.
10. Kita T, Nagano Y, Yokode M, Ishii K, Kume N, Ooshima A, Yoshida H and Kawai C, Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc Natl Acad Sci USA* **84**: 5928–5931, 1987.
11. Daugherty A, Zweifel BS and Schonfeld GS, Probucol attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Br J Pharmacol* **98**: 612–618, 1989.
12. Slater TF, The inhibitory effects "*in vitro*" of phenothiazines and other drugs on lipid peroxidation systems in rat liver microsomes and their relationship to liver necrosis produced by carbon tetrachloride. *Biochem J* **106**: 155–160, 1968.
13. Janero DR and Burghardt B, Prevention of oxidative injury to cardiac phospholipids by membrane-active "stabilizing agents". *Res Commun Chem Pathol Pharmacol* **63**: 163, 1989.
14. Buonassisi V and Ventner JC, Hormone and neurotransmitter receptors in an established vascular endothelial cell line. *Proc Natl Acad Sci USA* **73**: 1612–1616, 1976.
15. Havel RJ, Eder HA and Bragdon JH, The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* **34**: 1345–1353, 1955.
16. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–175, 1951.
17. Hessler JR, Morel DW, Lewis LJ and Chisolm GM, Lipoprotein oxidation and lipoprotein-induced cytotoxicity. *Arteriosclerosis* **3**: 215–222, 1983.
18. Steinbrecher UP, Oxidation of human low density lipoproteins results in derivatization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. *J Biol Chem* **262**: 3603–3608, 1987.
19. Mazière C, Mazière JC, Mora L and Polonovski J, Rapid analysis of cellular lipids without extraction. *J Biochem Biophys Methods* **14**: 267–272, 1987.
20. Bilheimer DW, Eisenberg S and Levy RI, The metabolism of very low density lipoproteins. I. Preliminary *in vivo* and *in vitro* observations. *Biochim Biophys Acta* **260**: 212–221, 1972.
21. Edelson PJ and Cohen ZA, Purification and cultivation of monocytes and macrophages. In: *In Vitro Methods in Cell-mediated and Tumor Immunity*. (Eds. Bloom BR and David JR), pp. 333–340. Academic Press Inc., New York, 1976.
22. Goldstein JL and Brown MS, Binding and degradation of low density lipoprotein by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J Biol Chem* **249**: 5153–5162, 1974.
23. Jore D and Ferradini C, Radiolytic study of alpha-tocopherol oxidation in ethanolic solution. *FEBS Lett* **183**: 299–303, 1985.
24. Witting IA, Vitamin E and lipid antioxidants in free-radical-initiated reactions. In: *Free Radicals in Biology* (Ed. Pryor WA), Vol. 4, pp. 295–319. Academic Press, New York, 1980.
25. Aust SD, Morehouse LA and Thomas CE, Role of metals in oxygen radical reactions. *J Free Rad Biol Med* **1**: 3–25, 1985.
26. Van Hinsberg VWM, Scheffer M, Hevekes L and Herman JM Kempen, Role of endothelial cells and their products in the modification of low density lipoproteins. *Biochim Biophys Acta* **878**: 49–64, 1986.
27. Esterbauer H, Striegl G, Puhl H and Rotheneder M, Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Rad Res Commun* **6**: 67–75, 1989.
28. Cathcart MK, Morel DW and Chisolm GM, Monocytes and neutrophils oxidize low density lipoprotein making it cytotoxic. *J Leukocytes Biol* **38**: 341–350, 1985.
29. Jessup W, Rankin SM, De Whalley CV, Hoult JR, Scott J and Leake DS, Alpha-tocopherol consumption during low density lipoprotein oxidation. *Biochem J* **265**: 399–405, 1990.
30. Katsuoka M and Ohnishi ST, Pharmacological protection of perfused rat heart against global ischemia. *Prostaglandins, Leukotrienes Essential Fatty Acids* **38**: 151–156, 1989.
31. Nagatsuka S and Nakazawa T, Effects of membrane-stabilizing agents, cholesterol and cepharantin, on radiation-induced lipid peroxidation and permeability in liposomes. *Biochim Biophys Acta* **691**: 171–177, 1982.
32. Lee AG, Local anesthesia: the interaction between phospholipids and chlorpromazine, propranolol and practolol. *Mol Pharmacol* **13**: 474–487, 1977.
33. Giraud F, Claret M, Bruckdorfer KR and Chailley B, The effects of membrane lipid order and cholesterol on the internal and external cationic site of the Na^+–K^+ pump in erythrocytes. *Biochim Biophys Acta* **647**: 249–258, 1981.
34. Ogiso T, Masahiro L and Mori K, Fluidity of human erythrocyte membranes and effect of chlorpromazine on fluidity and phase-separation of membrane. *Biochim Biophys Acta* **649**: 325–335, 1981.
35. Lee DM and Downs D, A quick and large-scale density gradient subfractionnation method for low density lipoproteins. *J Lipid Res* **23**: 14–27, 1982.
36. Barnhart RL, Busch SJ and Jackson RL, Concentration-dependent antioxidant activity of probucol in low density lipoproteins *in vitro*: probucol degradation precedes lipoprotein oxidation. *J Lipid Res* **30**: 1703–1710, 1989.
37. Urien S, Riant P, Albengres E, Brioude R and Tillement JP, *In vitro* studies of the distribution of probucol among human plasma lipoproteins. *Mol Pharmacol* **26**: 322–327, 1984.
38. McLean LR and Hagaman KA, Effect of probucol on the physical properties of low density lipoproteins oxidized by copper. *Biochemistry* **28**: 327, 1989.
39. Goldberg RB and Mendez A, Probucol enhances cholesterol efflux from cultured human fibroblasts. *Am J Cardiol* **62**: 57B–59B, 1988.