Biochemical Pharmacology, Vol. 36, No. 6, pp. 847–849, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

COMPARATIVE STUDY OF THE EFFECT OF β-BLOCKERS WITH DIFFERENT PHARMACOLOGICAL PROPERTIES ON CHOLESTERYL ESTER FORMATION IN MOUSE PERITONEAL MACROPHAGES

SHAMSUL ISLAM,* NACER EDDINE HOUTIA, JEAN-CLAUDE MAZIÈRE, CÉCILE MAZIÈRE and JACQUES POLONOVSKI

Laboratoire de Biochimie & CNRS UA 524, Faculté de Médecine Saint-Antoine, 27 rue Chaligny, 75012 Paris, France

(*Received* 10 *July* 1986; *accepted* 23 *September* 1986)

Abstract—The effect of three β-blockers: non-selective (propranolol), β1-selective (metoprolol), and with intrinsic sympathomimetic activity (pindolol), was investigated on ^{14}C-oleic acid incorporation into cholesteryl esters in mouse peritoneal macrophages. Incorporation of ^{14}C-oleic acid into cholesteryl esters was reduced about 10-fold by propranolol at 10^{-4} M while incorporation into triacylglycerols was only 30% decreased at the same concentration. Metoprolol and pindolol had no significant effect on ^{14}C-oleic incorporation into cholesteryl esters or triacylglycerols. Finally, propranolol inhibited the acyl-coenzyme A:cholesterol-O-acyltransferase activity, measured *in vitro* on macrophages homogenates, while the other studied β-blockers were ineffective. These results suggest that propranolol could antagonize cholesteryl ester accumulation by macrophages, one of the main processes involved in atherogenesis.

Massive cholesteryl ester accumulation in foam cells derived from monocytes is probably one of the main features involved in the atherosclerotic lesions [1]. Storage of cholesteryl esters is observed when macrophages are incubated with chemically modified low density lipoproteins (LDL), such as acetylated LDL [2, 3] or LDL which have been in contact with endothelial cells [4, 5]. This is a consequence of a strong induction of the acyl-coenzyme A:cholesterol-O-acyltransferase (ACAT) activity by modified LDL [2].

Previous studies from our laboratory have shown that propranolol strongly inhibits cholesterol esterification in cultured fibroblasts [6, 7]. β-Blockers are widely used in the treatment of hypertension [8] and angina pectoris [9]. More and more hypertensive patients are on β-blockers, and it is interesting to note that propranolol has been reported to alter lipid and lipoprotein plasma levels, whereas β1-selective compounds such as metoprolol, or β-blockers with intrinsic sympathomimetic activity such as pindolol affect it to a lesser extent [10, 11]. It was thus of interest to investigate the effect of β-blockers on lipid metabolism in macrophages, especially on cholesteryl ester formation induced by modified LDL, in view of the fact that long term treatment by these drugs could affect this process and thus influence the course of atherosclerosis. In the present work, we present data concerning the effects of propranolol, metoprolol and pindolol, on cholesteryl ester formation in mouse peritoneal macrophages. Triacylglycerol metabolism was also comparatively studied.

* To whom all correspondence should be addressed.

MATERIALS AND METHODS

Chemicals. Propranolol was from Sigma (St. Louis, MO). Metoprolol and pindolol were generous gifts of Searle and Sandoz Laboratories, respectively. [1-^{14}C] oleic acid, 52 mCi/mmol and [1-^{14}C] oleyl Coenzyme A, 55 mCi/mmol, were from Amersham, Buckinghamshire, U.K.

Mouse peritoneal macrophages. They were collected by peritoneal lavages with 5 ml of sterile phosphate buffer saline pH 7.4, from unstimulated adult female Swiss mice, according to Cohn *et al.* [12]. Cells were centrifuged at 400 *g* for 10 min, and washed twice with 30 ml of Dulbecco's-MEM medium (Gibco) containing antibiotics (penicillin 100 Ui/ml + streptomycin 100 μg/ml). They were then resuspended in Dulbecco's-MEM medium and plated in 35 mm Petri dishes at about 2×10^6/dish. Following a 2 hr incubation at 37° in a 5% CO_2 humidified atmosphere the dishes were washed twice with 2 ml of the same medium to get rid of the red blood cells and other non-adherent cells. The medium was then replaced by Dulbecco's-MEM medium supplemented with antibiotics (see above) and 10% foetal calf serum (Gibco).

Preparation of acetylated LDL (LDLac). LDLac was used for induction of macrophagic ACAT. LDL was prepared from normal human serum by three step ultracentrifugation according to Havel *et al.* [13], in an L5.50 Beckman instrument. LDL was taken as the 1.024–1.050 fraction. After extensive dialysis against Tris–HCl buffer pH 7.4, LDL was acetylated by the technique of Basu *et al.* [14], using pure acetic anhydride (Merck). LDLac thus obtained was dialysed against 5 l. of Tris–HCl 0.1 M buffer pH 7.4, and stored at 4°. LDL acetylation was

checked by agarose gel electrophoresis (Electrophoresis Universal Film Agarose 1% Corning, Palo Alto, U.S.A.). Protein determination was done by the method of Lowry [15].

Incorporation of oleic acid into cholesteryl esters and triacylglycerols. Cells were preincubated 24 hr in Dulbecco's-MEM medium supplemented with 2% Ultroser G and LDLac 100 μg/ml (to induce ACAT activity), in the absence or in the presence of the drugs (10^{-5} to 10^{-4} M). Incubation with [1-^{14}C] oleic acid (1 μCi/ml) was then performed 4 hr at 37°. Cells were then washed four times with a phosphate-buffered solution pH 7.4, harvested with rubber policemen, centrifuged and resuspended in NaCl 0.15 M. Lipid analysis was performed by thin layer chromatography on silicagel plates F 1500 (Schleicher & Schuell) by direct application of an aliquot of the cell suspension as described by Dosado *et al.* [16]. The solvent phase used was hexane/diethylether/acetic acid 70/30/2 (v/v). After autoradiography, spots corresponding to cholesteryl esters and triacylglycerols (identified by comparison with purified standards Sigma) were cut out and the radioactivity measured by liquid scintillation with an Intertechnique instrument. Results are expressed in nmol/mg of cell protein.

ACAT activity measured in vitro. ACAT activity was measured on sonicated macrophage homogenates, by an adaptation of the method of Brown *et al.* [17]. Each assay, containing 150 μg of protein, phosphate buffer 10^{-1} M pH 7.4, $MgCl_2$ 5×10^{-3} M, bovine serumalbumin 0.2 mg/ml, was preincubated 30 min at 37° under gentle shaking in the presence or in the absence of drugs. Final concentrations of propranolol, metoprolol or pindolol were 10^{-4} to 10^{-3} M, in order to achieve the same ratio of the absolute number of drug molecules/cell proteins which was used for *in situ* experiments (e.g. for *in situ* incorporations of ^{14}C-oleic acid, 10^{-4} M in 1 ml of medium = 10^{-7} molecules of drug/150 μg of cell protein; for *in vitro* ACAT measurement, 10^{-3} M in 100 μl incubation mixture = 10^{-7} molecules of drug/150 μg of cell proteins). After preincubation with drugs, the reaction was initiated by addition of [1-^{14}C] oleyl Coenzyme A (0.1 μCi) and non labeled oleyl-Coenzyme A (final concentration 10^{-5} M). The final incubation volume was 100 μl. The reaction was carried out for 30 min at 37°. An aliquot of the incubation mixture was then put on a silica gel plate and separation of neutral lipids was achieved by thin layer chromatography with hexane/diethylether/acetic acid 70/30/2 (v/v). Spots corresponding to cholesteryl esters were cut out, and the radioactivity was counted by liquid scintillation. ACAT activity was expressed as % of controls.

RESULTS AND DISCUSSION

Table 1 shows that propranolol decreased in a dose-dependent manner ^{14}C-oleic acid incorporation into cholesteryl esters: a 50% decrease was observed at 10^{-5} M, and at 10^{-4} M oleic acid incorporation was about 10-fold reduced. By contrast, neither metoprolol nor pindolol significantly affected oleic acid incorporation into cholesteryl esters, at all the concentrations studied.

It can also be seen in Table 1 that propranolol poorly affected ^{14}C-oleic acid incorporation into triacylglycerols. No effect was found at 10^{-5} M, whereas at this concentration oleic acid incorporation into cholesteryl ester was about 2-fold reduced. The maximal effect on triacylglycerol for-

Table 1. Effect of propranolol, metoprolol and pindolol on [^{14}C]-oleic acid incorporation into cholesteryl esters and triacylglycerols by mouse peritoneal macrophages

Addition	[^{14}C]-oleic acid incorporation into	
	Cholesteryl esters	Triacylglycerols
None	7.2 ± 1.1 (100%)	15.5 ± 2.5 (100%)
Propranolol		
10^{-5} M	3.4 ± 0.7 (47%)	15.2 ± 1.8 (98%)
5×10^{-5} M	1.4 ± 0.3 (19%)	14.4 ± 1.9 (93%)
10^{-4} M	0.8 ± 0.2 (11%)	10.8 ± 2.0 (70%)
Metoprolol		
10^{-5} M	7.4 ± 1.3 (103%)	15.2 ± 2.6 (98%)
5×10^{-5} M	7.0 ± 1.4 (97%)	15.7 ± 2.1 (101%)
10^{-4} M	6.9 ± 0.9 (96%)	15.5 ± 2.8 (100%)
Pindolol		
10^{-5} M	7.0 ± 1.1 (97%)	15.8 ± 1.9 (102%)
5×10^{-5} M	7.1 ± 1.0 (99%)	16.1 ± 2.5 (104%)
10^{-4} M	6.7 ± 0.8 (93%)	18.7 ± 2.1 (121%)

Cells were preincubated 24 hr in Dulbecco's-MEM medium supplemented with 2% Ultroser G and 100 μg/ml acetylated LDL, in the presence or in the absence of the drugs at indicated concentrations. Incorporation of [^{14}C]-oleic acid (1 μCi/ml) was then performed for 4 hr at 37°. Cells were then washed four times with a phosphate-buffered solution, harvested, and lipid analysis performed by TLC. Results are expressed in nmol of [^{14}C]-oleic acid incorporated/mg of cell protein ± SD (mean of three experiments).

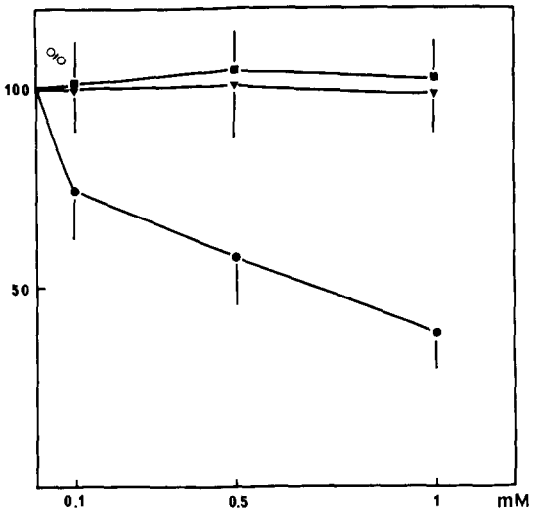

Fig. 1. Effect of propranolol (●), metoprolol (▼) and pindolol (■) on ACAT activity of mouse peritoneal macrophages. Cells were preincubated 24 hr in Dulbecco's-MEM medium supplemented with 2% Ultroser G and acetylated LDL 100 μg/ml. After harvesting, cell suspension was sonicated and ACAT activity measured on homogenates after 30 min preincubation in the absence (control) or in the presence of drugs at indicated concentrations. Results are expressed as % of controls (100% = 23.0 ± 3.6 nmol/hr/mg cell protein; mean of three experiments.

mation was about 30% reduction at 10^{-4} M. Metoprolol and pindolol had no significant effect.

Thus, propranolol strongly and quite specifically inhibited cholesterol esterification. In order to elucidate the mechanism by which the drug can reduce oleic acid incorporation into cholesteryl esters, we investigated the effect of the drug on ACAT activity, measured *in vitro* on macrophage homogenates.

Figure 1 shows that when homogenates of cells preincubated 24 hr with LDLac were treated *in vitro* by propranolol for 30 min prior to the ACAT activity measurement, a dose-dependent inhibition was observed, with about 2- to 3-fold reduction at 10^{-3} M. In contrast, neither metoprolol nor pindolol significantly modified ACAT activity.

ACAT has been reported to be very sensitive to modifications of its membrane micro-environment [18]. β-Blockers, as other amphipatic compounds, interact with membranes, especially with phospholipids [19]. Among β-blockers, propranolol has the most potent "stabilizing" effect on membranes [20]. It thus may be supposed that propranolol inhibits ACAT activity by affecting microsomal membrane physico-chemical properties. It must be noted that the reduction of cholesteryl ester formation by propranolol is not restricted to macrophages, as we also observed it in cultured human fibroblasts [6, 7]. In this latter experimental model, propranolol affected LDL catabolism and sterol metabolism in a manner very close to that of other amphiphilic compounds such as phenothiazines or the hypocholesterolemic agent AY 9944 [7].

The decrease of cholesterol esterification by propranolol in macrophages could be of interest in view of the fact that cholesteryl ester accumulation by monocyte-derived macrophages is probably one of the main factors involved in atherosclerosis. Several authors recently suggested that propranolol, which reduced high density lipoprotein level, could have an unfavorable influence on atherogenesis [10, 11]. But it must be emphasized that there is, at present, no study of the actual effect of propranolol on atherogenesis in man. It must be noted that, in our experimental conditions, propranolol 10^{-5} M was required to obtain a 50% reduction of cholesterol esterification. Although plasma concentrations in man generally do not exceed 3×10^{-7} M (about 100 ng/ml), long term treatment might, however, result in a significant reduction of cholesteryl ester accumulation. It is of note that Pick and Glick reported that, in some cases, propranolol decreased the involvement of atherogenesis in stumptail macaques fed with atherogenic diet [21]. This could be explained, in the light of our observation, by an inhibition of cholesteryl ester accumulation in macrophages, and subsequent delaying of the appearance of atherosclerotic lesions.

REFERENCES

1. S. Fowler, H. Shio and N. J. Haley, *Lab. Invest.* **41**, 372 (1979).
2. J. L. Goldstein, Y. K. Ho, S. K. Basu and M. S. Brown, *Proc. natn. Acad. Sci. U.S.A.* **76**, 333 (1979).
3. R. W. Mahley, T. L. Innerarity, K. H. Weisgraber and K. H. Ho, *J. clin. Invest.* **64**, 743 (1979).
4. T. Henriksen, E. M. Mahoney and D. Steinberg, *Proc. natn. Acad. Sci. U.S.A.* **78**, 6499 (1981).
5. T. Henriksen, E. M. Mahoney and D. Steinberg, *Atherosclerosis* **3**, 149 (1983).
6. J. C. Mazière, C. Mazière, J. Gardette, L. Mora and J. Polonovski, *Biochem. biophys. Res. Commun.* **112**, 795 (1983).
7. J. Polonovski, J. C. Mazière, C. Mazière, L. Mora, F. Gallié, J. Gardette, V. Barbu and C. Roux, in *Lipid Metabolism and Its Pathology* (Ed. M. J. Halpern), p. 19. Elsevier, North Holland, Amsterdam (1986).
8. B. N. C. Prichard and P. M. S. Gillam, *Am. J. Cardiol.* **18**, 387 (1966).
9. B. N. C. Prichard and P. M. S. Gillam, *Br. Heart J.* **33**, 473 (1971).
10. W. Krone, D. Müller-Wieland and H. Greten, *Klin. Wochenschr.* **62**, 193 (1984).
11. P. Van Brummelen, *J. Cardiovasc. Pharmac.* **5**, 551 (1983).
12. Z. A. Cohn and B. Benson, *J. exp. Med.* **119**, 153 (1964).
13. R. J. Havel, H. A. Eder and J. H. Bragdon, *J. clin. Invest.* **34**, 1345 (1955).
14. S. K. Basu, J. L. Goldstein, R. G. W. Anderson and M. S. Brown, *Proc. natn. Acad. Sci. U.S.A.* **73**, 3178 (1976).
15. O. M. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
16. E. A. Dosado, A. W. Hsie and F. Snyder, *J. Lipid Res.* **17**, 285 (1976).
17. M. S. Brown, S. E. Dana and J. L. Goldstein, *J. biol. Chem.* **250**, 4025 (1975).
18. A. G. Lee, *Molec. Pharmac.* **13**, 474 (1977).
19. K. A. Mitropoulos, S. Balasubramian, S. Venkatesan and B. E. A. Reeves, *Biochim. biophys. Acta* **530**, 99 (1978).
20. L. Wislicki, *Archs. Int. Pharmacodyn. Ther.* **182**, 310 (1969).
21. R. Pick and G. Glick, *Atherosclerosis* **27**, 71 (1977).