Pravastatin inhibits cellular cholesterol synthesis and increases low density lipoprotein receptor activity in macrophages: *in vitro* and *in vivo* studies

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- 1 Pravastatin, a 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) inhibitor, is a highly selective inhibitor of hepatic cholesterol synthesis. We studied the *in vivo* and *in vitro* effects of pravastatin on macrophage cholesterol metabolism.
- 2 The effects of incubating pravastatin with human monocyte derived macrophages (HMDM), mouse peritoneal macrophages (MPM) and a J-774 A.1 macrophage-like cell line, on macrophage cholesterol synthesis, cellular degradation of native low density lipoprotein (LDL) and modified LDL, cholesterol efflux from these cells and the cholesterol esterification rate were determined.
- 3 Pravastatin was administered either as one 40 mg dose or 40 mg daily for 8 weeks to normocholesterolaemic and hypercholesterolaemic individuals. The effects on cholesterol synthesis and degradation in monocytes derived from these subjects were studied.
- 4 *In vitro*, pravastatin resulted in a dose-dependent inhibition of macrophage cholesterol synthesis. Cellular degradation of native LDL increased by 119% in the presence of 0.1 mg ml⁻¹ pravastatin. Degradation of both acetyl LDL and oxidized LDL was unaffected. Small concentrations of pravastatin (up to 0.19 µg ml⁻¹) increased the cellular cholesterol esterification rate after incubation with LDL, but higher concentrations resulted in an inhibition of the esterification.
- 5 Single dose pravastatin administration caused a reduction in cholesterol synthesis by the subjects own HMDM by 62% and 47% in normocholesterolaemic and hypercholesterolaemic individuals, respectively. Chronic administration resulted in a 55% inhibition of cholesterol synthesis and a 57% increase in LDL degradation.
- 6 The results indicate that the selective uptake of pravastatin shown for hepatocytes can be extended to macrophages. Pravastatin can inhibit cholesterol accumulation in cells of the arterial wall and by so doing exhibits an additional anti-atherogenic characteristic.

Keywords cholesterol macrophages pravastatin monocytes LDL receptor metabolism

Introduction

Pravastatin is a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis [1]. The drug has been shown to reduce plasma low density lipoprotein (LDL) cholesterol concentrations

in experimental animals [2, 3] as well as in humans [4, 5]. Pravastatin, like the other HMG-CoA reductase inhibitors, inhibits cellular cholesterol synthesis, with a resulting increase in synthesis of LDL receptors on hepatocytes and a consequent acceleration of

the uptake and degradation of plasma LDL in the liver [6].

Pravastatin, unlike other HMG-CoA reductase inhibitors, has an hydroxyl group in the 6-position of the decalin ring and is an open ring acid molecule [7]. This structural difference makes pravastatin uniquely hydrophilic. Pravastatin possesses the ability of highly selective inhibition of hepatic as well as intestinal cholesterol biosynthesis [2]. It has been shown that the drug is taken up by the liver cell, by carrier-mediated transport and by passive diffusion mechanisms that are facilitated by the hepatic sinusoidal network [7].

Foam cells in atherosclerotic lesions contain large amounts of cholesterol esters and there is considerable evidence that a substantial number of these foam cells originate from monocyte-derived macrophages [8]. Previous studies have indicated that human monocyte derived macrophages (HMDM) demonstrate a pattern of cholesterol homeostasis similar to cholesterol metabolism in the liver [9, 10].

The aim of this study was to examine the effect of pravastatin on cellular cholesterol metabolism in macrophages both *in vitro* and *in vivo*. This study demonstrates that the selectivity of pravastatin to hepatocytes can be extended to macrophages, a characteristic which may contribute to the antiatherogenic potential of pravastatin.

Methods

Patients

Five normocholesterolaemic (control) and five hypercholesterolaemic (subjects) were selected. There were five men and five women aged 20–50 years. The patients were maintained before and during the study on the standard low fat and low cholesterol diet (with fat not exceeding 30% of the daily caloric intake and less than 300 mg cholesterol per day). The patients had plasma cholesterol levels of 260–300 mg% (plasma LDL concentrations 190–220 mg%) and all had normal thyroid, renal and hepatic function, and none had diabetes mellitus. Two separate study protocols were employed to investigate the effects of pravastatin on cholesterol metabolism in HMDM *in vivo*:

Acute pravastatin administration: Pravastatin at a dose of 40 mg was administered to the patients and the controls as a single dose in a fasting state. Blood was collected before drug administration and again 2 h later, and HMDM were then separated.

Chronic pravastatin administration: Pravastatin was administered to the five hypercholesterolaemic patients for 8 weeks. At the end of this period fasting blood was collected 2 h after 40 mg administration of pravastatin.

Lipoproteins

LDL was obtained from human plasma (in 4 mM EDTA) drawn from fasted normolipidaemic volun-

teers. The LDL ($d = 1.019\text{--}1.063\text{ g ml}^{-1}$) was prepared by discontinuous density gradient ultracentrifugation in a Beckman VT 50.1 vertical rotor (Beckman Instruments, Fullerton, CA, USA) as described previously [11]. The lipoprotein was washed at $d = 1.063\text{ g ml}^{-1}$ and dialyzed against 150 mM NaCl, 1 mM EDTA, pH 7.4. LDL was then sterilized by filtration and was used within 2 weeks.

LDL was iodinated by the method of McFarlane as modified for lipoproteins [12]. LDL was acetylated by repeated additions of acetic anhydride to 10 mg ml^{-1} LDL diluted 1:1 (vol/vol) with saturated ammonium acetate at 4°C . Acetic anhydride was added to a 40-fold molar excess with regard to total lysines in LDL, and the formation of acetyl LDL (Ac-LDL) was confirmed by electrophoresis on cellulose acetate at pH 8.6 in barbital buffer. For the preparation of oxidized LDL (Ox-LDL), the LDL was diluted in phosphate-buffered saline (PBS) to $300\text{ }\mu\text{g protein ml}^{-1}$ and dialyzed overnight against PBS at 4°C . LDL was then oxidized by incubation in the presence of $10\text{ }\mu\text{M CuSO}_4$ at 37°C for 24 h. Oxidation was terminated by refrigeration and addition 0.1 mM EDTA. The degree of oxidation was determined by malondialdehyde (MDA) analysis using the thiobarbituric acid reactive-substances (TBARS) assay [13]. [^{125}I]-Ox-LDL was prepared by iodination performed before the modification. This procedure did not interfere with the modification, as analyzed by the electrophoretic mobility pattern. The electrophoretic characteristics of the lipoproteins were determined on cellulose acetate as previously described [14]. Gradient (3–12%) SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of the lipoproteins was performed under reducing conditions using β -mercaptoethanol. Protein bands were identified by Coomassie blue staining [15]. To determine the relative size of lipoproteins, electrophoresis was also performed in non-denaturing gradient polyacrylamide gels (3–10%) as described by Krauss & Burke [16]. The protein content of lipoproteins was determined with the folin phenol reagent by the method of Lowry *et al.* [17]. Cholesterol was analyzed by the ferric chloride assay [18].

Solid phase competitive binding radioimmunoassay

LDL, drug associated LDLs and LDL incubated with the appropriate solvents (control LDLs) were assayed in competitive displacement assays on microtitre plates [19]. The plates were coated with $140\text{ }\mu\text{l}$ of 10 mg l^{-1} purified monoclonal antibody B1B6 (this antibody is directed towards the LDL receptor binding domains on apolipoprotein B-100 and mapped to amino acid residues 3114–3606 (it was a generous gift from Drs G. Shonfeld and E. Krul, Washington U., St Louis, MO, USA)).

Cells

The J-774A.1 murine macrophage-like cell line was purchased from American Type Culture Collection, (ATCC, Rockville, MD, USA). The cells were plated

at 5×10^5 cells per 16 mm dish in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum and were fed every 3 days.

Human mononuclear cells were isolated by density gradient centrifugation [20] from blood drawn from fasting normolipidaemic subjects and patients with hypercholesterolaemia.

Blood (20 ml anticoagulated with 100 units heparin ml^{-1}) was layered over 15 ml Ficoll-Paque and centrifuged at 500 g for 30 min at 23°C. The mixed mononuclear cell band was removed by aspiration, and cells were washed twice in RPMI-1640 culture medium containing 100 units ml^{-1} penicillin, 160 $\mu\text{g ml}^{-1}$ streptomycin, and 2 mM glutamine. The cells were plated at 10^6 mononuclear cells per 16 mm dish (Premaria, Falcon Labware, Becton Dickinson, Oxnard, CA, USA) in the same medium in the presence of 20% fetal calf serum. After 2 h of incubation at 37°C in 5% $\text{CO}_2/95\%$ air, nonadherent cells were removed (by three washes with serum-free medium), placed in a similar fresh medium in the presence of 20% autologous serum, and were fed twice weekly with the same medium. Macrophages were used 7–10 days after plating and contained approximately 5×10^5 cells per dish.

Mouse peritoneal macrophages were harvested from the peritoneal fluid of Balb/c mice (weight, 15–25 g) 4 days after intraperitoneal injection of 3 ml 24 g l^{-1} thioglycolate in saline into each mouse. The cells (20×10^6 per mouse) were pooled, treated with 3 ml 8.3 g l^{-1} NaCl (pH 7.4), and incubated for 5 min at 37°C to remove red blood cell contamination. The cells were washed and centrifuged three times with phosphate-buffered saline at 400 g for 10 min and then resuspended to 10^9 l^{-1} in DMEM containing 10% horse serum (heat inactivated at 56°C for 30 min). The cell suspension was dispensed into 16 mm plastic dishes and incubated for 2 h. The dishes were washed once with DMEM under similar conditions for 18 h before the experiment [21, 22].

Lipoprotein metabolism by macrophages

LDL cellular degradation was measured after incubation of [^{125}I]-LDL (150–250 counts $\text{min}^{-1} \text{ ng}^{-1}$) with the cells for 5 h at 37°C. The hydrolysis of LDL protein was assayed in the incubation medium by measurement of trichloroacetic acid soluble, noniodide radioactivity. Cell-free LDL degradation was minimal and was subtracted from total degradation. The cell layer was washed three times with phosphate-buffered saline and incubated with 0.1 N NaOH for 1 h at room temperature for measurement of cellular protein content. Macrophage cholesterol esterification rate was measured as the incorporation of [^3H]-oleic acid into cellular cholesteryl [^3H]-oleate as previously described [22]. The cells were incubated in the presence of the lipoproteins for 18 h at 37°C. During the last 2 h of incubation, [^3H]-oleate in complex with albumin (2.7 mM, 83 mmol oleate mg^{-1} albumin, 10 $\mu\text{Ci ml}^{-1}$) was added to the medium. At the end of the incubation, cellular lipids were extracted with hexane/isopropanol (3:2, vol/vol), and the cholesteryl ester

was separated by thin-layer chromatography (t.l.c.) using hexane/ether/acetic acid (130:30:1.5, vol/vol/vol), scraped into vials containing 15 ml scintillation fluid, and counted in a beta scintillation counter.

Cellular cholesterol efflux was determined in J-774 A.1 macrophages that were preloaded with cholesterol by 18 h of incubation with acetylated LDL (100 μg acetylated LDL protein ml^{-1}). Then cells were incubated with [^3H]-cholesterol (Amerisham Corp., Arlington Heights, IL; 0.5 $\mu\text{Ci ml}^{-1}$, 55 Ci mmol^{-1} , added in ethanol) for 2 h. At the end of the incubation, the cells were washed three times in serum-free medium and further incubated with high density lipoprotein subfraction 3 (HDL3) (25 μg protein ml^{-1}) for 5 h at 37°C. Radioactivity in the cells and the media (after centrifugation to remove detached cells) was measured. Macrophage cholesterol synthesis was measured in cells after 5 h of cell incubation with 1,2 sodium [^{14}C]-acetate (1.25 mM, 10 $\mu\text{Ci ml}^{-1}$). At the end of the incubation, the cells were washed with phosphate-buffered saline, and the radioactivity incorporated into the cholesterol moiety was determined by t.l.c. analysis. The binding of [^{14}C]-labeled pravastatin (1 $\mu\text{g ml}^{-1}$) to LDL or macrophages was assayed in the absence or presence of excess concentrations of unlabeled drug after incubation of 1 h with LDL or 18 h with the cells. In macrophages, cell-associated [^{14}C]-pravastatin was measured after three washes in PBS. In LDL, lipoprotein associated [^{14}C]-pravastatin was measured after LDL precipitation with heparin-manganese (HDL Precipitation Kit, Sigma Co., St Louis, MO, USA), after assuring the conditions for total LDL precipitation. [^{14}C]-labeled pravastatin was provided by Bristol Myers Squibb, Princeton, New Jersey.

Statistics

The nonpaired Student's *t*-test was used. Results are given as mean $\pm$ s.d.

Results

Upon incubation of LDL (1 mg protein ml^{-1}) with 1 $\mu\text{g ml}^{-1}$ of [^{14}C]-pravastatin, a significant drug binding to the lipoprotein was found (157 pmol mg^{-1} LDL protein). This binding was competitively inhibited $10 \pm 3\%$, $23 \pm 7\%$ and $39 \pm 6\%$, respectively, in the presence of 10, 50 and 100 μg of unlabeled pravastatin ml^{-1} . After LDL (1 mg of protein ml^{-1}) incubation with pravastatin (0.01–10 $\mu\text{g ml}^{-1}$) for up to 3 h at 37°C, analysis of the physicochemical properties of pravastatin-treated LDL revealed no significant change in either the lipoprotein electrophoretic mobility or the immunoreactivity of the drug-treated LDL (data not shown). Pravastatin was found to bind to macrophages after 18 h of incubation. On using 1 μg of [^{14}C]-labeled-pravastatin ml^{-1} , $0.28 \pm 0.03\%$ and $0.34 \pm 0.06\%$ of the drug was cell-bound to MPM and to J-774 A.1 macrophages, respectively. In a similar experiment with [^{14}C]-

labeled lovastatin, $2.27 \pm 0.16\%$ and $3.43 \pm 0.23\%$ of the added drug was cell bound, respectively. Using MPM, we have performed a drug competition study with 10, 50 and $100 \mu\text{g ml}^{-1}$ of unlabeled pravastatin. A dose dependent reduction in cell-associated drug by 22%, 39% and 65%, respectively was noted. The effect of pravastatin on macrophage cholesterol metabolism was analyzed both *in vitro* and *in vivo* studies. Upon incubation of three types of macrophages, J-774 A.1 macrophage-like cell line, human monocyte derived macrophages (HMDM) and mouse peritoneal macrophages (MPM) with increasing concentrations of pravastatin for 5 h at 37°C , a dose dependent reduction in cellular cholesterol biosynthesis was noted (Figure 1).

The pravastatin IC_{50} (drug concentration required to reduce cellular cholesterol synthesis by 50%) in J-774 A.1 macrophages, HMDM and MPM were $0.08 \mu\text{g ml}^{-1}$, $6.3 \mu\text{g ml}^{-1}$ and $7.8 \mu\text{g ml}^{-1}$, respectively (Figure 1). Since both cholesterol influx into cells and efflux from cells contribute to the macrophage cellular cholesterol content, the effect of pravastatin on these parameters was examined.

The effect of pravastatin on cellular cholesterol influx was studied in J-774 A.1 macrophages following cells incubation for 5 h at 37°C with $25 \mu\text{g protein ml}^{-1}$ of either native LDL, acetyl LDL (Ac-LDL) or oxidized LDL (Ox-LDL) in the presence of increasing concentrations of pravastatin. Figure 2 demonstrates that only the cellular degradation of native LDL was affected by pravastatin, up to 115% increment at high concentrations of pravastatin ($100 \mu\text{g ml}^{-1}$).

The effect of pravastatin on cellular cholesterol influx was further studied by analysis of cellular cholesterol esterification rates following incubation of J-774 A.1 macrophages with $25 \mu\text{g protein ml}^{-1}$ of native LDL in the presence of increasing concentrations of pravastatin for 5 h at 37°C (Figure 3). On using up to $0.1 \mu\text{g pravastatin ml}^{-1}$, the cellular cholesterol esterification rate was increased by up to 30% following cell incubation with native LDL (Figure 3). At higher pravastatin concentrations, however, the cholesterol esterification rates were similar to those obtained in the absence of pravastatin (Figure 3).

Similar results were found when mouse peritoneal macrophages (MPM) were used (data not shown). Upon incubation of J-774 A.1 macrophages, that were prelabeled with $[^3\text{H}]$ -cholesterol (2 h of preincubation of the cells with $1 \mu\text{Ci ml}^{-1}$ of the isotope) in the absence or presence of $50 \mu\text{g}$ of protein ml^{-1} of high density lipoprotein-3 (HDL-3), no significant effects of increasing pravastatin concentration on cholesterol efflux were found (Figure 4).

The *in vivo* effect of pravastatin on macrophage cholesterol metabolism was also studied using monocytes and monocyte-derived macrophages (HMDM) derived from five healthy volunteers and from five hypercholesterolaemic patients. Blood samples were collected before and 2 h after drug administration and cellular cholesterol synthesis was analyzed in monocytes as well as in monocyte-derived macrophages

Figure 1 The effect of pravastatin on cellular cholesterol synthesis in macrophages. J-774 A.1 macrophage-like cell line ($\circ$), human monocyte derived macrophages HMDM ($\bullet$) and mouse peritoneal macrophages (MPM) ($\triangle$) were incubated with increasing concentrations of pravastatin for 1 h at 37°C . Cellular cholesterol synthesis was determined as described under Methods. Results represent the mean of two experiments, each performed in triplicate.

Figure 2 The effect of pravastatin on macrophage degradation of native LDL ($\circ$), acetylated LDL (Ac-LDL, $\bullet$) and oxidized LDL (Ox-LDL, $\triangle$). J-774 A.1 macrophages were incubated with $25 \mu\text{g protein ml}^{-1}$ of LDL, Ac-LDL or Ox-LDL for 5 h at 37°C in the presence of increasing concentrations of pravastatin. Cellular degradation of the lipoproteins was determined as described under Methods. Results represent the mean of two experiments (for Ac-LDL and Ox-LDL) and three experiments $\pm$ s.d. for LDL.

Figure 3 The effect of pravastatin on cellular cholesterol esterification rate in J-774 A.1 macrophages following incubation with native LDL. Cells were incubated for 5 h at 37°C with $25 \mu\text{g protein ml}^{-1}$ of native LDL in the presence of increasing concentrations of pravastatin and the macrophage esterification rate was analyzed as described under Methods. Results are given as the mean $\pm$ 5.0 ($n = 3$).

Figure 4 The effect of pravastatin on cellular cholesterol efflux from J-774 A.1 macrophages. J-774 A.1 macrophages were labelled with $1 \mu\text{Ci ml}^{-1}$ of $[^3\text{H}]$ -cholesterol for 2 h at 37°C followed by cell wash and a further incubation of the cells for 5 h at 37°C in the presence of increasing concentrations of pravastatin without (●) or with (○) $50 \mu\text{g protein ml}^{-1}$ of HDL-3. At the end of the incubation, medium and cell radioactivity were measured and cholesterol efflux was calculated. Results are given as the mean $\pm$ s.d. ($n = 3$).

(that were obtained 10 days after monocyte culturing in the presence of autologous serum, which contains the drug). Table 1 demonstrates that in the healthy subjects (control) pravastatin reduced cellular cholesterol synthesis rates in monocytes by 22% and in HMDM by 62%. Similarly, in the patients, pravastatin reduced cellular cholesterol synthesis in monocyte and HMDM by 18% and 47%, respectively (Table 1).

Analysis of cellular cholesterol synthesis was also performed in HMDM obtained from 5 hypercholesterolemic patients that were on long term pravastatin therapy (for 8 weeks). The monocytes were separated from the blood 2 h after the administration of 40 mg of the drug after 8 weeks of treatment, and macrophages were obtained following 10 days of monocyte culturing in the presence of autologous serum. Macrophage cholesterol synthesis was reduced from 782 ± 39 (prior to the beginning of drug therapy) to 349 ± 37 counts $\text{min}^{-1} \text{mg}^{-1}$ cell protein ($n = 5$), $P < 0.01$. Lipoprotein degradation rates by HMDM obtained from these patients were also analyzed. Only macrophage degradation of $[^{125}\text{I}]$ -LDL, but not that of Ac-LDL or Ox-LDL was significantly increased (57%) by pravastatin therapy (Table 2).

Discussion

This study has indicated that pravastatin can influence cholesterol metabolism not only in liver and intestinal (ileum) cells [2], but also in monocyte-derived macrophages. Pravastatin differs from other HMG-CoA reductase inhibitors in two aspects. In pravastatin, the 6-position on the decalin ring is occupied by an hydroxyl group, whereas, in lovastatin and simvastatin, this same position is occupied by a

Table 1 The effect of pravastatin administration for a short term on cellular cholesterol synthesis in human monocytes and in monocyte-derived macrophages (HMDM)

Cells	Cellular cholesterol synthesis (counts $\text{min}^{-1} \text{mg}^{-1}$ cell protein)	
	Before	After drug administration
Control		
Monocytes	4035 ± 132	$3152 \pm 103^*$
HMDM	9453 ± 314	$3571 \pm 349^*$
Patients		
Monocytes	4091 ± 116	$3382 \pm 143^*$
HMDM	$13,690 \pm 695$	$7337 \pm 353^*$

Pravastatin (40 mg) was given to five fasting healthy volunteers (control) and to 5 fasting hypercholesterolaemic patients (patients). Blood samples were taken before and 2 h after drug administration. Monocytes were separated and divided into two groups: one was directly analyzed for cellular cholesterol synthesis, and the other group of monocytes was cultured in the presence of autologous serum for 10 days prior to analysis of cholesterol synthesis rate. Results are given as mean $\pm$ s.d. ($n = 5$) $*P < 0.01$ (vs Before).

Table 2 The effect of a long term pravastatin administration on cellular degradation of LDL, Ac-LDL and Ox-LDL by human monocyte derived macrophages (HMDM)

Pravastatin Therapy	Macrophage lipoprotein degradation (ng mg^{-1} cell protein)		
	LDL	Ac-LDL	Ox-LDL
Before	485 ± 391	1973 ± 177	1044 ± 99
After	$760 \pm 53^*$	2120 ± 153	1099 ± 111

Blood was collected from five hypercholesterolaemic patients prior to pravastatin (40 mg day^{-1}) administration (Before) and 8 weeks after (After) drug therapy. (At this point, blood was collected 2 h after pravastatin administration). Monocytes were separated and cultured for 10 days in the presence of 10% autologous serum (which contain the drug). The cellular degradation of $25 \mu\text{g protein ml}^{-1}$ of $[^{125}\text{I}]$ -LDL, $[^{125}\text{I}]$ -Ac-LDL and $[^{125}\text{I}]$ -Ox-LDL by HMDM were then determined. Ac-LDL—acetylated LDL, Ox-LDL—oxidized LDL. Results are given as the mean $\pm$ s.d. ($n = 5$) $*P < 0.01$ (vs Before).

methyl group. This difference in structure is responsible for the different physicochemical properties of these drugs and confers on pravastatin its hydrophilic characteristics. Lovastatin and simvastatin are hydrophobic and unselective. Pravastatin is administered as a sodium salt of an open acid and is the active inhibitor of HMG-CoA reductase; lovastatin and simvastatin are prodrugs and are given as inactive lactones that, following oral administration, are hydrolyzed to an active inhibitor.

Since the major site of cholesterol removal for the body is the liver, where cholesterol is converted into bile acids, the liver selectivity of pravastatin may be an advantage [3]. The ability of macrophages to respond to pravastatin may also be important since macrophages play a protective role in removing

excess plasma LDL or modified forms of LDL. This stimulatory role on macrophage receptors is probably secondary to the inhibitory effect of the pravastatin on macrophage cholesterol synthesis as demonstrated both *in vivo* and *in vitro*. As pravastatin did not effect cholesterol efflux from macrophages, the hypocholesterolaemic action of the drug must be related to increased cellular cholesterol influx.

In vivo, statins are known to concentrate massively in the biliary tract with resulting low levels in the plasma. The relevancy of the *in vitro* experiments, in which the inhibitory effect on macrophages was achieved at high pravastatin concentrations may be questioned. However, the drug, when administered to patients, appeared to inhibit *in vivo* cholesterol synthesis in both the monocytes and HMDM of these patients. It is possible that, *in vivo*, lower concentrations of pravastatin are sufficient for inhibition of monocyte/macrophage cholesterol because of the involvement of some other humoral factor(s) in the cellular cholesterol synthesis. Alternatively, high pravastatin concentrations are indeed required and the monocytes/macrophages may be able to increase the local concentration of the drug following its specific binding to the cells.

There is, as yet, no definite data suggesting the presence of specific pravastatin receptors on circulating mononuclear cells. However, some receptor-mediated internalization of pravastatin was suggested by the competition experiments where unlabeled drug competed with [¹⁴C]-labeled pravastatin for macrophage uptake. Doubtless, pravastatin entry into human monocyte derived macrophages is also related to the relative, but not absolute, hydrophilicity of the drug.

The inhibition by pravastatin of macrophage cholesterol synthesis, both *in vivo* and *in vitro*, was shown to be associated with enhanced cellular uptake of LDL but not Ac-LDL or Ox-LDL. The effect was obtained only at drug concentrations which resulted in substantial inhibition (70%) of cellular cholesterol synthesis, suggesting that the signal for LDL receptor synthesis was dependent on a very low cellular capacity for cholesterol synthesis.

In both monocytes (obtained 2 h after drug administration) and HMDM (obtained 10 days after monocyte culturing in the presence of autologous serum containing the drug), similar pravastatin induced effects were observed. However, the effects on cellular cholesterol synthesis were 2–3 fold more potent in the macrophages compared with the monocytes and probably reflect the long interaction time (10 days) between the drug and macrophages *in vitro*. Similar results have been found with lovastatin. Incubation of HMDM with plasma from lovastatin-treated patients reduced cellular cholesterol synthesis by 58% [24]. Hagemenas & Illingworth [25] have shown that 2–4 h after administration of lovastatin, monocytes derived from hypercholesterolaemic patients demonstrated a 50% reduction in cholesterol synthesis. Similarly, monocytes from normocholesterolaemic patients showed a 57% reduction in cholesterol synthesis. In the present study, no differences in the effects of

pravastatin on macrophage cholesterol synthesis were observed. Thus, macrophages from the hypercholesterolaemic patients, although possessing some different characteristics, when compared with normocholesterolaemic derived cells, interact in a similar fashion with pravastatin.

The effect of pravastatin on macrophage cholesterol esterification is of particular interest. At low dosages (up to 0.1 µg ml⁻¹), macrophage cholesterol esterification was increased, probably secondary to pravastatin mediated increased LDL receptor activity. At higher pravastatin concentrations, the cellular cholesterol esterification rate fell, probably as a result of a direct inhibition of the transport of unesterified cholesterol to the cholesterol esterification cellular compartment. Recently, such a mechanism was suggested in explaining the action of the various statins on acetyl CoA cholesterol acetyl transferase (ACAT) activity [26].

Although pravastatin has been shown to inhibit cholesterol synthesis in various types of macrophages [2], this study has demonstrated that the J-774 A.1 macrophage-like cell line, as evidenced by the reduced IC-50% value of pravastatin for these cells, was more sensitive to the drug than the other types of macrophages. Differences in membrane binding sites for pravastatin in the various cells could explain these findings. Similarly, pravastatin was shown to bind differently to a Hep G-2 cell line and to primary rat hepatocytes [26].

The effect of pravastatin on macrophage cholesterol metabolism could be mediated via interaction of the drug with LDL and/or through binding of the drug to macrophages. Certainly, binding of pravastatin to LDL contributes to the cellular entry of pravastatin *in vitro*. The relative contribution of this route, compared with direct binding of the drug to the macrophages, in the cellular uptake of pravastatin is not known. Unlike lovastatin and simvastatin [27], incubation of pravastatin with LDL had no effect on the lipoprotein electrophoretic mobility or immunoreactivity with mAb B1B6 (which reacts with the LDL receptor binding domains on the LDL apo B100). Pravastatin, unlike simvastatin, also did not affect LDL composition [27]. LDL may well be an important carrier of statin agents in the circulation [29]. This issue awaits classification when data relating statin half-lives and LDL turnover become available.

Pravastatin, thus, inhibits cellular cholesterol synthesis in HMDM both *in vitro* and *in vivo*. Pravastatin, like lovastatin, and some other hypocholesterolaemic drugs have been shown to reduce LDL propensity for lipid peroxidation and this characteristic may contribute to the anti-atherogenicity of these drugs [28]. Pravastatin is a selective HMG-CoA reductase inhibitor drug, not only in liver and ileal cells, but, on the basis of our data, also in arterial wall macrophages. Pravastatin may contribute as an anti-atherogenic protective agent by preventing LDL accumulation in the plasma and by preventing cholesterol accumulation and the transformation of macrophages into foam cells.

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