Biochemical Pharmacology, Vol. 46, No. 12, pp. 2333–2336, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

SHORT COMMUNICATIONS

Effect of lovastatin on cell surface expression of Fc receptors or CD14 antigen in human monocytes

(*Received* 2 *July* 1993; *accepted* 14 *September* 1993)

Abstract—Lovastatin is a widely used anticholesterolemic drug which exercises its effect by inhibiting hepatic cholesterol synthesis and up-regulating low density lipoprotein (LDL) receptors. In the present study, we determined that the drug has no adverse effects on the expression of three cell surface antigens of human monocytes, i.e. high affinity Fc receptors (FcγRI), low affinity Fc receptors (FcγRII) and CD14 antigen. We have shown previously these antigens are regulated by cholesterol and lipoproteins. At 0.5 μg/mL of culture medium, lovastatin did not reduce the percentage of receptor-positive cells or the average number of receptor molecules per cell. These observations add to the attractiveness of the drug as an anticholesterolemic agent and also indicate that endogenous cholesterol biosynthesis by monocytes is not required for expression of FcγRI, FcγRII, or CD14.

Key words: monocytes, lovastatin, Fc receptors, CD14 antigen, cholesterol, lipoproteins

Lovastatin is a potent and highly effective competitive inhibitor of β-hydroxy-β-methyl-glutaryl coenzyme A reductase [1]. This drug also up-regulates hepatic low density lipoprotein (LDL)* receptors. These two actions lead to lowering of plasma cholesterol [2]. We have reported that expression of CD14 antigen and Fc receptors in human monocytes is regulated by cholesterol and lipoproteins [3–6]. Lovastatin by inhibiting cholesterol biosynthesis by human monocytes/macrophages [7] might be expected to perturb the plasma membrane by lowering its cholesterol contents and impairing expression of these antigens. Indeed, modulation of cell surface expression of Fc receptors by altering cellular cholesterol content has been reported in a mouse leukemic cell line [8]. We, therefore, investigated the effect of incubation of human monocytes with lovastatin on the surface expression of CD14 antigen and Fc receptors. Our results showed that this drug even at a concentration as high as 0.5 μg/mL did not impair cell surface expression of high affinity Fc receptors (FcγRI), low affinity Fc receptors (FcγRII), or CD14 antigen. These observations add to the attractiveness of lovastatin as an anticholesterolemic agent and, in addition, indicate that endogenous cholesterol synthesis is not required for regulation of expression of these antigens.

Materials and Methods

Reagents. Human interferon-γ in phosphate-buffered saline (PBS) (IFN-γ, 10^6 U/mg of protein, adjusted to a total protein concentration of 2–4 mg/mL with human serum albumin), and bovine serum albumin (BSA) were products of Sigma (St. Louis, MO). Heat-inactivated fetal bovine serum (FBS) was purchased from GIBCO (Grand Island, NY) and RPMI 1640 was obtained from JRH Biosciences (Lenexa, KS). Ficoll-Paque was purchased from Pharmacia (Piscataway, NJ). All other chemicals were reagent grade and were obtained from various commercial sources.

Immunological reagents. Anti-CD64 (32.2) against FcγRI [9] and anti-CDw32 (IV.3) against FcRγRII [10] were gifts from Dr. Paul M. Guyre of the Dartmouth Medical School. Anti-CD14 (Leu M3) was a product of Becton Dickinson (San Jose, CA). Fluorescein-conjugated goat $F(ab')_2$ anti-murine immunoglobulin g (IgG) was obtained from Tago Inc. (Burlingame, CA).

Isolation of human monocytes. The procedure of Passwell *et al.* [11] with modification was used for isolation of human monocytes. Approximately 60 mL of heparinized blood from a healthy adult male individual was separated by Ficoll-Paque density gradient centrifugation. The mononuclear cells were washed and resuspended in RPMI containing 10% FBS and aliquots of 4–5 mL were transferred to 50-mL culture flasks (Falcon). After 1 hr of incubation at 37° in a CO_2 incubator, the non-adherent cells were discarded, and the adherent cells were washed two or three times with PBS. Five milliliters of the indicated growth media (see below) was added to monolayers in 50-mL flasks. The flasks were incubated for 42–43 hr at 37° in a 5% CO_2 incubator.

Growth media. Basal growth medium consisted of RPMI 1640 with L-glutamine (2 mM), sodium pyruvate (1 mM), penicillin (100 U/mL), streptomycin (78 U/mL), and IFN-γ (500 U/mL), IFN-γ did not have any significant effects on CD14 antigen expression. IFN-γ was added to augment FcγRI expression [12]. This medium was supplemented with 10% heat-inactivated FBS. Lovastatin in the lactone form was converted to dihyroxy-open acid as follows. Four milligrams of the drug was dissolved in 1 mL of ethanol, and then 0.15 mL of 0.1 N NaOH was added. After heating at 50° for 2 hr, the mixture was neutralized to a pH of approximately 7.2 and brought up to a volume of 1 mL with water and diluted with ethanol. The concentration of ethanol in the culture medium was 0.2%.

Immunofluorescence and flow cytometry. Antibody labeling and flow cytometry were carried out as described previously [3] except that the adherent cells were harvested with a rubber policeman and nonspecific binding of antibody was blocked by adding 50 μL of 50% human serum in PBS followed by incubation at 4° for 20 min. Values for the percentage of reactive cells and fluorescence intensity are expressed as means ± range of duplicate experiments. In each experiment, two replicates were analyzed and averaged.

* Abbreviations: LDL, low density lipoprotein; PBS, phosphate-buffered saline; FBS, fetal bovine serum; FcγRI, high affinity Fc receptors; FcγRII, low affinity Fc receptors; and IgG, immunoglobulin G.

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Table 1. Effect of lovastatin on the expression of high affinity Fc receptors (FcγRI) on human monocytes

Lovastatin in medium (μg/mL)	Donor I		Donor II	
	Receptor⁺ cells* (%)	MFI†	Receptor⁺ cells* (%)	MFI†
0.00	94 ± 0	100 ± 18	83 ± 8	74 ± 2
0.05	94 ± 1	110 ± 23	92 ± 3	85 ± 13
0.01	94 ± 1	108 ± 17	84 ± 6	65 ± 4
0.50	88 ± 0	104 ± 20	84 ± 0	82 ± 3

Values are means ± range of duplicate experiments. For details, see Materials and Methods.
* Percentage of cells that were FcγRI-positive.
† Mean fluorescence intensity (MFI) is a function of the average number of receptor molecules per cell.

Results

The effects of incubation of human monocytes for 42–43 hr with increasing concentrations of lovastatin on the expression of FcγRI are shown in Table 1. Two parameters were measured. The first one was the percentage of receptor-positive cells (cells that have FcγRI on the surface). The second was mean value fluorescence intensity which is proportional to the average number of receptor molecules per cell. Neither parameter was adversely affected by lovastatin. In donor I, the percentage of FcγRI-positive cells was 94 ± 0 in the absence of lovastatin and 88 ± 0 in the presence of 0.5 μg of the drug per ml of culture. The corresponding values for donor II were 83 ± 8 and 84 ± 0% respectively. Likewise, the mean fluorescence intensity was 100 ± 18 in the absence of lovastatin and 104 ± 20 in the presence of 0.5 μg/mL of the drug for donor I and 74 ± 2 and 82 ± 3, respectively, for donor II. Thus, lovastatin did not reduce the percentage of receptor-positive cells or the average number of receptor molecules per cell.

The effects of incubation of human monocytes for 42–43 hr with lovastatin on the expression of FcγRII are shown in Table 2. For donor I, the percentage of FcγRII-positive cells was 98 ± 0 in the absence of lovastatin and 96 ± 0 in the presence of 0.5 μg/mL of the drug. The corresponding values for donor II were 94 ± 5 and 96 ± 1%, respectively. The mean fluorescence intensity for donor I was 416 ± 27 in the absence and 390 ± 53 in the presence of 0.5 μg lovastatin/mL of culture. The corresponding values for donor II were 272 ± 67 and 265 ± 28, respectively. Thus, lovastatin did not impair FcγRII expression either.

The effects of lovastatin on the expression of CD14 antigen in human monocytes are shown in Table 3. In donor I, in the absence of lovastatin the percentage of receptor-positive cells was 97 ± 2 and in the presence of 0.5 μg/mL of the drug the percentage of CD14 positive cells was 93 ± 2. The corresponding values for donor II were 94 ± 4 and 94 ± 1%. Likewise, lovastatin did not have any effects on the mean fluorescence intensity. The value was 742 ± 57 for cells without lovastatin and 799 ± 8 in the presence of 0.5 μg/mL of drug for donor I. The corresponding values for donor II were 548 ± 24 and 565 ± 151. Therefore, lovastatin did not impair expression of CD14 antigen.

Discussion

We have reported previously that cholesterol and lipoproteins regulate cell surface expression of Fc receptors and CD 14 antigen [3–6]. Since lovastatin inhibits cholesterol biosynthesis in human monocytes/macrophages [7], and since cholesterol modulates expression of Fc receptors in a mouse leukemic cell line [8], it was expected that lovastatin would inhibit these antigens and consequently impair their function. In contrast, we did not observe any inhibitory effects when the cells were incubated for 42–43 hr with the very high concentration of 0.5 μg/mL. At 1 μg/mL, the cells aggregated and incubation for 4 days resulted in massive loss of viability, both conditions making the experiments technically impossible to carry out. Since the K_i of β-hydroxy-β-methyl-glutaryl CoA reductase of rat liver is 0.6 nM [1] and in the rat orally administered drug inhibits cholesterol synthesis with a 50% inhibitory

Table 2. Effect of lovastatin on the expression of low affinity Fc receptors (FcγRII) on human monocytes/macrophages

Lovastatin in medium (μg/mL)	Donor I		Donor II	
	Receptor⁺ cells* (%)	MFI	Receptor⁺ cells* (%)	MFI
0.00	98 ± 0	416 ± 27	94 ± 5	272 ± 67
0.05	97 ± 1	393 ± 49	98 ± 1	307 ± 18
0.01	97 ± 1	408 ± 24	98 ± 1	218 ± 14
0.50	96 ± 0	390 ± 53	96 ± 1	265 ± 28

Values are means ± range of duplicate experiments. For details, see Materials and Methods.
* Percentage of cells that were FcγRII-positive.
† MFI is a function of the average number of receptor molecules per cell.

Table 3. Effect of lovastatin on the expression of CD14 antigen on human monocytes/macrophages

Lovastatin in medium (μg/mL)	Donor I		Donor II	
	Receptor$^+$ cells* (%)	MFI†	Receptor$^+$ cells* (%)	MFI†
0.00	97 ± 2	742 ± 57	94 ± 4	548 ± 24
0.05	96 ± 2	771 ± 32	97 ± 1	580 ± 6
0.10	96 ± 2	816 ± 23	96 ± 3	394 ± 39
0.50	93 ± 2	799 ± 8	94 ± 1	565 ± 151

Values are means ± range of duplicate experiments. For details, see Materials and Methods.
* Percentage of cells that were CD14-positive.
† MFI is a function of the average number of CD14 molecules per cell.

dose of 46 μg/kg [1], the concentration used should almost totally block cholesterol biosynthesis. In humans, a daily dose of 20–80 mg/day for 48 weeks has been employed to lower plasma cholesterol [13]. We, therefore, used a substantially higher concentration than recommended for its therapeutic effect. It should also be noted that mean plasma concentration for total plasma β-hydroxy-β-methylglutaryl CoA reductase inhibitory activity is less than 50 ng/mL [14].

Fc receptors mediate clearance of particles opsonized with IgG [15], antibody-dependent cell mediated lysis [15], release of inflammatory mediators [16], and superoxide ion production [17]. Fc receptors have also been implicated in the etiology of atherosclerosis [18]. CD14 is a glycophosphatidylinositol-anchored antigen. The gene encoding this glycoprotein maps on chromosome 5 in a region containing genes for a number of growth factors and receptors [19, 20]. Its chromosomal location and its frequent deletion in certain myeloid leukemias have prompted the suggestion that CD14 may represent a new receptor important for myeloid differentiation [19, 20]. This antigen has also been implicated in gram-negative endotoxin-induced shock syndrome [19, 20]. CD14 plays a role in the clearance of gram-negative pathogens [19, 20]. Although expression of Fc receptors and CD14 is regulated by cholesterol and lipoproteins that deliver cholesterol to monocytes [3–6], it is not inhibited by lovastatin. We propose that lovastatin diffuses into the cells, inhibits cholesterol synthesis, and lowers cellular cholesterol. Cellular cholesterol is then replaced by cholesterol derived from plasma lipoproteins. The exogenously derived cholesterol then prevents perturbation of expression of these receptors while its removal contributes to the lowering of plasma cholesterol. The mechanism by which lipoproteins regulate expression of CD14 antigen and Fc receptors has not been elucidated. It remains to establish whether this regulation has a transcriptional or translational basis.

Acknowledgements—This work was supported by a grant from the Merck Research Laboratories and by Grant AR-40404 from the National Institute of Arthritis Musculoskeletal and Skin Diseases. Eric Gressen was the recipient of a summer research award from Hahnemann University School of Medicine.

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REFERENCES

1. Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopes M, Joshua H, Harris E, Pachett A, Monaghan R, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J and Springer J, Mevinolin: A highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc Natl Acad Sci USA* **77**: 3957–3961, 1980.
2. Bilheimer DW, Grundy SM, Brown MS and Goldstein JL, Mevinolin stimulates receptor-mediated clearance of low density lipoprotein from plasma in familial hypercholesterolemia heterozygotes. *Trans Assoc Am Physicians* **96**: 1–9, 1983.
3. Bigler RD, Brown HM, Guyre PM, Lund-Katz S, Scerbo, L and Esfahani M, Effect of low-density lipoprotein on the expression of high affinity Fc receptors. *Biochim Biophys Acta* **1011**: 102–109, 1989.
4. Bigler RD, Khoo M, Lund-Katz S, Scerbo L and Esfahani M, Identification of low-density lipoprotein as a regulator of Fc receptor-mediated phagocytosis. *Proc Natl Acad Sci USA* **87**: 4981–4985, 1990.
5. Esfahani M, Bigler RD, Alfieri JL, Baum JD, Lund-Katz S and Scerbo L, Cholesterol regulates the cell surface expression of phosphatidylinositol-anchored CD14 antigen on human monocytes. *Biochim Biophys Acta* **1149**: 217–223, 1993.
6. Esfahani M, Bigler RD, Alfieri JL, Gressen E, Lund-Katz S and Scerbo L, Lipoproteins upregulate high affinity Fc receptors in human monocytes. *Biochem Biophys Res Commun* **191**: 610–616, 1993.
7. Traber MG and Kayden HJ, Inhibition of cholesterol synthesis by mevinolin stimulates low density lipoprotein receptor activity in human monocyte derived macrophages. *Atherosclerosis* **52**: 1–11, 1984.
8. Kyoizumi S, Kannagi R and Masuda T, Membrane expression of Fc-receptors in cultured leukemic cell lines. I. Induction of Fc receptor in undifferentiated types of cells after passive modulation of lipid viscosity. *J Immunol* **127**: 2252–2256, 1981.
9. Anderson CL, Guyre PM Whitin JC, Ryan RH, Rooney RJ and Fanger MW, Monoclonal antibodies to Fc receptors for IgG on human mononuclear phagocytes. Antibody characterization and induction of superoxide production in a monocyte cell line. *J Biol Chem* **261**: 12856–12864, 1986.
10. Rosenfeld SL, Looney RJ, Leddy JP, Phipps DC,

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Abraham GN and Anderson CL, Human platelet Fc receptor for immunoglobulin G. Identification of a 40,000-molecular weight membrane protein shared by monocytes. *J Clin Invest* **76**: 2317–2322, 1985.

11. Passwell J, Rosen FS and Merler E, The effect of Fc fragments on human mononuclear cell responses. *Cell Immunol* **52**: 395–403, 1980.
12. Guyre PM, Morganelli PM and Miller R, Recombinant immune interferon increases immunoglobulin G Fc receptors on cultured human mononuclear phagocytes. *J Clin Invest* **72**: 393–397, 1983.
13. Bradford RH, Shear CL, Chremas AN, Dujovne C, Downton M, Frankin FA, Good AL, Hesney M, Higgins J, Hurley DP, Langendorfer A, Nash DT, Pool JL, and Schnaper H, Expanded clinical evaluation of lovastatin (EXCEL) study results. I. Efficacy in modifying plasma lipoproteins and adverse event profile in 8245 patients with moderate hypercholesterolemia. *Arch Intern Med* **151**: 43–49, 1991.
14. Pentikainen PJ, Saraheimo M, Schwartz JI, Amin RD, Schwartz MS and Brunner-Ferber F, Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. *J Clin Pharmacol* **32**: 136–140, 1992.
15. Unkeless J, Scigliano E and Freedman VH, Structure and function of human and murine receptors for IgG. *Annu Rev Immunol* **6**: 251–281, 1988.
16. Rouzer CA, Scott WA, Kempe J and Cohn ZA, Prostaglandin synthesis by macrophages requires a specific receptor–ligand interaction. *Proc Natl Acad Sci USA* **77**: 4279–4282, 1980.
17. Yamamoto K and Johnson RB, Dissociation of phagocytosis from stimulation of oxidative metabolic burst in macrophages. *J Exp Med* **159**: 405–416, 1984.
18. Steinberg D, Parthasarathy S, Crew TE, Khoo JC and Witztum JL, Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* **320**: 915–924, 1989.
19. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ and Mathison JC, CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* **249**: 1431–1433, 1990.
20. Raetz CRH, Ulevich RJ, Wright SD, Sibley CH, Ding A and Nathan CF, Gram-negative endotoxin: An extraordinary lipid with profound effects on eukaryotic signal transduction. *FASEB J* **5**: 2652–2660, 1991.