Inhibition of Acyl Coenzyme A: Cholesterol Acyltransferase by 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Inhibitors

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Relatively high concentrations of MK-733 (simvastatin) and MK-803 (lovastatin, mevinolin), which are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, were found to inhibit acyl coeznyme A: cholesterol acyltransferase (ACAT) of rabbit intestinal microsomes with IC_{50}'s of 2.0×10^{-5} and 3.6×10^{-5} M, respectively. Dihydroxy acid forms of both MK-733 and MK-803 did not inhibit ACAT activity. A kinetic analysis using a Lineweaver–Burk plot indicated that MK-733 is a competitive inhibitor of ACAT, with a K_i value of 1.2×10^{-5} M.

Keywords 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor; acyl coenzyme A: cholesterol acyltransferase (ACAT); cholesterol esterase; lecithin: chlolesterol acyltransferase (LCAT)

MK-733 (simvastatin) and MK-803 (lovastatin, mevinolin) are potent inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.[1,2] Both compounds are lactones. Their dihydroxy acid forms, L-654,969 and L-154,819, inhibit HMG-CoA reductase even more potently.[1,2] These HMG-CoA reductase inhibitors are effective hypocholesterolemic agents in several animal species and man.[1,3,4] In our previous study, it was demonstrated that MK-733 dose-dependently suppressed the increase of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol levels in cholesterol-fed rabbits, and prevented the development of atherosclerosis.[5] We have found that one mechanism of the cholesterol-lowering effect of MK-733 is interference with the intestinal absorption of exogenous cholesterol.[6] It is generally accepted that cholesterol is absorbed as free cholesterol[7] and then predominantly esterified before it reaches the lymph.[8] There are two major cholesterol esterification enzymes in the intestine-mucosal cells,[9] acyl CoA : cholesterol acyltransferase (ACAT)[10] and cholesterol esterase.[11] Each enzyme plays an important role in the absorption of exogenous cholesterol. The present study was undertaken to clarify the effect of MK-733 on these cholesterol esterification enzymes.

Materials and Methods

[4-^{14}C]Cholesterol (53.2 mCi/mmol) was obtained from New England Nuclear. [1-^{14}C]Oleoyl CoA (52 mCi/mmol) was purchased from Amersham International plc. MK-733, L-654,969, MK-803 and CS-514[12] were prepared by Merck Sharp and Dohme Research Laboratories. L-154,819 was prepared from MK-803 in our laboratories. Progesterone was purchased from Sigma. Melinamide was prepared from commercial capsules (Artes®, Sumitomo Rharmaceutical Co.). All other chemicals used were standard high-purity commercial materials. Test compounds were dissolved in dimethylsulfoxide (DMSO). Rabbit intestinal microsomes were prepared by the method of Heider *et al.*[13] The supernatant ($107000 \times g$) fraction was taken for cholesterol esterase assay and the microsomal fraction suspended in 0.154 M phosphate buffer (pH 7.4) was used in the ACAT assay. ACAT activity was determined according to the method of Heider *et al.*[13] Endogenous cholesterol of the microsomal fraction and exogenous [1-^{14}C]oleoyl CoA were used as substrates. The supernatant from the microsomal preparation was taken for cholesterol esterase activity essentially by the method of Heider *et al.*[13] Lecithin : cholesterol acyltransferase (LCAT) activity was determined by using a commercial clinical assay kit (Nippon Shoji). Human serum was used as an enzyme source. Protein was determined by the method of Lowry *et al.*[14]

Results

The formation of cholesteryl [1-^{14}C]oleate from mucosal microsomes from rabbits fed a 1% cholesterol diet was linear up to 4 min and there was a linear relationship between ACAT activity and microsomal protein content up to 0.4 mg of protein (data not shown). The presence of MK-733 and MK-803 in the standard ACAT assay mixture reduced the rate of cholesteryl oleate synthesis in a dose-related manner (Fig. 1). As shown in Table I, MK-733 and MK-803 caused 50% inhibition of cholesteryl oleate formation at 2.0×10^{-5} and 3.6×10^{-5} M, respectively, while their open acid forms, L-654,969 and L-154,819 showed almost no inhibition even at 1×10^{-4} M. Another HMG-

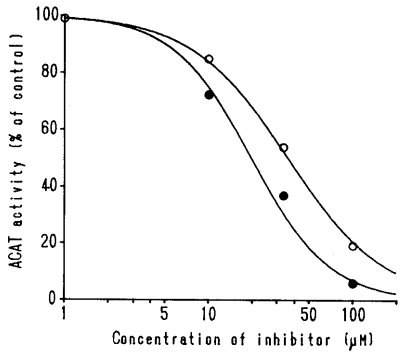

Fig. 1. Effects of MK-733 and MK-803 on the Rabbit Intestinal ACAT Activity

Both compounds dissolved in 5 μl of DMSO were added to 0.5 ml of the reaction mixture for ACAT assay. ACAT reaction was done for 4 min. The control reaction was done with 5 μl of DMSO. Each value shows the mean of duplicate assays. ●—●, MK-733; ○—○, MK-803.

TABLE I. Effects of HMG-CoA Reductase Inhibitors on ACAT, Cholesterol Esterase and LCAT Activities

Inhibitor	IC_{50} (M)		
	ACAT	Cholesterol esterase	LCAT
MK-733	2.0×10^{-5}	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$
L-654,969	$>1.0 \times 10^{-4}$ (15.3%)	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$
MK-803	3.6×10^{-5}	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$
L-154,819	$>1.0 \times 10^{-4}$ (10.5%)	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$
CS-514	$>1.0 \times 10^{-4}$ (0.8%)	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$
Melinamide	1.8×10^{-7}	—	—
Progesterone	2.5×10^{-5}	—	—

Each compound dissolved in DMSO was added to the assay mixture. Final concentration of DMSO was less than 1% in the assay solution. —, not tested. ( ), inhibition (%) at 10^{-4} M.

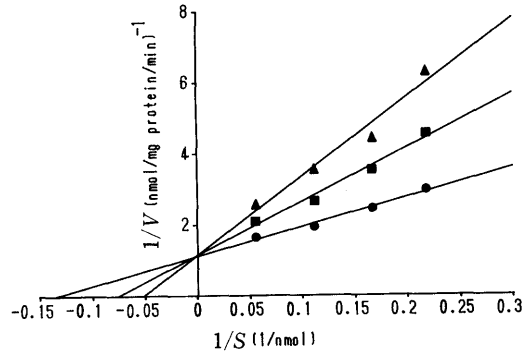

Fig. 2. Lineweaver–Burk Plot of Inhibition of ACAT Activity by MK-733

ACAT reaction was done for 2 min. ●---●, 5 μl of DMSO; ■---■, 1×10^{-5} M MK-733; ▲---▲, 2×10^{-5} M MK-733; *V*, nmol cholesteryl oleate formed/mg microsomal protein/min; *S*, [1-^{14}C]oleoyl CoA nmol/incubation mixture; each value shows the mean of triplicate assays.

CoA reductase inhibitor, CS-514, which is also an open acid, did not inhibit ACAT. Melinamide and progesterone were both used as positive controls, and their IC_{50} values were 1.8×10^{-7} and 2.5×10^{-5} M, respectively. Inhibitory activities of these HMG-CoA reductase inhibitors were tested with cholesterol esterase and LCAT, which is a cholesterol esterification enzyme in serum. None of these compounds inhibited rabbit cholesterol esterase or human serum LCAT even at 1×10^{-4} M. Phenylmethylsulfonyl fluoride as a positive control inhibited cholesterol esterase activity, and 5,5′-dithiobis(2-nitrobenzoic acid) inhibited LCAT activity (data not shown). A kinetic analysis using a Lineweaver–Burk plot indicated that MK-733 was a competitive inhibitor of ACAT (Fig. 2). The apparent K_m value for oleoyl CoA was 1.4×10^{-5} M, and K_i was 1.2×10^{-5} M. These data indicate that MK-733 acts as a competitive inhibitor, whereas melinamide inhibited rabbit intestinal ACAT uncompetitively (data not shown).

Discussion

The present study has clearly demonstrated that MK-733 and MK803, which are lactone compounds, inhibited ACAT in rabbit intestinal mucosa. This is the first report that HMG-CoA reductase inhibitors have ACAT inhibitory activity in the micromolar range. The dihydroxy acid forms of MK-733 and MK-803, L-654,969 and L-154,819, showed almost no effect on ACAT activity. Another HMG-CoA reductase inhibitor, CS-514,[12] also a dihydroxy acid form, did not inhibit ACAT. These results suggest that the lactone forms of HMG-CoA reductase inhibitors may be essential for the inhibition of ACAT. The dihydroxy acid forms of these compounds are potent inhibitors of HMG-CoA reductase, are active in the nanomolar range and are somewhat more potent than their lactone forms.[1,2] MK-733 and MK-803 are easily converted to their dihydroxy acid forms *in vivo*.[3] Three hypolipidemic acylamide compounds, melinamide,[15] 57-118[13] and 58-035,[16] are potent ACAT inhibitors and reduce the transpotation of cholesterol into the mesenteric lymph. MK-733 also inhibited the absorption of exogenous cholesterol in cholesterol-fed rabbits.[6] From these results, a dual mechanism of the hypolipidemic effect of high dose MK-733 and MK-803 in the cholesterol-fed rabbit model is postulated.

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References and Notes

1) A. W. Alberts, J. Chen, G. Kuron, V. Hunt, J. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, E. Harris, A. Patchett, R. Monaghann, S. Currie, E. Stapley, G. Albers-Schonberg, O. Hensens, J. Hirshfield, K. Hoogsteen, J. Liesch and J. Springer, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 3957 (1980).
2) W. F. Hoffman, A. W. Alberts, P. S. Anderson, J. S. Chen, R. L. Smith and A. K. Willard, *J. Med. Chem.*, **29**, 849 (1986).
3) A. W. Alberts, J. Chen, J. Huff, V. Hunt and G. Kuron, IXth Int. Symp. Drugs Affect. Lipid Metab., Florence, 1986, Abstracts, p. 8.
4) J. A. Tobert, G. Hitzenberger, W. R. Kukovertz, I. B. Holmes and K. H. Jones, *Atherosclerosis*, **41**, 61 (1982).
5) M. Kobayashi, F. Ishida, T. Takahashi, K. Taguchi, K. Watanabe, I. Ohmura and T. Kamei, *Jpn. J. Pharmacol.*, **49**, 125 (1989).
6) F. Ishida, A. Sato, Y. Iizuka, Y. Sawasaki, A. Aizawa and T. Kamei, *Biochim. Biophys. Acta*, **963**, 35 (1988).
7) T. Shiratori and D. S. Goodman, *Biochim. Biophys. Acta*, **106**, 625 (1965).
8) G. V. Vahouny and C. R. Treadwell, *Am. J. Physiol.*, **191**, 179 (1957).
9) L. L. Gallo, S. B. Clark, S. Myers and G. V. Vahouny, *J. Lipid. Res.*, **25**, 604 (1984).
10) R. Haugen and K. R. Norum, *Scand. J. Gastroenterol.*, **11**, 615 (1976).
11) L. Swell, J. E. Byron and C. R. Treadwell, *J. Biol. Chem.*, **186**, 543 (1950).
12) Y. Tsujita, M. Kuroda, Y. Shimada, K. Tanzawa, M. Arai, I. Kaneko, M. Tanaka, H. Masuda, C. Tarumi, Y. Watanabe and S. Fujii, *Biochim. Biophys. Acta*, **877**, 50 (1986).
13) J. G. Heider, C. E. Pickens and L. A. Kelly, *J. Lipid Res.*, **24**, 1127 (1983).
14) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
15) H. Fukushima, S. Aono, Y. Nakamura, M. Endo and T. Imai, *J. Atherosclerosis Res.*, **10**, 403 (1969).
16) S. B. Clark and A. M. Tercyak, *J. Lipid Res.*, **25**, 148 (1984).