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 coenzyme A: cholesterol acyltransferase (ACAT) of rabbit intestinal microsomes with IC_{50} s of 2.0 \times 10^{-5} and 3.6 \times 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 \times 10^{-5} M.

Keywords: 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor; acyl coenzyme A: cholesterol acyltransferase (ACAT); cholesterol esterase; lecithin: cholesterol 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.\(^{3,4,5}\) 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.\(^{6}\) We have found that one mechanism of the cholesterol-lowering effect of MK-733 is interference with the intestinal absorption of exogenous cholesterol.\(^{9}\) It is generally accepted that cholesterol is absorbed as free cholesterol\(^{10}\) and then predominantly esterified before it reaches the lymph.\(^{11}\) There are two major cholesterol esterification enzymes in the intestine-mucosal cells,\(^{12}\) acyl CoA: cholesterol acyltransferase (ACAT)\(^{13}\) and cholesterol esterase.\(^{14}\) 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

\[^{[14]}C\text{Cholesterol}\] \( (53.2 \text{mCi/mmol})\) was obtained from New England Nuclear. \(^{[14]}C\text{Oleoyl CoA}\] \( (52 \text{mCi/mmol})\) was purchased from Amersham International plc. MK-733, L-654,969, MK-803 and CS-514\(^{15}\) 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. Melanamide was prepared from commercial capsules (Artes®, Sumitomo Rharapeutical 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 \((100,000 \times g)\) fraction was taken for cholesterol esterase assay and the microsomal fraction 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]}C\text{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.\(^{16}\)

Results

The formation of cholesteryl \(^{[1]}C\text{oleate}\) 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 \(2.0 \times 10^{-5}\) and \(3.6 \times 10^{-5}\) M, respectively, while their open acid forms, L-654,969 and L-154,819 showed almost no inhibition even at \(1 \times 10^{-4}\) M. Another HMG-CoA reductase inhibitor, Lovastatin, also inhibited ACAT activity, but to a lesser extent (Fig. 2).

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. 0—0, MK-733; O—O, MK-803.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC_{50} (M)</th>
<th>Cholesterol esterase</th>
<th>LCAT</th>
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<tbody>
<tr>
<td>MK-733</td>
<td>2.0 \times 10^{-5}</td>
<td>&gt;1.0 \times 10^{-4}</td>
<td>&gt;1.0 \times 10^{-4}</td>
</tr>
<tr>
<td>L-654,969</td>
<td>&gt;1.0 \times 10^{-4} (15.3%)</td>
<td>&gt;1.0 \times 10^{-4}</td>
<td>&gt;1.0 \times 10^{-4}</td>
</tr>
<tr>
<td>MK-803</td>
<td>3.6 \times 10^{-5}</td>
<td>&gt;1.0 \times 10^{-4}</td>
<td>&gt;1.0 \times 10^{-4}</td>
</tr>
<tr>
<td>L-154,819</td>
<td>&gt;1.0 \times 10^{-4} (10.5%)</td>
<td>&gt;1.0 \times 10^{-4}</td>
<td>&gt;1.0 \times 10^{-4}</td>
</tr>
<tr>
<td>CS-514</td>
<td>&gt;1.0 \times 10^{-4} (8.0%)</td>
<td>&gt;1.0 \times 10^{-4}</td>
<td>&gt;1.0 \times 10^{-4}</td>
</tr>
<tr>
<td>Melanamide</td>
<td>1.8 \times 10^{-7}</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Progesterone</td>
<td>2.5 \times 10^{-5}</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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.
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.\textsuperscript{1,2} MK-733 and MK-803 are easily converted to their dihydroxy acid forms \textit{in vivo}.\textsuperscript{3} Three hypolipidemic acylamide compounds, melaminide,\textsuperscript{5,7-11} 57-118\textsuperscript{13} and 58-035,\textsuperscript{16} are potent ACAT inhibitors and reduce the translocation of cholesterol into the mesenteric lymph. MK-733 also inhibited the absorption of exogenous cholesterol in cholesterol-fed rabbits.\textsuperscript{59} 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

Discussion

The present study has clearly demonstrated that MK-733 and MK-803, 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,\textsuperscript{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