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Inhibition of cholesterol synthesis ex vivo and in vivo by fluvastatin, a new inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase

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Abstract. The inhibitory effect of fluvastatin sodium (fluvastatin), a new type of 3-hydroxy-3-methylglutaryl (HMG) coenzyme A inhibitor, on de novo cholesterol synthesis was investigated and compared with that of pravastatin. Fluvastatin at a concentration of 12.5 mg/kg inhibited sterol synthesis ex vivo from [^{14}C]acetate in rat liver and ileum by 97–99% with respect to the control, while the inhibition in kidney was 55%. The inhibition by fluvastatin in the liver and ileum persisted for approximately 9 h after administration. Significant differences between fluvastatin also had an inhibitory effect on cholesterol synthesis in vivo in various tissues of rats given [^{14}C]acetate intraperitoneally. Sterol synthesis in the liver, ileum and kidney was inhibited by over 95% 3 h after administration of 6.25 mg/kg of fluvastatin. Significant differences between fluvastatin and pravastatin were found in the liver and ileum. Fluvastatin was more potent than pravastatin in inhibiting both ex vivo and in vivo sterol synthesis in the ileum (but not in kidney) and liver.

Key words. Fluvastatin; hydroxymethylglutaryl-CoA reductase; hydroxymethylglutaryl-CoA reductase inhibitor; cholesterol synthesis; rat peripheral organ.

Fluvastatin sodium (Sandoz compound XU 62-630, [R, S-(E)]-(±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indole-2-yl]-3,5-dihydroxy-6-heptenoic acid monosodium salt) (fig. 1) is a new inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase; EC1.1.1.88), the rate-limiting enzyme in cholesterol biosynthesis, and is under investigation as a candidate hypolipidemic and antiatherosclerotic agent[1,2]. Cholesterol synthesis in the liver and intestine, the primary organs supplying serum cholesterol, is regulated by induction and suppression of HMG-CoA reductase[3–5]. Moreover, when dietary cholesterol derived from the intestine is delivered to the liver in the form of lipoproteins, it suppresses the activity of HMG-CoA reductase[3–5]. In humans and dogs these inhibitors selectively reduce low-density lipoprotein (LDL)[6,7]. In a recent study, fluvastatin sodium (as fluvastatin) showed significant induction of LDL receptor on HepG2 cells and a strong plasma cholesterol-lowering effect[5]. Moreover, fluvastatin is more potent than compactin and lovastatin in inhibiting HMG-CoA reductase in vitro and cholesterol biosynthesis in vivo[8–11].

The present study was therefore undertaken to investigate the inhibitory effect of fluvastatin on cholesterol synthesis in various tissues of rats ex vivo and in vivo, and to compare its effect with that of another HMG-CoA reductase inhibitor, pravastatin.

Materials and methods

Materials. [1-^{14}C]Sodium acetate (56–60 mCi/mmol) was purchased from the Radiochemical Centre (Amer-

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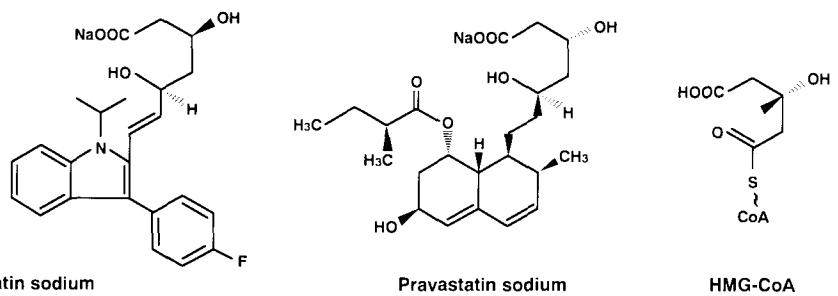

Figure 1. Chemical structures of fluvastatin and pravastatin.

sham, England). Fluvastatin sodium (XU62-320) was a generous gift from Sandoz Pharmaceuticals Co. (Japan). Digitonin was purchased from Sigma. Other reagents were of analytical grade.

Animals and treatment. Wistar-Imamichi male rats weighing 150–160 g were used. The animal room was maintained at 23 ± 1 °C, with 50 ± 5% relative humidity and a 12 h light-dark cycle (lights on from 06.00 to 18.00 h). They were housed three per cage and freely given water and commercial laboratory chow (MF; Oriental Yeast Co., Japan) for at least 1 week prior to use. The rats were divided into three treatment groups. The first group served as a control and received the vehicle alone (0.1 ml/100 g b. wt/day). The second group received fluvastatin at doses of 6.25 or 12.5 mg/kg. The third group received pravastatin at doses equivalent to 6.25 or 12.5 mg/kg of fluvastatin. All drugs were administered orally. [^{14}C]Acetic acid was used as a precursor for sterol synthesis in both ex vivo and in vivo experiments. The animals were starved after drug administration.

Determination of various concentrations of fluvastatin ex vivo and in vivo on sterol synthesis in liver, ileum and kidney. Fluvastatin was orally administered at doses of 6.25, 12.5 or 25.0 mg/kg to rats. 2 h after drug administration, the various tissues were rapidly removed and sterol synthetic activity in their tissues was determined.

Time-course of inhibition by fluvastatin of sterol synthesis in liver, ileum and kidney in rat ex vivo. Rats were given fluvastatin orally at a single dose of 6.25 mg/kg. The tissues were collected 0.25, 1, 2, 6, 9, 12, 16 and 24 h after drug administration, and their sterol synthetic activity was determined.

Determination of ex vivo sterol synthesis in various organ slices. Rats were killed (10.00 h) by decapitation 2 h after oral administration of fluvastatin (12.5 mg/kg) or pravastatin (12.5 mg/kg), and the various organs were rapidly removed and washed with ice-cold saline. Tissue slices (0.5-mm thick) were prepared with a tissue slicer (McIlwain Mechanical Tissue Chopper, Neuroscience, Inc., Tokyo). Sterol synthetic activity in various tissues was determined according to the method of Endo et al.[8] with slight modifications. Tissue slices (100 mg) were placed in a test tube (ST 16.5–125, NEG Co.) containing 1 ml of Kreb's/Ringer/phosphate buffer and a radioactively labeled substrate (100 µl of 4 µCi [^{14}C]acetic acid) for sterol synthesis. The tube was gassed with 95% O_2/5% CO_2 for 10 s, stoppered with a serum cap, and incubated at 37 °C in a metabolic shaker at 150 oscillations/min for 2 h. At the end of the incubation period, 1 ml of 15% alcoholic KOH was added to the test tube, and the mixture was saponified for at least 2 h at 80 °C. A portion of the mixture was assayed for protein concentration by the method of Lowry et al.[12] and the remainder was extracted three times with 2 ml of light petroleum. The light petroleum extracts were dried and dissolved in 1 ml of acetone-ethanol (1:1). This solution was added to a mixture of 0.5% digitonin and 50% ethanol, and the mixture was centrifuged at 3,000 rpm for 10 min.

The digitonin-precipitable sterols (DPS) were isolated[13], washed with acetone-ether (1:2) and diethylether, dissolved in 0.5 ml of Soluene-350 (Packard) and counted.

Determination of in vivo sterol synthesis in various organ slices. Rats received oral administration (08.00 h) of fluvastatin (6.25 mg/kg) or pravastatin (6.25 mg/kg) 2 h before i.p. injection of [^{14}C]acetate (20 µCi/kg). 1 h after the injection of [^{14}C]acetate, the rats were killed by decapitation, and each tissue was rapidly excised and washed with ice-cold saline. Tissue slices were prepared and saponified at 75 °C for 4 h in 20% alcoholic KOH, then DPS were isolated and counted in the same way as in the ex vivo study[5,6,8–11].

Statistical analysis. Statistical analysis was performed by using Student's t-test.

Results

The effects of various concentrations of fluvastatin ex vivo and in vivo on the sterol synthesis in liver, ileum and kidney. A single administration of fluvastatin ex vivo and in vivo at three doses, 6.25, 12.5 and 25.0 mg/kg to rats caused a significant suppression of sterol synthesis in liver and ileum, compared with the control rats (fig. 2A and B). However, no significant inhibition by fluvastatin (6.25 mg/kg) ex vivo was observed in the kidney (fig. 2A). This suggested that 25.0 mg/kg was a high dose; therefore 6.25 (in vivo) and 12.5 (ex vivo) mg/kg were used as the dose of fluvastatin in this study.

Time-course of fluvastatin inhibition of sterol synthesis ex vivo. As shown in figure 3, potent inhibitory effects on sterol synthesis in liver and ileum were seen 0.25 h after fluvastatin, reached a maximum at 1 or 2 h, and lasted for at least 9 h. However, the inhibition in kidney was less effective. Fluvastatin inhibited the sterol synthesis of liver and ileum by 62–99% for 0.25–9 h after its oral administration at 6.25 mg/kg, but caused no inhibition at 12 h. In this study, the value at 2 h was adopted, because the 1-h value was subject to more variation.

Inhibition of sterol synthesis ex vivo. Table 1 shows the inhibitory activity of fluvastatin on the ex vivo incorporation or [^{14}C]acetate into sterols in several organs of rats. When fluvastatin was orally administered to rats at a dose of 12.5 mg/kg, sterol synthesis in tissue slices of liver and ileum was reduced by 97% or more, whereas the inhibition in kidney was 55%. Among the tissues tested, liver and ileum were most sensitive to the administration of fluvastatin. On the other hand, 12.5 mg/kg of pravastatin significantly reduced the sterol synthesis in liver, ileum and kidney by 47–91%. Fluvastatin was more potent than pravastatin in inhibiting sterol synthesis in liver.

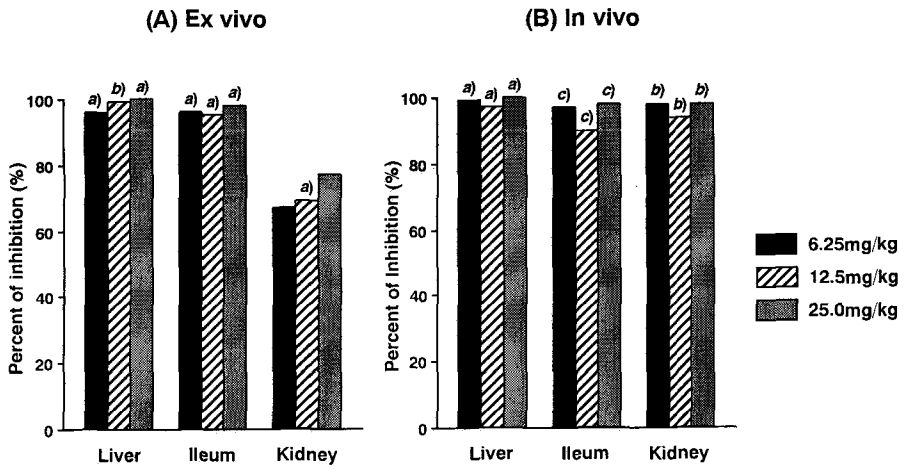

Figure 2. Effects of various concentrations of fluvastatin ex vivo and in vivo on sterol synthesis in liver, ileum and kidney of rat.
A Fluvastatin was orally administered at doses of 6.25–25.0 mg/kg to rats. Rats were killed by decapitation 2 h after drug administration, and the various organs were rapidly removed. Each value represents the mean ± SEM of 3–4 animals. The inhibition in liver, ileum and kidney are shown as percentage of control. Control values of liver, ileum and kidney were 3644 ± 946, 654 ± 196 and 389 ± 105 dpm/mg/h, respectively. *a*) $p < 0.05$, *b*) $p < 0.01$ vs control.
B Fluvastatin was orally administered at doses of 6.25–25.0 mg/kg to rats. After 2 h, 200 μCi of [^{14}C]acetate was injected i.p. into rats which were killed by decapitation 60 min after labeled acetate injection. Each value represents the mean ± SEM of 4 animals. The inhibition in liver, ileum and kidney are shown as percentage of control. Control values of liver, ileum and kidney were 645 ± 200, 590 ± 11 and 94 ± 19 dpm/100 mg tissues, respectively. *a*) $p < 0.05$, *b*) $p < 0.01$, *c*) $p < 0.001$ vs control.

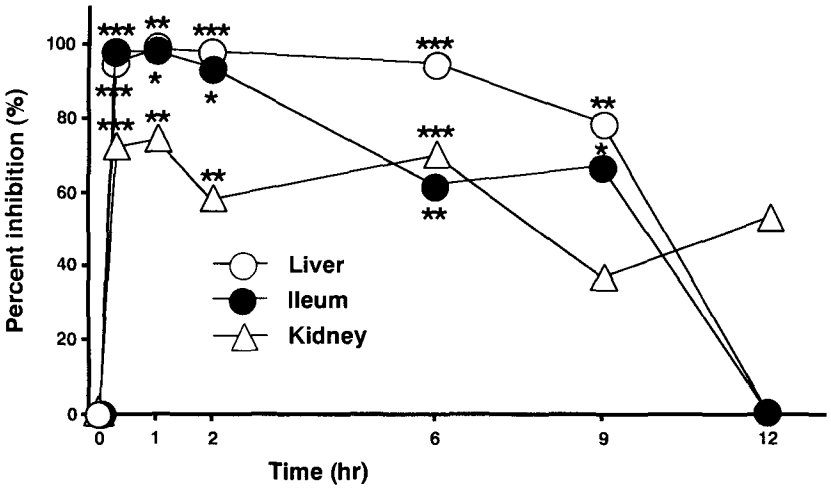

Figure 3. Time-course of inhibition by fluvastatin of sterol synthesis in liver, ileum and kidney of rat ex vivo. The animals were killed by decapitation 0.25, 1, 2, 6, 9, 12, 16 and 24 h after oral administration of fluvastatin (6.25 mg/kg), and sterol synthetic activity in their tissues was determined. Each value represents the mean ± SEM of 5–6 animals. Each value shows inhibition as a percentage with respect to the control. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs control.

Inhibition of sterol synthesis in vivo. When fluvastatin was orally administered to rats at a dose of 6.25 mg/kg at 2 h before [^{14}C]acetate injection, sterol synthesis was inhibited by over 90% in liver, ileum and kidney (table 2). On the other hand, although 6.25 mg/kg of pravastatin did not cause a significant reduction of sterol synthesis in kidney tissue, inhibition in liver and ileum amounted to 43–62%. The difference between the effects of fluvastatin and pravastatin on inhibitory activity of sterol synthesis was statistically significant in liver and ileum.

Discussion

As shown in table 1, fluvastatin strongly inhibited ex vivo sterol synthesis in liver and ileum, but the inhibition in kidney tissue was weaker. In addition, in vivo sterol synthesis in liver, ileum and kidney was markedly inhibited (table 2). The liver and/or intestine (which delivers dietary cholesterol to the liver in the form of specific lipoproteins) are the major sources of serum cholesterol, and little sterol synthesis occurs in nonhepatic peripheral tissues[8,10]. In the present study, since the

Table 1. Inhibitory activity of fluvastatin on sterol synthesis in various tissues of rat ex vivo.

Organ	Synthetic activity (dpm/mg/hr)		
	Control	Fluvastatin (12.5 mg/kg)	Pravastatin (12.5 mg/kg)
Liver	1732 ± 129	22 ± 2***(99)	488 ± 102***(72)[a)]
Ileum	416 ± 128	14 ± 1*(97)	38 ± 12*(91)
Kidney	163 ± 21	73 ± 6**(55)	87 ± 13*(47)

Male Wistar Imamichi rats each weighing 150–160 g were housed in colony cages under controlled lighting conditions (18.00–06.00 h dark phase) for 1 week and starved after administration of drugs. 2 h after oral administration of fluvastatin (12.5 mg/kg) or pravastatin (12.5 mg/kg), the animals were killed by decapitation, and tissues were excised. Each value represents the mean ± SEM of 6 animals. Inhibition as a percentage with respect to the control is shown in parenthesis. Significant difference from control value: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Significant difference between fluvastatin and pravastatin: a)$p < 0.01$.

Table 2. Inhibitory activity of fluvastatin on sterol synthesis in various tissues of rat in vivo.

Organ	Synthetic activity (dpm/100 mg tissue)		
	Control	Fluvastatin (6.25 mg/kg)	Pravastatin (6.25 mg/kg)
Liver	711 ± 143	8 ± 2***(99)	272 ± 28*(62)[a)]
Ileum	972 ± 137	50 ± 14***(95)	557 ± 52*(43)[a)]
Kidney	118 ± 17	4 ± 1***(97)	80 ± 10(32)

Male Wistar Imamichi rats each weighing 150–160 g were housed in colony cages under controlled lighting conditions (18.00–06.00 h dark phase) for 1 week and starved after administration of drugs. Fluvastatin and pravastatin were orally administered at the dose of 6.25 mg/kg to rats. After 2 h, 200 μCi of [^{14}C]acetate was intraperitoneally injected into the rats, which were killed by decapitation 60 min thereafter. Each value represents the mean ± SEM of 5–10 animals. Inhibition as a percentage with respect to the control is shown in parenthesis. Significant difference from control value: *$p < 0.05$, ***$p < 0.001$. Significant difference between fluvastatin and pravastatin: a)$p < 0.001$.

incorporation of [^{14}C]acetate into sterols in rat liver was inhibited by fluvastatin, it was expected that serum cholesterol would be reduced as a result of inhibition of cholesterol biosynthesis in the liver. The reduced cholesterol levels in nonhepatic tissues tested might be explained by a poor supply of cholesterol to nonhepatic tissues from the blood. In this study, therefore, the difference between the ex vivo and in vivo effects on the inhibition of de novo sterol synthesis may indicate that cholesterol in nonhepatic tissues such as kidney is partly derived from the liver.

Recently, Hayashi et al.[5] and Ma et al.[14] suggested that HMG-CoA reductase inhibitors lower plasma cholesterol through induction of hepatic LDL receptor mRNA by hepatic cholesterol depletion. In the present study, the sterol synthesis in hepatic and nonhepatic tissues was markedly inhibited by fluvastatin. Fluvastatin-induced inhibition of both ex vivo and in vivo sterol synthesis was more potent that that of pravastatin in liver and ileum (but not in kidney).

Moreover, repeated administration of fluvastatin (6.3 mg/kg) to rats caused a significant decrease of serum cholesterol, although a single administration of fluvastatin (100 mg/kg) had no effect (data not shown). Therefore, our results may indicate that the lowering of serum cholesterol is due to an increase in the number of LDL receptors and that the induction of LDL receptors in the liver, by lowering intracellular free cholesterol, is a time-consuming process.

Koga et al.[11] and Tsujita et al.[15] have shown that pravastatin-induced inhibition of sterol synthesis was less potent in nonhepatic peripheral tissues, and they suggested that pravastatin is tissue-selective with respect to lens and liver in its ability to inhibit cholesterol synthesis[10]. In our experiments, fluvastatin exhibited higher inhibitory activity than pravastatin in peripheral organs, such as liver, ileum and kidney. Fluvastatin may be better able to penetrate the cell membrane than pravastatin in these tissues. In this connection, the hydrophilicity of the β-hydroxy group at the 6th position of decaline may contribute to the tissue-selectivity[10,16]. On the other hand, fluvastatin has a 4-fluorophenyl group at the 3 position of indole. Recently, Tse and Labbadia[2] reported that fluvastatin was subject to a considerable first-pass effect, its absolute bioavailability being 46 per cent. The structure-activity relationship for tissue-selective inhibition of cholesterol synthesis by fluvastatin is being studied further.

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