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Effect of YM-53601, a novel squalene synthase inhibitor, on the clearance rate of plasma LDL and VLDL in hamsters

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1 To better understand how it decreases plasma cholesterol and triglyceride, we evaluated the effect of YM-53601 ((*E*-2-[2-fluoro-2-(quinuclidin-3-ylidene) ethoxy]-9*H*-carbozole monohydrochloride) on the clearance rate of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in hamsters.

2 Treatment with YM-53601 at 50 mg kg^{-1} for 5 days in hamsters fed a normal diet enhanced the disappearance of 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI)-VLDL and DiI-LDL. This effect on DiI-LDL was lost in the early phase after DiI-methyl(met)-LDL, chemically modified to block LDL receptor binding, was injected in hamsters, but was retained in the late phase. Pre-treatment with protamine sulphate, which inhibits the activity of LPL, also failed to enhance DiI-VLDL clearance rate by YM-53601.

3 Even on single oral administration at 30 mg kg^{-1}, YM-53601 enhanced the disappearance of the high concentration of plasma triglyceride after injection of intrafat, an emulsion of fat. Plasma triglyceride was significantly decreased as soon as 1 h after single administration of YM-53601 in hamsters fed a normal diet.

4 These results indicate that the decrease in plasma total cholesterol and triglyceride after the treatment with YM-53601 is due to its enhancement of the clearance rate of LDL and VLDL, respectively. Moreover, YM-53601 may be effective in decreasing plasma triglyceride levels early in the course of treatment of hypertriglyceridaemia in humans.

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Abbreviations: DiI, 1, 1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein; LPL, lipoprotein lipase; VLDL, very low density lipoprotein; WHHL, Watanabe heritable hyperlipidaemic

Introduction

In addition to plasma LDL cholesterol, plasma triglyceride has recently come to be considered of increasing importance as an independent risk factor for coronary heart disease (Cullen, 2000). Most plasma triglyceride occurs as very low density lipoprotein (VLDL) and chylomicron particles in circulating blood. The former derives from the liver and the latter from lymph vessels through the intestine. These particles are rapidly remodelled by lipoprotein lipase (LPL), which hydrolyzes triglyceride into fatty acid and monoacylglycerol. Chylomicrons are metabolized to chylomicron remnants. VLDL is first remodelled to intermediate low density lipoprotein (IDL) and then converted to low density lipoprotein (LDL), which incorporate most plasma cholesterol, and are taken into the liver by the LDL or non-LDL receptor pathways (Slater *et al*., 1984). A significant amount of IDL is also removed by the liver. Fibrates, which stimulate peroxisome proliferator-activated receptor alpha, reduce plasma triglyceride in hyperlipidaemia. Moreover, some inhibitors of squalene synthase (farnesyl-diphosphate: farnesyl-diphosphate farnesyl transferase, EC 2.5.1.21), an enzyme vital for cholesterol biosynthesis, show a similar effect to fibrates on plasma triglyceride in several animal species (Amin *et al*., 1997; Hiyoshi *et al*., 2001; Ugawa *et al*., 2000). It has been shown that squalene synthase inhibitor suppresses triglyceride biosynthesis in hepatocytes isolated from homozygous Watanabe heritable hyperlipidaemic (WHHL) rabbits *in vitro* (Hiyoshi *et al*., 2001), but the mechanism by which squalene synthase inhibitors act on triglyceride metabolism is not precisely known. YM-53601 may belong to a novel class of lipid-lowering agents that inhibit squalene synthase, leading to reduced cholesterol biosynthesis in animals. In pre-clinical studies in rodents and rhesus monkeys, YM-53601 significantly decreased plasma concentrations of cholesterol and had a more potent triglyceride-lowering effect than a fibrate (Ugawa *et al*., 2000). To more closely examine the mechanism by which YM-53601 reduces plasma cholesterol and triglyceride, we measured the effect of YM-53601 on the clearance rate of plasma LDL and VLDL in hamsters. It was previously reported that fibrates induce an increase in plasma VLDL clearance rate from circulating blood (Arakawa *et al*., 1995). However, this is the first report to demonstrate the enhancement of LDL and VLDL clearance by a squalene synthase inhibitor. Further, we also

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evaluated the participation of LPL in the enhancement of VLDL clearance by YM-53601. Finally, we examined whether YM-53601 acts on LDL receptor, non-LDL receptor or both pathways in LDL clearance in hamsters.

Methods

Materials

YM-53601 was synthesized at the Chemistry Laboratories, Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan). Clofibrate was purchased from Tokyo Kasei (Tokyo, Japan). 1, 1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), potassium bromide and sodium azide were purchased from Wako Pure Chemical Industries (Osaka, Japan). Protamine sulphate and human lipoprotein-deficient serum were obtained from Sigma (St Louis, MO, U.S.A.). Intrafat was purchased from Takeda Chemical Industries Ltd (Osaka, Japan).

Plasma lipid lowering effect in hamsters

Male Syrian golden hamsters (from Hamri, Ibaraki, Japan) weighing 140–170 g were fed a standard diet (CE-2 from CLEA Japan Inc., Tokyo, Japan). YM-53601 was suspended in 0.5% methylcellulose. Hamsters were given a single oral administration of YM-53601 at a dose of 50 mg kg^{-1}. The control group was administered an equal volume of the 0.5% methylcellulose vehicle solution. Blood specimens were obtained from the femoral vein using a glass capillary at 0, 1, 2, and 4 h after administration. Plasma triglyceride was measured enzymatically with the Triglyceride-G Test Wako (Osaka, Japan).

Isolation of VLDL and LDL, and methylation of LDL

VLDL and LDL were isolated from plasma obtained from hamsters that had been fed a standard rodent diet. Sodium azide was added to hamster plasma at a final concentration of 0.1% to prevent the denaturation of protein. VLDL was obtained by centrifugation of 4 ml plasma at a density of 1.006 g ml^{-1} in a Hitachi ultracentrifuge at $114{,}000 \times g$ for 16 h at 4°C after removal of chylomicron. The top layer was harvested and pooled as a part of VLDL. Potassium bromide was added to the plasma residue to adjust density to 1.063 g ml^{-1}. LDL was isolated by ultracentrifugation at $114{,}000 \times g$ for 20 h at 4°C and harvested (Gofman *et al*., 1949). LDL fraction was dialyzed for 24 h at 4°C using a dialysis membrane (Spectrum Medical Industries, Los Angeles, CA, U.S.A.). Proteins of VLDL and LDL were assayed by the method of Lowry *et al*. (1951). Protein concentration of VLDL and LDL was adjusted to 1–2 mg ml^{-1} with 150 mM sodium chloride/1 mM EDTA, pH 7.4.

Methylated LDL (met-LDL) was prepared by treatment of the LDL fraction with formaldehyde plus sodium borohydride as described previously (Weisgraber *et al*., 1978). The extent of methylation of lysine residues of met-LDL was 50% by amino acid analysis using the trinitorobenzenesulphonic acid method (Habeeb, 1966). A 50% modification is sufficient to abolish LDL binding activity on LDL receptor (Weisgraber *et al*., 1978).

DiI labelling of lipoproteins

Fluorescent DiI labelling of native VLDL, LDL and met-LDL was performed as described by Innerarity *et al*. (1986). The lipoproteins were mixed with human lipoprotein-deficient serum and DiI in DMSO. The solution was then filtered (0.45 μm for LDL and met-LDL; 0.8 μm for VLDL) through Millex-PF filters (Millipore Corp., Bedford, MA, U.S.A.) and incubated for 15 h at 37°C. Potassium bromide was added to the mixture to adjust the density to 1.006 g ml^{-1}. The lipoproteins were reisolated from the incubation mixture by ultracentrifugation at a density of 1.063 g ml^{-1} for LDL and met-LDL and 1.006 g ml^{-1} for VLDL at $114{,}000 \times g$ for 24 h at 4°C. The lipoproteins were harvested, dialyzed and filtered as described above.

Plasma lipoprotein clearance

Male Syrian golden hamsters weighing 140–180 g were fed CE-2 chow. Clofibrate was used as a positive control agent as it is well known to increase the clearance rate of VLDL triglyceride and has been proved to have a similar activity to other fibrates such as gemfibrozil in humans (Kesaniemi & Grundy, 1984; Kesaniemi *et al*., 1985). Hamsters were orally dosed with YM-53601 at 50 mg kg^{-1} for 5 days or clofibrate at 150 mg kg^{-1} for 7 days. The dose of clofibrate was established on the basis of a previous report (Wada *et al*., 1981). At 0.5 or 4 h after the last administration of YM-53601 or clofibrate, respectively, DiI-VLDL, DiI-LDL or DiI-met-LDL at the concentration of 100 μg protein per hamster was intravenously injected into a brachial vein. At 2 and 30 min and 1, 2, 4 and 6 h after injection of DiI-VLDL, the femoral vein was cut with a needle and fresh blood from the cut was carefully collected into glass capillaries. The collected blood was centrifuged to obtain plasma. Fluorescent intensities of plasma was measured at 530 nm excitation and 590 nm emission using CytofluorII (PE Biosystems, Foster City, CA, U.S.A.). The amount of DiI circulating at 2 min was set as 100%. For DiI-LDL or DiI-met-LDL, blood samples were collected at 2 min and 3, 6, 9, 24 and 48 h after injection. In the experiment to evaluate the participation of lipoprotein lipase (LPL), protamine sulphate at the concentration of 26 mg kg^{-1} was intravenously injected into a brachial vein 5 min before the last dose of YM-53601. DiI-VLDL at the concentration of 100 μg protein per hamster was intravenously injected into a brachial vein 30 min after the last dose of YM-53601. Subsequent treatment was as described for the DiI-VLDL clearance experiment.

Intrafat clearance

Ten-week-old male Syrian golden hamsters were fed CE-2 chow and water *ad libitum*. Hamsters were allotted to one of two groups depending on their plasma triglyceride level after fasting for 16 h. They were given a single oral dose of YM-53601 suspended in a 0.5% methylcellulose vehicle solution at 30 mg kg^{-1} body weight. The control group was given an equal volume of the methylcellulose vehicle. After 30 min, intrafat at the concentration of 1.5 ml kg^{-1} was intravenously injected into a brachial vein. Blood specimens were obtained from the femoral vein using a glass capillary at 5, 25, 45, 65 and 85 min after the administration of intrafat.

Plasma triglyceride was measured enzymatically with a Triglyceride-G Test Wako. The concentration of plasma triglyceride at 5 min was set as 100%.

Statistical analysis

Results are presented at the mean ± s.e.mean. The effect of drugs was evaluated by two-way repeated analysis of variance (ANOVA) using the Statistical Analysis System (SAS) in all experiments. When a significant change was noted in any experiment, the following analyses were performed. To study the effect of YM-53601 and clofibrate on DiI-LDL and DiI-VLDL clearance, the drug-treatment values were compared with the vehicle-treatment control values using Dunnett's multiple comparison test for each hour. The effect of YM-53601 on DiI-met-LDL clearance and plasma triglyceride in hamsters fed a normal diet was analysed using Student's *t*-test for each hour. Elimination half-life of plasma triglyceride after the injection of intrafat was calculated using the approximated curve and the effect of YM-53601 analysed using Student's *t*-test. $P<0.05$ was considered to be significant.

Ethical considerations

All experiments were performed in accordance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical.

Results

To assess the relationship between plasma lipid lowering effect and clearance rate of LDL and VLDL, the effect of YM-53601 on the clearance of DiI-LDL and DiI-VLDL was investigated in hamsters. DiI-LDL and DiI-VLDL were injected intravenously 30 min or 4 h after the last administration of YM-53601 or clofibrate, respectively. Treatment with YM-53601 at 50 mg kg^{-1} for 5 days in hamsters fed a normal diet enhanced the disappearance of DiI-LDL from hamster plasma (Figure 1a). Indeed, YM-53601 enhanced DiI-LDL clearance by 2.2 times and by 60% at 3 and 6 h after injections of DiI-LDL, respectively. In contrast, clofibrate at 150 mg kg^{-1} for 7 days had little effect on the clearance rate of DiI-LDL in hamsters. As shown in Figure 1b, YM-53601 at the above dose for 5 days in hamsters fed a normal diet increased the disappearance of DiI-VLDL. Clofibrate also increased clearance, although not as profoundly as YM-53601. Table 1 shows plasma total cholesterol and triglyceride changes during the DiI-VLDL disappearance experiment. YM-53601 given the day before DiI-VLDL injection in hamsters fed a normal diet decreased plasma total cholesterol and triglyceride by 35 and 64%, respectively, compared to control group animals. In contrast, clofibrate tended to reduce them by 12 and 15%, respectively.

The clearance rate of LDL *via* the LDL receptor pathway can be estimated as the differences between the clearance of labelled native LDL and that of met-LDL, in which the receptor recognition site in apoB has been blocked by chemical modification (Weisgraber *et al*., 1978). DiI-met-LDL was injected into control and YM-53601-treated hamsters 30 min after the last administration of YM-53601.

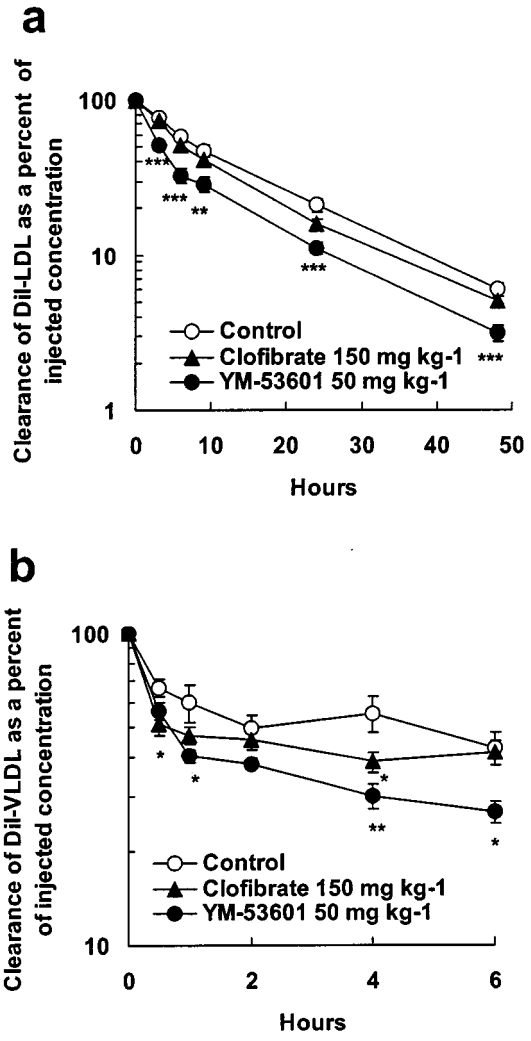

Figure 1 Plasma clearance of labelled hamster LDL and VLDL in hamsters treated with clofibrate (150 mg kg^{-1} for 7 days) and YM-53601 (50 mg kg^{-1} for 5 days). The fluorescent intensities of DiI-LDL (a) and DiI-VLDL (b) were evaluated in plasma of hamsters. See Methods section for details. Statistical analaysis versus control was carried out using Dunnett's multiple comparison test. $*P<0.05$, $**P<0.01$, $***P<0.001$ versus control. Each value represents the mean ± s.e.mean of data obtained in six animals.

The concentration of DiI-met-LDL in the plasma of control hamsters fed a normal diet decreased more slowly than that of DiI-LDL. YM-53601 at 50 mg kg^{-1} for 5 days increased the clearance of DiI-met-LDL from the plasma of hamsters fed a normal diet at 24 and 48 h after injection of DiI-met-LDL, although this effect was not seen until 9 h after YM-53601 administration (Figure 2).

With regard to the enhancement of VLDL clearance rate by YM-53601 in Figure 1b, the participation of lipoprotein lipase (LPL) in this increase was confirmed using hamsters treated with protamine sulphate, which inhibits the activity of LPL. The enhanced clearance of DiI-VLDL by YM-53601 in Figure 1b disappeared in hamsters injected with protamine sulphate at 26 mg kg^{-1} 5 min before the last administration of YM-53601 (Figure 3).

To evaluate the clearance of plasma triglyceride from plasma, intrafat, a fat emulsion, was administered to

Table 1 Plasma total cholesterol and triglyceride the day before DiI-VLDL injection in hamsters

Treatment	*Dose* (mg kg^{-1})	*Total cholesterol* (mg dl^{-1})	*Triglyceride* (mg dl^{-1})
Control		188±13	337±37
YM-53601	50	122±6*** (−35%)	121±10*** (−64%)
Clofibrate	150	167±8 (−12%)	286±27 (−15%)

Data are represented as mean±s.e.mean ($n=6$). Each compound was administered once daily. Blood specimens were obtained from animals shown in Figure 1b on the day before DiI-VLDL injection, with the duration of dosage of YM-53601 and clofibate being 4 and 6 days, respectively. Statistical analysis versus control was carried out using Dunnett's multiple comparison test. ***$P<0.001$ versus control. Per cent changes from respective control values are given in parentheses.

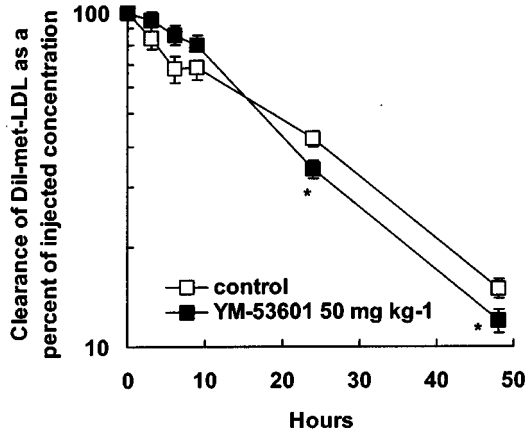

Figure 2 Plasma clearance of labelled methylated hamster LDL in hamsters treated with YM-53601 (50 mg kg^{-1} for 5 days). The fluorescent intensities of DiI-met-LDL were evaluated in plasma of hamsters. See Methods section for details. Statistical analysis versus control at each time point was carried out using Student's t-test. *$P<0.05$ versus control. Each value represents the mean±s.e.mean of data obtained in five or six animals.

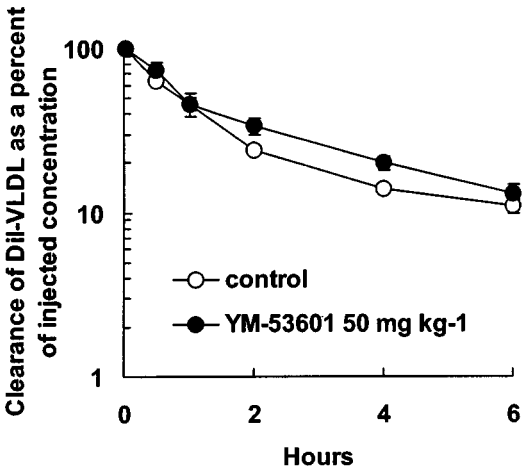

Figure 3 Effect of protamine sulphate on plasma clearance of labelled hamster VLDL in hamsters treated with YM-53601 (50 mg kg^{-1} for 5 days). The fluorescent intensities of DiI-VLDL were evaluated in plasma of hamsters. See Methods section for details. There was no significant difference between control and YM-53601 groups using two-way repeated ANOVA test. Each value represents the mean±s.e.mean of data obtained in four to six animals.

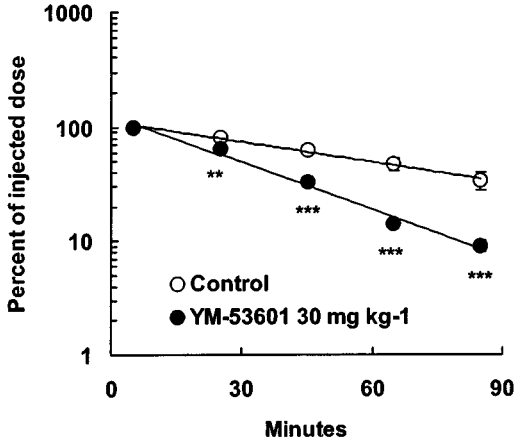

Figure 4 Plasma triglyceride clearance from plasma after injection of intrafat in hamsters treated with YM-53601. Plasma triglyceride level was evaluated in hamsters. See Methods section for details. Statistical analysis versus control at each time point was carried out using Student's t-test. **$P<0.01$, ***$P<0.001$ versus control. $t_{1/2}$ value was calculated using approximated curve. Each value represents the mean±s.e.mean of data obtained in seven animals.

hamsters. A single injection of intrafat at 1.5 ml kg^{-1} increased plasma triglyceride levels by 2.8 times in hamsters fed a normal diet (plasma triglyceride level pre-injection, 301±50 mg dl^{-1}; 5 min after injection, 833±48 mg dl^{-1}). In Figure 4, the high level after injection of intrafat decreased time-dependently in control hamsters. When YM-53601 was given by single oral administration at 30 mg kg^{-1} 30 min before injection of intrafat, plasma triglyceride disappearance was increased at each time point compared to control hamsters, and $t_{1/2}$ values (elimination half-life of plasma triglyceride) were shorter for treated hamsters (30±1 min) than those given vehicle solution (64±9 min).

To confirm whether the enhancement of plasma triglyceride clearance by YM-53601 in Figure 4 was connected with the plasma triglyceride changes, plasma triglyceride level in hamsters was measured after single oral administration of YM-53601. Concentration of plasma triglyceride was significantly decreased as soon as 1 h after administration at 50 mg kg^{-1} in hamsters fed a normal diet, by 67% compared to control hamsters (Figure 5). This decrease was maintained until 4 h after administration of YM-53601.

Discussion

This study set out to evaluate the mechanism by which the squalene synthase inhibitor YM-53601 decreases plasma cholesterol and triglyceride. It is well known that plasma cholesterol and triglyceride levels are maintained by a balance between lipids biosynthesis in the liver, uptake into the liver, secretion and excretion from the liver, absorption from the

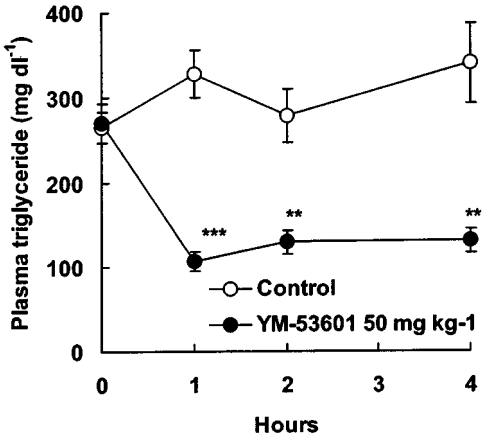

Figure 5 Time course of triglyceride-lowering effect on single administration of YM-53601 in hamsters. Plasma triglyceride level was evaluated in hamsters. See Methods section for details. Statistical analysis versus control at each time point was carried out using Student's *t*-test. $^{**}P<0.01$, $^{***}P<0.001$ versus control. Each value represents the mean ± s.e.mean of data obtained in six animals.

small intestine and catabolism in the blood. In this report, we focused on the effect of YM-53601 on the latter factors in hamsters.

Previous results showed that fenofibrate and YM-53601 act in different ways in their effect on plasma lipid changes in hamsters fed a high fat diet: for YM-53601, a positive correlation was seen in the decrease in plasma cholesterol and triglyceride, whereas somewhat surprisingly, a negative correlation was seen with fenofibrate (Ugawa *et al*., 2000). In other experiments in hamsters, moreover, YM-53601 showed a different character to clofibrate, an activator of peroxisome proliferator-activated receptor (PPAR)-alpha that increases the activity of lipoprotein lipase (LPL). These results suggested that YM-53601 might enhance VLDL clearance by a pathway other than the LPL pathway.

In the present study, YM-53601 given the day before DiI-VLDL injection in hamsters fed a normal diet decreased plasma total cholesterol and triglyceride. Further, 5 days' treatment with YM-53601 at 50 mg kg^{-1} per day decreased levels in hamsters fed a normal diet by 57 and 81%, respectively (Ugawa *et al*., 2000). Taken together, these results show that the enhancement of plasma VLDL and LDL clearance was consistent with the decrease in plasma total cholesterol and triglyceride in hamsters. They also show the existence of a relationship between the degree of enhancement of VLDL and LDL clearance and that of decreases in plasma lipids for both YM-53601 and clofibrate, with the effect of YM-53601 being stronger than of clofibrate for both.

The enhancement of LDL clearance by YM-53601 might have resulted from the up-regulation of LDL receptor. Zaragozic acid A, one of the squalene synthase inhibitors, increased LDL receptor expression in a similar way to HMG-CoA reductase inhibitor (Ness *et al*., 1994; 1996), a characteristics which may be common to inhibitors of cholesterol biosynthesis through sterol-regulatory element-binding protein (SREBP) 2 (Brown & Goldstein, 1997). VLDL are rapidly remodelled by LPL and metabolized to IDL, which are finally converted to LDL. The enhancement of VLDL clearance may arise from the active incorporation of IDL and LDL metabolized from VLDL into the liver by the enhanced LDL receptor. In addition, as IDL are known to bind remnant receptors, such as LDL receptor-related protein (LRP), which binds to apolipoprotein E (Chappell & Medh, 1998), the remnant receptor might take up IDL efficiently in hamsters treated with YM-53601. In WHHL rabbits treated with NK-104, an HMG-CoA reductase inhibitor, LRP mRNA expression levels were increased by 49.5%, although this effect was not significant (Suzuki *et al*., 2000).

On the other hand, as with VLDL, chylomicrons are also remodelled by LPL and then converted to the chylomicron remnant. Previous studies have generally been interpreted to show that, with regard to plasma triglyceride at least, metabolism of intrafat proceeds as for chylomicrons (Carlson & Hallberg, 1963; Hultin *et al*., 1995). Similar to its effect on VLDL particle, YM-53601 enhanced the clearance rate of triglyceride in intrafat from the blood circulation. It seems that chylomicron remnant converted from chylomicron are positively taken into the liver through the remnant receptor. However, YM-53601's effect was seen even at 30 min after a single administration of YM-53601 in hamsters injected intrafat. These findings indicate that the effect of YM-53601 on intrafat clearance is direct, and not due to any change in the expression of remnant receptor. These enhancements were reflected by the plasma triglyceride changes induced by YM-53601. Plasma triglyceride was decreased as soon as 1 h after single administration, with maximum effect in fact seen at 1 h. The maximum concentration of YM-53601 was detected at 1 h after single administration in hamsters fed a normal diet (data not shown). YM-53601's effect on plasma triglyceride changes paralleled this pharmacokinetic finding. This relationship suggests that YM-53601 acts directly on the degradation pathway of triglyceride, in addition to the possibility that YM-53601 increases the expression of LDL and remnant receptors.

Protamine sulphate inhibits plasma LPL activity, which leads to an increase in plasma triglyceride (Tsutsumi *et al*., 1993). Pre-treatment with protamine sulphate failed to increase the clearance rate of VLDL by YM-53601 in hamsters. This result suggests that YM-53601 might enhance the clearance of VLDL through the LPL degradation pathway. However, YM-53601 did not stimulate LPL activity in the post-heparin plasma of hamsters (data not shown). Therefore, although the enhancement of VLDL clearance rate by YM-53601 is through the LPL pathway, YM-53601 does not act on the enzyme directly. The target molecule of YM-53601 might be VLDL particle; a substrate of LPL, and YM-53601 might strengthen the affinity of LPL binding to VLDL particles. Probucol, an anti-oxidant agent, is well known to act on the LDL particle and to strengthen the affinity of LDL binding to LDL receptor followed by the enhancement of uptake into the liver (Naruszewicz *et al*., 1984). Therefore, it may be possible that YM-53601 acts directly on the lipoprotein. In contrast, VLDL receptor, which plays a role in VLDL-triglyceride metabolism, might participate in YM-53601's enhancement of VLDL clearance rate. If this receptor is stimulated, VLDL clearance may be increased directly without the LPL degradation pathway.

Comparing the clearance rate of DiI-LDL and DiI-met-LDL, the latter was obviously cleared more slowly from

plasma than the former, owing to met-LDL's well-known lack of an uptake pathway from blood into liver *via* LDL receptors. The results of Figures 1a and 2 show that the effect of YM-53601 on LDL clearance was lost on methylation of LDL for 9 h after injection of DiI-LDL or DiI-met-LDL, whereas at 24 h no inhibition by methylation was detected. We therefore speculate that the LDL receptor pathway is dominant over the non-LDL receptor pathway in the enhancement of LDL clearance by YM-53601 in the first 9.5 h after YM-53601 administration, and that this is followed by the accumulation of cholesterol in the liver and then down-regulation of LDL receptor through SREBP 2. From 24 h, YM-53601 enhanced the non-LDL receptor pathway, which participates in LDL uptake into the liver, in addition to the LDL receptor pathway. YM-53601 may therefore enhance not only the LDL receptor but also the non-LDL receptor pathway. On continuous administration, such as once daily, however, participation of the non-LDL receptor pathway may be lost owing to domination by the LDL receptor pathway during the 24 h period after each administration. ER-28448, a squalene synthase inhibitor, reduced plasma cholesterol after single daily treatment for 4 days in WHHL rabbit heterozygotes but failed to do so in homozygotes, meaning that the non-LDL receptor pathway did not participate in the plasma cholesterol reduction (Hiyoshi *et al*., 2001). In hamsters, the liver is the major site for the removal of plasma LDL, accounting for 73% of the degradation that takes place, and only 6.3% of LDL uptake in this organ is due to the non-LDL receptor pathway (Spady *et al*., 1983). These results indicate that the decrease in plasma cholesterol by YM-53601 may derive largely from up-regulation of the LDL receptor pathway, with the outcome that the non-LDL receptor pathway is not detectable, even though YM-53601 characteristically acts on non-LDL receptor 24 h after administration. On the other hand, as ER-28448 reduced plasma triglyceride not only in WHHL rabbit heterozygotes but also in homozygotes (Hiyoshi *et al*., 2001), it is probable that squalene synthase inhibitor influences the function of the non-LDL receptor pathway.

These results indicate that the decrease in plasma total cholesterol and triglyceride after treatment with YM-53601 in hamsters fed a normal diet is due to enhancement of the clearance rate of LDL and VLDL, respectively. YM-53601 seems to stimulate not only the LDL receptor pathway but also the non-LDL receptor pathway. The increase in VLDL clearance by YM-53601 was completely blocked by protamine sulphate, which indicates that the LPL degradation pathway might contribute to the increase, although such action would not seem to be a direct effect on LPL. These effects resulted in an acute decrease in plasma triglyceride even 1 h after single administration of YM-53601 in hamsters. YM-53601 may therefore provide an effective and rapid decrease in plasma triglyceride level in the treatment of hypertriglyceridaemia in humans.

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