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YM-53601, a novel squalene synthase inhibitor, reduces plasma cholesterol and triglyceride levels in several animal species

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1 The aim of this study was to evaluate the potency of YM-53601 ((*E*)-2-[2-fluoro-2-(quinuclidin-3-ylidene) ethoxy]-9*H*-carbazole monohydrochloride), a new inhibitor of squalene synthase, in reducing both plasma cholesterol and triglyceride levels, compared with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor and fibrates, respectively.

2 YM-53601 equally inhibited squalene synthase activities in hepatic microsomes prepared from several animal species and also suppressed cholesterol biosynthesis in rats (ED_{50}, 32 mg kg^{-1}).

3 In guinea-pigs, YM-53601 and pravastatin reduced plasma nonHDL-C (=total cholesterol−high density lipoprotein cholesterol) by 47% ($P<0.001$) and 33% ($P<0.001$), respectively (100 mg kg^{-1}, daily for 14 days). In rhesus monkeys, YM-53601 decreased plasma nonHDL-C by 37% (50 mg kg^{-1}, twice daily for 21 days, $P<0.01$), whereas the HMG-CoA reductase inhibitor, pravastatin, failed to do (25 mg kg^{-1}, twice daily for 28 days).

4 YM-53601 caused plasma triglyceride reduction in hamsters fed a normal diet (81% decrease at 50 mg kg^{-1}, daily for 5 days, $P<0.001$). In hamsters fed a high-fat diet, the ability of YM-53601 to lower triglyceride (by 73%, $P<0.001$) was superior to that of fenofibrate (by 53%, $P<0.001$), the most potent fibrate (dosage of each drug: 100 mg kg^{-1}, daily for 7 days).

5 This is the first report that a squalene synthase inhibitor is superior to an HMG-CoA reductase inhibitor in lowering plasma nonHDL-C level in rhesus monkeys and is superior to a fibrate in significantly lowering plasma triglyceride level. YM-53601 may therefore prove useful in treating hypercholesterolemia and hypertriglyceridemia in humans.

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Keywords: YM-53601; squalene synthase inhibitor; hypocholesterolemic effect; hypotriglyceridemic effect; fenofibrate; HMG-CoA reductase inhibitor; rhesus monkeys

Abbreviations: FPP, farnesyl pyrophosphate; HDL-C, high density lipoprotein cholesterol; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; nonHDL-C, non HDL cholesterol; TC, total cholesterol

Introduction

Squalene synthase (farnesyl-diphosphate:farnesyl-diphosphate farnesyl-transferase, EC 2.5.1.21) is an enzyme vital for cholesterol biosynthesis. It catalyzes the dimerization of two farnesyl pyrophosphate molecules to form squalene, a key cholesterol precursor (Popjak & Agnew, 1979). Unlike HMG-CoA reductase inhibitors squalene synthase inhibitors do not lower the levels of ubiquinone and dolicol *in vivo* (Ciosek *et al*., 1993; Keller, 1996; McTaggart *et al*., 1996; Thelin *et al*., 1994), both essential for cell growth and viability (Folkers *et al*., 1990; Ghirlanda *et al*., 1993; Willis *et al*., 1990). Since they lower not only plasma cholesterol but also plasma triglyceride levels in rodents and marmosets (Amin *et al*., 1996; 1997; Bergstrom *et al*., 1995; Ciosek *et al*., 1993), squalene synthase inhibitors might prove better cholesterol lowering agents than the currently used HMG-CoA reductase inhibitors. Recently, YM-53601, a novel squalene synthase inhibitor was discovered in a search of compounds able to reduce plasma cholesterol. This is the first report demonstrating a greater cholesterol lowering effect of YM-53601 compared with that of a HMG-CoA reductase inhibitor in rhesus monkeys, whose lipid metabolism closely resembles that of humans (Nicolosi & Zannis, 1984; Zannis *et al*., 1985). Although squalene synthase inhibitors reduce plasma triglyceride levels in animals, their efficacies have not been directly compared with those of fibrates, the plasma triglyceride lowering agents in clinical use. Therefore, this study also directly compares the plasma triglyceride lowering efficacy of YM-53601 with that of fenofibrate, the most potent fibrate in clinical use.

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Methods

Materials

YM-53601 and Zeneca's squalene synthase inhibitor (3-[4′-fluoro-4-biphenylyl]-3-quinuclidinol) (FBQ) which was used as reference compound were synthesized at the Chemistry Laboratories, Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan). Figure 1 shows the chemical structures of these compounds. The HMG-CoA reductase inhibitor, pravastatin, and fenofibrate were purchased from Sankyo Co., Ltd.

(Tokyo, Japan) and Sigma (MO, U.S.A.), respectively. Farnesyl pyrophosphate (FPP), [^{3}H]-FPP (15 Ci mmol^{-1}) and [^{14}C]-acetate (55 mCi mmol^{-1}) were obtained from American Radiolabelled Chemicals Inc, (MO, U.S.A.). Aquasol-2, scintillation fluid, was purchased from Packard (Groningen, Netherlands). NB-598 (Horie *et al*., 1990), a squalene epoxidase inhibitor, was synthesized at the Chemistry Laboratories, Yamanouchi Pharmaceutical Co., Ltd.

Preparation of microsomes from the livers of several species and HepG2 cells

Microsomes were prepared from the livers of rats, hamsters, guinea-pigs, beagle dogs and a rhesus monkey as well as from HepG2 cells, a human hepatoma cell line previously described (Lindsey & Harwood, 1995). The tissues or harvested cells were homogenized in 50 mM HEPES buffer using a glass homogenizer. Homogenates were centrifuged at $500 \times g$ for 5 min at 4°C and the resulting supernatants were further centrifuged at $8000 \times g$ for 15 min at 4°C. Microsomes were then isolated from this second supernatant by ultra-centrifugation at $100{,}000 \times g$ for 60 min at 4°C. The microsome precipitates were suspended in HEPES buffer (1–5 mg ml^{-1}) and stored in aliquots at −80°C for up to 2 months. Protein was assayed by the method of Lowry *et al*. (1951).

Squalene synthase activities

Squalene synthase activities of these microsomes were assayed using the technique of Amin *et al*. (1992) with modifications. Briefly, the test compounds were dissolved in DMSO and the assay carried out in 50 mM HEPES buffer, pH 7.5, containing (in mM): NaF 11, $MgCl_2$ 5.5, DTT 3, NADPH 1, FPP 5 μM, [^{3}H]-FPP (0.017 μM, 15 Ci mmol^{-1}), NB-598 10 μM and sodium pyrophosphate decahydrate 1 mM. After pre-incubation of these components at 30°C for 5 min, the reaction was started by the addition of microsomes (10 μg protein). The reaction was carried out at 30°C for 20 min then terminated by the addition of 100 μl of 1 : 1 solution of 40% (w v^{-1}) KOH:ethanol. Synthesized [^{3}H]-squalene was extracted in petroleum ether and counted in Aquasol-2 using a Beckman liquid scintillation counter.

Cholesterol biosynthesis in rats

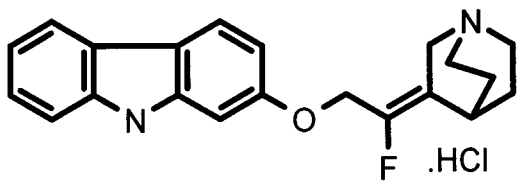
De novo cholesterol biosynthesis was assayed as previously described (Tsujita *et al*., 1986). Sprague-Dawley (SD) rats weighing 150–170 g were fed standard rodent diet (CE-2 from CLEA Japan Inc., Tokyo, Japan). To increase hepatic cholesterol biosynthesis in the daytime, rats were housed with the lights off from 07:30 h to 20:30 h for a week (Higgins *et al*., 1971). YM-53601 and FBQ were suspended in 0.5% methylcellulose. Rats were given a single oral administration of YM-53601 or FBQ at 13:00 h (either 6.25, 12.5, 25 or 50 mg kg^{-1}, followed by an intraperitoneal injection of [^{14}C]-acetate (40.5 μCi per animal) 1 h later. The rats were anaesthetized with diethyl ether and sacrificed 2 h after the drug treatment. One milliliter of the plasma was saponified in 15% (w v^{-1}) KOH in EtOH at 75°C for 2 h. Samples were extracted with petroleum ether in alkaline conditions and the amount of [^{14}C]-cholesterol was measured by scintillation counting following separation by thin layer chromatography in petroleum ether:diethylether:acetate (80:20:1).

Plasma lipids lowering effect on various animal species

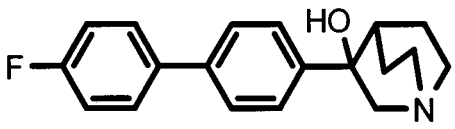
Rat study Five-week-old male SD rats (from SLC, Shizuoka, Japan) were fed a high-fat diet consisting of CE-2 feed supplemented with 1.5% cholesterol, 0.5% cholate and 10% coconut oil for 7 days. The normal dietary group were fed CE-2 without the high-fat supplements during the experiment. Rats received YM-53601 or pravastatin orally once daily at doses of 12.5, 25 and 50 mg per kg of body weights, and 50 mg per kg of body weight, respectively. These compounds were suspended in a 0.5% methylcellulose vehicle solution. Normal dietary and no-treatment-high-fat diet control groups were administered equal volumes of the 0.5% methylcellulose vehicle solution.

Guinea-pig study Four-week-old male Hartley guinea-pigs (from Charles River, Kanagawa, Japan) were fed GC-4 diet (CLEA Japan Inc., Tokyo, Japan), and water *ad libitum*. Guinea-pigs were orally given YM-53601 at doses of 10, 30 and 100 mg per kg of body weight, or pravastatin 100 mg per kg of body weight once a day for 14 days. YM-53601 and pravastatin were suspended in a 0.5% methylcellulose vehicle solution. The no-treatment control group was given an equal volume of the 0.5% methylcellulose vehicle solution.

Hamster study Male Syrian golden hamsters were purchased from Hamri (Ibaraki, Japan). At the start of the study, the 8-week-old animals weighed approximately 140 g. They were kept for a week under reverse diurnal light cycles with the lights off from 07:30 h to 20:30 h. The animals were fed either a standard low cholesterol diet (CE-2) or a high-fat diet (CE-2 supplemented with 0.5% cholesterol and 5% coconut oil). Water was provided *ad libitum*. Animals fed the normal diet were given a 12.5, 25 or 50 mg oral dose of YM-53601 per kg of body weight once a day for 5 days. YM-53601 was suspended in a 0.5% methylcellulose vehicle solution. The no-treatment control group was given an equal volume of the 0.5% methylcellulose vehicle solution. Animals fed the high-fat diet were placed on this diet for 11 days. Oral administration of YM-53601 or fenofibrate, at doses of 10, 30 or 100 mg per kg of body weight once a day, started on day 5 and continued throughout the study.



YM-53601



3-[4'-fluoro-4-biphenylyl]-3-quinuclidinol (FBQ)

Figure 1 Chemical structures of YM-53601 and FBQ.

Rhesus monkey study Adult male rhesus monkeys weighing approximately 4 kg (from Hamri., Ibaraki, Japan) were individually caged and fed a commercial diet (Primate Chow No. 5048, from Oriental Yeast Co., Ltd., Tokyo, Japan). Each monkey received approximately 100 g of banana each day as a dietary supplement. In the first experiment, YM-53601 at doses of 12.5, 25 or 50 mg per kg of body weight was given twice a day (09:00 h and 17:00 h) by diet admixture. Monkeys were allotted to one of four groups depending on their plasma lipid level measured over a 3-week period. YM-53601 was administered for a further 3 weeks. Blood specimens were obtained at 09:00 h after a 16-h fast before (at −2, −1 and 0 weeks), during (1, 2 and 3 weeks) and after the cessation of drug treatment (1 and 2 weeks after, indicated in Figures 4 and 5 as R1 and R2, respectively). In the second experiment, YM-53601 at doses of 25 or 50 mg per kg of body weight, or pravastatin at a dose of 25 mg per kg of body weight was administered to rhesus monkeys twice a day by diet admixture. In this experiment, the stability of plasma lipid levels was confirmed for 2 weeks and drug treatment continued for 4 weeks. Blood specimens were obtained at 09:00 h after a 16-h fast before (at −1 and 0 weeks), during (1, 2, 3 and 4 weeks) and after the cessation of drug treatment (at 1 and 2 weeks after, indicated in Figures 4 and 5 as R1 and R2, respectively). In both experiments, the no treatment control group was fed the same diet without YM-53601 or pravastatin for the same period.

Blood collection and analysis

In all experiments except those with the rhesus monkeys, blood specimens were obtained 2 h after the last compound dose from animals which had fasted 18 h. In rhesus monkeys, blood was obtained from femoral 16 h after the last compound dose as mentioned above. All plasma samples were analysed for total cholesterol, HDL cholesterol, triglyceride and other clinical parameters such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using a Hitachi 7250 Automatic analyzer (Tokyo, Japan).

Statistical analysis

Results are presented as mean ± s.e.mean. Figures in parentheses show the per cent decrease compared to the vehicle-treated mean control value. For study of the effects of drugs on plasma lipids, the drug-treatment values were compared with the vehicle-treatment control values using Dunnett's multiple comparison test. The effects of high-fat diet and the comparisons between YM-53601 and the reference compound in each experiment were analysed using Student's *t*-test. IC_{50} and ED_{50} values were calculated using linear regression analysis by probit method in RS/1 computer program (DOMAIN solutions corporation, U.S.A.). The statistical difference in ED_{50} between compounds was estimated by parallel line assay in Statistical Analysis System (SAS). In rhesus monkey experiments, the effects of YM-53601 and pravastatin were compared using two-way repeated analysis of variance (ANOVA). $P<0.05$ 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

YM-53601 inhibits in vitro *squalene synthase activities and* in vivo *cholesterol biosynthesis*

YM-53601 inhibited squalene synthase activity in a dose-dependent manner in microsomes from liver tissue and the human hepatoma cell line HepG2 (Table 1). The degree of inhibition was equal to or greater than that activated with FBQ.

In the second experiment, rats were given a single p.o. Treatment with YM-53601 or FBQ at doses of 6.25, 12.5, 25 and 50 mg kg^{-1} followed by i.p. injection of [^{14}C]-acetate 1 h later. YM-53601 inhibited cholesterol biosynthesis from acetate in a dose-dependent manner in rats (Figure 2). The ED_{50} value for YM-53601 and FBQ cholesterol biosynthesis inhibition is 32 and 18 mg kg^{-1}, respectively.

YM-53601 lowers plasma cholesterol in rats fed a high-fat diet

In order to examine whether the inhibition of cholesterol biosynthesis elicited by YM-53601 causes changes in plasma lipid levels, YM-53601 was administered at doses of 12.5, 25 or

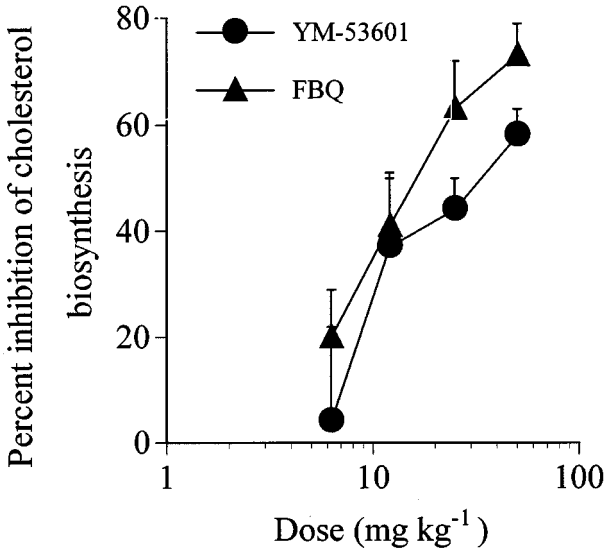


Figure 2 *In vivo* inhibition of *de novo* cholesterol biosynthesis from acetate by YM-53601 in rats. Results are represented as mean ± s.e.mean ($n=4$) of per cent inhibition versus the untreated control group. The ED_{50} values for YM-53601 and FBQ of 32 mg kg^{-1} and 18 mg kg^{-1} respectively, were calculated by RS/1 program. The difference between these compounds was statistically significant by parallel line assay in the Statistical Analysis System (SAS).

Table 1 Inhibition of squalene synthase activities by YM-53601 in hepatic microsomes from several species of animals and human-derived HepG2 cells

	Inhibition of squalene synthase activities IC_{50} (nM)				
	Rat	*Hamster*	*Guinea-pig*	*Rhesus monkey*	*HepG2 cell*
YM-53601	90	170	46	45	79
FBQ	170	380	110	67	84

Data are represented as average IC_{50} values ($n=2$). IC_{50} values were calculated using the RS/1 program.

50 mg kg^{-1}, or pravastatin at a dose of 50 mg kg^{-1}, for 1 week to rats fed a high-fat diet. Table 2 shows that high-fat diet caused increases in plasma nonHDL-C and triglyceride levels of 23 and 2 times, respectively. However, there was almost no change in plasma HDL-C level due to diet. The changes of plasma nonHDL-C and triglyceride by administration of 50 mg kg^{-1} YM-53601 compared to control values were −44% and −33%, respectively. In contrast, pravastatin treatment had little effect on plasma lipid levels.

YM-53601 lowers plasma cholesterol in guinea-pigs fed a normal diet

To confirm the plasma cholesterol lowering efficacy of YM-53601, its effect was compared to that of pravastatin, a widely used hypercholesterolemia treatment. Table 3 shows that guinea-pigs treated for 2 weeks with YM-53601 at doses 10 to 100 mg kg^{-1} had a significant and dose-dependent reduction in total and nonHDL-C concentration (*c.f.* 44% and 47% reduction respectively at highest dose, compared with control group). Animals treated with pravastatin (100 mg kg^{-1}) also exhibited significant reduction of 32 and 33%, respectively, in total and nonHDL cholesterol compared to controls. YM-53601 did not affect plasma HDL-C level, whereas pravastatin significantly decreased this level. However, the changes of plasma triglyceride levels following administration of YM-53601 or pravastatin at doses of 100 mg kg^{-1} were −30% or +17% respectively, compared with control value.

YM-53601 lowers plasma concentrations of cholesterol and triglyceride in hamsters regardless of diet

Hamsters have a plasma lipid composition very similar to humans (Spady & Dietschy, 1985). Therefore, in order to assess the potential clinical efficacy of YM-53601 in lowering plasma cholesterol and triglyceride levels, the compound was orally administered to hamsters fed a normal diet. YM-53601 markedly lowered plasma triglyceride levels at doses of 12.5 to 50 mg kg^{-1} (Table 4). Indeed, YM-53601 at doses of 12.5–50 mg kg^{-1}, lowered plasma concentrations of TC by 39 to 57% and nonHDL-C by 57 to 74%. In contrast, YM-53601 had a less pronounced effect on plasma HDL-C. FBQ lowered both plasma cholesterol and triglyceride, although not as profoundly as YM-53601.

Table 2 Effects of YM-53601 and pravastatin on plasma cholesterol and triglyceride concentrations in rats fed a high-fat diet

Treatment	*Dose* (mg kg^{-1})	*Total cholesterol*	*nonHDL cholesterol* (mg dl^{-1})	*HDL cholesterol* (mg dl^{-1})	*Triglyceride*
Normal diet		52±4	2.4±1	50±4	33±4
Control		102±8###	46±7###	57±2	68±7###
YM-53601	12.5	99±8 (−3%)	40±7 (−13%)	59±2 (+5%)	60±6 (−12%)
	25	100±5 (−2%)	38±4 (−17%)	62±1 (+10%)	60±6 (−12%)
	50	79±2 (−23%)	26±3 (−44%)	53±2 (−6%)	46±4 (−33%)
Pravastatin	50	114±15 (+12%)	53±12 (+17%)	61±3 (+7%)	70±8 (+5%)

Data are represented as mean±s.e.mean ($n=6$ or 7). Each compound was administered once a day. All groups received a high-fat diet except for the normal diet group. By feeding the high-fat diet, total cholesterol, nonHDL cholesterol and triglyceride were increased significantly (using Student's *t*-test, ###$P<0.01$, ###$P<0.001$) compared with the normal diet group. Per cent changes from the respective control values are given in parentheses. There was no significant change of any parameter in YM-53061 and pravastatin groups by using Dunnett's multiple comparison test compared with control.

Table 3 Effects of YM-53601 and pravastatin on plasma cholesterol and triglyceride concentrations in normal guinea-pigs fed a normal diet

Treatment	*Dose* (mg kg^{-1})	*Total cholesterol*	*nonHDL cholesterol* (mg dl^{-1})	*HDL cholesterol* (mg dl^{-1})	*Triglyceride*
Control		54±10	50±4	4.5±0.2	59±6
YM-53601	10	47±7 (−13%)	42±3 (−15%)	3.5±0.2 (+5%)	75±26 (+27%)
	30	43±8* (−21%)	38±2* (−22%)	4.7±0.5 (0%)	43±9 (−26%)
	100	30±8*** (−44%)	26±1*** (−47%)	4.5±0.2 (−11%)	41±7 (−30%)
Pravastatin	100	37±8*** (−32%)	33±3*** (−33%)	4.0±0.2* (−22%)	69±11 (+17%)

Data are represented as mean±s.e.mean ($n=7$ to 10). Each compound was administered once a day. Per cent changes from respective control values are given in parentheses. Statistical analysis was carried out using Dunnett's multiple comparison test. $P<0.05$, **$P<0.001$ versus control. There were no differences of total cholesterol and nonHDL cholesterol between YM-53601 and pravastatin at 100 mg kg^{-1} by using Student's *t*-test.

Table 4 Effects of YM-53601 and FBQ on plasma cholesterol and triglyceride concentrations in hamsters fed a normal diet

Treatment	*Dose* (mg kg^{-1})	*Total cholesterol*	*nonHDL cholesterol* (mg dl^{-1})	*HDL cholesterol* (mg dl^{-1})	*Triglyceride*
Control		191±11	122±13	70±2	87±19
YM-53601	12.5	116±6*** (−39%)	53±6*** (−57%)	63±2 (−9%)	30±7** (−65%)
	25	88±8*** (−54%)	37±5*** (−69%)	51±3*** (−27%)	22±4*** (−75%)
	50	83±2***### (−57%)	32±3***### (−73%)	50±2***# (−28%)	17±2***## (−81%)
FBQ	50	105±3*** (−45%)	47±2*** (−61%)	58±3* (−16%)	35±5** (−59%)

Data are represented as mean±s.e.mean ($n=7$ or 8). Each compound was administered once a day. Per cent changes from respective control values are given in parentheses. Statistical analysis was carried out using Dunnett's multiple comparison test in order to compare with control, or Student's *t*-test to compare between YM-53601 and FBQ at each 50 mg kg^{-1}. *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus control. #$P<0.05$, ##$P<0.01$, ###$P<0.001$ versus FBQ.

In order to assess the efficacy of YM-53601 in lowering plasma triglyceride levels of hamsters fed a high-fat diet, YM-53601 or fenofibrate was administered (Table 5 and Figure 3). YM-53601 at doses of 10–100 mg kg^{-1} reduced plasma triglyceride levels in a dose-dependent manner, showing significantly greater effects than fenofibrate. The effects of YM-53601 were also upon plasma TC and nonHDL-C significantly superior to those of fenofibrate. For YM-53601, a positive correlation ($r^2=0.6393$, $P<0.001$) was seen between the percentage reduction in plasma nonHDL-C and triglyceride; however somewhat surprisingly, a negative correlation ($r^2=0.2976$, $P<0.05$) was seen for fenofibrate treatment (Figure 3).

YM-53601 lowers plasma cholesterol more than pravastatin in rhesus monkeys fed a normal diet

In order to predict the efficacy of YM-53601 in treating human hypercholesterolemia, rhesus monkeys were used to compare the effects of YM-53601 and pravastatin. Figure 4 shows the dose-dependency curves for YM-53601, while Figure 5 shows a comparison with pravastatin. Figure 4b shows that after 3 weeks treatment with YM-53601 at doses of 12.5, 25 and 50 mg kg^{-1} plasma concentrations of nonHDL-C were reduced by 23% ($P<0.05$), 17% (not significant) and 37% ($P<0.01$) respectively compared with untreated animals. The time-course shows nonHDL-C levels decreased by 27, 27 and 37% at the end of 1-, 2- and 3-weeks in rhesus monkeys treated with YM-53601 at 50 mg kg^{-1}. TC levels also fell due to treatment with YM-53601 (Figure 4a) but to a lesser extent compared with nonHDL cholesterol reduction. Plasma HDL-C levels were unaffected (Figure 4c). To compare the efficacies of YM-53601 and pravastatin in lowering plasma cholesterol levels, each compound was given to rhesus monkeys and the changes in cholesterol levels were determined during a 4-week treatment course. The efficacy of YM-53601 (50 mg kg^{-1}) in lowering plasma nonHDL-C was significantly greater than that of pravastatin (25 mg kg^{-1}) ($P<0.001$), the former showing a 21% reduction and the latter 13% reduction after 4 weeks compared with the non-treatment control (Figure 5b). Each experiment showed that YM-53601 did not affect plasma HDL-C (Figures 4c and 5c).

Discussion

This study set out to evaluate two characteristics of the squalene synthase inhibitor YM-53601. The first was to assess the effects of YM-53601 on plasma cholesterol in rodents and rhesus monkeys, which are Old World monkeys whose lipid metabolism is almost the same as humans (Nicolisi & Zannis, 1984; Zannis *et al*., 1985), the second was to compare the efficacies of YM-53601 and fibrates in lowering plasma triglyceride levels.

Previous studies on squalene synthase inhibitors used marmosets (Amin *et al*., 1996; 1997; Bergstrom *et al*., 1995), which are New World monkeys with a lipid metabolism somewhat dissimilar to that of humans. However, HMG-CoA reductase inhibitors, which are in widespread clinical use in treating hypercholesterolemia, were evaluated using Old World monkeys (Corsini *et al*., 1995). Therefore, to assess the actions of YM-53601 accurately and fairly, rhesus monkeys were used in comparison tests with pravastatin, a widely used HMG-CoA reductase inhibitor. YM-53601, at doses of 50 mg kg^{-1} twice a day for 1 week, decreased plasma cholesterol levels in rhesus monkeys. The effect was equal to or greater than pravastatin at doses of 25 mg kg^{-1} twice a day. This dosage of pravastatin is at the upper safety limit since increased plasma alanine aminotransferase, an indicator of

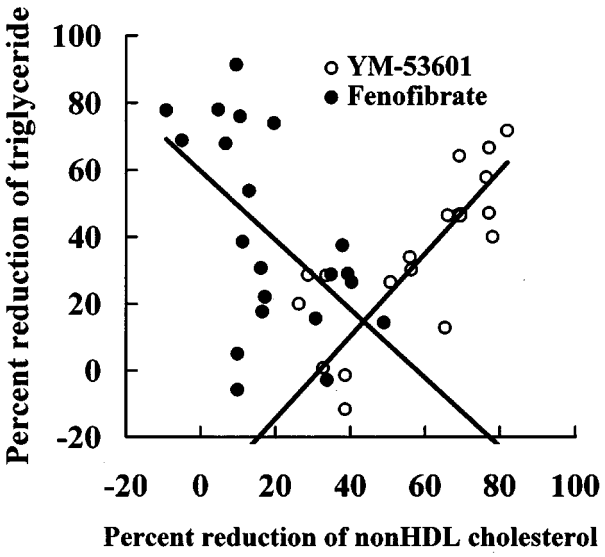


Figure 3 Correlation between the percentage reduction in plasma nonHDL cholesterol and triglyceride in high-fat fed hamsters administered YM-53601 and fenofibrate. YM-53601; $f(x)=1.24x-39.2$, $r^2=0.6393$ ($P<0.001$), fenofibrate; $f(x)=-1.03x+59.7$, $r^2=0.2976$ ($P<0.05$). Data were taken from Table 5.

Table 5 Effects of YM-53601 and fenofibrate on plasma cholesterol and triglyceride concentrations in hamsters fed a high-fat diet

Treatment	*Dose* (mg kg^{-1})	*Total cholesterol*	*nonHDL cholesterol* (mg dl^{-1})	*HDL cholesterol* (mg dl^{-1})	*Triglyceride*
Normal diet		195±6	117±5	78±2	320±46
Control		387±17###	289±15###	99±2###	545±67#
YM-53601	10	295±9*** (−24%)	199±8*** (−31%)	96±2 (−3%)	356±26** (−35%)
	30	207±9*** (−46%)	110±8*** (−62%)	97±1 (−2%)	238±25*** (−56%)
	100	159±6*** (−59%)	70±5*** (−76%)	89±2* (−10%)	145±24*** (−73%)
Fenofibrate	10	372±10 (−4%)	276±9 (−4%)	96±2 (−3%)	463±35 (−15%)
	30	343±8* (−12%)	241±9** (−16%)	101±4 (+3%)	369±41* (−32%)
	100	295±12*** (−24%)	185±10*** (−36%)	110±4* (+12%)	256±27*** (−53%)

Data are represented as mean±s.e.mean ($n=7$). Each compound was administered once a day. All groups received a high-fat diet except for a normal diet group. ED_{50} values of YM-53601 were 49, 22 and 23 mg kg^{-1} for total and nonHDL cholesterol and triglyceride, respectively. Those of fenofibrate were 2221, 297 and 84 mg kg^{-1}, respectively. These differences between compounds were statistically significant by using parallel line assay in SAS. By feeding high-fat diet, total cholesterol, nonHDL cholesterol, HDL cholesterol and triglyceride were increased significantly. Statistical analysis versus control was carried out using Dunnett's multiple comparison test. *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus control. Student's *t*-test, #$P<0.05$, ###$P<0.001$ compared with normal diet group. Per cent changes from respective control values are given in parentheses.

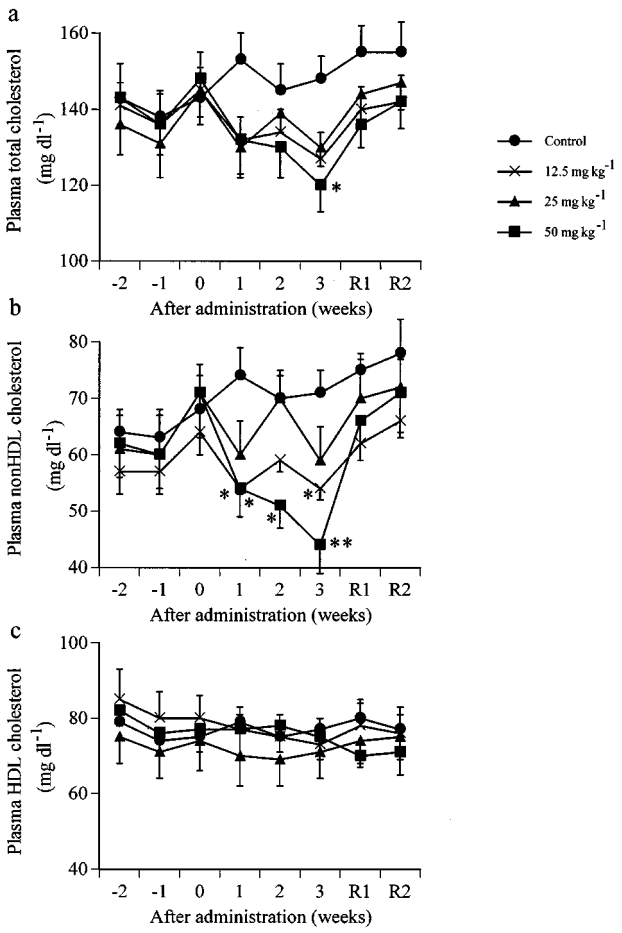


Figure 4 Time course for cholesterol lowering dose-dependent effects by YM-53601 in rhesus monkeys. Results are represented as mean ± s.e.mean ($n=5$). Each compound was administered twice a day. R1 and R2 are the values for 1 and 2 weeks after the cessation of drug treatment, respectively. Statistical analysis versus control was carried out using Dunnett's multiple comparison test. $^{*}P<0.05$, $^{**}P<0.01$ versus control.

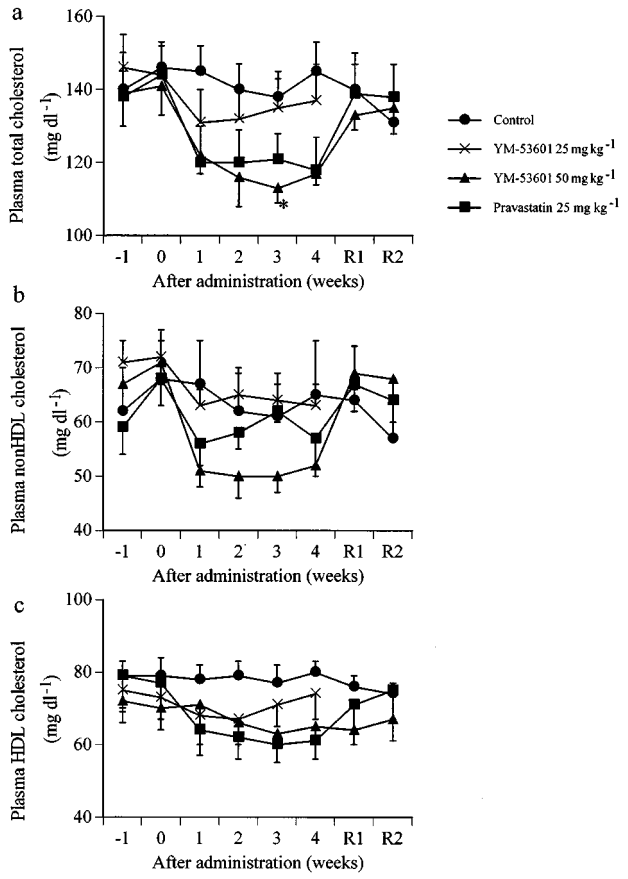


Figure 5 Time course for cholesterol lowering effects by YM-53601 and pravastatin in rhesus monkeys. Results are represented as mean ± s.e.mean ($n=5$). Each compound was administered twice a day. R1 and R2 are the values for 1 and 2 weeks after the cessation of drug treatment, respectively. Statistical analysis was carried out using Dunnett's multiple comparison test versus control group in each week. $^{*}P<0.05$ versus control. Resulted from two-way repeated ANOVA between YM-53601 at dose of 50 mg kg^{-1} and pravastatin at dose of 25 mg kg^{-1} about plasma total, nonHDL and HDL cholesterol levels for 4 weeks treatment, all parameters were statistically significant ($P<0.001$).

liver damage, was observed (data not shown). No indications of liver damage were observed following administration of YM-53601 at doses of 50 mg kg^{-1}. Therefore, YM-53601 has superior safety to pravastatin with similar efficacy.

The effects of YM-53601 were also examined in guinea-pigs with a high nonHDL-C to HDL-C ratio. This is the only rodent model which responds to administration of HMG-CoA reductase inhibitors (Matsunaga *et al*., 1991) and therefore suitable for comparison between YM-53601 and pravastatin. In these animals YM-53601 lowered plasma cholesterol levels much more effectively than pravastatin. A third set of experiments used hamsters, which are commonly used in preclinical studies of antihyperlipidemic drugs because their plasma lipoprotein composition closely resembles that of humans. In these animals YM-53601 drastically reduced plasma cholesterol levels. The results of these three groups of experiments clearly show YM-53601 has a high plasma cholesterol lowering equal to or greater than pravastatin.

The study group of the European Atherosclerosis Society recently issued guidelines that pay more attention to hypertriglyceridemia as a risk factor for the development of coronary heart disease, and proposed a stepwise approach to treat patients with elevated triglyceride levels (Study group, European Atherosclerosis Society, 1988; Pyorala *et al*., 1994). Patients with elevated plasma cholesterol and triglyceride levels can be classified as type IIb or IV by the WHO classification of hyperlipidemia; these types of patients are 41% of the total hyperlipidemic patients (Yamanura *et al*., 1990). It is known that HMG-CoA inhibitors generally have little to no effect on plasma triglyceride level. Although atorvastatin, a widely used inhibitor of this type, is more effective in reducing plasma triglyceride levels in hyperlipidemic patients than existing HMG-CoA reductase inhibitors, it is still less effective than that of fibrates (Ooi *et al*., 1997), the primary hypertriglyceridemia treatment drugs to date. Consequently, treatment with both an HMG-CoA reductase inhibitor and a fibrate is commonly performed. However, this type of therapy is occasionally accompanied by severe adverse effects such as rhabdomyolysis and must be used carefully (Naser *et al*., 1995). It would therefore be desirable if new drugs were available that could reduce both plasma cholesterol and triglyceride levels with less side-effects. YM-53601 was found to lower not only plasma cholesterol but also plasma triglyceride levels during initial screening in rodents, and to have an effect on triglyceride greater than that of fenofibrate, the most potent fibrate, in hamsters (Nagayama *et al*., 1995). However, the current study is the first to report that a squalene synthase inhibitor is superior to a fibrate in lowering plasma triglyceride levels directly. In rhesus monkeys, this triglyceride lowering effect could not be detected clearly because of

unstable plasma triglyceride values, even in control group (data not shown). One possible explanation for this may be admixture administration of drugs, although the amount of food intake was not significantly different among the groups. Since there are few reports which clearly show the effect of fibrates in primates, YM-53601 appears to be as potent an agent as fibrates indicating its ability to treat hypertriglyceridemia as well as hypercholesterolemia.

In order to explain the plasma triglyceride lowering effect by YM-53601, Figure 3 shows the correlation curves between per cent reduction of plasma nonHDL-C and triglyceride in YM-53601- or fenofibrate-treated hamsters fed a high-fat diet. These individual data were taken from Table 3. It is known that fenofibrate has a strong inhibitory effect not on cholesterol biosynthesis but on triglyceride directly (Kloer, 1987). Therefore, the triglyceride content of lipids particles such as very low density lipoprotein (VLDL) would be lower relative to cholesterol. If YM-53601 affects the biosynthesis of both cholesterol and triglyceride, lipid particles would have a much lower total lipid content. On the other hand, fibrates are known to act on peroxisome proliferator-activated receptors (PPARs) and beta-oxidation (Staels *et al*., 1998). Fibrates lower hepatic apoC3 production and increase lipoprotein lipase mediated lipolysis *via* PPAR-alpha, and reduce fatty acid contents in liver by the beta-oxidation pathways. It has not been examined whether YM-53601 acts on these pathways, although Figure 3 shows the different character between fenofibrate and YM-53601. The precise mechanism by which YM-53601 acts remains unknown.

YM-53601 may belong to a novel class of lipid-lowering agents which inhibit squalene synthase activity, leading to reduced cholesterol biosynthesis in animals. Preclinical studies in rats, guinea-pigs, hamsters and rhesus monkeys demonstrated that YM-53601 significantly reduced the plasma concentrations of nonHDL cholesterol to a greater degree than did pravastatin. These results also demonstrate that YM-53601 has a more potent triglyceride lowering effect than fenofibrate. From these results, we conclude that YM-53601 holds promise as a lipid-lowering therapy for the treatment of hypercholesterolemia and hypertriglyceridemia in humans.

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References

AMIN, D., CORNELL, S.A., GUSTAFSON, S.K., NEEDLE, S.J., ULLRICH, J.W., BILDER, G.E. & PERRONE, M.H. (1992). Bisphosphonates used for the treatment of bone disorders inhibit squalene synthase and cholesterol biosynthesis. *J. Lipid Res.,* **33,** 1657–1663.

AMIN, D., RUTLEDGE, R.Z., NEEDLE, S.N., GALCZENSKI, H.F., NEUENSCHWANDER, K., SCOTESE, A.C., MAGUIRE, M.P., BUSH, R.C., HELE, D.J., BILDER, G.E. & PERRONE, M.H. (1997). RPR 107393, a potent squalene synthase inhibitor and orally effective cholesterol-lowering agent: comparison with inhibitors of HMG-CoA reductase. *J. Pharmacol. Exp. Ther.,* **281,** 746–752.

AMIN, D., RUTLEDGE, R.Z., NEEDLE, S.J., HELE, D.J., NEUENSWANDER, K., BUSH, R.C., BILDER, G.E. & PERRONE, M.H. (1996). RPR 101821, a new potent cholesterol-lowering agent: inhibition of squalene synthase and 7-dehydrocholesterol reductase. *Naunyn Schmiedebergs Arch. Pharmacol.,* **353,** 233–240.

BERGSTROM, J.D., DUFRESNE, C., BILLS, G.F., NALLIN-OMSTEAD, M. & BYRNE, K. (1995). Discovery, biosynthesis, and mechanism of action of the zaragozic acids: potent inhibitors of squalene synthase. *Annu. Rev. Microbiol.,* **49,** 607–639.

CIOSEK, JR., C.P., MAGNIN, D.R., HARRITY, T.W., LOGAN, J.V.H., DICKSON, JR., J.K., GORDON, E.M., HAMILTON, K.A., JOLIBOIS, K.G., KUNSELMAN, L.K., LAWRENCE, R.M., MOOKHTIAR, K.A., RICH, L.C., SLUSARCHYK, D.A., SULSKY, R.B. & BILLER, S.A. (1993). Lipophilic 1,1-bisphosphonates are potent squalene synthase inhibitors and orally active cholesterol lowering agents in vivo. *J. Biol. Chem.,* **268,** 24832–24837.

CORSINI, A., MAGGI, F.M. & CATAPANO, A.L. (1995). Pharmacology of competitive inhibitors of HMG-CoA reductase. *Pharmacol. Res.,* **31,** 9–27.

FOLKERS, K., LANGSJOEN, P., WILLIS, R., RICHARDSON, P., XIA, L., YE, C. & TAMAGAWA, H. (1990). Lovastatin decreases coenzyme Q levels in humans. *Proc. Natl. Acad. Sci. U.S.A.,* **87,** 8931–8934.

GHIRLANDA, G., ORADEI, A., MANTO, A., LIPPA, S., UCCIOLI, L., CAPUTO, S., GRECO, A.V. & LITTARRU, G.P. (1993). Evidence of plasma CoQ10-lowering effect by HMG-CoA reductase inhibitors: a double-blind, placebo-controlled study. *J. Clin. Pharmacol.,* **33,** 226–229.

HIGGINS, M., KAWACHI, T. & RUDNEY, H. (1971). The mechanism of the diurnal variation of hepatic HMG-CoA reductase activity in the rat. *Biochem. Biophys. Res. Commun.,* **45,** 138–144.

HORIE, M., TSUCHIYA, Y., HAYASHI, M., IIDA, Y., IWASAWA, Y., NAGATA, Y., SAWASAKI, Y., FUKUZUMI, H., KITANI, K. & KAMEI, T. (1990). NB-598, a potent competitive inhibitor of squalene epoxidase. *J. Biol. Chem.,* **265,** 18075–18078.

KELLER, R.K. (1996). Squalene synthase inhibition alters metabolism of nonsterols in rat liver. *Biochim. Biophys. Acta,* **1303,** 169–179.

KLOER, H.U. (1987). Structure and biochemical effects of fenofibrate. *Am. J. Med.,* **83** (Suppl. 5B): 3–8.

LINDSEY, S. & HARWOOD, JR., H.J. (1995). Inhibition of mammalian squalene synthetase activity by zaragozic acid A is a result of competitive inhibition followed by mechanism-based irreversible inactivation. *J. Biol. Chem.,* **270,** 9083–9096.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193,** 265–275.

MATSUNAGA, A., SASAKI, J., TAKADA, Y., HIDAKA, K. & ARAKAWA, K. (1991). Effect of simvastatin on receptor mediated metabolism of low density lipoprotein in guinea pigs. *Atherosclerosis,* **90,** 31–37.

MCTAGGART, F., BROWN, G.R., DAVIDSON, R.G., FREEMAN, S., HOLDGATE, G.A., MALLION, K.B., MIRRLEES, D.J., SMITH, G.J. & WARD, W.H.J. (1996). Inhibition of squalene synthase of rat liver by novel 3′ substituted quinuclidines. *Biochem. Pharmacol.,* **51,** 1477–1487.

NAGAYAMA, T., TSUCHIYA, A., ARAKAWA, R., SAKAMOTO, Y., KATAYAMA, K., TANAKA, T. & SAITOH, K. (1995). Hypolipidemic action of fenofibrate and bezafibrate in normo- and hyperlipidemic animals. *Jpn. Pharmacol. Ther.,* **23** (suppl): 181–188.

NASER, U.A.M.A., ABDUL-GHAFFAR, M.R.C.P. & MOHAMMED RIAD EL-SONBATY, M.R.C.P. (1995). Pancreatis and rhabdomyolysis associated with lovastatin-gemfibrozil therapy. *J. Clin. Gastroenterol.,* **21,** 340–341.

NICOLOSI, R.J. & ZANNIS, V.I. (1984). Apolipoprotein A-I of primates. *J. Lipid Res.,* **25,** 879–887.

OOI, T.C., HEINONEN, T., ALAUPOVIC, P., DAVIGNON, J., LEITER, L., LUPIEN, P.J., SNIDERMAN, A.D., TAN, M.H., TREMBLAY, G., SORISKY, A., SHURZINSKE, L. & BLACK, D.M. (1997). Efficacy and safety of a new hydroxymethlglutaryl-coenzyme A reductase inhibitor, atorvastatin, in patients with combined hyperlipidemia: comparison with fenofibrate. *Arterioscler. Thromb. Vasc. Biol.,* **17,** 1793–1799.

POPJAK, G. & AGNEW, W.S. (1979). Squalene synthase. *Mol. Cell. Biochem.,* **27,** 97–116.

PYORALA, K., BACKER, G.D.E., GRAHAM, I., POOLE-WILSON, P. & WOOD, D. (1994). Prevention of coronary heart disease in clinical practice: recommendations of the task force of the European Society of Cardiology, European Atherosclerosis Society and European Society of Hypertension. *Eur. Heart J.,* **15,** 1300–1331.

SPADY, D.K. & DIETSCHY, J.M. (1985). Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster. *Proc. Natl. Acad. Sci. U.S.A.,* **82,** 4526–4530.

STAELS, B., DALLONGEVILLE, J., AUWERX, J., SCHOONJANS, K., LEITERSDORF, E. & FRUCHART, J.C. (1998). Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation,* **98,** 2088–2093.

STUDY GROUP, EUROPEAN ATHEROSCLEROSIS SOCIETY. (1998). The recognition and management of hyperlipidaemia in adults: a policy statement of the European Atherosclerosis Society. *Eur. Heart J.,* **9,** 571–600.

THELIN, A., PETERSON, E., HUTSON, J.L., McCARTHY, A.D., ERICSSON, J. & DALLNER, G. (1994). Effect of squalestatin 1 on the biosynthesis of the mevalonate pathway lipids. *Biochim. Biophys. Acta,* **1215,** 245–249.

TSUJITA, Y., KURODA, M., SHIMADA, Y., TANZAWA, K., ARAI, M., KANEKO, I., TANAKA, M., MASUDA, H., TARUMI, C., WATANABE, Y. & FUJII, S. (1986). CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species. *Biochim. Biophys. Acta,* **87,** 50–60.

WILLIS, R.A., FOLKERS, K., TUCKER, J.L., YE, C., XIA, L. & TAMAGAWA, H. (1990). Lovastatin decreases coenzyme Q levels in rats. *Proc. Natl. Acad. Sci. U.S.A.,* **877,** 8928–8930.

YAMAMURA, T., TAJIMA, S., MIYAKE, Y., NOMURA, S., YAMAMOTO, A., HAZE, K. & HIRAMORI, K. (1990). Hyperlipoproteinemia as a risk factor for ischemic heart disease. *Jpn. Circ. J.,* **54,** 448–456.

ZANNIS, V.I., NICOLOSI, R.J., JENSEN, E., BRESLOW, J.L. & HAYES, K.C. (1985). Plasma and hepatic apoE isoproteins of nonhuman primates. Differences in apoE among humans, apes, and New and Old World monkeys. *J. Lipid Res.,* **26,** 1421–1430.

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