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Species specificity in the blood cholesterol-lowering effect of YM-16638

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1 The compound YM-16638, [[5-[[3-(4-acetyl-3-hydroxy-2-propylphenoxy)propyl]thio]-1,3,4-thiadiazol-2-yl]thio] acetic acid was developed in a series of *in vitro* and *in vivo* studies as a leukotriene D_4 receptor antagonist.

2 In a clinical trial as a leukotriene antagonist drug, this compound was found to have a potent serum cholesterol lowering effect in normolipidaemic healthy male volunteers.

3 In the present study, we investigated the serum cholesterol lower effect of this compound in various species of experimental animals.

4 Administration of YM-16638 did not cause a significant decrease in serum total cholesterol (TC) in mice (up to 200 mg kg^{-1}, body weight per day for 28 days), rats (200 mg kg^{-1} for 15 days) or rabbits (90 mg kg^{-1} for 18 days). In hamsters, administration of YM-16638 orally or by peritoneal injection at 50 mg kg^{-1} or more daily for 7 days caused a significant decrease in serum TC and the rate of body weight gain. In monkeys, serum TC did not change in YM-16638-administered squirrel monkeys (50 mg kg^{-1} daily for 3 weeks), but a significant decrease in serum TC was observed in cynomolgus monkeys (33% decrease at 30 mg kg^{-1} for 4 weeks) and rhesus monkeys (27% decrease at 30 mg kg^{-1} for 3 weeks) without any serious decrease in body weight. These results were consistent with those in a phase I study in human subjects. In contrast, serum alanine aminotransferase (ALT) level decreased in all animals after YM-16638 treatment.

5 From these results, we conclude that YM-16638 has a potent hypocholesterolaemic effect, but that this effect if species-specific and is only recognized clearly in human subjects and old-world monkeys.

Keywords: YM-16638; leukotriene antagonist; hypocholesterolaemic effect; alanine aminotransferase; species specificity; human subjects; old-world monkeys

Introduction

The compound YM-16638 was found in preclinical study to be one of a series of orally active leukotriene D_4 receptor antagonists of potential use in the treatment of asthma and related respiratory disorders (Tomioka *et al.*, 1988; 1989). In a clinical phase I study, we unexpectedly found that this compound demonstrated a strong hypocholesterolaemic effect in normolipidaemic human subjects (unpublished data). No serious side effects were seen, but serum alanine aminotransferase (ALT) activity decreased during administration. The purpose of the present study was therefore to confirm the hypocholesterolaemic effect of YM-16638 in various experimental animals in combination with investigation of its species-specificity to help elucidate its cholesterol-lowering mechanism.

Methods

Test compound

The test compound YM-16638 was prepared by Yamanouchi Pharmaceutical Co. (Toyko, Japan). Purity was greater than 98%.

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Study protocol

Mouse study Thirty-two male ICR mice (purchased from SLC, Inc., Shizuoka, Japan) were divided into 4 groups of similar body weight (approximately 33–34 g, 6 weeks of age at the start of the study), and fed a standard low cholesterol diet (CE-2, CLEA Japan, Inc., Tokyo, Japan) for 28 days. Throughout the experimental period, each group received 50, 100 or 200 mg kg^{-1} body weight of YM-16638 in 0.5% methylcellulose solution by oral administration daily.

Rat study Seventeen male Fisher-344 rats (purchased from Charles River Japan Inc., Kanagawa, Japan) were randomly assigned to control and YM-16638 treatment groups. The rats were 6 weeks of age and approximately 200 g at the start of the study. They were housed in stainless steel cages and were fed CE-2 diet containing YM-16638 at a concentration of 0.2% to provide an average daily dose for the 15-day treatment period of approximately 200 mg kg^{-1} body weight. Water was provided *ad libitum*.

Rabbit study Twelve male Japanese white rabbits (purchased from Oriental Bioservice Kanto Inc., Ibaraki, Japan) aged 7 weeks and weighing approximately 1–1.5 kg at the start of the study were divided into 2 groups of similar body weight and fed a normal low cholesterol diet (RC-4, Oriental Yeast Co., Shizuoka, Japan) for 18 days. Throughout the experimental period, each group received 90 mg YM-16638 per kg body weight in 0.5% methylcellulose solution by oral administration daily.

Hamster study Seven-week-old Syrian-golden hamsters (purchased from Hamri Co., Ltd., Ibaraki, Japan) weighing approximately 100 g were used. In the first experiment, groups of 4 male hamsters were fed either a standard low cholesterol CE-2 diet for 28 days as a control group or the same diet containing YM-16638 at a concentration of 0.05 or 0.2% (wt/wt) to provide a daily dosage of approximately 40 and 160 mg kg^{-1} body weight, respectively. In a second experiment, groups of 8 male hamsters were fed a low cholesterol CE-2 diet for 28 days. Throughout the experimental period, groups received YM-16638 by oral gavage of 0.5% methylcellulose solution to provide YM-16638, 50 or 100 mg kg^{-1} body weight per day. Animals injected with an equal volume of 0.5% methylcellulose solution were used as control. In the third experiment, groups of 8 male hamsters were fed CE-2 supplemented with 0.5% (wt/wt) cholesterol and 10% (wt/wt) coconut oil for 7 days. Throughout the experimental period, groups received YM-16638 by oral gavage of 0.5% methylcellulose solution to provide YM-16638 50 or 100 mg kg^{-1} body weight per day. Control group was injected with an equal volume of 0.5% methylcellulose solution.

In the fourth experiment, groups of 8 male hamsters were fed CE-2 supplemented with 0.5% (wt/wt) cholesterol and 10% (wt/wt) coconut oil for 7 days. Throughout the experimental period, groups received YM-16638 by intraperitoneal injection of 0.5% methylcellulose solution to provide YM-16638 5 or 50 mg kg^{-1} body weight per day. A control group was injected with an equal volume of 0.5% methylcellulose solution.

Squirrel monkey study Fourteen male common squirrel monkeys (*Saimiri sciurea*; purchased from Hamri Co., Ltd., Japan) were individually caged. Monkeys weighing approximately 0.8–1.0 kg were randomly assigned to two groups consisting of seven monkeys each. They were fed a commercial diet (Primate Chow No. 5048, PMI Feeds, Inc., St. Louis, Mo, U.S.A.). One group received YM-16638 by oral gavage of 0.5% methylcellulose solution to provide YM-16638 50 mg kg^{-1} body weight daily for 21 days. Animals injected with an equal volume of 0.5% methylcellulose solution were used as control.

Cynomolgus monkey study In the first experiment, ten adult male cynomolgus monkeys (*Macaca irus*; purchased from Hamri Co. Ltd., Japan) weighing approximately 4.5–5.2 kg were individually caged and divided into two groups with similar body weight. They were fed a commercial diet (Primate Chow No. 5048) or the same diet containing YM-16638 at a concentration of 0.25% to provide an average daily dose of approximately 30 mg kg^{-1} body weight for 28 days. In the second experiment, 32 monkeys were used. They were randomly divided into five experimental groups and fed a control diet or experimental diet (containing YM-16638 at 3.75, 7.5, 15 and 30 mg kg^{-1} body weight day^{-1} each) for 28 days.

Rhesus monkey study Six adult male rhesus monkeys (*Macaca mulatta*; purchased from Hamri Co., Ltd., Japan) weighing 4.8–5.6 kg were individually caged and divided into two groups with a similar mean body weight. Animals were fed a commercial diet (Primate Chow No. 5048) or the same diet containing YM-16638 to provide an average daily dose of 45 mg kg^{-1} body weight for 28 days.

Laboratory methods

In all animals studies, blood samples were obtained after 12 h overnight fasting before treatment (prevalue) (in rabbit and monkey studies) and at the end of the experimental period (in all animal experiments). Serum cholesterol, triglyceride, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined by enzymatic colorimetric methods (Allain *et al.*, 1974; Siedel *et al.*, 1981) (Boehringer Mannheim Mono-test cholesterol, TRIGLI-color, GOT-opt and GPT-opt, respectively, using a Hitachi 736–10 automatic analyser). HDL cholesterol in the supernatant after precipitation of LDL and VLDL cholesterol fractions was determined (Burnstein *et al.*, 1970).

Incorporation experiments

Rhesus monkey liver homogenate was prepared from fresh liver of control ($n=3$) and YM-16638-administered (30 mg kg^{-1} day^{-1} for 28 days) monkeys ($n=3$) and frozen at −80°C until assay. DL-[2-^{14}C]-mevalonic acid DBED salt was obtained from DuPont-NEN Corp. All other chemicals were commercially obtained. Assay was carried out according to the method of Endo *et al.* (1976). The reaction mixture had the following composition (mM) (total 1.0 ml): ATP 1, glucose-1-phosphate 10, $MgCl_2$ 6, NAD + 0.25, NADP + 0.25, potassium phosphate buffer 100 (pH 7.4), 2.5 mg protein of liver homogenate and DL-[2-^{14}C]-mevalonic acid DBED-salt 0.6 (22.0 MBq mol^{-1}). After incubation at 37°C for 45 min, the reaction was terminated by the addition of 1 ml of 15% KOH-EtOH solution. The synthesized nonsaponifiable lipids were extracted and radioactivity was measured in a liquid scintillation counter.

Analysis of data

Statistical analyses were carried out by the paired *t* test, ANOVA test, simple correlation test and simple regression test using the RS/1 computer analysis system.

Results

Changes in body weight and serum cholesterol in mice, rats and rabbits

Effect of YM-16638 on body weight gain and serum cholesterol levels is shown in Table 1. In mice and rabbits given YM-

Table 1 Body weight and serum cholesterol levels in mice, rats, rabbits administered YM-16638

Species	*Administration period*	*Daily dose* (mg kg^{-1} day^{-1})	*Body weight gain* (g)	*TC* (mg dl^{-1})	*HDL-C* (mg dl^{-1})	*VLDL-C + LDL-C* (mg dl^{-1})
Mouse	28 days	0	4.4 ± 0.2	121 ± 6	102 ± 5	17 ± 3
		50	4.1 ± 0.6	123 ± 10	99 ± 4	16 ± 3
		100	3.3 ± 1.2	116 ± 9	95 ± 6	20 ± 4
		200	3.3 ± 0.5**	143 ± 7**	119 ± 6	24 ± 4
Rat	15 days	0	49.1 ± 2.2	57.1 ± 0.6	47.9 ± 0.7	9.3 ± 0.5
		200	51.1 ± 1.5	55.2 ± 5.0	47.1 ± 1.6	8.1 ± 0.7
Rabbit	18 days	0	323 ± 24	23.0 ± 1.6	–	–
		90	161 ± 35*	23.0 ± 1.7	–	–

TC: serum total cholesterol, HDL-C: HDL-cholesterol, VLDL-C: VLDL-cholesterol, LDL-C: LDL-cholesterol.
*$P<0.05$; **$P<0.01$ vs. control.

16638, body weight gain was less than that in control groups (mice; 200 mg kg^{-1} day^{-1} for 28 days: 75% of control, rabbits; 90 mg kg^{-1} day^{-1} for 28 days: 50% of control). In rats, in contrast, no difference in body weight was observed between the control and YM-16638 groups at the end of the experiment. With regard to cholesterol, although serum total cholesterol significantly increased (18% increase vs. control) in mice administered YM-16638 at 200 mg kg^{-1}, no effect on total cholesterol or HDL-cholesterol was seen in rats and rabbits given YM-16638. As for serum triglyceride levels, no significant difference between YM-16638 and control groups was seen in any of the three species.

Changes in body weight, liver weight and serum cholesterol in hamsters

We next investigated the effects of YM-16638 on serum cholesterol level in Syrian-golden hamsters, a strain in which lipid metabolism is considered closer to that of man than other experimental rodent species (Spady & Dietschy, 1985). Table 2 shows the results in a series of studies. In animals given a normal diet with or without YM-16638 (Exp.1 and 2) hamsters administered YM-16638 tended to demonstrate a lower body weight gain than the control group. In animals given a high fat diet with or without drug (Exp.3 and 4), a slight decrease in mean body weight was seen in all animals during the 7-day experimental period, with this effect being greater and dose-dependent in YM-16638-treated animals. Relative liver weight (g 100 g^{-1} body weight) was significantly increased in the 100 and 200 mg kg^{-1} administration group in exp.2, but no significant difference was seen in any group in the other experiment. In Exp.1, no significant differences were seen between the control and YM-16638 groups in serum total cholesterol, HDL-cholesterol and VLDL + LDL-cholesterol level. In the other three experiments, however, serum total cholesterol at the end of the test periods was significantly lower in animals receiving YM-16638 at 50 mg kg^{-1} or more, principally due to changes in VLDL + LDL fractions.

Changes in body weight and serum cholesterol in non-human primates (Table 3)

In squirrel monkeys, mean body weight slightly decreased in both the control and YM-16638 groups during the experimental period (baseline, 804 ± 62 and 807 ± 40 g; at 3 weeks, 770 ± 80 and 769 ± 23 g; control and YM-16638 groups, respectively). However, these differences between pre- and 3-week values were not statistically significant. Further, no statistically significant difference in serum cholesterol concentration at 3 weeks compared to the prevalues was noted for either group. In cynomolgus monkeys, although body weight changes were not significant between the two groups (baseline, 4.61 ± 0.15 and 4.82 ± 0.14 kg; at 4 weeks, 4.57 ± 0.24 and 4.75 ± 0.31 kg; control and YM-16638 groups, respectively), serum cholesterol concentration was significantly decreased (33%) in animals administered YM-16638 for 4 weeks but not in the control group. Also in rhesus monkeys, changes in mean body weight during the experimental period in animals administered YM-16638 were not significantly different from values of the control group (baseline, 5.57 ± 0.41 and 5.45 ± 0.53 kg; at 4 weeks, 5.83 ± 0.40 and 4.90 ± 0.31 kg; control and YM-16638 groups, respectively). After 3 weeks of

Table 2 Body weight gain, liver weight and serum cholesterol level in hamsters administered YM-16638

Exp.[a]	*Route, Administration*	*Daily dose* (mg kg^{-1})	*Body weight gain* (g)	*LW* (g (100 g BW)$^{-1}$)	*TC* (mg dl^{-1})	*HDL-C* (mg dl^{-1})	*VLDL-C + LDL-C* (mg dl^{-1})
I	in diet	0	15.5 ± 1.2	3.97 ± 0.14	159 ± 11	65 ± 3	92 ± 8
	28 days	40	23.3 ± 0.3**	4.27 ± 0.07	183 ± 10	65 ± 1	118 ± 11
		160	6.0 ± 3.1*	4.38 ± 0.11	130 ± 8	68 ± 4	61 ± 12
II	p.o.	0	27.8 ± 1.3	3.97 ± 0.07	203 ± 6	76 ± 2	127 ± 6
	28 days	50	15.6 ± 2.3***	4.05 ± 0.08	180 ± 5*	74 ± 2	105 ± 3**
		100	13.4 ± 2.3***	4.28 ± 0.10*	166 ± 8**	70 ± 2	96 ± 6**
		200	4.9 ± 2.8***	4.74 ± 0.17**	177 ± 5*	83 ± 8	94 ± 5**
III	p.o.	0	−1.1 ± 0.8	4.20 ± 0.06	405 ± 41	82 ± 2	323 ± 42
	7 days	50	−3.8 ± 1.1	4.26 ± 0.08	259 ± 17**	80 ± 2	179 ± 16*
		100	−6.7 ± 0.5***	4.38 ± 0.06	288 ± 13*	92 ± 2**	196 ± 12*
IV	i.p.	0	−0.4 ± 1.5	3.76 ± 0.10	415 ± 66	61 ± 3	353 ± 66
	7 days	5	−1.8 ± 0.9	3.91 ± 0.09	387 ± 20	65 ± 3	322 ± 18
		50	−12.9 ± 1.5***	3.51 ± 0.07	256 ± 29*	37 ± 2***	219 ± 29

LW: liver weight, TC: serum total cholesterol, HDL-C: HDL-cholesterol, VLDL-C: VLDL-cholesterol, LDL-C: LDL-cholesterol.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control.
[a] Experimental diet; commercial chow CE-2 (Clea Japan Inc.) (Exp. I, II) or CE-2 + 0.5% cholesterol, 10% coconut oil (Exp. III, IV).

Table 3 Effect of YM-16638 on serum cholesterol level in monkeys

Monkey	*Daily dose,* (mg kg^{-1} day^{-1}) *Administration period*	*Group*	*Serum total cholesterol* (mg dl^{-1}) *pre*	*post*	*% Decrease vs. pre-value*
Squirrel	50	Control	168 ± 8	174 ± 13	
	3 weeks	YM-16638	164 ± 6	153 ± 9	6.7
Cynomolgus	30	Control	111 ± 8	109 ± 8	
	4 weeks	YM-16638	112 ± 11	75 ± 8	33*
Rhesus	30	Control	157 ± 8	163 ± 8	
	3 weeks	YM-16638	148 ± 18	108 ± 18	27*

*$P < 0.05$ vs. mean pre-values.

administration, serum cholesterol concentration in animals administered YM-16638 was significantly decreased by (27%) while no change was seen in the control group.

Effect of YM-16638 on serum ALT level in experimental animals

Changes in serum ALT levels in YM-16638-administered animals during the present series of studies are shown in Table 4. Values are represented as percentage compared to mean values of control groups in the mouse, rat, rabbit and hamster studies, and as percentage compared to mean values of baseline in the monkey studies. In all animal experiments, mean serum AST and ALT activities in control groups showed no significant changes throughout the experimental period (data not shown).

In contrast, strong decreases were observed in serum ALT but not AST levels in all YM-16638-administered animals irrespective of species. Further, in cynomolgus monkeys, a positive correlation ($r=0.5514$, $P=0.0001$) was seen between the percentage of TC decrease and that of ALT decrease (Figure 1).

Effect of YM-16638 on incorporation of mevalonic acid into cholesterol in rhesus monkey hepatocytes

In the liver homogenate of rhesus monkeys administered YM-16638 (30 mg kg^{-1} day^{-1} in diet for 28 days administration), potency of incorporation of radiolabelled mevalonate into nonsaponifiable lipids was dramatically lower than that in control group (Figure 2).

Discussion

From the results of the *in vivo* study presented here, the strong hypocholesterolaemic effect of YM-16638 demonstrated in a human phase I study (24% and 36% decrease in serum total cholesterol at 60 mg and 120 mg day^{-1} per man, respectively, after 2 weeks of treatment, mainly due to decreases in LDL- and VLDL-cholesterol not HDL-cholesterol, unpublished data) reappeared only in experiments that used cynomolgus monkeys and rhesus monkeys, classified as 'Catarrhini' (known as 'old-world monkeys'), and not in mice, rats, rabbits or squirrel monkeys. In the case of hamsters, in which lipid metabolism is thought to be comparatively close to that of man (Spady & Dietschy, 1985), a statistically significant decrease in serum TC concentration appeared in two kinds of experimental food conditions and by two methods of administration of YM-16638. At the same time, however, the percentage body weight gain in the YM-16638 groups was smaller than in the control group in all cases, and this effect was assumed to be mainly due to a slight decrease in food intake in YM-16638-administered hamster group (data not shown). As the loss of food intake may reasonably be considered as one cause of the decrease in serum TC, these conditions are, as in hamsters, hardly the same as in human subjects and cynomolgus or rhesus monkeys. On the other hand, the remarkable serum

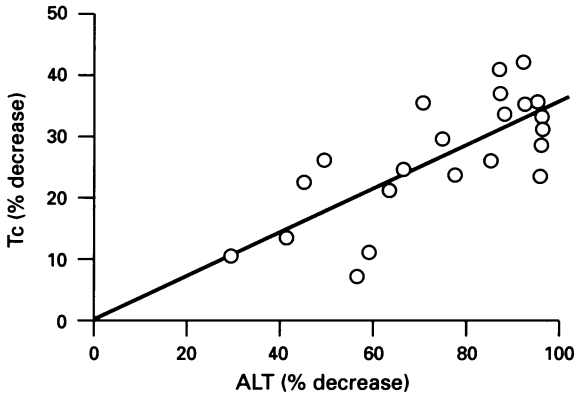

Figure 1 Correlation between the percentage decrease in serum total cholesterol (TC) concentration and percentage decrease in serum alanine-aminotransferase activity in cynomolgus monkeys administered YM-16638. f(X) = 0.3455x + 0.6944, r = 0.5514 ($P<0.001$).

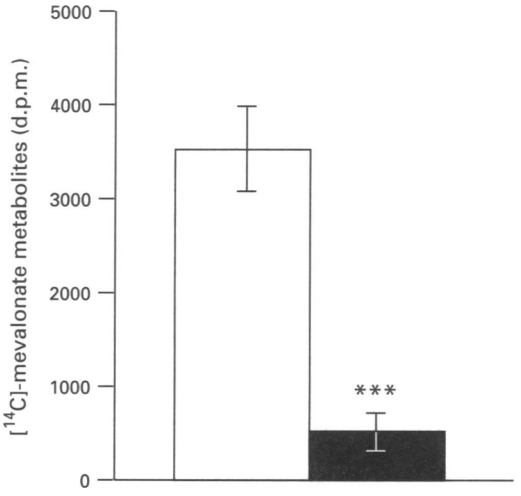

Figure 2 Effect of YM-16638 on the incorporation of mevalonic acid into nonsaponifiable lipids in rhesus monkey liver homogenate. The liver homogenate was prepared from rhesus monkeys untreated (open column) or treated (closed column) with YM-16638 (30 mg kg^{-1} day^{-1} for 28 days). Incorporation of radiolabelled mevalonic acid into nonsaponifiable lipids was measured accordingly to method of Endo *et al.* (1976).

Table 4 Effect of YM-16638 on serum ALT activity in experimental animals

Species	*Daily dose* (mg kg^{-1} day^{-1})	*Administration period*	*% decrease in serum ALT*
Mouse	200	28 days	11.3[a]
Rat	200	15 days	74.8***[a]
Hamster	100	28 days	62.7**[a]
Rabbit	90	18 days	49.1*[b]
Monkey			
Squirrel	50	21 days	80.9*[b]
Cynomolgus	30	28 days	96.0**[b]
Rhesus	30	21 days	75.0**[b]

*$P<0.05$, **$P<0.01$, ***$P<0.001$.
[a] % decrease compared to mean values of control group; [b] % decrease compared to mean pre-value.

ALT-lowering effects of YM-16638 were observed without distinction among species; this effect seems specific to ALT probably because no significant changes in serum AST level, equally an indicator of hepatopathy, were observed in any species. Although a positive correlation between the percentage TC decrease and percentage ALT decrease was observed in the cynomolgus monkey study, it appears that, on the basis of the species specificity of the TC decrease observed in this study and the independence of the metabolic pathways of cholesterol and ALT, the respective effects of YM-16638 appeared independently.

We speculate through the investigation of a series of chemically modified compounds with similar structure (not shown) and from other reports (Feuer, 1977; 1981; Dhai, 1979) that the serum ALT lowering effect is due to the thiadiazol structure at 3-methyl position of the YM-16638 molecule; however, we still have insufficient data to confirm this point. LY-171883 (Eli Lilly Co., Ltd., U.S.A.), a potent and leukotriene antagonist with a similar structure to YM-16638, (Marshall *et al.*, 1987; Fleisch *et al.*, 1985) is known to decrease serum triglyceride level in rats and monkeys, but not serum cholesterol level and ALT activity (Eacho *et al.*, 1985; Foxworthy *et al.*, 1990; Hoover *et al.*, 1990).

As far as we know, very few reports give scientific support to the relevance of serum ALT activity or to leukotriene-antagonism in cholesterol metabolism.

In liver from cynomolgus monkeys administered YM-16638, the potency of sterol synthesis from mevalonate, the precursor of cholesterol, was markedly suppressed. The results of our preliminary studies showed that the activity of enzymes mevalonate kinase, isopentenyl pyrophosphate isomerase and farnesyl pyrophosphate synthase, which catalyze the reaction steps between mevalonate and squalene in the cholesterol biosynthetic pathway, was suppressed in YM-16638-administered cynomolgus monkeys (unpublished data). These results indicate that the blood cholesterol lower effect of YM-16638 occurs via a different mechanism from that of existing cholesterol lowering drugs HMG-CoA reductase inhibitors, squalene synthase inhibitors, fibrates, etc., and that the potency of the hypocholesterolaemic effect of YM-16638 is equal to or greater than that of HMG-CoA reductase inhibitors such as pravastatin and lovastatin (Grundy, 1986; Endo, 1992). At present, however, many points concerning the mechanism of the serum TC lowering effect of YM-16638 remain uncertain, and more detailed investigations *in vivo* and *in vitro* are required.

In this paper, we describe the strong serum cholesterol lowering effect of YM-16638 in monkeys. We conclude from this *in vivo* study that YM-16638 is a potent and novel hypocholesterolaemic drug.

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