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Influence of the new antihypertensive drug, SM-2470 (a quinazoline derivative), on cholesterol metabolism in rats

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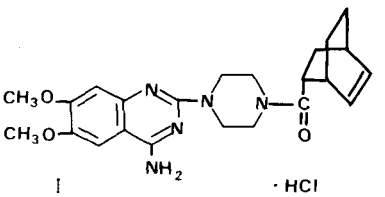
The influence of SM-2470 (4-amino-2-{4-[bicyclo(2,2,2)oct-2-ene-5-carbonyl]-1-piperazinyl}-6,7-dimethoxyquinazoline), a new antihypertensive agent, on cholesterol metabolism was investigated in hypercholesterolaemic rats, using the dual isotope method (cholesterol absorption) and the intestinal ligated loop method (cholesterol uptake). In the hypercholesterolaemic model, 1–30 mg kg^{-1} doses of SM-2470 significantly inhibited the elevation of the total serum cholesterol and very low and low density lipoproteins (VLDL + LDL)-cholesterol, without causing any change in the hepatic cholesterol level. In a dual isotope model experiment, SM-2470 (10, 30 mg kg^{-1}) inhibited the intestinal absorption of cholesterol, but did not affect biliary excretion of sterol and/or bile acids, nor did it affect cholesterol movement from the liver to blood. In the intestinal ligated loop method, SM-2470 remarkably inhibited the mucosal uptake of cholesterol in a dose-dependent manner in 0·5–2·0 mg mL^{-1} of micellar solution. In addition, SM-2470 inhibited micellar formation in-vitro, which increased the distribution of large sized micelles as well as cholesterol absorption inhibitors. From these results, it can be assumed that a possible mechanism behind the hypocholesterolaemic effect of SM-2470 is the inhibition of cholesterol absorption related to the reduction of cholesterol solubilization, occurring in the gut micelles, similar to the action of plant sterol.

It has been well documented that the metabolic effect of antihypertensive drugs on lipids during therapy for hypertension is a determinant of therapeutic effectiveness (Johnson 1982; Cutler 1983). In recent years, there has been increasing clinical evidence that many drugs, such as thiazide diuretics (Grimm et al 1981; Ames & Hill 1982), β-blockers (Tanaka et al 1976; Helgeland et al 1978; Leons et al 1984) and α_1-blockers (Leren et al 1980; Kokubo et al 1982; Leren et al 1982; Kather & Sauberlich 1984; Mauersberger 1984; Takabatake et al 1984), affect lipid profiles. Tanaka et al (1976) and others (Helgeland et al 1978; Leren et al 1980; Kather & Sauberlich 1984; Leons et al 1984; Mauersberger 1984; Takabatake et al 1984) reported an unfavourable effect of β-blockers on lipid profiles, such as increasing plasma triglyceride and/or total cholesterol and low density lipoprotein cholesterol. On the other hand, it is evident from several clinical trials that prazosin, an α_1-blocker, favourably affects lipid profiles by increasing the HDL-cholesterol and/or the ratio of total cholesterol to HDL-cholesterol, and decreasing the triglyceride level. It is therefore suggested that prazosin may be more effective in preventing atherosclerosis than antihypertensive drugs, which worsen lipid profiles.

In view of these circumstances, the purpose of the present study has been to investigate whether SM-2470 (I, 4-amino-2-{4-[bicyclo(2,2,2)oct-2-ene-5-carbonyl]-1-piperazinyl}-6,7-dimethoxyquinazoline), a new antihypertensive agent which is a quinazoline derivative, could modify cholesterol metabolism in rats.



I

SM-2470

MATERIALS AND METHODS

Experimental animals and diet

Male Sprague-Dawley rats were purchased from Charles River Japan, Inc. The rats were fed either

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standard diet pellets (CE-2, Nippon Clea) or given a high fat diet composed of CE-2 93·8%, olive oil 5%, cholesterol 1% and sodium cholate 0·2%. They were allowed free access to food and tap water during the experimental period.

Chemicals

Materials were purchased from the following sources: cholesterol, olive oil, sodium cholate, β-sitosterol, triolein, sodium taurodeoxycholate and cholestyramine (Nakarai Chemicals Co. Kyoto, Japan); sodium glycochenodeoxycholate and monooleoyl glycerol (Sigma Chemical Co. USA); clofibrate (Fluka Switzerland); [4-^{14}C]cholesterol (sp. act., 57·5 mCi mmol^{-1}), [1α,2α-^{3}H]cholesterol (sp. act., 60 Ci mmol^{-1}), Aquasol-2 (scintillation solution) and Protosol (tissue solubilizer) [New England Nuclear]. SM-2470 was supplied by Sumitomo Chemical Co. Ltd (Osaka Japan). Hydrochlorothiazide and melinamide were isolated from commercial drugs and identified in Research Laboratories, Morishita Pharmaceutical Co. Ltd (Shiga, Japan).

Cholesterol-induced hyperlipidaemic rats

Rats weighing 120–160 g were allowed free access to a high fat diet and tap water for 10 days. Drugs were suspended in 0·5% methylcellulose and given orally to the animals through a stomach tube once a day during the experimental period. The animals were killed 4 h after withdrawal from the diet and drug administration. Control rats received an equal volume of vehicle. The blood samples for lipid analysis were obtained from the abdominal aorta under anaesthesia with sodium pentobarbitone (40 mg kg^{-1} i.p.) The liver was immediately removed, washed, blotted, weighed and kept at −20 °C, until analysed.

Cholesterol was measured by an enzymatic method using a commercial reagent set (T-CHO reagent, International Reagent Co. Kobe, Japan) with semi-autoanalyser (Model 105 system, Hitachi). Serum lipoprotein fractionation was performed by an isoelectric method (Isopol, International Reagent Co.) as described previously (Seki et al 1985). The very low and low density lipoproteins (VLDL + LDL)-cholesterol levels were estimated as the difference between total cholesterol and high density lipoprotein (HDL)-cholesterol levels.

For the determination of hepatic cholesterol, 0·2 g of wet tissue was extracted with 3·0 mL of isopropanol using a Polytron (Kinematica GmbH, Switzerland), and the amount of cholesterol in the extracts was determined by the above method.

Absorption, distribution and excretion of cholesterol

Cholesterol absorption. Cholesterol absorption was measured by a dual-isotope method as described previously (Zilversmit 1972). The rats were maintained on commercial laboratory chow for 10 days. The drugs were suspended in 0·5% methylcellulose and given orally through a stomach tube once a day during the experiment. On the 9th day, [4-^{14}C]-cholesterol (0·381 μCi/100 g) was injected into the caudal vein under light anaesthesia with ethyl ether. This was immediately followed by the [^{3}H]cholesterol (1·712 μCi/100 g), given via a stomach tube. The rats were fasted for 18 h before and for 6 h after treatment with the two isotopes. Samples of whole blood were taken at 1, 3, 6, 9, 12, 24 and 48 h from the caudal vein using heparinized capillary glass tubes. Aliquots (0·1 mL) of whole blood were placed in liquid scintillation vials and decolourized with small amounts of hydroperoxide. The radioactivity was determined by a liquid scintillation spectrometer in a Packard model LSC-900. Cholesterol absorption was calculated from the ^{3}H/ ^{14}C ratio of radioactivity in whole blood and the dose solution.

Radioactivity in the liver and bile. Immediately after the last sampling of blood by the dual isotope method, rats were anaesthetized with sodium pentobarbitone and placed on an animal board in the spinal position. The abdomen was opened along the midline and a polyethylene tube (PE-10, Intramedie, Clay Adams) was inserted centrally into the common bile duct. Bile volumes were determined using disposable syringes for 2 h, after which the liver was removed for radioactivity measurement. An aliquot of bile samples (0·25 mL) was added to 12 volumes of 15% alcoholic KOH, and hydrolysed at 85 °C for 24 h. After cooling to room temperature (20 °C), the hydrolysates were added to 2 mL of water and extracted with three 4 mL portions of ethyl ether before and after acidification with 6 M HCl to separate sterols and bile acids fractions. Each extract was evaporated to dryness in a scintillation vial under N_2 gas and counted.

For the determination of hepatic radioactive materials, 0·2 g of wet tissue was dissolved with 1 mL of Protosol under 60 °C and counted as above.

Preparation of micellar solution and measurement of micellar sizes

The micellar solution was prepared according to the method of Shidoji et al (1980) and Watanabe et al

(1981). This consisted of 5 mM 2-monooleoyl-glycerol, 5 mM Na-glycochenodeoxycholate, 100 μg of non-radioactive cholesterol and 0·1 μCi of [4-^{14}C]cholesterol in 0·5 mL of 50 mM sodium phosphate buffer (pH 6·3). Drugs in amounts ranging from 0·5–2·0 mg were added to 1·0 mL of the micellar solution in a screw vial and the air displaced with N_2 gas, the solutions were then incubated at 37 °C overnight in the dark. Aliquots (0·2 mL) of the micellar solutions were transferred to scintillation vials for counting as described. Radioactivity determined in these solutions (d min^{-1}) was used as the index of micellar formation. Samples of micellar solution (0·2 mL) were filtered under centrifugation through millipore filters of 450 nm, 220 nm (FM-45, FM-22; Fuji Film Co.) and 100 nm (Brunswick Tech. Co.) with a micropartition system (MPS-1, Amicon Co.), respectively. The radioactivity of each filter and 100 nm-filtrate was then determined in 4 mL of the scintillation solution using a liquid scintillation spectrometer in a Packard model LSC-900. The micellar size distribution in the micellar solution, as a percentage, was obtained by dividing each value by the sum of individual values.

Cholesterol uptake (intestinal ligated-loop method)

Measurement of the mucosal uptake of cholesterol was carried out in-situ by the ligated loop method. Male rats, 7 weeks old, were fasted overnight before the experiment unless otherwise specified. After the animals were anaesthetized with sodium pentobarbitone, 4 segments of the jejunum, 4–6 cm in length were isolated with an intact blood supply. Before the segments were completely closed, 100 μCi of [4-^{14}C]cholesterol in 0·5 mL of the dose solution, prepared as described above, was injected through one end of each segment. The animals were kept at body temperature by means of a floor heater during the experiment.

After a 30 min incubation, the segments were resected and washed with 2 mL of ice-chilled physiological saline solution. The tissue was further washed 5 times with 2 mL of ice-chilled 5 mM sodium deoxycholate in saline solution to remove the radioactive cholesterol remaining on the mucosal surface. Each sample of washed tissue was weighed and transferred into a glass scintillation vial. The tissue was solubilized with 2 mL of Protosol and then decolourized with a small amount of hydroperoxide. The radioactivity was determined in 10 mL of the scintillation solution. The mucosal uptake of non-radioactive cholesterol was calculated and expressed as μg/100 mg tissue/30 min.

Table 1. Effect of SM-2470 on the serum cholesterol levels of hypercholesterolaemic rats fed high cholesterol diet. The rats were maintained on a high cholesterol diet for 10 days with oral administration of drugs once a day. The very low and low density lipoproteins (VLDL + LDL)-cholesterol levels were estimated as the difference between total cholesterol and high density lipoprotein (HDL)-cholesterol level. The values represent the mean ± s.e. of 6 rats, excluding the control group (n = 12).

Treatment		Serum cholesterol (mg dL^{-1})		
Drug	Dose (mg kg^{-1})	Total	VLDL + LDL	HDL
Normal	—	59 ± 2[b]	17 ± 2[c]	43 ± 1[b]
Control	—	334 ± 20	269 ± 20	65 ± 6
SM-2470	1	258 ± 20[a]	206 ± 22	51 ± 5
	3	220 ± 18[b]	157 ± 18[b]	63 ± 6
	10	210 ± 12[c]	156 ± 12[b]	54 ± 3
	30	241 ± 15[b]	187 ± 13[b]	54 ± 5
Hydrochlorothiazide	10	330 ± 19	269 ± 19	61 ± 5
β-Sitosterol	400	239 ± 34[a]	185 ± 32[a]	54 ± 5
Clofibrate	200	361 ± 29	320 ± 28	41 ± 2[b]

Significantly different from the control by Student's *t*-test: [a] $P < 0·05$, [b] $P < 0·01$, [c] $P < 0·001$.

RESULTS

Effect of SM-2470 on cholesterol-induced hypercholesterolaemia

The efficacy of SM-2470 was compared with that of hypolipidaemic drugs (β-sitosterol, clofibrate) and hydrochlorothiazide, an antihypertensive drug, in cholesterol-induced hypercholesterolaemia. As can be seen from Table 1, the total serum cholesterol in rats fed a high fat diet increased to five times that of normal rats that had received the normal chow diet, and this change was accompanied by marked changes in cholesterol distribution of serum lipoproteins. In this model, SM-2470 significantly reduced the total serum cholesterol level even at a dose of 1 mg kg^{-1}. SM-2470 in the range of 1 to 10 mg kg^{-1} caused a dose-dependent decrease in total serum cholesterol, but there was no further reduction at a high dose (30 mg kg^{-1}). Under the same conditions, β-sitosterol caused a significant decrease in total serum cholesterol at a fairly high dose (400 mg kg^{-1}) but clofibrate and hydrochlorothiazide had no such effect. The cholesterol levels of lipoproteins (VLDL + LDL and HDL) in the control group were elevated in comparison with those of the normal group. SM-2470 and β-sitosterol markedly reduced the cholesterol level in the VLDL + LDL fraction but the HDL-cholesterol level did not change. In contrast, clofibrate at 200 mg kg^{-1} reduced only the HDL-cholesterol level and then showed a tendency to increase the cholesterol level in the VLDL + LDL fraction.

Table 2 shows the liver weight and liver cholesterol in rats treated with SM-2470 and reference drug for

Table 2. Effect of SM-2470 on body weight increase, liver weight and cholesterol level in hypercholesterolaemic rats.

Treatment				
Drug	Dose (mg kg^{-1})	Weight gain (g/10 days)	Liver wt (g)	Liver cholesterol (mg)
Normal	—	81 ± 3	11·6 ± 0·3[b]	25 ± 1[c]
Control	—	85 ± 2	13·8 ± 0·4	387 ± 15
SM-2470	1	83 ± 3	13·3 ± 0·4	375 ± 28
	3	89 ± 4	15·4 ± 0·5[a]	424 ± 12
	10	85 ± 3	14·5 ± 0·4	427 ± 12
	30	78 ± 3	13·6 ± 0·3	353 ± 27
Hydrochlorothiazide	10	87 ± 2	13·1 ± 0 3	396 ± 15
β-Sitosterol	400	79 ± 2[a]	13·7 ± 0 2	370 ± 12
Clofibrate	200	89 ± 4	17·2 ± 0·6[c]	451 ± 23[a]

For details concerning the experimental condition see Table 1 or the experimental section. The values represent the mean ± s.e. of 6 rats, excluding the control (n = 12). Significantly different from the control by Student's *t*-test; [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.

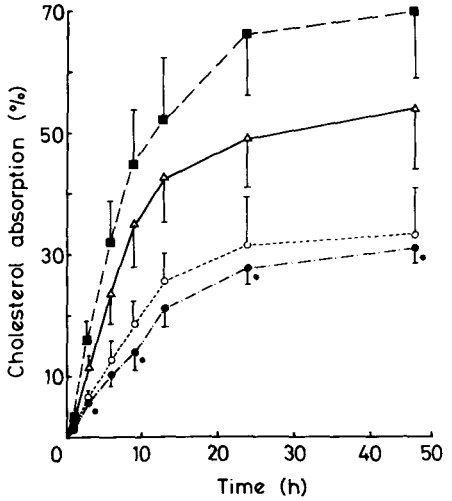

FIG. 1. Effect of SM-2470 on cholesterol absorption in rats fed a normal diet (dual isotope method). △——△ control, ○- - -○ SM-2470 10 mg kg^{-1}, ●- · - · -● SM-2470 30 mg kg^{-1}, ■ — — — ■ clofibrate 300 mg kg^{-1}. Cholesterol absorption (%) was calculated from $^{3}H/^{14}C$ ratio of radioactivity in whole blood and dose solutions. The values represent the mean ± s.e. of 4–5 rats. Significantly different from the control by Student's *t*-test: * $P < 0.05$.

10 days. SM-2470 showed a hepatomegalic effect at a daily oral dose of a 3 mg kg^{-1} alone, though the effect was not dose-related. SM-2470 and β-sitosterol did not significantly influence the liver cholesterol level whereas clofibrate, at a 200 mg kg^{-1} dose, increased the liver weight and liver cholesterol level.

Effect of SM-2470 on cholesterol absorption, hepatic level and biliary excretion of sterols

Cholesterol absorption measured by the dual isotope ratio method. The effect of SM-2470 on cholesterol absorption measured by the dual isotope ratio method is shown in Fig. 1. At doses of 10 and 30 mg kg^{-1} it clearly inhibited cholesterol absorption, but the inhibitory effect at a lower dose was not statistically significant. At 2 days after the injection of radioactive cholesterol, the mean percentage of cholesterol absorbed was 53·9% for the controls, and 33·2% at 10 mg kg^{-1} and 30·7% at 30 mg kg^{-1} of SM-2470, respectively. In contrast, clofibrate at 300 mg kg^{-1} did not significantly alter cholesterol absorption.

In addition, the ^{3}H radioactive materials in serum derived from the oral dose of [^{3}H]cholesterol were significantly decreased in SM-2470-treated rats, whereas ^{14}C-radioactivity in serum derived from the intravenous dose of [^{14}C]cholesterol did not change (Fig. 2).

Radioactivity in the liver and bile. Bile from rats with common bile duct fistulas was collected after cannulation and as shown in Table 3, the excreted radioactive material derived from [^{3}H]cholesterol in bile acid as well as the sterol fractions in bile, was markedly reduced by SM-2470, whereas radioactive materials derived from [^{14}C]cholesterol did not change. Clofibrate increased bile flow significantly, but SM-2470 did not.

As shown in Table 4, SM-2470 reduced the ^{3}H-radioactive material derived from [^{3}H]cholesterol and did not change the ^{14}C-radioactivity level derived from [^{14}C]cholesterol in the liver. These findings were in accordance with the changes in the radioactivity of serum and bile.

Effect of SM-2470 on micellar formation and size in-vitro

Table 5 shows the effect of SM-2470 on micellar formation and distribution of micellar formation and distribution of micellar size in-vitro. While it inhibited micellar formation, β-sitosterol, cholestyramine and melinamide, which are typical inhibitors of cholesterol absorption, had no such effect. Furthermore, SM-2470, as well as inhibitors of cholesterol absorption, increased the distribution of large micelles in a dose-dependent manner.

Effect of SM-2470 on cholesterol uptake via the intestinal ligated loop method

Fig. 3 shows the effect of SM-2470 on the mucosal uptake of cholesterol, measured by the ligated loop method in-situ. SM-2470 significantly inhibited the mucosal uptake of cholesterol in a dose-related fashion. The uptake after 0·5, 1·0 and 2·0 mg doses of SM-2470 mL^{-1} of the dose solution, was about

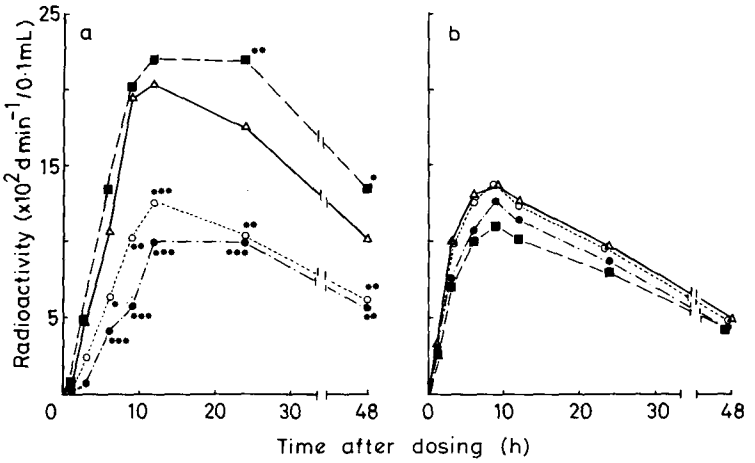

FIG. 2. Effect of SM-2470 on radioactivity in whole blood from 1 to 48 h after oral administration of (a) [^{3}H]cholesterol and intravenous administration of (b) [^{14}C]cholesterol in normal rats. △——△ control, ○ - - - ○ SM-2470 10 mg kg^{-1}, ●-·-·-● SM-2470 30 mg kg^{-1}, ■ — — — ■ clofibrate 300 mg kg^{-1}. The values represent the mean ± s.e. of 4–5 rats. Significantly different from the control group by Student's *t*-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3. Effect of SM-2470 on biliary volume and excretion of radioactive materials into bile of normal rats. Immediately after completion of the cholesterol absorption experiment, rats with common bile-duct fistulas were prepared 48 h after administration of radioactive cholesterol, and then bile was collected for 2 h. Bile samples were hydrolysed under alkaline conditions, and extracted with ethyl ether before and after acidification with 6 M HCl to separated sterols and bile acids fractions.

Treatment			^{3}H, ^{14}C radioactivities (×10^3 dmin^{-1}) in bile			
			Bile acids fraction		Sterols fraction	
Drug	Dose (mg kg^{-1})	Bile volume (mL/2 h)	^{3}H	^{14}C	^{3}H	^{14}C
Control	—	1·1 ± 0·1	7·1 ± 0·6	3·4 ± 0 6	48·7 ± 6·0	23·1 ± 4·3
SM-2470	10	0·9 ± 0·1	5·1 ± 0·9	2·9 ± 0·5	25·9 ± 5·1[a]	19·1 ± 2·8
	30	1·0 ± 0·1	4·4 ± 0·5	3·2 ± 0·6	25·2 ± 2·1[b]	20·3 ± 4·0
Clofibrate	300	1·6 ± 0·1[b]	7·5 ± 1·1	2·8 ± 0·7	42·3 ± 7·3	17·6 ± 5·2

The values represent the mean ± s.e. of 3–5 rats.
Significantly different from the control by Student's *t*-test: [a] $P < 0.05$, [b] $P < 0.01$.

70% ($P < 0.05$), 49% ($P < 0.01$) and 38% ($P < 0.001$) in the controls, respectively. This effect was stronger than the effects of melinamide and cholestyramine at the 2 mg mL^{-1} level, though the potency of SM-2470 was inferior to that of β-sitosterol.

DISCUSSION

Recently there has been increasing clinical evidence demonstrating that many antihypertensive drugs affect the lipid or lipoprotein profiles (e.g. Leren et al 1982; Leons et al 1984; Mauersberger 1984; Kather & Sauberlich 1984; Takabatake et al 1984). Even though the effects of those drugs on lipid metabolism have been extensively studied in man, so far there have been only a limited number of reports indicating the metabolic effect of antihypertensive drugs on lipids in animal models (Helgeland et al 1984). We have evaluated the effect of SM-2470, a new antihypertensive agent, on cholesterol metabolism in rats. We would first like to emphasize its hypocholesterolaemic effect on dietary-induced hyperlipidaemic rats which have been widely used for evaluating the efficacy of hypolipidaemic drugs. In this model, SM-2470 markedly reduced the total serum cholesterol level even at a dose of 1 mg kg^{-1}, which indicates the drug's hypotensive activity (unpublished data). Though a definite dose-response relationship could not be obtained, SM-2470 at 3 mg kg^{-1} reduced total serum cholesterol more than

Table 4. Effect of SM-2470 on radioactivity in liver after administration of radioactive cholesterol in normal rats. Immediately after completion of the bile collection, the liver was removed for measurement of radioactivity.

Treatment		Liver	Radioactivities in whole liver ($\times 10^5$ dmin^{-1})	
Drug	Dose (mg kg^{-1})	wt (g)	^{3}H	^{14}C
Control	—	8·0 ± 0·4	2·20 ± 0·08	1·09 ± 0·12
SM-2470	10	8·4 ± 0·3	1·17 ± 0·18[a]	0·96 ± 0·18
	30	8·6 ± 0·5	1·15 ± 0·17[a]	0·95 ± 0·21
Clofibrate	300	10·0 ± 0·4[a]	3·14 ± 0·13[b]	1·16 ± 0·14

The values represent the mean ± s.e. of 4–5 rats.
Significantly different from the control by Student's *t*-test: [a] $P < 0·01$, [b] $P < 0·001$.

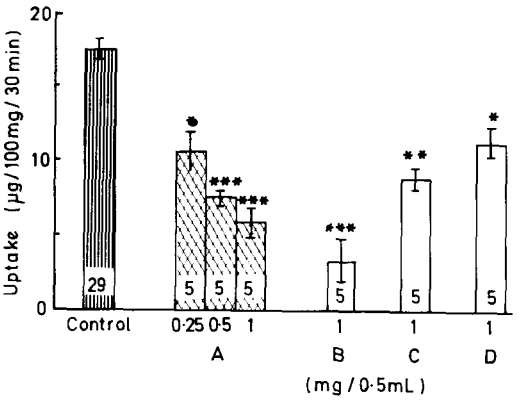

FIG. 3. Effect of SM-2470 on mucosal uptake of cholesterol in the ligated loop method. Four segments 4–6 cm in length were prepared in the jejunums of rats that were anaesthetized with pentobarbitone. The micellar solution without drug (control) and solutions with drugs were injected simultaneously into different segments of the same rats. Figures within the columns represent the number of segments. Significantly different from the control group by Student's *t*-test: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$. Key: A, SM-2470; B, β-sitosterol; C, cholestyramine; D, melinamide.

a higher dose (400 mg kg^{-1}) of β-sitosterol, which is a typical inhibitor of cholesterol absorption (Hernandez et al 1953). The influence of SM-2470 on the lipoprotein cholesterol distribution in this model was examined by the method described previously (Seki et al 1985). It is apparent from the data presented that the hypocholesterolaemic effect of SM-2470 is due to a decrease in serum low density lipoproteins (VLDL + LDL) rather than high density lipoprotein (HDL). In view of the general acceptance of the atherogenicity of VLDL + LDL, the prevention of a marked increase in VLDL + LDL following a high fat diet by a low dose of SM-2470 is noteworthy. One possibility is that the direct effect of SM-2470 on cholesterol metabolism may account for the observed results. Another possibility to be considered would be an indirect effect through its hypotensive and/or other pharmacological actions. However, this appears unlikely since there was no significant difference in growth and diet-intake between control and SM-2470-treated rats. In addition, hydrochlorothiazide used as a reference drug did not show a positive effect in the same experiment. It suggests that the hypocholesterolaemic effect of SM-2470 may be achieved without mediation of its hypotensive action. Accordingly, to clarify the functional mechanism of SM-2470 in our hyperlipidaemic model, its effect on cholesterol absorption in rats was then examined using the dual isotope method. SM-2470 at 10 and 30 mg kg^{-1} clearly inhibited cholesterol absorption from the gut. Thus, it exerted a specific effect on cholesterol metabolism in the two

Table 5. Effect of SM-2470 on micellar formation and distribution of micellar size in-vitro. The micellar solution consisted of monooleoyl-glycerol (5 mM), Na-glycochenodeoxycholate (5 mM), non-radioactive cholesterol (100 μg) and [^{14}C]-cholesterol (0·01 μCi) in 0·5 mL of 50 mM sodium phosphate buffer (pH 6·3). Drugs were added to 0·5 mL of the micellar solution in amounts ranging from 0·25 to 1·0 mg. Radioactivity determined in these solutions was used as the index of micellar formation. Measurement of micellar sizes were performed using three millipore filters (450, 220, 100 nm) with a micropartition system.

Treatment		Radioactivity in micellar sol. (dmin^{-1}/0·1 mL)	Distribution of micellar size (%)			
Drug	Concn (mg mL^{-1})		>450 nm	<450–220 nm	<220–100 nm	<100 nm
Control (n = 6)	—	4652 ± 88	6·2 ± 0·2	0	6·0 ± 0·7	87·7 ± 0·6
SM-2470	0·50	4182	28·8	3·4	0	67·9
	1·00	3215	44·8	0	13·5	41·8
	2·00	2611	75·9	0	5·7	18·5
β-Sitosterol	2·00	4669	93·4	0	2·3	4·2
Melinamide	2·00	4794	50·5	0	0	49·5
Cholestyramine*	2·00	4933	58·0	8·0	0	34·0

The values represent the mean of duplicate determinations, excluding the control group (n = 6).
* Dowex 1-X2-Cl.

models, although the connection between hypocholesterolaemic and hypotensive actions remains unclear.

In addition, this agent did not influence the level of radioactive materials in bile or the liver, derived from an intravenous dose of [^{14}C]cholesterol in this model.

In view of these results, it is very unlikely that the hypocholesterolaemic effect of SM-2470 is due to the increase in biliary excretion of sterol and/or bile acids and the block of cholesterol movement from the liver to the blood.

Further experiments, using the ligated loop method in-situ, were carried out to determine whether SM-2470 itself had a direct effect on cholesterol absorption. This model requires only ½ h, and it is also possible to evaluate the effect of the drug on cholesterol uptake, which is one of the limiting steps in the process of cholesterol absorption (Shidoji et al 1980). The drug inhibited the mucosal uptake of cholesterol in a dose-related manner, at 0·15–2·0 mg mL^{-1} of micellar solution. Thus, the findings clearly rule out the possibility that the effects produced by SM-2470 are the result of the direct inhibition of cholesterol absorption.

Additionally, the distribution of micellar size in the dose solution used in the ligated-loop method was determined by millipore-filters with a micropartition system. SM-2470 inhibited micellar formation in-vitro, and the distribution of large micelles also increased in micellar solution containing SM-2470.

In a preliminary study, the large micelles showed poor uptake in the intestine compared with that of small-sized micelles (unpublished data). The inhibitory effect observed in the in-situ experiment may be related to the reduction in cholesterol solubility, disturbing micellar formation and the production of large micelles at a stage before absorption. Watanabe et al (1981), using the ligated-loop method, have indicated that the radioactive cholesterol incorporated into the mucosa was not detected in the lymph or blood during a ½ h incubation, and that the radioactive cholesterol in the extract from intestinal mucosa was mostly of the free form.

More recently, the role of mucosal ACAT (acylCoA : cholesterol acyltransferase) as a possible mediator of cholesterol movement across the intestine has received much attention (Norum et al 1979; Heider et al 1983; Bennett Clark & Tercyak 1984; Erickson 1984; Field 1984). Heider et al (1983) and Bennett Clark & Tercyak (1984) have shown that ACAT plays an essential role in cholesterol absorption. They conducted studies, using available agents such as the compounds 57-118 [(2)-*N*-(1-oxo-9-octadecenyl)-DL-tryptophan ethyl ester] and 58-035 [3-(decyldimethylsilyl)-*N*(2-(4-methylphenyl)-1-phenethyl)propanamide], which are competitive inhibitors of ACAT.

However, the inhibitory effect of SM-2470 in the ligated loop method does not seem likely to be associated with the process of esterification of absorbed cholesterol before lymphatic transport on the basis of the above explanation. Further observations are necessary to clarify the functional mechanism of SM-2470 on cholesterol metabolism. The results of this study appear to indicate that a possible mechanism for the hypocholesterolaemic effect of SM-2470 is the inhibition of cholesterol absorption related to the reduction of cholesterol solubilization in micelles in the gut. This is similar to the action of β-sitosterol. Furthermore, the action of SM-2470 on cholesterol metabolism differs completely from that of clofibrate.

From the findings it may be assumed that SM-2470 possesses both the beneficial effects of reducing blood pressure and improving the blood lipid profile, which suggest its application in coronary and vascular diseases.

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REFERENCES

Ames, R. P., Hill, P. (1982) J. Cardiovasc. Pharmacol. 4: 206–212

Bennett Clark, S., Tercyak, A. M. (1984) J. Lipid Res. 25: 148–159

Cutler, A. (1983) Am. J. Cardiol. 51: 628–631

Erickson, S. K. (1984) J. Lipid Res. 25: 411–415

Field, F. J. (1984) Ibid. 25: 389–399

Grimm, R. H., Leons, A. S., Hunninghake, D. B., Lenz, K., Hannan, P., Blackburn, H. (1981) Ann. Int. Med. 94: 7–11

Heider, J. G., Pickens, C. E., Kelly, L. A. (1983) J. Lipid Res. 24: 1127–1134

Helgeland, A., Hjermann, I., Leren, P., Enger, S., Holme, I. (1978) Br. Med. J. 2: 403

Helgeland, A., Eirksen, K., Foss, P. O., Nafstad, I., Solberg, L. A., Tollersrud, S. (1984) Acta Pharmacol. Toxicol. 54: 270–272

Hernandez, H. H., Peterson, D. W., Chaikoff, I. L., Dauben, W. G. (1953) Proc. Soc. Exp. Biol. Med. 83: 498–499

Johnson, B. F. (1982) J. Cardiovasc. Pharmacol. 4: S213–S221

Kather, H., Sauberlich, P. (1984) Am. J. Med. 76: 89–93

Kokubo, T., Itoh, I., Kurita, H., Ochi, T., Murata, K., Yuba, I. (1982) J. Cardiovasc. Pharmacol. 4: S228–S232

Leons, A. S., Agre, J., McNally, C., Bell, C., Neibling, M., Grimm, R. H., Hunninghake, D. B. (1984) J. Clin. Pharmacol. 24: 209–217

Leren, P., Foss, O. P., Helgeland, A., Hjermann, H., Holme, I., Lund-Larsen, P. G. (1980) Lancet ii: 4–6

Leren, P., Eide, I., Foss, O. P., Helgeland, A., Hermann, I., Holme, I., Kjeldsen, S. E., Lund-Larsen, P. G. (1982) J. Cardiovasc. Pharmacol. 4: 222–224

Mauersberger, H. (1984) Am. J. Med. 76: 101–104

Norum, K. R., Lilljeqvist, A. G., Helgerud, P., Normann, A. Mo., Selbekk, B. (1979) Eur. J. Clin. Invest. 9: 55–62

Seki, K., Fukuda, M., Ohki, M. (1985) Chem. Pharm. Bull. 33: 5036–5041

Shidoji, Y., Watanabe, M., Oku, T., Muto, Y., Hosoya, N. (1980) J. Nutr. Sci. Vitaminol. 26: 183–188

Takabatake, T., Ohta, H., Maekawa, M., Yamamoto, Y., Ishida, Y., Hara, H. (1984) Am. J. Med. 76: 113–116

Tanaka, N., Sakaguchi, K., Oshige, K., Niimura, T., Kanehisa, T. (1976) Metabolism 25: 1071–1075

Watanabe, M., Oku, T., Shidoji, Y., Hosoya, N. (1981) J. Nutr. Sci. Vitaminol. 27: 209–217

Zilversmit, D. B. (1972) Proc. Soc. Exp. Biol. Med. 140: 862–865