The Synergistic Hypocholesterolemic Activity of the Potent Cholesterol Absorption Inhibitor, Ezetimibe, in Combination With 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors in Dogs

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Ezetimibe (SCH 58235) and SCH 48461 are potent cholesterol absorption inhibitors, which cause significant decreases in plasma cholesterol levels in cholesterol-fed animals and in humans with hypercholesterolemia. These compounds selectively block intestinal uptake and absorption of cholesterol. These cholesterol absorption inhibitors cause modest, inconsistent reductions in plasma cholesterol levels in animals fed cholesterol-free chow diets. Although, these compounds block cholesterol absorption and increase neutral sterol excretion, chow-fed animals compensate for the loss of biliary cholesterol by increasing hepatic cholesterol synthesis. Therefore, we determined the effect of SCH 48461 and ezetimibe in combination with 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors in chow-fed dogs. A synergistic reduction in plasma cholesterol was observed in chow-fed dogs given SCH 48461 (0.1 mg/kg/d) and the HMG CoA reductase inhibitor, lovastatin (5 mg/kg/d). Neither SCH 48461 nor lovastatin alone affected plasma cholesterol levels. Their combination for 14 days caused a 36% reduction in plasma cholesterol levels from 129 mg/dL to 83 mg/dL ($P < .05$). Ezetimibe (0.007 mg/kg/d) also caused synergistic reductions in plasma cholesterol levels in chow-fed dogs when combined with HMG CoA reductase inhibitors for 2 weeks (5 mg/kg lovastatin -50%; 2.5 mg/kg pravastatin -41%; 5 mg/kg fluvastatin -60%, and -30% with low doses of simvastatin and atorvastatin 1 mg/kg). The combination of this class of cholesterol absorption inhibitors with an HMG CoA reductase inhibitor should be very effective clinically at reducing plasma cholesterol levels, even with reduced dietary intake of cholesterol.

EZETIMIBE, SCH 58235, 1-(4-fluorophenyl)-3(R)-[3(S)-hydroxy-3-(4-fluorophenyl)propyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone) and SCH 48461, (3R,4S)-1,4-S-(4-methoxyphenyl)-3-(3-phenylpropyl)-2-azetidinone are potent inhibitors of both dietary and biliary cholesterol absorption leading to decreased plasma cholesterol levels in several cholesterol-fed animal species.[1-4] These compounds are very selective inhibitors of intestinal cholesterol absorption that do not affect the absorption of triglycerides, bile acids, or vitamins A and D.[1,4,5] SCH 48461 treatment does not consistently lower plasma cholesterol levels in hamsters and monkeys fed cholesterol-free diets.[1] We previously reported that the inability of SCH 48461 to significantly reduce plasma cholesterol levels was a result of increases in hepatic cholesterol biosynthesis in chow-fed hamsters in response to the inhibition of biliary cholesterol absorption.[1]

The inhibition of cholesterol biosynthesis by 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (EC1.1.1.34) inhibitors has been shown clinically to be an effective way to reduce plasma cholesterol[6] and reduce atherosclerosis.[7-9] Combination therapy with HMG CoA reductase inhibitors and bile acid sequestrants has been demonstrated to be more effective in human hyperlipidemic patents than either agent in monotherapy.[10] Ezetimibe caused a significant 18.5% lowering of low-density lipoprotein (LDL)-cholesterol in humans,[11] and it is thought that this class of cholesterol absorption inhibitors will be used clinically in combination with the HMG CoA reductase inhibitors.

The beagle dog is a standard model to study the hypocholesterolemic activity of HMG CoA reductase inhibitors.[12] We first determined the potency (ED_{50}, dose causing a 50% inhibition of increase in plasma cholesterol levels) of SCH 48461 and ezetimibe in cholesterol-fed dogs and the effect of a high dose of SCH 48461 on hepatic HMG CoA reductase activity in dogs. We then determined if the cholesterol absorption inhibitors, SCH 48461 and ezetimibe, would have increased hypocholesterolemic efficacy when administered in combination with HMG-CoA reductase inhibitors in noncholesterol-fed dogs.

MATERIALS AND METHODS

Inhibition of Dietary-Induced Hypercholesterolemia

Male, dogs (beagles, 9 to 14 kg, 1 to 4 years old) were fed Purina Dog Chow (#5006; St Louis, MO) supplemented with 5.5% lard, 0.2% cholate, and 1% cholesterol with or without added SCH 48461 or ezetimibe (SCH 58235). The diets were formulated to deliver intended daily doses of 0.3, 1, and 3 mg/kg SCH 48461 and 0.003, 0.01, and 0.03 mg/kg ezetimibe. The compounds were dissolved in an excess of ethanol to insure uniform mixing, and diets were periodically extracted and assayed for compound stability and concentrations. All diets were prepared by Research Diets (New Brunswick, NJ). Baseline blood samples were drawn from fasted dogs prior to initiating the study to obtain reference values for plasma cholesterol. Dogs were then randomized to groups with equivalent plasma cholesterol levels (n = 5/group) and consumed the appropriate diet for 7 days (300 g/day). Blood samples were obtained 24 hours after the last dose of SCH48461 or ezetimibe for plasma cholesterol determinations. Plasma cholesterol levels were determined by a modification of the cholesterol oxidase method of Allain et al,[13] in which the reagents were available in a kit form from Wako Pure Chemicals Industries (Osaka, Japan).

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Effect on Hepatic HMG CoA Reductase Activity

Twenty male beagles were randomly divided into 2 groups of 10 dogs each. One group was fed normal dog chow, and the other group was fed dog chow supplemented with 5.5% lard, 0.2% cholate, and 1% cholesterol. Half of the chow-fed or cholesterol-fed dogs received SCH 48461 in polyethylene glycol (PEG_{8000}/Tween 80) capsules (300 mg/kg), and half the dogs in each treatment group received PEG_{8000}/Tween 80 capsules as a placebo treatment.

Dogs were dosed once daily for 7 days. Twenty-four hours after receiving the last dose, dogs were anesthetized with pentobarbital sodium (50 mg/kg intravenou [IV]) and were killed via exsanguination. Blood samples were drawn prior to initiating the study and at sacrifice to obtain values for plasma lipids. Hepatic samples were obtained for use in microsome preparations for HMG CoA reductase activity determinations and for determination of hepatic lipid content.

To determine the hepatic cholesterol content, samples were lipid extracted according a modification of the procedure of Folch et al.[14] Samples were analyzed for cholesterol content by high-performance liquid chromatography (HPLC) as described previously.[15]

To determine hepatic HMG CoA reductase activity, liver microsomes were prepared from fresh tissue according to the method described by Burrier et al[16] and frozen at -80°C until utilized for assay. The assay for liver microsomal HMG CoA reductase activity was performed as reported by Goldstein et al.[17]

Effect of SCH 48461 and Ezetimibe in Combination With the HMG CoA Reductase Inhibitors in Chow-Fed Dogs

A series of noncholesterol-fed dog studies were performed with a similar experimental design. The doses of the HMG CoA reductase inhibitors were determined from previous reports and preliminary dose ranging studies.[12,18-23] In each study, 20 male beagles were divided into 4 groups with equivalent body weights (9 to 14 kg, 1 to 4 years old) and baseline plasma cholesterol levels (n = 5/group). Mean baseline plasma cholesterol levels were all in the normal range for dogs and varied from 119 mg/dL to 153 mg/dL among the studies. The dogs were fed Purina Dog Chow (#5006) containing maltodextrin and either 0.1 mg/kg SCH 48461 or 5mg/kg lovastatin or the combination of SCH 48461 (0.1 mg/kg) and lovastatin (5 mg/kg) for 14 days (300 g/d). This SCH 48461 dose was equivalent to the ED_{50} for inhibiting the increase in plasma cholesterol levels in cholesterol-fed dogs (Fig 1A). Nonfasting plasma samples were obtained at day 0, 3, 7, 11, and 14, and plasma total cholesterol levels were measured as described above.

In the next study, dogs were fed the chow containing either 0.007 mg/kg ezetimibe or 5 mg/kg lovastatin or the combination of ezetimibe (0.007 mg/kg) and lovastatin (5 mg/kg) for 14 days. This ezetimibe dose was equivalent to the ED_{50} for inhibiting the increase in plasma cholesterol levels in cholesterol-fed dogs (Fig 1B). Nonfasting plasma samples were obtained, and total plasma cholesterol levels were measured as described above. On day 15, a pharmacokinetic study was performed after the dogs had been fasted overnight. The above treatments were administered orally as suspensions in corn oil delivered in gelatin capsules. Plasma samples were obtained 1, 2, and 4 hours postdosing for determination of fasting plasma lovastatin and lovastatin hydroxy acid levels by a validated liquid chromatography/mass spectrophotometer/mass spectrophotometer (LC/MS/MS) method.

A lovastatin dose sparing study was evaluated in dogs fed chow containing either 0.007 mg/kg ezetimibe alone (drug alone control group) or 0.007 mg/kg ezetimibe in combination with lovastatin at 0.625, 1.25, or 2.5 mg/kg for 14 days. Nonfasting plasma cholesterol levels were measured periodically as above.

The hypocholesterolemic activity of pravastatin in combination with ezetimibe was evaluated in dogs fed chow containing either 0.007 mg/kg ezetimibe or 2.5 mg/kg pravastatin or the combination of ezetimibe (0.007 mg/kg) and pravastatin (2.5 mg/kg) for 14 days.[23] On day 15, a pharmacokinetic study was performed after the dogs had consumed the above treatments in the diets. Plasma samples were obtained 1, 2, 4, 6, and 8 hours postdosing for quantitation of pravastatin levels by a validated LC/MS/MS assay.

The hypocholesterolemic activity of fluvastatin in combination with ezetimibe was evaluated in dogs fed chow containing either 0.007 mg/kg ezetimibe or 5 mg/kg fluvastatin or the combination of ezetimibe (0.007 mg/kg) and fluvastatin (5 mg/kg) for 14 days. On day 15, a pharmacokinetic study was performed after the dogs had consumed the above treatments in the diets. Plasma samples were obtained 1, 2, 4, 6, 8, 12, and 24 hours postdosing for the determination of fluvastatin levels by a validated HPLC assay with fluorescence detection.

The hypocholesterolemic activity of simvastatin (1 mg/kg/d) and atorvastatin (1 mg/kg/d) in combination with ezetimibe (0.007 mg/kg/d) was evaluated in dogs with the same 2-week experimental design as above.

Results of these noncholesterol-fed dog studies are presented as means ± SE (n = 5/group). Statistical significance among responses in drug-treated and placebo-treated dogs was assessed using analysis of variance and Scheffe's F-statistic. *P* values less than .05 were considered significant.

The experiments described in this report were approved by the Schering-Plough Research Institute's Animal Care and Use Committee. They were also performed in accordance with the NIH *Guide to the Care and Use of Laboratory Animals* and the Animal Welfare Act in a program accredited by the American Association for Accreditation of Laboratory Animal Care.

RESULTS

Inhibition of Diet-Induced Hypercholesterolemia

The ability of SCH 48461 to inhibit the increase in plasma cholesterol induced by feeding beagles a cholesterol-cholic acid diet is shown in Fig 1A. In control animals, plasma cholesterol was increased by 126 ± 23 mg/dL ($P < .05$) from a basal value of 121 ±9 mg/dL after 1 week on the cholesterol-cholic acid diet. There was a marked, dose-dependent decrease in the diet-induced increase in plasma cholesterol in dogs that consumed a similar cholesterol-cholic acid diet with SCH 48461. The mean values for the inhibition of the increase in plasma cholesterol levels were 63% ± 8%, 76% ± 4% and 83% ± 3% at daily doses of 0.3, 1, and 3 mg/kg, respectively. An extrapolated ED_{50} of 0.1 mg/kg/d for SCH 48461 in inhibiting the increase in plasma cholesterol in cholesterol-fed beagles was determined using log-linear regression analysis.

Figure 1B also summarizes the ability of ezetimibe (SCH 58235) to inhibit the increase in plasma cholesterol induced by feeding beagles a cholesterol-cholic acid diet. In control animals, plasma cholesterol was increased by 165 ± 20 mg/dL ($P < .05$) from a basal value of 119 ± 13 mg/dL after 1 week on the cholesterol-cholic acid diet. In dogs that received daily doses of 0.003, 0.01, and 0.03 mg/kg ezetimibe, there was a dose-dependent decrease in the diet-induced increase of plasma cholesterol in these animals (Fig 1B). Using log-linear regression analysis, an ED_{50} of 0.007 mg/kg/d was extrapolated for ezetimibe in inhibiting the increase in plasma cholesterol in cholesterol-fed beagles.

Hepatic HMG CoA Reductase Activity

SCH 48461 was previously found to induce hepatic HMG CoA reductase activity in chow-fed hamsters, an observation

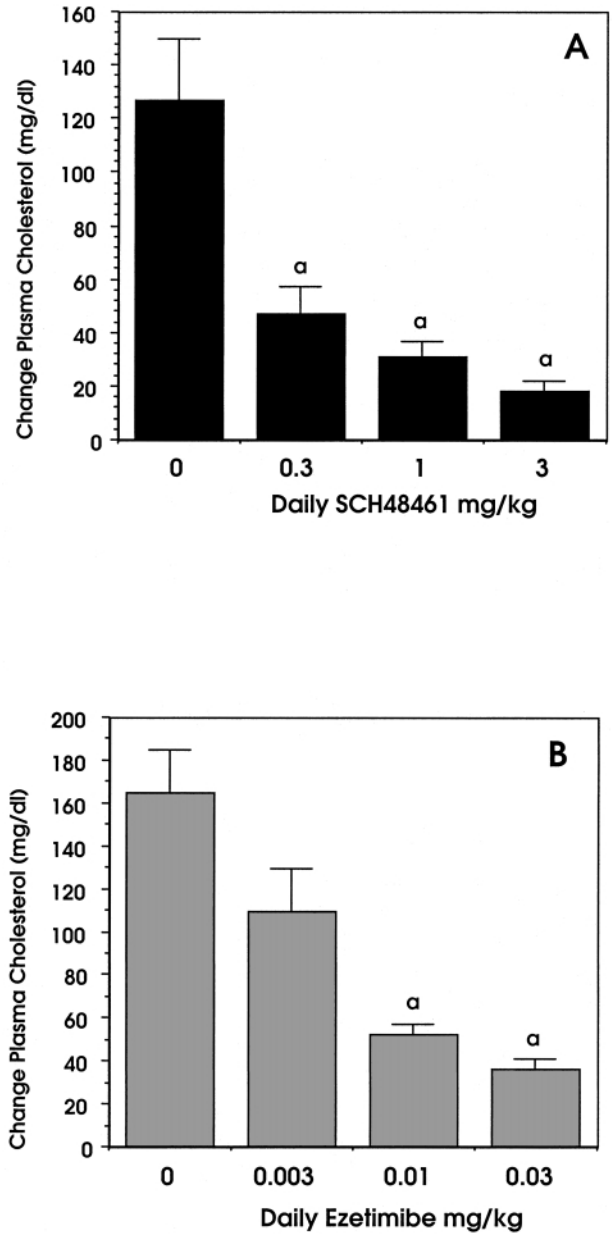


Fig 1. Effects of SCH 48461 and ezetimibe on diet-induced increases in plasma cholesterol in cholesterol-fed male beagles. Dogs were fed a chow diet supplemented with 5.5% lard, 0.2% cholate, and 1% cholesterol with or without added SCH 48461 (A) or ezetimibe (B) (SCH 58235). Blood samples were obtained 24 hours after the last dose of SCH 48461 or ezetimibe on day 7 for plasma cholesterol determinations. Values denote 7-day changes in plasma cholesterol levels (means ± SEM) for 5 dogs/treatment, $^{a}P < .05$ compared with cholesterol-fed control group.

that attributed to this compound's modest hypocholesterolemic activity.[1] The only study in which the effect of SCH48461 on dog hepatic HMG CoA reductase activity could be assessed was in a high-dose 7-day safety study. Beagles were fed a cholesterol-cholic acid enriched diet or dog chow and dosed with SCH 48461 at 300 mg/kg daily for 7 days. Plasma lipids were assessed at the beginning and at the end of the 7-day dosing interval in all the treatment groups. Table 1 shows that in the chow-fed group of dogs, total plasma cholesterol was unchanged in the nondrug-treated animals. The total plasma cholesterol in chow-fed beagles that received the high dose of SCH 48461 (300 mg/kg) was slightly (14%), although significantly less ($P < .05$, paired t test) after 1 week of drug treatment. In contrast, dogs that received the cholesterol-cholic acid diet experienced a significant 100 mg/dL elevation in their plasma total cholesterol levels (Table 1). SCH 48461 administration at 300 mg/kg/d completely blocked the increase in plasma cholesterol due to the ingestion of cholesterol-cholic acid diet, and there was no significant difference in their plasma cholesterol values before or after 7 days on diet/drug treatment (Table 1).

Hepatic total cholesterol content was not different in chow-fed placebo dogs or in chow-fed SCH 48461-treated dogs (Table 1). Hepatic total cholesterol content tended to be less in drug-treated dogs, but this difference did not attain statistical significance. An approximate 4-fold increase in hepatic cholesterol content in cholesterol-fed, placebo-treated dogs compared with control, chow-fed animals was significant ($P < .05$). This diet-induced hepatic cholesterol loading was significantly attenuated by SCH 48461 treatment. In fact, the hepatic cholesterol load in these cholesterol-fed, drug-treated dogs was not significantly different than the cholesterol content of placebo-treated, chow-fed dogs.

The effect of dietary cholesterol and SCH 48461 (300 mg/kg/d) treatment on hepatic HMG CoA reductase activity was assessed in isolated hepatic microsome preparations. As shown in Table 1, in livers of the chow-fed dogs treated with SCH 48461, there was a 3.8-fold higher microsomal HMG CoA reductase activity than that of chow-fed control animals. Dietary cholesterol supplementation, as expected, resulted in a dramatic decrease in the activity of HMG CoA reductase compared with those of chow-fed, placebo-treated dogs. Treatment of cholesterol-fed animals with 300 mg/kg/d SCH 48461 produced a 31-fold increase in hepatic HMG CoA reductase activity above the levels observed in the cholesterol-fed, placebo-treated beagles and equivalent levels to the chow-fed, SCH 48461-treated group. These results suggest that SCH 48461 upregulates hepatic cholesterol biosynthesis due to the inhibition of intestinal absorption of biliary and dietary cholesterol.

Effect of SCH 48461 and Ezetimibe in Combination With the HMG CoA Reductase Inhibitors in Chow-Fed Dogs

SCH 48461 was studied in normocholesterolemic dogs to evaluate whether SCH 48461 alone would be effective in this species and if the combination with the cholesterol biosynthesis inhibitor, lovastatin, was required to demonstrate a hypocholesterolemic effect. Male beagles fed chow containing either SCH 48461 at 0.1 mg/kg/d or lovastatin at 5 mg/kg/d resulted in plasma cholesterol levels, which were unchanged from baseline over the 14-day treatment period (Fig 2A). The combination of SCH 48461 at 0.1 mg/kg/d and lovastatin at 5 mg/kg/d caused a 36% reduction in total plasma cholesterol levels at day 14 compared with baseline at day 0 (Fig 2A). The plasma cholesterol levels in the group given the combination were also

Table 1. Effect of SCH 48461 (300 mg/kg) in Chow-Fed and Cholesterol-Fed Dogs

Treatment Group (n = 5/group)	Baseline Plasma Cholesterol (mg/dL)	Terminal Plasma Cholesterol (mg/dL)	Hepatic HMG CoA Reductase (pmol/min/mg protein)	Hepatic Cholesterol (mg/g)
Chow-fed control	147 ± 18	146 ± 23	19.0 ± 4.1	2.11 ± 0.34
Chow-fed SCH 48461	152 ± 19	126 ± 14*	72.0 ± 12.9†‡	1.59 ± 0.22
Cholesterol-fed Control	147 ± 11	247 ± 26*†	1.8 ± 0.6†	8.81 ± 1.82†
Cholesterol-fed SCH 48461	153 ± 12	146 ± 7‡	56.2 ± 11.5†‡	1.87 ± 0.40‡

NOTE. Values are means ± SEM.
*$P < .05$ compared with baseline values.
†$P < .05$ compared with chow-fed control group.
‡$P < .05$ compared with cholesterol-fed control group.

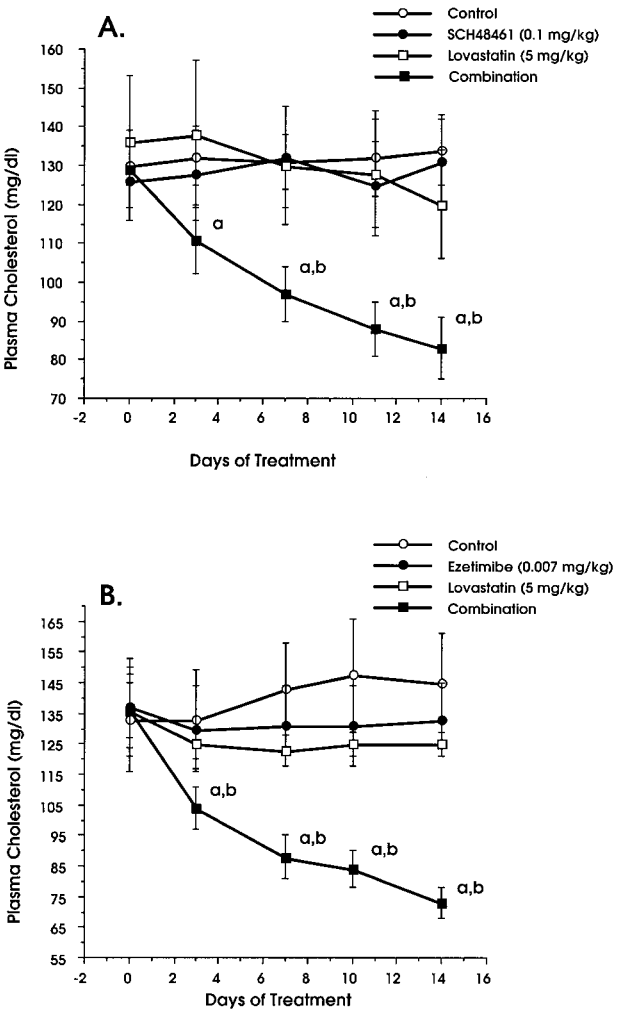


Fig 2. Effect of SCH 48461 and ezetimibe alone or in combination with lovastatin on plasma cholesterol levels in chow-fed dogs. Dogs were fed a chow diet containing SCH 48461 (0.1 mg/kg/d) (A) or ezetimibe alone (0.007 mg/kg/d) (B), HMG CoA reductase inhibitor alone (lovastatin 5 mg/kg/d), or their combination for 14 days. The a signifies that the change in plasma cholesterol is significantly ($P < .05$, ANOVA) different from that in the control group, and the b indicates the response is also significantly different from that of dogs treated with SCH 48461 (A) or ezetimibe (B) or lovastatin alone. Values are means ± SEM with 5 dogs/group.

significantly lower than levels in either group administered SCH 48461 or lovastatin alone at day 14 (Fig 2A). SCH 48461 and lovastatin alone fail to reduce plasma cholesterol levels in normocholesterolemic, chow-fed dogs at these doses. When these compounds were given in combination, a significant, synergistic hypocholesterolemic effect occurred.

Ezetimibe was also administered for 14 days at its ED_{50} for inhibiting the increase in plasma cholesterol levels in cholesterol-fed dogs (0.007 mg/kg/d) to dogs fed a chow diet either alone or in combination with lovastatin (5 mg/kg/d). Neither ezetimibe nor lovastatin alone had significant effects on plasma cholesterol levels in the chow-fed dogs. In contrast, their combination caused a 50% reduction in plasma cholesterol levels at day 14 (Fig 2B). To determine if ezetimibe had a pharmacokinetic interaction with lovastatin, on day 15, the dogs were fasted and given the compounds in capsules. Both the inactive lactone prodrug lovastatin and the active hydroxy acid lovastatin fasting plasma levels were determined over 4 hours. Drug exposure as assessed by fasting plasma area under the curve (AUC) for 0 to 4 hours was compared between the treatment groups. Plasma lovastatin AUCs over 4 hours were 53 ± 19 and 94 ± 26 ng/h/mL, and lovastatin hydroxy acid levels were 167 ± 37 and 119 ± 14 ng/h/mL for the lovastatin alone and the ezetimibe plus lovastatin groups, respectively. The total plasma lovastatin exposures were not significantly different between the lovastatin alone group and the combination group.

The dose of lovastatin required to demonstrate hypocholesterolemic synergy with ezetimibe was determined. Because ezetimibe at 0.007 mg/kg/d did not reduce plasma cholesterol levels (Fig 2B), this dose was used as an ezetimibe alone control group. The dogs were fed Purina Dog Chow containing either 0.007 mg/kg/d ezetimibe alone or 0.007 mg/kg/d ezetimibe in combination with lovastatin at 0.625, 1.25, or 2.5 mg/kg/d for 14 days. All doses of lovastatin in combination with ezetimibe caused significant reductions in plasma cholesterol levels when compared with the ezetimibe alone control group by day 14 (Fig 3).

To determine if ezetimibe would demonstrate synergistic activity in dogs with other HMG CoA reductase inhibitors with different metabolic excretion patterns, ezetimibe was combined with pravastatin or fluvastatin.[19-21,23] Both ezetimibe at 0.007 mg/kg/d and pravastatin at 2.5 mg/kg/d alone caused modest reductions in plasma cholesterol levels (Fig 4A). Pravastatin at 2.5 mg/kg/d combined with ezetimibe at 0.007 mg/kg/d for 14 days caused a 41% synergistic reduction in plasma cholesterol

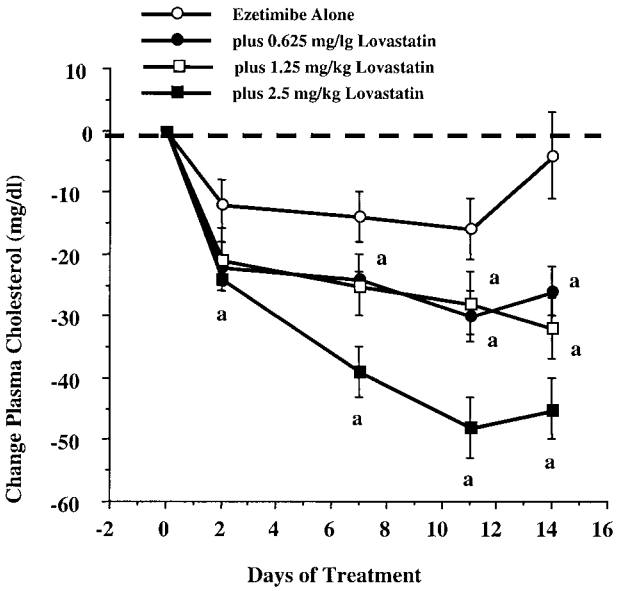


Fig 3. Effect of ezetimibe alone or in combination with varying doses of lovastatin on plasma cholesterol levels in chow-fed dogs. Dogs were fed a chow diet containing ezetimibe alone (0.007 mg/kg/d) or the combination with the HMG CoA reductase inhibitor lovastatin at 0.625, 1.25, or 2.5 mg/kg/d for 14 days. The a signifies that the change in plasma cholesterol is significantly ($P < .05$, ANOVA) different from that in the dogs treated with ezetimibe alone control group. Values are means ± SEM with 5 dogs/group.

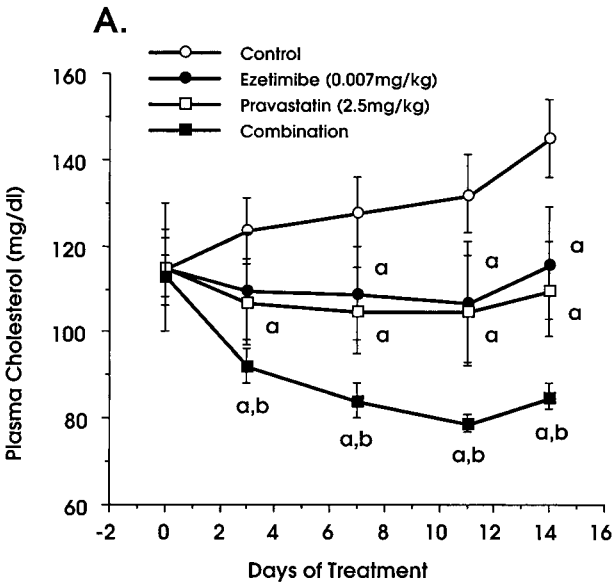
levels in the chow-fed dogs. On day 15, a pharmacokinetic study was performed after the dogs had consumed the treatments in the diets. Plasma pravastatin exposures were determined over 8 hours postdosing and were found not to be significantly different between the pravastatin alone (264 ± 62 ng/h/mL, 0 to 8 hours AUC) and the ezetimibe combined with pravastatin groups (344 ± 40 ng/h/mL, 0 to 8 hours AUC).

Ezetimibe was also combined with the HMG CoA reductase inhibitor, fluvastatin, which undergoes limited metabolism in dogs.[20] Fluvastatin alone at 5 mg/kg/d caused a 38% ($P < .05$) reduction in plasma cholesterol levels at day 14 in the chow-fed dogs. The addition of 0.007 mg/kg/d of ezetimibe to the fluvastatin (5 mg/kg/d) caused a synergistic plasma cholesterol decrease of 60% relative to the control group (Fig 4B). On day 15, a pharmacokinetic study was performed after the treatments were given in the diet. Plasma fluvastatin exposures were determined over 24 hours postdosing, and no significant differences were found between the exposures in the ezetimibe combination group (16,260 ± 2,244 ng/h/mL, 0 to 24 hours AUC) and the fluvastatin alone group (10,749 ± 2,901 ng/h/mL, 0 to 24 hours AUC).

Simvastatin and atorvastatin at 1 mg/kg/d were also combined with ezetimibe at 0.007 mg/kg/d in noncholesterol-fed dogs. Simvastatin and ezetimibe did not significantly reduce plasma cholesterol levels alone, but their combination caused a 30% reduction relative to the control group at 14 days of treatment (Fig 5A). Atorvastatin caused a modest reduction in plasma cholesterol alone at 1 mg/kg/d. When atorvastatin was combined with ezetimibe, plasma cholesterol dropped 30% relative to the control group at day 14 (Fig 5B).

DISCUSSION

This class of cholesterol absorption inhibitors, which include ezetimibe and SCH 48461, prevent the absorption of choles-



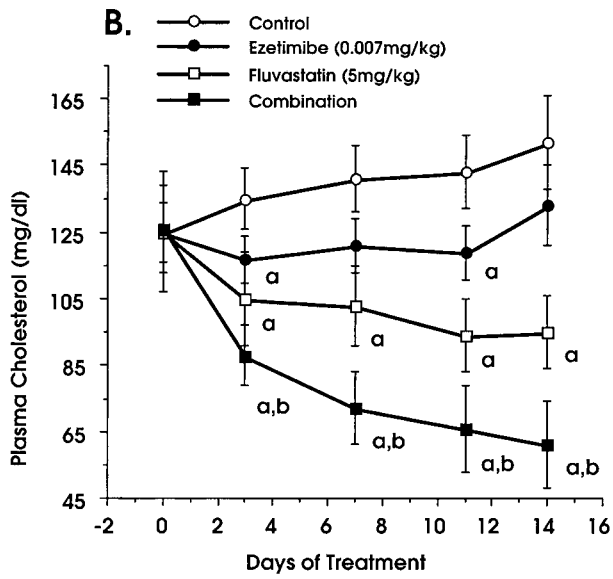


Fig 4. Effect of ezetimibe alone or in combination with pravastatin (A) or fluvastatin (B) on plasma cholesterol levels in chow-fed dogs. Dogs were fed a chow diet containing ezetimibe alone (0.007 mg/kg/d), HMG CoA reductase inhibitor alone (A) pravastatin 2.5 mg/kg/d, (B) fluvastatin 5 mg/kg/d, or their combination for 14 days. The a signifies that the change in plasma cholesterol is significantly ($P < .05$, ANOVA) different from that in the control group, and the b indicates the response is also significantly different from that of dogs treated with ezetimibe or statin alone. Values are means ± SEM with 5 dogs/group.

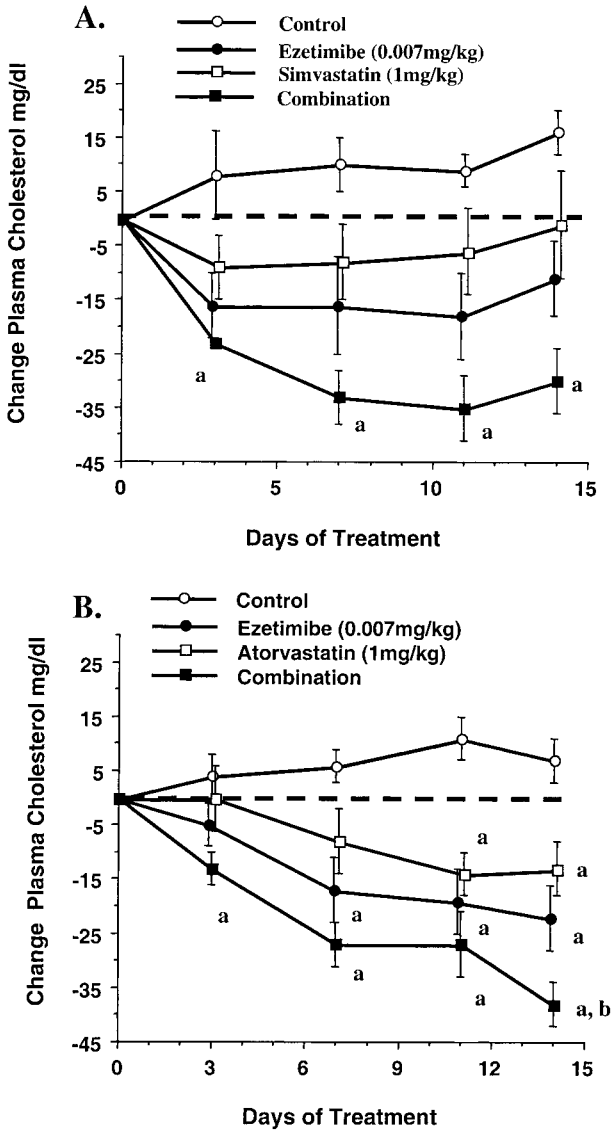


Fig 5. Effect of ezetimibe alone or in combination with simvastatin (A) or atorvastatin (B) on plasma cholesterol levels in chow-fed dogs. Dogs were fed a chow diet containing ezetimibe alone (0.007 mg/kg/d), HMG CoA reductase inhibitor alone (A) simvastatin 1 mg/kg/d, (B) atorvastatin 1 mg/kg/d, or their combination for 14 days. The a signifies that the change in plasma cholesterol is significantly ($P < .05$, ANOVA) different from that in the control group, and the b indicates the response is also significantly different from that of dogs treated with ezetimibe or statin alone. Values are means ± SEM with 5 dogs/group.

terol by inhibiting the passage of dietary and biliary cholesterol across the intestinal wall. These compounds are potent, selective cholesterol absorption inhibitors, which are efficacious in hypercholesterolemic animal models.[1-5] In animals fed diets without the addition of cholesterol, these cholesterol absorption inhibitors cause modest reductions in plasma cholesterol levels.[1] It was previously reported that noncholesterol-fed hamsters upregulate their hepatic HMG CoA reductase activity in response to SCH 48461 treatment.[1] In the present study, SCH 48461 was found to upregulate hepatic HMG CoA reductase activity 3.8-fold in chow-fed dogs and only reduce plasma cholesterol levels 14%. The upregulation of cholesterol biosynthesis in response to the chronic inhibition of biliary cholesterol absorption may be responsible for the modest plasma cholesterol lowering activity seen with these compounds in noncholesterol-fed animals. We investigated whether the inhibition of hepatic cholesterol synthesis with HMG CoA reductase inhibitors (statins) would block this compensatory increase in synthesis and a greater reduction in plasma cholesterol would result in combination with the cholesterol absorption inhibitors SCH 48461 and ezetimibe.

The beagle dog is a widely used model to study hypocholesterolemic drugs and was used to evaluate the activity of the cholesterol absorption inhibitors and HMG CoA reductase inhibitors.[12] The ability of SCH 48461 and ezetimibe to inhibit the increase in plasma cholesterol levels in dogs fed a diet containing 1% cholesterol/0.5% cholate/5.5% lard was established. It was found that SCH 48461 had an ED_{50} of 0.1 mg/kg/d, and ezetimibe had an ED_{50} of 0.007 mg/kg/d in the cholesterol-fed dogs. Knowing the ED_{50} for inhibition of diet-induced hypercholesterolemia allowed for the examination of the effect of these cholesterol absorption inhibitors in combination with HMG CoA reductase inhibitors at comparable levels of activity in dogs. Ezetimibe had also been found to be approximately 50-fold more potent than SCH 48461 in cholesterol-fed hamsters and 400-fold more potent in cholesterol-fed rhesus monkeys.[2-4]

The HMG CoA reductase inhibitor, lovastatin, was evaluated in combination with SCH 48461 to determine if inhibiting the upregulation of cholesterol synthesis was required to demonstrate a hypocholesterolemic effect. SCH 48461 was administered to noncholesterol-fed dogs at its hypocholesterolemic ED_{50} found in cholesterol-fed dogs (0.1 mg/kg/d). Lovastatin was given at 5 mg/kg/d, a dose reported to have no effect on plasma cholesterol levels in dogs,[18] which resulted in plasma cholesterol levels that were unchanged from baseline over the 14-day treatment period. The combination of SCH 48461 and lovastatin at 5 mg/kg/d caused a 36% synergistic reduction in total plasma cholesterol levels. Ezetimibe was also administered for 14 days at its ED_{50} for inhibiting the increase in plasma cholesterol levels in cholesterol-fed dogs (0.007 mg/kg/d) to dogs fed a chow diet either alone or in combination with lovastatin (5 mg/kg/d). Both ezetimibe and lovastatin alone had no significant effect on plasma cholesterol levels in the chow-fed dogs. Their combination caused a synergistic 50% reduction in plasma cholesterol levels. Lovastatin at high doses will reduce plasma cholesterol levels in dogs.[12,18] Lovastatin is an inactive prodrug lactone, which is converted in vivo to the active beta-hydroxy acid form.[18,19] Thus, we investigated if ezetimibe increased plasma lovastatin exposure by a pharmacokinetic interaction to reduce plasma cholesterol levels. The results of the study show that plasma lovastatin and lovastatin hydroxy acid exposures were not significantly different between the lovastatin alone and the combination group. Ezetimibe is primarily metabolized by glucuronidation without significant cytochrome P450 enzyme metabolism, therefore, a pharmacokinetic interaction with lovastatin or other com-

pounds metabolized by cytochrome P450 enzymes would not be expected.[3,24-26]

Additional dog combination studies with ezetimibe and lovastatin were performed to see if a hypocholesterolemic synergy could be observed at lower doses of lovastatin. Combinations with lovastatin at 0.625, 1.25, or 2.5 mg/kg/d with ezetimibe caused significant reductions in plasma cholesterol levels when compared with the ezetimibe alone. These findings suggest that ezetimibe may result in lovastatin dose sparing when used clinically in combination.

The combination of ezetimibe with pravastatin caused a synergistic reduction in plasma cholesterol levels in the chow-fed dogs. Pravastatin is not metabolized by the cytochrome P450 enzymes that metabolize lovastatin, and pravastatin is primarily excreted in the urine, while lovastatin is excreted in the bile.[19,21,23] Plasma exposures to pravastatin were similar when pravastatin was given alone or in combination with ezetimibe in the dogs. These results indicate that the hypocholesterolemic synergy with pravastatin and ezetimibe was not associated with a pharmacokinetic interaction between the compounds. The combination of ezetimibe with fluvastatin resulted in a 60% reduction in plasma cholesterol in chow-fed dogs. Fluvastatin is primarily excreted in the feces after limited oxidative metabolism in dogs.[20] The large synergistic hypocholesterolemic effect with ezetimibe combined with fluvastatin was not associated with an increased plasma exposure to fluvastatin.

Ezetimibe (0.007 mg/kg/d) was also combined with simvastatin and atorvastatin at 1 mg/kg/d. At these low combination doses, a synergistic reduction in plasma cholesterol levels was found in the chow-fed dogs. Simvastatin's metabolism is similar to lovastatin, with a conversion of the lactone to the active hydroxy acid form and excretion primarily through the feces.[19,21] Atorvastatin is metabolized primarily through cytochrome P450 enzyme-mediated aromatic hydroxylation and is also primarily excreted in the feces.[22] Preliminary clinical results with the combination of ezetimibe and simvastatin have been presented.[27] Individuals with hypercholesterolemia were given 10 mg/d of ezetimibe and 10 mg/d of simvastatin for 14 days and an additive 52% reduction in LDL cholesterol levels was reported.[27] In this clinical study, ezetimibe had no effect on the pharmacokinetics of simvastatin. Additional clinical studies have shown that ezetimibe does not alter the metabolism of a variety of agents metabolized by cytochrome P450 enzymes.[26]

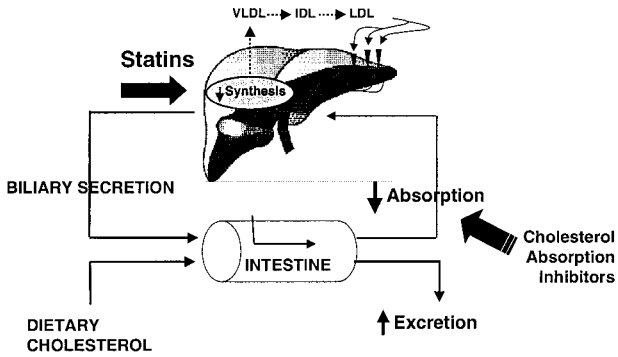


Fig 6. Complementary actions of HMG CoA reductase inhibitors (statins) and selective cholesterol absorption inhibitors (ezetimibe) to reduce plasma cholesterol levels.

Overall, ezetimibe caused a synergistic reduction in plasma cholesterol in dogs when combined with 5 different HMG CoA reductase inhibitors with differing routes of excretion and metabolism.[18-23] HMG CoA reductase inhibitors (statins) act primarily in the liver by inhibiting cholesterol biosynthesis, which results in an upregulation of LDL receptors and a reduction in LDL-cholesterol levels. It is our hypothesis that the synergistic hypocholesterolemic activity occurs through the inhibition of both biliary and dietary cholesterol absorption by ezetimibe and the inhibition of the compensatory increase in hepatic cholesterol biosynthesis by the HMG CoA reductase inhibitors as outlined in Fig 6. This hypocholesterolemic synergy was not associated with any plasma pharmacokinetic interaction with the HMG CoA reductase inhibitors. Ezetimibe should be effective clinically in combination with HMG CoA reductase inhibitors (statins) in reducing plasma cholesterol to target levels, even under restricted dietary intake of cholesterol.

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