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Ezetimibe: a selective inhibitor of cholesterol absorption

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Ezetimibe is a novel selective cholesterol absorption inhibitor that effectively blocks intestinal absorption of dietary and biliary cholesterol. Ezetimibe undergoes glucuronidation to a single metabolite and is localized in the intestinal wall, where it prevents cholesterol absorption. Enterohepatic recirculation of ezetimibe and the glucuronide ensures repeated delivery to the site of action and limits peripheral exposure. Ezetimibe does not affect the absorption of fat-soluble vitamins or triglycerides. Results from pre-clinical studies in various animal models have shown the lipid-lowering and anti-atherosclerotic properties of ezetimibe as a single agent, and a synergistic effect when combined with a statin. In cholesterol-fed rhesus monkeys, ezetimibe reduced both plasma cholesterol (ED_{50}= 0·0005 mg . kg^{-1} . day^{-1}) and low-density lipoprotein cholesterol levels in a dose-dependent manner. In apo E knockout mice, ezetimibe reduced serum cholesterol more than 50% and decreased carotid (97%) and aortic (47–87%) atherosclerosis. Ezetimibe inhibited the rise of plasma cholesterol in cholesterol-fed dogs (ED_{50}=0·007 mg.kg^{-1}.day^{-1}). Co-administration of ezetimibe and lovastatin in chow-fed dogs synergistically reduced plasma cholesterol to levels lower than those achieved with either agent alone (P<0·05). These results suggest that ezetimibe combined with a statin may similarly reduce plasma cholesterol levels in patients with hypercholesterolaemia.

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Key Words: Cholesterol absorption inhibitor, ezetimibe, pre-clinical studies, atherosclerosis, hypercholesterolaemia, statins.

Introduction

Ezetimibe (SCH 58235) (Fig. 1) is a highly potent and selective cholesterol absorption inhibitor that prevents absorption of cholesterol from dietary and biliary sources by preventing transport of cholesterol through the intestinal wall[1,2]. Ezetimibe prevents intestinal absorption of cholesterol without affecting absorption of triglycerides, fatty acids, bile acids or fat-soluble vitamins[3], unlike pancreatic lipase inhibitors (e.g. orlistat), which affect the absorption of triglycerides[4,5] and may decrease the absorption of fat-soluble vitamins[6]. Furthermore, ezetimibe also differs from the bile acid sequestrants (e.g. cholestyramine) which interfere with absorption of vitamins A, D, E and K, taurocholate and bile acids[7,8].

Ezetimibe undergoes phase II metabolism[9], yielding a glucuronide that appears to be pharmacodynamically active. Following intraduodenal delivery to rodents, ezetimibe is rapidly and extensively glucuronidated in the intestinal wall, is absorbed into the portal plasma and passes through the liver into the bile within minutes[2]. Once glucuronidated, ezetimibe circulates enterohepatically, repeatedly returning the drug back to the primary site of action (the intestine), thus limiting peripheral exposure. This recirculation may explain the long duration of action of ezetimibe.

Ezetimibe localizes in the intestinal wall, mainly as the phenolic glucuronide (SCH 60663)[2]. Comparative studies suggest that the glucuronide is a more potent inhibitor of cholesterol absorption than ezetimibe[2], and the localization of the glucuronide at the intestinal villi may explain its apparent increase in potency compared with ezetimibe.

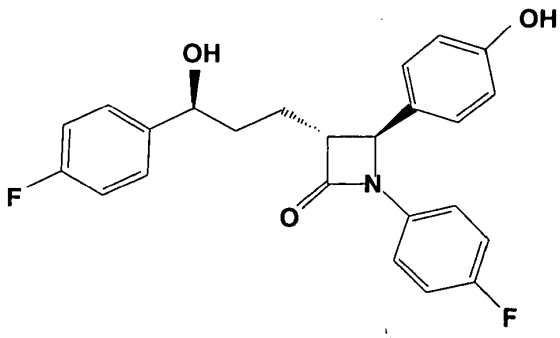

Figure 1 **Structure of ezetimibe.**

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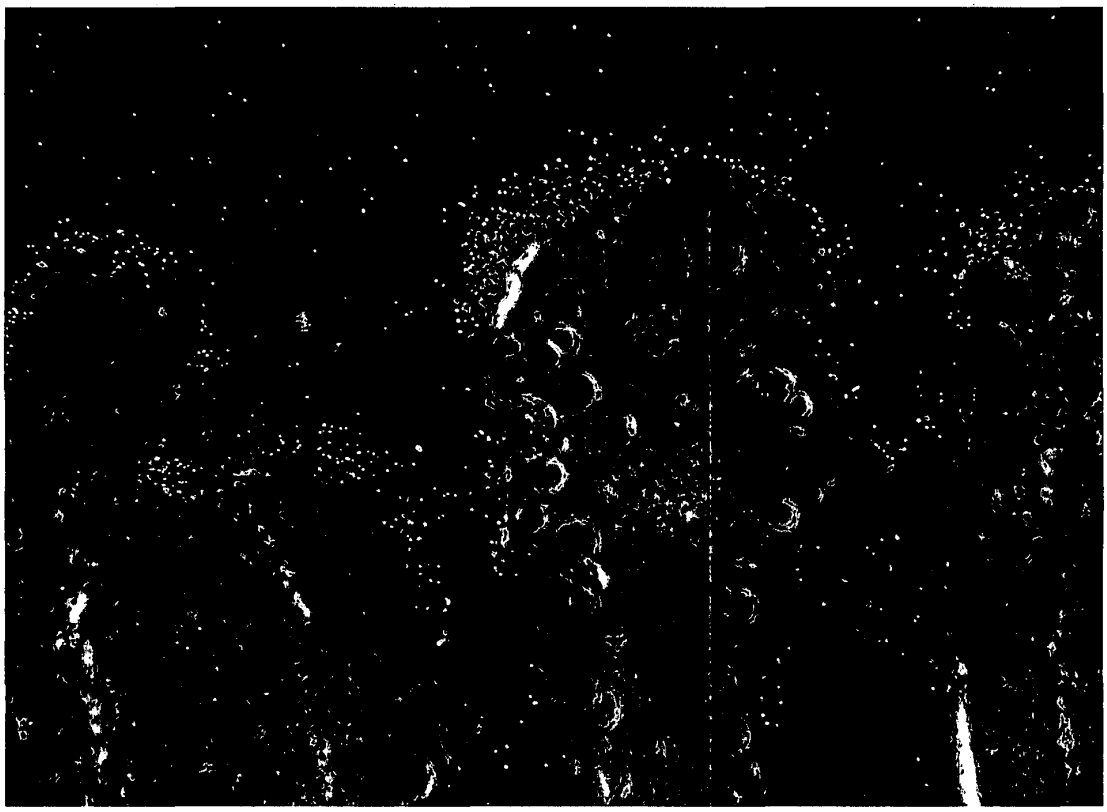

Figure 2 **Autoradiographic analysis of intestinal wall after intravenous administration of ^{3}H-ezetimibe in bile-cannulated rats. Three distinct villi are shown. The dark background is the intestinal lumen. ^{3}H-ezetimibe concentrates on the surface of the enterocytes at the tips of the villi (dark-field microscopy, original magnification 250 ×). (Reproduced with permission[2].)**

Specific localization of radiolabelled ezetimibe to the intestinal villi, its site of action, has been reported in bile-cannulated rats intravenously injected with ^{3}H-ezetimibe[2]. Autoradiographic analysis of cross-sections of the intestines of these rats shows that ezetimibe is located throughout the intestinal villi and is concentrated in the tips of the intestinal villi (Fig. 2).

This paper reviews data from several pre-clinical studies in various animal models. The first series of studies evaluated the lipid-lowering effects of ezetimibe in cholesterol-fed rhesus monkeys, as well as the effects of an ezetimibe analogue on postprandial chylomicron cholesterol content[10]. Another study in apo E knockout mice examined the lipid-lowering effects of ezetimibe, as well as its effects on atherosclerosis in this animal model[11]. Finally, data from two studies in dogs are reviewed[12]. The first study was designed to determine whether ezetimibe would decrease plasma cholesterol levels in cholesterol-fed dogs, and the second evaluated whether the combination of ezetimibe with lovastatin would decrease plasma total cholesterol levels in animals fed a cholesterol-free chow diet.

Effects of ezetimibe in cholesterol-fed monkeys

A series of studies was conducted to determine the effects of ezetimibe on plasma lipids and lipoprotein composition in monkeys with diet-induced hypercholesterolaemia[10]. All monkeys were fed a chow-based, high-fat, high-cholesterol (i.e. 'western') diet containing 0·25% cholesterol and 22·5% fat (15% coconut oil and 7·5% olive oil).

In one series of studies, groups of five rhesus monkeys received either the western diet alone or with ezetimibe (0·3, 1, 3 or 10 $\mu g \cdot kg^{-1} \cdot day^{-1}$) admixed in the diet. After 3 weeks, plasma cholesterol was significantly ($P<0·05$) lower in ezetimibe-treated monkeys compared with control animals at all doses tested. Ezetimibe inhibited the expected increase in both plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C) in a dose-dependent fashion (ED_{50}= 0·5 $\mu g \cdot kg^{-1} \cdot day^{-1}$, or 0·0005 $mg \cdot kg^{-1} \cdot day^{-1}$). Ezetimibe did not affect plasma high-density lipoprotein cholesterol (HDL-C) or triglyceride levels[10].

In another experiment in rhesus monkeys, a crossover design was used to evaluate the duration of action of ezetimibe and the time course of the reversal of pre-established hypercholesterolaemia. Control animals and ezetimibe-treated animals ($100\ \mu g \cdot kg^{-1} \cdot day^{-1}$ admixed in the diet; five per group) were fed their respective diets for 20 days. Monkeys receiving the cholesterol-containing diet without drug had a marked increase in cholesterol concentration over the 20-day period, but there was a complete inhibition of the dietary effect in animals receiving the diet with ezetimibe. Treatments were switched at 20 days, i.e. the monkeys that had received the cholesterol diet with ezetimibe then received the diet without the drug, and those that had received the cholesterol diet only were given the diet with ezetimibe. Despite a daily intake of 375 mg of dietary cholesterol for 20 days, plasma cholesterol remained unchanged from baseline levels for 3 days after discontinuation of ezetimibe. Withdrawal of ezetimibe eventually did reverse the cholesterol-lowering effect, with significant increases in plasma cholesterol levels seen 10 days after cessation of the drug. Ezetimibe had a rapid onset of action after administration and a reversal of effect after withdrawal[10].

A single $10\ mg \cdot kg^{-1}$ dose of an analogue of ezetimibe (SCH 48461)[1] was given to a small group of cynomolgus monkeys to evaluate postprandial chylomicron cholesterol content. The animals were fasted for 20 h and then fed the high-fat, high-cholesterol diet in one single meal with or without the ezetimibe analogue. Blood samples were collected 5 h later. Chylomicrons and chylomicron remnants were isolated and analyzed for cholesteryl ester, free cholesterol and triglyceride content. A single dose of the ezetimibe analogue decreased postprandial chylomicra cholesterol content by 69% ($P<0.05$), with no significant effect on the triglyceride content[10].

Effects of ezetimibe in apo E knockout mice

The apo E knockout (KO) mouse is a widely used model to study the development and progression of atherosclerosis. Apo E is an LDL-C receptor ligand that is important in the regulation of serum cholesterol and lipid levels. Inactivation of the gene coding for apo E is associated with high serum cholesterol levels and premature, spontaneous development of atherosclerosis in mice. In one study conducted in this animal model, lipoprotein and atherosclerotic changes were determined in apo E KO mice fed either a low-fat diet (10 kcal% corn oil, 0·15% cholesterol) or a high-fat (western) diet (40 kcal% butter fat, 0·15% cholesterol)[11]. Ezetimibe ($5\ mg \cdot kg^{-1} \cdot day^{-1}$) reduced plasma cholesterol levels by more than 60% in apo E KO mice fed either diet for 6 months. These reductions occurred in the very-low-density lipoprotein and low-density lipoprotein fractions. Ezetimibe increased HDL-C levels more than twofold in animals fed either the low-fat or western diet. Moreover, ezetimibe inhibited the development and progression of aortic atherosclerosis in both the western and low-fat groups of apo E KO mice. Ezetimibe also reduced carotid artery atherosclerosis in the apo E KO mice (Fig. 3). The drug's anti-atherosclerotic effect was demonstrated by a 97% reduction in the atherosclerosis in the intimal area of the carotid artery as well as a 47–87% reduction in surface areas of aortic lesions.

Co-administration of ezetimibe with a statin in dogs

Before evaluating the co-administration of ezetimibe and a statin in the dog, investigators wished to verify whether ezetimibe blocked cholesterol absorption in this animal model. Beagle dogs were fed a diet containing 1% cholesterol, 0·2% cholic acid and 5·5% lard alone (control) or with ezetimibe (0·003, 0·01 or $0·03\ mg \cdot kg^{-1}$) for 7 days. Ezetimibe significantly inhibited the rise in plasma total cholesterol levels in these cholesterol-fed dogs ($ED_{50}=0·007\ mg \cdot kg^{-1} \cdot day^{-1}$) ($P<0·05$ compared with controls)[12].

A study in normocholesterolaemic dogs was then conducted to assess the effects of the co-administration of ezetimibe and lovastatin[12]. Beagle dogs were fed a cholesterol-free chow diet and given ezetimibe ($0·007\ mg \cdot kg^{-1}$), lovastatin ($5\ mg \cdot kg^{-1}$) or a combination of ezetimibe and lovastatin for 14 days. Neither ezetimibe nor lovastatin alone significantly affected plasma cholesterol levels. The combination of ezetimibe with lovastatin, however, synergistically reduced plasma total cholesterol levels by 50% ($P<0·05$ compared with control and either agent alone) from day 3 of treatment to day 14. Thus, combined therapy with ezetimibe and lovastatin decreased plasma cholesterol concentrations significantly more than either agent alone.

Discussion

Ezetimibe is the first of a new class of lipid-modifying drugs, the selective cholesterol absorption inhibitors. Ezetimibe inhibited cholesterol absorption and reduced plasma total cholesterol concentrations in a number of pre-clinical models. Ezetimibe also increased HDL-C levels in apo E knockout mice given either a low-fat or western diet; furthermore, ezetimibe inhibited the progression of atherosclerosis in apo E knockout mice under both dietary conditions, suggesting an effect independent, at least in part, from the type of diet.

Ezetimibe effectively reduced plasma cholesterol and LDL-C levels in animals fed a high-cholesterol diet. Ezetimibe prevented diet-induced hypercholesterolaemia and reduced pre-established hypercholesterolaemia in rhesus monkeys fed a high-fat, western diet. Because of the long duration of action of ezetimibe, the effect of

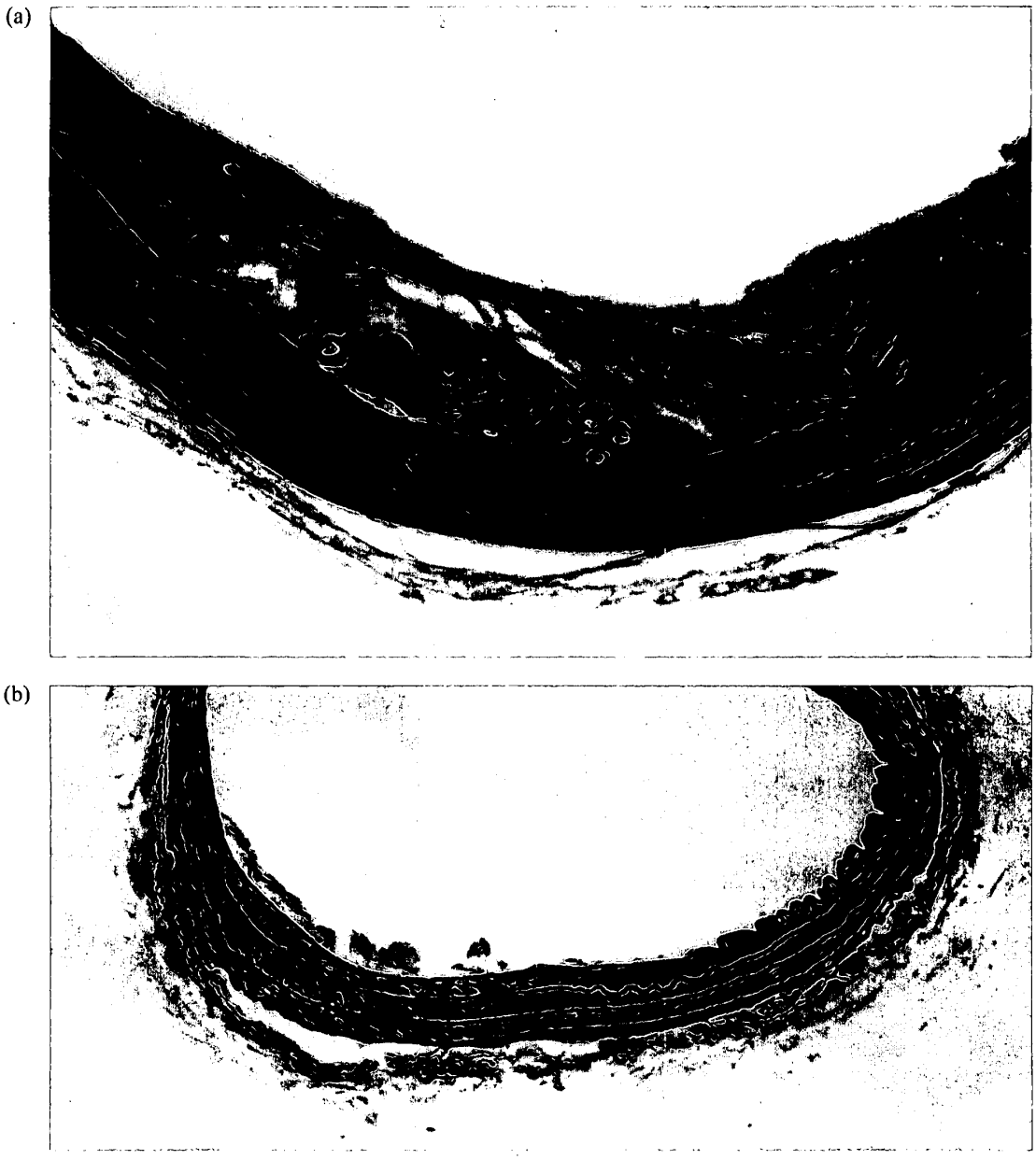

Figure 3 **Ezetimibe reduces carotid artery atherosclerosis in apo E knockout mice fed a 0·15% cholesterol diet for 6 months[11]. (a) Control, (b) 5 mg . kg^{-1} . day^{-1} ezetimibe.**

treatment persisted for several days after its cessation, supporting the contention that once-daily dosing should be sufficient for an adequate therapeutic effect. In addition, ezetimibe rapidly reversed pre-existing hypercholesterolaemia, despite a continued high-cholesterol diet.

Cynomolgus monkeys fed a single high-cholesterol meal and treated with an ezetimibe analogue also had a significant reduction in the chylomicron cholesterol content, with no effect on the triglyceride content. Because chylomicrons and chylomicron remnants may be atherogenic[13–16], further investigation of this phenomenon might shed more light on the mechanism of the anti-atherogenic effect of ezetimibe. It should be noted that while statins may increase the clearance of chylomicron remnants they may not decrease cholesterol content of chylomicrons. Therefore, the combination of a statin and ezetimibe should be highly effective in reducing the atherogenic potential of chylomicrons.

Ezetimibe caused only modest reductions in plasma cholesterol levels in animals fed cholesterol-free diets, which was probably the result of an up-regulation of hepatic cholesterol synthesis[12]. Ezetimibe reduces the delivery of dietary and biliary cholesterol to the liver from the intestine, resulting in a decrease of hepatic cholesterol stores and an up-regulation of hepatic HMG-CoA reductase activity. Ezetimibe has a mode of action complementary to that of the statins in lowering plasma cholesterol concentrations as it inhibits the absorption of cholesterol, while statins decrease the synthesis of cholesterol. The synergistic reduction in plasma total cholesterol concentration in dogs treated with ezetimibe ($0{\cdot}007\ mg\ .\ kg^{-1}\ .\ day^{-1}$) and lovastatin ($5\ mg\ .\ kg^{-1}\ .\ day^{-1}$) has also been reported with ezetimibe and pravastatin ($2{\cdot}5\ mg\ .\ kg^{-1}\ .\ day^{-1}$) or fluvastatin ($2{\cdot}5\ mg\ .\ kg^{-1}\ .\ day^{-1}$)[17].

The synergistic or additive result of combining ezetimibe with a statin is particularly significant because of the rule of six[18], which has been demonstrated with all of the statins. According to the rule of six, each doubling of the statin dose achieves a further reduction in LDL-C of only 6%.

However, at the same time, increasing the statin dose increases the potential for toxicity. Ezetimibe has no significant effect on the activity of major drug-metabolizing enzymes, and the potential for ezetimibe to cause a drug interaction involving the cytochrome P450 substrates is unlikely[19]. Clinical benefit could derive from co-administration of ezetimibe and a statin in cases in which a single agent is not sufficiently effective or in which there is concern about higher doses of an effective agent.

In conclusion, studies of hyperlipidaemia and atherosclerosis in animal models have shown that ezetimibe, given alone or combined with a statin, has significant lipid-lowering and anti-atherosclerotic properties. Ezetimibe lowered plasma total cholesterol and LDL-C levels in cholesterol-fed animals. Ezetimibe also inhibited the development of aortic and carotid artery atherosclerosis in apo E knockout mice. Co-administration of ezetimibe with a statin has a synergistic cholesterol-lowering effect, suggesting that such combination therapy for patients with hypercholesterolaemia may reduce plasma cholesterol more than is attainable with either drug alone.

References

[1] van Heek M, France CF, Compton DS *et al.* In vivo metabolism-based discovery of a potent cholesterol absorption inhibitor, SCH58235, in the rat and rhesus monkey through the identification of the active metabolites of SCH48461. J Pharmacol Exp Ther 1997; 283: 157–63.

[2] van Heek M, Farley C, Compton DS *et al.* Comparison of the activity and disposition of the novel cholesterol absorption inhibitor, SCH58235, and its glucuronide, SCH60663. Br J Pharmacol 2000; 129: 1748–54.

[3] van Heek M, Farley C, Compton D, Hoos L, Davis H. The novel cholesterol absorption inhibitor, ezetimibe, selectively inhibits the intestinal absorption of free cholesterol in the presence and absence of exocrine pancreatic function (Abstr). Atherosclerosis 2000; 151: 155.

[4] Guerciolini R. Mode of action of orlistat. Int J Obes Relat Metab Disord 1997; 21 (Suppl 3): S12–23.

[5] Toplak H, Marhardt K. Reduction of obesity and improvement in metabolic parameters by inhibition of intestinal lipases: current results with orlistat. Acta Med Austriaca 1998; 25: 142–5.

[6] Heck AM, Yanovski JA, Calis KA. Orlistat, a new lipase inhibitor for the management of obesity. Pharmacotherapy 2000; 20: 270–9.

[7] West RJ, Lloyd JK. The effect of cholestyramine on intestinal absorption. Gut 1975; 16: 93–8.

[8] Matsui MS, Rozovski SJ. Drug-nutrient interaction. Clin Ther 1982; 4: 423–40.

[9] Zbaida S, Alton K, Shannon D *et al.* In vitro metabolism of SCH 58235, 1-(4-fluorophenyl)-3*R*-[3-(4-fluorophenyl)-3*S*-hydroxypropyl]-4*S*-(4-hydroxyphenyl)-2-azetidinone, by liver and kidney slices (Abstr). 12th International Symposium on Microsomes and Drug Oxidations (ISMDO), Montpellier, France, July 20–24, 1998.

[10] van Heek M, Compton DS, Davis HR. The cholesterol absorption inhibitor, ezetimibe, decreases diet-induced hypercholesterolemia in monkeys. Eur J Pharmacol 2001; 415: 79–84

[11] Davis HR, Compton DS, Hoos L, Tetzloff G. The cholesterol inhibitor ezetimibe inhibits the development of atherosclerosis in apo E knockout (−/−) mice fed low fat and western diets (Abstr). Atherosclerosis 2000; 151: 133.

[12] Davis HR, Watkins RW, Compton DS *et al.* The cholesterol absorption inhibitor ezetimibe (SCH 58235) and lovastatin synergistically lower plasma cholesterol and inhibit the development of atherosclerosis. Metabolism; in press.

[13] Zilversmit DB. Atherogenic nature of triglycerides, postprandial lipemia, and triglyceride-rich remnant lipoproteins. Clin Chem 1995; 41: 153–8.

[14] Cohn JS. Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. Can J Cardiol 1998; 14 (Suppl B): 18B–27B.

[15] Mamo JC, Proctor SD, Smith D. Retention of chylomicron remnants by arterial tissue; importance of an efficient clearance mechanism from plasma. Atherosclerosis 1998; 141 (Suppl 1): S63–9.

[16] Havel RJ. Remnant lipoproteins as therapeutic targets. Curr Opin Lipidol 2000; 11: 615–20.

[17] Davis HR, van Heek M, Watkins RW *et al.* The hypocholesterolemic activity of the potent cholesterol absorption inhibitor SCH58235 alone and in combination with HMG CoA reductase inhibitors (Abstr). Drugs Affecting Lipid Metabolism (DALM); Houston, TX; November 1995.

[18] Knopp RH. Drug treatment of lipid disorders. N Engl J Med 1999; 341: 498–511.

[19] Zhu Y, Statkevich P, Kosoglou T *et al.* Effect of ezetimibe (SCH 58235) on the activity of drug metabolizing enzymes in vivo (Abstr). Clin Pharmacol Ther 2000; 67: 152.