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Pharmacology of ezetimibe

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Ezetimibe is the first of the cholesterol absorption inhibitors, a novel class of lipid modifying drugs, which potently inhibit the absorption of biliary and dietary cholesterol from the small intestine without affecting the absorption of fat-soluble vitamins, triglycerides or bile acids. Ezetimibe localizes in the brush border of the small intestinal enterocytes and reduces the uptake of cholesterol into the enterocytes. This has the net effect of inhibiting cholesterol absorption by keeping the cholesterol in the intestinal lumen, allowing it to be excreted.

Pre-clinical studies demonstrated that ezetimibe was glucuronidated to a single metabolite localized at the intestinal wall, where it prevented cholesterol absorption. It was found that enterohepatic re-circulation of ezetimibe and/or its glucuronide ensured repeated delivery to the site of action and limited peripheral exposure. Ezetimibe had no effect on the activity of major drug metabolizing enzymes (CYP450), which reduces any potential drug-drug interactions with other medications.

Pre-clinical models also demonstrated the lipid-lowering and anti-atherosclerotic properties of ezetimibe as a single agent, and showed its synergistic effect in combination with HMG CoA reductase inhibitors ('statins'). In cholesterol-fed rhesus monkeys, ezetimibe reduced both plasma cholesterol (ED_{50} = 0·0005 mg . kg^{-1} . day^{-1}) and LDL cholesterol in a dose-dependent manner. Plasma cholesterol levels were also reduced in dogs (ED_{50} = 0·007 mg . kg^{-1} . day^{-1}) and in hamsters (ED_{50} = 0·04 mg . kg^{-1} . day^{-1}) fed cholesterol-containing diets. In apo E knockout mice, a model of atherosclerosis, ezetimibe reduced plasma cholesterol levels over 60% primarily through the reduction in VLDL and LDL cholesterol. Ezetimibe inhibited the development of carotid artery (decrease of 97%) and aortic (decrease of 71–87%) atherosclerosis. In non-cholesterol fed dogs, co-administration of ezetimibe with statins resulted in a synergistic reduction in plasma cholesterol levels that was significantly lower than with monotherapy with either agent.

Clinically, ezetimibe (10 mg . day^{-1}) was found to inhibit cholesterol absorption by an average of 54% in hypercholesterolemic individuals ($P < 0·001$). Plasma total and LDL cholesterol levels were significantly reduced and cholesterol synthesis was increased. These results suggest that in patients with hypercholesterolemia, ezetimibe, in combination with a statin, may produce clinically important additional reductions in plasma cholesterol levels.

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Key Words: Cholesterol-lowering drugs, ezetimibe, LDL cholesterol, cholesterol absorption inhibitor, biliary cholesterol.

General overview of cholesterol absorption

Intestinal cholesterol absorption begins with the micellar solubilisation of both dietary and biliary cholesterol in the lumen of the small intestine. In general, approximately two thirds of intestinal cholesterol absorbed is from the bile and the other one third is derived from the diet. The cholesterol is then transferred from the micelles to the surface of the brush border membrane of the enterocyte, and into the cytoplasmic compartment. Cholesterol moves to the endoplasmic reticulum where it may be esterified by acyl-CoA : cholesterol acyltransferase (ACAT) to form cholesteryl ester. Free cholesterol and cholesteryl esters are packaged into chylomicrons, which are then secreted into the mesenteric lymph. Once in the circulation, the liver rapidly clears chylomicrons and their remnants. The consequences of cholesterol absorption inhibition include decreased cholesterol delivery to the liver, reduced hepatocyte cholesterol stores, decreased low-density lipoprotein (LDL) production, increased LDL clearance and, subsequently, decreased LDL cholesterol levels.

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Discovery of ezetimibe

Ezetimibe (1-(4-fluorophenyl)-(3R)-[3-(4-fluorophenyl)-(3S)-hydroxypropyl]-(4S)-(4-hydroxyphenyl)-2-azetidinone; SCH 58235) was discovered through the identification of the active biliary metabolites of its predecessor, SCH 48461[1,2], and extensive structure-activity relationship information obtained from a seven-day cholesterol-fed hamster model[3]. Ezetimibe potently inhibits cholesterol absorption in the intestine, thereby reducing plasma cholesterol in a number of pre-clinical models of hypercholesterolemia, including mice, hamsters, rats, rabbits, dogs and monkeys. Clinical trials have demonstrated that ezetimibe lowers LDL cholesterol and triglycerides, and raises HDL cholesterol in humans[4].

Efficacy of ezetimibe in preclinical models of hyperlipidemia

Monotherapy

Ezetimibe has demonstrated efficacy in a variety of pre-clinical models. Ezetimibe dose-dependently inhibited diet-induced hypercholesterolemia in hamsters with an effective dose at which 50% inhibition occurs (ED_{50}) of 0·04 mg . kg^{-1} [5]. Ezetimibe attenuated hypercholesterolemia by 60–94% at doses of 0·1–3 mg . kg^{-1} in rats. In an acute model of intestinal absorption using radiolabeled cholesterol in rats, ezetimibe inhibited the appearance of radiolabeled cholesterol in plasma with an ED_{50} of 0·0015 mg . kg^{-1} 90 minutes after dosing, indicating that the onset of activity is rapid[2]. Ezetimibe also dose-dependently reduced intestinal cholesterol absorption in wildtype, apolipoprotein (apo) E knockout ($^{-/-}$) and scavenger receptor class B, type I (SR-BI)$^{-/-}$ mice [6,7].

In the pre-clinical models described above, ezetimibe effectively lowered plasma cholesterol, but had little effect on plasma triglycerides, most likely because these models exhibited normotriglyceridemia. The effect of ezetimibe on other dyslipidemias, particularly hypertriglyceridemia, was also investigated in a hamster model of combined hyperlipidemia[8]. Hamsters were fed a high-fat, cholesterol-containing diet with or without ezetimibe for 3 months. Hamsters maintained on high-fat diets became obese, hyperinsulinemic, hyperleptinemic, hypercholesterolemic and hypertriglyceridemic. Ezetimibe ablated the combined hypercholesterolemia and hypertriglyceridemia induced by high-fat diets. Ezetimibe normalized very low density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) cholesterol and triglyceride, and significantly decreased LDL cholesterol to below chow-fed levels. The ratio of HDL cholesterol to LDL cholesterol increased significantly with the addition of ezetimibe. Ezetimibe was very effective in correcting the combined dyslipidemia in diet-induced obese, hyperinsulinemic hamsters, an animal model that is partially representative of the metabolic syndrome in humans. It will be interesting to determine how effective ezetimibe is in ameliorating combined dyslipidemia in the obese, insulin-resistant and/or type 2 diabetic human population.

Of all the pre-clinical species studied, ezetimibe has proven to be most potent in monkeys. In rhesus monkeys fed a diet containing 375 mg . day^{-1} of cholesterol, 0·1 mg . kg^{-1} of ezetimibe completely prevented the doubling of plasma cholesterol normally induced under these dietary conditions (ED_{50} = 0·0005 mg . kg^{-1})[2]. LDL cholesterol was dose-dependently reduced[9]. A single dose of the ezetimibe analog, SCH 48461, administered to cynomolgus monkeys fed a single cholesterol-containing meal caused a significant reduction of cholesterol in chylomicrons and chylomicron remnants during the postprandial phase without affecting triglyceride content. In rhesus monkeys, LDL apo B-100 was reduced by nearly 50% after treatment with the ezetimibe analog. Combined, these data indicate that these cholesterol absorption inhibitors reduce cholesterol content in chylomicrons, which indirectly leads to a decrease in LDL cholesterol and particle number[9].

In combination with statins

Ezetimibe blocks intestinal uptake and absorption of cholesterol but causes modest, inconsistent reductions in plasma cholesterol levels in animals fed cholesterol-free chow diets. Although this class of compounds blocks cholesterol absorption and increases neutral sterol excretion, chow-fed animals compensate for the loss of biliary cholesterol by increasing hepatic cholesterol synthesis. Therefore, the effect of ezetimibe in combination with 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors (statins) was determined in chow-fed dogs[10]. A synergistic reduction in plasma cholesterol was observed in chow-fed dogs given ezetimibe (0·007 mg . kg^{-1} . day^{-1}) and the HMG CoA reductase inhibitor lovastatin (5 mg . kg^{-1} . day^{-1}). Neither ezetimibe nor lovastatin alone affected plasma cholesterol levels. Their combination for 14 days caused a synergistic 50% reduction in plasma cholesterol levels. Ezetimibe (0·007 mg . kg^{-1} . day^{-1}) also caused synergistic or additive reductions in plasma cholesterol levels in chow-fed dogs when combined with other HMG CoA reductase inhibitors for 2 weeks (–41% with 2·5 mg . kg^{-1} pravastatin; –60% with 5 mg . kg^{-1} fluvastatin and –30% with low doses of simvastatin and atorvastatin 1 mg . kg^{-1}). The combination of the cholesterol absorption inhibitor ezetimibe with an HMG CoA reductase inhibitor may be very effective clinically at reducing plasma cholesterol levels, even with reduced dietary intake of cholesterol.

Effect of ezetimibe on atherosclerosis

The effect of ezetimibe on plasma cholesterol levels and atherogenesis was determined in apo $E^{-/-}$ mice, an

atherosclerosis model with chylomicron remnant hypercholesterolemia[6]. Cholesterol absorption was inhibited by >90% at doses of ezetimibe greater than 3 mg . kg^{-1} in the apo $E^{-/-}$ mice. Atherosclerosis and lipoprotein changes were determined in apo $E^{-/-}$ mice fed a high-fat, 0·15% cholesterol 'western' diet; a low-fat, 0·15% cholesterol diet or a semi synthetic cholesterol-free diet with or without ezetimibe (5 mg . kg^{-1} . day^{-1}) for 6 months. Ezetimibe reduced plasma cholesterol levels from 964 to 374 mg . dl^{-1}, 726 to 231 mg . dl^{-1} and 516 to 178 mg . dl^{-1} in the western, low-fat and cholesterol-free diet groups, respectively. The reductions occurred primarily in the VLDL/chylomicron remnant and LDL fractions, while HDL cholesterol levels were increased by ezetimibe treatment. Ezetimibe reduced aortic atherosclerotic lesion surface area from 20·2 to 4·1% in the western diet group and from 24·1 to 7·0% in the low-fat diet group. Ezetimibe reduced carotid artery atherosclerotic lesion cross-sectional area by 97% in both the western and low-fat groups, and by 91% in the cholesterol-free group. In summary, ezetimibe inhibited cholesterol absorption, reduced plasma cholesterol levels, increased HDL cholesterol levels and inhibited the progression of atherosclerosis under western, low-fat, and cholesterol-free dietary conditions in apo $E^{-/-}$ mice. Although apo $E^{-/-}$ mice have more severe hypercholesterolemia and more pronounced LDL cholesterol reductions with ezetimibe than humans, these animal data suggest that ezetimibe may inhibit atherogenesis in individuals consuming restricted-fat or western diets.

Selectivity

Using radiolabeled molecules, it has been demonstrated that ezetimibe inhibits the transport of cholesterol across the intestinal wall. Experiments were conducted in rodents to determine whether ezetimibe would affect the absorption of molecules other than free cholesterol, namely cholesteryl ester, triglyceride, ethinylestradiol, progesterone, vitamins A and D and taurocholic acid[5]. Using cholesteryl esters labeled on either the cholesterol or the fatty acid moiety, it was shown that ezetimibe did not affect cholesteryl ester hydrolysis or the absorption of fatty acid thus generated in either hamsters or rats. The free cholesterol from this hydrolysis, however, was not absorbed in the presence of ezetimibe. Ezetimibe did not affect the absorption of triglyceride, ethinylestradiol, progesterone, vitamins A and D, or taurocholic acid in rats[5]. These studies indicate that ezetimibe does not affect pancreatic lipase and therefore shares no properties with orlistat (Xenical). Ezetimibe also does not sequester bile acids and thus differs from cholestyramine (resins).

More recently, a study in humans demonstrated that ezetimibe effectively reduced serum plant sterols in patients with sitosterolemia[11], indicating that ezetimibe also inhibits the absorption of these molecules that are highly structurally related to cholesterol. Additional studies in humans have indicated that ezetimibe does not affect serum fat-soluble vitamin status[12].

Metabolism

A number of studies were conducted in animal models to understand the disposition and metabolism of ezetimibe[13]. Ezetimibe is rapidly metabolized in the intestine to its phenolic glucuronide; once glucuronidated, it is excreted in the bile, thereby delivering the drug back to the site of action. Cholesterol absorption studies indicated that the glucuronide appeared more potent than ezetimibe itself, and this is likely because glucuronidated ezetimibe localizes more avidly to the intestine. Autoradiographic analysis demonstrated that drug-related material was located throughout the intestinal villi but concentrated in the villus tip.

In humans, ezetimibe is rapidly absorbed and primarily metabolized in the small intestine and liver to its glucuronide, with little oxidative cytochrome P450 mediated metabolism[14]. Ezetimibe and its glucuronide undergo enterohepatic recycling and have a half-life of approximately 24 hours in humans. Ezetimibe and/or the glucuronide metabolite are excreted in the feces (90%) and urine (10%). Since ezetimibe does not influence the activities of cytochrome P450 enzymes, significant pharmacokinetic interactions with many medications have not been noted. Pharmacokinetic interaction studies of ezetimibe in humans have found no significant changes in the plasma levels of other medications including statins (atorvastatin, simvastatin, pravastatin, lovastatin, and fluvastatin), fibrates (gemfibrozil and fenofibrate), digoxin, glipizide, warfarin and oral contraceptives (ethinyl estradiol and levonorgestrel)[15–24].

Effect of ezetimibe on cholesterol absorption in humans

As described above, ezetimibe has been shown to inhibit cholesterol absorption in many animal models. The effect of ezetimibe (10 mg . day^{-1}) on human cholesterol absorption as well as cholesterol synthesis, sterol excretion and plasma concentrations of cholesterol and noncholesterol sterols was investigated in mild to moderate hypercholesterolemic individuals[25]. Treatment periods lasted 2 weeks with an intervening 2-week washout period. Fractional cholesterol absorption rate was measured by the continuous dual-isotope feeding method using deuterium-labeled cholesterol and sitostanol. Fractional cholesterol absorption rates averaged 49·8% on placebo and 22·7% on ezetimibe, indicating a reduction of 54% ($P < 0·001$). Cholesterol synthesis increased by 89% from 931 mg . day^{-1} (mean) on placebo to 1763 mg . day^{-1} on ezetimibe ($P < 0·001$), while the ratio of lathosterol-to-cholesterol, an indirect marker of cholesterol synthesis, was increased by 72% from 1·103 µg . mg^{-1} on placebo to 1·895 µg . mg^{-1} on ezetimibe ($P < 0·001$). Bile acid synthesis was slightly but not significantly increased. LDL and total cholesterol levels following ezetimibe treatment were reduced 20·4 and 15·1%, respectively, whereas campesterol and sitosterol were decreased by 48 and 41%, respectively. The reduction of plasma concentrations of the noncholesterol sterols,

sitosterol and campesterol, which are not endogenously synthesized, suggests a direct effect on the absorption of these sterols by ezetimibe.

In humans, ezetimibe inhibits cholesterol absorption and promotes a compensatory increase in cholesterol synthesis, which leads to clinically relevant reductions in plasma LDL and total cholesterol concentrations. The combination of ezetimibe with a statin may inhibit the compensatory increase in cholesterol synthesis and lead to additional reductions in plasma cholesterol levels.

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