C31

LEADING ARTICLE

Drugs 2002; 62 (16): 2333-2347
0012-6667/02/0016-2333/$30.00/0



# Cholesterol Absorption Inhibitors for the Treatment of Hypercholesterolaemia

*Thomas Sudhop* and *Klaus von Bergmann*

Department of Clinical Pharmacology, University of Bonn, Bonn, Germany

## Abstract

The benefits of lipid lowering therapy on coronary heart disease have been clearly established in many clinical trials on primary and secondary prevention. Despite the availability of potent lipid lowering drugs, many patients do not reach the current treatment goals. This paper reviews new therapeutic approaches in lipid lowering drugs focusing on compounds which lower cholesterol absorption. The role of plant sterols and stanols, new acyl-CoA:cholesterol O-acyl transferase (ACAT) inhibitors, microsomal triglyceride transfer protein (MTP) inhibitors, and ezetimibe are summarised.

Although the lipid lowering effect of plant sterols and plant stanols is only moderate, their use as functional foods is beneficial for patients with mild hypercholesterolaemia and is able to enhance the lipid lowering effect of HMG-CoA reductase inhibitors (statins). The role of ACAT inhibitors that might also inhibit cholesterol absorption remains unclear. Avasimibe, the first oral bioavailable ACAT inhibitor, has entered phase III trials. However, the presently available data in humans do not indicate a clear clinical benefit. The role of MTP inhibitors, which exhibit remarkable effects on all plasma lipids, also remains unclear, as safety concerns must first be addressed. Ezetimibe, the first available 2-azetidinone, succeeded in phase III trials showing remarkable effects in inhibition of cholesterol absorption as well as cholesterol lowering. The synergistic effect of co-administration of ezetimibe with statins seemingly offers a new approach in reaching the therapeutic goals.

Elevated serum cholesterol levels is the leading cause of morbidity and mortality from coronary heart disease (CHD).[1-4] The benefits of lipid lowering therapy, especially with HMG-CoA reductase inhibitors (statins), on CHD have been clearly established in several clinical trials for primary and secondary prevention.[5-9] The Third National Cholesterol Education Program (NCEP) has recently issued new recommendations for the treatment goal of patients with hypercholesterolaemia and additional risk factors for CHD.[10] Although statins, the most potent cholesterol lowering drugs, have been shown to be effective in lowering total and low density lipoprotein (LDL) cholesterol, not all patients achieve treatments goals, with a larger proportion of patients remaining at higher risk.[10-14] Doubling the daily dose of statins usually results in an additional LDL cholesterol lowering benefit of 5–7% but also increases the risk of adverse effects.[15] Combining statins with fibric acid derivatives (fibrates) increases efficacy, but also increases the risk of myopathy and rhabdomyolysis.[16-21] In particular, the latter adverse effect led to the withdrawal of cerivastatin from the world

market in August 2001.[22] Although other lipid agents, such as fibrates, bile acid sequestrants, nicotinic acid (niacin), etcetera, are available, the demand for additional lowering compounds has directed research towards mechanisms such as the inhibition of intestinal cholesterol absorption.[23-28]

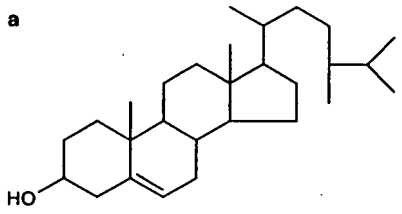
Intestinal cholesterol absorption shows great inter-individual variation and ranges from 20–80%.[29-33] Dietary supplements, such as plant sterols and plant stanols, soy lecithin, and the synthetic sucrose polyester olestra, are known to reduce intestinal cholesterol absorption.[34-38] Whereas the effect of soy lecithin has been discussed controversially,[39] the effect of olestra seems to be clinically relevant only when administered in very high dosages of up to 50 g/day. These doses are accompanied by unwanted adverse effects.[37,38] Although neomycin, an aminoglycoside antibiotic, reduces cholesterol absorption by 44–49% and total cholesterol by 20–27%, it is unsuitable for long-term treatment because of renal and oto-toxicity.[40-44] It is therefore not licensed as lipid lowering drug.

## 1. Plant Sterols and Plant Stanols

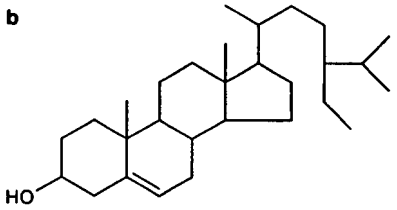
Plant sterols are very similar to cholesterol in their chemical structure. More than 40 different plant sterols have been identified. The most common plant sterols found in the diet are (β-)sitosterol, the 24-ethyl analogue, and campesterol, the 24-methyl analogue of cholesterol (figure 1a and 1b). The amount of cholesterol and plant sterols in the diet varies with food composition. The usual Western diet contains between 200 and 600mg cholesterol and approximately 200–300mg plant sterols per day.[45,46] Despite their chemical similarity, plant sterols are less absorbed than cholesterol in the intestine.[47] Serum levels of plant sterols are usually less than one-hundredth those of cholesterol.[48] After absorption plant sterols are not metabolised and are excreted unchanged into the bile.[49]

Almost 50 years ago, Pollak[50] reported that administration of a plant sterol mixture which contained 75% of sitosterol lowered total serum cholesterol on average by 25% (range +5 to –53%) via reduction of the intestinal cholesterol absorption. The dose in these experiments ranged from 5–10 g/day. In later studies, using sitosterol in doses between 10–15 g/day, total cholesterol was lowered by 10–20%.[51,52] Even with lower amounts of plant sterols (3 g/day), a significant decrease in total cholesterol by 12% was observed and was accompanied by a reduction in cholesterol absorption by approximately 50%.[35,51] Animal studies showed that sitostanol (figure 1c), the Δ5 saturated product of sitosterol, lowered cholesterol more than sitosterol.[53] The first study in humans revealed that a low dose of sitostanol (0.5g three times a day) administered for four weeks, reduced LDL cholesterol by 15%.[54] These authors also compared the inhibition of intestinal cholesterol absorption by sitosterol and sitostanol. Using an intestinal perfusion method, Heineman et al.[47] showed that cholesterol absorption was reduced by 50% with sitosterol. The reduction was even greater with sitostanol (85%).

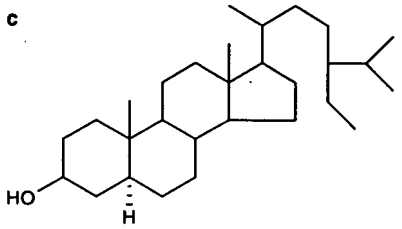
Because of their potent cholesterol lowering effect sitostanol and later sitosterol were incorporated into margarine and other dietary products, and introduced as functional foods in many countries. Since plant sterols/stanols are difficult to solubilise even in food containing fat, esterification with fatty acids, such as oleic acid, was necessary to incorporate greater amounts of plant sterols in dietary products. Miettinen and colleagues[55] reported on a randomised, placebo controlled trial in 153 patients with hypercholesterolaemia who had been treated for 1 year with a sitostanol-oleate margarine (1.8 or 2.6 sitostanol per day). They observed a LDL cholesterol reduction of 14%. Triglycerides and HDL cholesterol did not change significantly and no adverse events occurred. In patients treated with simvastatin for 1 year, margarine containing sitostanol esters, administered in a dose of 3 g/day led to a further reduction of LDL cholesterol by 16%.[56] Similar results were also reported by other authors.[57,58] Since their commercial availability, many studies with plant sterol/stanol fortified products have been performed to estimate the cholesterol lowering effect (see



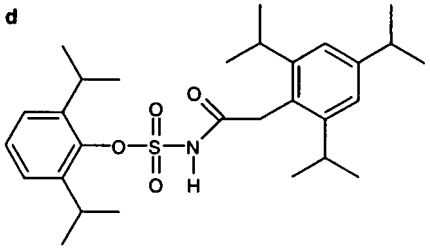

Campesterol (24α -methyl-cholest-5en-3β-ol)



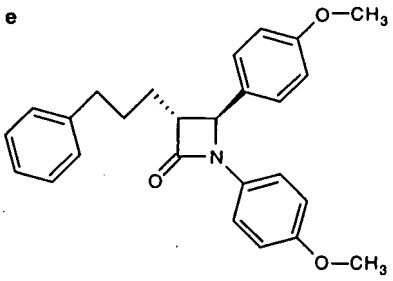

Sitosterol (24α-ethyl-cholest-5en-3β-ol)



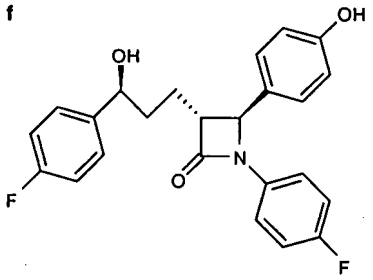

Sitostanol (24α-ethyl-5α-cholestan-3β-ol)




Avasimibe (CI-1011)




SCH-48461




Ezetimibe (SCH-58235)

**Fig. 1.** Chemical structures of the plant sterols campesterol (**a**) and sitosterol (**b**), the plant stanol sitostanol (**c**), the ACAT inhibitor avasimibe (**d**), and the 2-azetidinones SCH-48461 (**e**) and ezetimibe (**f**). **ACAT** = acyl-CoA:cholesterol O-acyl transferase.

Law[59] and Moghadasian and Frohlich[60] for reviews). Using ≥2.0g sterol/stanols per day, a mean LDL cholesterol reduction of 21 mg/dl was observed with a greater benefit in elder persons.[59]

Although a small reduction in fat soluble vitamins was reported in some studies, the overall effect seems to be negligible.[61-63] The 1-year trial in 102 patients with sitostanol-ester margarine showed no compound related adverse effects.[55]

Although the lipid lowering effect of plant sterols/stanols is only moderate, their use as functional food is beneficial for patients with mild hypercholesterolaemia and is able to enhance the lipid lowering effect of statins in these patients. Although clinical endpoint studies are not yet available, plant sterols may reduce atherosclerotic lesions, as observed in the apoliprotein (apo) E-deficient mice model,[64,65] and may play an important role as

add-on therapy in the treatment of hypercholesterolaemia.

## 2. ACAT Inhibitors

Acyl-coenzyme A:cholesterol O-acyl transferase (ACAT) is a membrane-bound enzyme involved in balancing intracellular cholesterol homeostasis. ACAT is located in the rough endoplasmic reticulum of various tissues, such as macrophages, liver, vessel wall, intestine and adrenal cortex. It catalyses the re-esterification of cholesterol and long fatty acids to intracellular cholesterol esters.[66] In the intestine, ACAT catalyses the re-esterification of free cholesterol allowing further transport as chylomicrons. In the liver, ACAT is involved in the secretion of very low-density lipoprotein (VLDL) particles. In addition, in the vessel wall, ACAT is responsible for macrophage-mediated storage of cholesterol esters which play an important role in the development of plaque lesions. Thus, inhibition of ACAT may not only lower intestinal absorption of cholesterol but it may also inhibit accumulation of cholesterol esters in atherosclerotic lesions.[67] ACAT exists at least in two iso-enzyme forms, ACAT1 and ACAT2,[68] which are present in different locations. Whereas ACAT1 is present in the microsomal fraction of different types of cells throughout the whole body, ACAT2 is found only in the intestine and in the liver.[69-71]

Some ACAT inhibitors, such as avasimibe (CI-1011), CL-277082 and DuP-182, were evaluated in humans in the past. Whereas CL-277082 revealed no effect on cholesterol absorption, faecal sterol excretion and plasma lipoproteins,[72] DuP-128, a poorly absorbed compound, inhibited cholesterol absorption by approximately 14% but only with a marginal LDL cholesterol lowering effect of approximately 5%.[73] Other ACAT inhibitors, such as CS-505 and F-1394, are currently under early clinical development[74] but clinical data are only available for avasimibe.

### 2.1 Avasimibe

Avasimibe, developed by Parke Davis, is the first orally bioavailable ACAT inhibitor currently in phase III clinical trials.[75] The synthetic acyl sulfamate (figure 1d) was derived in structure-activity relationship studies and inhibits ACAT both *in vitro* and *in vivo*.[76-78]

When added to HepG2 cell cultures in a concentration of 10 μmol/L, avasimibe inhibits ACAT activity by 79% resulting in a cholesteryl ester mass reduction of 32% and a reduction of apo B secretion of 43% without affecting apo B synthesis.[79]

In normal chow-fed rats avasimibe reduces plasma total cholesterol in a dose of 3–10 mg/kg by 16–30% and plasma triglycerides by 44–66%.[78] In cholesterol-free casein diet fed rabbits, avasimibe 1–10 mg/kg reduced plasma cholesterol levels by 15–37%.[78] In normal chow-fed cynomolgus monkeys, avasimibe 30 mg/kg reduced plasma total cholesterol and lipoprotein (a) levels by approximately 27 and 32%, respectively, and also decreased HDL cholesterol by 23%.[80] In mini pigs, fed a high fat, high cholesterol diet, avasimibe 10–25 mg/kg led to a reduction of total triglycerides, VLDL triglycerides and VLDL cholesterol of 31–40%, 39–48% and 31–35%, respectively, whereas significant reductions in total (35%) and LDL cholesterol (51%) were only observed in animals receiving higher doses (25 mg/kg).[81] Turnover studies in these animals showed that avasimibe reduces both the hepatic VLDL apo B secretion rate and the VLDL apo B pool size without affecting the fractional catabolism. It also decreases the LDL apo B pool size presumably mainly by reducing LDL apo B production.

The direct anti-atherosclerotic effect of avasimibe was examined in several experiments. In one study with cholesterol-fed ApoE*3-Leiden transgenic mice, which represent a good model for defective postprandial lipoprotein clearance, avasimibe reduced plasma cholesterol by 56% and atherosclerotic lesion area in the aorta by 92% compared with untreated animals.[82] Similar results were seen in the prevention and regression of aortic fatty streaks in the hypercholesterolaemic hamster model.[83] Treatment with daily doses of 3–30 mg/kg over 10 weeks reduced the aortic fatty

streak area by 68–93%. Studies in hypercholesterolaemic rabbits showed that avasimibe reduces both the number of monocyte-macrophages and the activity of matrix metalloproteinases in athero-sclerotic lesions. This led to a shift from more fibrofoamy to more fibromuscular lesions in these animals, possibly resulting in a plaque-stabilising effect of avasimibe.[84] Similar results were also reported in combined treatment with statins.[85-87]

Short- and long-term toxicology studies revealed significant toxicological effects only in doses higher than 300 mg/kg.[88] Most common findings were loss in bodyweight, emesis, salivation, changes in red blood cell morphology and hepatic toxicity. Mortality due to hepatic toxicity occurred only in animals treated with doses of 1000 mg/kg. Adrenal effects, such as moderate cortical cytoplasmic vacuolisation and fibrosis, were seen in long-term studies with doses ≥300 mg/kg.

Efficacy and tolerability in humans were investigated in 130 patients with hypercholesterolaemia and low HDL cholesterol levels in a double-blind, randomised, placebo controlled, parallel-group trial over 8 weeks.[89] After an 8-week placebo and dietary controlled run-in period, patients received either placebo, 50, 125, 250 or 500mg avasimibe once daily. Avasimibe reduced triglycerides by 16–23% and VLDL cholesterol by 20–30%, whereas total, LDL and HDL cholesterol, and apo B levels were not significantly altered. Interestingly, apoA-I was also significantly reduced in the 500mg group. All dosage levels were well tolerated with no significant laboratory abnormalities. Most commonly reported adverse events were headache (4.8%), gastrointestinal symptoms (3.8%) and musculoskeletal symptoms (2.9%).

Although the results of phase III studies have not been published yet, it seems clear that the lipid lowering effect on total and LDL cholesterol in humans is marginal compared with other lipid lowering drugs. However, the possible anti-atherosclerotic effect of ACAT inhibition in the vessel wall, which still has to be proven in humans, might be useful, especially in the combined treatment with statins.

## 3. MTP Inhibitors

The microsomal triglyceride transfer protein (MTP), a heterodimeric lipid transfer protein, catalyses the transport of triglyceride, cholesteryl ester and phosphatidylcholine between membranes, and is essential in the assembly of VLDL and chylomicrons in liver and intestine, respectively (for review see Gordon and Jamil[90]). Defects in MTP encoding genes lead to abetalipoproteinaemia, a rare inherited disease, associated with the absence of apo-B containing lipoprotein particles and malabsorption of fat soluble vitamins causing severe spinocerebellar and retinal degeneration.[91-94] The inhibition of MTP reduces intestinal chylomicron secretion and hepatic VLDL secretion resulting in a decrease of plasma LDL, VLDL cholesterol and triglycerides, but also bearing the risk of accumulation of fatty acids in liver and gut.

Various experimental MTP inhibitors have been developed and tested in different cell types and animal models.[94-102] BMS-201038, a benzimidazole-based MTP inhibitor, showed a remarkable reduction of plasma cholesterol and plasma triglycerides in the WHHL rabbit (10 mg/kg) within the range of –84 to –92% and –60 to –90%, respectively.[97] Structure-activity relationship studies led to the discovery of BMS-212122, an even more potent MTP inhibitor with an 50% effective dose ($ED_{50}$) of 0.04 mg/kg in rats after intravenous administration.[101]

Although currently under clinical development, only very limited data are available on MTP inhibitors in humans. Implitapide, a MTP inhibitor which was also shown to be efficient in the WHHL rabbit,[100] was examined in 188 patients with primary hypercholesterolaemia in a dose range of 20–160 mg/day versus placebo in a randomised, double-blind trial. It reduced LDL cholesterol by 8–55%.[103] Increases in plasma transaminases and gastrointestinal adverse events were the most often reported adverse effects, especially in the higher dose levels. Serum levels of vitamin A and

DOCKET ALARM

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

**LAW FIRMS**
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

**FINANCIAL INSTITUTIONS**
Litigation and bankruptcy checks for companies and debtors.

**E-DISCOVERY AND LEGAL VENDORS**
Sync your system to PACER to automate legal marketing.