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Squalene Synthase Inhibitors
Clinical Pharmacology and Cholesterol-Lowering Potential

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Abstract

HMG-CoA reductase inhibitors (statins) reduce cardiovascular disease morbidity and mortality with a high level of safety. Nonetheless, there are substantial numbers of people who either do not tolerate statins or whose low-density lipoprotein (LDL) levels are not lowered adequately. For these reasons, there is a need to develop other cholesterol-lowering drugs. A target for these new agents is provided by the enzymes distal to HMG-CoA reductase in the cholesterol biosynthesis pathway. Two classes of drugs have been developed: (i) squalene synthase inhibitors, which act at the first committed step in cholesterol biosynthesis, distal to the mevalonate-farnesyl diphosphate pathway; and (ii) oxidosqualene cyclase inhibitors, which act distal to the squalene intermediate. Of these, squalene synthase inhibitors have received more attention and are the subject of this review. Squalene synthase inhibitors decrease circulating LDL-cholesterol by the induction of hepatic LDL receptors in a similar manner to statins. They have fewer secondary effects mediated by a decrease in non-cholesterol products of mevalonate metabolism distal to HMG-CoA reductase, but have the potential to increase intermediates proximal to squalene. Squalene synthase inhibitors are just now entering clinical trials and data on how effectively they lower LDL-cholesterol and how they compliment the actions of statins and other agents is awaited with considerable interest.

The serum cholesterol level of an individual is one of the most important factors in predicting[1,2] and preventing[3] coronary heart disease (CHD). Statin drugs are competitive inhibitors of HMG-CoA reductase (figure 1), the primary rate-limiting enzyme in the hepatic biosynthesis of cholesterol. The resulting statin-induced decrease in intrahepatic cholesterol concentrations leads to the upregulation of hepatic receptors for low-density lipoprotein (LDL), an effect mediated by the transcription factor SREBP (sterol response element binding protein). The increase in LDL receptor expression enhances the fractional catabolic rate of circulating LDL, thus lowering its concentration. Despite the upregulation of HMG-CoA reductase that ensues,[4] the more potent statins can lower LDL by ≥50%.[5] Statins have been consistently shown to reduce both CHD- and stroke-related morbidity and mortality,[6] and with the exception of cerivastatin, do so with a wide margin of safety.[7]

1. Statins

The main problems with the use of statins relate to dose and response. There is considerable inter-individual variation in the magnitude of the effect of statins on LDL-cholesterol. There is evidence that

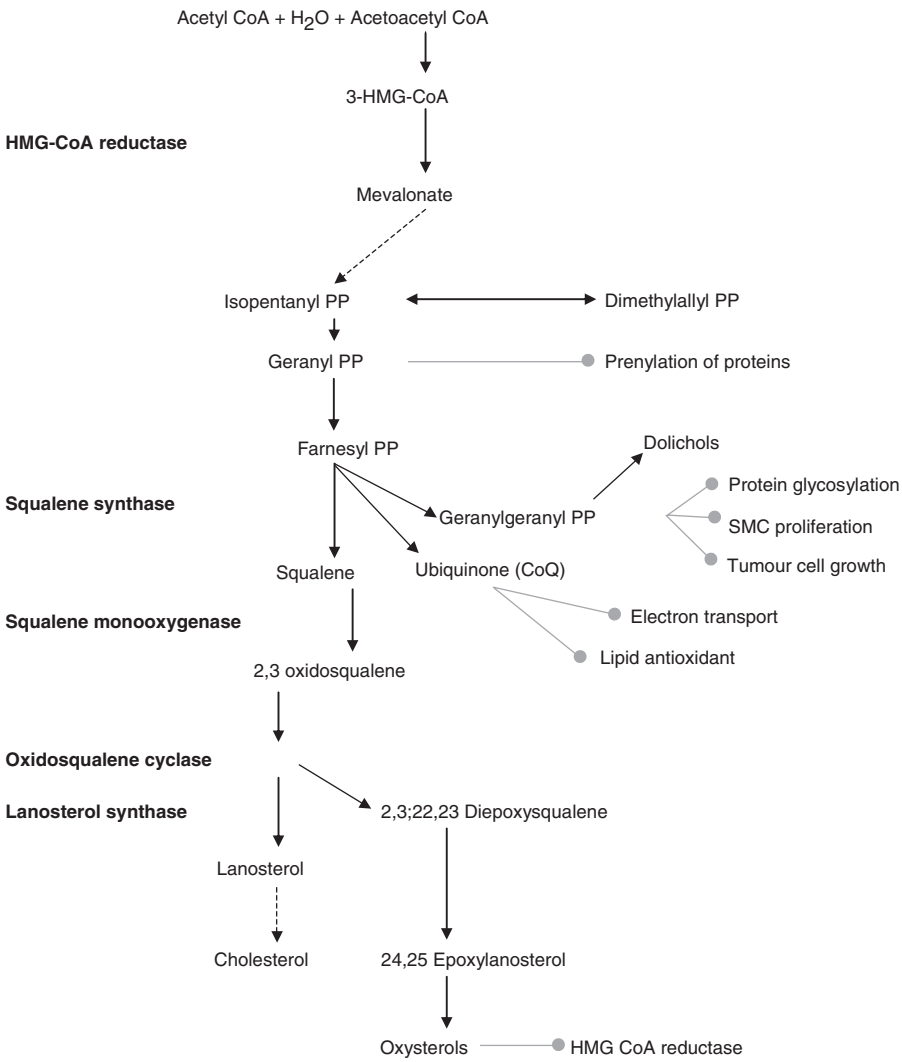

Fig. 1. The sites of action of HMG-CoA reductase inhibitors, squalene synthase inhibitors and oxidosqualene cyclase inhibitors in the hepatic biosynthesis of cholesterol. The putative functions of intermediates and derivatives of intermediates up to the first branch point in the biosynthetic pathway for cholesterol synthesis are shown by the dashed arrows, and the stages involving multiple reactions are shown by the green lines. **CoQ** = coenzyme Q (ubidecarenone); **PP** = pyrophosphate; **SMC** = smooth muscle cell.

people with low rates of cholesterol synthesis,[8] perhaps due to increased absorption of dietary cholesterol,[9] may be most resistant. Accordingly, an individual whose LDL-cholesterol is insufficiently lowered with any one statin at a given dose may not benefit from an increased dose of that statin or from treatment with a different statin. Even in people whose initial response to statin therapy is good, the dose response remains relatively flat,[10] with further decreases of LDL-cholesterol of only approximately 6% resulting from doubling doses.[5,11]

1.1 Safety of Statins

Although the margin of safety for most statins approved for clinical use is very wide,[7,12-14] elevation of liver aminotransferases (ALT and AST) and

myositis or myopathy can occur. Their likelihood, although low, relates to the statin dose rather than to the degree of reduction in LDL-cholesterol.[14] Studies on rat and human myotube cultures showed that statin-induced myopathy is probably due to decreased geranylgeranylation of proteins secondary to decreased mevalonate synthesis.[15,16]

Potentially, statins could have other effects downstream of HMG-CoA reductase (figure 1). Their consequences might prove favourable, enhancing the protection of statins against cardiovascular disease by mechanisms other than LDL-cholesterol lowering (pleiotropic effect), or unfavourable, leading to adverse events as in the case of myositis. An example of a potentially favourable statin effect, as yet unexplained, is the decrease in C-reactive protein associated with their use. [17] Important effects of statins downstream of HMG-CoA reductase include prenylation (post-translational modification of proteins by farnesyl pyrophosphate or geranylgeranyl pyrophosphate), which regulates the subcellular location of G-proteins influencing many signalling cascades within the cell.[18] Oxysterols and farnesyl pyrophosphate derived from the cholesterol biosynthetic pathway after mevalonate, but before squalene synthase, also affect the activity of nuclear orphan receptors such as liver X receptor (LXR) and farnesoid X-activated receptor (FXR), which are important in biliary cholesterol metabolism, lipoprotein metabolism and excretion, and in macrophage foam cell formation.[19]

2. Squalene Synthase Inhibitors

Squalene synthase is another enzyme in the cholesterol biosynthetic pathway (figure 1). Important differences between cholesterol-independent effects of squalene synthase inhibitors and those of HMG-CoA reductase might be expected because squalene synthase, which acts downstream of mevalonate, is the first committed step of hepatic cholesterol biosynthesis at the final branch point of the cholesterol biosynthetic pathway (figure 1).[20,21]

2.1 Safety and Efficacy of Squalene Synthase Inhibitors Compared with Statins

Preliminary studies with the squalene synthase inhibitors BMS-187745 and BMS-188494 showed that, at concentrations that markedly decreased cholesterol synthesis, no myotoxicity was found *in vitro*.[22] Thus, inhibition of squalene synthase did not result in myotoxicity *in vitro* because intermediates formed before squalene and responsible for prenylation of proteins were not depleted.[16,22] It was later shown that the squalene synthase inhibitors ER-27856[23] and TAK-475[24] and EP2302[25] decreased circulating LDL levels, as with statins, by inducing LDL receptors, assessed using HepG2 cells in culture. It was also shown in rhesus monkeys that ER-27856 lacked the hepatotoxicity found with atorvastatin,[23] and the same was shown for TAK-475, in cynomolgus monkeys.[24] More recently, two new potent squalene synthase inhibitors (EP2306 and EP2302) have been described.[25] EP2302 inhibited cholesterol synthesis dose-dependently with a similar potency to that of simvastatin. In tests so far, the degree of inhibition observed *in vitro* has not been seen *in vivo*, probably because of upregulation of HMG-CoA reductase, in the case of statins, or through a compensatory increase in intestinal cholesterol uptake with both statins and squalene synthase inhibitors. Tavidrou et al.[25] also showed that oleate-induced apolipoprotein B secretion by HepG2 cells was more markedly inhibited by simvastatin than by EP2306 or EP2302.

2.2 Other Potential Toxicity of Squalene Synthase Inhibitors

HMG-CoA reductase is the site of physiological regulation of cholesterol biosynthesis, making it unlikely that accumulation of metabolites earlier in the pathway (figure 1) would be toxic, but this is not necessarily true of squalene synthase inhibition. Triparanol, another inhibitor of cholesterol biosynthesis downstream of mevalonate, was found to cause cataract formation.[26] More recently, an association between lanosterol synthase (figure 1) mutations and cholesterol deficiency resulting in cataract formation in a rat model was described.[27] However,

in the rats with cataracts, the cholesterol deficiency was confined largely to the lens. In contrast, hepatic and serum cholesterol levels were not decreased. Since vertebrate eye lenses are not vascularised, *de novo* cholesterol synthesis in the lens is necessary for normal proliferation of epithelial cells in the lens. This suggests that the gene or isoform of the gene regulating lens cholesterol synthesis differs from the gene regulating hepatic cholesterol synthesis. There is evidence that statins, in much higher doses than used in the clinic, can cause cataract formation in rats;[28] this would appear to be due to a failure of upregulation of lens HMG-CoA reductase, and decreased ubiquinone (ubidecarenone) levels with statin treatment may also be a factor.[29]

3. Oxidosqualene Cyclase Inhibitors

Another enzyme target in cholesterol biosynthesis is 2,3-oxidosqualene cyclase (figure 1). Selective inhibitors of oxidosqualene cyclase have been reported to decrease cholesterol biosynthesis[21,30] without influencing LDL catabolism,[31] unlike statins, which principally decrease circulating LDL-cholesterol by upregulating hepatic LDL uptake. Inhibition of oxidosqualene cyclase with U18666A also, unlike HMG-CoA reductase inhibition, consistently induces cataract formation in rats. However, this may not be wholly due to inhibition of cholesterol synthesis because U18666A has been shown to have a direct toxic effect of lens epithelial cells.[31] Inhibition of oxidosqualene cyclase results in redirection of 2,3-oxidosqualene, which in turn results in increased formation of oxysterols, and downregulates HMG-CoA reductase;[21] this would not occur with inhibition of squalene synthase (figure 1). These findings suggest that squalene synthase inhibitors will prove to have less toxicity than either oxidosqualene cyclase inhibitors[21,30,31] or statins.[28,29] This is because inhibition of squalene synthase would not result in increased oxysterol formation or decreased ubiquinone levels. However, it remains for more detailed clinical assessment to be undertaken to determine whether the newer squalene synthase inhibitors, such as TAK-475 and EP2302 cause lens opacities or other toxicity.

4. Clinical Trials with Squalene Synthase Inhibitors

Squalene synthase inhibitors are now entering clinical trials and, thus, it will be fascinating to see how effectively they lower LDL-cholesterol levels and the extent to which their role might complement those of statins and other lipid-lowering drugs. Pharmacokinetic, and more especially pharmacodynamic and toxicological studies, will be required in humans to determine whether squalene synthase inhibitors do in reality offer advantages over statins. Thus far, one pharmacokinetic and pharmacodynamic study with the squalene synthase inhibitor BMS-188494 has been performed,[32] in which squalene synthase inhibition was quantified indirectly by assaying dicarboxylic acids in urine. This elegant approach to quantifying the pharmacodynamic response revealed encouragingly that farnesyl pyrophosphate, which might be expected to accumulate when squalene synthase was inhibited, was in fact metabolised through a series of oxidative steps to dicarboxylic acids, readily excreted in urine.

5. Future Directions

On a final note, another source of cholesterol is from intestinal absorption. Ezetimibe is a selective inhibitor of intestinal cholesterol absorption and is increasingly finding use in the clinic as an LDL-cholesterol-lowering agent, although randomised, clinical events trials are yet to be reported. Ezetimibe has a limited LDL-cholesterol-lowering effect of around 20% either alone or in the presence of a statin.[33] It acts by decreasing the intestinal cholesterol supply to the liver, lowering hepatic cholesterol levels and thus inducing LDL-receptor expression. It is effective because it not only decreases the absorption of dietary cholesterol but also interrupts the enterohepatic circulation of cholesterol entering the intestine in bile. Its limitation is the upregulation of hepatic cholesterol biosynthesis. Nonetheless, the 20% additional decrease over and above that achieved with a statin is clinically worthwhile now that therapeutic targets for LDL-cholesterol have been lowered.[34,35] The LDL-cholesterol-lowering goal can also be achieved with lower doses of the

statin, potentially avoiding the adverse effects associated with high statin doses. Squalene synthase development will thus occur against this background.

6. Conclusions

Squalene synthase inhibitors represent an interesting group of drugs for lowering LDL-cholesterol. The potential will depend on their LDL-lowering efficacy compared with and in combination with existing treatment, particularly the statin group. The inhibition of squalene synthase is likely to have fewer downstream effects on other pathways than seen with statins. This might mean that some of the adverse effects associated with statin therapy can be avoided. However, they might also lack some of the favourable pleiotropism of statins and could have other adverse effects related to accumulation of metabolites upstream of squalene synthase. The outcome of clinical trials is eagerly awaited.

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