DOI: 10.1002/cmdc.200700165

The Treatment of Dyslipidemia—What's Left in the Pipeline?

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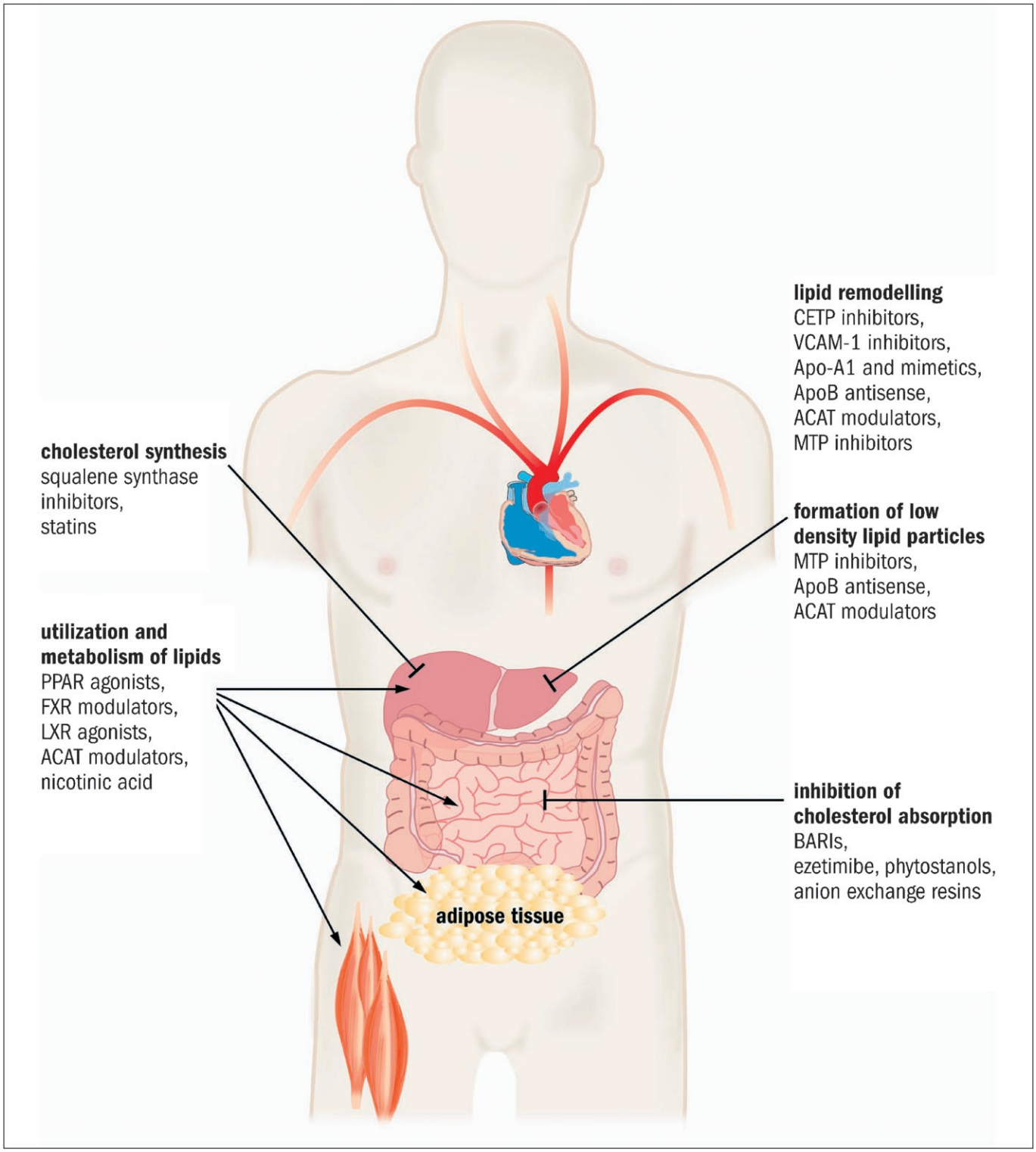

Dyslipidemia is a pathological alteration of serum lipid levels. The most common forms are either elevations of triglycerides or low density lipoprotein cholesterol associated with a reduction of high density lipoprotein cholesterol. Most frequently both forms of lipid disorders are combined. Elevations of free fatty acid blood levels are commonly not subsumed under the term dyslipidemia. However, free fatty acids should also be considered, as they are frequently associated with dyslipidemia and represent a risk factor for cardiovascular diseases. Dyslipidemias are among the major etiologic factors for arterial occlusive diseases. Resulting in fatal implications such as stroke and coronary heart disease, dyslipidemias contribute to the most prevalent causes of death. Lowering of low density lipoprotein and raising of high density lipoprotein cholesterol levels have been shown in both epidemiologic and intervention studies to decrease mortality. Established treatments of dyslipidemias are statins and fibrates. However, recent research has established some new potential therapeutic targets which are currently investigated in clinical trials. New therapeutic approaches include subtype selective, dual, and pan-agonists of the peroxisome proliferator activated receptor, inhibitors of the cholesterol ester transfer protein, Acyl-CoA-cholesterol-acyltransferase, squalene synthase, microsomal triglycerid-transfer-protein, and cholesterol absorption. Clinical implications of new drugs under investigation are discussed in this review.

Introduction

Dyslipidemia is a pathological increase of lipid and lipoprotein levels in the blood. Most prominent among these are the hyperlipidemic diseases hypercholesterolemia and hypertriglyceridemia. Elevated levels of cholesterol do mainly appear associated with low-density lipopoprotein (LDL) and less with very low-density lipoprotein (VLDL) particles in the blood, whereas elevated triglycerides are mainly related to VLDL and chylomycrones. As high-density lipoprotein (HDL) has a protective effect on the endothelium, lowered levels of those lipoprotein particles are associated with an increased risk for cardiovascular disease. Besides some rare genetically determined or familial forms, most disturbances of lipid homeostasis are associated with a more sedentary lifestyle, excessive caloric intake, and age, and do frequently appear combined with each other.[1,2] Another alteration of lipid levels, which is commonly not subsumed under the term dyslipidemia is raised levels of free fatty acids (FFA). Elevations of free fatty acid levels in the blood cause enhanced triglyceride synthesis in the liver and therefore lead to increased VLDL levels. Furthermore, free fatty acids induce insulin resistance, which is another risk factor for atherosclerosis.[3,4]

In both epidemiologic and intervention studies, lower or lowered levels of LDL and triglycerides and increased levels of HDL have been shown to be associated with a decreased risk for cardiovascular events.[5–12] Therefore the reduction of lipid levels or the increasing of HDL seems to be a valuable therapeutic approach. Indeed, several drugs (for example, statins) reduce cardiovascular events and mortality. However, lowering LDL or increasing HDL does not always seem to be an appropriate surrogate parameter for the prediction of a decreased cardiovascular mortality. Fibrates for example efficiently lower triglycerides and less extensively LDL, while increasing HDL. However, in some of the trials the overall mortality or cardiovascular mortality was not significantly lowered.[13–16]

Notably, for some of the newer drug candidates referred to in this review, it has clearly been demonstrated that they beneficially affect lipid parameters but increased mortality. In this review we give an overview about new lipid lowering agents and their targets and discuss their clinical implications and medicinal chemistry. Drugs acting by reducing food intake are not covered by this review.

Therapeutic Targets for the Treatment of Dyslipidemia

Nuclear receptors

Peroxisome proliferator-activated receptor

The peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear receptor family comprising the three subtypes PPARα, PPARδ (also referred to as PPARβ), and PPARγ.

PPAR is a key-player of lipid and glucose homeostasis and therefore an appropriate target for the treatment of dyslipidemias.[17,18] Activation of PPAR leads to dimerization with the retinoid X receptor (RXR) and subsequent transactivation of the expression of target genes especially those of lipid and glucose transport and metabolism for example, lipoproteinlipase (LPL), apolipoprotein A1 (apoA1), fatty acid binding protein (FABP), fatty acid transport protein (FATP), and glucose transport proteins (GLUT).[19–23] As the PPAR subtypes bind to the same cognate response elements (PPRE) on DNA in vitro, the selectivity in vivo seems to largely depend on the tissue specific expression pattern, with PPARα mainly expressed in the liver, PPARγ in the adipose tissue, and PPARδ ubiquitously.[24,25]

Endogenous activators of PPAR are fatty acids the activity of which increases with the degree of unsatuaration. Fatty acids show only minor subtype selectivity, at most it has been suggested that PPARα has the highest affinity to less unsaturated fatty acids.[26,27] In our own observations, PPARα was activated by the mono- and diunsaturated fatty acids oleic and linoleic acid at lower concentrations than required for activation of PPARγ. Eicosanoids derived from cyclooxygenase and lipoxygenase pathways have also to be considered as endogenous

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PPAR activators. As synthetic PPAR agonists mimic these natural ligands, they share the carboxyl group or a bioisosteric equivalent and a lipophilic backbone. Several structural variations have been identified that lead to increased affinity and subtype selectivity.[28,29]

There is also some prevailing preclinical evidence that PPAR activation modulates inflammatory responses, which might lead to some additional beneficial therapeutic effects for example, in endothelial dysfunction.[30,31]

Selective PPARα agonists—new fibrates?

Fibrates are well established in the therapy of dyslipidemias especially hypertriglyceridemia since the 1960s and also slightly increase HDL levels.[32–34]

A common structural element of the fibrates is the 2-hydroxy-*iso*-butyric acid residue. However in some preclinical high-affinity PPARα agonists, the oxygen linker has been replaced by sulfur.

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Prof. Dr. Dieter Steinhilber, born in 1959, studied pharmacy at the University of Tübingen. After an assistantship there he spent a postdoctoral period with Prof. Bengt Samuelsson, Karolinska Institute, Stockholm from 1989 until 1991. In 1994 he received an associated professorship and since 2000 he has been a full professor of pharmaceutical chemistry at the Johann Wolfgang Goethe University in Frankfurt. Since 1999 he has been the director of this institute. From 1999 to 2000 he was dean of the faculty Biochemistry, Pharmacy, and Food Chemistry. Prof. Steinhilber is the speaker of the European Graduate School "Roles of Eicosanoids in Biology and Medicine" funded by DFG and Land Hessen and is a member of the board of ZAFES.

Prof. Manfred Schubert-Zsilavecz was born in Austria in 1961. He studied pharmacy at Karl-Franzens University Graz where he received his PhD in 1989. After spending his postdoctoral sojourn at Bayreuth University, he joined the faculty of chemistry at Ulm as an Erwin Schrödinger Fellowship (1992–1993). Accepting a position at the Johan Wolfgang Goethe University, Frankfurt, he has been a professor since 1997 in the pharmaceutical chemistry department and currently directs a research group of 10–15 postdoctoral and graduate students. Since 2001 Prof. Schubert-Zsilavecz has been dean of the chemical and pharmaceutical science department at Johann Wolfgang Goethe University (responsible for teaching activities). He is member of the board of ZAFES. In 2003 Prof. Schubert-Zsilavecz was appointed scientific director of the Central Laboratory of German Pharmacists and he was recently elected president of the German Pharmaceutical Society.

In vitro the fibrates are ligands of PPARα with moderate affinity displaying EC_{50} values in the micromolar range and possessing about tenfold selectivity over PPARγ. Their moderate affinity in vitro is reflected by dosages starting from one hundred milligrams. Interestingly, bezafibrate activates all three PPAR subtypes in an equipotent fashion in vitro and therefore should be considered as a pan-PPAR agonist. Bezafibrate has been proven to delay the progression of type 2 diabetes, suggesting a close relationship between diabetes and inherent PPARγ agonism.[35–37] There has been an intensive search for new high-affinity fibrates, however to our knowledge only LY518674 (Figure 1) has reached phase II trials. LY518674 is a

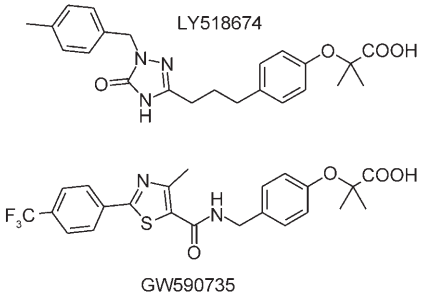

Figure 1. LY518674 and GW590735, selective agonists of PPARα.

highly selective and affine PPARα agonist with an EC_{50} of about 40 nM that displays more than 100-fold selectivity over PPARγ and PPARδ. In humanized transgenic apoA1 mice, LY518674 increased HDL levels and apoA1 synthesis. A first phase II trial with LY518674 has been completed but a subsequent trial has been suspended.[38,39]

Recently, the synthesis and structure of another selective PPARα agonist GW590735, which is currently in phase II clinical trials has been published.[40] In vitro, GW590735 showed an EC_{50} value of 4 nM for the activation of PPARα and a more than 100-fold selectivity compared to PPARγ and PPARδ. In a humanized apoA1 transgenic mouse model, GW590735 did significantly lower VLDL, LDL, and triglycerides whereas HDL was increased.

As fibrates are well established and effective in clinics, it might be questioned, whether new PPARα agonists with higher affinity and therefore presumably lower doses will show a higher therapeutic benefit and will be competitive on an economic basis. However, fibrates are essential drugs for the treatment of dyslipidemia. Fibrates are in use for more than two decades but there has been no real progress in the development of new fibrates. At least one of them, gemfibrozil is known for its strong potential for interactions, which promoted the development of rhabdomyolysis when it was combined with the cholesterol lowering drug cerivastatin. The introduction of new PPARα agonists with increased selectivity and affinity would enrich the spectrum of available fibrates and thus advance current therapies.

Selective PPARδ agonists—a new therapeutic approach

The activation of PPARδ is a rather new approach.[41] Although bezafibrate shows PPARδ agonistic activity, it is not clear at the moment whether selective PPARδ agonism is of any clinical relevance. There is preclinical evidence that PPARδ might have beneficial effects on lipid parameters.[41] GW501516 (Figure 2)

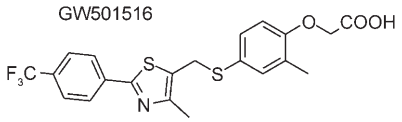

Figure 2. GW501516, a selective agonist of PPARδ.

is a highly selective and potent PPARδ agonist with an EC_{50} value of about 1 nM that displays 1000-fold selectivity over PPARα and PPARγ. GW501516 significantly reduced TG levels and increased HDL cholesterol in obese rhesus monkeys. Similar trends were observed in a small investigational study with nine healthy volunteers.[42,43] Phase II clinical trials seem to be ongoing, but have not been published so far. In addition, GW501516 led to weight loss in high fat diet-induced obese mice.[44]

However, preclinical observations also raise concerns, whether PPARδ activation might promote cancer.[45–51] Even if no such effects have been observed for the pan-agonistic bezafibrate in man, it cannot be excluded, that the selective PPARδ activation might promote angiogenesis and tumour progression.

Selective PPARγ agonists—affecting lipid levels with an antidiabetic drug

PPARγ selective glitazones are established in the treatment of type 2 diabetes.[52] However, clinical data show that the blood glucose level and lipid parameters are affected by rosiglitazone and pioglitazone.[34,53]

As PPARγ is most prominently expressed in the adipose tissue, it has to be considered as the major target organ. Activation of PPARγ leads to an enhanced uptake and storage of glucose and more relevant, free fatty acids in the adipose tissue.[19,54]

As mentioned above, glitazones, which are also referred to as thiazolidinediones (TZDs), do not only have an impact on blood glucose, but have also been shown to increase HDL, to lower TG, and for pioglitazone only, to lower LDL as well.[52]

A frequent side effect of glitazones is weight gain, which is caused by an increase of subcutaneous adipose tissue and fluid retention.[55] In a recent study, fluid retention has been suggested to be a PPARγ specific effect, caused by the increased expression of a renal sodium channel.[56] Weight gain alone is a typical side effect of antidiabetic drugs and also appears with insulin substitution and sulfonylurea therapy, although to a lesser extent. The increased incidence for edema and heart failure under glitazone therapy has lead to the con-

clusion that thiazolidinediones should not be used in patients with congestive heart failure or with edemas.[55]

Netoglitazone (MCC-555) (Figure 3), rivoglitazone (CS-011), and balaglitazone (DRF-2593) are examples of PPARγ selective agonists in clinical development, even though netoglitazone has also been suggested to affect other PPAR subtypes as well.[57–60]

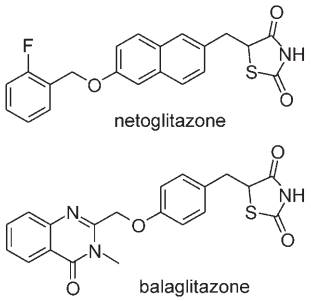

Figure 3. New glitazone type of PPARγ agonists.

A new focus in PPARγ research is the development of partial agonists such as halofenate (Figure 4). Some of these PPARγ modulators lower glucose levels as well, but might show a reduced fluid retention.[61–63] There are many factors influencing the balance between PPARγ agonism and antagonism that include the distribution of the drug in the body, the modulation of the interaction of PPARγ with co-activators and co-repressors by the drug, and the tissue distribution (kidney versus adipose tissue) of these proteins. Interestingly, recent reports have shown that some angiotensin II receptor antagonists are partial agonists of PPARγ with a moderate micromolar activity and one of them, telmisartan, is currently undergoing clinical trials to determine whether the PPARγ agonism shows any additional clinical benefit.[64,65]

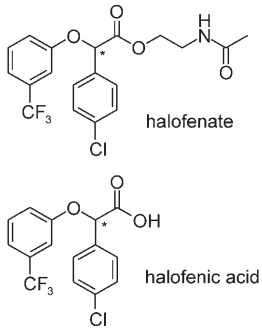

Figure 4. Halofenate and its active metabolite halofenic acid, a partial agonist of PPARγ.

Dual PPARα/γ agonists—two birds with one stone?

As hypertrigliyceridemia and type 2 diabetes are frequently associated with each other, the rationale for the combination of PPARα and PPARγ activity in one drug is obvious: PPARα agonism reduces triglyceride levels and activation of PPARγ hyperglycemia.[66–69] Hence, many efforts have been started to obtain dual agonists. However, so far, none of them has been approved although some of them have reached different stages of clinical trials up to phase III. Many of the drug candidates have been withdrawn from further trials for different reasons and there has been much controversy, whether the combined activation of PPARα and PPARγ might lead to distinct and additional toxicological problems.

The dual PPARα/γ agonist ragaglitazar (Figure 5) has been withdrawn from clinical studies after having reached phase III trials, due to the induction of bladder tumours in rats.[70,71] An animal study in rats suggests some changes in the rate of early growth response factor (EGF-1) expression in bladder urothelium, which depends on the concomitant activation of both PPARα and PPARγ. However the relevance of this observation is not clear. Interestingly, a recent publication shows that another dual PPARα/γ agonist, muraglitazar, did also lead to bladder tumours in rats, however the effect has been shown to depend on the formation of calcium and magnesium contain-

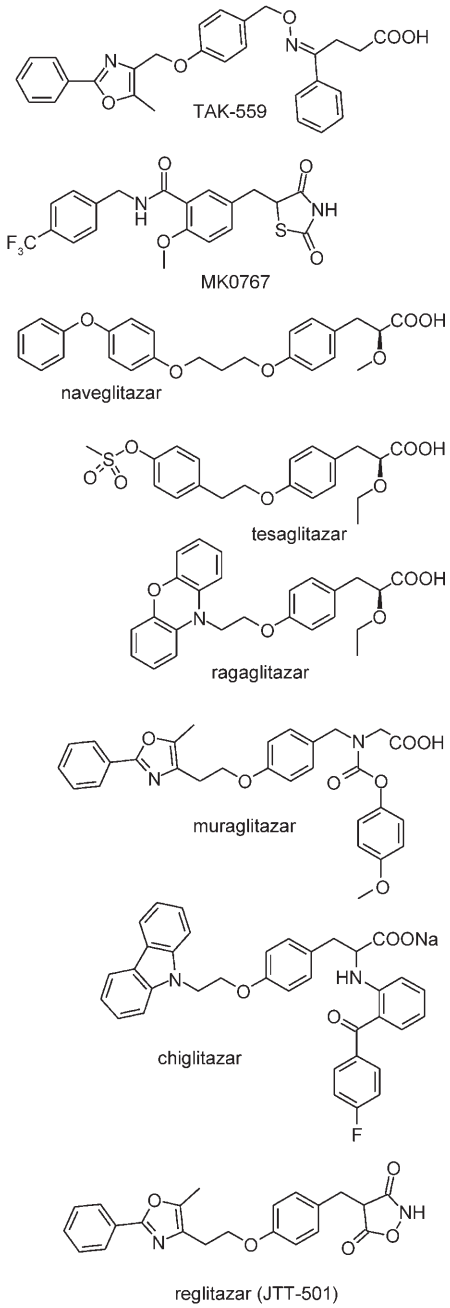

Figure 5. Dual PPARα/γ agonists.

ing precipitates and did not occur when the urine was acidified by diet, suggesting that muraglitazar is prolithogenic in rats and therefore promoting bladder tumours.[72,73] There are only two other publications in PubMed concerning bladder tumours in rodents after the administration of a PPAR activator, the PPARα agonist clofibrate.[74,75] However there is some data referred to by Samuel Cohen, which has been presented at the FDA or different symposia, suggesting that PPARγ activation might be promoting bladder tumours in rats.[76] The induction of bladder tumours by PPARγ and dual PPARα/γ agonists was exclusively observed in rats, preferentially in male ones. Cohen assumes that the bladder tumours in rats are initiated by lithiasis rather than activation of local PPARγ. That would explain why rats and especially male ones are more prone to that effect. As PPARγ activation leads to fluid retention in man (see above) and rats, this might be the triggering event for urolithiasis and subsequent tumour development in rats.

At present, it is unclear whether bladder tumour formation is relevant in man. One might argue, if the effect would be PPARγ mediated that there is a prevailing preclinical body of evidence that PPARγ is rather tumour protective.[77–79] Interestingly the PPARγ agonist rosiglitazone has recently been clinically investigated for the treatment of early stages of bladder carcinoma. The trial has been completed but not been published so far. At present, PPARγ agonistic glitazones did not show a clear benefit in clinical trials for the treatment of different types of cancer conducted so far.

Ragaglitazar is a dual agonist with a tenfold preference for PPARγ. In a 12 week dose ranging study, at single daily doses of 1 to 10 mg, ragaglitazar significantly decreased fasting plasma glucose, triglycerides, free fatty acids, LDL, total cholesterol, and glycosylated hemoglobin (HbA_{1c}) and increased HDL cholesterol compared to placebo. Reported side effects were weight gain, edema, leukopenia, and anemia.[80]

Muraglitazar is another example for a recent failure of dual PPARα/γ agonists. The new drug application (NDA) for muraglitazar had been filed to the US food and drug administration (FDA) at the end of 2005, but is still pending after the FDA has demanded additional cardiovascular safety data. The decision is related to an article by Nissen et al. in *JAMA*, where the authors analysed the data of the clinical trials and came to the conclusion that muraglitazar caused a significant excess in several composite end points including fatal events. Other combined or individual end points tended to be increased, however not significantly.[81]

It might be questioned whether the free post-hoc combination of individual endpoints and the comparison to combined control groups pioglitazone and placebo actually allows conclusion about significances. However the elevated relative risk for any of the individual end points for muraglitazar, whether significant or not, is convincing and supports the author's statement that approval should depend on additional trials concerning cardiovascular safety.

Muraglitazar is a nearly equipotent agonist of PPARα and PPARγ, with slight prevalence for PPARγ (Table 1). Muraglitazar has been investigated in phase II and III clinical trials, at dosages from 0.5 to 20 mg once a day, for its efficacy to lower lipids and glucose in the blood. At present, there are two published phase III clinical trials[82,83] and further clinical data can be extracted from the new drug application (NDA) 21-865 at the FDA homepage.[84] In CV168018, the effect of 2.5 and 5 mg muraglitazar monotherapy versus placebo over 24 weeks in three parallel groups of about 100 subjects each was studied. Muraglitazar significantly decreased fasting plasma glucose, free fatty acids, and HbA_{1c} levels but also significantly increased body weight.[82] CV168025 investigated the combined therapy of either 5 mg muraglitazar or 30 mg pioglitazone plus open-label metformin over 24 weeks in two parallel groups of over 500 subjects each with a 26 week follow-up control of weight gain, edema, cardiovascular events, and death. Muraglitazar was superior to pioglitazone in the reduction of HbA_{1c} (week 24), triglyceride, and non HDL-cholesterol (week 12) and in the increase in HDL-cholesterol (week 12).

Table 1. In vitro PPAR activation profile of dual PPARα/γ agonists.

	PPARα[a]	PPARγ[a]	Reference
ragaglitazar	0.98	0.09	[85]
tesaglitazar	1.2	1.3	[86]
muraglitazar	0.32	0.11	[87]
chiglitazar	1.2	0.08	[88]
reglitazar	5.4/1.9	0.28/0.08	[89], [90]
naveglitazar	2.86	0.36	[91]
MK0767	0.15/0.85	0.08	[90], [92]
TAK559	0.07	0.03	[93]

[a] EC_{50} values [μM] obtained in cellular transactivation assays.

Muraglitazar also showed a significantly higher weight gain and comparable rate of edema after week 50, compared to pioglitazone. The authors also provided data for cardiovascular events and deaths for both muraglitazar and pioglitazone, but no statistical analysis for these events, presumably due to low event rates that are related to the short duration of the trial.[83] What is striking is the lack of data comparing muraglitazar effects on triglycerides, non-HDL-, and HDL-cholesterol versus pioglitazone after 24 weeks, suggesting that the advantage of muraglitazar was lost.

Tesaglitazar is the third dual PPARα/γ agonist, which recently has been withdrawn because of decreased creatinine clearance and glomerular filtration rate (press release by Astra Zeneca). Tesaglitazar again is a dual PPARα/γ agonist. In a phase I study at doses of up to 1 mg per day for 12 weeks in 390 non-diabetics, tesaglitazar significantly decreased triglycerides, free fatty acids, non-HDL cholesterol, and fasting plasma glucose, and increased HDL cholesterol. At the 0.5 and 1 mg dosage, tesaglitazar also significantly increased body weight by approximately one kilogram, an effect similar to that of glitazones.[94] Another observation was increased serum creatinine levels in the first month of this study. This effect was confirmed in the yet unpublished ARMOR and GALLANT trials. This side effect and the associated reduction of the glomerular filtration rate led to the withdrawal of tesaglitazar.

Other examples of dual PPARα/γ agonists are TAK-559, KRP-297, and JTT-501, withdrawn because of unexpected toxic effects. In vitro data suggest TAK-559 to be a nearly equipotent

dual PPARα/γ agonist.[93] MK0767 has been reported to be a nearly equipotent dual PPARα/γ agonist.[92] However, other authors found that MK0767 (which has formerly been described as KRP-297) is a dual agonist with tenfold preference for PPARγ with EC_{50} values of 0.85 and 0.083 μM for PPARα and PPARγ, respectively.[90] In a recent small phase I study at doses of up to 25 mg once daily over 14 days in healthy males, MK-0767 led to significant reductions in triglycerides, free fatty acids, LDL, and total cholesterol and at the 25 mg dosage in fasting glucose.[95] The compound farglitazar, despite its name is at least in vitro a selective PPARγ agonist with a 1000-fold preference for PPARγ compared to PPARα.[96]

The development of naveglitazar is reported to be discontinued.[97] So today only chiglitazar seems to be left in clinical development. Shenzhen Chipscreen is announcing chiglitazar as a PPAR pan-agonist, suggesting that it might activate PPARδ as well, however there is no data available supporting that statement. Furthermore, Astra Zeneca reported that a PPARα/PPARγ-partial agonist has entered phase II clinical studies.

Notably, the in vitro PPAR activation profile of the referred dual PPARα/γ agonists with the exception of tesaglitzar and TAK559 shows an up to tenfold preference for PPARγ. At present, it is not clear, whether a preference in PPARα activity would lower the risk for edema and cardiovascular events.

Dual PPARα/δ agonists?

A rather new concept is the combined activation of PPARα and PPARδ, which is expected to combine the beneficial effects of PPARα and PPARδ activation on lipid metabolism, without being restricted to type 2 diabetics. However there seems to be no such compound in clinical development yet (Figure 6).

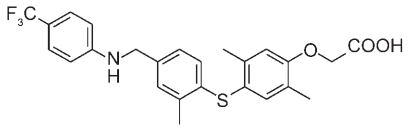

Figure 6. T0913659 a preclinical dual PPARα/δ agonist.[98]

Dual PPARγ/δ agonists?

Another concept is the simultaneous activation of PPARγ and PPARδ. The concomitant activation of both receptors is hoped to counteract the PPARγ induced weight gain. However, so far it does not seem that any compound has entered clinical trials.[99–101]

Pan-PPAR agonists

At last, when combining the activation of PPAR subtypes, then why not combine all three subtypes?[102] Well-known examples for such types of PPAR agonists are fatty acids as endogenous PPAR ligands, which are rather unselective and can be considered as pan-PPAR agonists. Another example of a pan-PPAR agonist is bezafibrate, which at least in vitro is an almost equipotent activator of all three PPAR subtypes. This drug positively influences glucose homeostasis and is well established and proven safe in decades of clinical use.[35,36,90] Bezafibrate also leads to a minor, however significant decrease in body mass index.[37] Candidates in early clinical development are GW625019, sodelglitazar (GW677954) (Figure 7), and PLX204.

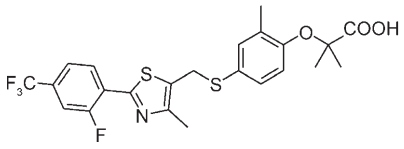

Figure 7. Sodelglitazar (GW677954).

Summary of PPAR agonists

In conclusion, today only the fibrates and the glitazones are established as PPAR agonistic drugs. Since the discovery of PPAR as the molecular target for these types of drugs in the early 1990s, there has been a lot of research on selective and combined PPAR agonists. However none of these new compounds has reached the market so far.

PPARδ seems to be a new promising approach. As it is expressed ubiquitously and its physiological effects are less understood, it might not be such a straightforward target as PPARα or PPARγ.

Dual PPARα/γ agonists seemed to be a promising strategy as well. However, there are concerns, whether dual PPARα/γ agonists might not only show the beneficial effects, but also show the combined or even superadditive toxicological profile by activating PPARα and PPARγ. So far, we cannot definitively answer this question. In the case of muraglitazar, it seems pretty likely that PPARγ specific side effects such as edema induction and an increased risk for coronary heart failure counterbalance the beneficial effects. As these effects are well known, they encourage the development of PPARγ partial agonists, also in combination with PPARα agonism. Several other dual PPARα/γ agonists failed due to toxic side effects, but so far it cannot be concluded whether these effects depend on the concomitant activation of PPARα and PPARγ or whether they are unrelated to the mode of action.

However, it has to be considered that many patients concomitantly receive fibrate and glitazone therapy, but so far there are only very limited clinical trials dealing with combination of both types of drugs.[103,104] With respect to the concern about enhanced toxicity of concomitant activation of PPARα and PPARγ, there is of course an urgent need for such a study. However, it should be kept in mind that endogenous ligands such as free fatty acids act as pan-PPAR agonists. Furthermore long experience with the pan-PPAR agonist bezafibrate does not provide evidence for such undesired events.

Agonists of the farnesoid X and the liver X receptor

The liver X receptor (LXR) and the farnesoid X receptor (FXR) are both members of the nuclear hormone receptor superfami-

ly. Activation of both receptors occurs by the formation of heterodimers with the retinoid X receptor (RXR).[31,105–107] FXR functions as a bile acid sensor and is highly expressed in tissues with high concentrations of bile acids such as liver, kidney, and small intestine.[108] FXR is an important regulator of lipid and cholesterol metabolism. FXR agonists lower serum triglyceride levels whereas FXR antagonists are predicted to lower cholesterol levels. Thus the challenge is to design FXR modulators (partial agonists).

The natural product guggulipid, an extract of the resin of *Commiphora mukul*, has been reported to lower lipid levels. The *E* and *Z* isomers of the compound guggulsterone (Figure 8), which are considered as the active ingredients, have been shown to be antagonists of FXR with an efficacy in the micromolar range.[109] However, more recent research raises doubt whether guggulsterone containing extracts are truly lowering lipid levels.[110] Furthermore it has been shown that guggulsterones do not only affect FXR but other nuclear receptors as well and even more potently.[111]

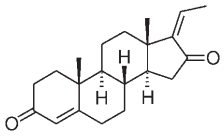

Figure 8. *Z*-guggulsterone.

The LXRs (LXRα and LXRβ) are oxysterol-activated transcription factors which play an important role in regulating the expression of the ABCA1 (ATP binding cassette A1) gene.[112] ABCA1 mediates the efflux of free cholesterol from peripheral tissues to nascent HDL. Thus, upregulation of ABCA1 expression is expected to promote reverse cholesterol transport. Several small molecule LXR agonists have been synthesized for example, the sulphonamide T0901317 and the acetic acid derivative GW3965 (Figure 9). For both compounds, preclinical studies in mice showed increased expression of ABCA1 and increased plasma HDL levels.[113,114]

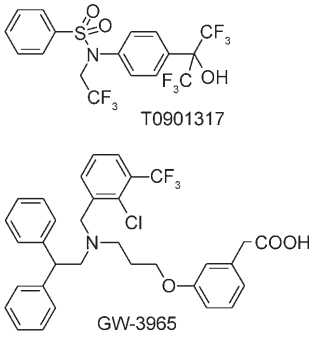

Figure 9. LXR-agonists.

CETP Inhibition

Based on the well-established inverse relation between the risk for cardiovascular disease and the level of HDL cholesterol, new pharmacological strategies to raise HDL have become interesting options.[115,116] The cholesteryl ester transfer protein (CETP) is a promising target, because the cholesteryl ester transfer process lowers HDL and contributes to an atherogenic lipoprotein profile, particularly when plasma triglycerides are high. CETP facilitates the transfer of cholesteryl ester from HDL to proatherogenic lipoproteins like LDL and VLDL with a balanced exchange of triglycerides.[117] Thus, CETP-inhibition is expected to raise HDL, lower LDL and provide a therapeutic benefit for patients with coronary heart disease (CHD). Based on results from pharmacological interference with reverse cholesterol transport, observations in animal models and CETP-deficient human populations, pro- as well as antiatherogenic effects of CETP-inhibition are still in discussion.[118] So far the most pronounced increase in HDL has been achieved by CETP inhibition.

Two substances, JTT-705 and torcetrapib (Figure 10), have been tested in phase III studies. JTT-705 is a thiol-based inhibitor.[50] To improve chemical stability and oral absorption, JTT-705 has been developed as a prodrug which shows an inhibition of CETP with an IC_{50} value of 6 μM in human plasma. An increase in activity occurs by hydrolysis of the isobutyryl thioester moiety (Figure 11), leading to the free thiol ($IC_{50} = 3$ μM) which is almost twice as potent as JTT-705. This relation in human plasma is consistent with calculated IC_{50} values in different animal species such as rabbits (1.0 μM versus 0.44 μM) and cynomolgus monkeys (2.4 μM versus 1.3 μM).[118] The suggested mechanism is the formation of a disulfide bond between the free thiol and a cysteine residue at position 13 of CETP.[119]

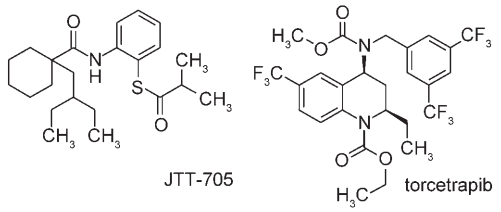

Figure 10. Structures of JTT-705 and torcetrapib.

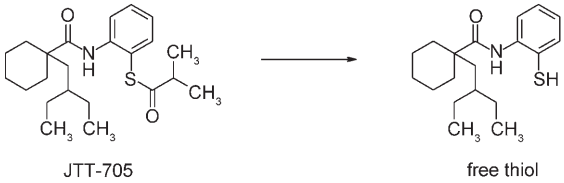

Figure 11. Bioactivation of JTT-705.

Currently, data for phase I and phase II studies of JTT-705 are published. A phase II study included 298 healthy individuals with mild dyslipidaemia, who were treated with JTT-705 at doses of 300–900 mg day^{-1} for 4 weeks.[120] The optimal dose was 600 mg day^{-1}, whereas 900 mg day^{-1} only slightly enhanced the effects. JTT-705 900 mg day^{-1} increased HDL by 34% whereas LDL was decreased by 7% and plasma triglycerides were not altered. The drug was well tolerated and the ef-

fects were readily reversible after washout. Another phase II study in which JTT-705 was combined with pravastatin 40 mg day^{-1} maintained these observations.[121] JTT-705 has been licensed in by Roche and the results of phase III studies are eagerly awaited.[122]

Torcetrapib belongs to a class of inhibitors based on 1,2,3,4-tetrahydroquinoline which shows a higher potency than JTT-705 with an IC_{50} of 0.05 μM. The molecule has two chiral centres, the 2 *R*-ethyl and 4 *S*-amino orientation was identified as optimal and thus used for further development. Torcetrapib specifically binds to CETP in an 1:1 stoichiometry and acts as a reversible inhibitor of CETP.[119] Notable physicochemical parameters are the very poor water solubility and high hydrophobicity (log*P* > 4.5) requiring atypical formulation technologies to ensure oral bioavailability.[118] The first human studies have been very promising: The maximal effect observed in a multidose study with 40 healthy subjects was an increase of HDL by 91% and a decrease of LDL by 42% with 120 mg torcetrapib twice a day. Based on these results, Pfizer decided not to await the outcome of two phase II studies but to start in parallel the large-scale phase III ILLUMINATE-trial (15000 patients) which was terminated abruptly after approximately one year and torcetrapib was withdrawn in December 2006.

Recently, the outcomes of the above mentioned phase II studies have been published.[123,124] As a parameter for the efficacy in the treatment of atherosclerosis the studies used maximum carotid intima-media thickness and normalised total atheroma volume, respectively.[123,124] The study carried out by Kastelein et. al included 850 patients with heterozygous familial hypercholesterolemia receiving either atorvastatin alone or atorvastatin combined with 60 mg torcetrapib for 2 years.[123] Despite an increase of HDL in the atorvastatin plus torcetrapib group by 54.4% (atorvastatin alone: 2.5%) and a decrease of LDL in the atorvastatin plus torcetrapib group by 14.4% (atorvastatin alone: +6.3%), both subgroups showed nearly no effect on the maximum carotid intima-media thickness as primary end point. Additionally, a slight increase in carotid intima-media thickness of the common carotid artery provided evidence of accelerated atherosclerosis in the atorvastatin plus torcetrapib group, which cannot be explained by the observed increased blood pressure of 2.8 mmHg in this group.[123] The results obtained in this study are consistent with results from the study carried out by Nissen et al. which included 1188 patients with CHD.[124] Besides the fact that mortality was raised significantly in the atorvastatin plus torcetrapib group, no additional information about the outcome of the ILLUMINATE trial is available at this point in time. There is an ongoing controversial discussion about the reasons for the failure of torcetrapib, raising the questions if torcetrapib is a "dirty drug" with several undiscovered off-target effects or if the approach of CETP-inhibition has no therapeutic benefit.[122]

Squalene Synthase Inhibitors

One of the rate limiting steps of cholesterol synthesis is the formation of squalene from trans-farnesyldiphosphate which is catalysed by the enzyme squalene synthase. An inhibition of this enzyme leads to a reduction of LDL, similar to the statins. Statins lower plasma mevalonate concentrations by the inhibition of HMG-CoA-reductase, therefore reducing cholesterol synthesis and the formation of nonsteroidal isoprenoids such as dolichol and ubiquinone.[125,126] The most important safety concern associated with statin therapy is myalgia, which might be caused by the depletion of mitochondrial ubiquinone levels.[127] Squalene synthase acts downstream of mevalonate and thus an inhibition might be able to lower LDL, avoiding the effects associated with decreased formation of nonsteroidal isoprenoids. TAK-475 (Figure 12), an orally active squalene

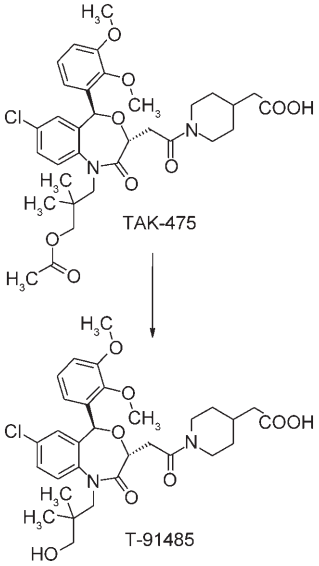

Figure 12. The squalene synthase inhibitor TAK-475 and its active metabolite T-91485.

synthase inhibitor derived from a series of 4,1-benzoxazepine-3-acetic acid derivatives is currently undergoing several phase III studies. One of these studies determining the efficacy and safety of TAK-475 compared to placebo in subjects with primary hypercholesterolemia was expected to be completed in June 2006, but no data from any human trial is available so far.[128,129]

Other ongoing studies are designed to investigate several combination regimens, for example, with atorvastatin, simvastatin, and ezetimibe.

The evaluation of TAK-475 in a variety of animal models showed a decrease in hepatic VLDL production and an increase in LDL clearance, but no influence on plasma HDL levels. The IC_{50} value of TAK-475 measured in vitro using squalene synthase from HepG2 cells is 79 nM.[130] TAK-475 is a prodrug and its pharmacologically active compound (T-91485) is generated by hydrolysis of the acetylated hydroxy group. According to the anticipated improved safety profile compared to the statins, the in vitro myotoxicity in human RD cells and skeletal myocytes was investigated. Compared to atorvastatin and simvastatin, the in vitro myotoxicity of T-91485 was at least 100- and 50-fold lower, respectively.[131]

Intestinal Cholesterol Absorption Inhibitors

Several direct approaches to inhibit the cholesterol uptake from the intestine are possible; one prominent example is ezetimibe. Currently, FM-VP4 (Figure 13) which is a water-soluble

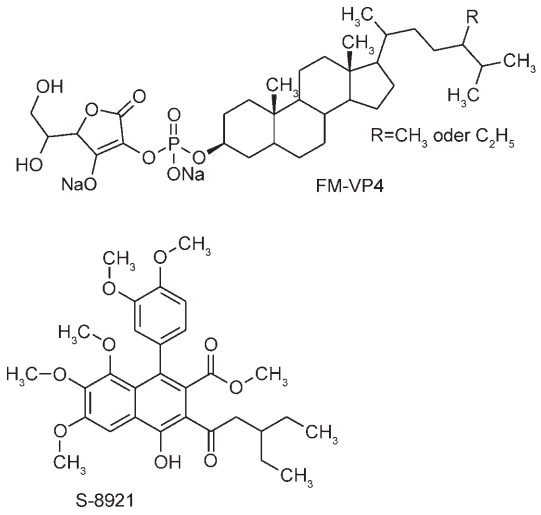

Figure 13. Cholesterol absorption inhibitors.

phytostanol analogue is under clinical investigation.[132] FM-VP4 is based on hydrogenated campesterol and sitostanol, each of them is covalently linked to ascorbic acid by a phosphodiester bond. Animal studies carried out with gerbils and apoE-deficient mice yielded very promising results. In fact, a significant decrease in both total cholesterol and total triglycerides, an increase in HDL cholesterol, and a profound effect on body weight was observed.[132] Notably, no apparent toxic effects were seen. The mechanism by which FM-VP4 prevents the absorption of cholesterol from the gastrointestinal tract is unknown. A phase II study that started in November 2005, investigating the safety and efficacy of FM-VP4 in subjects with primary hypercholesterolemia was completed, but no results have been published so far.

Another approach to treat hypercholesterolemia by modulating the cholesterol absorption process is by using bile acid reabsorption inhibitors (BARI), for example, the long established bile acid sequestrants. A new class of BARIs acts through inhibition of the ileal bile acid transporter (IBAT) which is responsible for the reabsorption of bile acids into the enterohepatic circulation.[133] In the liver, bile acids inhibit the cholesterol-7α-hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids. Thus, an interruption of this circulation leads to an enhanced formation of bile acids which goes along with an upregulation of LDL-receptors and a decrease of serum LDL.[134] S-8921, a lignan derivative, is a specific IBAT-inhibitor which is currently in phase II trials.[134] Efficacy of this compound in lowering serum cholesterol and reducing atherosclerosis was shown in a variety of animal studies.[135,136] Interestingly, because of the action of BARIs at the luminal side of the intestine, these compounds do not need to be systemically available which could be advantageous with regard to the safety profile of these drugs.[134]

Vascular Protectants—VCAM-1 Inhibitors

Vascular cell adhesion molecule-1 (VCAM-1) is an early inducible inflammatory response gene which is upregulated in chronic inflammatory diseases such as atherosclerosis. Thus, besides its eligibility as a biomarker, VCAM-1 is a very promising target for the treatment of these diseases.[137] Succinobucol (AGI-1067) (Figure 14) is the monosuccinate ester of probucol,

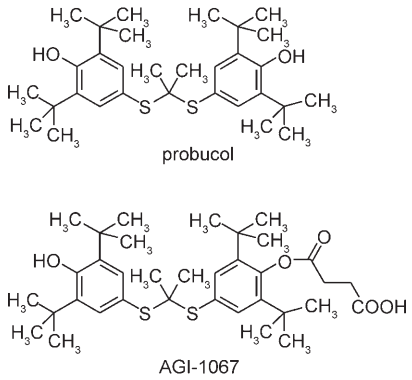

Figure 14. Vascular protectants.

an antioxidant which was discovered in the 1960s and which has been available on the US market as a lipid-lowering agent before the establishment of statins.[138] In addition to its action as an antioxidant and in contrast to probucol, succinobucol shows potent inhibitory activity on VCAM-1 expression and has improved physicochemical properties.[139] Furthermore, there have been safety concerns due to a QTc prolongation caused by probucol. In the case of succinobucol this effect is missing. A large scale phase III study (ARISE: aggressive reduction of inflammation stops events) with approximately 6600 subjects worldwide started in June 2003 and was estimated to be completed in December 2006. The aim of the study was the evaluation of the safety and efficacy of succinobucol in the treatment of vascular inflammation and atherosclerosis compared to placebo, by assessing the reduction in cardiovascular events. Results of this study are not published yet.

However, the results of a smaller study named CART-2 were recently published.[140] In this placebo-controlled, randomised trial the effects of 280 mg succinobucol per day on coronary atherosclerosis were investigated for 12 months ($n = 232$). Patients treated with succinobucol showed an atherosclerosis regression of $-4.0\ mm^3$ (assessed by coronary vascular ultrasound - IVUS), although this was not statistically significant. The reason for the lack of significance might be the study size and the relatively short duration. Thus, the outcome of the ARISE study has to answer the remaining question about the efficacy of succinobucol. LDL was decreased, whereas HDL was increased significantly in the verum group of CART-2, although the clinical relevance of these changes is not clear. Notably, the

observation of reduced levels of myeloperoxidase in CART-2 supports the assigned anti-inflammatory activity of succinobucol.

ApoA1 and Mimetics

Apolipoprotein-A1 (apoA1) is the major protein component of HDL, consisting of 243 amino acids. ApoA1 is able to interact with lipids and aqueous environments because of its amphiphilic properties. Similiar to HDL, apoA1 levels are inversely correlated to the risk of atherosclerosis and other vascular diseases.[141] The protective effect of apoA1 is based on a number of mechanisms.[142] Firstly, there is a major role of apoA1 in reverse cholesterol transport, partly mediated by activation of the lecithin cholesteryl acyltransferase (LCAT), which converts cholesterol from peripheral cells to cholesteryl ester. Secondly, anti-inflammatory properties have been shown for apoA1. Several peptide and small molecule mimetics of apoA1 are currently under clinical investigation. $ApoA1_{milano}$ is a naturally occurring mutant protein which is characterized by a cysteine to arginine mutation at position 173 in the α-helix of apoA1.[143] This structural change allows the formation of homodimers and heterodimers with apoA2. A first clinical study investigating the weekly intravenous administration of a recombinant $apoA1_{milano}$/phospholipid complex (ETC-216) to patients with acute coronary syndromes has been published.[144] After 5 weeks, ETC-216 led to a significant regression of coronary atherosclerosis as measured by IVUS. In 2006, another study was published in which the relationship between atheroma regression and arterial wall remodelling after administration of ETC-216 was investigated by IVUS.[145] The authors showed a significant reduction of atheroma volume, especially in those arterial subsegments with the greatest plaque burden. The regression of atherosclerosis was accompanied by a reduction of the external elastic membrane volume, but interestingly there were no changes in luminal dimensions observed. Thus, it can be supposed that atherosclerotic plaque regression goes along with a reverse remodelling of the external elastic membrane and is achievable by infusion of $apoA1_{milano}$.

The finding that the lipid binding properties of apoA1 are largely related to its class A amphiphatic helices led to the design of synthetic 18 amino acid-peptides with a similar secondary structure.[146] D-4F is an apoA1 mimetic that exclusively contains D-amino acids and thus can be given orally. The description 4F reflects the fact that four of the 18 amino acids are phenylalanine residues. Early human clinical trials are in progress, in mice the combination of D-4F and pravastatin was able to regress already established atherosclerotic lesions.[147] Another compound which has reached clinical trials is AZD-2479 (Figure 15), a small molecule apoA1 mimetic. AZD-2479 is an orally bioavailable drug that contains a biphenylalanine residue linked with glutamate and arginine.[148]

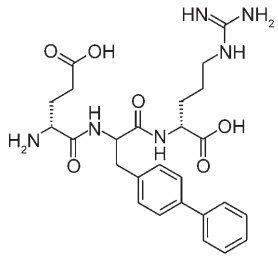

Figure 15. AZD-2479 a small molecular entity as an apoA1 mimetic.

Antisense Inhibitors of ApoB-100

Apolipoprotein-B (apoB) is the major protein component of LDL and plays a key role in LDL transport and removal. High levels of apoB seem to correlate with cardiovascular risk.[149] Thus, direct inhibition of apoB production might be an attractive therapeutic strategy. The antisense inhibitor ISIS 301012 is a 20-mer oligonucleotide, complementary to the coding region for human apoB-100 mRNA. Several clinical trials up to phase II were carried out. In 2006, the FDA granted orphan drug status to ISIS 301012 for the treatment of homozygous familial hypercholesterolemia.[150] Kastelein et al. investigated the short-term administration of ISIS 301012 to humans with mild dyslipidaemia.[151] ISIS 301012 was given weekly subcutaneous and led to a significant, prolonged, and dose dependent reduction in apoB-100 and LDL, with maximum reductions of 50% for apoB-100 and 35% for LDL reached with the largest dose of 200 mg. The most prominent side effect was mild erythema at the injection site. Further phase II studies assessing the safety and efficacy of ISIS 301012 are ongoing.

ACAT Inhibitors

Acyl-CoA cholesterol transferase (ACAT) is an intracellular enzyme which catalyzes the esterification of cholesterol with free fatty acids into cholesteryl esters. Two isoforms of ACAT are known: ACAT-1, which is expressed ubiquitously, and ACAT-2, which can be found in the liver and the small intestine.[127,152] The accumulation of cholesterol esters is a pivotal process during the formation of foam cells from macrophages and is associated with the formation of atherosclerotic plaques. Inhibition of this enzyme is supposed to reduce the accumulation and deposit of cholesterol esters in macrophages and the vessel wall. Furthermore, the clearance of free cholesterol should be increased.

Avasimibe and pactimibe (Figure 16) are nonselective ACAT inhibitors that reached phase III clinical trials. However, both compounds did not show the expected therapeutic effects and further development was discontinued in 2003 (avasimibe, Pfizer) and 2005 (pactimibe, Sankyo), respectively. In several animal studies, atherosclerotic lesions were reduced with ACAT inhibitors.[153] However, the subsequent clinical trials showed the opposite result: a proatherogenic effect was shown for avasimibe and pactimibe.[154,155] There are several issues that are discussed in the context of the failure of both compounds.[156] There have been large dosage differences between the animal and the human studies. The pharmacological explanation faces two issues: The accumulation of free cholesterol in macrophages leads to macrophage cell death, instead of an enhanced reverse cholesterol transport.[157] The other fact is

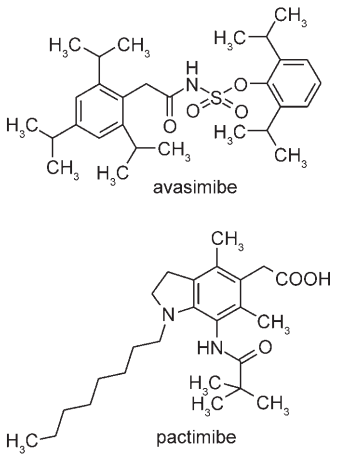

Figure 16. The ACAT inhibitors avasimibe and pactimibe.

that ACAT-1 depletion alters ABCA1 protein stability, thereby reducing reverse cholesterol transport.[158] Although the attractiveness of unspecific ACAT inhibitors in cardiovascular prevention is quite low after these findings, there are other approaches such as the design of specific ACAT-2 inhibitors and the combination of ACAT-inhibitors with drugs that stimulate reverse cholesterol transport.[156]

Novel Approaches in Nicotinic Acid Therapy?

In 1954, the Canadian pathologist Rudolf Altschul discovered that gram doses of nicotinic acid (Figure 17) lowers plasma

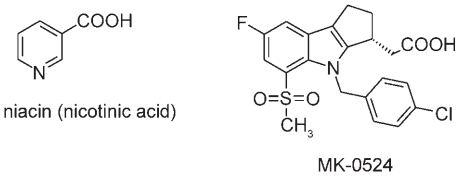

Figure 17. Structures of niacin and the prostaglandin D2 receptor antagonist, suppressing niacin induced flush.

levels of cholesterol.[159] This was the beginning of the career of one of the most effective drugs for the treatment of dyslipidemia. Niacin lowers the levels of all atherogenic lipoproteins such as VLDL, LDL, and lipoprotein A, accompanied by an increase of HDL. Notably the elevation of HDL is the most prominent among the approved drugs.[160] Beneficial effects of the combination of niacin with statins have also been proven in several clinical studies.[161] There are two major side effects associated with niacin therapy, flush and an increase of uric acid in blood. Flush is a cutaneous vasodilatation occurring rapidly after the oral administration of niacin and takes about half an hour. This very unpleasant effect limiting the clinical use could be reduced but not prevented by a prolonged release formulation of niacin.[162] As flush is induced by cyclooxygenase dependent secretion of prostaglandins by immune cells of the skin, the use of cyclooxygenase inhibitors like acetyl salicylic acid is another therapeutic option to reduce flush.[163]

The discovery that niacin-induced vasodilatation could be suppressed by antagonizing the prostaglandin D_2 receptor 1 (DP1, also called DP) by Cheng et al. has provided a new strategy for a niacin therapy with suppressed flushing.[164] MK-0524, an indole-based acetic acid derivative originally designed to treat allergic rhinitis, is a potent and selective DP receptor antagonist which inhibits the PGD_2-induced cAMP production in platelet-rich plasma with an IC_{50} of 4.0 nM.[165] In healthy subjects MK-0524 reduced niacin-induced flushing symptoms by 68% and the increase in malar skin perfusion by 76% compared to placebo when administered one hour before niacin.[164] Currently, MK-0524 is undergoing a phase III clinical trial to evaluate the efficacy to improve the tolerability of extended-release niacin therapy.

MTP Inhibitors

The microsomal triglyceride transfer protein (MTP) is one of the key enzymes in the formation of apolipoprotein B (apoB) containing lipoproteins such as chylomicrons in the intestinum and VLDL in the liver.[166] An inhibition of this enzyme showed antiatherosclerotic efficacy in animal models.[167] The first drug candidate of this class which reached clinical trials was implitapide (Figure 18), that inhibits both hepatic and intestinal MTP. In dyslipidemic subjects, implitapide decreased plasma total cholesterol, LDL and apoB. However, due to increased serum liver enzymes and gastrointestinal side effects the development of implitapide was discontinued. BMS-201038 (synonym AEGR-733) is another MTP-inhibitor which is currently undergoing clinical trials.[168] The results of a first dose-escalation study with BMS-201038 given to patients with homozygous familial hypercholesterolemia have been published recently.[169] BMS-201038 was highly effective in reducing plasma levels of all apoB containing lipoproteins. However, the therapy was associated with hepatic fat accumulation and elevated liver aminotransferase levels. A larger ongoing study for the evaluation of

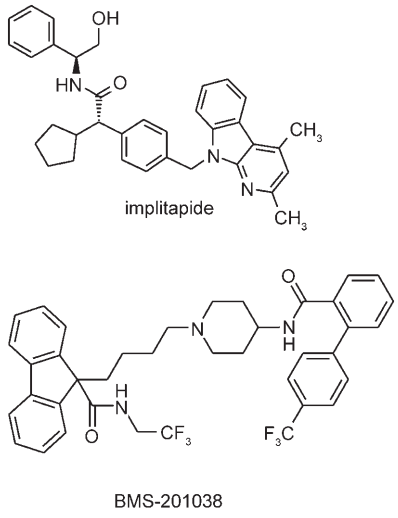

Figure 18. MTP inhibitors.

BMS-201038 and its combination with ezetimibe might deliver a more detailed picture of the safety of this drug.

Summary

In recent years a variety of potential new targets for the treatment of dyslipidemia have been discovered, including nuclear receptors and enzymes involved in lipid metabolism. For some of these targets potential drugs reached the clinic but none of these potential new drugs came on the market so far, as they have been withdrawn or failed in clinical trials. The most well developed ones for example, tesaglitzar, muraglitazar, and torcetrapib effectively modulated lipid metabolism but had toxicological problems.

Outlook

In this review, we discussed several drug candidates which seemed to be beneficial in early stages of clinical development and which significantly affected surrogate parameters. However, most of them failed in later stages of clinical development. The reasons for the withdrawals were unexpected toxic effects or discouraging outcomes in clinical endpoints such as cardiovascular events. That was the case for muraglitazar and torcetrapib, which were well effective in cholesterol lowering, but showed an increase in cardiovascular events.

This raises the question, whether the selected surrogate parameters are appropriate and sufficient for the development of safe and effective lipid lowering drugs. The data obtained from the clinical trials with compounds that address various enzymes or receptors involved in lipid metabolism underline the fact that the interplay between alterations in lipid metabolism and the modulation of disease development is complex. At present, it seems that much more work has to be done to define pharmacological profiles that lead to new, therapeutically effective lipid modulating drugs.

Abbreviations

ABCA1: ATP binding cassette A1; apoA1: apolipoprotein A1; CETP: cholesteryl ester transfer protein; CHD: coronary heart disease; EGF-1: early growth response factor 1; FA: fatty acid; FABP: fatty acid binding protein; FATP: fatty acid transport protein; FDA: Food and Drug Administration; FFA: free fatty acid; FXR: farnesoid X receptor; GFR: glomerular filtration rate; GLUT: glucose transporter; HDL: high density lipoprotein; LDL: low density lipoprotein; LXR: liver X receptor; PPAR: peroxisome proliferator-activated receptor; PPRE: PPAR response element; RXR: retinoid X receptor; TG: triglycerides; TZD: thiazoidinedione; VLDL: very low density lipoprotein.

Keywords: fatty acids · lipids · lipoproteins · PPAR

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Received: July 12, 2007
Revised: September 11, 2007
Published online on October 26, 2007

