REVIEW

Next generation multifunctional angiotensin receptor blockers

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Angiotensin receptor blockers (ARBs) are well-tolerated drugs that are known to be useful for inhibiting activity of the renin–angiotensin (RAS) system, treating hypertension and reducing the risk for cardiovascular disease. However, inhibition of the RAS does not control all pathophysiological mechanisms of hypertension or cardiovascular risk and many patients continue to suffer from cardiovascular events and metabolic disturbances despite being treated with an ARB, an angiotensin-converting enzyme inhibitor or both, in addition to other standard therapies for cardiovascular disease. Recently, it has become apparent that bifunctional molecules can be designed that do more than just block AT_1 receptors and that can target additional mechanisms of hypertension, cardiovascular disease and diabetes besides just increased activity of the renin–angiotensin system. Specifically, next generation ARBs are becoming available that are intended to not only antagonize AT_1 receptors, but also block endothelin receptors, function as nitric oxide donors, inhibit neprilysin activity and increase natriuretic peptide levels, or stimulate the peroxisome proliferator-activated receptor γ (PPARγ). In this review, we: (1) discuss the potential importance of multifunctional ARBs that can reduce cardiovascular and metabolic risk through multiple mechanisms that go beyond just inhibition of the renin–angiotensin system and (2) describe specific examples of next generation ARBs in development that are intended to do more than simply block AT_1 receptors.

INTRODUCTION

The discovery and clinical use of first generation nonpeptide angiotensin II receptor antagonists represents a major success story in the history of cardiovascular medicine.[1,2] These molecules were designed to block angiotensin II type 1 (AT_1) receptors with the goal of reducing blood pressure and the risk for cardiovascular events. Angiotensin II receptor blockers (ARBs) in use today are very well-tolerated antihypertensive drugs and are considered to offer protection against stroke, renal damage and cardiovascular disease.[2] Angiotensin-converting enzyme inhibitors (ACEis) can provide all the same clinical benefits if not more than those provided by ARBs.[3,4] However, ARBs are generally better tolerated as they are associated with a significantly lower risk of cough and angioedema than ACE inhibitors.[4–7] Renin inhibitors represent a more recently developed class of molecules also designed to inhibit activity of the renin–angiotensin system (RAS) and lower blood pressure. However, there is a paucity of cardiovascular outcome data for this group of drugs. Results from large-scale comparative clinical trials will be required to determine whether renin inhibitors provide any greater benefits than other available drugs that inhibit the RAS through different mechanisms.

THE MOTIVATION FOR NEXT GENERATION ANGIOTENSIN RECEPTOR BLOCKERS

Given the well-established ability of currently available ARBs to safely block AT_1 receptors and reduce blood pressure, little motivation might seem to exist for developing next generation ARBs for use in clinical practice. However, on the basis of the results of controlled clinical trials together with recent advances in our understanding of the pathophysiology of cardiovascular disease and diabetes, it has become increasingly clear that currently available ARBs leave much to be desired when it comes to reducing cardiovascular and metabolic risk in patients with hypertension. In this review, we discuss some of the major unmet needs of hypertensive patients being treated with existing ARBs and highlight new efforts underway to develop next generation ARBs that are expected to provide superior control of blood pressure and associated cardiovascular and metabolic risk factors in patients with hypertension. The ability of medicinal chemists to design bifunctional molecules that not only block AT_1 receptors but also target other pathways involved in the regulation of blood pressure and the pathogenesis of cardiovascular and metabolic diseases has opened the door for the development of next generation ARBs that do more than just inhibit the renin–angiotensin system (Table 1).

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Table 1 Next generation ARBs with actions beyond AT$_1$ receptor blockade

Molecule	Stage	Special action	References
Azilsartan	Phase III	Increase PPARγ gene expression[a]	35–39
K-868	Preclinical	PPARγ partial agonism[b]	43
PF-03838135	Preclinical	PPARγ partial agonism[b]	44
ARNI	Preclinical	Neprilysin inhibition[b]	55–58
LCZ-696	Phase II/III	Neprilysin inhibition[c]	59–62
PS433540	Phase IIb	Endothelin receptor antagonism[b]	74, 76
WB1106	Preclinical	Nitric oxide donor[b]	86
3-[(nitrooxy)methyl] benzoate ester of losartan	Preclinical	Nitric oxide donor[b]	84, 85

[a]Feature observed by chance—not deliberately sought in the AT$_1$ receptor antagonist.
[b]Feature deliberately sought and achieved together with AT$_1$ receptor-blocking activity in a single molecule.
[c]Feature deliberately sought and achieved by linking a neprilysin inhibitor to an AT$_1$ receptor antagonist (two molecules).

ANGIOTENSIN RECEPTOR BLOCKERS: CURRENT LIMITATIONS AND UNMET NEEDS

All clinically approved angiotensin receptor antagonists can block AT$_1$ receptors and reduce blood pressure. As increased activity of the renin–angiotensin system is believed to contribute to end organ damage, considerable emphasis has been placed on the still controversial notion that ARBs can afford major benefits on cardiovascular outcome beyond their ability to reduce blood pressure. Although the clinically used ARBs can differ with respect to pharmacokinetic characteristics such as half life, potency for AT$_1$ receptor blockade, degree of insurmountable antagonism, etc, one can usually minimize the practical impact of these differences on control of RAS activity and antihypertensive efficacy by making dose adjustments.[8] Thus, if one simply administers the appropriate doses required to control blood pressure and effectively inhibit the renin–angiotensin system, it is generally assumed that all ARBs will yield equally satisfactory protection against risk for cardiovascular disease.

Unfortunately, administration of ARBs in doses considered sufficient to robustly block AT$_1$ receptors often fails to provide effective blood pressure control and to protect against adverse cardiovascular events.[7,9] Furthermore, a recent report by a panel of experts assembled by the American Heart Association points to the increasing problem of difficult-to-treat and outright drug-resistant hypertension,[10] which is most pronounced in certain subpopulations including African Americans, patients with isolated systolic hypertension, and those with concomitant cardiovascular and renal disease, metabolic syndrome or diabetes. Even if one administers a powerful ARB in combination with an ACEi to fully inhibit the renin–angiotensin system, many high-risk patients, including those getting their blood pressure controlled and those taking statins and aspirin, still suffer from adverse cardiovascular events.[7] In addition, notwithstanding evidence that the risk for new onset diabetes is lower in patients taking ARBs or ACEis than in patients taking other antihypertensive drugs known to cause disturbances in glucose metabolism,[11] many patients still develop diabetes despite taking ARBs, ACEis or both. Moreover, most ARBs have never been compared against placebo in randomized, double-blind trials and it is unknown whether ARBs as a class are capable of reducing the risk for new onset diabetes. As hypertension itself is associated with increased risk for diabetes and is often accompanied by multiple metabolic disturbances that strongly promote diabetes,[12–14] it is important to reduce the risk for diabetes linked to hypertension rather than to simply avoid the risk for diabetes caused by certain kinds of antihypertensive drugs. Furthermore, regardless of the ongoing controversy over whether the diabetes that develops during hypertension treatment is associated with increased cardiovascular risk, diabetes per se is a highly undesirable problem that negatively affects the quality of life in many ways and significantly increases health-care costs. Thus, the availability of multifunctional ARBs that are more effective than existing ARBs in lowering blood pressure and enabling patients to reach blood pressure goals, protecting against new onset diabetes, and reducing overall cardiovascular risk would be of considerable clinical and financial value.

MULTIFUNCTIONAL THERAPEUTICS: GOING BEYOND AT$_1$ RECEPTOR BLOCKADE

It could be argued that greater therapeutic benefits might be gained by administration of higher doses of existing ARBs, and several studies have been conducted to test this notion (for example, Shargorodsky et al.[15] and Hollenberg et al.[16]). However, such a strategy fails to address the need to control the other pathogenetic determinants of hypertension, diabetes and cardiovascular disease that do not depend solely on increased activity of the renin–angiotensin system. It is widely recognized that effective control of these multifactorial disorders requires the therapeutic modulation of multiple pathways involved in disease pathogenesis. For example, when given as monotherapy for hypertension, ARBs often fail to sufficiently lower blood pressure.[9] Thus, many patients require two or more drugs from different classes with different pharmacologies to control hypertension and combination therapy is becoming more and more the mainstay of treatment.[17]

COMBINATION THERAPIES VS. MULTIFUNCTIONAL MOLECULES: PROS AND CONS

There are principally three different approaches to targeting multiple pharmacologies when treating multifactorial disorders such as hypertension.[18] Most commonly, patients are given a cocktail of two or more different drugs. Although this option allows for maximal flexibility with respect to possible drug combinations and different dosages, it suffers from (a) potentially poor adherence to treatment by the patient because of the increased pill burden and differing dosing schedules for the individual drugs, (b) possible incompatibilities because of drug–drug interactions, and (c) increased risk of drug side effects. These problems can reduce the effectiveness of therapy and hamper the attainment of treatment goals particularly in the management of chronic, asymptomatic disorders such as hypertension.

The second approach relies on the use of fixed-dose combinations of drugs in a single dosage form. In patients with hypertension, fixed-dose combinations have become increasingly popular in recent years because they reduce pill burden, result in improved patient compliance and often improve blood pressure-lowering and patient responder rates.[19] Fixed-dose combinations of angiotensin-converting

enzyme inhibitors or angiotensin receptor blockers with diuretics have increased significantly in market share, and the recent FDA approval of Exforge HCT (a combination of the ARB valsartan, the calcium channel blocker amlodipine and the diuretic hydrochlorothiazide marketed by Novartis, East Hanover, NJ, USA) now provides the first treatment option for hypertension with triple pharmacology in a single tablet. The disadvantage of fixed-dose combinations is primarily the limited choice of possible dose ratios between the active ingredients, which makes it more difficult to properly titrate the individual patient to maximum efficacy with minimal adverse effects. In case of treatment-emergent adverse effects, it may be difficult to determine which of the individual components is responsible. In addition, different pharmacokinetic properties of the components in the combination might lead to a complex temporal mismatch in pharmacodynamic effects at the individual targets thereby compromising overall efficacy.

The third approach to the treatment of multifactorial disorders is the use of multifunctional drugs that combine two or more pharmacologies in a single compound. Although the discovery of such multifunctional compounds is more complex and requires substantial investigation into the optimal ratio of target activities in the molecule, both their development and clinical use are simplified because of the unified pharmacokinetics, resulting in matched pharmacodynamic activities at the molecular targets. Although therapeutic approaches that involve the administration of multiple drugs in combination are certainly of value, advances in medicinal chemistry, rational drug design and high throughput screening have made it possible to discover multifunctional molecules that modulate more than one molecular target, show two or more pharmacologies and meet several therapeutic objectives with a single agent. It should be noted that multifunctional molecules may also be amenable to fixed dose combination with other drugs thereby combining three or even four pharmacologies in a single pill to produce further increments in efficacy.

A PROTOTYPE FOR NEXT GENERATION ARBS

Recent studies by Benson et al.[20] and Schupp et al.[21] provided a clue that it might be possible to develop multifunctional ARBs that not only block angiotensin II receptors but also stimulate the activity of a separate molecular target useful for the prevention of diabetes. These investigators observed that the angiotensin II receptor antagonist telmisartan not only blocked AT_1 receptors but could also partially activate the peroxisome proliferator-activated receptor-γ (PPARγ), an established target of thiazolidinedione drugs with known efficacy for the prevention and treatment of type II diabetes.[20,21] This raised the possibility that in addition to being useful for treating hypertension, telmisartan might be capable of reducing the risk for new onset diabetes. In addition, as telmisartan activates PPARγ less robustly and differently than do thiazolidinediones, the use of telmisartan is not associated with the kinds of side effects that have plagued the use of full PPARγ agonists, such as rosiglitazone or pioglitazone.[22]

The latest results from large-scale, randomized, double-blind clinical trials are consistent with the possibility that telmisartan might have true antidiabetic properties (that is, be capable of decreasing the risk for developing new onset diabetes when compared with placebo, not just when compared with other antihypertensive drugs that are associated with increased risk for diabetes). Figure 1 shows the results of a meta-analysis of two randomized, double-blind, placebo-controlled trials with telmisartan in which the incidence of new onset diabetes was measured as a pre-specified secondary end point. This meta-analysis of the TRANSCEND[23] and PRoFESS trials[24] indicates that telmisartan can reduce the risk for new onset diabetes by
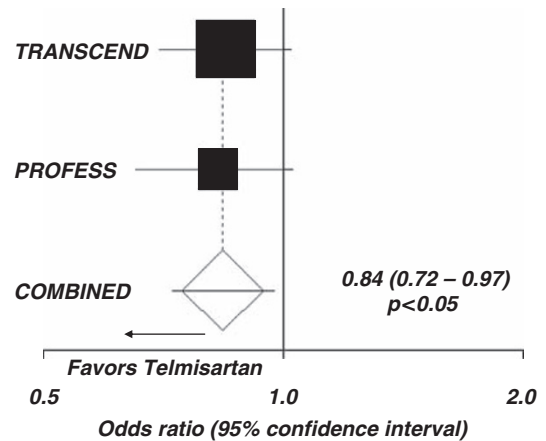

Figure 1 Meta-analysis of the antidiabetic effects of telmisartan. Meta-analysis showing the effects of telmisartan treatment on risk for new onset diabetes compared with placebo treatment in the TRANSCEND and PRoFESS trials. The results show a significant antidiabetic effect of telmisartan with a point estimate reduction in risk for new onset diabetes of 16%.
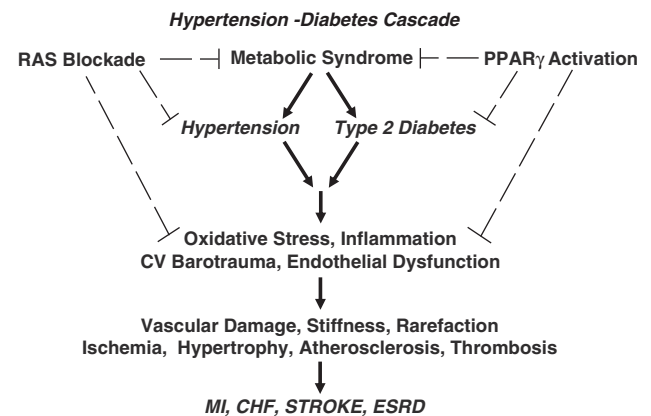
Figure 2 The hypertension—diabetes cascade. Diagram showing that inhibitors of the renin–angiotensin system (RAS) and activators of PPARγ interrupt at multiple points early in the hypertension—diabetes cascade.

approximately 16% compared with placebo in high cardiovascular risk patients without heart failure. This antidiabetic effect of telmisartan is similar in overall magnitude to that observed with ACE inhibitors in placebo-controlled clinical trials of patients without heart failure.[25,26] Few randomized, double-blind, placebo-controlled trials have been performed that have examined the antidiabetic effects of other ARBs in patients without heart failure.[27]

Just as ACE inhibitors are believed to improve glucose metabolism through at least two or more primary mechanisms (for example, inhibition of the renin–angiotensin system and activation of the bradykinin/nitric oxide pathways),[28–31] it is possible that telmisartan might also be improving glucose metabolism and cardiovascular protection through two or more key pathways (for example, inhibition of the renin–angiotensin system and activation of PPARγ). By the use of multifunctional ARBs that have the potential to target multiple points in the hypertension–diabetes cascade (Figure 2), it is conceivable that one might achieve greater metabolic and cardiovascular protection than that attained with the angiotensin receptor blockade
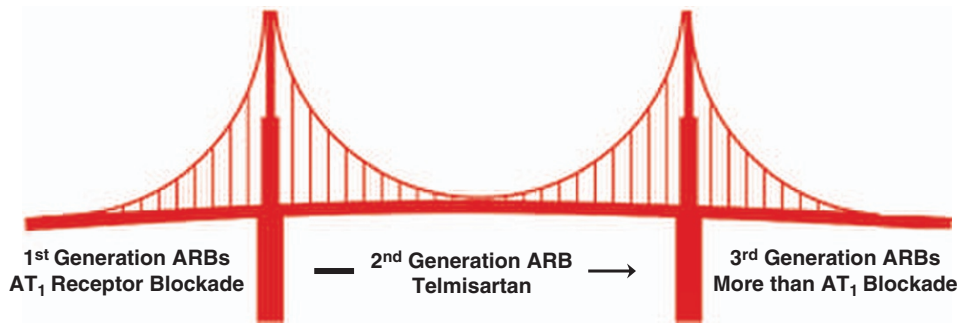
Figure 3 Bridge to the future of next generation ARBs. Illustration depicting telmisartan as a bridge to the future between first generation ARBs focused almost entirely on the AT_1 receptor blockade and next generation ARBs intended to do more than just block AT_1 receptors.

alone. In addition, the antidiabetic properties of multifunctional ARBs such as telmisartan may be useful in counteracting the pro-diabetic effects of diuretic agents that are often used in combination with AT_1 receptor antagonists. It should be noted, however, that telmisartan was not specifically developed for the purpose of activating PPARγ and that it is not a highly potent PPARγ agonist. The doses of telmisartan used in the TRANSCEND and PRoFESS trials were not optimized with the intent of achieving PPARγ activation. Thus, the administration of higher doses of telmisartan or the use of next generation ARBs with more potent effects on PPARγ activity will likely be required to achieve greater antidiabetic effects than those revealed by the meta-analysis of the TRANSCEND and PRoFESS trials. In light of these observations, telmisartan might be viewed as a bridge to the future between first generation ARBs focused almost entirely on AT_1 receptor blockade and next generation ARBs that are intended to do more than just inhibit activity of the RAS (Figure 3). Although the work on telmisartan has suggested the feasibility of creating new ARBs with effects on receptor pathways beyond the renin–angiotensin system, structure-activity relationship studies of telmisartan may also prove useful in guiding the future development of next generation PPARγ ligands as well.[32]

As noted, inhibition of the renin–angiotensin system itself is believed to have a positive effect on glucose metabolism; however, the magnitude of any such effect is likely to depend on the nature of the underlying disturbance in glucose homeostasis. In some circumstances, inhibition of the RAS is thought to improve glucose handling at least in part by enhancing tissue blood flow and the delivery of glucose to skeletal muscle beds where it undergoes extraction and metabolism.[31,33] Thus, in situations such as congestive heart failure where peripheral glucose metabolism may be significantly impaired owing to reduced tissue perfusion, any drug that inhibits the RAS and improves the microcirculatory function might be anticipated to improve glucose metabolism. This could partly explain why the ARB candesartan was observed to have antidiabetic effects in heart failure patients studied in the CHARM trial but did not reduce the risk for new onset diabetes in a randomized, double-blind, placebo-controlled study conducted in patients without heart failure.[27,34] In contrast, ARBs that have the ability to improve glucose metabolism through mechanisms such as partial PPARγ agonism and not just RAS blockade may hold greater promise for protecting against new onset diabetes in patients without heart failure. The meta-analysis results showing the antidiabetic effects of telmisartan in high CV risk patients without heart failure suggest that it might be feasible to develop next generation ARBs with greater PPARγ potency and greater efficacy than existing ARBs for reducing the risk of new onset diabetes in patients with hypertension.

NEXT GENERATION ARBS THAT AFFECT PPARγ

Several major pharmaceutical companies have identified new ARBs that can increase PPARγ gene expression or receptor activity in a manner that holds promise for enhancing the antidiabetic potential of these agents (Table 1). Takeda Pharmaceuticals (Deerfield, IL, USA) has been conducting Phase II and Phase III clinical trials of the next generation ARB azilsartan (TAK-536) and its prodrug azilsartan medoxomil (TAK-491) in patients with hypertension.[35–37] Azilsartan is a potent AT_1 receptor antagonist that is structurally similar to candesartan except for bearing a 5-oxo-1,2,4-oxadiazole moiety in place of a tetrazole ring (Figure 4).[38] Azilsartan is less acidic and more lipophilic than candesartan and in preclinical studies has been shown to be significantly more potent than candesartan in improving glucose tolerance and tissue glucose metabolism.[39] In cell-based receptor transactivation studies *in vitro*, azilsartan appears to have little or no direct effect on PPARγ activity.[39] However, in a mouse model of type II diabetes, oral administration of very low doses of azilsartan has been found to significantly increase PPARγ gene expression in adipose tissue.[39] Dose response studies *in vivo* showed that azilsartan is much more potent in stimulating PPARγ gene expression than candesartan.[39] These observations raise the possibility that azilsartan may have stronger antidiabetic effects than candesartan and perhaps other ARBs as well. Takeda is also conducting Phase III clinical trials of azilsartan medoxomil in combination with chlorthalidone, a benzenesulfonamide diuretic that has been shown to have significant cardioprotective effects in large-scale clinical trials.[40,41] Depending on the extent to which azilsartan shows enhanced antidiabetic properties, its metabolic benefits might offset or even override the adverse effects of chlorthalidone on glucose metabolism.[42] If so, the combination of azilsartan and chlorthalidone could become particularly attractive from both a metabolic and hemodynamic perspective.

Recently, Kyowa Hakko Kirin pharmaceuticals (Tokyo, Japan) has reported the development of a next generation ARB-designated K-868 with high binding affinity for AT_1 receptors and that also functions as a partial agonist of PPARγ with improved metabolic properties relative to other ARBs.[43] In a variety of preclinical models of hypertension, K-868 has been shown to have antihypertensive properties similar to candesartan. At the same time, K-868 appears to have more robust metabolic effects than do candesartan and other ARBs. In the prediabetic Zucker fatty rat model of obesity and insulin resistance, oral administration of K-868 improved glucose tolerance, reduced insulin levels and inhibited increases in glucose and hemoglobin A1c levels, whereas oral administration of similar doses of candesartan did not. In the Zucker diabetic fatty rat, the administration of a relatively low dose of K-868 ($1\,mg\,kg^{-1}$) reduced proteinuria
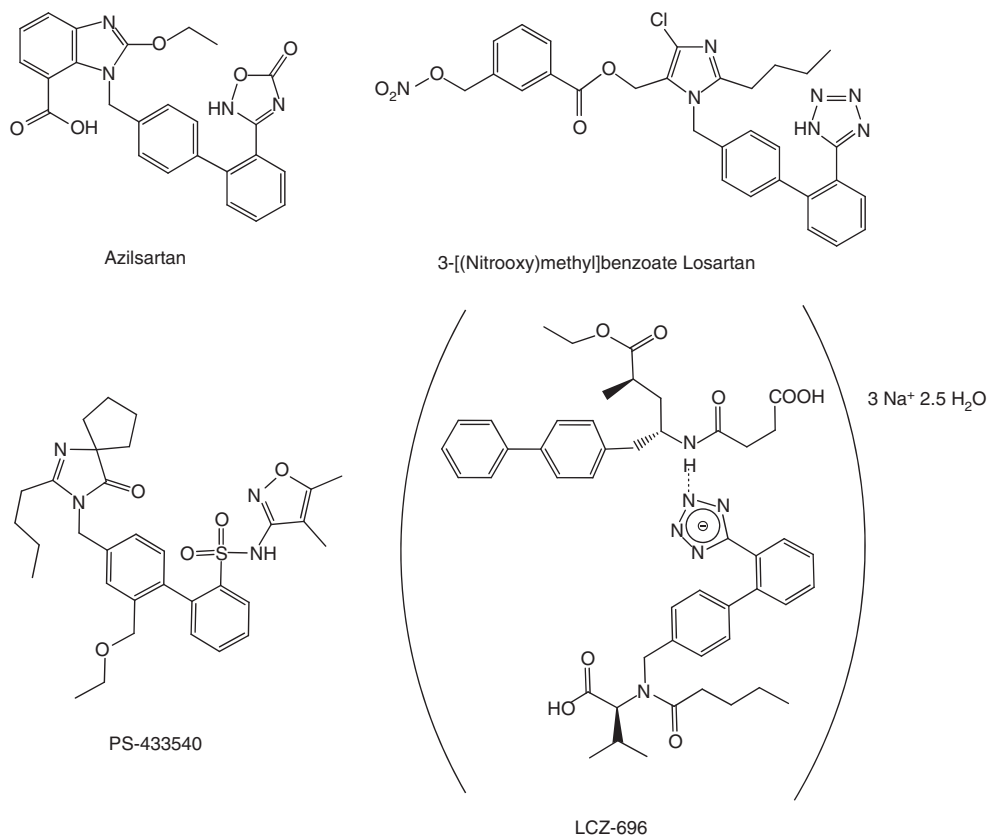
Figure 4 Structures of next generation ARBs. Examples of chemical structures of some next generation ARBs.

more effectively than did a 30-fold greater dose of valsartan.[43] Although the mechanisms that mediate the potent renoprotective effects of K-868 remain to be determined, its ability to limit kidney damage might be in part due to its ability to inhibit inflammatory cytokine production through the partial activation of PPARγ. As PPARγ activation can also reduce C-reactive protein levels, another inflammatory mediator of risk for target organ damage and diabetes, it will also be of interest to determine whether K-868 has any special capacity to reduce C-reactive protein levels compared with other ARBs that do not affect PPARγ-related pathways. Given the potential metabolic and renoprotective benefits of K-868, this molecule holds promise not only for preventing new onset diabetes in hypertensive patients more effectively than existing ARBs, but also for improving glucose metabolism and limiting kidney damage in patients with diabetes and hypertension.

In addition to K-868 that is under development by Kyowa Hakko Kirin, another compound with bivalent pharmacology of AT_1 receptor antagonism and partial PPARγ agonism has been studied by Pfizer (New York, NY, USA). Pfizer has identified an AT_1 antagonist PF-03838135 that has been available for out-licensing and that has blood pressure-lowering effects in spontaneously hypertensive rats comparable with those of telmisartan but with greater potency than telmisartan on PPARγ activation.[44] In the Zucker diabetic fatty rat model, PF-03838135 has been reported to show insulin-sensitizing effects that are greater than those of telmisartan and comparable with those of the full PPARγ agonist pioglitazone while inducing less weight gain than pioglitazone. Taken together, the observations with telmisartan, K-868 and PF-03838135 support the feasibility of identifying next generation ARBs with the potent ability to antagonize AT_1 receptors and

simultaneously activate PPARγ with the goal of providing greater metabolic benefits than those afforded by first generation AT_1 receptor antagonists with little or no effect on PPARγ activity.

NEXT GENERATION ARBS THAT INHIBIT NEPRILYSIN ACTIVITY

Neprilysin (also called neutral endopeptidase or NEP) is a membrane-bound zinc metallopeptidase that metabolizes a number of small secreted vasoactive peptides involved in blood pressure homeostasis, including the natriuretic peptides and bradykinin. Additional bioactive peptide substrates for neprilysin include Leu- and Met-enkephalin, vasointestinal polypeptide and amyloid-β peptide. For a review on neprilysin and its function, see Turner et al.[45]

As the natriuretic peptides have vasodilatory, natriuretic and diuretic properties, it was hypothesized and shown experimentally that the pharmacological inhibition of neprilysin to potentiate natriuretic peptide activity results in blood pressure-lowering effects. A number of companies pursued the discovery and development of neprilysin inhibitors for the treatment of hypertension and heart failure, and modest antihypertensive activities of such molecules were shown in a number of clinical trials. However, the full potential of neprilysin inhibition was only realized in combination with concomitant inhibition of the angiotensin-converting enzyme (ACE), which culminated in the discovery and development of the vasopeptidase inhibitors, compounds that combined both neprilysin and ACE inhibitory activity in a single small molecule.[46]

The clinically most advanced vasopeptidase inhibitor was omapatrilat, which was discovered and developed by Bristol-Myers Squibb (New York, NY, USA). Clinical hypertension trials have shown broadly

superior antihypertensive efficacy of omapatrilat relative to enalapril,[47] and good efficacy was also shown in heart failure.[48,49] However, the 3-fold higher incidence of angioedema observed for omapatrilat relative to enalapril, and the incidence of life-threatening angioedema in the late-stage clinical studies led to the discontinuation of omapatrilat in 2002 because of the unfavorable risk-benefit profile.[50] As both ACE and neprilysin are key enzymes in the metabolism of bradykinin, it is hypothesized that dual inhibition of ACE and neprilysin by omapatrilat and other vasopeptidase inhibitors results in a significantly greater elevation of bradykinin levels relative to ACE inhibition alone, which in a subset of patients can lead to excessive vascular leakage, fluid extravasation and angioedema, explaining the unfavorable tolerability issues observed for omapatrilat.[51]

The objective of two current drug research and development programs conducted by Theravance (South San Francisco, CA, USA) and Novartis is the discovery of compounds that replicate the superior efficacy of omapatrilat through dual inhibition of the RAS and neprilysin, but without elevation of bradykinin levels and associated high risk for angioedema. This is achieved through inhibition of the RAS at the level of the AT_1 receptor rather than at the level of the converting enzyme, which is the rate-limiting enzyme in bradykinin metabolism. The concept was first shown experimentally in two separate studies in the spontaneously hypertensive rat model of hypertension.[52,53] For example, in the study of Pu et al.,[52] it was shown that treatment with the ARB valsartan and a neprilysin inhibitor in combination resulted in equivalent blood pressure-lowering and comparable effects on vascular remodeling and cardiac fibrosis compared to treatment with a vasopeptidase inhibitor. The group of Hegde et al.[54] at Theravance has recently described a rat model for tracheal plasma extravasation. Omapatrilat and lisinopril produced robust and dose-dependent increases in plasma extravasation in the anesthetized rat, which is consistent with their demonstrated clinical risk for angioedema. Increased plasma extravasation by those two compounds in this model appears to be mediated by bradykinin, as pre-treatment with the bradykinin receptor antagonist HOE-140 completely blocked the response. In contrast to the effect of low doses of omapatrilat, even high doses of the angiotensin receptor blocker valsartan, a neprilysin inhibitor or their combination failed to produce a measurable increase in tracheal plasma extravasation. Taken together, these data suggest that dual inhibition of the AT_1 receptor and neprilysin has the potential to produce similar antihypertensive effects as omapatrilat, but without the risk of angioedema.[54]

Theravance is pursuing the discovery of novel dual-acting antihypertensive agents that have AT_1 receptor antagonist and neprilysin inhibitory activity in the same molecule (AT_1 Receptor Blocker and Neprilysin Inhibitor, (ARNIs)). The initial results of Theravance's ARNI program have appeared recently in a series of published patent applications.[55–58] Theravance describes a structurally diverse series of compounds that generally contain an aryl acid moiety and a zinc-chelating group such as a hydroxamate or thiol. These bifunctional compounds are reported to bind to the AT_1 receptor and also inhibit neprilysin activity.

Although the compounds discovered by Theravance combine the AT_1 blocking and neprilysin inhibitor activities in single small bifunctional molecules, Novartis describes their drug candidate LCZ-696 as a 1:1 molar complex of the ARB valsartan with the neprilysin inhibitor prodrug AHU-377 (Figure 4). The patent literature characterizes the complex as a multicomponent salt between valsartan, the neprilysin inhibitor, one or more cations and solvent molecules.[59–61] Although this complex is not a bifunctional single molecule, it is described as a distinct crystal form that has different properties from the

simple physical mixture of the two active compounds. However, as a fixed-dose combination of two drugs, the complex would not result in matched DMPK properties and might still lead to temporal dissociation in pharmacodynamic activity at the two targets and with this to suboptimal efficacy. Novartis recently reported encouraging data from a phase II study, showing superior blood pressure reduction of the combination relative to valsartan alone in patients with mild to moderate hypertension, without any reported cases of angioedema, which clinically validates the concept of dual inhibition of the AT_1 angiotensin receptor and neprilysin.[62] At the high dose of 400 mg, LCZ-696-treated patients showed a 20 mm Hg reduction in SBP relative to baseline. This compares very favorably with the 14 mm Hg reduction seen for 320 mg valsartan in the study, considering that the effective dose of valsartan in the 400 mg dose of the LCZ-696 combination is about 200 mg, assuming a 1:1 molar complex between the ARB and the neprilysin inhibitor. LCZ-696 is also being studied for the treatment of heart failure.

NEXT GENERATION ARBS THAT BLOCK ENDOTHELIN RECEPTORS

Endothelin-1 is a very potent vasoconstricting peptide and its dysregulation can contribute to the development of high blood pressure. The actions of endothelin-1 are mediated by two G-protein coupled receptors, the ET_A and ET_B endothelin receptors, which are expressed in smooth muscle and in the endothelial cells of the vasculature.[63] Three endothelin receptor antagonists are approved and clinically used for the treatment of pulmonary arterial hypertension: bosentan, sitaxentan and ambrisentan.[64] Recent clinical data for another endothelin antagonist, darusentan, which is developed by Gilead Sciences (Foster City, CA, USA), also establishes the use of this class of compounds for the treatment of hypertension.[65]

A number of preclinical studies have established that dual blockade of AT_1 and endothelin receptors results in greater blood pressure-lowering effects than does the blockade of either receptor alone.[66–69] An early series of dual AT_1 and ET receptor antagonists was described by Merck,[70] following the recognition of structural and functional similarities between the endothelin and AT_1 receptors, and similar structure-activity relationships seen for their peptide agonist ligands. A starting point for the discovery of bifunctional AT_1/ET_A antagonists was the existing Merck library of compounds blocking the AT_1 receptor. Subsequent structure-activity relationship studies resulted in the identification of L-746,072, which showed potent binding affinity toward all four receptor subtypes (IC_{50}s for AT_1=13 nM, AT_2=32 nM, ET_A=24 nM, ET_B=60 nM). However, no preclinical or clinical data have been reported for this compound.

A program at Bristol-Myers Squibb pursued the converse approach, starting with lead discovery efforts with potent and selective ET_A receptor antagonists. It was realized that the common core structure (a biphenyl sulfonamide) in endothelin antagonist leads was bioisosteric with the biphenyl carboxylates and biphenyl tetrazoles found in most of the marketed sartan ARBs.[71] A dedicated medicinal chemistry program focused on increasing the AT_1 activity of the endothelin receptor antagonist scaffold, while maintaining and further enhancing activity at the ET_A receptor. This effort led to the discovery of a number of dual action receptor antagonist compounds, which were shown to be potent and selective for inhibition of the AT_1 and ET_A receptors, with only low affinities for the AT_2 and ET_B receptors. BMS-248360, a first generation dual action receptor antagonist lead compound, showed high affinity for the ET_A receptor, with somewhat lower affinity at the AT_1 receptor (ET_A K_i=1.9 nM, AT_1 K_i=10 nM). The compound was orally bioavailable in rats with a good half life,

and produced potent inhibition of angiotensin II and big ET-1 pressor responses in rats, showing its bifunctional *in vivo* activity for inhibition of both targets.[72]

Following the work on BMS-248360, further optimization studies led to the discovery of the second generation dual action receptor antagonist compound PS-433540 (BMS-346567) (Figure 4) with improved affinity at the AT_1 receptor (ET_A $K_i=9.3$ nM, AT_1 $K_i=0.8$ nM), better pharmacodynamic activity at the two targets after oral dosing, and superior pharmacokinetic properties in higher species (dog and monkey).[73] PS-433540 was shown to be more efficacious than irbesartan in the spontaneously hypertensive rat, which is considered a model for renin-dependent hypertension.[71] PS-433540 was also effective in the DOCA-salt rat,[74] which is a model of low-renin hypertension in which AT_1 receptor antagonists show relatively little or no efficacy.[75]

Bristol-Myers Squibb licensed PS-433540 to Pharmacopeia in 2006, and phase I clinical studies were initiated in early 2007. Ligand Pharmaceuticals (San Diego, CA, USA), which acquired Pharmacopeia in late 2008, recently reported results from a phase IIb study, in which three doses of PS-433540 (200, 400 and 800 mg) were directly compared with 300 mg of the ARB irbesartan or placebo after 12 weeks of treatment.[76] PS-433540 at all doses resulted in a significant lowering of mean-seated SBP and DBP relative to baseline, with higher doses resulting in more blood pressure lowering. The efficacy of the 800 mg dose was significantly different from that of the 300 mg dose of irbesartan, with SBP/DBP reductions of 23/14 mm Hg for PS-433540, and 11/7 mm Hg for irbesartan, respectively. Importantly, the blood pressure goal of less than 140/90 mm Hg was reached by 62 and 52% of patients treated with the 800 and 400 mg doses of PS-433540, whereas only 32% of patients reached this goal when treated with irbesartan. PS-433540 was generally well tolerated; however, about 11% of patients experienced peripheral edema at the 800 mg dose, relative to 2% of patients treated with placebo and 3% of patients treated with irbesartan. Although the data presented for this compound to date suggest superior efficacy relative to ARBs alone, the relative contribution of endothelin receptor blockade toward overall efficacy is unclear at present.

NEXT GENERATION ARBS THAT ARE NO DONORS

Nitric oxide (NO) is a potent gaseous signaling molecule with complex chemistry and biology. It is generated by a number of cell types in response to various stimuli, and because of its very short half life and spatially limited range of activity is considered a local autocrine and paracrine signaling molecule with both beneficial and deleterious downstream effects, depending on its localization and effective concentration.[77] NO is produced by the endothelial cells of the blood vessels, and was identified in 1986 as the elusive endothelium-derived relaxing factor.[78,79] Its actions are mainly mediated by activation of the enzyme soluble guanylate cyclase, which catalyzes the production of cyclic guanosine monophosphate (cGMP) from GTP, resulting in the activation of a number of cGMP-dependent protein kinases that mediate the downstream effects. In humans, NO has been shown to have a major role in modulating vascular tone. It also has pronounced antithrombotic activity, as well as protective effects in the heart and the kidneys.[80] Dysregulation of the NO system is associated with cardiovascular disease, and reduced NO bioactivity is considered to be an important determinant of endothelial dysfunction and hypertension.[81]

A number of academic groups and biopharmaceutical companies are pursuing the discovery and development of NO donor hybrid drugs, which are molecules that contain a NO-donor moiety linked to an existing drug with well-established pharmacological properties.[82] The additional NO-releasing activity of such bifunctional compounds can either improve the efficacy of the compounds and/or counteract the adverse effects. An example for the latter is naproxcinod, which is the hybrid of an NO donor with the nonsteroidal anti-inflammatory drug naproxen. The compound should carry the anti-inflammatory properties of naproxen, but without its adverse effects on blood pressure and the gastrointestinal system.[83]

A group from the University of Pisa in Italy has described the synthesis and characterization of NO-donor analogs of losartan and its active metabolite EXP-3174.[84,85] The bifunctional nature of the compounds was assessed using *in vitro* organ bath preparations of rat endothelium-denuded aortic rings. ARB-NO hybrids mediated concentration-dependent relaxation of aortic rings pre-contracted with KCl, which was blocked in the presence of ODQ, an inhibitor of guanylate cyclase, showing the activation of the NO–cGMP pathway by the compound. AT_1 antagonist activity of compounds was confirmed in a similar assay by assessing the inhibition of contractile responses to angiotensin II. The lead compound, the 3-[(nitrooxy)-methyl]benzoate ester of losartan (Figure 4), also showed a significant lowering of SBP in spontaneously hypertensive rats after 4 weeks of oral or subcutaneous dosing, with equivalent effects to losartan at equimolar doses. Importantly, this compound was shown to be cardioprotective in a model of myocardial ischemia and reperfusion, whereas losartan essentially showed no activity. Also, it inhibited platelet aggregation to a greater extent than losartan. Both activities can be attributed to the additional NO-releasing group of the bifunctional compound, differentiating it pharmacologically from the monofunctional ARB.

A group from the China Pharmaceutical University in Nanjing, China, has explored NO-releasing derivatives of telmisartan using an approach similar to that of Breschi *et al.*[84–86] The lead compound WB1106 was found to have dual activity in isolated rat aortic preparations and significantly reduced angiotensin II-mediated pressor responses in normotensive rats. WB1106 lowered blood pressure in spontaneously hypertensive rats to the same extent as telmisartan, but, in contrast to telmisartan, increased aortic cGMP levels, with similar magnitude as the ACE inhibitor, fosinopril. In high-fat and carbohydrate-fed rats, WB1106 effectively reduced body weight gain and fasting serum glucose levels and significantly improved oral glucose tolerance, all more effectively than did equimolar doses of telmisartan, possibly reflecting the synergistic effects of NO and telmisartan on glucose metabolism. Several ARBs containing one or more NO-donor groups have also been described in a series of published patent applications by Merck (Whitehouse Station, NJ, USA) and NiCox (Sophia Antipolis, France).[87–89]

CONCLUSIONS AND PERSPECTIVE

Although the renin–angiotensin system has an important role in the pathogenesis of hypertension and related cardiovascular and metabolic disorders, it is clear that the use of ARBs to inhibit RAS activity is not sufficient to control blood pressure and the risk for cardiovascular disease and diabetes in many patients. On the basis of advances in medicinal chemistry, it has become possible to design new classes of ARBs that are multifunctional and show pharmacological activities in addition to AT_1 receptor antagonism. Such molecules can target multiple pathophysiological pathways in cardiovascular disease and diabetes besides just the upregulation of the renin–angiotensin system. Although the discovery of such multifunctional compounds is more complex and the optimal ratio of potencies for the different

targets needs to be found, the unified pharmacokinetics should allow for better-matched pharmacodynamic activities at the two targets, compared with simple combination therapy. Furthermore, a multifunctional molecule is also amenable to a fixed-dose combination with other drugs, which allows for three or more pharmacologies in a single pill. An important consideration in the design of multifunctional ARBs is the kind of activity added to the molecule, as maximal efficacy benefits will be achieved when adding a target mechanism that is complementary to, or synergistic with, RAS blockade. By going beyond inhibition of the RAS, the next generation ARBs described herein could be of considerable clinical value and provide greater therapeutic benefits than molecules that work only through the AT_1 receptor blockade.

CONFLICT OF INTEREST

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