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REVIEW ARTICLE

VASOPEPTIDASE INHIBITION – SOLVING THE CARDIOVASCULAR PUZZLE?

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SUMMARY

The therapeutic blockade of the renin–angiotensin–aldosterone system (RAAS) using angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, aldosterone receptor antagonists and, more recently, renin inhibitors has been a mainstay for the treatment of hypertension, the main risk factor for cardiovascular disease. Blood pressure and fluid homeostasis are regulated by the interconnected pathways of the RAAS, the endothelin system, the natriuretic peptide system and the kallikrein–kinin system. The simultaneous modulation of several neurohumoral mediators has been an attractive approach that has received much attention. The dual ACE/neutral endopeptidase (NEP) and triple ACE/NEP/endothelin-converting enzyme (ECE) inhibitors are the most celebrated vasopeptidase inhibitors, but their progress to the clinic has been stalled by kinin-mediated adverse effects. NEP/ECE and ACE/ECE inhibitors and compounds targeting the angiotensin receptor and NEP are other attractive options that have been investigated. In this review, we discuss the systems that regulate the concentrations of vasoactive peptides, the current therapies and the molecular basis for the design and action of vasopeptidase inhibition.

INTRODUCTION

According to data released by the World Health Organization, stroke is one of the main causes of major disability and approximately 15 million people worldwide are afflicted by stroke annually, with two-thirds of cases resulting in either death or permanent disability (http://www.who.int/cardiovascular_diseases/resources/atlas). Major risk factors for stroke and coronary heart disease are high blood pressure, diet and physical inactivity. Currently, 30% of all deaths worldwide are due to cardiovascular disease (CVD), with the vast majority of mortalities occurring in developing countries. The global burden of CVD continues to increase, with the likelihood that it will rank as the leading cause of death 20 years from now (1). To curb this growing epidemic, novel drugs are required that are safe, effective, affordable and lessen the need for polypharmacy, which is currently the norm. The main challenge in developing countries remains the accessibility of chronic disease medicines (2).

The renin–angiotensin–aldosterone system (RAAS), kallikrein–kinin system (KKS) and endothelin system (ES) play key roles in the pathogenesis of cardiovascular disease, and thus drugs that target components of these systems are used to control blood pressure. Among these components, peptidases have been important drug targets for CVD for 30 years. The most celebrated and longest-serving peptidase inhibitor drugs are the angiotensin-converting enzyme (ACE) inhibitors, while the most recent addition is the renin inhibitor aliskiren (Rasilez®). Unfortunately, poorly controlled hypertension is the norm rather than the exception, and progression of established heart failure, nephropathy and retinopathy can at best be slowed but not halted. The economic burden of CVD and end-organ damage is staggering, with estimated annual costs of $149 billion in the U.S., €169 billion in the E.U. and £29 billion in the U.K. (3-5). Thus, novel approaches to hypertensive therapy, such as inhibiting more than one peptidase, may help bridge current treatment gaps and enable more patients to maintain normotensive blood pressure. Here we review some of the more recent approaches that involve peptidase inhibitors as modulators of vascular tone and regulators of blood pressure homeostasis, and which therefore have promise in meeting the demands of blood pressure control.

BRIEF OVERVIEW OF SYSTEMS INVOLVED IN THE MAINTENANCE OF BLOOD PRESSURE

Regulators of cardiovascular function, and therefore appropriate targets for therapeutic intervention, can be summarized in four overlapping yet distinct vasoactive systems, as outlined below (Fig. 1).

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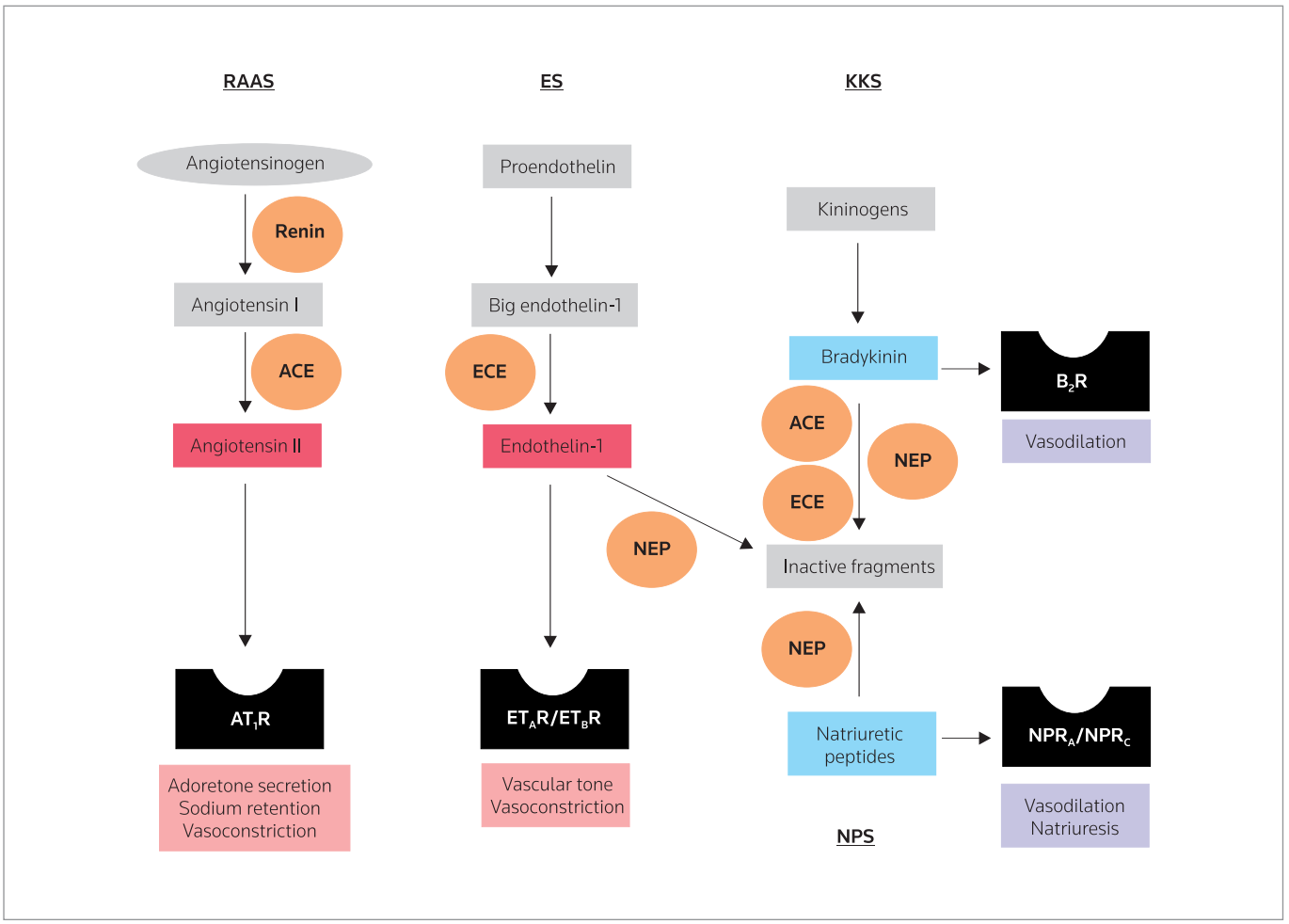


Figure 1. Outline of the systems involved in vasoaction. Production of angiotensin II and endothelin-1 in the renin–angiotensin–aldosterone system (RAAS) and endothelin system (ES), respectively, results ultimately in vasoconstrictive events. Production of nartiuretic (within the natriuretic peptide system, NPS) and kinin peptides (within the kallikrein–kinin system, KKS) results in vasodilation. Peptides of the NPS and KKS can be degraded by several vasopeptidases. Vasoconstrictive peptides and their consequences are shown in red, vasodilators and their effects are shown in blue and inactive hormones are indicated in grey. ACE, angiotensin-converting enzyme; ECE, endothelin-converting enzyme; NEP, nepriysin.

Control of cardiovascular function is regulated by a fine balance of the enzyme-catalyzed generation and degradation of a diverse set of vasoactive peptides that mediate both vasoconstriction and vasodilation.

The renin–angiotensin–aldosterone system (RAAS)

Angiotensinogen, a 55-kDa plasma protein secreted by the liver, is cleaved at its *N*-terminal region by the specific aspartyl protease renin, to produce the decapeptide angiotensin I (Ang I) (6). Ang I, a physiologically inactive peptide, undergoes subsequent hydrolysis of the penultimate peptide bond by ACE to yield the octapeptide angiotensin II (Ang II) (7, 8). Ang II is the most active form of the angiotensin peptides, eliciting downstream effects via binding to the widely expressed angiotensin AT_1 receptor, resulting in vasoconstriction, aldosterone release and sodium retention, and thus ultimately increasing arterial blood pressure (9). Although Ang II can also bind to the AT_2 receptor mediating vasodilatory effects, minimal expression of the AT_2 receptor in the adult cardiovascular system accounts for the net increase in systemic blood pressure (10). More recent work has shown the involvement of peptide Ang(1-7), a product of angiotensin-converting enzyme 2 hydrolysis and substrate for ACE, in the regulation of the RAAS due to its being a vasodilating and antifibrotic agent (11, 12). The centrality of the RAAS in blood pressure regulation has been demonstrated in hypotensive ACE and renin knockout mice (13-15).

The endothelin system (ES)

The more recently elucidated endothelin-1 (ET-1) system involves the production of preproendothelin-1, a 212-amino-acid peptide produced mainly by endothelial cells (16). After removal of the signal sequence, proendothelin-1 is converted to a 38-amino-acid peptide (known as big endothelin-1) by furin convertase (17). Subsequently,

big endothelin-1 undergoes proteolytic cleavage by endothelin-converting enzyme (ECE), yielding a 21-amino-acid peptide hormone (ET-1) (18-20). The vasoconstrictive effects of ET-1 are mediated through the ligand–receptor interaction with the G protein-coupled receptors ET_A and ET_B (21). ET-1 vasoactivity is considerably higher (more than 100-fold) than big endothelin-1, implicating production of ET-1 as the critical step in the potent action of this system (22, 23). Another metalloproteinase, neprilysin (neutral endopeptidase 24.11, NEP), is able to degrade ET-1 into inactive fragments (24). The recent demonstration of reduced vascular tone and blood pressure in ET-1 knockout mice emphasizes the contribution of this hormone to vascular function (25).

Natriuretic peptide system (NPS)

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are the principal members of the natriuretic peptide family involved in cardiovascular function in humans (reviewed in 26). These peptides elicit their vasodilating effects through the natriuretic peptide receptors A and C, both of which have a wide tissue distribution. ANP and BNP are cleaved into inactive fragments by NEP (27, 28).

The kallikrein–kinin system (KKS)

Kininogens can be converted to the kinins bradykinin (BK) and kallidin by kallikrein enzymes. BK and kallidin bind strongly to the widely expressed B_2 receptor, resulting in vasodilatory events (29). In addition to aminopeptidase P, all three of the enzymes described above (ACE, ECE and NEP) are able to cleave BK into physiologically inactive fragments (30-32).

THERAPEUTIC APPROACHES INVOLVING THE RAAS AND RELATED VASOPEPTIDASES

ACE inhibitors

The design, development and clinical use of ACE inhibitors is often described as a triumph of rational drug design, although the development of these drugs in the 1970s and 1980s predates modern structure-guided design as we know it today. Nevertheless, the introduction of ACE inhibitors in the early 1980s revolutionized the treatment of hypertension, heart failure, myocardial infarction and nephropathy, as reviewed extensively elsewhere (see reference 9 and references cited therein). The success of these drugs drove the search for inhibitors of each of the other components of the RAAS and also stimulated an interest in the inhibition of other vasopeptidases, most notably NEP and ECE, spawning an array of drug classes, many of which are in clinical use today.

Angiotensin receptor blockers (ARBs) and combination therapies

Strictly speaking, the ARBs are not peptidase inhibitors, but the AT_1 receptor is an important target in the RAAS and ARBs are the biggest-selling class of antihypertensive drugs, and hence worth commenting on. Furthermore, ARBs are sometimes combined with ACE inhibitors, and there is also interest in combining them with renin and NEP inhibitors.

Following the success of ACE inhibitors in the clinic, there was enormous interest in finding additional RAAS targets, partly because side effects limit ACE inhibitor use in a significant proportion of patients (reviewed in 33), and partly for commercial reasons. During the 1980s, significant progress was made in defining the Ang II receptors, with the discovery of AT_1 and AT_2 receptors and the realization that the AT_1 receptor is the principal mediator of Ang II-induced vasoconstriction and aldosterone release. Extensive medicinal chemistry efforts led to the development of potent, nonpeptide antagonists of the AT_1 receptor, giving rise to ARBs such as **losartan** and **valsartan** (reviewed in 34) (Fig. 2).

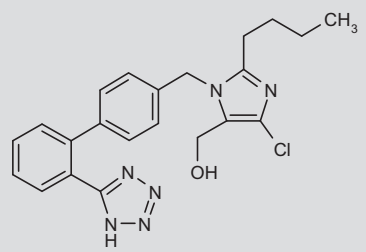
Although there was an expectation that ARBs might be more effective in the clinic than ACE inhibitors (based on theoretical considerations), this has not been borne out. In general, ARBs appear to be noninferior to ACE inhibitors in the treatment of hypertension and heart failure (33, 35), but it remains controversial whether ARBs are equivalent to ACE inhibitors in myocardial infarction (36, 37). For these and other reasons, ACE inhibitors remain first-line therapy for heart failure, myocardial infarction and nephropathy, with ARBs to be used if ACE inhibitors are not tolerated (or added to ACE inhibitors; see below).

In recent years, there has been considerable interest in combining an ACE inhibitor with an ARB for heart failure, myocardial infarction and nephropathy. The rationale for this is based on the notion that Ang II levels can remain elevated despite ACE inhibitor therapy due to alternative enzymatic pathways, such as chymases, and hence AT_1 receptor activation can be blocked more completely by addition of an ARB. Alternatively, the therapeutic efficacy of ACE inhibitors may depend in part on increased BK levels, and therefore combination with an ARB may provide true synergy (38). The Val-HeFT and CHARM-Added trials showed benefits of the combination in the setting of heart failure (39, 40), but the VALIANT and ONTARGET trials showed no added benefit in patients with myocardial infarction, established cardiovascular disease or diabetes (37, 38). The combination also raised safety concerns, with an increased incidence of hypotension, hyperkalemia and renal impairment compared with monotherapy. Therefore, at present, the clinical utility and safety of combining an ARB with an ACE inhibitor is uncertain (41, 42).

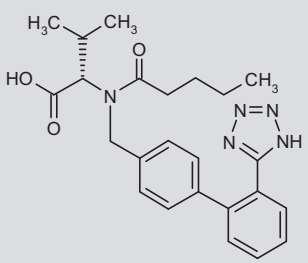
Recently, unexpected safety concerns have been raised related to the use of ARBs, in both cases triggering ongoing safety reviews by the FDA. In two phase III studies of **olmesartan medoxomil** (Fig. 2) (ROADMAP and ORIENT), designed to evaluate the ability of the drug to slow the progression of renal disease in type 2 diabetes, there was an excess risk of cardiovascular death in the treatment group. The second safety concern emerged after a meta-analysis of trials involving more than 60,000 patients revealed that ARBs are associated with a modest but significant increase in the incidence of cancer (43), and these data are currently under review at the FDA (FDA Drug Safety Communication, 2010).

Renin inhibitors

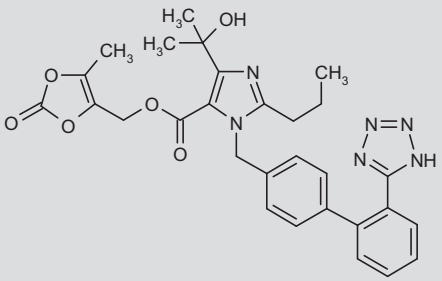
Renin inhibitors, also called direct renin inhibitors, are the newest class of drugs inhibiting the RAAS. The only approved renin inhibitor is **aliskiren** (Fig. 2). It has been argued that proximal inhibition of the RAAS should lead to more complete suppression of Ang II production, and hence greater efficacy (42, 44). However, a recent meta-analysis



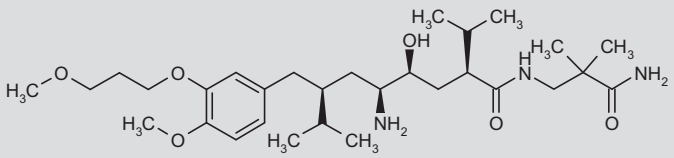
Losartan

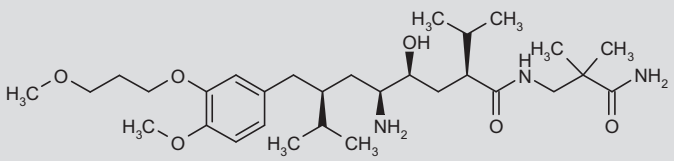


Valsartan

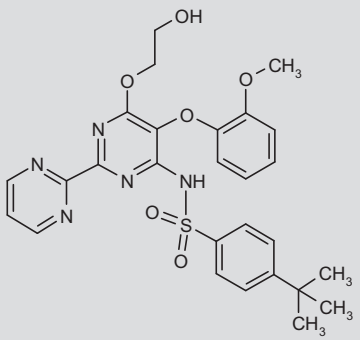


Olmesartan medoxomil

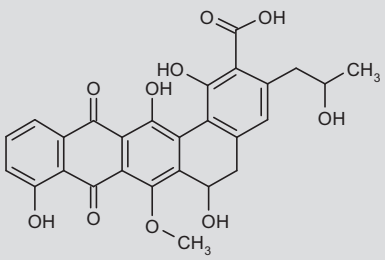




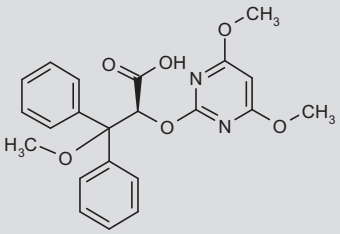
Aliskiren



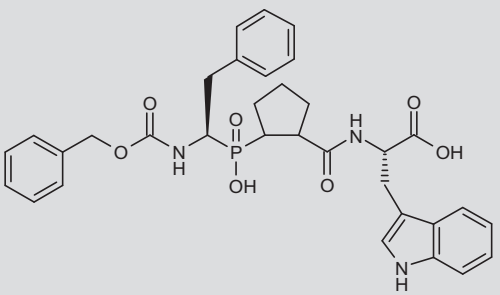
Bosentan



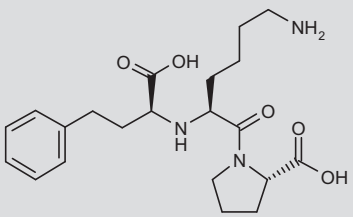
FR-901533



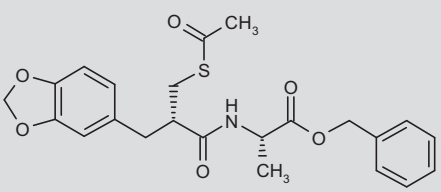
Darusentan



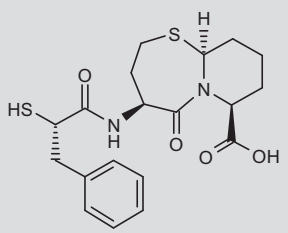
RXPA-380



Lisinopril



Alatriopril



Omapatrilat

Figure 2. Drugs and inhibitors designed for the treatment of cardiovascular disease.

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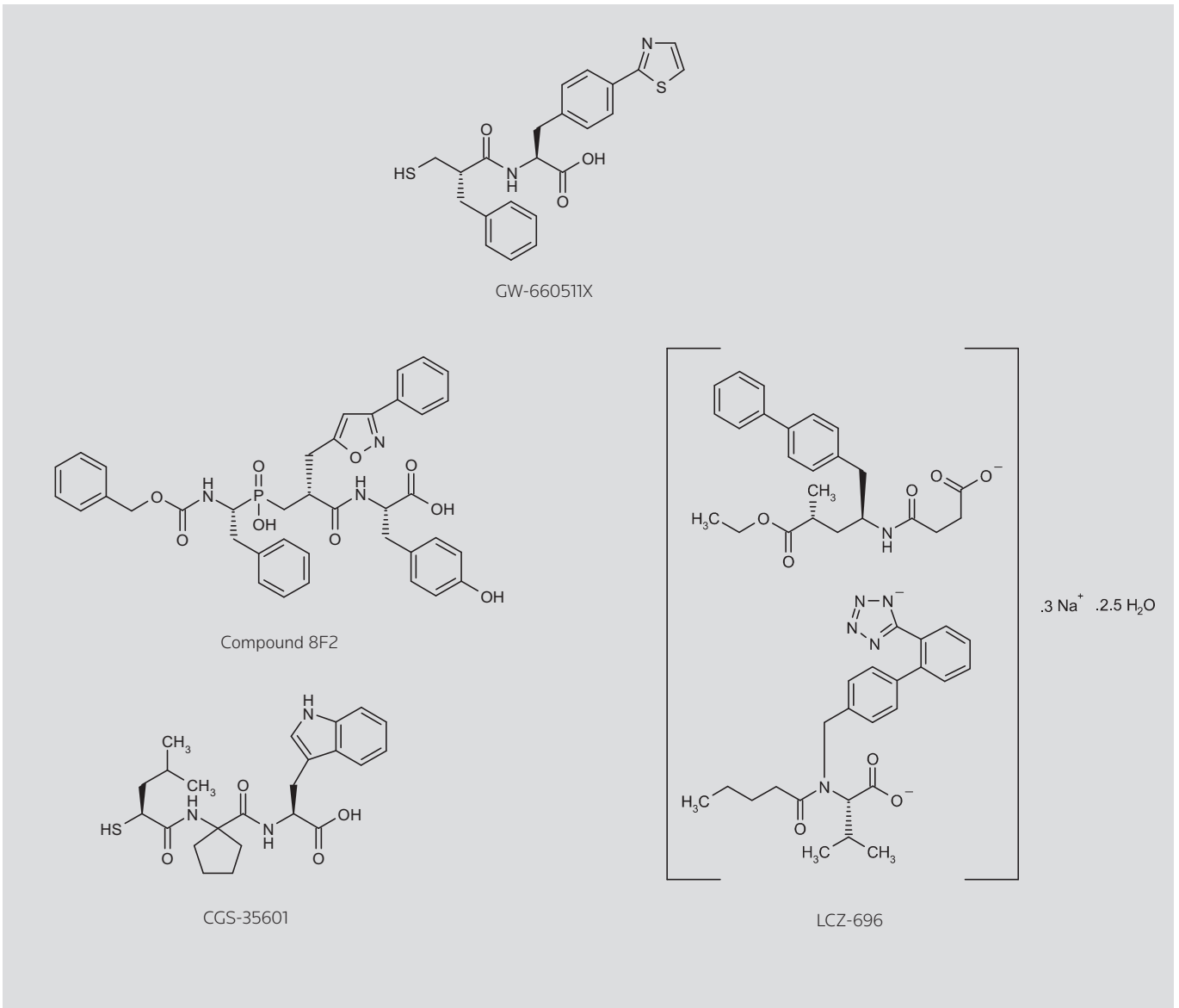

Figure 2. *(Cont.)*. Drugs and inhibitors designed for the treatment of cardiovascular disease.

of all clinical trials involving aliskiren, many of which included ARB and ACE inhibitor comparator arms, concluded that the magnitude of the blood pressure-lowering effect of aliskiren was no different from that of the ARBs and ACE inhibitors (45). The adverse event profile of aliskiren appears to be favorable. To date, no long-term morbidity and mortality trials have been completed, and therefore the true cardiovascular benefits or harm of aliskiren therapy are unknown. A theoretical concern involves the aliskiren-induced feedback elevation of plasma renin levels, because of controversial data linking renin and prorenin to pathophysiological events unrelated to RAAS activation (46).

As in the case of ARBs and ACE inhibitors, combination therapies with aliskiren are also being investigated. Dual aliskiren/ACE inhibitor or aliskiren/ARB therapy is theoretically appealing because both ARBs and ACE inhibitors increase plasma renin activity (PRA), whereas aliskiren decreases PRA (as opposed to total renin protein levels). Thus far, relatively short-term studies (~3 months) of aliskiren combined with an ACE inhibitor or ARB using surrogate endpoints have been encouraging, but longer-term studies with clinical endpoints are required to evaluate the true safety and efficacy of this approach (*42*, 44).

NEP and ECE inhibitors

Following the success of the RAAS inhibitors at various points in the cascade, there has been tremendous interest in identifying related pathways of vasoactive peptides and associated vasopeptidases that might be amenable to pharmacological manipulation.

This interest is driven in part by the realization that, despite maximal suppression of the RAAS, significant numbers of patients with chronic heart and kidney pathology continue to progress towards end-stage disease (42). The two neurohumoral systems that have garnered the most attention are the endothelins and the ANPs.

Plasma ET-1 levels are elevated in heart failure and predict morbidity and mortality. A number of endothelin receptor antagonists (selective for ET_A or nonselective for ET_A and ET_B) are in development for heart failure. Thus far, however, only a single drug, the dual ET_A and ET_B receptor blocker **bosentan** (Fig. 2), has been approved for the orphan disease idiopathic pulmonary arterial hypertension. Despite intensive efforts over the past 10-15 years involving a number of selective and nonselective antagonists in a variety of indications, including heart failure and hypertension, these agents have failed to demonstrate sustained clinical efficacy and are bedeviled by significant adverse events (AEs) (47). The disappointing efficacy has been ascribed in part to increased circulating ET-1 levels during endothelin receptor blockade (48). The most frequent AEs are related to the dominant pharmacological effect of these drugs, namely vasodilation, and include postural hypotension, flushing, headache and, more significantly, fluid retention with occasional heart failure (47, 49). Furthermore, endothelin receptor blockers are also associated with elevations in hepatic transaminases and are potent teratogens (47). This AE profile would effectively disqualify this class of drugs from the largest potential market, uncomplicated hypertension, even if sustained efficacy could be demonstrated.

An alternative approach to blocking the endothelin pathway is inhibition of ECE, which converts the inactive precursor big endothelin-1 to the active vasoconstrictor ET-1. This approach has the advantage that ET-1 levels are decreased rather than increased by feedback mechanisms, analogous to ACE inhibition versus AT_1 receptor blockade in terms of plasma Ang II levels. In a dog heart failure model, the specific ECE inhibitor **FR-901533** (Fig. 2) showed modest efficacy, but the magnitude of the effect on hemodynamic parameters was generally less than for the selective ET_A receptor antagonist FR-139317 (50). FR-901533 treatment resulted in an expected increase in big ET-1 levels, but, surprisingly, ET-1 levels were unchanged, which may account for the modest efficacy. Unresolved at present is the expected AE profile for ECE inhibitors (selective or nonselective) in the clinic, and whether this will resemble the profile observed with ET receptor blockers. Interestingly, FR-901533, but not FR-139317, decreased PRA, Ang II and aldosterone levels, which would mitigate the fluid retention effects observed with ET receptor antagonists. Interestingly, work on the development of pure ECE inhibitors has been sparse, in contrast to dual ECE/NEP inhibitors or triple ECE/NEP/ACE inhibitors (which will be considered further below).

Work on NEP inhibitors dates back to the 1980s and 1990s. These inhibitors were considered to be very promising for heart failure, in particular because sodium and water retention, which are counteracted by the natriuretic peptides, are a prominent feature. However, many studies have shown that NEP inhibitors have only modest effects in animal models and patients with heart failure (reviewed in 48, 51, 52). This lack of efficacy has been ascribed to an increase in ET-1 (ET-1 is degraded by NEP), feedback activation of the RAAS and downregulation of ANP receptors (52). Attention has therefore focused on incorporating NEP inhibition into dual or triple vasopeptidase inhibitor compounds.

Resistant hypertension and unmet treatment needs

Despite treatment, millions of patients worldwide fail to reach blood pressure goals. Although in many cases this is due to inadequate dosing or poor compliance, in a substantial number it is due to "resistant hypertension": failure to reach target pressures despite optimum doses of at least three classes of drugs (53). Generally, patients with resistant hypertension are older, overweight and diabetic, which is precisely the demographic that is growing fastest. Hence, there is an unmet need to develop additional treatment modalities that are not only more effective than current options but also offer the prospect of reducing the need for polypharmacy.

A novel approach that was recently evaluated in a controlled trial is the addition of **darusentan** (Fig. 2), an ET_A receptor antagonist, to existing therapy in patients with resistant hypertension. Darusentan was clearly effective, producing a further fall in systolic pressure of 10 mmHg in difficult-to-treat patients who were already on three and in most cases four or more drugs (49). However, as already noted for this class of drug, fluid retention with occasional heart failure was a significant safety concern. Nevertheless, given limited options in this setting, ET_A receptor blockers may establish themselves as drugs of last resort in patients with resistant hypertension, although approval will likely require adequately powered morbidity and mortality trials showing clear clinical benefit.

An attractive alternative, although as yet untested in the clinic, are dual and triple vasopeptidase (ACE/NEP/ECE) inhibitors. Such compounds would not only target multiple neurohumoral pathways simultaneously (RAAS, natriuretic peptides and endothelins) with additive and even synergistic effects, but would also reduce the need for polypharmacy. Alternatively, a dual NEP/ECE inhibitor is designed instead and combined with an ARB (54), but this has the disadvantage of requiring addition of a second drug (unless a fixed-dose combination is developed) and will be subject to the emerging safety concerns around ARBs (43).

Finally, another approach that has not been fully explored in resistant hypertension is more extensive inhibition of the RAAS by addition of aliskiren to a regimen that already includes an ACE inhibitor or an ARB. Conceivably, aliskiren could also be added to a triple vasopeptidase inhibitor.

NOVEL INHIBITORS OF THE RAAS, NPS AND ES

C-domain-selective ACE inhibitors

While ACE blockade alone is often sufficient to reduce arterial blood pressure, AEs remain a major reason for patients discontinuing treatment (55, 56). Such AEs are believed to be the result of increased plasma BK (57-59). Original ACE inhibitors were designed assuming a structural similarity to carboxypeptidase A and therefore lacked proper structure-based design (60). In addition, ACE inhibitors were designed without the knowledge that ACE comprises two domains, each containing an intact active site (designated N- and C-domains depending on polypeptide chain location) (61, 62). Bernstein and colleagues later used gene targeting to show that the C-domain was the predominant active site of Ang I conversion in mice (63, 64). BK is

cleaved efficiently by both domains and increased BK levels by dual domain blockade has been shown to increase the activity of mast cell chymase, an enzyme capable of producing Ang II independently of ACE, thus restoring or increasing circulating Ang II levels (65). Selective inhibition of the C-domain has therefore been proposed to reduce circulating Ang II while still allowing the N-domain to attenuate the adverse buildup of plasma BK levels (9, 66). The high-resolution determination of both N- and C-domain crystal structures has allowed for structure-based design of C-domain-selective inhibitors (67, 68).

Prior to structure-based approaches, **RXPA-380** was the first inhibitor developed that selectively inhibited the C-domain active site (Fig. 2) (69). Subsequent cocrystallization with the C-domain active site revealed the importance of the P2′ tryptophan moiety in conferring C-domain selectivity (70, 71). Furthermore, RXPA-380 inhibition in vivo showed decreased Ang II/Ang I ratios and decreased BK concentrations compared to dual domain inhibition (69). Modification of the inhibitors **lisinopril** and keto-ACE by replacement of the P2′ proline with the bulkier residues tryptophan and phenylalanine resulted in increased C-domain selectivity (Fig. 2) (72, 73). These promising modified drug templates are currently being investigated in the preclinical setting.

Dual inhibitors

ACE/NEP inhibitors

By inhibiting both the RAAS and potentiating the NPS, dual ACE/NEP inhibitors reduce vasoconstriction, increase vasodilation and reduce sodium retention. Vasopeptidase inhibitors that vary in their relative potency for the inhibition of ACE and NEP include **alatriopril** (fasidotrilat), gemopatrilat, **omapatrilat**, sampatrilat, MDL-100240 and **GW-660511X** (Fig. 2) (74).

The first vasopeptidase inhibitors developed were alatriopril and glycopril (75). The potency of these molecules was attributed to interaction of the methylenedioxy group with the S1 pocket of ACE and the aromatic ring with the S1′ pocket of NEP. Alatriopril has a free C-terminal alanine, unlike most ACE inhibitors, which have a proline, due to NEP's preference for dipeptides with an unsubstituted bond between the P1′ and P2′ groups (76). The presence of the bulky P1′ phenylalanine substituted with a methylenedioxy group in alatriopril was based on the assumption that the S1′ subsite of NEP should be able to accommodate a larger hydrophobic group (75). The P1′-P2′ bonding requirement was addressed by designing mercaptodipeptide mimetics like omapatrilat and gemopatrilat.

Molecular docking of 11 vasopeptidase inhibitors in the crystal structures of the ACE C-domain (PDB 1O86), N-domain (PDB 2C6N) and NEP (PDB 1R1H) provides important insights for the development of second-generation vasopeptidase inhibitors. Interestingly, such an analysis revealed different modes of zinc coordination in ACE and NEP (77). In terms of subsite contacts, vasopeptidase inhibitors exhibit similar interactions with ACE and NEP (Fig. 3). Furthermore, all 11 vasopeptidase inhibitors were predicted to bind more tightly to the C-domain of ACE than the N-domain, with an average difference of the free energy of binding of 2-3 kcal/mol. This is consistent with the structural differences in the S1 and S1′ subsites, where, for example, the more hydrophobic interactions between C-domain Val379 and Val380 and the P1′

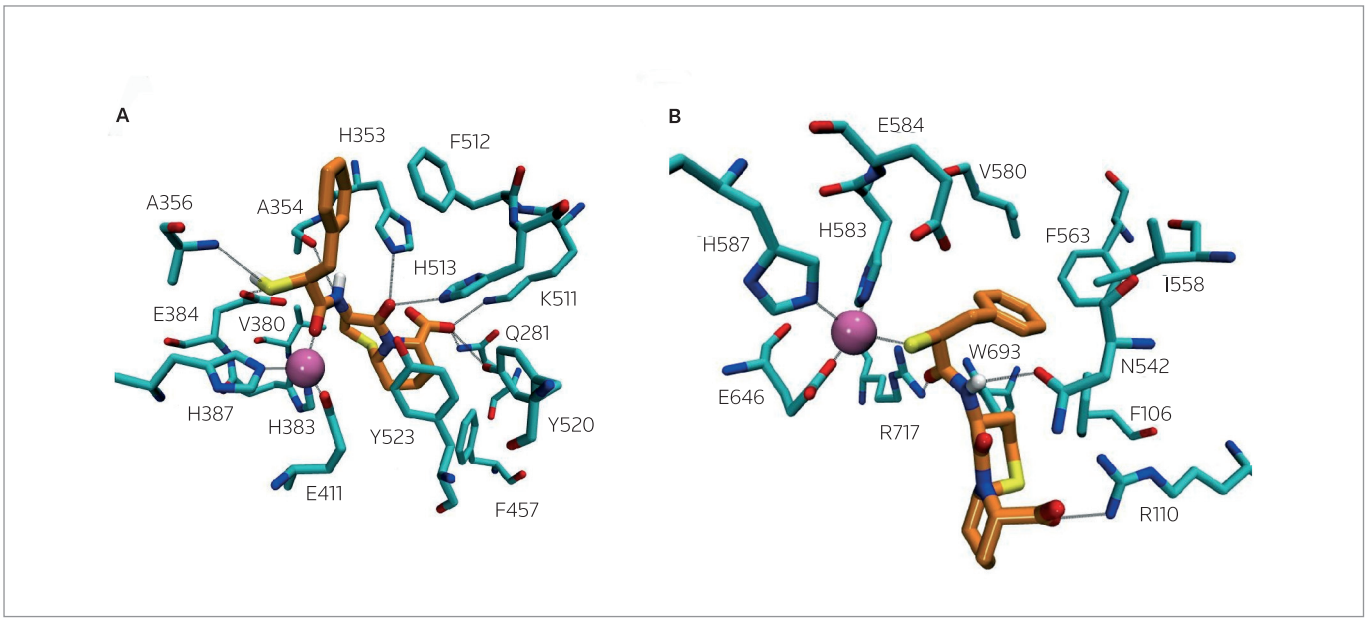

Figure 3. Omapatrilat docked with the C-domain of ACE (**A**) and NEP (**B**). The inhibitor and surrounding residues are shown with carbon atoms in orange and cyan sticks, respectively. For both enzymes and inhibitors, nitrogen atoms are blue, oxygen atoms are red, sulfur atoms are yellow and the catalytic zinc is shown as a mauve sphere. Adapted with permission from N. Dimitropoulos, A. Papakyriakou, G.A. Dalkas, E.D. Sturrock, G.A. Spyroulias. *A computational approach to the study of the binding mode of dual ACE/NEP inhibitors*. J Chem Inf Model 2010, 50(3): 388-396. ©2010 American Chemical Society.

residue are lost due to substitution with more polar residues in the N-domain (Ser357 and Thr358).

Omapatrilat inhibits ACE and NEP with K_i values of 0.5 and 8.9 nM, respectively, and showed long-lasting efficacy in low-, normal- and high-renin rodent models of hypertension (78). In the OCTAVE trial, patients on omapatrilat had a greater reduction in blood pressure than those treated with enalapril (79). However, there is no convincing evidence that omapatrilat is clearly superior to an ACE inhibitor in the treatment of heart failure (52). The increased risk of angioedema, likely due to increased bradykinin or one of its metabolites, led to the discontinuation of further development of the drug.

The ACE/NEP inhibitor GW-660511X, originally known as Z-13752A, is a potent vasopeptidase inhibitor with IC_{50} values of 3.2 nM for ACE and 1.8 nM for NEP (80). Intravenous and oral administration of GW-660511X in spontaneously hypertensive rats (SHR) and DOCA-salt rats resulted in long-lasting antihypertensive effects (81). Moreover, the inhibitor was effective in protecting the heart from the consequences of ischemia in a canine model, and this protection was primarily due to bradykinin potentiation (82). More recently, it was demonstrated to be an effective agent for the treatment of patients with mild to moderate hypertension, with NEP inhibition contributing more to blood pressure reduction than ACE inhibition (83). The safety profile of GW-660511X was comparable to that of placebo and no AEs related to angioedema were reported. However, longer-term studies in larger populations and in different ethnic groups are required to provide more comprehensive information on the compound's side effect profile. In general, changing the relative degree of ACE and NEP inhibition may alter the efficacy and safety profile of this class of compounds and deserves further study.

Dual C-domain-selective ACE/ECE inhibitor: compound 8F2

With vasopeptidase inhibitor treatment increasing the risk of angioedema due to increased concentrations of BK, other strategies have been designed. Dive and coworkers developed novel phosphinic dual inhibitors of the ACE C-domain and ECE, thus leaving the N-domain and NEP to clear BK buildup and thereby lessen the risk of adverse effects (84, 85). Compound development took the form of structure-based design and compound refinement: a free tryptophan moiety in the P2′ position was common to both RXPA-380 and phosphoramidon, one of the first phosphinic peptidomimetic inhibitors of ECE. Modification of the P1′ substituent was required in order to discriminate between the active sites of ECE and NEP, with differences in the stereochemistry of the P1′ position ultimately affecting inhibitor selectivity towards ECE. Although the compound showed good inhibition in vitro, nonspecific binding to albumin in rat plasma studies required removal of the tryptophan group. The final compound, **8F2**, displays low nanomolar inhibition of ACE C-domain and ECE, with only micromolar inhibition of NEP (Fig. 2). Further tests with SHR indicated that treatment with 8F2 significantly reduced blood pressure, making this inhibitor an important template for the design of novel ACE/ECE inhibitors (85).

Triple vasopeptidase inhibitors (ACE/NEP/ECE)

The alternative to dual inhibitors is triple ACE/NEP/ECE inhibitors, where the reduction in ET-1 levels may confer additional benefits. Triple vasopeptidase inhibitors exert antihypertensive, antifibrotic and antiinflammatory actions by reducing the production of Ang II and ET-1, while increasing levels of BK and natriuretic peptides. In the dual ACE/NEP inhibitors, inhibition of NEP was responsible for an increase in ET-1 levels, and thus the ECE-inhibitory activity of the triple inhibitors gives them a significant advantage over dual inhibitors. One of the first effective triple vasopeptidase inhibitors capable of inhibiting ACE, NEP and ECE simultaneously with IC_{50} values in the low nanomolar range was **CGS-35601** (Fig. 2). This compound contained a central cyclic non-natural amino acid and a bulky P2′ tryptophan that were previously shown to be important for α-mercaptodipeptide inhibition of ACE and NEP (86). Moreover, the terminal tryptophan was also important for ECE inhibition.

In normal (SHR) and low-renin (Dahl salt-sensitive) animal models of hypertension in which RAAS blockers are ineffective, systolic and diastolic blood pressures were significantly decreased by CGS-35601 (87, 88). While BK was elevated by 80%, nitric oxide (NO) was considerably reduced when compared to during treatment with omapatrilat (78). Thus, it seems that the ET-1-induced NO production predominates during ACE/NEP vasopeptidase inhibition and may contribute to the increased incidence of angioedema. In contrast, during ACE/NEP/ECE vasopeptidase inhibition, the decrease in ET-1 plasma concentrations can compensate for the overproduction of BK and the associated release of NO.

Nevertheless, the AEs associated with triple vasopeptidase inhibitors may include persistent cough and angioedema. The effect of triple inhibitors, such as CGS-35601, on vascular permeability and edema has not been investigated. No toxic effects were observed in the preclinical safety profile carried out on CGS-35601 in SHR (89). However, it is imperative to assess their inhibition of other enzymes involved in the metabolism of kinins, such as aminopeptidase P and carboxypeptidase N, to further evaluate the potential for angioedema. An attractive approach might be to develop a triple ACE/NEP/ECE inhibitor that only targets the C-domain active site in ACE, allowing the N-domain site to continue to degrade BK (as in the dual ACE/ECE inhibitor 8F2). To date, this idea has not been tested.

Dual ARB/NEP inhibitor: LCZ-696

Although not strictly defined as a vasopeptidase inhibitor (90), **LCZ-696** (Novartis) (Fig. 2) is a new drug in development for the treatment of hypertension that could reach the market in the next few years. In response to the increased risk with ACE/NEP dual inhibition (as seen with omapatrilat), LCZ-696 is a compound that incorporates both ARB (valsartan) and NEP prodrug inhibitor (AHU-377) moieties. This design reduces AT_1 receptor stimulation and increases ANP concentrations, while leaving ACE free to clear BK buildup associated with NEP inhibition. In healthy volunteers, inhibitor administration increased levels of cGMP (a biomarker for NEP inhibition) and renin and Ang II concentrations, and the drug was well tolerated (91). A phase II clinical trial in mild to moderate hypertensive patients revealed improved blood pressure reduction compared to valsartan. Importantly, no cases of angioedema or other major AEs related to the drug were reported, further suggesting the potentially superior safety profile of this inhibitor com-

pared to omapatrilat (92). However, the long-term safety and efficacy of LCZ-696 remain to be established in both hypertension and heart failure.

CONCLUSION

Recent advances in our understanding of the molecular basis for the modulation of components of the RAAS, NPS, KKS and ES, together with meta-analyses of data from clinical trials, have led to the development of new classes of compounds, such as renin and ARB/NEP inhibitors, for the treatment of hypertension and CVD. Moreover, there are several lead candidates in the development pipeline that have increased peptidase selectivity and/or simultaneously inhibit two or three targets. These are likely to result in more efficient blockade of the formation of vasoactive peptides and could reduce the need for polypharmacy. The ongoing challenge is to improve the efficacy of these compounds without increasing the incidence of significant AEs. The unrelenting increase in the prevalence and burden of CVD, in part due to a rapidly growing number of patients with resistant hypertension that does not respond well to traditional medical therapy, will continue to drive the development of new therapeutic antihypertensive approaches that involve vasopeptidase inhibition.

DISCLOSURES

The authors state no conflicts of interest.

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