ACE Inhibitor-Angiotensin Receptor Blocker Combinations: A Clinician's Perspective

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Abstract: ACE Inhibitors (ACEI) and angiotensin receptor blockers (ARB) inhibit the renin-angiotensin system, but ACEI may do so incompletely when administered as monotherapy at conventional doses. In theory, combining an ACEI and ARB might be beneficial, whereas clinical evidence for this approach in hypertension is lacking. An ACEI-ARB combination is likely to be useful in proteinuric renal disease, but recent experimental evidence suggests that very high dose monotherapy with an ARB may be the best approach. However, the results of large outcome studies for combinations vs. ACEI or ARB monotherapy are still awaited.

Keywords: Renin-angiotensin system, angiotensin converting enzyme (ACE) inhibitor, angiotensin-receptor blocker, hypertension, diabetic nephropathy, chronic kidney disease, proteinuria.

THE RENIN-ANGIOTENSIN SYSTEM

The principle determinants of renin release by the juxtaglomerular apparatus (JGA) of the renal afferent arterioles, and major mediators of the "classical" circulating renin-angiotensin system (RAS), are outlined in (Fig. **1**). The terminal His-Leu dipeptide of Angiotensin I (Ang I) is cleaved by membrane-bound angiotensin converting enzyme (ACE) in the pulmonary circulation to form the octapeptide Angiotensin II (Ang II), the major effector molecule of the RAS [1]. ACE is a chloride-dependent metallopeptidase (MW 146 kDa) and possesses two homologous arms, both of which have an active site containing a zinc moiety. ACE is widely distributed throughout all mammalian species in both membrane-bound and soluble forms [1,2]. There are a number of substrates for ACE besides Ang I including the vasodilator peptide bradykinin [1]. Furthermore, there is emerging evidence of the importance of novel, "non-classical" RAS components, for example the presence of all components of a functional RAS within individual tissues (e.g. kidney, brain, heart, vasculature), generation of Angiotensin (1-7) by peptidases, and an alternative angiotensin-converting enzyme ACE2 [3].

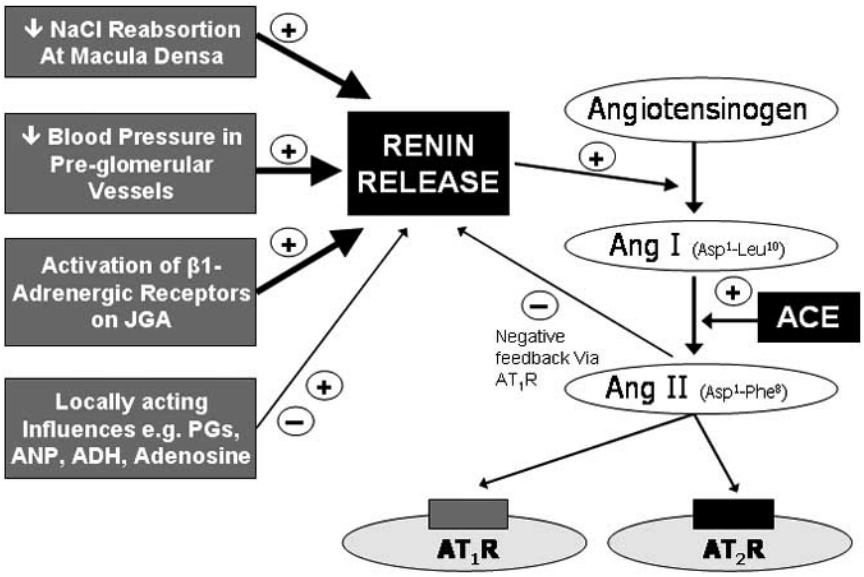

Fig. (1). Overview of the "classical" circulating renin-angiotensin system. Key: ACE =angiotensin converting enzyme; ADH =antidiuretic hormone (vasopressin); ANP =atrial natriuretic peptide; Ang I/II =angiotensin I/II; AT1R/AT2R =angiotensin receptors type 1 and 2; PGs =prostaglandins.

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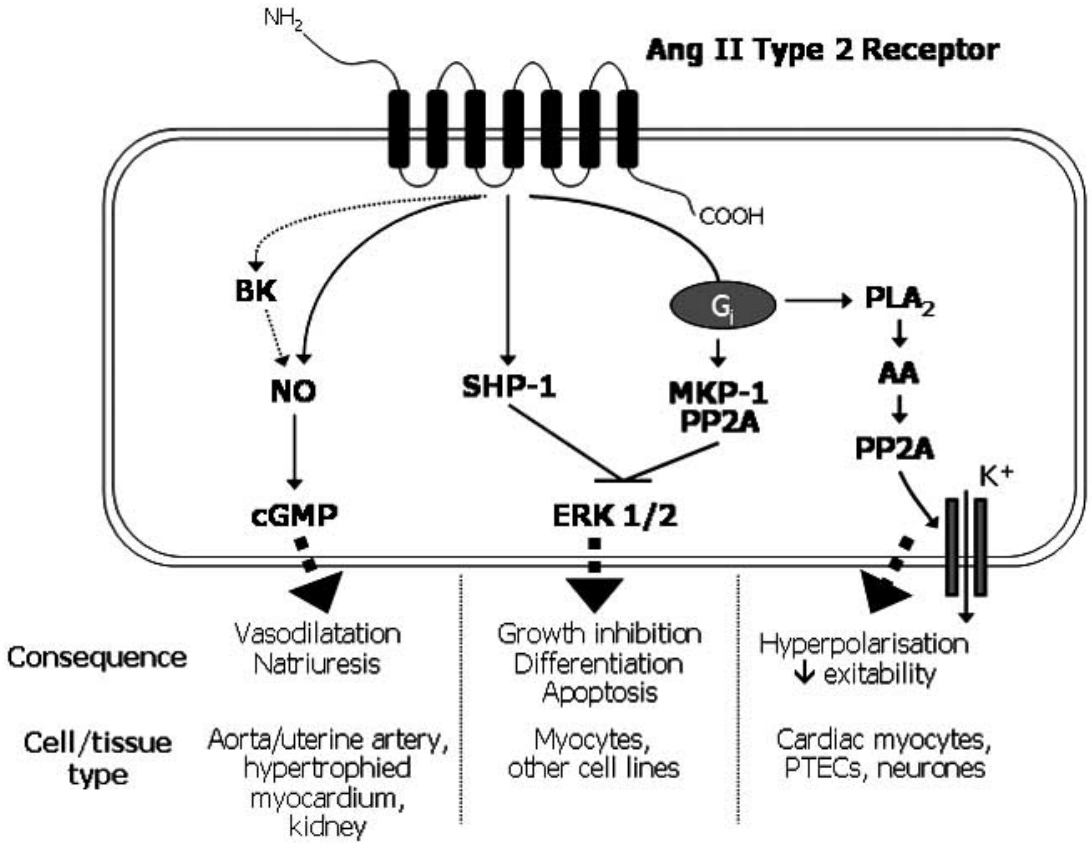


Fig. (2). Intracellular signal transduction pathways of the Angiotensin II Type 2 Receptor. AA =arachidonic acid; MPK-1 =mitogen-activated protein kinase phosphatase-1; PP2A =serine/threonine phosphatase 2A; SHP-1 = Src homology 2 domain phosphatase-1; ERK 1/2 =extracellular-regulated kinase 1 and 2; PLA_2 =phospholipase A_2; NO = nitric oxide; G_i =inhibitory G-protein. Adapted from [5].

The major physiological and pathological effects of Ang II are mediated by two distinct receptor subtypes – AT_1R and AT_2R – which have been extensively studied by pharmacological and genetic techniques, and are summarised in Table **1** [4,5]. Both receptors, which share 34% amino acid sequence homology, belong to the G-protein-coupled receptor superfamily and possess seven transmembrane spanning domains [6,7]. Although AT_1R stimulation mediates the major known actions of Ang II, it is increasingly recognised that stimulation of AT_2R opposes functions mediated by AT_1R [5]. In particular AT_2R stimulation results in vasodilatation, inhibits cellular growth and differentiation, promotes apoptosis [8], and contributes to sodium excretion and pressure natriuresis in the renal tubules [9]. Binding of Ang II to AT_1R leads to G protein-coupled activation of phospholipase C, A2 and D, increased inositol 1,4,5-triphosphate levels and cytoplasmic calcium, and up-regulation of a variety of other intracellular pathways; additionally stimulation of AT_1R attenuates the production of cyclic adenosine 3',5'-monophosphate (cAMP) [10]. The intracellular signal transduction pathways coupled to AT_2R depend on the cell type and are summarised in (Fig **2**). In brief, binding of Ang II to AT_2R stimulates phospholipase A2 activity, arachidonic acid formation and serine/threonine phosphatase A2 (PP2A) activation. In certain cell lines, activation of mitogen-activated protein kinase phosphatase-1 (MKP-1) occurs, which along with PP2A activation inhibits extra-cellular regulated kinase 1 and 2 via inhibitory G-proteins. This results in inhibition of cell growth, increased cell differentiation and stimulation of apoptosis. Additionally, Ang II binding leads to increased cellular cGMP levels by nitric oxide/bradykinin dependent pathways [5].

ANGIOTENSIN CONVERTING ENZYME INHIBITORS AND ANGIOTENSIN II RECEPTOR BLOCKERS

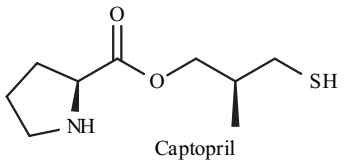
The first orally active ACE inhibitor (ACEI) captopril was developed in the late 1970's by Cushman and co-workers who realised the necessity of designing an inhibitor that would bind to the zinc moiety of ACE [11]. Whilst captopril contained a sulfhydryl group (Fig. **3**), subsequent ACEIs employed dicarboxyl groups (e.g. enalapril, Fig. **3**) and phosphinyl groups (e.g. fosinopril, Fig. **3**) that varied in terms of potency, pharmacokinetics, and whether inhibition of ACE is due to the drug itself or conversion of a prodrug to an active metabolite [12]. Captopril and lisinopril are active drugs, whereas other ACEI are lipophilic prodrugs with ester groups that increase absorption via the gastro-intestinal tract; these require de-esterification by the gut wall and liver to the active metabolite. Apart from lisinopril, which is excreted unchanged, other ACEI undergo extensive metabolism in the body. Following a distribution phase, most ACEI undergo an initial elimination phase lasting 2 to 6 hours, followed by a long terminal washout

Table 1. Function and Distribution of Angiotensin Receptors [Refs. 5, 61]. NO =Nitric Oxide; cGMP =Cyclic Guanidine Monophosphosphate

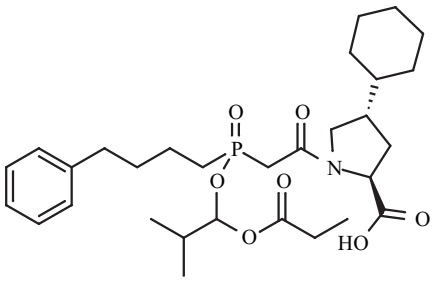
Receptor	Actions	Location
Type 1	Vasoconstriction ↑ aldosterone secretion ↓ renin secretion ↑ sodium retention (direct effects on proximal renal tubular cells, and via aldosterone and effects on intra-renal haemodynamics) ↑ vasopressin (anti-diuretic hormone) synthesis/release Stimulation of hypothalamic thirst centres Initiates sympathetic activity ↑ myocardial contractility Promotes myocyte hypertrophy Stimulates vascular & cardiac fibrosis Induces arrhythmias Stimulates superoxide formation Stimulates production of transforming growth factor-α platelet derived growth factor, endothelin, epidermal growth factor, plasminogen activator inhibitor-1 Lymphocyte activation Foetal renal tract development	Vasculature Myocardium Central & Peripheral Nervous System Adrenal gland Kidney Lymphocytes
Type 2	Kidney and urinary tract development ↓ AT_1R expression in vasculature, myocardium and kidney ↓ cell proliferation / ↑ cell differentiation during foetal development ↓ tissue remodelling after injury ↑ apoptosis Vasodilatation (mediated by ↑ NO and ↑ cGMP, possibly via bradykinin) ↓ baroreceptor sensitivity ↑ pressure-natriuresis ↑ intra-renal bradykinin, prostaglandin $F_{2\alpha}$, NO and cGMP	Vasculature Myocardium Kidney Central nervous system Adrenal Gland Myometrium Foetus Sites of tissue injury

(often >24 hours), due to the slow dissociation of the drug from ACE. The majority of ACEI are renally excreted, both by glomerular filtration and tubular secretion. Although some – ramipril and benazepril – undergo hepatic inactivation, the doses of nearly all ACEI require dose adjustment in renal failure [13].

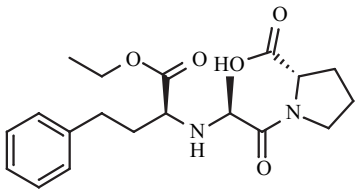
The prototypical non-peptide orally active specific AT_1R antagonist, losartan, was first approved for use in 1995 (Fig. **4**) [14]. Other antagonists have subsequently been developed, which are either biphenylmethyl or trienylmethylacrylic acid derivatives. All approved AT_1R antagonists bind competitively to AT_1R with high affinity, and are approximately 10,000-fold more selective for AT_1R than AT_2R. Although binding of angiotensin receptor blockers (ARB) to AT_1R is competitive, this binding is, in reality, insurmountable (i.e. biological responses to Ang II are in general completely blocked regardless of Ang II levels); this is probably due to slow dissociation kinetics of the drugs from AT_1R, although other factors may contribute [15]. Oral bioavailability of ARBs, with the exception of irbesartan, is generally low (<50%), and protein binding is high (>90%). ARBs are predominantly cleared from the plasma by hepatic metabolism and, with the exception of



Captopril



Fosinopril



Enalapril

Fig. (3). Structures of ACE inhibitors: captopril, enalapril, fosinopril.

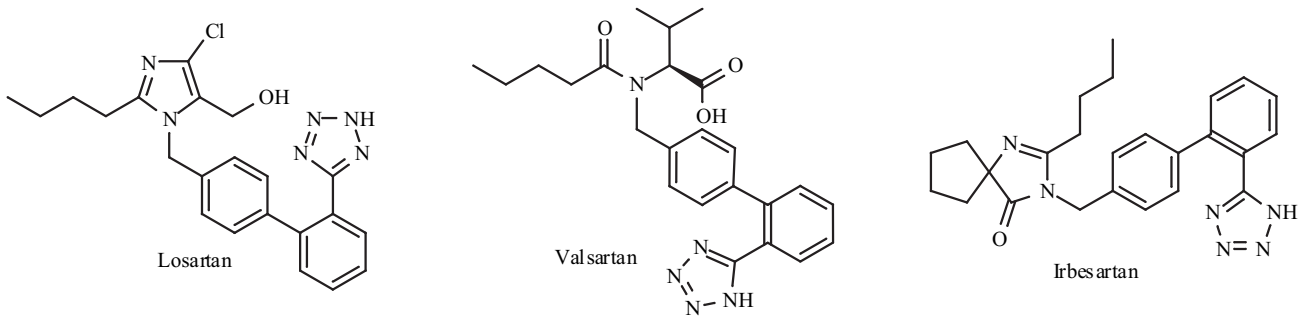


Fig. (4). Structures of angiotensin receptor (Type 1) blockers: losartan, irbesartan, valsartan.

candesartan, do not require dose adjustment in renal failure. Plasma half-life of ARBs vary from between 3 to 24 hours, with the majority being towards the upper end of this range [15].

Although the biological and physiological effects of ARBs have been largely attributed to inhibition of AT_1R (Table **1**), loss of the negative feedback of Ang II on the JGA results in increased levels of Ang I and Ang II and, thereby, increased and unopposed stimulation of AT_2R. There is experimental and clinical evidence to suggest that this increased stimulation of AT_2R during AT_1R blockade is responsible for some of the beneficial effects of ARBs, including lowering of blood pressure, via bradykinin, NO and cGMP pathways. For example, the hypotensive action of the AT_1R blockade with losartan is completely blocked by the specific AT_2R inhibitor PD123319 in rats with renovascular hypertension [16]. Also, the AT_2R mediates the hypotensive response to AT_1R blockade with Valsartan in conscious salt-restricted normal rats [17]. Furthermore, in a study of forearm vascular resistance in elderly women receiving treatment with the ARB candesartan, Ang II infused into the brachial artery produced dose-dependent reductions in forearm vascular resistance, which was partly attenuated by co-administration of PD-123319 [18]. These observations have important implications in a clinical setting, where ACEI and ARBs are increasingly being used in combination; this will be discussed in more detail below.

CLINICAL APPLICATIONS OF ACEIS AND ARBS

ACEIs and ARBs lower BP in hypertensive individuals, and are routinely employed in the treatment of raised BP [19]. ACEIs have greater anti-proteinuric and nephroprotective properties when compared to other antihypertensive drug classes such as calcium-channels blockers or beta-blockers, as measured by reductions in rate of decline in glomerular filtration rate (GFR) or reduced progression to end-stage kidney disease; such properties have been demonstrated in people with both diabetic [20] and non-diabetic nephropathies, over a range of urinary protein excretions [21, 22]. These effects are independent of reductions in BP achieved [23,24]. More recently, ARBs have been shown to prevent progression from micro- to microalbuminuria [25], and slow progression of established nephropathy [26,27] in type 2 diabetes mellitus (DM); again such benefits appear to be independent of reductions in BP. Additionally both ACEIs and ARBs have cardioprotective properties in high risk individuals [27,28].

THE CONCEPT OF "ACE ESCAPE"

ACEIs may become less effective as antihypertensive agents when used chronically due to inadequate blockade of the RAS, and in the longer-term it is likely that the BP lowering actions of ACEIs are mediated, at least in part, by inhibition of metabolism of the potent vasodilator bradykinin [29, 30]. This phenomenon, by which inadequate RAS blockade may occur with long-term ACEI administration, has been termed "ACE escape" , and probably arises by a number of mechanisms. Firstly, inadequate ACEI dosage may be responsible: high-dose ACE inhibition is more effective at suppressing Ang II generation than low dose [31]. Secondly, loss of negative feedback of Ang II on the JGA cells results in reactive hyperrenineamia, and thereby increased generation of Ang I [32]. This provides greater substrate for ACE, which will partially overcome competitive ACE inhibition. Thirdly, there is some evidence that Ang I may be converted to Ang II by non-ACE enzymatic pathways including the serine protease chymase, which is present in the myocardium, vasculature and kidney [33-35], and by cathepsin D. In consequence, whereas acute administration of ACEIs results in undetectable plasma Ang II, chronic ACEI administration results in significant but incomplete suppression of plasma Ang II at peak effect and low-normal Ang II levels at trough [36]. As well as the possibility that ACEIs may lose some antihypertensive efficacy with chronic administration, the presence of residual proteinuria in individuals with diabetic and non-diabetic nephropathies receiving an ACEI independently predicts progression of chronic kidney disease (CKD) [37]. For these reasons (i.e. partial loss of antihypertensive efficacy, and incomplete suppression of proteinuria) there is considerable interest amongst clinicians treating diabetic and non-diabetic CKD and essential hypertension, in therapeutic approaches that may circumvent the problems posed by "ACE escape". To this end, clinical studies have been conducted to determine whether adding an ARB to an ACEI, or vice versa, confers benefits over-and-above monotherapy in terms of reducing proteinuria, preventing progression of kidney disease and lowering blood pressure [38-40].

COMBINATION RAS BLOCKADE IN HYPERTENSION

Early, small studies produced conflicting results as to whether an ACEI-ARB combination lowered BP more than either agent alone. Some larger, more recent studies suggested benefit from a combination in terms of BP

lowering [38], whereas others did not [39,40]. In a recent meta-analysis (Table **2**) it was found that the combination of an ACEI and ARB lowered 24 hour ambulatory BP by 4.7/3.0 mmHg when compared with ACEI monotherapy, and by 3.8/2.9 mmHg compared to ARB alone [41]. Similarly, the combination reduced clinic BP by 3.8/2.7 mmHg and 3.7/2.3 mmHg, compared to ACEI and ARB monotherapy respectively [41]. Whilst this additive effect was statistically significant, the clinical utility of a ~4/3 mmHg reduction in BP is questionable. More importantly, the majority of the studies included in this analysis used sub-maximal dosing of ACEI, or administered shorter-acting ACEIs once-daily, and either measured the effect on BP at trough (i.e. 24 hours after the previous drug dose) or on 24 hour ambulatory monitoring [42]. It is well recognized that many ACEIs have a lower peak:trough BP ratio than ARBs, which are generally longer acting [43-45]. Therefore it is likely that the apparent additive effect of an ACEI-ARB combination on BP simply reflected a pharmacodynamic interaction of the two classes of drugs, rather than a genuine synergistic effect. Some evidence from clinical studies supports the idea that if sufficiently high dosage of monotherapy were to be given at an appropriate dose frequency, there appears to be no advantage to using the combination. For example in the COOPERATE study, in which the longest acting ACEI trandolapril (3 mg once daily) was combined with the ARB losartan (100 mg daily in divided doses), no additive effect of the combination was seen compared to monotherapy with either agent alone [39]. Similarly, Morgan *et al*. found that for BP measured in the clinic setting a combination of lisinopril 20 mg once daily and candesartan 16 mg once daily was only superior to lisinopril 20 mg once daily, but not lisinopril 40 mg once daily or candesartan 16 mg and 32 mg once daily [45]. Ambulatory BP was reduced by approximately 4/3 mmHg with the ACEI-ARB combination compared to monotherapy in this study, but the authors concluded that this was likely, at least in part, to recruitment of non-responders i.e. some individuals will have a more marked response to an ACEI than an ARB, and vice versa [45]. Finally, Forclaz and co-workers showed that a supra-maximal dose of losartan achieves equivalent RAS inhibition to a combination of losartan plus lisinopril, particularly when administered twice daily [46].

COMBINATION RAS BLOCKADE IN PROTEINURIC KIDNEY DISEASES

In contrast, the evidence that an ACEI-ARB combination may reduce proteinuria more than monotherapy is more compelling. A combination of an ACEI and ARB reduces proteinuria by 30% compared with ACEI monotherapy, and by 39% compared with ARBs alone [41]. Furthermore, as has been observed with ACEI and ARB monotherapy, these anti-proteinuric properties appear to be independent of BP reductions [24, 47]. Importantly, the COOPERATE Study investigators, who administered trandolapril and losartan to people with non-diabetic CKD, demonstrated that the combination also slowed clinical progression of nephropathy more than monotherapy, in addition to reducing proteinuria [39].

What is currently unclear is why an ACEI-ARB combination should have a more profound impact on proteinuria compared to the relatively insignificant additive effect of dual therapy on BP. There is considerable evidence that the "classical" circulating RAS is responsible for maintaining BP in both normotensive and hypertensive humans [48, 49]. On the other hand there is evidence of the presence of local RAS within some tissues, including the kidney, that may mediate other physiological or pathological functions of this system, such as urinary protein excretion, independently of changes in BP [3,50]. Within the kidney, angiotensinogen mRNA and protein is localised in the proximal tubular endothelial cell (PTEC), and is secreted into the tubular lumen. Ang I is formed by the action of renin, which is also produced by PTECs, which is then converted to Ang II by ACE located on the PTEC brush border [3,50]. AT_1R are expressed widely within the kidney, especially on vascular smooth muscle cells of the afferent and efferent arterioles, mesangial cells, apical and basolateral membranes of PTECs and elsewhere. Ang II acts in a paracrine fashion within the kidney, and is present at concentrations 1000-fold higher in the renal interstitium compared with plasma [3,50]. Given that Ang II levels within the kidney are significantly higher than in the circulation it is conceivable that, whereas ACEIs or ARBs given as monotherapy at "conventional" doses will maximally inhibit the circulating RAS and thereby maximally reduce BP, they will not fully inhibit intra-renal generation of Ang II. Since Ang II is a key promoter of renal

Table 2. Summary of Main Findings in Meta-Analysis of Combination ACEI and ARB vs. Monotherapy [Ref. 41] $^{+}$Data not provided for all studies; * "Significant hyperkalaemia" Generally defined as serum potassium >5.0 to 5.2 Mmol/L; ** CrCl =Creatinine Clearance, GFR = Glomerular Filtration Rate

Area of interest	vs. ACEI	vs. ARB
ACEI + ARB vs. monotherapy – BP	Clinic (office) BP ↓ by 3.8/2.7 mmHg Ambulatory BP ↓ by 4.7/3.0 mmHg	Clinic (office) BP ↓ by 3.7/2.3 mmHg Ambulatory BP ↓ by 3.8/2.9 mmHg
ACEI + ARB vs. monotherapy – nephropathy	Proteinuria ↓ by 30%	Proteinuria ↓ by 39%
ACEI + ARB vs. monotherapy – safety^{+}	Significant hyperkalaemia* occurred in 19/434 subjects ↑ in serum potassium concentration of 0.3 mmol/L reported in 3/12 studies ↓ in renal function in 2/12 studies (CrCl/GFR** ↓ by 4.4 mL/min and 5 mL/min in 2 studies) ↓ haemoglobin 0.4 g/dL in 2/4 studies	

damage, this would result in continuing proteinuria, inflammation, cellular proliferation, fibrosis and ultimately glomerulosclerosis. Support for this hypothesis comes from the work of Komine *et al*. who compared the effects of captopril (C), losartan (L) and their combination (C+L) on plasma and kidney Ang II generation in Wistar rats [51]. Whereas C+L did not lower plasma Ang II or BP more than C or L alone, kidney Ang II was reduced significantly more by C+L than C or L monotherapy, thereby confirming the potential for greater attenuation of intrarenal Ang II levels when an ACE-ARB combination is used [51].

Human studies of the anti-proteinuric effects of ACEIs and ARBs have tended to employ doses of these agents no higher than that maximally recommended for lowering BP, and as a consequence the optimal anti-proteinuric doses of these drugs have yet to be clearly defined. For example, in one study, proteinuria was reduced by lisinopril in a stepwise manner up to 40 mg, but whether higher doses would have reduced proteinuria further is unknown [52]. Conversely, the maximal anti-proteinuric dose of trandolapril appears to be 3 mg in a study of dose titration up to 6 mg [39]. Stepwise reductions in proteinuria have also been found with the ARB irbesartan up to a dose of 300 mg, but again whether higher doses would have additional benefits are unknown [26]. Studies using a 5/6 nephrectomy model in Munich-Wistar rats demonstrated that very high doses of ARBs (losartan 500 mg/kg) provide greater attenuation of renal Ang II content, AT_1R expression, macrophage infiltration and glomerulosclerosis compared to standard dose of ARBs (losartan 50 mg/kg) [53]. In addition the animals treated with high-dose ARB experienced a significant reduction in albuminuria compared to animals treated with hydralazine and hydrochlorothiazide, despite identical reductions in BP [53]. Early experience of adverse events – for example, membranous glomerulonephritis – in humans administered high dose captopril, and the risk of significant hyperkalaemia, is likely to discourage the use of very high dose ACEIs, whereas there are fewer concerns about higher-than-normal dosages of ARBs. Indeed a recent pilot study has demonstrated that candesartan up to 160 mg daily (i.e. 10-fold higher than the current maximal licensed dose in the U.K.) did not cause hyperkalaemia or a significant reduction in creatinine clearance [54]. Two multi-centre studies of large numbers of patients with proteinuric renal disease are currently ongoing (SMART, candesartan up to 128 mg; DROP, Valsartan up to 640 mg), and will provide valuable data on whether high dose angiotensin receptor blockade is superior to standard treatment in these patient populations [54].

SAFETY OF COMBINATION RAS BLOCKADE

Before a particular therapy can be recommended for widespread use, there should be convincing evidence for the safety of such an approach. Combination RAS blockade has significant detrimental effects in the context of salt and water depletion in experimental animals [55], and in human subjects with chronic heart failure excess morbidity and mortality was observed in those co-prescribed three RAS blocking drugs (a β-blocker, an ACEI and an ARB) compared with those just receiving a β-blocker and an ACEI [56]. Although the number of significant adverse events in clinical studies of patients receiving an ACEI-ARB combination has been very low, with significant hyperkalaemia occurring in 4.4% and acute renal failure in 0.2% of subjects, this observation is based upon small studies of less than 500 subjects in total [41].

PERSPECTIVES AND FUTURE DIRECTIONS

Although there are theoretical reasons as to why a combination of an ACEI and ARB may lower blood pressure more than either class of drug alone, there is currently a lack of good evidence to support the co-administration of these agents in uncomplicated essential hypertension. Further studies in this area are warranted, and need to determine the anti-hypertensive efficacy of the combination compared to maximal or supra-maximal dose monotherapy. Such research also needs to determine both peak and trough effects of different drugs and drug combinations.

In chronic proteinuric (>1 gram protein/24 hours) renal failure an ACEI-ARB combination may have advantages over standard dose monotherapy, both in terms of reducing proteinuria and preventing progression of nephropathy. As with ACEI and ARB monotherapy such benefits appear to be independent of falls in BP. Greater reductions in proteinuria are seen with the combination than with monotherapy in diabetic nephropathy [38], an effect that may also be independent of reductions in BP [57]. Although it seems likely, there is as yet no direct evidence that an ACEI-ARB combination prevents progression of diabetic nephropathy, and this is an area that should be subjected to further study.

Limited experimental data suggests that very high dose ARB monotherapy may confer greater nephroprotection than standard dose ARBs, and accompanying clinical evidence of improved renal outcomes in individuals given high-dose ARBs is awaited. If the latter is proven to be the case, there will be a requirement to compare combination RAS blockade with high-dose ARB monotherapy, thereby allowing determination of optimal anti-proteinuric and nephroprotective treatment regimes. This is all the more important given the knowledge that some beneficial effects of ARBs arise from unopposed stimulation of AT_2R by high levels of Ang II. In other words, it is possible that the potential effects of high-dose ARBs would be attenuated by the co-administration of an ACEI and, thereby, partial diminution of Ang II levels. Conversely, ACE inhibition increases circulating bradykinin levels which may account for some of the cardioprotective properties of this drug class, mediated via bradykinin B1 and B2 receptors [58, 59]. Such properties would argue in favour of retaining ACE inhibition. Finally, there is more limited evidence to suggest that AT_2R stimulation may in some settings be detrimental, in particular resulting in cardiac hypertrophy and suppressing angiogenesis [60]. Until further data on safety and efficacy of combination RAS blockade is available, the use of ACEI-ARB combinations should probably be restricted to those with progressive proteinuric non-diabetic CKD, and be accompanied by close monitoring of renal function and plasma electrolytes.

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