

Optimal Blockade of the Renin Angiotensin System in Cardiorenal Dysfunction

Ruud van de Wal

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Optimal Blockade of the Renin Angiotensin System in Cardiorenal Dysfunction

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CHAPTER 1

General introduction Outline of the thesis

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Urinary albumin excretion

Interest in the value of low levels of urinary albumin excretion (UAE) dates back to 1981, when it was shown that concentrations of urinary albumin that could not be detected by the standard dipsticks at that moment predict development of overt proteinuria in patients with diabetes mellitus.^{1,2} It was described that in such patients a urinary albumin excretion (UAE) between 30 and 140 mg/min had a 24 fold higher risk to develop nephropathy. A few years later, in 1984, it was also shown that such low levels of albuminuria predict mortality in patients with diabetes.³ Since, the term “microalbuminuria” has been introduced, reflecting the interest that rose in the clinical value of such slightly elevated levels of UAE.

In adults the normal mean value for UAE is 10 mg per day.⁴ However, UAE can be slightly increased under certain physiological circumstances, such as upright posture, exercise, pregnancy, and fever. Therefore, it is generally recommended in case an abnormal test result is obtained to confirm this on two separate occasions. The present definitions of microalbuminuria were described by the seventh report of the Joint National Committee (JNC 7) in 2002 and summarized in Table 1.

	Urine collection method	Normal	microalbuminuria	macroalbuminuria
Albumin	24-hour excretion	<30 mg/day	30-300 mg/day	>300 mg/day
	Spot urine albumin-Specific Dipstick	<20 mg/L	>20-200 mg/L	>200 mg/L
	Spot urine albumin-to creatinine ratio	<17 mg/g (♂) <25 mg/g (♀)	17-250 mg/g (♂) 25-355 mg/g (♀)	>250 mg/g (♂) >355 mg/g (♀)

Table 1. Definitions of albuminuria according to JNC 7 [4]

The glomerular capillary wall consists of fenestrated endothelium, the glomerular basement membrane, and the interdigitated foot processes of podocytes expressing the transmembrane protein nephrin.⁵ Under physiological conditions, the structural integrity of this filtration barrier prevents the abnormal passage of albumin (molecular mass 66 kDa) and high-molecular-weight proteins (>66 kDa), whereas low-molecular-weight proteins (<66 kDa) can pass without restriction. In addition, the transglomerular passage of macromolecules is regulated by the charge-selective properties of the

glomerular capillary membrane and the hemodynamic forces operating across the capillary wall.^{6;7}

In a healthy subject the amount of albumin excreted with urine normally represents less than 1% of the albumin filtered at the glomerular level.⁸ The remaining albumin is reabsorbed predominantly by the proximal tubuli through cellular mechanisms, by means of the synergistic receptors megalin and cubilin.⁹ This mechanism usually works quite close to saturation. As a consequence, any further increase in the amount of albumin filtered at the glomerular level will inevitably be accompanied by an increase in UAE.

In subjects with renal disease proteinuria is a good marker for renal disease progression.^{10;11} However, in renal disease urinary protein loss usually results from a specific lesion within the kidney, not per se reflecting the “health” status of the overall vasculature. Therefore, it remains unclear whether in these patients UAE can be utilized as a predictor of cardiovascular risk.

In hypertensive and diabetic patients albumin leakage is more often considered to be a reflection of generalized endothelial or vascular dysfunction,^{12;13} which is supported by the observation that elevated UAE is associated with elevated levels of high sensitivity C-reactive protein, von Willebrand factor and with impaired arterial dilatory capacity.¹⁴⁻¹⁶ It is hypothesized that damaged endothelium may lead to increased leakage of plasma albumin and lipids through the vessel walls and evoke an inflammatory response, thus linking elevated UAE to atherosclerosis.¹⁷ Since this process is not restricted to the kidney, microalbuminuria may be an indication of widespread vascular leakage of albumin. In addition to this hypothesis, increased intraglomerular pressure can also cause local renal leakage of albumin.

The evidence that albumin leakage is associated with cardiovascular risk is overwhelming. In recent studies on the potential prognostic value of microalbuminuria for cardiovascular events, the threshold value indicating increased risk has even been found to be well below the UAE values presently defining microalbuminuria regardless of the population included.¹⁸ A continuous cardiovascular risk spectrum has been observed starting from albumin excretion rates as low as 10 mg/24h.^{19;20}

Subsequent clinical evidence documented an association between UAE and other cardiovascular risk factors, target organ damage and risk of cardiovascular disease in the general population,²⁰⁻²³ and in specific clinical contexts, including essential hypertension.^{19;24;25} Accordingly, recent evidence suggests that reduction of UAE is associated with a lower cardiovascular risk.^{26;27}

Urinary albumin excretion and cardiovascular risk factors

Although UAE is a powerful predictor of cardiovascular morbidity and mortality, even today it continues to be unclear whether UAE is a risk factor per se, or merely a reflection of the effects of other risk factors (Figure 1). The fact is, that UAE is associated with biological factors like age and gender, but also often coincides with other risk factors, such as hypertension, insulin resistance, and increased levels of low-density lipoprotein.²⁸⁻³⁰

Hypertension

Over 30 years ago Parving et al. were the first to find an association between UAE and blood pressure in a hypertensive population.³¹ Since then several authors have confirmed this finding, and even in patients with high-normal blood pressure the prevalence of microalbuminuria has been shown to be increased.³² The prevalence of elevated UAE in hypertensive populations has been reported to be up to 46%, depending on the technique of measurement and the definition used.³³ Recent work suggests that hypertensive patients with increased UEA are more inclined to show early signs of other end-organ disease as well, including left ventricular hypertrophy and increased carotid artery thickness.³⁴ In addition, blood pressure lowering reduces UAE.

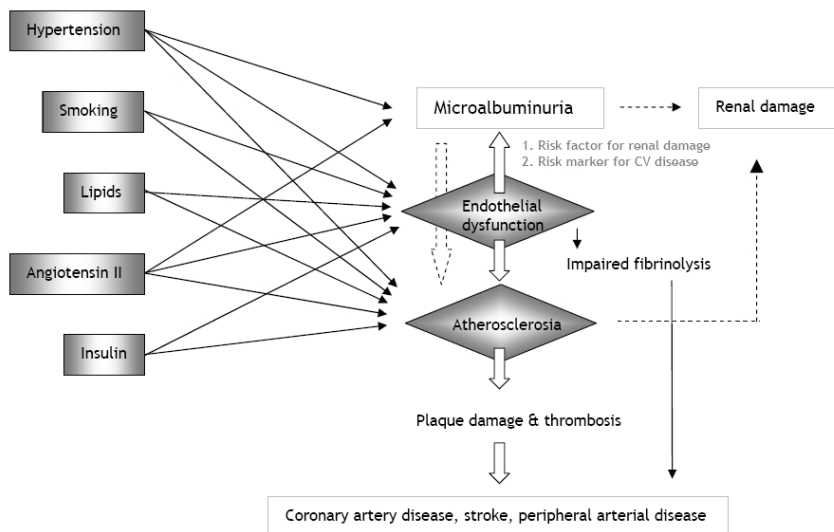


Figure 1. Possible factors involved in the pathogenesis of microalbuminuria (adapted from Verdecchia and Reboldi).³³

The relation between insulin resistance and primary hypertension was described almost two decades ago.⁴⁶ Since then, several authors have investigated and discussed the association between insulin resistance and elevated UAE, but conflicting results were obtained.^{47;48} In addition, it remains uncertain whether their presumed association is independent of other factors, such as obesity and hypertension.

Lipids

In patients with hypertension and diabetes, the combined presence of elevated UAE and hyperlipidemia is frequent. In diabetic populations several authors reported an association between UAE and atherogenic lipids.⁴⁹⁻⁵¹ In two non-diabetic hypertensive populations microalbuminuric subjects had a significantly worse lipid profile than their non-albuminuric counterparts.^{52;53} In a large general population study, plasma cholesterol was an independent correlate of microalbuminuria.⁵⁴

Several explanations for the association between hyperlipidemia and elevated UAE have been proposed.⁵³ First, the rise in serum lipids could be caused, direct or indirect, by the loss of proteins that are involved in lipid production. Second, lipid abnormalities may contribute to glomerulosclerosis by a mechanism similar to atherosclerosis.⁵⁵ In support of this theory is the finding that lipid-lowering medication also ameliorates UAE,⁵⁶ although these results are not supported by others.²⁶

Smoking

Intrarenal hemodynamics can be influenced by smoking.⁵⁷ In smokers, the response of the kidney to increased systemic blood pressure may be impaired, possibly leading to increased intraglomerular capillary pressure.⁵⁸ The fact is, that smoking type I and type II diabetics excrete more albumin than non-smoking patients.^{59;60} In non-diabetics, hypertensive or not, smoking also seems to be independently associated with elevated UAE, even in the range defined as normal.⁶¹⁻⁶³ For this reason, other non-hemodynamic mechanisms must be involved in the pathophysiology of smoking-induced albumin leakage. Importantly, damage induced by smoking is probably not limited to renal endothelium, but throughout the body the endothelial function may be affected. Although several mechanisms have been proposed (e.g. alteration of prostaglandin/thromboxane pathway, generation of reactive oxygen species, carbon monoxide-induced hypoxia, tubulotoxicity, increased clotting of platelets, increased creatinin resistance), the exact underlying mechanism remains to be determined.⁶⁴

The underlying renal mechanism of hypertensive nephropathy has been the focus of research over the last years. At present, the common view is that due to failure of autoregulatory preglomerular constrictive response, the systemic hemodynamic load is conducted to the renal glomeruli.³⁵ The sympathetic nervous system may play a crucial role in this process.³⁶ Increased intraglomerular perfusion pressure will induce hyperfiltration and subsequently lead to a strain-related elevation of UAE.³⁷ Several studies have shown that agents that lower systolic and diastolic blood pressure reduce albuminuria, which establishes a strong argument in favor of this theory.³⁸⁻⁴¹ However, the finding that elevated UAE in hypertensive patients is an independent risk factor for cardiovascular disease, also suggests a link between vascular albumin leakage in the glomeruli, and systemic vascular damage. On the other hand, the pathogenesis of hypertensive nephropathy is multimodal and intrarenal mechanisms additional to blood pressure are involved, as the correlation with blood pressure can only partly explain urinary albumin loss. Interestingly, Brantsma et al recently reported that UAE may not always be merely the consequence of high blood pressure, but may also predict the development of hypertension.⁴²

Diabetes mellitus and insulin resistance

UAE, as a component of diabetic nephropathy, is a common complication in diabetic subjects with a prevalence between 10 to 42%. In the early stages the patient will show hyperfiltration, represented by a high glomerular filtration rate, and the occasional occurrence of microalbuminuria. Later, a gradual decline can be observed, while microalbuminuria progresses to macroalbuminuria.⁴³ Accordingly, an association between elevated UAE and disease duration has been reported.

Hyperglycemia induces renal cells to produce cytokines and growth factors that are thought to be responsible for structural changes and for functional changes, such as increased permeability of the glomerular basement membrane. Several humeral factors have been proposed to contribute to these modifications, such as transforming growth factor- β_1 (TGF- β_1), platelet-derived growth factor, connective tissue growth factor, protein kinase C, and vascular permeability factor.⁴⁴ Advanced glycosylation end products, which are abundantly present in the diabetic kidney, have also been recognized to induce extracellular matrix production and hence contribute to glomerular sclerosis.⁴⁵ According to the Steno hypothesis, this detrimental process is not restricted to renal tissue, but will, to a certain degree, also affect extrarenal matrix components.¹²

The renin angiotensin system

The renin angiotensin system (RAS) is an endocrine pathway which provides a homeostatic control mechanism for sodium balance, intravascular volume, and therefore blood pressure (Figure 2).^{65;66} The potent effector peptide, angiotensin II, is an octapeptide that is generated by cleavage from angiotensinogen through an action of two different peptides, renin and angiotensin converting enzyme (ACE).⁶⁷ The latter enzyme also links the RAS and the kallikrein-kinin system (KKS), as it breaks down bradykinin.⁶⁸

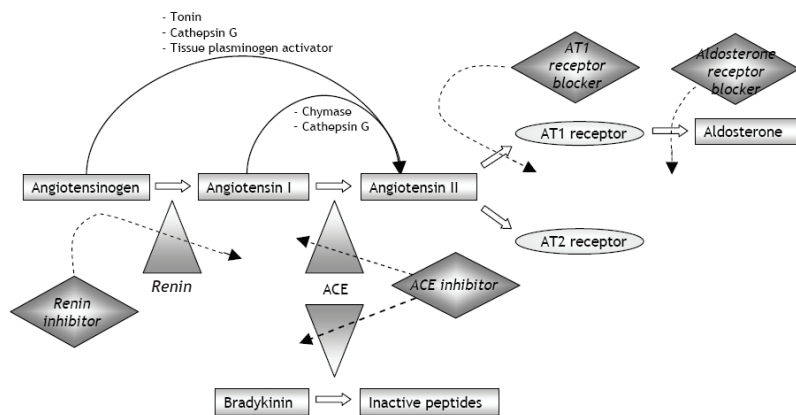


Figure 2. Simplified renin angiotensin system and targets for pharmacological intervention.

The role of angiotensin II in cardiovascular disease is well documented and nowadays this peptide is considered to be a key mediator of cardiovascular damage. The classical actions of angiotensin II include vasoconstriction, facilitation of sympathetic neurotransmission, and water and sodium retention. The latter can be accomplished directly or indirectly via aldosterone, a mineralocorticoid, which is considered to be an important mediator of angiotensin II-induced damage.⁶⁹

Angiotensin II mediates its effects through two main receptor subtypes: the angiotensin II type 1 receptor (AT1R) and the angiotensin II type 2 receptor (AT2R). These two receptors are expressed and distributed heterogeneously throughout the human body, and can be found in peripheral tissues, several organs (e.g. in the kidney) and the brain.⁷⁰ Both receptors are seven transmembrane

glycoproteins with only 32-34 % homology and they both have been cloned.^{71;72} The classical, in general detrimental, peripheral actions of angiotensin II are mediated by the AT1R (Table 2). Although knowledge is expanding rapidly, the (patho)physiological role of the AT2R is still poorly understood. Due to these effects angiotensin II contributes to the pathogenesis of vascular, cardiac, renal and cerebral pathologies, such as atherosclerosis, post-infarction remodeling, left ventricular hypertrophy, heart failure, stroke, and possibly diabetes.

AT1 receptor	AT2 receptor
Vasoconstriction	Vasodilatation (?)
Na ⁺ reabsorption/ H ₂ O retention	Apoptosis
Renin suppression	Tissue regeneration/repair
Inotropic effects	Inhibition of tissue proliferation
Vascular and cardiac hypertrophy (TGF- β_1)	Neuroprotection
Vascular injury and myocardial fibrosis	Cell differentiation
Proarrhythmic effects	Stimulation of bradykinin production
Prothrombotic effects (PAI-I \uparrow)	
Free radical formation (aging?)	
Facilitation of sympathetic transmission	
Endothelin secretion	
Proinflammatory effects	
Inhibition of Cell differentiation	
Facilitation of LDL transport	

Table 2. Angiotensin II mediated effects.
Abbreviations: TGF- β_1 , transformin growth factor β_1 ; PAI-1, plasminogen activator inhibitor 1

The traditional view of the RAS being a hormonal system, whereby angiotensin II is exclusively formed in the circulation and transferred to peripheral tissues, has long been rejected.^{73;74} A vast amount of evidence confirms that peripheral tissues are an important site of generation of this peptide, and local independent renin angiotensin systems have been found in many tissues, including brain, kidney, adrenal gland, pancreas, testis, blood vessels, and the heart.⁷⁰ Importantly, the autocrine and paracrine effects contribute to the regulation of local cellular and tissue functions, independent of the circulating endocrine system.⁷⁵ Furthermore, It is important to note that even within organs tissue RAS can be locally compartmentalized. For instance, within the kidney overall angiotensin II concentrations are higher in the medulla compared with the cortex.⁷⁶

Renal and vascular endothelial effects of the RAS

RAS and Kidney

Sodium retention and excretion, resulting from aldosterone which is released after activation of AT1R in the kidney, provokes change of blood pressure and controls volume status. Other effects mediated via AT1R include regulation of endocrine functions, and stimulation of mitogenic pathways.

Angiotensin II exerts specific actions on intrarenal hemodynamics, thus contributing to a higher perfusion pressure. Angiotensin II-induced constriction of the efferent arteriole reduces renal blood flow and raises glomerular capillary pressure, consequently augmenting glomerular filtration rate and filtration fraction.^{77;78} The changes in oncotic (\uparrow) and hydrostatic (\downarrow) pressure which are thus created in the peritubular vessels, are transduced to the interstitium, and promote a shift of sodium and water from the proximal tubule into the interstitium and systemic circulation. The reduction of medullary perfusion and diminished interstitial pressure simultaneously lower sodium and water excretion.⁷⁸ Beside these hemodynamic effects, the RAS increases water and sodium resorption by acting directly on several ion pumps (e.g. Na^+/H^+ -antiporter, Na^+/K^+ -ATPase, $\text{Na}^+/\text{HCO}_3^-$ -cotransporter) located throughout the tubular system.^{79;80} Thus, the RAS plays a pivotal role in maintaining normovolumic state and a normal ion balance. However, derangement of this hormonal cascade is thought to induce renal dysfunction (Figure 3).

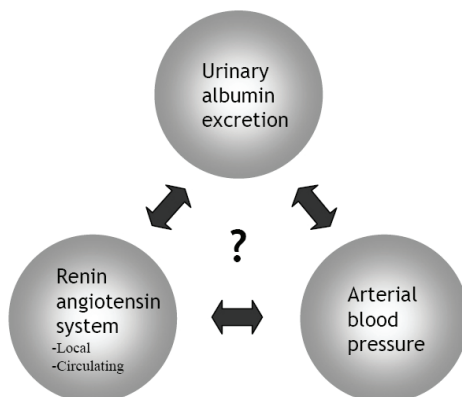


Figure 3. Our knowledge of the relation between the local and circulating renin angiotensin system, arterial blood pressure and urinary albumin excretion remains incomplete.

Furthermore, angiotensin II is responsible for the production of nephrotoxic oxygen species, profibrotic cytokins, and growth factors, consequently inducing cell proliferation and tissue remodeling. Together these effects promote the development of glomerulosclerosis and tubulointerstitial fibrosis.^{81;82} Renal remodeling is augmented by angiotensin-induced aldosterone synthesis, as this hormone, besides being an important regulator of water and salt homeostasis, shares the ability to provoke mitogenic and profibrotic changes.⁸³ TGF- β_1 , plasminogen activator inhibitor-1, reactive oxygen species, endothelial dysfunction, upregulation of the AT1R have all been proposed as possible mediators of angiotensin II- and aldosterone-mediated renal injury and scarring (Table 2).⁸³ However, only few reports have been published showing a direct relationship between angiotensin II and glomerular permselectivity.^{84;85}

The above-mentioned effects may induce and maintain glomerular leakiness, and therefore play a role in UAE. Conversely, urinary albumin may also stimulate the RAS in proximal tubular cells.⁸⁶

Hemodynamic effect	Non-hemodynamic effect
Increased glomerular capillary pressure:	Induction of renal hypertrophy and cell proliferation
1. Post glomerular vasoconstriction (direct or indirect via endothelins \uparrow or NO \downarrow)	Modulation of extracellular matrix synthesis (\uparrow) and degradation (\downarrow)
2. Systemic hypertension	Stimulation of cytokine (e.g. TGF- β_1 , VEGF, PAI-1)
Filtration surface area reduction due to mesangial cell contraction	Production of free oxygen radicals

Table 3. Summary of proposed angiotensin II-mediated mechanisms leading to increased urinary albumin excretion (adapted from Leehey et al.⁸⁷).
Abbreviations: TGF- β_1 , transforming growth factor- β_1 ; VEGF, vascular endothelial growth factor; PAI-1, plasminogen activator inhibitor-1; NO; nitric oxide

RAS and vascular endothelium

Angiotensin II exerts its proinflammatory effects through the AT1R (Table 2).⁸⁸ One of the most important, angiotensin II-modulated steps, in the initial stage of inflammation is the increase in vascular permeability.⁸⁹ Increased permeability of the vascular endothelium probably results from pressure-mediated mechanical injury, but also from angiotensin II-induced second mediators that may influence permeability (VEGF, prostaglandins).⁸⁸ This important early manifestation of inflammation and atherosclerosis will subsequently lead to cell infiltration and

exudation of protein-rich fluid. In line with this theory, an association between plasma renin activity and UAE was described in a small study performed in a population with essential hypertension.⁹⁰ In addition, a few authors found an association between the angiotensin converting enzyme DD genotype and the presence of elevated UAE.⁹¹⁻⁹³ For other genetic variants within the RAS (AGT M235T, and AT1 A1266C gene polymorphisms) this relationship remains to be determined.^{93;94}

Pharmacological inhibition of the RAS in renal dysfunction

The cumulative incidence of diabetic nephropathy after duration of either type I or type II diabetes of 25 years has been reported to be 25-40%.⁹⁵ As diabetic and hypertensive nephropathy are the leading causes of end-stage renal disease in Europe and the United States, it is important to prevent, or at least to delay, in such subjects disease progression. Moreover, in non-diabetics the degree of UAE is related to cardiovascular prognosis. Considering this and the rising incidence of type II diabetes mellitus and its complications, it is imperative to develop new and to optimize old pharmacological strategies. Subsequently, the level of UAE may be used to monitor treatment efficacy,⁹⁶ although heterogeneity of changes in UAE during treatment may complicate the interpretation of efficacy.

ACE inhibitors

The antihypertensive effects of the first compound blocking the RAS were described in the early 1970s.^{97;98} A few years later the first orally available ACE inhibitor, captopril, was marketed. Nowadays, more than 10 different ACE inhibitors are available for human use, all having specific pharmacological properties. ACE inhibitors block circulating and tissue ACE, and therefore lower systemic and local angiotensin II production and raise bradykinin levels (Figure 1). Besides lowering blood pressure, ACE inhibitors have clearly demonstrated to reduce proteinuria in both diabetic and non-diabetic nephropathy.⁹⁹ Several meta-analyses, suggest that this antiproteinuric effect is partly independent of blood pressure reduction, and greater than can be achieved with calcium blocking agents, diuretics and beta-blockers.⁹⁹ This suggests additional RAS-related effect up-and-above hemodynamic effects.

The Diabetic Nephropathy Trialist Group published a meta-analysis in which they demonstrate that ACE inhibitors significantly slow down the progression from microalbuminuria to macroalbuminuria in (type I) diabetic patients.¹⁰⁰

In patients with overt nephropathy of type 1 diabetes, ACE inhibitors not only lowered UAE, but in addition significantly delayed the decline in glomerular filtration rate that is commonly observed in diabetic patients suffering from nephropathy.^{101;102}

Data from the diabetic population (primarily type II diabetes mellitus) of the Heart Outcomes Prevention Evaluation (HOPE) trial suggested the ACE inhibitor ramipril, compared with placebo, significantly reduced the risk of developing nephropathy in these subjects.¹⁰³ Recently, the Bergamo Nephrologic Diabetes Complications Trial (BENEDICT) indicated that in hypertensive, normo-albuminuric type II diabetics the ACE inhibitor trandolapril was even more effective in preventing the development of microalbuminuria than the non-dihydropyridine calcium channel blocker verapamil.¹⁰⁴ In contrast, a small study in hypertensive type II diabetics did not reveal a significant difference between the antialbuminuric effects of ramipril and lercanidipine.¹⁰⁵

ACE inhibitors have a clear renoprotective effect in non-diabetic renal disease, when compared to compounds not interfering with the activity of the RAS.¹⁰⁶ In patients with non-diabetic renal disease use of ramipril was even associated with an improvement of glomerular filtration rate.¹⁰⁷ The renoprotective effects of ACE inhibitors are probably mediated by their specific ability to block angiotenin II-induced constriction of the post-glomerular capillaries. The resulting fall in glomerular capillary pressure consequently reduces glomerular filtration rate and filtration fraction.

Beside their specific renoprotective effect, ACE inhibitors also protect the cardiovascular system. There is much debate whether ACE inhibitors offer more cardiovascular protection than the older blood pressure lowering agents, diuretics and beta-blockers. The meta-regression analysis by Staessen et al suggests that the efficacy of blood pressure lowering agents to prevent cardiovascular accidents and myocardial infarction is predominantly dependent on the reduction in blood pressure that is achieved.¹⁰⁸ Some recent individual studies suggests however, that ACE inhibitors may confer a cardiovascular protective effect that goes beyond blood pressure reduction per se.^{103;109-112} Interestingly, a number of post-hoc analyses attributed the superior CV effect of ACE inhibition to be dependent on the level of albuminuria, for instance in the HOPE study, in which patients at high risk for cardiovascular events were randomised to either ramipril or placebo. Subgroup analysis of this study showed that in those patients with high baseline UAE, intervention with an ACE inhibitor is of particular value and improves cardiovascular prognosis.¹⁰³ The potency of

ACE inhibitors to induce cardiovascular benefits is also illustrated by the results from the Prevention of Vascular and Endstage Renal Disease Intervention Trial (PREVEND-IT). In this trial performed in non-hypertensive microalbuminuric subjects, fosinopril reduced UAE significantly and in addition, treatment was associated with a trend in reducing cardiovascular events.²⁶ Interestingly, the efficacy of fosinopril in this study to prevent cardiovascular events was shown to be dependent on baseline UAE. In subjects with UAE >50 mg/24hr the ACEi induced a 60% relative risk reduction in CV endpoints, whereas in subjects with baseline UAE 15-50 mg/24hr almost no cardioprotective effect was noted.

By blocking angiotensin II generation, ACE inhibitors induce a postglomerular vasodilation as stated above. This will reduce intraglomerular pressure and may consequently lead to a reversible reduction of GFR. In turn, this will lead to renoprotection in the long run (“short term pain, is long term gain”). Unfortunately, this may force a small selection of patients to suspend or discontinue therapy. Hypotension, cough and hyperkalemia are relatively common side effects, whereas angioneurotic edema is a rare but potentially dangerous side effect of ACE inhibitors.

In summary, ACE inhibitors have demonstrated to be valuable antiproteinuric and renoprotective agents in both diabetic and non-diabetic subjects. Over the last few years evidence for their cardiovascular protective effects is mounting as well. Some evidence even suggests that these agents exert an extra beneficial cardiovascular profile in comparison to other blood pressure lowering agents, especially in albuminuric subjects.

Angiotensin II receptor blockers

The first peptide angiotensin type I receptor blockers (ARB), saralazin, was described in 1971,¹¹³ but it took another 20 years before the first non-peptide ARB, losartan, became available for patient use. Since then, several ARBs have proven to lower blood pressure, slow down the progression of diabetic and non-diabetic renal disease, reduce proteinuria irrespective of the type of renal disease, and also reduce the risk of overt nephropathy.^{114;115}

	Treatment	Patients	Follow up	Primary endpoint	Outcome
Parving HH, 2001	Irbesartan 150 mg 300 mg Placebo	590 patients, MA Hypertension	2y	Time to onset diabetic nephropathy	I150 vs P HR=0.56 I300 vs P HR=0.32
Lewis EJ, 2001	Irbesartan 200 mg Amlodipine 10 mg Placebo	1715 patients Proteinuria Hypertension	2.6y	Doubling serum creat Development of ESRD Death from any cause	I vs A RR=0.77 I vs P RR=0.80
Brenner MB, 2001	Losartan 50- 100 mg Placebo	1513 patients Proteinuria	3.4y	Doubling serum creat Development of ESRD Death from any cause	L vs P RR=0.84
Viberti G, 2002	Valsartan 80 mg Amlodipine 5 mg	332 Patients, MA	1y	% Change UAE from baseline	V:56% UAE ↓ A: 8% UAE ↓

Table 4. Randomized controlled trials performed with angiotensin II receptor blockers in type II diabetic patients.

Abbreviations: MA; microalbuminuria, creat; creatinine, ESRD; end-stage renal disease, HR; hazard ratio, RR; relative risk

In contrast to ACE inhibitors, the evidence for a beneficial ARB-induced renoprotective effect in type 1 diabetic patients is limited.¹¹⁶ Although ACE inhibitors and ARBs seem to have comparable effects on renal hemodynamics,¹¹⁷ only small randomised placebo-controlled trials have actually studied the renoprotective properties of ARBs in type 1 diabetes.¹¹⁸

In type 2 diabetic patients, however, the evidence is compelling. Four large randomised clinical trials (IRMA-2,¹¹⁹ IDNT,¹²⁰ RENAAL,¹²¹ MARVAL¹²²) established the beneficial (dose-dependent) effects of ARBs on a variety of renal endpoints in early and more advanced diabetic nephropathy (Table 4). Data from several other studies support these results.¹¹⁵ Importantly, treatment with an ARB is not inferior to ACE inhibition, when renoprotective efficacies are compared.¹²³

The antiproteinuric effects of ARBs in early non-diabetic hypertensive nephropathy have been described in animal experiments.^{124;125} Initially, only smaller randomised clinical trials were performed in hypertensive non-diabetic subjects with¹²⁶ and without advanced renal disease.¹²⁷⁻¹³² Later, post-hoc analysis of a larger cohort of 918 patients with isolated systolic hypertension demonstrated that telmisartan reduced UAE irrespective of baseline UAE,

and to a greater extent than hydrochlorothiazide.¹³³ The most convincing evidence was provided by the Losartan Intervention For Endpoint Reduction in Hypertension (LIFE) study.¹³⁴ In 8602 hypertensive patients with ECG-ascertained left ventricular hypertrophy, treatment with losartan significantly reduced UAE. In addition, the reduction was significantly greater than the one achieved with atenolol, as was the reduction in cardiovascular endpoints. The authors concluded that part of the mechanism behind the superiority of losartan is related to its greater UAE-reducing effect.¹³⁴

Interestingly, ARBs seem to have pleiotropic effects. For example, telmisartan has the ability to interact with the nuclear peroxisome proliferator-activated receptor gamma (PPAR- γ),¹³⁵ and olmesartan has been reported to exhibit anti-inflammatory effects in hypertensive patients.¹³⁶ The clinical value of these findings needs further exploration.

In summary, both ACE inhibitors and ARBs are first choice components of an antihypertensive treatment regimen in patients with diabetic or non-diabetic renal disease with albuminuria.^{114;137} However, the predicted specific cardiovascular benefits of RAS-inhibitors in terms of survival and morbidity in subjects with renal damage need further verification.

Aldosterone receptor antagonists

The role of aldosterone in the development and progression of renal injury has been studied in variety of experimental models of progressive (hypertensive) renal disease.¹³⁸⁻¹⁴⁰ Rodent experiments suggest that local overproduction of the mineralocorticoid receptor may be a significant factor in the predisposition to hypertension and the subsequent vascular and renal injuries.¹⁴¹ In agreement with these findings, patients with an aldosterone-secreting tumour often have proteinuria.¹⁴² Furthermore, several studies provide evidence that spironolactone, a non-selective aldosterone receptor antagonist, has a renovascular protective effect.¹⁴³⁻¹⁴⁵ Spironolactone also improved left ventricular remodeling in patients experiencing a first anterior myocardial infarction,¹⁴⁶ and reduced cardiovascular mortality in severe chronic heart failure patients.¹⁴⁷ Recent data suggest that spironolactone may also improve acetylcholine induced vasodilation and thus protect endothelial function in patients with mild (NYHA class I or II) heart failure.¹⁴⁸ However, due to the lack of selectivity for the mineralocorticoid receptor and its endocrine side effects, the widespread use of spironolactone in humans is limited.

The selective aldosterone receptor antagonist eplerenone has recently demonstrated to have renoprotective properties that go beyond its antihypertensive effects as well. Compared with enalapril¹⁴⁹ and amlodipine,¹⁵⁰ the antiproteinuric properties of eplerenone were significantly better. In addition, combination of eplerenone and enalapril reduced UAE even to a greater extent than therapy with either drug alone.¹⁴⁹ However, whether this translates into long-term cardiovascular protection remains to be established. Although eplerenone has less antiandrogenic side effects than spironolactone, both drugs increase serum potassium, especially in patients using an ACE inhibitor or an ARB. Ensuing hyperkalemia, although troublesome, is reversible, and depends on dose and creatinine clearance.¹⁵¹ The fact that eplerenone is better tolerated than spironolactone offers the possibility to use eplerenone alone, or combined with an ACE inhibitor or ARB as a new renoprotective strategy. In the EPHESUS trial eplerenone demonstrated to improve post-infarction left ventricular remodeling, and subsequently reduce cardiovascular mortality.¹⁵² Initial reports, although performed in rodents, suggest that the cardioprotective properties of this agent may be mediated, in part, by stimulating endothelial NO synthase.¹⁵³ Extrapolation of these results to patients without heart failure is complex, and currently unfeasible. However, these data again indicate that agents that interfere in the renin-angiotensin-system may result in reno- and cardiovascular protection that goes beyond what might be expected from blood pressure lowering alone.

Renin inhibitors

The latest addition to the RAS-blocking armamentarium are the renin inhibitors.¹⁵⁴ After a few disappointing predecessors, Aliskiren (SPP100) seems to be the first potent orally active alkane carboxamide renin inhibitor and the first reports suggest that its blood pressure lowering effects are comparable to other RAS blocking agents.^{155;156} In addition, aliskiren is well-tolerated.¹⁵⁵ By actively antagonizing the rate-limiting step of the RAS cascade a general down-regulation of RAS activity can be observed.¹⁵⁶⁻¹⁵⁸ Interestingly, Pilz et al. demonstrated that both low and high dose aliskiren reversed albuminuria and normalized serum creatinine in transgenic rats.¹⁵⁹ New renin inhibitors may therefore become an alternative to ACE inhibitors and ARBs in the treatment of hypertension, and possibly also provide end organ protection. Other renin inhibitors, for example zalkiren,¹⁶⁰ are currently under investigation. To date, none of these agents has proven to be cardioprotective.

Conclusion

Increased UAE is a common finding in diabetic patients, as well as in hypertensive patients without renal disease, which negatively affects prognosis. Even in the general population subjects with increased UAE experience more cardiovascular events than subjects with normal UAE. Although increased UAE seems to be interrelated with several other cardiovascular risk factors and markers, evidence emerges that albuminuria is also an independent risk factor. As blocking the RAS induces a specific reduction of UAE that is greater than might be expected from blood pressure lowering alone, a causal relationship between RAS and UAE is suggested. Beside ACE inhibitors and blockers of the AT1R, (selective) aldosterone receptor antagonists and renin inhibitors may also be able to reduce UAE. Yet, whether the efficacy of these compounds to lower albuminuria is a specific renal mechanism, or a reflection of a superior efficacy of these drugs to ameliorate generalized endothelial dysfunction is yet unknown. Recent evidence suggests however, that these compounds may have a specific cardioprotective effect in subjects with higher levels of albuminuria, besides their proven renoprotective value.

Outline of this thesis

This present thesis concerns dysfunction of the cardiorenal axis, with a special focus on elevated urinary albumin excretion and the renin angiotensin system. The effect of pharmacological blockade of the renin angiotensin system on the activity of the renin angiotensin system and on urinary albumin excretion will also be discussed.

The value of pre-operative renal function as a predictor of long-term outcome in patients undergoing coronary artery bypass graft surgery will be described in **Chapter 2**. In **Chapter 3** we describe the prevalence of microalbuminuria in a cohort of severe chronic heart failure patients. We also present the neurohormonal variation that coincides with elevated urinary albumin excretion in these patients. The renin angiotensin system can be blocked by ACE inhibitors and angiotensin II type 1 receptor blockers. **Chapter 4** elaborates on the renin angiotensin system in chronic heart failure patients and the effect of ACE inhibition on the activity of this cascade. One of the benefits attributed to angiotensin II type 1 receptor blockers is parallel stimulation of the angiotensin II type 2 receptor. For this reason, we analyse the vasoactive effects of the type 2 receptor in human arteries in **Chapter 5**. Several studies suggest that combination of angiotensin II type 1 receptor blockers and ACE inhibitors can provide a more complete protection against the detrimental effects of the renin angiotensin system. In **Chapter 6** we discuss the pros and cons of this strategy. Finally, in **Chapter 7** we present data from the PREVEND intervention trial. In otherwise healthy, microalbuminuric subjects we determine which parameters affect baseline urinary albumin excretion. In an additional analysis we verify the antialbuminuric effect of ACE inhibition, and we describe which parameters determine the efficacy of treatment.

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CHAPTER 2

Mild preoperative renal dysfunction as a predictor of long-term clinical outcome after coronary bypass surgery

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Abstract

Background Renal dysfunction is a prognostic marker in patients with cardiovascular disease. However, no long-term follow-up studies on the influence of mild renal dysfunction on mortality in patients undergoing coronary bypass surgery have been reported. Therefore, we aimed to identify the significance of preoperative (mild) renal dysfunction as a long-term predictor of clinical outcome after coronary bypass surgery.

Methods In 358 patients who underwent isolated saphenous vein aorta-coronary artery bypass surgery, estimated glomerular filtration rates were calculated with the Cockcroft-Gault equation (GFRc). Patients were categorized into two groups (group 1, GFRc > 71.1 ml/min; group 2, GFRc < 71.1 ml/min). Multivariate Cox proportional hazard analyses were performed to determine the independent prognostic value of GFRc.

Results During a median follow-up of 18.2 years 233 patients (65.1%) died. Patients who died had lower GFRc and were older. Multivariate analysis demonstrated that total mortality in patients with lower GFRc was significantly increased (lower GFRc-group vs. normal GFRc-group; hazard ratio 1.44; $p=0.019$). Lower GFRc was also an independent predictor of cardiac mortality (hazard ratio 1.51, $p=0.032$). No significant differences between groups were observed in the occurrence of myocardial infarction and need for reintervention.

Conclusion Our study demonstrates that after long-term follow-up, preoperative mild renal dysfunction is an independent predictor of long-term (cardiac) mortality in patients who undergo coronary artery bypass grafting.

Introduction

Several prognostic risk markers for mortality, re-intervention and the occurrence of cardiac events after coronary bypass surgery have been identified over the last two decades. We have already demonstrated that age and left ventricular function are continuous incremental risk factors for mortality. Left ventricular function and completeness of revascularisation are independent predictors of cardiac death, and age and vessel disease are independent predictors of re-intervention. Preoperative risk markers for cardiac events are hypertension, diabetes mellitus, hypertriglyceridemia, obesity and smoking.¹

Patients with chronic renal failure are known to have an increased risk of cardiovascular disease.^{2,3} In addition, outcome after coronary bypass surgery of patients with end-stage renal disease is poor, and in this group a 5-year survival of less than 50 % has been observed.⁴ Anderson et al. demonstrated that patients with a mild renal dysfunction are predisposed to adverse 30-day outcomes after coronary artery bypass surgery.⁵ However, no studies assessed the influence of mild preoperative kidney dysfunction on long-term outcome after coronary bypass surgery. Therefore, we aimed to identify the significance of renal function as a predictor of long-term clinical outcome after coronary bypass surgery.

Methods

Patients

The clinical and angiographic definitions as well as the surgical technique and patient characteristics have been previously described in detail.⁶ Between April 1, 1976, and April 1, 1977, a series of 446 consecutive patients underwent isolated saphenous vein aorta-coronary bypass surgery in our hospital. Thirteen patients died within 30 days after surgery and they were excluded from the present analysis. Another 18 patients in whom the revascularization was combined with valve replacement surgery were also excluded. Of the remaining 415 patients in 57 (13.7%), the estimated glomerular filtration rates according to Cockcroft-Gault (GFRc) could not be calculated. This group was excluded as well. Therefore, this study population consisted of 358 patients. Several follow-up methods were used simultaneously to provide the most complete information possible. All patients were followed by using the anniversary method at our outpatient clinic or the outpatient clinic of referring cardiologists.

Measurement of serum creatinine

Serum creatinine was measured using standard techniques in the clinical chemistry laboratory of our institution from blood samples drawn in the week before surgery. Serum creatinine then was used to calculate GFRc by using the Cockcroft-Gault equation ($[(140 - \text{age in years}) \times (\text{weight in Kg})] / (72 \times \text{serum creatinine in mg/dL})$, multiplied by 0,85 in women). This equation is closely correlated with measured creatinine clearance (correlation coefficient 0.83) and gives a more accurate assessment of renal function than serum creatinine alone.⁷

Statistical analysis

In our analysis we categorized GFRc into quintiles and the hazard ratios were calculated for the lower GFRc quintiles compared with the (reference) highest GFRc group. Univariate Cox proportional hazard analysis demonstrated that the lowest 2 quintiles and the highest 3 quintiles showed similar hazard ratios. Subsequently, the patients were categorized into 2 groups (group 1, GFRc > 71.1 ml/min, quintiles 3, 4 and 5; group 2, GFRc < 71.1 ml/min, quintiles 1 and 2). Age was dichotomized in a similar manner; this resulted in 2 groups with a cut-off point of 54.7 years.

To identify prognostic covariates that might have been responsible for a difference in survival time between groups, survival curves were estimated by the method described by Kaplan and Meier from the following variables: presence of left main vessel disease, left ventricular function, number of vessels involved, completeness of revascularization, preoperative diabetes mellitus, preoperative cholesterol, number of distal anastomosis, preoperative smoking behaviour, and hypertension. The log-rank test was used to calculate the statistical significance of differences in survival curves between groups. All variables with a significance level of $p < 0.10$ in this univariate test were included into a multivariate model proposed by Cox. The clinical events studied were overall mortality, cardiac mortality, acute myocardial infarction and re-intervention. Cardiac death was defined as death from a documented cardiac cause or death from an unknown cause. End points were scored in an hierarchical matter.⁸ All statistical analyses were performed with SPSS software (version 11.0), and all reported p-values are 2-sided.

	Group 1 (n=215, GFR>70.1 mL/min)	Group 2 (n=143 GFR<70.1 mL/min)	Significance (2-tailed)
Mean age (years)	49.7 ± 6.5	57.2 ± 6.0	< 0.001 ^b
Sex			
Male	96.3 %	78.3 %	< 0.001 ^a
Female	3.7 %	21.7 %	
BMI (kg/m ²)	24.1 ± 1.5	23.1 ± 1.7	< 0.001 ^b
Serum creatinine (mmol/mL)	97.2 ± 11.1	109.0 ± 18.1	< 0.001 ^b
GFR _c (mL/min)	85.1 ± 11.0	60.8 ± 7.9	< 0.001 ^b
Diabetes mellitus			0.274 ^a
Yes	98.6 %	96.5 %	
No	1.4 %	3.5 %	
Smoking			0.186 ^a
Yes	41.9 %	35.0%	
No	57.7 %	65.0 %	
Unknown	0.4 %	0.0 %	
Cholesterol (mmol/L)	7.61 ± 1.42	7.60 ± 1.37	0.939 ^b
Triglyceride (mmol/L)	1.70 ± 1.01	1.70 ± 1.37	0.968 ^b
BP syst (mm Hg)	129 ± 12	132 ± 12	< 0.007 ^b
BP dias (mm Hg)	85 ± 11	84 ± 8	0.602 ^b
Angina pectoris			< 0.001 ^a
CCS class I	3.3 %	0.7 %	
II	45.6 %	30.8 %	
III	37.7 %	44.1 %	
IV	13.5 %	24.5 %	
Family History			0.561 ^a
Positive	9.3 %	7.0 %	
Negative	79.1 %	76.9%	
Unknown	11.6 %	16.1 %	
LV function (CASS-WMS score)			0.904 ^a
5-7	64.2 %	62.9 %	
8-10	19.1 %	21.0 %	
> 10	16.7 %	16.1 %	
1-vessel disease	16.3 %	9.1 %	0.034 ^a
2-vessel disease	38.1 %	36.4 %	
3-vessel disease (>50% stenosis)	45.6 %	54.5 %	
Extra corporal circulation time (minutes)	111 ± 49	115 ± 49	0.695 ^a

Table 1. Baseline characteristics of both groups at time of surgery.
^a Chi-square test; ^b t-test; ^c Mann-Whitney
Abbreviations: BP, blood pressure; CASS, Coronary artery surgery study; CI, confidence intervals;
CCS, Canadian Cardiovascular Society; HR, hazard ratio; LV, left ventricular; WMS, wall motion
score

Results

Baseline characteristics

The mean age of the total study population was 52.6 years (range, 20-74 years, SD 7.35), and 10.9% were women. The mean serum creatinine concentration (SD) in the 2 GFRc groups was 97.2 (11.1) and 109.0 (18.1) $\mu\text{mol/L}$, respectively. Only 3 patients had a GFRc < 40 ml/min (minimum 32.1 ml/min). Mean GFRc of both groups was within two standard deviations of normal GFR values in the general population.⁹ Other baseline clinical characteristics are shown in Table 1. Thirty-day operative mortality for the study population was 2.9%. Only 1 patient was lost to follow-up. The median duration of follow-up was 18.2 years.

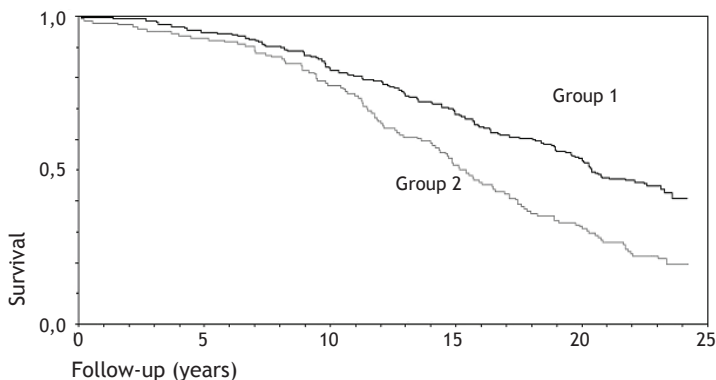


Figure 1. All cause mortality after coronary artery bypass grafting in patients, based on their calculated glomerular filtration rate (Cockcroft-Gault): Group 1, cGFR greater than 70.1 ml/min; group 2, cGFR less than 70.1 ml/min.

All cause mortality

Cumulative survival of the 2 groups is shown in Figure 1. In the high GFRc group, 120 patients (55.8%) died, whereas in the low GFRc group 113 patients (79.0%) died. The variables with a significance level of $p < 0.10$ in the univariate tests were age, left ventricular function, preoperative diabetes mellitus, number of anastomoses, number of vessels involved, left main coronary artery disease, completeness of revascularization and categorized preoperative GFRc (Table 2). These values were included into a multivariate analysis model. Multivariate analysis identified three preoperative risk factors that were related to long-term mortality: age, left ventricular function, and preoperative renal function (Table 3). Patients in group 2 had a significantly increased risk of mortality

during follow-up (group 2 vs. group 1, hazard ratio 1.44, 95% CI 1.06-1.96, $p=0.019$).

	From 30 days after surgery to 18.2 years after surgery			
	Total mortality		Cardiac mortality	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Age (>54.7) years	2.08 (1.60-2.69)	<0.001	1.73 (1.26-2.38)	0.001
Completeness of revascularization	1.35 (1.04-1.75)	0.026	1.73 (1.26-2.37)	0.001
Diabetes mellitus	2.29 (1.08-4.88)	0.031	2.00 (0.74-5.54)	0.169
Body mass index	1.00 (0.92-1.08)	0.928	0.99 (0.90-1.10)	0.895
Number of diseased vessels				
2-vessel disease	1.23 (0.79-1.91)	0.366	2.04 (1.07-3.89)	0.031
3-vessel disease	1.78 (1.16-2.71)	0.008	3.03 (1.62-5.68)	0.001
LMCA involved	1.63 (1.13-2.35)	0.009	1.66 (1.07-2.59)	0.024
LV function (Cass-WMS)				
<8 vs. 8-10	2.02 (1.37-3.00)	<0.001	1.78 (1.29-2.45)	<0.001
<8 vs. >10	3.59 (2.45-5.25)	<0.001	2.63 (1.90-3.65)	<0.001
Sex (m/f)	1.23 (0.83-1.82)	0.311	1.25 (0.77-2.02)	0.365
Smoking	1.17 (0.90-1.52)	0.235	1.10 (0.79-1.51)	0.581
Cholesterol	0.97 (0.88-1.08)	0.581	1.00 (0.88-1.13)	0.978
Triglycerides	1.05 (0.93-1.18)	0.422	1.09 (0.95-1.24)	0.245
Number of anastomoses	1.13 (1.03-1.24)	0.013	1.20 (1.07-1.34)	0.001
Glomerular Filtration Rate (≤ 70.1 mL/min)	1.82 (1.41-2.36)	<0.001	1.74 (1.27-2.39)	0.001

Table 2. Univariate Hazard Ratios (95% confidence limits) for total mortality and cardiac mortality of statistically significant and other predictors for either total mortality or cardiac mortality
Abbreviations: CASS, Coronary artery surgery study; CI, confidence intervals; HR, hazard ratio; LMCA, left main coronary artery; LV, left ventricular; WMS, wall motion score

Cardiac mortality

Cumulative survival of the 2 groups is shown in Figure 2. In the high GFRc group, 83 patients (38.6%) died of cardiac cause, whereas in the other group, 74 patients (51.7%) died of cardiac causes. The variables with a significance level of $p < 0.10$ in the univariate tests were age, left ventricular function, number of anastomoses, number of diseased vessels, left main coronary artery disease, completeness of revascularization, and preoperative GFRc (Table 2). If only cardiac death was taken into account categorized preoperative kidney function was still a significant risk factor (group 2 vs. group 1, hazard ratio 1.51, 95% CI 1.04-2.19, $p=0.032$). The 2 most powerful predictors of cardiac mortality after multivariate analysis were left ventricular function and preoperative kidney function (Table 3).

	Total mortality		Cardiac mortality	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Age (>54.7) years	1.72 (1.27-2.33)	<0.001	1.43 (0.99-2.08)	0.058
Completeness of revascularization	1.16 (0.88-1.53)	0.298	1.40 (1.00-1.95)	0.050
Diabetes mellitus	2.02 (0.93-4.41)	0.077	-	
Number of diseased vessels				
2-vessel disease	0.97 (0.59-1.60)	0.895	1.43 (0.71-2.90)	0.316
3-vessel disease	1.03 (0.58-1.84)	0.914	1.50 (0.69-3.28)	0.308
LMCA involved	1.57 (1.08-2.29)	0.020	1.53(0.97-2.42)	0.070
LV function (Cass-WMS)				
<8 vs. 8-10	1.68 (1.22-2.33)	0.002	1.43 (0.71-2.90)	0.001
<8 vs. >10	2.52 (1.80-3.53)	<0.001	3.10 (2.10-4.57)	<0.001
Number of anastomoses	1.06 (0.93-1.21)	0.366	1.09 (0.94-1.27)	0.275
Glomerular Filtration Rate (≤ 70.1 mL/min)	1.44 (1.06-1.96)	0.019	1.51 (1.04-2.19)	0.032

Table 3. Multivariate Hazard Ratios (95% confidence limits) for total mortality and cardiac mortality of statistically significant and other predictors for either total mortality or cardiac mortality
Abbreviations: CASS, Coronary artery surgery study; CI, confidence intervals; HR, hazard ratio; LV, left ventricular; LMCA, left main coronary artery; WMS, wall motion score

Myocardial infarction and reintervention.

No significant differences were found in the occurrence of (re)myocardial infarction (group 2 vs. group 1, hazard ratio 1.29, $p=0.219$), and reintervention rate (group 2 vs. group 1, hazard ratio 1.38, $p=0.198$) between groups. These data are not shown.

Discussion

In this long-term follow-up study, we demonstrated that mild preoperative renal dysfunction is an independent predictor of long-term mortality. Ischemic heart disease is the most common cause of death in patients with chronic kidney disease,^{10;11} and renal dysfunction has now been generally recognized as a risk marker in a wide variety of patients with cardiovascular disease.¹² It has been well established that patients with severe chronic renal failure have a high risk for adverse outcome after coronary bypass surgery.^{5;13;14} Several studies showed that patients with mild renal dysfunction have an increased risk of dying within 30 days after coronary surgery.^{5;13;15} Patients with decreased renal function (serum creatinine ≥ 2.0 mg/dL) carry significant operative risks, require

prolonged hospital stay, and have a higher risk of dying within 3 years after coronary surgery.¹⁵ Nakayama et al. demonstrated that in a group of patients with a preoperative serum creatinine level ≥ 1.5 mg/dL (≥ 133 $\mu\text{mol/L}$) the 10-year actuarial survival was significantly lower than in a group of patients with normal serum creatinine levels (< 1.0 mg/dL, < 88 $\mu\text{mol/L}$). Most patients in our population had a milder preoperative renal dysfunction than described in these studies, yet this parameter was still an independent predictor for mortality. It is interesting to note that Kaplan-Meier analysis clearly demonstrated that differences between GFR groups only occurred after approximately 10 years of follow-up. Possibly, this mirrors an accelerated rate of atherosclerosis, although in our population no difference in myocardial infarction was observed between groups.

Several explanations have been proposed for the association between renal dysfunction, cardiovascular disease and mortality.^{3;16;17} First, renal dysfunction is often associated with the presence of other cardiovascular risk factors, such as hypertension and diabetes. In these cases, renal dysfunction and cardiovascular morbidity would both be the results of end-organ damage. However, the effect of renal function on mortality in this study was independent of other known cardiovascular risk factors. Second, renal dysfunction might be the direct effect of cardiac dysfunction. In patients with a reduction of cardiac output there is a decline in renal perfusion and an activation of compensatory mechanisms, which leads to renal function impairment.^{18;19} In these cases renal dysfunction is a reflection of the cardiac function and may therefore be used as a risk marker for cardiovascular morbidity and mortality.²⁰

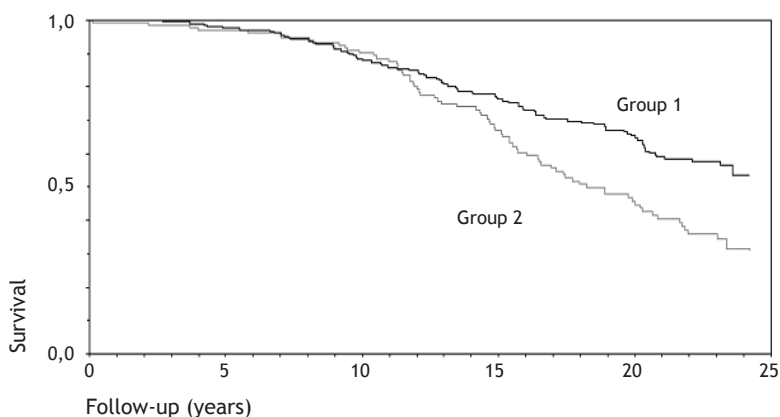


Figure 2 Cardiac mortality after coronary artery bypass grafting in patients, based on their calculated glomerular filtration rate (Cockcroft-Gault): Group 1, cGFR greater than 70.1 ml/min; group 2, cGFR less than 70.1 ml/min.

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CHAPTER 3

High prevalence of microalbuminuria in chronic heart failure patients

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Abstract

Background Microalbuminuria is associated with increased risk for cardiovascular morbidity and mortality. However, the relation between microalbuminuria and chronic heart failure has not been well described yet. In this cross-sectional study, we aimed to evaluate the prevalence of microalbuminuria and the association with neurohormonal parameters in severe chronic heart failure patients.

Methods We studied 94 stable chronic heart failure patients (New York Heart Association class III/IV) receiving therapy with angiotensin-converting enzyme inhibitors for over three months. In all patients, renal function and neurohormonal status were evaluated and correlated with urinary albumin/creatinine ratio.

Results The studied population consisted of 70 men and 21 women (mean age 69 ± 12 years). Ischemia was the underlying cause of heart failure in 61 patients. Overall, 100% of the patients were treated with an ACE inhibitor, 72% with a β -blocker, and 47% with spironolactone. In 32% (95% confidence interval: 22-42) of the patients, microalbuminuria was present, which is significantly higher than in the general population. However, we found no significant association between the presence of microalbuminuria and renal function. Plasma NT-proBNP, active renin protein, angiotensin I, angiotensin II and aldosterone did not differ significantly between groups with and without microalbuminuria.

Conclusion In 32% of the patients microalbuminuria was present. No association was found with either renal, or neurohormonal parameters.

Introduction

Urinary albumin excretion (UAE) is a predictor of cardiovascular mortality in patients with diabetes and hypertension, but also in the general population.^{1;2} The prevalence of microalbuminuria in the general population is 6-8%, whereas in patients with hypertension and diabetes this percentage increases to 10-15% and 15-20%, respectively.^{3;4} However, literature data on the prevalence of microalbuminuria in advanced chronic heart failure (CHF) patients are scarce.⁵ The etiology of microalbuminuria remains uncertain, but a large body of evidence proves that treatment with an angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker reduces microalbuminuria.⁶ In the present cross-sectional study, we therefore evaluate the prevalence of microalbuminuria in advanced chronic heart failure patients and its association with neurohormones and renal function.

Methods

We cross-sectionally evaluated 96 patients CHF New York Heart Association (NYHA) functional class III or IV, who visited the Heart Failure Clinic of the St. Antonius Hospital (Nieuwegein), the University Medical Center Groningen (Groningen), and the Deventer Hospital (Deventer), the Netherlands. All subjects were maintained on a stable dose of ACE inhibitor. Diagnosis had been made on the basis of medical history, ongoing symptoms, and physical examination. In all patients, left ventricular ejection fraction (LVEF) was assessed by echocardiography or radio nucleotide measurement. Patient had been clinically stable for at least three months before the study. Patients who used an angiotensin II receptor blocker were excluded from this study. The study was approved by each hospital's ethics committee and written informed consent was obtained from all patients.

Venous blood samples and random spot urine samples were taken at the outpatient clinic while the patient was in an upright position. The blood and urine samples were transported to the local laboratory immediately and each aliquot was processed and stored according to protocol for later batched analysis. The concentration of aldosterone was measured by a sandwich radio immunoassay (Diagnostic Products Corporation, Breda, the Netherlands). Active renin protein was measured by an immunoradiometric assay (Nichols Institute Diagnostics, Middlesex, United Kingdom) and serum ACE activity was measured by an enzymatic assay (Bühlmann Laboratory AG, Schönenbuch, Switzerland).

Analyses were performed in a routine setting according to the guidelines of the manufacturer. Angiotensin I and II were measured by specific radioimmunoassays after SepPak extraction of plasma. NT-probrain natriuretic peptide (NTproBNP) was measured by an Elecsys NT-proBNP immunoassay (Roche Diagnostics, Mannheim, Germany). Urinary creatinine and albumin were measured using a turbidimetric assay (Cobas Integra, Roche Diagnostics, Mannheim, Germany). Microalbuminuria was defined as a urinary albumin/creatinine ratio of 3.5 - 25 mg/mmol in male patients and 2.5 -25 mg/mmol in female patients. Serum creatinine was measured using standard techniques. Serum creatinine, age, weight and gender were used to calculate glomerular filtration rate (GFRc) using the Cockcroft-Gault equation.⁷ The prevalence of microalbuminuria was compared to the prevalence of microalbuminuria in patients between 60 and 74 years old in the PREVEND study.⁸ The PREVEND (Prevention of Renal and Vascular ENDstage Disease) study was designed to investigate the natural course of microalbuminuria and its relation with renal and cardiovascular disease in the general population as described previously.⁴

Values are expressed as mean values \pm SD. Neurohormonal levels and urinary albumin/creatinine ratios were log-transformed before statistical comparison. Differences between groups were investigated by using the unpaired t-test for independent samples and the chi-square test, when appropriate.

Results

Average age in our population was 69 years (\pm 12), and 22% were female. Ninety-two patients were classified as CHF NYHA class III, whereas the remaining 4 patients were class IV. All patients used an ACE inhibitor, 72% used a beta-blocking agent, and 47% used spironolactone. Median albumin/creatinine ratio was 1.89 mg/mmol (interquartile range 1.29-3.72) and 5 patients (5.2%) were macroalbuminuric (all male, 2 diabetics). Because macroalbuminuria is often the result of intrinsic renal disease, we excluded these patients from further analysis. Microalbuminuria was present in 29 patients (31.9%; 95% confidence interval: 22.3- 41.5). Patient characteristics are presented in Table 1. In 10 622 patients between 60 and 74 years old in the PREVEND study the prevalence of microalbuminuria was 10.4% (95% confidence interval: 9.8-11.0), which is significantly lower ($p < 0.001$) than the prevalence we found in advanced chronic heart failure patients (Figure 1). GFRc was generally impaired, and tended to be slightly higher in patients without microalbuminuria (Table 1) However, this difference was small and not statistically significant. Other patient characteristics were not different between groups. In addition, no statistically

significant differences in active renin protein, angiotensin II, and aldosterone plasma levels were found in normoalbuminuric and microalbuminuric patients (Figure 2). Plasma NT-proBNP levels were slightly higher in the microalbuminuric group, but this difference did not reach statistical significance.

	No microalbuminuria (n = 62)	Microalbuminuria (n = 29)	Significance (two-tailed) p value
Sex male (%)	74.2	82.8	0.366*
Age (years)	68.2 ± 12.9	70.8 ± 11.2	0.325 [#]
Body mass index (kg/m ²)	27.4 ± 4.1	28.8 ± 5.0	0.179 [#]
ACE inhibitor (%)	100.0	100.0	
Beta Blocker	74.2	69.0	0.603*
Diuretic	91.9	96.6	0.408*
Spirolactone	41.9	55.2	0.238*
Anti arrhythmic	12.9	6.9	0.393*
Calcium Blocker	11.3	13.8	0.733*
Ischemic cause (%)	66.1	62.1	0.705*
Diabetes (%)	20.9	37.9	0.204*
Smoking	16.1	13.8	0.774*
Blood pressure (mm Hg)			
Diastolic	74 ± 11	75 ± 8	0.676 [#]
Systolic	121 ± 21	122 ± 22	0.816 [#]
LVEF (%)	28 ± 11	30 ± 11	0.409 [#]
Creatinine (µmol/L)	117 ± 29	125 ± 27	0.217 [#]
GFRc (ml/min/1.73m ²) ^a	64.6 ± 30.5	59.6 ± 25.2	0.444 [#]
Diet			
Fluid restriction	29.0	27.6	0.887*
Low sodium	56.5	69.0	0.255*

Table 1. Patient characteristics at baseline

^aGlomerular filtration rate calculated using the Cockcroft-Gault equation

[#]independent samples t-test *chi-square test

Abbreviation: LVEF, left ventricular ejection fraction

Discussion

In the present study we demonstrate that almost one third of a group of advanced CHF patients had microalbuminuria despite ACE inhibition. This finding demonstrates that the prevalence of microalbuminuria is much higher in CHF patients than in the comparable cohort of the PREVENT population, which was selected from the same geographical area as our CHF population.

The prevalence we found was also higher than in hypertensive patients and diabetics.^{1,2} It is well described that microalbuminuria is a risk factor for developing CHF and for cardiovascular mortality.^{1,9;10} However, published data on the prevalence of microalbuminuria in advanced CHF patients are scarce. Almost 25 years ago Carrie et al. suggested that either the fractional clearance for anionic albumin was disproportionately enhanced or the glomerular electrostatic barrier function was impaired in CHF patients.¹¹ Since then, several pathophysiological mechanisms for the development of microalbuminuria have been proposed. First, microalbuminuria might be an early sign of renal damage. While the kidney function remains clinically stable, a reduced number of nephrons are trying to maintain normal homeostasis. The resulting augmented glomerular blood flow and hydraulic pressure lead to hyperfiltration and excretion of protein.¹² Yet, the effects of an impaired left ventricular function on this compensation mechanism remain unclear. As most of the patients already had a mild to moderate renal dysfunction, we can merely speculate on the contribution of this mechanism to the occurrence of microalbuminuria in the present study.

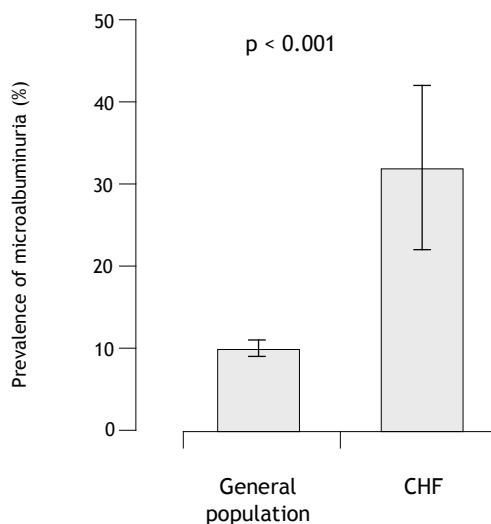


Figure 1. The prevalence of microalbuminuria in 96 severe chronic heart failure patients is significantly higher than the prevalence in patients between 60 and 74 years old from the general population in the PREVEND study.

Abbreviations: CHF, chronic heart failure.

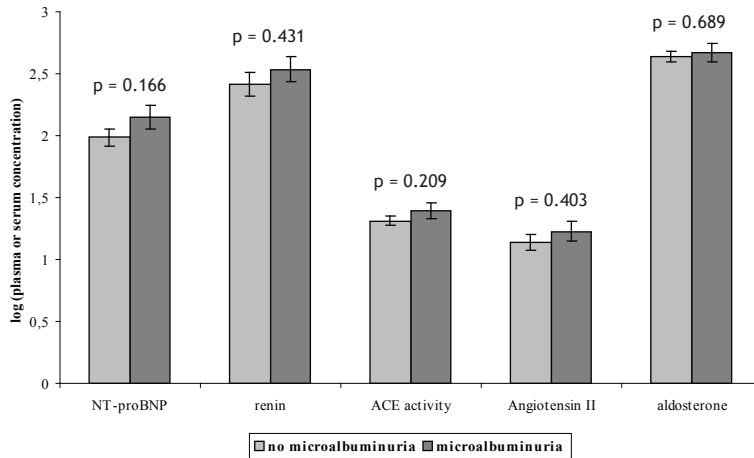


Figure 2. Neurohormonal concentrations and activities in patients without and with microalbuminuria.

Second, microalbuminuria is thought to be a reflection of generalized endothelial dysfunction, which results in leakage of albumin through the endothelium and glomerular basement membrane. Endothelial function is abnormal in CHF, and this hypothesis therefore provides a plausible explanation for the high prevalence of microalbuminuria in CHF patients.^{13;14}

The Strong Heart study demonstrated that increased UAE is associated with systolic dysfunction in diabetic patients.^{15;16} The authors hypothesize that the urinary loss of albumin reflects cardiac systolic dysfunction and that this is mediated by extensive endothelial and vascular changes. However, our results do not confirm this, as left ventricular ejection fractions are comparable in both groups. Furthermore, another marker of systolic function, NT-proBNP, was not significantly increased in patients with microalbuminuria.

Because beneficial effects of both ACE inhibitors and angiotensin II receptor blockers on UAE have been described, activation of the renin angiotensin system seems to play an important role in the process.^{17;18} In CHF patients the renin angiotensin system is activated.¹⁹ Nevertheless, despite the use of ACE inhibitors a large number of our patients had microalbuminuria. Furthermore, the renin angiotensin system tended to be slightly, yet non-significantly, more activated in patients with microalbuminuria. However, the absence of statistical significance may have been caused by the wide range of medication used and by the relatively small number of patients. Theoretically, microalbuminuria could arise from the supplementary effect of various mildly activated neurohormonal

systems. In this theory the renin angiotensin system is not the sole contributor to the development of microalbuminuria, which would explain the small differences we found.

Limitations

There are several limitations to the study. First, this was an observational, hypothesis-generating study in an out-patient clinical setting. Consequently, analysis revealed a wide variety of neurohormonal concentrations. This might be due to different levels of activation of the RAS under the circumstances we used. However, we believe that for revealing mechanisms within the RAS in a population-based study, it is a valuable tool. Secondly, we analyzed random spot urine samples. Previous reports indicate that the albumin/creatinine ratio in spot urine sample is a good screening test for microalbuminuria, but a poorer predictor of quantitative UAE than 24-hour UAE.²⁰ Yet, this method can be easily used in daily clinical practice, and therefore our data are relevant.

Conclusion

In conclusion, in patients with advance heart failure UAE was increased. Furthermore, microalbuminuria was present in almost one third of the patients, despite ACE inhibition and normal blood pressures. These findings favor further studies into the natural course of microalbuminuria in CHF patients, and microalbuminuria as a treatment target in these patients.

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CHAPTER 4

Determinants of increased angiotensin II levels in severe chronic heart failure patients despite ACE inhibition

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Abstract

Introduction The beneficial effects of ACE inhibitors are generally ascribed to blockade of neurohormonal activation. However, especially in chronic heart failure patients plasma angiotensin II and aldosterone levels can be elevated despite ACE inhibition, the so-called ACE escape. In the present study, we aimed to identify the frequency and determinants of ACE escape in CHF patients.

Methods We studied 99 stable chronic heart failure patients (NYHA class III and IV, 66% ischemic etiology) receiving long-term therapy with ACE inhibitors. In all patients, cardiac, renal, and neurohormonal parameters were measured. ACE escape was defined as plasma angiotensin level ≥ 16 pmol/L.

Results Mean (\pm SD) left ventricular ejection fraction of our 99 patients (79 men and 20 women, age 69 ± 12 years) was $28 \pm 10\%$. In addition to an ACE inhibitor, 93% of patients received diuretics, 71% a β -blocker, and 49% spironolactone. None of the patients used an angiotensin receptor blocker. In our population, 45% of the patients had an angiotensin II plasma concentration higher than 16 pmol/L (median concentration was 14.1 pmol/L). Spironolactone use was an independent predictor of elevated plasma angiotensin II levels. Furthermore, spironolactone users had significantly higher plasma active renin protein and aldosterone levels. Plasma angiotensin II concentration was positively correlated to active renin, plasma angiotensin I and plasma aldosterone. No correlation was found between plasma angiotensin II levels and serum ACE activity, dose of ACE inhibitor, or duration of use.

Conclusion In a group of severe chronic heart failure patients, 45% had elevated plasma angiotensin II levels independent of serum ACE activity despite long-term ACE inhibitor use. Although a causal link could not be proven, an association was found between spironolactone use and active renin protein, angiotensin II and aldosterone levels, suggesting that escape from ACE is mainly caused by a feedback mechanism.

Introduction

Inhibition of the renin angiotensin system with angiotensin-converting enzyme (ACE) inhibitors proved to be beneficial in patients with chronic heart failure (CHF).^{1,2} The clinically favorable outcome of CHF patients using an ACE inhibitor is mainly explained by reduction of angiotensin II formation. However, in patients on chronic ACE inhibition the angiotensin II and aldosterone levels will often rise again, even though plasma ACE levels remain suppressed and the antihypertensive effect does not disappear.³⁻⁶ Escape from ACE inhibition occurs particularly in patients with an activated renin angiotensin system (RAS). Activation of the RAS depends on several factors such as medication, salt intake, physical activity, posture and genetic preposition.⁷⁻⁹ In addition, activation of the RAS is observed in CHF patients and in these patients the activity is related to the severity of CHF. Plasma angiotensin II levels under ACE inhibition vary from less than 10 pg/ml in mild CHF patients to 70 pg/ml in patients with severe CHF.^{10,11} Angiotensin II has been viewed as a primary factor causing target organ damage in the cardiovascular system, and aldosterone exacerbates its tissue-damaging properties.^{12,13} Moreover, both elevated angiotensin II concentrations and elevated plasma aldosterone levels are associated with poorer prognosis.^{14,15} However, predictors of ACE and aldosterone escape have not been well described. Therefore, the present study was designed to measure plasma angiotensin II levels and other neurohormones in heart failure patients on chronic ACE inhibitor therapy in a routine clinical setting, and to identify which factors are related to ACE escape.

Methods

Between February 2003 and November 2003, we evaluated 106 patients with congestive heart failure New York Heart Association (NYHA) functional class III or IV, as a result of idiopathic dilated cardiomyopathy, ischemic or valvular heart disease, who presented at the Heart Failure Clinic of St. Antonius Hospital (Nieuwegein), University Hospital Groningen (Groningen), and Deventer Hospital (Deventer). All had been followed by the outpatient clinic of one of the participating hospitals and all subjects were being treated with stable doses of ACE inhibitor for at least three months. Diagnosis had been made on the basis of medical history, ongoing symptoms and physical examination. All patients had a left ventricular ejection fraction (LVEF) <45%, as assessed by echocardiography or radionuclide measurement. Every patient had been in a stable clinical condition for at least three months before the study.

Patients who used an angiotensin II receptor blocker were excluded from this study. Various ACE inhibitors were used and the dose of each ACE inhibitor was expressed as a percentage of the maximum recommended dose (Table 1).¹⁶

The study was approved by each hospital's ethics committee and written informed consent was obtained from all patients.

Hormonal measurements

Venous blood samples and urine samples were taken at the outpatient clinic while the patient was in an upright position. The blood and urine samples were transported to the local laboratory immediately, and each aliquot was processed and stored according to protocol for later batched analysis. The concentration of aldosterone was measured by a sandwich radioimmunoassay (Diagnostic Products Corporation, Breda, the Netherlands). Active renin protein was measured by an immunoradiometric assay (Nichols Institute Diagnostics, Middlesex, United Kingdom) and serum ACE activity was measured by an enzymatic assay (Bühlmann Laboratory AG, Schönenbuch, Switzerland). Analyses were performed in a routine setting according to the guidelines of the manufacturer. Angiotensin I and II were measured by specific radioimmunoassays after SepPak extraction of plasma as described previously.¹⁷ ACE escape was defined as an angiotensin II plasma concentration of ≥ 16 pmol/L (≥ 16.7 pg/ml), which is twice the upper limit of the reference value used in our laboratory, and comparable to the definition used by Roig et al.¹⁴

NT-probrain natriuretic peptide (NTproBNP) was measured by an Elecsys NT-proBNP immunoassay (Roche Diagnostics, Mannheim, Germany).

	Angiotensin II < 16 pmol/L (n = 54)	Angiotensin II \geq 16 pmol/L (n = 45)	Significance (two-tailed) p-value
Captopril (% mdd)	27.8 (94 mg)	15.7 (59 mg)	<0.001*
Enalapril (% mdd)	33.1 (18 mg)	37.8 (13 mg)	0.677*
Fosinopril (% mdd)	1.9 (10 mg)	6.7 (23 mg)	0.327*
Lisinopril (% mdd)	27.8 (13 mg)	20.0 (15 mg)	0.481*
Perinodpril (% mdd)	3.7 (2 mg)	17.8 (3.7 mg)	0.040*
Quinapril (% mdd)	5.6 (23 mg)	2.2 (30 mg)	0.624*
Dose ACE-inhibitor (% of recommended daily dose)	72 \pm 37	68 \pm 37	0.568 [#]
Duration of ACE-i use (months)	46 \pm 28	36 \pm 30	0.110 [#]

Table 1. ACE-inhibitor characteristics
[#] independent samples t-test, * chi-square test
 Abbreviations: MDD, median daily dose

Statistics

Values are expressed as mean values \pm SD, and neurohormone levels or activity are expressed as median values (25th-75th percentile). Differences between groups were investigated by using the unpaired t-test for independent samples and the chi-square test, when appropriate. Stepwise multiple regression analysis was performed to identify the independent predictors of increased plasma angiotensin II levels. Neurohormonal data were log-transformed before statistical comparison in order to correct for skewness. Linear and logistic regression analysis were performed to identify relations between variables. A p-value <0.05 was considered statistically significant.

Results

Of the 106 patients in our study, seven patients were excluded because they either failed to give informed consent (n=2), because inadequate venous blood samples were collected (n=3), or because the LVEF was higher than 45% (n=2). Therefore, the study population consisted of 99 patients (age 68.5 ± 11.7 years), 65 of whom had ischemic heart disease, 14 had a dilated cardiomyopathy, and the remaining patients had CHF due to other causes. Ninety-four patients (95%) were in NYHA functional class III and 5 patients were in functional class IV. Mean daily dose of captopril, enalapril, and lisinopril were 82.9 mg, 15.2 mg, and 13.9 mg respectively (Table 1). Detailed patient characteristics are listed in Table 2. Despite chronic ACE inhibitor treatment, 45 patients (45%) had plasma angiotensin II levels ≥ 16 pmol/L (median 35.4 pmol/L), while levels were within normal range in the remaining 54 patients (median 7.7 pmol/L). The variables in Table 2 with a significance level of $p < 0.10$ in the univariate tests were age, LVEF, spironolactone use, and systolic blood pressure. These values were included into a multivariate model. Multivariate analysis identified only spironolactone use and dose to be related to elevated plasma angiotensin II levels ($p = 0.020$). Patients on spironolactone had significantly higher levels of angiotensin II (40.5 vs. 16.5 pmol/L, $p < 0.001$, Figure 1) and of aldosterone (755.9 vs. 491.2 pmol/L, $p = 0.005$, Figure 1) compared to patients not using spironolactone. Of the patients on spironolactone 60% had elevated angiotensin II levels (versus 31% in non-users, $p = 0.005$), while 54% had aldosterone > 650 pmol/L (versus 17% in non-users, $p < 0.001$). Potassium levels were comparable in both spironolactone users and non-users (4.4 ± 0.4 mmol/L versus 4.5 ± 0.5 mmol/L). Median plasma levels of neurohormones are listed in Table 3. Correlations were found between plasma angiotensin II levels and active renin protein ($r = 0.574$, $p < 0.001$), plasma angiotensin I level ($r = 0.445$, $p < 0.001$), and plasma aldosterone level ($r = 0.337$, $p = 0.016$). No significant

		Angiotensin II < 16 pmol/L (n = 54)	Angiotensin II ≥ 16 pmol/L (n = 45)	Significance (two-tailed) p-value
Sex	male (%)	78.8	82.2	0.583*
	female (%)	22.2	17.8	
Age (years)		70.5 ± 10.0	66.0 ± 13.3	0.055 [#]
Body mass index (kg/m ²)		27.6 ± 4.6	27.8 ± 4.5	0.859 [#]
ACE inhibitor (%)		100.0	100.0	
Beta Blocker		75.9	64.4	0.211*
Diuretic		90.7	95.6	0.352*
Spironolactone		35.2	64.4	0.004*
Dose (mg/day)		20.6	26.2	0.039 [#]
Anti arrhythmic		11.1	15.6	0.514*
Calcium Blocker		11.1	13.3	0.565*
Acetyl salicylic acid		24.1	22.2	0.966*
Oral anticoagulant		68.7	68.9	0.972*
Digoxin		20.4	24.4	0.627*
Ischemic cause (%)		66.7	64.6	0.817*
Diabete	No	72.2	72.3	0.121*
	Type 1	0	6.7	
	Type 2	27.8	20.0	
Smoking	No	85.2	85.1	0.647*
	Yes	14.8	14.9	
Blood pressure (mm Hg)				
Diastolic		76 ± 11	74 ± 10	0.303 [#]
Systolic		126 ± 19	119 ± 22	0.089 [#]
LVEF (%)		30 ± 10	26 ± 10	0.074 [#]
Hemoglobine (mmol/L)		8.4 ± 0.8	8.6 ± 0.7	0.311 [#]
Cholesterol (mmol/L)		4.66 ± 1.06	4.93 ± 0.98	0.217 [#]
AF or flutter (%)		16.7	15.6	0.887*
cGFR (ml/min) ^a		60.0 ± 22.9	64.4 ± 23.8	0.670 [#]
Diet	Fluid restriction	24.1	26.7	0.768*
	Low sodium	59.3	57.8	0.882*

Table 2. Patient characteristics

^a Glomerular filtration rate calculated using the Cockcroft-Gault equation

[#] independent samples t-test * chi-square test

Abbreviations: AF, atrial fibrillation; LVEF, left ventricular ejection fraction

correlation was found with serum ACE activity ($r=-0.036$, $p = 0.755$, Table 4). Furthermore, no significant correlation was found between plasma angiotensin II levels and plasma NT-proBNP levels, or duration of ACE inhibitor use, while a weak association was found with LVEF ($r=-0.243$, $p=0.019$). Subgroup analyses demonstrated that the 22 patients receiving captopril had higher serum ACE activity than the patients using another ACE inhibitor (68.2 U/L vs. 18.7 U/L, $p<0.001$). However, the frequency of ACE escape tended to be lower in these patients (32% versus 49%, $p=0.145$).

Discussion

In the present study, we evaluated 99 patients with severe CHF who used an ACE inhibitor for at least three months. We found that 45 patients (45%) had elevated angiotensin II levels despite the long-term use of an ACE inhibitor. No clinical characteristic was found to be related to ACE escape. The use of a non-selective aldosterone antagonist was the only predictor of elevated angiotensin II levels. Moreover, elevated plasma angiotensin II levels were related to increased active renin protein and plasma angiotensin I levels. A previous study suggested that betablockers reduce the activity of the renin angiotensin system.¹⁸ Our data support this suggestion (Figure 2).

	Angiotensin II < 16 pmol/L	Angiotensin II ≥ 16 pmol/L	p-value (two-tailed)
Angiotensin II (pmol/L)	7.7 (4.3-11.2)	35.4 (2.5-61.5)	<0.001 [#]
Angiotensin I (pmol/L)	618 (264-1377)	2358 (881-5137)	<0.001 [#]
Active renin (μU/mL)	138 (53-299)	770 (260-2400)	<0.001 [#]
ACE activity (U/L)	15.8 (12.6-22.0)	16.5 (11.6-20.9)	0.627 ^{#u}
Aldosterone (pmol/L)	385 (200-585)	690 (390-1135)	0.001 [#]
NT-proBNP	111.4 (50.0-236.9)	128.0 (50.5-281.9)	0.624 [#]

Table 3. Median neurohormonal levels (25th-75th percentile)
[#] independent samples t-test, ^u captopril users excluded

It is firmly established that ACE inhibitor use does not completely block angiotensin II and aldosterone production, and in some patients, angiotensin II and aldosterone levels remain high.^{3;10;14;19} This might be the result of insufficient suppression of ACE due to an inadequate dose of ACE inhibitor. The most obvious explanation for this ACE escape is the existence of alternative enzymes for the formation of angiotensin II.²⁰ Non-ACE mediated pathways (e.g. tissue chymase mediated angiotensin II formation) may become activated when the activity of ACE is reduced by an ACE inhibitor.

Remarkably, in our study ACE escape was not affected by either dose or duration of ACE inhibitor use, or with serum ACE activity. In a previous study it was suggested that during storage captopril dissociates from ACE, which might lead to an overestimation of serum ACE activity, whereas other studies suggested that captopril is not able to suppress the activity of ACE as profoundly as other ACE inhibitors.^{21;22} Therefore, in the present study captopril users were excluded from analyses involving serum ACE activity. Active renin protein and angiotensin I levels were elevated in the patients with elevated angiotensin II levels. It has been well described that renin and angiotensin I accumulate

during short-term and long-term ACE inhibition.²³⁻²⁵ Since all our patients had been on a steady dose of ACE inhibitor for at least three months, this does not account for the different hormone levels we found in our population.

Particularly in patients that used spironolactone, plasma angiotensin II and aldosterone levels were elevated. In addition, these patients had higher plasma levels of active renin protein and angiotensin I. These results indicate that blockade of the aldosterone receptor on top of ACE inhibition in CHF patients induces further activation of the renin angiotensin system, likely through intensification of the positive renin feedback mechanism (Figure 3). Due to activation of renin and increased availability of angiotensin I, more angiotensin II and aldosterone will be formed. The activity of ACE seems to be of minor importance in this process.

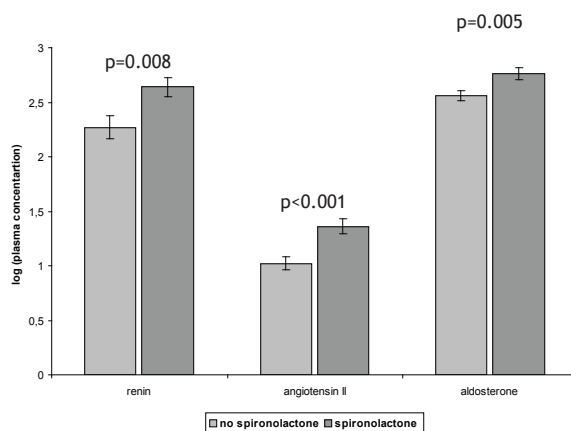


Figure 1. Plasma concentrations of active renin protein, angiotensin II, and aldosterone in patients using spironolactone versus patients not using spironolactone

Our data are consistent with the results of Rousseau et al. who performed a neurohormonal substudy in the RALES population.^{26;27} Fifty-four patients on spironolactone had significantly higher plasma angiotensin II levels and plasma aldosterone levels after three and six months of treatment, than 53 patients who were treated with placebo. In their study 97.2% of the patients received an ACE inhibitor. After 48 months of follow-up cardiac mortality in the spironolactone group was significantly lower (21% vs. 38%, $p=0.05$) than in the placebo group. They suggested that the mechanism underlying the Angiotensin II and aldosterone escape reflected activated feedback mechanisms on the renin angiotensin system.²⁷ The results of this RALES substudy are in contrast

with the conclusion drawn by Roig et al.¹⁴ They identified a group of patients with increased levels of angiotensin II within a selected group of patients using ACE inhibitors, and compared this group to the patients with normal levels of angiotensin II. Both groups had similar left ventricular ejection fractions and no patients used spironolactone. After 3 years, death or new heart failure episodes occurred in 43 % of the patients with increased angiotensin II levels at baseline, compared with 13 % of the patients with normal angiotensin II levels ($p=0.002$). Therefore, Roig et al. stated that beside a marker for the severity of heart failure, an increased plasma angiotensin II level under ACE inhibition is an independent predictor for increased mortality and morbidity in heart failure patients as well.¹⁴ Patients using spironolactone appear to be an exception to this statement.²⁸

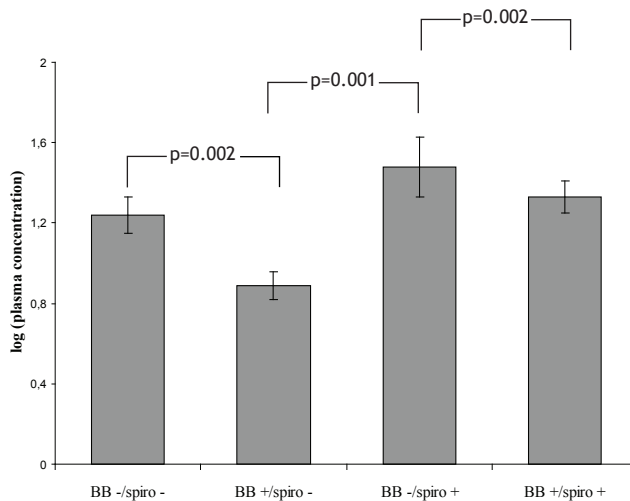


Figure 2. Comparison of angiotensin II plasma levels in severe CHF according to medication profile
Abbreviations: BB, betablocker; spiro, spironolactone

Recently, beneficial effects were described of angiotensin receptor blockers in combination with ACE inhibitors in CHF patients in the CHARM-trial.²⁹ Although in CHARM no subgroup analysis was performed, we hypothesize that especially CHF patients with elevated plasma angiotensin II and plasma aldosterone levels despite ACE inhibitor use might benefit from additional treatment aimed at receptor blockade within the RAS. Both angiotensin receptor blockers and aldosterone receptor blockers are candidates for neutralizing the detrimental effects of angiotensin II and aldosterone.

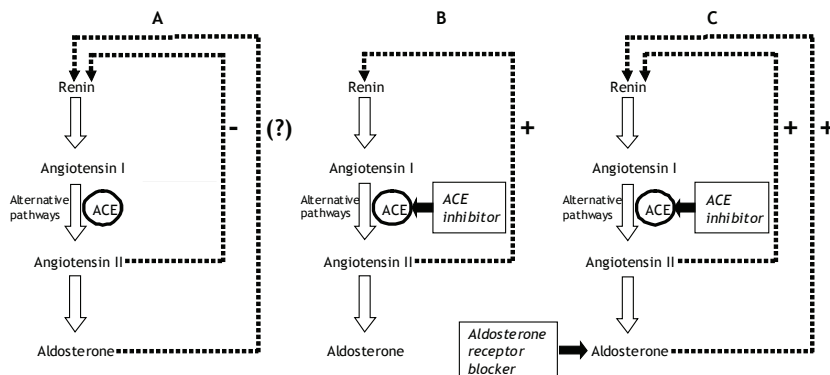


Figure 3. Hypothesis: A: negative renin feedback of angiotensin II in untreated patients. B: positive renin feedback (or lack of negative feedback?) when only ACE is blocked, resulting from lower angiotensin II levels; C: intensified renin feedback when both ACE and aldosterone receptors are blocked.

Limitations

This was an observational, hypothesis-generating study in an out-patient clinical setting. Consequently, analysis revealed a wide variety of neurohormonal concentrations. This might be due to different levels of activation of the RAS under the circumstances we used. However, we believe that for revealing mechanisms within the RAS in a population-based study, it is a valuable tool. Secondly, the cut-off values for plasma angiotensin II concentrations were partly arbitrary. The cut-off value of 16 pmol/L is comparable to the value used by Roig et al.¹⁴ Approximately half of their patients escaped from ACE inhibitor therapy and had elevated plasma angiotensin II levels, which is comparable to the percentage (45%) we found. Finally, we did find different daily doses of individual ACE inhibitors in both groups (Table 1). However, when all ACE inhibitors were grouped the mean dose (expressed as percentage of the recommended dose) was not statistically different. Consequently, we cannot draw conclusions on the effect of individual ACE inhibitors on RAS activity.

Parameter A	Parameter B	Pearson r	p-value
Dose	Log (ACE-activity)*	0.112	0.277
Log (angiotensin II)	Dose	0.027	0.794
Log (ACE-activity)*	Log (angiotensin II)	-0.036	0.755

Table 4. Correlation between ACE inhibitor dose, ACE activity and angiotensin II levels
* *captopril users excluded*

Conclusion

In a group of severe chronic heart failure patients 45% had elevated plasma angiotensin II levels despite long-term ACE inhibitor use. Elevated levels were associated with the use of spironolactone, and with increased levels of active renin protein and angiotensin I. No association was found between angiotensin II levels and serum ACE activity, or dose or duration of ACE inhibitor use. Therefore, we believe ACE escape is related to a feedback mechanism, leading to increased levels of active renin protein and angiotensin I. This mechanism is intensified when patients use both an ACE inhibitor and an aldosterone receptor antagonist.

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CHAPTER 5

Angiotensin II type 2 receptor vasoactivity in internal mammary arteries of patients with coronary artery disease

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Abstract

Objectives Several animal studies suggested that the angiotensin II type 2 (AT2) receptor subtype mediates vasodilation, yet in human arteries the results are less well-described and inconsistent. Therefore, we evaluated the role of the AT2 receptor stimulation on the vasotonus of human internal mammary arteries.

Methods and results Human internal mammary arteries were obtained from 50 patients undergoing coronary bypass surgery. The expression of angiotensin II type 1 receptor and AT2 receptor mRNA was determined by using real time polymerase chain reaction. In addition, angiotensin II and CGP42112A concentration-response curves (concentration range: 10^{-10} M to 10^{-6} M) were constructed in absence or presence of candesartan (10^{-5} M) and/or the AT2 receptor antagonist PD-123319 (10^{-6} M) and/or the α receptor antagonist phentolamine.

Both AT1 and AT2 receptor protein and mRNA were present in human internal mammary arteries, and higher AT2 receptor mRNA expression levels were associated with increased contractile response to angiotensin II. Angiotensin II caused vasoconstriction up to $41.1 \pm 2.0\%$ of the maximal response to phenylephrine, and PD123319 significantly reduced this response ($28.6 \pm 1.0\%$, $p < 0.001$). Candesartan completely blocked the angiotensin II mediated response ($1.4 \pm 1.2\%$, $p < 0.001$ versus control), and additional blockade of the AT2 receptor with PD123319 did not change this effect ($1.8 \pm 1.3\%$). Phentolamine (10^{-5} M) caused an attenuation and a rightward shift of the angiotensin II concentration response curves. The AT2 receptor agonist CGP42112A did not induce a significant response.

Conclusion Although AT2 receptor mRNA is present in human internal mammary arteries, AT2 receptor stimulation does not mediate vasodilation in these arteries.

Introduction

As the key effector peptide of the renin angiotensin system (RAS), angiotensin II exerts a potent role in the control of cardiovascular homeostasis. Over the last 15 years several receptors for angiotensin II have been identified of which the angiotensin II type 1 (AT1) receptor and the angiotensin II type 2 (AT2) receptor are the two most important subtypes.¹ The classic hormonal actions on blood pressure and fluid homeostasis are being attributed to stimulation of the AT1 receptor. Moreover, through this receptor angiotensin II plays a part in inflammation and in cell proliferation. In contrast to the AT1 receptor, the effect of stimulation of the AT2 receptor remains less well-defined. In general, it is presumed that the AT2 receptor has effects opposing the AT1 receptor. AT2 receptors are abundantly expressed in foetal tissues and they predominantly disappear in most tissues after birth, which suggests a role in growth and development.^{2,3} In addition, the antiproliferative and apoptotic effects of the AT2 receptor have been well-established.⁴⁻⁶ Several *in vitro* and animal studies suggested that stimulation of the AT2 receptor mediates signalling pathways associated with vasodilation.⁷⁻¹¹ Furthermore, a recent study in spontaneously hypertensive rats demonstrated that reduction of high blood pressure reversed AT2 receptor mediated vasoconstriction into vasodilation.¹² Extrapolation of these data to the human species is highly questionable, and for that reason unwarranted. Data concerning the vasoactivity of AT2 receptor stimulation in humans are scarce and also inconsistent.¹³⁻¹⁵ Therefore, the aim of the present study was to examine the effect of AT2 receptor stimulation on the vascular tonus of the human internal mammary artery.

Methods

Human tissue collection

Segments of human internal mammary arteries (IMA) were obtained from 50 patients who underwent coronary bypass surgery (CABG) in St Antonius Hospital in Nieuwegein, or in the University Medical Center Groningen, the Netherlands. After removal, the tissue was stored in cold (4 °C) RPMI 1640 medium (GIPCO, Paisley, UK) and prepared for organ bath studies. In addition, IMA segments from 14 of the patients were immediately stored at -80 °C for mRNA determination. Patient receiving therapy with ACE-inhibitors or AT1 receptor antagonists at the time of surgery were excluded. The segments were processed within 24 hours after removal. Studies with excess human arteries are approved by the Ethics Review Committee, and conform with the principles outlined in the Declaration of Helsinki.

AT1 and AT2 receptor mRNA isolation and real-time polymerase chain reaction

For assessment of vascular gene expression, IMA samples were cleaned of surrounding tissue, quickly frozen in liquid nitrogen, and homogenized with a motorized homogenizer. RNA from the homogenates was isolated with RNA-clean according to the manufacturer's protocol, and 1 µg aliquots were electrophoresed through 1.2% agarose/0.67% formaldehyde gels and stained with ethidium bromide to verify the quantity and quality of the RNA. 1 µg of the isolated total RNA was reverse transcribed using random primers and MMLV reverse transcriptase for 60 min at 42 °C and 10 min at 75 °C. The single stranded cDNA was amplified by real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) with the TaqMan system (ABI-Prism 7700 Sequence Detection System, Applied Biosystems, Weiterstadt, Germany) using SYBR Green dye. For human AT1 receptors, the primers were 5'-CTG-GAA-GGC-ATA-ATT-ACA-TAT-TTG-TCA-3' and 5'-GCC-ACA-GTC-TTC-ACG-TTC-ATA-TAA-AA-3', for human AT2 receptors, the primers were 5'-TCC-CCT-TGT-TTG-GTG-TAT-GGC-C-3' and 5'-CAC-TGC-GGA-GCT-TCT-GTT-GGA-A-3', for GAPDH, the primers were 5'-ACC-ACA-GTC-CAT-GCC-ATC-3' and 5'-TTC-ACC-ACC-CTG-TTG-CTG-TA-3'. For quantification, AT1 receptor and AT2 receptor mRNA expression were normalized to the expressed housekeeping gene GAPDH.

Quantification of protein expression

Frozen tissue samples were homogenized in lysis buffer and Western blot analysis was performed as described by Adams et al.¹⁶ To detect specific proteins the following antibodies were applied: AT1 receptor and AT2 receptor (both Santa Cruz Biotechnology, Heidelberg, Germany). This antibody was diluted 1:500 in Tris buffered salt solution containing Tween 20 (TTBS). The antibody was incubated overnight at 4 °C. The second antibody used was a goat anti-rabbit coupled to peroxidase (Dako, Hamburg, Germany), which was diluted 1:10.000. After an incubation time of 2 hours at room temperature, this peroxidase-coupled secondary antibody was detected by chemiluminescence. The amount of protein loaded on the gel (70 µl in each lane) was checked in two ways. First, equal loading was evaluated by Ponceau red (Sigma-Aldrich Chemie GmbH, Munich, Germany) staining. Second, to control for loading differences the blots were reprobbed with an antibody against GAPDH (Hytest, Turku, Finland).

Organ chamber studies

The vessels were dissected free, cleaned of surrounding tissues, and cut into several rings (\pm 2mm), while care was taken not to damage the endothelium.¹⁷ Rings were mounted in 15 ml organ baths, containing a buffer solution of the following (Krebs) composition (mM): NaCl (120.4), KCl (5.9), CaCl₂(2.5),

MgCl₂(1.2), NaH₂PO₄(1.2), Glucose (11.5) and NaHCO₃ (25.0). The medium was continuously aerated with 95% O₂-5% CO₂ and kept at 37°C. The rings were connected to an isotonic displacement transducer by 5-0 braided, uncoated polyester suture. We performed isotonic measurements of vascular contraction; that is, vessel rings were subjected to a constant tension of 1.4 g and changes in vessel diameter were registered in μm . Both the isotonic transducer, the recording system, and the software were custom made and calibrated by the University Medical Center Groningen, the Netherlands.

In every single experiment, we studied several arterial rings from one donor in a parallel fashion. Two of the rings were always used to obtain control responses (incubated with vehicle) in each experiment. The artery rings were allowed to equilibrate for at least 60 minutes, during which regular washing periods were performed. Rings were primed and checked for viability by repeated stimulation (2-3 times) with 10 μM phenylephrine followed by in-between washing and stabilization periods. Rings that failed to reach a contractile response of at least 100 μm to phenylephrine were not included in the experiment. In experiments 2A, 2B and 4, results were excluded if control rings did not reach a contraction response to angiotensin II of 15% of the final response to 10 μM phenylephrine. In experiment 3, rings were stimulated with a single high concentration of 10 mM sodium nitrite at the end of the experiment. In these two experiments, vessel segments were excluded from analyses if the contractile response of control rings to 10 μM phenylephrine minus the dilatory response to 10 mM sodium nitrite was less than 100 μm . In order to exclude procedure-related endothelial damage, data of 187 consecutive CABG patients were analysed. In these vessel segments both endothelial dependent and endothelial independent were tested by constructing an acetylcholine (10⁻⁸-10⁻⁴ M) concentration-response curve and by a single bolus injection of sodium nitrite (10mM), respectively. Vessel segments were excluded from further analysis if the contractile response to 10 μM phenylephrine was less than 100 μm .

Experiment 1

In 14 IMA segments AT₁ receptor mRNA and AT₂ receptor mRNA expression was analysed. In addition, the contractile response to increasing concentrations (0.1 nM to 1 μM) of angiotensin II of segments from the same patients was determined.

Experiment 2A

In the second experiment, rings were preincubated (30 minutes) with either vehicle, an AT₁ receptor antagonist (candesartan, 10 μM), an angiotensin II-AT₂ receptor antagonist (PD123319, 1 μM), or a combination of both AT receptor antagonists. They were then stimulated with increasing concentrations (0.1 nM to 1 μM) of angiotensin II.

Experiment 2B

Rings were preincubated (30 minutes) with either vehicle, an AT1 receptor antagonist (candesartan, 10 μM), an AT2 receptor antagonist (PD123319, 1 μM), or a combination of both AT receptor antagonists. Furthermore, for each condition all the rings were additionally incubated with 10 μM of the α -receptor blocking agent phentolamine. Subsequently, rings were stimulated with increasing concentrations (0.1 nM to 1 μM) of angiotensin II.

Experiment 3

In the third experiment, after preincubation (30 minutes) with either vehicle, 10 μM candesartan, 1 μM PD123319, or a combination of both AT receptor antagonists, all rings were precontracted by administering 10 μM phenylephrine. Then they were stimulated with increasing concentrations (0.1 nM to 1 μM) of angiotensin II.

Experiment 4

In the final experiment rings were preincubated (30 minutes) with either vehicle, an angiotensin II-AT1 receptor antagonist (candesartan, 10 μM), an AT2 receptor antagonist (PD123319, 1 μM), or a combination of both AT receptor antagonists. Then they were stimulated with increasing concentrations (0.1 nM to 1 μM) of the AT2 receptor agonist CGP42112A.^{18;19}

Drugs and Reagents

Candesartan was a gift from Astra Pharmaceutica BV (Zoetermeer, The Netherlands). PD123319 (1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(di phenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid ditrifluoroacetate), phenylephrine, sodium nitrite, phentolamine, and CGP42112A (nicotinic acid-Tyr-N-benzoxyl-carbonyl-Arg-Lys-His-Pro-Ile-OH) were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands). Angiotensin II was obtained from CIBA-Geigy Ltd. (Basle, Switzerland). The drugs were dissolved in saline and freshly prepared each day from stock solutions.

Data Analyses

All data are expressed as means \pm SEM. To avoid non-specific differences between subjects, we evaluated the effect of the inhibitors by comparing concentration-response curves obtained with parallel rings from the same patient. To control for non-specific differences between rings from the same patient, contractile responses of individual rings to angiotensin II are expressed as a percentage of the contractile response to 10 μM phenylephrine, whereas relaxant responses are expressed as a percentage of the difference between the response to 10 μM phenylephrine and 10 mM sodium nitrite. Comparisons between the complete

concentration-response curves were made by repeated measures analysis of variance. Analysis was performed according to recommendations by Ludbrook.²⁰ To correct for multisample asphericity, the Huynh-Feldt adjustment was always made. Calculations were done using the GLM procedure of the SAS-system (SAS Institute Inc., Chicago, IL, USA, version 8.2). When maximum response was reached, results from each dose-response curve were fitted to Hill's equation. The negative log of the concentration that would give 50% constriction or dilation (-log EC50), and the maximum response (contraction or dilation, Emax) was calculated from this curve. If during an experiment no contraction or dilation was observed, -log EC50 could not be calculated (indicated as not determined, ND, in the tables). Shifts in -log EC50 and Emax between rings from different experiments were compared by 2-sided Student's t-test. A probability level of <0.05 was considered to indicate statistical significance.

Results

Functional measurements.

Internal mammary arteries were obtained from 50 patients undergoing coronary bypass surgery. The patient population was a representative group of patients undergoing coronary bypass surgery with a mean age of 65.0 years (range 44-82); patient characteristics are shown in Table 1.

Age (years)	65.0 ± 1.3
Male gender, (%)	74.0
Body mass index (kg/m ²)	27.4 ± 0.5
NYHA functional class	2.4 ± 0.1
Diabetes mellitus (%)	12.0
Family history of CAD (%)	48.0
Myocardial Infarction (%)	32.0
Current smoker (%)	20.0
SBP (mm Hg)	147 ± 3
DBP (mm Hg)	81 ± 1
Aspirin (%)	82.0
B-blocker (%)	76.0
Calcium antagonist (%)	52.0
Lipid lowering drugs (%)	52.0
Nitrates (%)	58.0

Table 1. Baseline characteristics

Values are mean ± SEM or percentage. Aspirin was discontinued 1 week before surgery.

Abbreviations: CAD, coronary artery disease; NYHA, New York Heart Association; DBP, diastolic blood pressure; SBP, systolic blood pressure

AT1 and AT2 receptor mRNA expression and contraction to angiotensin II

Both AT1 and AT2 receptor mRNA were identified in human internal mammary arteries of 14 different patients. AT1 receptor mRNA expression (ratio versus GAPDH expression) was significantly higher than the expression of AT2 receptor mRNA (3.91 ± 0.65 versus 1.72 ± 0.42 , $p < 0.001$).

Median AT2 receptor mRNA level was used to stratify these 14 patients into a low and a high AT2 receptor mRNA expression group (mean AT2 receptor mRNA expression ratio 0.48 ± 0.23 versus 2.95 ± 1.31 respectively, $p < 0.001$). The contractile response to increasing concentrations of angiotensin II was significantly higher in the high AT2 receptor mRNA expression group (Emax: $34.4 \pm 3.3\%$ versus $7.7 \pm 1.5\%$, $p < 0.001$), while $-\log EC_{50}$ values were comparable in both groups (-7.6 ± 0.3 mol/L versus -8.0 ± 0.2 mol/L, $p = 0.341$). Comparable results were found for AT1 receptor mRNA expression. Furthermore, a linear relation between AT2 receptor mRNA expression and angiotensin II mediated maximal vasoconstriction was observed ($r = 0.62$, $p = 0.018$), whereas the correlation between contraction and AT1 receptor mRNA expression almost reached statistical significance ($r = 0.46$, $p = 0.098$, Figure 1).

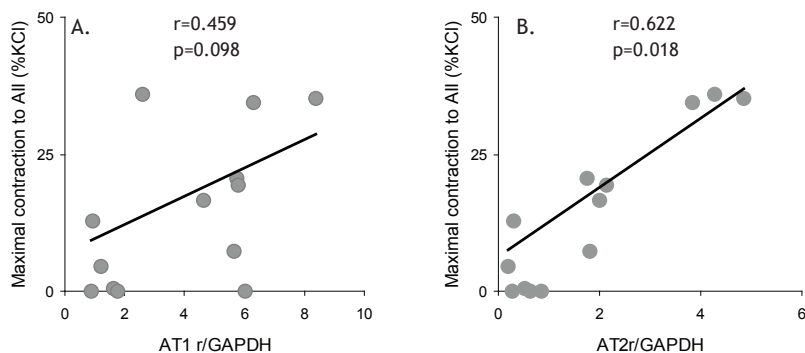


Figure 1. Correlation between AT1 (panel A) and AT2 (panel B) receptor mRNA expression (normalized to the expressed housekeeping gene GAPDH), and Emax of the angiotensin II concentration response curve in human internal mammary arteries (n=14).

AT1 and AT2 receptor protein expression

Both AT1 and AT2 receptor protein expression were determined in segments from 4 different patients. AT1 and AT2 receptor protein expression did not differ significantly in these patients (ratio versus GAPDH). The results of the Western blot analysis are shown in Figure 2.

Endothelial dependent and independent relaxation

Of the 187 patients analysed, segments of 86 patients (mean age 63.3 ± 1.2 , 86.7% male) showed a contractile response to $10 \mu\text{M}$ phenylephrine. In these vessel segments endothelium dependent and independent relaxation was tested. The acetylcholine concentration-response curve in Figure 3 shows that acetylcholine cause a maximum dilation of 34.2% when expressed as percentage of the maximum response to phenylephrine. By subsequently exposing the vessel segments to 10 mM sodium nitrite an additional dilation was observed. Maximum endothelium independent relaxation was determined to be $91.1\% (\pm 4.2\%)$.

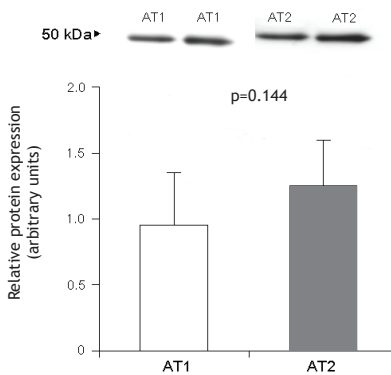


Figure 2. Protein expression of AT1R and AT2R subtypes in left internal mammary arteries ($n=4$). To determine total protein content of AT1R and AT2R western blot analyses was performed. Top; representative western blot results

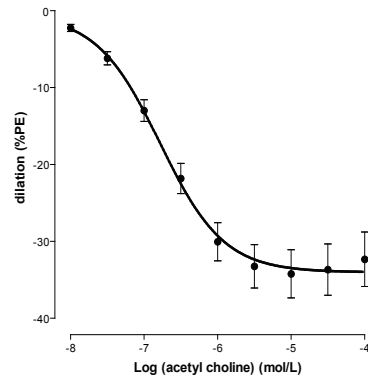


Figure 3. Dilations of human internal mammary artery rings to increasing doses of acetylcholine to test endothelial dependent relaxation. Responses (mean \pm SEM, $n = 86$) are expressed as a percentage of the response to $10 \mu\text{M}$ PE.

Effect of AT1 and AT2 receptor blockade on angiotensin II induced responses in arteries under baseline conditions

Organ bath experiments demonstrated that in control vessels, increasing doses of angiotensin II caused vasoconstriction up to 41.1% of the maximal response to $10 \mu\text{M}$ phenylephrine (Figure 4 and Table 2). Presence of $1 \mu\text{M}$ PD123319 significantly reduced maximal contraction and shifted the concentration-response curve to the right (although repeated measures analysis of variance did not reveal a significant difference), but did not abolish the response to angiotensin II. In contrast, contractions to angiotensin were almost completely abolished in the presence of $10 \mu\text{M}$ candesartan. Presence of PD123319 did not affect this effect of candesartan.

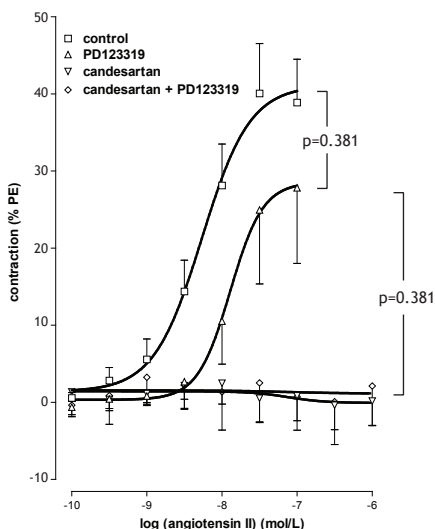


Figure 4. Contractions of human internal mammary artery rings to increasing doses of angiotensin II in the presence and absence of 10 μM candesartan and /or 1 μM PD123319. Data are mean \pm SEM, (n = 7 to 10). Concentration response curves were compared using repeated measures analysis of variance.

Effect of α -receptor blockade on angiotensin II induced contraction

Under control conditions, blockade of the α -receptors by phentolamine caused a significant rightward shift of the concentration-response curves to angiotensin II ($-\log EC_{50}$ 8.26 ± 0.07 mol/L vs 7.34 ± 0.14 mol/L for vehicle vs phentolamine, $p < 0.001$), as well as a significant reduction in maximal contraction (E_{max} $41.1 \pm 2.0\%$ vs $27.7 \pm 2.6\%$ for vehicle vs phentolamine, $p < 0.001$, Figure 5A).

	n	$-\log EC_{50} \pm SEM$	p^*	$E_{\text{max}} \pm SEM$	p^*
Control	10	8.26 ± 0.07		$41.1 \pm 2.0\%$	
10 μM candesartan	8	ND		$1.4 \pm 1.2\%$	<0.001
1 μM PD123319	7	7.89 ± 0.03	<0.001	$28.6 \pm 1.0\%$	<0.001
10 μM candesartan + 1 μM PD123319	7	ND		$1.8 \pm 1.3\%$	<0.001

Table 2. Concentration response characteristics of human internal mammary arteries to increasing concentrations of angiotensin II in the presence or absence of 10 μM candesartan and/or 1 μM PD123319

* versus control

• $p < 0.001$ versus PD123319

Data are expressed as mean \pm standard error of the mean (SEM).

Abbreviations: $-\log EC_{50}$, concentration at which 50% of the maximum response was reached;

E_{max} , maximum contraction response; ND, not determined

In contrast, under conditions of AT₂ receptor blockade the main effect of phentolamine seemed to be a reduction in maximal contraction (E_{max} $28.6 \pm 1.0\%$ vs $19.6 \pm 0.8\%$ for vehicle vs phentolamine, $p < 0.001$) rather than a change in sensitivity to angiotensin II ($-\log EC_{50}$ 7.98 ± 0.03 mol/L vs 7.65 ± 0.07 mol/L for vehicle vs phentolamine, $p = 0.008$, Figure 5B). It should be reminded, however, that sensitivity and maximal contraction to angiotensin II already are reduced to some extent during conditions of per se AT₂ receptor blockade (Figure 5 and Table 2). As a result, therefore, it appears that the sensitivity and maximal contraction to angiotensin II are similar during conditions of α -receptor blockade with phentolamine, irrespective of additional AT₂R blockade (Table 3). Finally, under conditions of AT₁ receptor blockade with candesartan the responses to angiotensin II were virtually abolished, and this remained unchanged by phentolamine (data not shown).

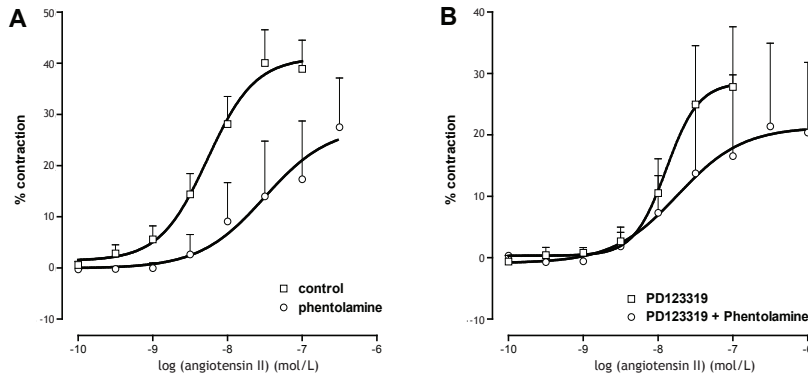


Figure 5. Effect of α -receptor blockade with $1 \mu\text{M}$ phentolamine on angiotensin-induced contraction in isolated human internal mammary artery rings under control conditions (panel A) and under conditions of AT₂ receptor blockade with $1 \mu\text{M}$ PD123319 (panel B). Data are mean \pm SEM.

Effect of AT₁ and AT₂ receptor blockade on angiotensin II induced responses in precontracted arteries

The mean absolute levels of precontraction did not differ significantly between the 4 groups; 330 ± 63 , 325 ± 66 , 289 ± 84 , and $308 \pm 64 \mu\text{m}$ (for the saline, candesartan, combination, and PD123319 group, respectively). After precontraction of the control rings, increasing doses of angiotensin II caused a non-significant further increase in contraction (E_{max} $10.5 \pm 1.5\%$, Figure 6). This response was significantly reduced by $1 \mu\text{M}$ PD123319 (E_{max} $1.7 \pm 1.1\%$, $p < 0.001$ versus control; although repeated measures analysis of variance did not reveal a significant difference), and reversed into relaxation in the presence of $10 \mu\text{M}$ candesartan (E_{max} $-12.9 \pm 0.5\%$, $p < 0.001$ versus control).

After blockade of both the AT1 and AT2 receptor, angiotensin II administration induced a similar response compared to AT1 receptor blockade only ($E_{max} - 10.3 \pm 0.6\%$, $p = ns$ versus candesartan only).

	n	$-\log EC_{50} \pm SEM$	p^*	$E_{max} \pm SEM$	p^*
Control	7	7.51 ± 0.18		$27.6 \pm 2.9\%$	
10 μM candesartan	7	ND		$-3.7 \pm 0.4\%^{\#}$	<0.001
1 μM PD123319	7	7.74 ± 0.08	0.260	$21.3 \pm 0.8\%$	0.048
10 μM candesartan + 1 μM PD123319	7	ND		$-4.4 \pm 0.5\%^{\#}$	<0.001

Table 3. Concentration response characteristics of human internal mammary arteries to increasing concentrations of angiotensin II in the presence or absence of 10 μM candesartan and/or 1 μM PD123319, after addition of 10 μM phentolamine

* versus control

maximal dilation was used

Data are expressed as mean \pm standard error of the mean (SEM).

Abbreviations: $-\log EC_{50}$, concentration at which 50% of the maximum response was reached;

E_{max} , maximum contraction response; ND, not determined

AT2 receptor stimulation in absence and presence of AT1 and AT2 receptor blockade

Increasing concentrations of CGP42112A did not induce significant dilation or contraction in any of the groups, irrespective of the antagonist used. Data not shown.

Discussion

In this study we demonstrated that the AT2 receptor is present in human internal mammary arteries, and did not mediate vasodilation in our in vitro model.

To our knowledge, the vasoactivity of the arterial AT2 receptor has been the focus of a human in vitro study only once before, when Batenburg et al. demonstrated that AT2 receptor mediated vasodilation in human coronary microarteries.¹⁴ An earlier publication from the same group did not specifically focus on the role of AT2 receptor, but in that study addition of PD123319 did not influence the angiotensin II induced concentration response curve in larger human coronary arteries.¹³ Unfortunately, quantification of the AT2 receptor was not part of the protocol in the latter study. Both studies used tissue that was collected from young heart-beating donors who died of non-cardiac causes. Furthermore, Borland et al. already demonstrated that stimulation of the AT2 receptor neither induces vasoconstriction, nor dilation in isolated human saphenous vein segments.¹⁵ In the present study, larger non-resistance arteries were studied and the segments were obtained from older patients with severe coronary

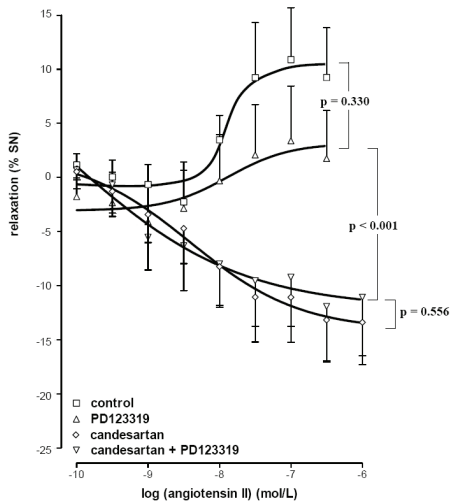


Figure 6. Response of phenylephrine-precontracted human internal mammary arteries to increasing concentrations of angiotensin II in the absence or presence of absence of $1 \mu\text{M}$ PD123319, $10 \mu\text{M}$ candesartan or a combination of both agents ($n = 10$ to 14). Concentration response curves were compared using repeated measures analysis of variance.

disease. In previous studies the distribution of the AT2 receptor appeared to be species-, tissue-, and disease specific, and the developmental stage of the organism studied also seemed to play a role.⁵ In various pathological states of the cardiovascular system the expression of the AT2 receptor is increased, but even under pathological conditions the AT1 receptor subtype seems to outnumber the AT2 receptor subtype.^{14;21;22} Although the AT2 receptor mRNA levels are lower than the AT1 receptor mRNA levels in the arterial segments we analysed, the AT2 receptor protein levels seem to be comparable to the AT1 receptor protein levels. Furthermore, Adams et al. identified both AT2 receptor mRNA and protein expression in human internal mammary arteries, and they demonstrated that expression increased after a regular preoperative physical activity program.¹⁶ In this study, the authors found a blunted response to high concentrations of angiotensin II in human internal mammary vessels. They speculated that the blunted response was caused by a shift in AT1/AT2 receptor expression, since they found a linear correlation between receptor protein expression ratio and the maximal angiotensin II induced vasoconstriction.¹⁶ We found a correlation between AT2 receptor mRNA expression and maximal angiotensin II induced constriction.

Besides its direct vasoconstrictor effects, angiotensin II also facilitates norepinephrine release from norepinephrine stores in presynaptic nerve terminals through AT1 receptor stimulation.²³⁻²⁵ As a result postsynaptic alpha-1

receptors are stimulated, mediating vasoconstriction.²⁶ With the intention of eliminating these indirect vasoconstrictor effects of angiotensin II, we repeated the first experiment after antagonizing α -receptors with phentolamine. This resulted in a rightward shift of the concentration response curve and into a reduction of E_{max} , suggesting that a significant percentage of the response to angiotensin II is actually an indirect norepinephrine-mediated effect. Despite attenuation of the angiotensin II induced response that occurs after elimination of α -mediated effects, blockade of the AT₂ receptor still seems to affect the concentration-response curve when compared to vehicle-treated rings. Unfortunately, in the absence of nerve stimulation, it is unclear how much alpha adrenergic nerve firing is occurring in isolated vessels. In the present study, the differences between concentration response curves are subtle, and consequently repeated measures analyses of variance can not uncover small changes. In contrast to common views, when we analyse E_{max} and $-\log EC_{50}$ values of the concentration response curves, our findings even suggest an AT₂ receptor-mediated vasoconstriction in human internal mammary arteries. Several explanations can be proposed. First, due to lack of selectivity, PD123319 in a concentration of 1 μ M might have partly blocked AT₁ receptors. The relatively high concentration of this AT₂ receptor antagonist may have induced aspecific effects, although previous studies suggest that PD123319 is highly selective.^{18;27} Second, stimulation of the AT₂ receptor could directly induce vasoconstriction. AT₂ receptor -mediated vasoconstriction has been reported before in cerebral arteries,²⁸ and in renal arteries.²⁹ In addition, experiments performed by Touyz et al. using small mesenteric arteries of young spontaneously hypertensive rats, demonstrated that blockade of the AT₂ receptor reduces angiotensin II-induced contractile response.²¹ A recent study performed by You et al. demonstrated that AT₂ receptor stimulation induced a vasoconstriction in untreated spontaneously hypertensive rats resistance vessels associated with a decrease in AT₂ receptor expression.¹² They also found that treatment of hypertension restored both AT₂ receptor expression and its vasodilator function. However, in our experiments direct stimulation of the AT₂ receptor using the AT₂ receptor agonist CGP42112A did not provoke dilation, nor contraction. Furthermore, in the curves constructed after precontraction with phenylephrine, no difference was found between the angiotensin II response in the presence of candesartan, and in the presence of the combination of candesartan and PD123319. This makes a direct contractile effect of the PD123319-sensitive receptor less likely.

A third explanation was proposed by Hong et al.³⁰ They already speculated that the sensitivity of the AT₁ receptor is revealed when the AT₂ receptor is blocked. This hypothesis advocates the existence of a dynamic cross-talk between the AT₁ and AT₂ receptor. The nature of this receptor-receptor interaction ought to

be present at the second messenger system level, or at the mRNA and protein level. In the first case, an intermediate enzyme of the AT2 receptor secondary messenger system interferes with the secondary messenger system of the AT1 receptor. In the second case, treatment with PD123319 would result in a decreased expression of the AT1 receptor, giving rise to a reduction of E_{max} . Preceding studies demonstrated that in vascular smooth muscle cells angiotensin II increased AT2 receptor protein levels. This effect was suppressed by the AT1 receptor antagonist losartan but not by the AT2 receptor antagonist PD123319, suggesting that angiotensin II influences AT2 receptor expression through the AT1 receptor.³¹ Additionally, Andresen et al. report that AT2 receptor cross-talk with AT1 receptors through a nitric oxide- and RhoA-dependent mechanism in a rodent preglomerular smooth muscle cell culture.³² These studies illustrate the complexity of the interplay between angiotensin II receptor subtypes.

Limitations

It has been reported that adrenergic response can be affected by atherosclerotic disease. The contractile response to potassium appears not to change with stage of disease. In this study, we found a strong correlation ($r=0.83$, $p<0.001$) between contractile response to phenylephrine and to potassium. Therefore, we feel we can express the response to angiotensin II as a percentage of the contractile response to phenylephrine.

Technically and logistically, it was not possible to perform the experimental protocols in all segments. Consequently, it was not possible to link endothelial function to other functional tests. However, it is important to notice that results from this paper and from previous other publications from our laboratory demonstrate that it is unlikely that manipulation of the arterial segments affects the functionality of the endothelium.^{17;33}

Conclusion

The present study demonstrated that although AT2 receptor is present in human internal mammary arteries, they do not mediate vasodilation in these arteries.

Acknowledgements

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CHAPTER 6

Addition of an Angiotensin Receptor Blocker to full-dose ACE-inhibition: Controversial or common sense?

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Abstract

Both angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers interfere with the activity of the renin angiotensin system in a different way. Theoretically, one might expect beneficial effects when they are used in combination, as a more complete suppression of the renin angiotensin system can be achieved. But can this additional effect still be seen in patients on full-dose ACE inhibition? Several controlled trials demonstrated that combination therapy can have additional benefits in hypertensive patients, chronic heart failure patients and in both diabetic and non-diabetic nephropathy. However, the clinical benefit was not always as pronounced as expected and not every patient will benefit from dual blockade of the renin angiotensin system. There is some evidence of a less pronounced effect of combination therapy when a full dose of the ACE-inhibitor is given. However, it is well known that ACE inhibitors cannot completely suppress the formation of angiotensin II, in particular when the renin-angiotensin system is activated. Indeed, clinical trials indicated that add-on therapy with an angiotensin receptor blocker was especially of use when the renin- angiotensin system remained activated despite full-dose ACE inhibitor treatment. In summary, combination of a full-dose ACE inhibitor and an angiotensin receptor blocker can be a rational choice in selected patients.

Introduction

One of the evolutionary goals of the development of the renin angiotensin system (RAS) is to protect the body by preserving salinity.¹ However, over the last few decades it has become clear that an activated RAS can provoke detrimental effects as well. Pharmacological blockade of the RAS has significantly improved prognosis of patients with cardiovascular disease.²⁻⁵ ACE inhibitors and angiotensin receptor blockers (ARBs) are capable of interfering with the activity of the RAS. At first sight, the modes of action of both ACE inhibitors and ARBs seem very similar, but after closer examination several differences are revealed. Theoretically, both groups could even have additional effects. Several studies confirmed this theoretical consideration. Others questioned whether the same effect is present when full-dose ACE inhibitors are used. Recent data from the ValHeFT trial indicated a trend towards a more pronounced effect of valsartan in patients with lower doses of the ACE inhibitor.⁶ However, this was a non-significant finding in a post-hoc analysis. Also, patients on lower doses of the ACE inhibitor might have reflected more diseased patients, with a higher activation of the RAS.

In the present paper, theoretical and practical considerations of add-on therapy with an ARB on top of an ACE inhibitor in hypertension, (non)-diabetic nephropathy, myocardial infarction, and heart failure will be discussed.

ACE inhibitors

The ACE, also known as kininase II exists in the entire human body in both free and membrane-bound form.^{7;8} ACE is one of the components of a complex system of regulating and counter-regulating mechanisms (Figure 1). It was originally thought that the majority of the effects of ACE inhibitors could be explained by the reduction of angiotensin II formation. However, in the early eighties, it was demonstrated that ACE inhibitors cannot fully suppress angiotensin II formation in hypertensive patients over a longer period of time.⁹ In normotensive male volunteers, ACE inhibition could not suppress angiotensin II increase during exercise.^{10;11} Interestingly, the chymase inhibitor nafamostat was able to significantly reduce angiotensin II formation in the same patients. In addition, increasing levels of both angiotensin II and aldosterone were eventually observed in a large proportion of patients.¹²⁻¹⁵ Jorde et al. demonstrated that even maximally recommended doses of ACE inhibitors could not completely prevent ACE-mediated angiotensin II formation.⁹ However, it is unknown whether

Bradykinin induces vasodilation by stimulating the formation of nitrogen oxide and metabolites of arachidonic acid in vascular endothelium.²⁶ Additionally, bradykinin induces natriuresis by direct tubular effects.²⁷ Thus, ACE regulates the balance between the vasodilative and natriuretic properties of bradykinin and the vasoconstrictive and salt-retaining properties of angiotensin II.

Beside destroying bradykinin, ACE also is the exclusive enzyme that catabolizes N-acetyl-Ser-Asp-Lys-Pro (AcSDKP). Results from a recent experimental study suggest that high levels of this peptide lower cardiac collagen content.²⁸ These findings explain the antifibrotic effects of ACE inhibitors.

Furthermore, ACE inhibitors lead to elevated angiotensin 1-7 levels.²⁹ This metabolite of both angiotensin I and II can stimulate the synthesis and excretion of vasodilatory prostaglandins, fortify the metabolic effects of bradykinin, and increase nitric oxide production. The clinical value of these final two additional effects needs to be clarified in future research.

Angiotensin II receptor blockers

Angiotensin II mediates its wide variety of effects through four angiotensin II receptors (AT1R, AT2R, AT3R en AT4R).³⁰ The functions of AT3R and AT4R remain unclear, but these receptors seem to be of secondary importance.^{31;32} In humans, the AT2R disappears after fetal stage, but expression increases in certain situations (e.g. coronary ischemia or heart failure).³³ In general, stimulation of AT2R and AT1R induces opposite effects. The AT1R is usually associated with the detrimental effects of angiotensin II. By selectively blocking AT1R, excess angiotensin II will stimulate AT2R. The currently available ARBs specifically block the AT1R. ARBs probably also block the stimulation of aldosterone release, since this pathway is mediated by the AT1R. Still, other stimulants, for example potassium, can also induce aldosterone release.³⁴ According to current views aldosterone is an important moderator of the unfavorable effects of angiotensin II.³⁵

Differences between ACE inhibitor and ARB

Previous findings demonstrate that ACE inhibitors and ARBs have a marked different pharmacology. Obviously, the most apparent difference is the primary point of action, which leads to a differentiated neurohormonal activation. Accordingly, in patients using an ACE inhibitor slightly reduced angiotensin II

concentration will be found, while in patients who are using an ARB, angiotensin II concentration will be increased.³⁶

The relatively high angiotensin II level in patients on ARB therapy offers the advantage of supplementary stimulation of AT2R. Since plasma angiotensin II levels are supposed to decrease in patients using an ACE inhibitor and consequently less AT2R stimulation will occur, this is the second distinction between both groups. A third difference is the increase in angiotensin 1-7 that will take place when an ACE inhibitor is used. However, in an animal model administration of losartan also induced a slight increase in angiotensin 1-7, but the clinical implications of this observation are not yet fully understood.³⁷

A fourth distinction between ACE inhibitor and ARB is their influence on bradykinin catabolism and the accompanying increase in nitric oxide. ACE inhibitors impede the breakdown of nitric oxide by a direct inhibiting effect.³⁸ In fact, some studies indicated that a substantial part of the effects of ACE inhibitors is contributed to the accumulation of bradykinin.²⁴ ARBs might also induce higher bradykinin levels, possibly through an AT2R-mediated mechanism, although the magnitude of this effect in clinical practice remains doubtful.³⁹

Dual RAS blockade and hypertension

Several studies demonstrated that the antihypertensive action of ACE inhibitors and ARBs is comparable to that of other blood pressure lowering compounds.^{40;41} Furthermore, it was demonstrated that blood pressure lowering using a dual RAS blockade strategy is more effective than using monotherapy.⁴²⁻⁴⁴ Another study showed that addition of an ARB to an ACE inhibitor is more effective than doubling ACE inhibitor dose.⁴⁵ This suggests that both medication groups exercise a different mode of action to achieve their goal. However, the most important question is whether the combination of ACE inhibitor and ARB is equally effective as combining an ACE inhibitor and another antihypertensive agent, for example a diuretic. Unfortunately, up till now no studies have addressed this question.

The combination of ACE inhibitor and ARB has shown to be more effective than the individual compounds in the treatment of microalbuminuric diabetics,⁴⁴ diabetic nephropathy^{46;47} and non-diabetic nephropathy.⁴⁸⁻⁵² In hypertensive patients, monotherapy was not as effective as the combination candesartan and lisinopril in reducing urinary albumin excretion.⁴⁴ These circumstantial findings link microalbuminuria to activation of the renin angiotensin system, but to our knowledge, no controlled studies have confirmed this linkage.

These studies suggest that both ACE inhibitor and ARBs are effective blood pressure lowering drugs when they are used as single-agent therapy. In the uncomplicated patient the combination of a diuretic agent and an ACE inhibitor or an ARB is advocated by the guidelines of the European Society of Hypertension⁵³. However, the complicated (diabetic) hypertension patient with (micro)albuminuria might benefit from dual RAS blockade as the combination is reportedly more effective as the use of either type of drug alone.

Dual RAS blockade after acute myocardial infarction

In VALIANT valsartan, captopril, and their combination were compared, when added to standard treatment within 10 days after acute myocardial infarction, complicated by heart failure.⁵⁴ Mortality and combined cardiovascular endpoints did not differ significantly in any of the groups, although significantly less patients were hospitalized in the combination group when compared to the patients on captopril. Therefore, in these patients, a substantial effect of combination therapy could not be demonstrated. Several explanations for these findings have been proposed.⁵⁵ The most likely explanation however, is the consistent finding that angiotensin II levels eventually rise when ACE inhibitors are administered chronically.^{9;56;57} For that reason, after myocardial infarction, during the early phase when cardiac remodeling occurs, ACE inhibitors might be able to suppress RAS activity adequately. Also, the untoward hypotensive effects of combination therapy might have counteracted possible beneficial effects. So acute myocardial infarction complicated by left ventricular dysfunction is not an indication for starting the combination ACE inhibitor and ARB. This might change when the condition progresses to chronic heart failure.

Dual RAS blockade and systolic heart failure

In systolic heart failure the RAS is activated.⁵⁸ Elevated levels of renin, angiotensin II, and aldosterone are accompanied by activation of the sympathetic nerve system, and by elevated blood levels of brain natriuretic peptide (BNP) and vasopressin. Despite current standard therapy (diuretic, beta-blocker, and ACE inhibitor) morbidity and mortality in heart failure remains high.⁵⁹ Roig et al. demonstrated that elevated angiotensin II concentrations despite ACE-inhibition in heart failure patients bears prognostic value.¹⁹ In the accompanying editorial we already suggested that combination of ARB and ACE inhibitor theoretically provides a more complete suppression of the RAS, while preserving the positive effects of bradykinin potentiation.^{60;61} This is probably

not true for every patient, but we hypothesize that especially patients with an activated RAS despite full-dose ACE-inhibition will benefit most from more complete suppression of the system, and thus from combination therapy.

Val-HeFT en CHARM demonstrated that by combining an ACE inhibitor and an ARB (valsartan and candesartan, respectively) a synergistic effect can be achieved, thus reducing the combined endpoint mortality and morbidity.⁶²⁻⁶⁴ A recent post-hoc analysis of Val-HeFT demonstrated a trend towards a more pronounced effect of combination therapy in patients with a left ventricular ejection fraction of <30%.⁶ This suggests that combination therapy is more effective in severe chronic heart failure, with an highly activated RAS. Both Val-HeFT and the ATLAS trial suggested that the difference in efficacy between intermediate and high dosed ACE inhibition is likely to be very small.⁶⁵ We recently demonstrated that increased angiotensin II levels in chronic heart failure patients were independent of dose, type or duration of ACE-inhibitor use.¹⁸

Dual RAS blockade in combination with beta blockade

Beta blockers are strong renin inhibitors.^{18;66-69} ELITE II already suggested that the combination of a beta blocker with an ACE inhibitor was better than the combination of a beta blocker and an ARB in patients with heart failure.⁷⁰ This might be due to residue angiotensin II formation despite ACE inhibition, which will not occur when the AT1R is blocked directly, and to positive feedback mechanisms that increase plasma renin activity. So, from a pharmacological point of view, the combination of an ACE-inhibitor with a beta blockers seems to be more appropriate than the combination of an angiotensin receptor blocker and a beta blocker. However, this hypothesis has not been tested in a randomized clinical trial. There have also been some concerns in using both an ACE-inhibitor, a beta blocker and an ARB ('triple therapy') in patients with chronic heart failure. Subgroup analyses of Val-HeFT suggested an increased mortality in the group of patients treated with 'triple therapy' compared to patients treated with an ACE-inhibitor and a beta blocker.⁶² However, both CHARM-added and VALIANT clearly demonstrated that 'triple-therapy' does not confer any additional risk.^{63;71}

Conceptual frame for combined use of ACE inhibitor and ARB

In the previous paragraphs, we concluded that combination of an ACE inhibitor and an ARB is not first choice treatment in all patients with hypertension, heart failure, or myocardial infarction. However, under certain circumstances the RAS will become progressively activated. In hypertensive patients, a small percentage (e.g. diabetic subgroup, renal dysfunction) may be at risk for developing high RAS activity. Several authors have demonstrated that in post-myocardial infarction patients, RAS activity remains higher when left ventricular dysfunction develops.^{72;73} In the majority of chronic heart failure patients RAS markers will rise as the disease advances. Particularly in these subgroups, dual RAS-blockade might be beneficial, whereas in the remaining subgroups more conventional therapies are preferred. This concept is illustrated in Figure 2.

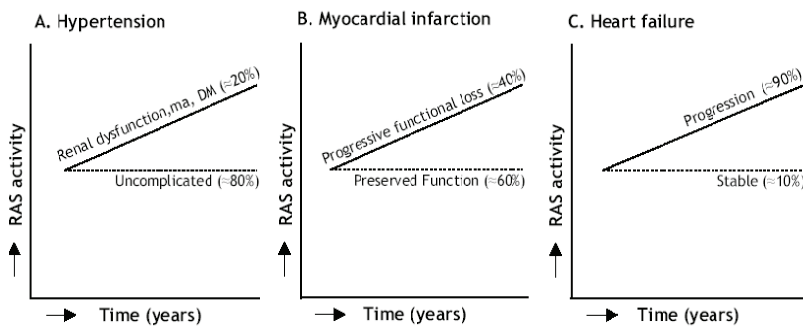


Figure 2. This conceptual figure illustrates that activity of the RAS in cardiovascular disease changes over time, and depends on severity of the disease and comorbidity. In uncomplicated hypertension RAS activity is fairly constant (panel A), but when hypertension occurs in combination with renal dysfunction, diabetes or microalbuminuria RAS activity might increase. Shortly after myocardial infarction the activity of the RAS will peak and then return back to baseline level. However, when progressive left ventricular dysfunction develops after myocardial infarction, RAS chronic heart failure patients elevated RAS parameters will be found (panel C). The higher the activity of the RAS, the more effect one might expect from dual RAS blockade.

Abbreviations: RAS, renin angiotensin system; ma, microalbuminuria; DM, diabetes mellitus

Indication	Indication for dual RAS inhibition?	References
Hypertension	Not standard, possibly in LVH, microalbuminuria, DM	42-44
Systolic heart failure	Yes, when symptoms persist despite diuretic, betablocker and ACE-inhibitor (when RAS remains activated despite ACE inhibition?)	62;63
Post-myocardial infarction	No	54;74
Nephroprotection (diabetics)	Yes	44;47
Nephroprotection (non-diabetics)	Yes	43;50-52

Table 1. Indications and evidence for combining ACE-inhibitor and Angiotensin Receptor Blocker
Abbreviations: RAS; Renin angiotensin system, CHF; chronic heart failure, LVH; left ventricular hypertrophy, DM; diabetes mellitus

Safety and risks

A few large trials indicated that when both ACE inhibitor and ARB were used, significantly more patients discontinued study medication.^{54;63} In these trials almost one in four patients discontinued study medication because of primarily hypotension, hyperkalemia, and renal dysfunction.⁷⁵ When a patient is treated with an ACE inhibitor and an ARB, the treating physician needs to be aware of these adverse effects and strict monitoring is warranted.

As dual RAS blockade is a relatively new concept, several questions remain unanswered. The duration of action of different ACE inhibitors and ARBs might influence the effect of different combinations. Furthermore, the effect of a combination might depend on the order and timing of intake of both agents.⁷⁶ Finally, more complete RAS-suppression and thus a higher dose or a shorter dosing interval might be needed to obtain organ protection, where lower doses are sufficient to treat hypertension.⁷⁷

Besides maintaining salt and fluid balance in the body, the RAS has undoubtedly more physiological implications of which we are not even aware of at the present time. Completely blocking the RAS might lead to side effects that will become evident only after a much longer time span than the duration of a scientific trial. These unknown potential side effects warrant a word of caution, in particular when triple blockade of the RAS is used.

Conclusion

First, ACE inhibitors and ARBs have a fundamentally different mode of action. ACE inhibitors initially reduce angiotensin II formation, but levels increase again over time, probably through non-ACE angiotensin II forming pathways (i.e. chymase). The persistent effects of ACE inhibitors, despite a rise in angiotensin II levels can be explained by several other modes of action of ACE inhibitors, such as the decreased breakdown of bradykinin. ARBs more effectively suppress angiotensin II mediated effects, but seem to have less pronounced bradykinin effects. Beneficial effects of combination therapy were demonstrated in hypertension, (non)-diabetic nephropathy, and chronic heart failure. Some studies indicated that the effects of ACE inhibitors were less pronounced when a full-dose ACE-inhibitor was given. In contrast, a large body of evidence suggests that ACE is not the rate-limiting step in the RAS cascade, and that elevated angiotensin II levels are not related to the dose of the ACE-inhibitor, but more to the activation of the RAS. Consequently, this leads to the concept that combination therapy will be more effective in conditions where the RAS is intensively activated. Clinically, combination therapy in uncomplicated hypertension is not a wrong choice, but other combinations may be better. In patients with an acute myocardial infarction, complicated by heart failure, combination therapy is not preferred. However, combination therapy seems to be a rational choice in patients with a chronic activation of the RAS, such as patients with renal disease and (severe) chronic heart failure.

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CHAPTER 7

Predictors of ACE inhibitor-induced reduction of urinary albumin excretion in non-diabetic patients

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Abstract

Urinary albumin excretion is considered to be a powerful predictor for cardiovascular mortality and morbidity. In the present study we investigated which parameters determine baseline urinary albumin excretion in non-diabetic subjects, without renal disease. In addition, we evaluated the parameters that predict the albuminuria lowering efficacy of an ACE inhibitor.

In this substudy of the Prevention of Renal and Vascular Endstage Disease Intervention Trial, 384 microalbuminuric patients were included. Patient and biochemical characteristics were obtained at baseline and after 3 months of double blinded, randomized treatment (fosinopril 20 mg or placebo).

Mean age was 51.1 ± 11.5 years and 65.6% was male. Median urinary albumin excretion was 22.2 (range 2.8 - 251.6) mg/24hr. Multivariate linear regression analysis demonstrated that at baseline only mean arterial pressure ($\beta_{\text{standardized}} = 0.161$, $p=0.006$), urinary sodium excretion ($\beta_{\text{standardized}} = 0.154$, $p=0.011$), and estimated renal function, were independently associated with urinary albumin excretion. In these predominantly normotensive to prehypertensive subjects, fosinopril reduced urinary albumin excretion with 18.5%, versus a 6.1% increase on placebo after 3 months ($p<0.001$). Fosinopril use and blood pressure reduction independently predicted the change in urinary albumin excretion. Baseline urinary albumin excretion independently predicted the antialbuminuric effect of fosinopril ($\beta_{\text{standardized}} = -0.303$, $p<0.001$). In conclusion, before ACE inhibition was started, sodium intake and blood pressure were positively associated with urinary albumin excretion. Fosinopril reduced urinary albumin excretion more than might be expected from its blood pressure lowering effect alone, and this effect was more outspoken in subjects with higher baseline urinary albumin excretion. Based on our data we hypothesize that ACE inhibition may result in superior cardiovascular protection when compared to other blood pressure lowering agents in subjects with higher baseline levels of albuminuria.

Introduction

In patients with primary renal disease and diabetic nephropathy it has been shown that proteinuria predicts future renal function decline.¹ Moreover, it has been shown that a decrease in proteinuria induced by ACE inhibitors in the short-term predicts long-term efficacy of such treatment on renal function outcome.² The more proteinuria is lowered, the better the prognosis with regard to renal function. These observations have led in clinical practice to pursue a maximal antiproteinuric response on angiotensin-converting enzyme (ACE) inhibitors in renal patients, by co-prescribing treatments that are known to increase the antiproteinuric efficacy of this class of drugs, e.g. sodium restricted diet and diuretics. Interestingly, it has also been shown that in the subjects with diabetes or hypertension and even in the general population urinary albumin excretion (UAE) predicts future cardiovascular events.³⁻⁵ It was also suggested that in such subjects a short-term decrease in UAE with a renin angiotensin system blocker predicts long-term cardiovascular outcome.⁶

Factors that determine the response to ACE inhibitors have been examined mainly in patients with renal disease. In subjects on a low sodium diet and with a high baseline plasma renin activity, for example, a more pronounced antiproteinuric response is obtained.^{7,8} Whether this is also the case in microalbuminuric subjects in the general population is yet unknown. The mechanism by which albuminuria originates in renal patients with renal disease and in subjects from the general population may well be different. Whereas in patients with renal disease urinary protein loss is the result of specific glomerular damage, in non-renal patients microalbuminuria has been mentioned to be a consequence of generalised endothelial dysfunction.⁹ Also, the efficacy of ACE inhibitors to lower urinary protein loss may be different between proteinuric patients with renal disease and microalbuminuric subjects. Whereas in patients with renal disease it is generally accepted that ACE inhibitors lower UAE more than may be expected from the blood pressure lowering effect of these drugs alone, this issue is debated in microalbuminuric subjects.¹⁰

Given these considerations we investigated in non-diabetic subjects from the general population, without renal disease, which patient characteristics are associated with UAE in the untreated situation, and which characteristics predict a reduction in UAE after 3 months of ACE inhibitor treatment. Such knowledge may be helpful to optimise treatment for the prevention of cardiovascular disease. For this purpose we used data from the Prevention of Renal and Vascular ENdstage Disease Intervention Trial (PREVEND IT).

Methods

Population

The study population was selected from the PREVEND IT study, an investigator initiated, randomized trial performed between November 2000 and February 2004 that used a two-by-two factorial design to compare the effect of statin therapy (40 mg of pravastatin orally per day) and placebo therapy and of ACE inhibitor therapy (20 mg of fosinopril orally per day) and placebo on urinary albumin excretion and the risk of cardiovascular events in microalbuminuric patients. In total, 864 subjects who presented with persistent microalbuminuria during the screening phase (once a urinary albumin concentration > 10 mg/l in a first morning void urine sample and at least once 15-300 mg/24 hours in two subsequent 24 hour urines), and who provided written informed consent were enrolled. Exclusion criteria were: hypertension and hypercholesterolemia, as defined according to prevailing guidelines for Dutch general practitioners at the design of the study (respectively, SBP \geq 160 mmHg, DBP \geq 100 mmHg, or use of antihypertensives, and a total cholesterol \geq 8.0 mmol/L, or \geq 5.0 in case of a previous myocardial infarction), as well as known diabetes. Details of the study design have been presented previously.^{11;12} Of note, subjects with known renal disease were excluded from participation, as well as subjects with UAE \geq 300 mg/24hr, as this might indicate presence of a primary renal disease. As part of the protocol, fasting blood samples were obtained at randomization. Plasma samples were stored at -80°C until assayed. For these laboratory assessments, that were not part of the initial protocol of the PREVEND intervention trial, we used plasma obtained at baseline, before subjects were randomized to treatment, and for albuminuria also after 3 months of double-blind treatment, a period assumed to be adequate for the effect of ACE inhibitor therapy on this variable to be established. For logistic reasons, we decided to restrict the analysis to plasma samples of 400 participants of the total cohort (from all 4 randomized groups we used the samples of the final 100 patients that were included) who had a plasma sample available at both baseline and 3 month visit. As the patients were randomly assigned to each treatment arm, this methodology guarantees that proper randomisation is maintained and that the results will not be biased. Since we wanted to study a non-diabetic population, all subjects not previously known with diabetes, but with a baseline fasting plasma glucose \geq 7.0 mmol/l were excluded from this analysis. Thus, 384 patients were eligible for the present study. All participants gave written informed consent. The PREVEND IT Study was approved by the local medical ethics committee, and was conducted in accordance with the guidelines of the Declaration of Helsinki.

Laboratory methods

Urinary albumin concentration was determined using nephelometry with a threshold of 2.3 mg/L, and intra- and inter-assay coefficients of less than 2.2% and 2.6%, respectively (Dade Behring Diagnostics, Marburg, Germany). High-sensitivity CRP was also analysed by nephelometry (BNII, Dada Behring, Marburg, Germany). Plasma glucose, serum cholesterol, and serum creatinine were determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York, USA). A validated assay was used to determine plasma renin activity.¹³ Urinary sodium and urea measurements were done with a MEGA clinical chemistry analyser (Merck, Darmstadt, Germany). Sodium was determined by indirect potentiometry and urea by a photometric test with the urease-GIDH method. Urinary albumin, sodium and urea are given as the mean of two 24 hr collections. At baseline, before study medication was started, each subject collected two sets of two 24 hour urine samples. To avoid spurious associations between dependent and independent variables, urinary sodium and urea were determined in the first two collections, whereas urinary albumin concentration was determined in the last two 24 hr urine collections.

Definitions and calculations

Systolic and diastolic blood pressure measurements were calculated as the mean of the last 2 out of 10 consecutive measurements with an automatic Dinamap XL model 9300 series device (Johnson-Johnson Medical Inc.). Mean arterial pressure (MAP) was calculated as $(1/3 \times \text{SBP}) + (2/3 \times \text{DBP})$. Renal function was estimated using the simplified MDRD formula (Modification of Diet in Renal Disease): $\text{eGFR (mL/min/1.73m}^2) = 186.3 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times 0.742$ if female.¹⁴ Body mass index (BMI) was calculated as the ratio of weight and the square of height (kg/m^2). Protein intake was estimated using the method proposed by Maroni.¹⁵

Statistical analysis

Baseline characteristics are given as means \pm standard error. In case of a skewed distribution the median (interquartile range) was used. Differences between groups were tested for continuous data by Student's t test or a Mann-Whitney rank test in the case of skewed distribution. Differences in prevalence or incidence were tested with a chi-square test. We initially used Pearson correlation coefficients to evaluate the relationship between UAE and other parameters at baseline. In case of a skewed distribution values were log-transformed. To explore the association between UAE and patient characteristics in detail, stepwise backward multivariate linear regression analysis was used with UAE as the dependent variable. What patient characteristics are associated with ACE inhibitor-induced changes in UAE after 3 months of treatment was studied

using the same statistical methods. For these latter analyses we corrected for assignment to pravastatine or placebo. To examine a possible non-linear relationship between dependent and independent variables, we also entered quadratic terms of each of the independent variables into each model, and tested these quadratic terms for statistical significance. Models were furthermore tested for interaction of independent variables by entering product-terms into the regression equation. Interactions were considered significant at $p < 0.10$. All p values were two-tailed. All analyses were performed using SPSS version 11.5 software (SPSS, Chicago, IL, USA).

Results

Population

Table 1 shows the mean baseline patient characteristics of the whole group and of both groups when stratified according to fosinopril use or placebo to which they were randomised. Beside a significant difference in serum total cholesterol and LDL levels, no differences were found between the two groups that later during the study were to receive fosinopril or matching placebo. Of note, our population consisted of subjects that are assumed to be relatively healthy, as can be concluded from their average blood pressure (MAP 94.6 ± 11.6 mmHg), serum cholesterol level (5.87 ± 1.04 mmol/L), and the fact that only 2 patients had a medical history showing a myocardial infarction, 4 a stroke and 4 patients peripheral vascular disease. Subjects were excluded from participation in the PREVEND IT study in case they had known diabetes, hypertension and or hypercholesterolemia. For the latter two criteria cut-offs were used as defined in prevailing guidelines for Dutch general practitioners at the design of the study (see methods section). However, when expressed as categories according to the present JNC-7 classification, it appeared that in our population 138 patients were normotensive, 123 prehypertensive, and 123 hypertensive.¹⁶ Of our patients, 16.2% were found to be obese (body mass index >30 kg/m²). In the groups that were to receive fosinopril or matching placebo during the study randomisation to pravastatin or matching placebo was well balanced, with 50.5% of subjects in the fosinopril that were to receive pravastatin and 50.0% in placebo group. Of note, the use of pravastatin did not induce a change in UAE (in patients not using fosinopril median UAE was 20.8 mg/24hr at baseline and 20.4 mg/24hr after 3 months of pravastatin treatment). Furthermore, no interaction was found between the effect of ACE inhibition and HMG CoA reductase inhibition, allowing us to study the effect of ACE inhibition separately. The effect of pravastatin on UAE in PREVEND-IT has recently been described by Aththobari et al.¹⁷

Variables	Total group (n=384)	Fosinopril (n=192)	Placebo (n=192)
Age	51.1±11.5	50.9±11.7	51.3±11.3
Male gender (%)	252 (65.6)	128 (66.7)	124 (64.6)
Caucasian (%)	369 (96.1)	179 (93.2)	190 (99.0)
Current smokers (%)	123 (32.0)	65 (33.9)	58 (30.2)
BMI (kg/m ²)	26.4±4.3	26.5±4.3	26.4±4.2
Blood pressure			
Systolic	131.3±18.4	130.7±17.7	131.9±19.1
Diastolic	76.3±9.7	76.3±9.6	76.3±10.0
Lipids (mmol/l)			
Total cholesterol	5.87±1.04	5.98±1.06 [‡]	5.75±1.01
LDL cholesterol	4.15±0.94	4.25±0.98 [‡]	4.05±0.90
HDL cholesterol	1.03±0.31	1.01±0.32	1.05±0.29
Triglycerides	1.55±1.06	1.60±1.01	1.50±1.11
Serum creatinine (µmol/l)	90.3±14.0	91.4±14.3	89.2±13.6
eGFR (ml/min/1.73m ²)	94.3±23.3	93.2±23.7	95.4±22.9
U _{Na/24hr} (mmol/24hr)	155.0 (45.0-386.9)	157.5 (56.2-372.9)	154.9 (45.5-386.9)
U _{Urea/24hr} (mmol/24hr)	404.3 (144.2-739.5)	410.9 (144.2-739.5)	393.3 (173.4-727.9)
UAE	22.2 (2.8-251.6)	23.2 (3.5-251.6)	20.2 (2.8-237.6)
Beta blocking agent (%)	1.6	0.5	2.6
Nitrate (%)	0.5	1.0	0.5
Diuretic (%)	0.5	1.0	0
Digoxin (%)	0.5	0.5	0.5
Anti-platelet agent (%)	1.8	1.0	2.6

Table 1. Baseline characteristics of all patients, and of patients allocated to placebo and fosinopril treatment.

Values are shown as mean ± SD or as percentage, when appropriate.

U_{Na/24hr}, U_{Urea/24hr}, and UAE are shown as median (range).

([‡] p<0.05, when compared to placebo)

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; U_{Na/24hr}, 24 hour urinary sodium excretion; U_{Urea/24hr}, 24 hour urinary urea excretion; UAE, urinary albumin excretion

Variables associated with baseline UAE

The results of the univariate and multivariate linear regression analyses are shown in Table 2. Only three baseline variables remained statistically significant in the multivariate model. Mean arterial pressure and urinary sodium excretion were positively associated with UAE (p=0.006 and 0.011, respectively), implying that higher baseline blood pressure and sodium intake are associated with higher levels of albuminuria. A negative association was found between eGFR

and UAE ($p=0.018$). Of the other variables BMI and CRP reached near statistical significance ($p=0.076$ and 0.077 , respectively). Estimated protein intake (as well as urinary urea excretion) did not univariately affect baseline UAE, but exhibited strong colinearity with urinary sodium excretion. Therefore this term was removed from the multivariate model. Of note, when estimated protein intake (or urinary urea excretion) was forced into the definite multivariate model, while deleting urinary sodium excretion, again no significant association was found between baseline estimated protein intake and UAE. No significant interactions were found and no curved relationships between dependent and independent variables were identified.

Baseline variable	Univariate linear regression		Multivariate linear regression	
	$\beta_{\text{standardized}}$	p-value	$\beta_{\text{standardized}}^*$	p-value*
Age	0.144	0.005		
Gender	0.002	0.976		
Smoking	0.067	0.191		
BMI	0.265	<0.001	0.117	0.076
MAP	0.259	<0.001	0.161	0.006
sMDRD	-0.118	0.021	-0.132	0.018
PRA	0.006	0.899		
CRP	0.205	<0.001	0.104	0.077
Cholesterol	0.069	0.179		
Glucose	0.014	0.783		
$U_{\text{Na}/24\text{hr}}$	0.154	0.003	0.154	0.011

Table 2. Predictors of baseline urinary albumin excretion in 384 non-diabetic microalbuminuric patients.

* $\beta_{\text{standardized}}$ and p value are only shown when $p < 0.10$

Abbreviations: BMI, body mass index; MAP, mean arterial pressure; sMDRD, simplified MDRD; PRA, plasma renin activity; CRP, C-reactive protein; $U_{\text{Na}/24\text{hr}}$, 24-hour urinary sodium excretion

UAE reduction by ACEi: relation with blood pressure

After 3 months of treatment we observed a median UAE reduction of 18.5% in patients using fosinopril, while in patients assigned to placebo a 6.1% increase was observed ($p < 0.001$). In the placebo treated patients MAP decreased by -0.7 mmHg, whereas with the ACE inhibitor a decrease in MAP of -5.0 mmHg was observed ($p < 0.001$). Both fosinopril use ($\beta_{\text{standardized}} = -0.185$, $p < 0.001$) and change in mean arterial pressure after 3 months ($\beta_{\text{standardized}} = 0.151$, $p = 0.004$) were independent predictors of change in UAE after bivariate linear regression analysis. Both remained significant after multivariate analysis, correcting for age and gender.

Baseline predictors of response to ACE inhibitor

The results of the multivariate linear regression analysis are shown in Table 3. Both in univariate and multivariate regression analysis baseline UAE appeared to be the only independent predictor of the change in UAE: the higher baseline albuminuria, the more the percentage decrease in UAE with ACE inhibition. Again, no significant interactions were found and no curved associations were identified.

Baseline variable	Univariate linear regression		Multivariate linear regression	
	$\beta_{\text{standardized}}$	p-value	$\beta_{\text{standardized}}^*$	p-value*
Age	-0.080	0.277		
Gender	0.003	0.963		
Smoking	0.032	0.666		
BMI	-0.038	0.611		
Pravastatin	0.129	0.078		
MAP	-0.097	0.188		
sMDRD	0.028	0.701		
PRA	0.090	0.219		
CRP	0.026	0.747		
Cholesterol	-0.018	0.802		
Glucose	0.016	0.830		
U _{Na/24hr}	-0.026	0.003		
UAE	-0.265	<0.001	-0.303	<0.001

Table 3. Baseline predictors of change in urinary albumin excretion after 3 months of fosinopril treatment in 192 non-diabetic microalbuminuric patients.

* $\beta_{\text{standardized}}$ and p value are only shown when p<0.10

Abbreviations: BMI, body mass index; MAP, mean arterial pressure; sMDRD, simplified MDRD; PRA, plasma renin activity; CRP, C-reactive protein; U_{Na/24hr}, 24-hour urinary sodium excretion; UAE urinary albumin excretion

Discussion

In the present study we demonstrate that in a non-diabetic population without renal disease baseline UAE is associated with mean arterial blood pressure, renal function, and, interestingly, urinary sodium excretion. Furthermore, we found that ACE inhibition decreased UAE more than might be expected from blood pressure reduction alone and that higher baseline UAE predicted a more outspoken albuminuria lowering response to ACE inhibition.

UAE has been shown to be an early risk marker of cardiovascular morbidity and mortality,^{18;19} and is thought to reflect widespread endothelial damage.⁹ For that reason reduction of UAE may be interpreted as a recovery of endothelial function, which is in line with the observation that lowering UAE by blocking

the renin angiotensin system improves cardiovascular prognosis in high risk patients.²⁰ The PREVEND IT study demonstrated that fosinopril reduced UAE effectively in microalbuminuric subjects.¹² In addition, fosinopril treatment was associated with a trend in reducing cardiovascular events in this population. As biological covariates of UAE, it is not surprising to see mean arterial pressure and baseline renal function arise as independently associated with baseline UAE, as these factors are widely acknowledged to be causally related to UAE.^{21;22} Only few studies have mentioned an association between UAE and sodium intake.^{23;24} This is an important finding as urinary sodium excretion is a reflection of sodium intake, which is, of course, modifiable. The strength of this finding is the fact that urinary sodium excretion was calculated as the mean of two 24 hour collections and UAE was calculated as the mean of two different 24 hour collections. This excludes the possibility that the observed association between baseline UAE and urinary sodium excretion is spurious and based on artefacts in 24 hour urine collections.

In addition to identifying independent predictors of baseline UAE, we also demonstrate that the use of fosinopril and the change in mean arterial pressure are independent predictors of the change in UAE after 3 months of treatment. This observation supports the view that blood pressure lowering obtained by intervention in the renin angiotensin system has an additive albuminuria lowering effect over reduction of blood pressure alone. The superior antiproteinuric effects of ACE inhibitors have been acknowledged for a long time. But only in diabetic patients and in patients with renal disease this characteristic of ACE inhibitors has been shown to be independent of blood pressure reduction.²⁵⁻²⁷ Our data suggest that in non-diabetic, nonrenal subjects the antialbuminuric effect of blockade of the renin angiotensin system cannot completely be attributed to the reduction in arterial blood pressure.

Besides these two important findings discussed so far, we also identified baseline UAE as the only independent predictor of the change in UAE after three months of fosinopril treatment. Before conclusions can be drawn, it is important to investigate whether the association we found was biased. For instance, a similar association can be found in case regression-to-the-mean plays a role. Subjects were included in case they had a baseline UAE ≥ 15 mg/24hr. Due to applying a cut-of value, it is to be expected that during follow-up on average a decrease in UAE will take place, due to regression-to-the-mean. We analysed whether such bias may have played a role. In Figure 1A we depicted changes in UAE at three months of follow-up separately for ACE inhibitor and placebo treated subjects, with these subjects divided into tertiles of baseline UAE.

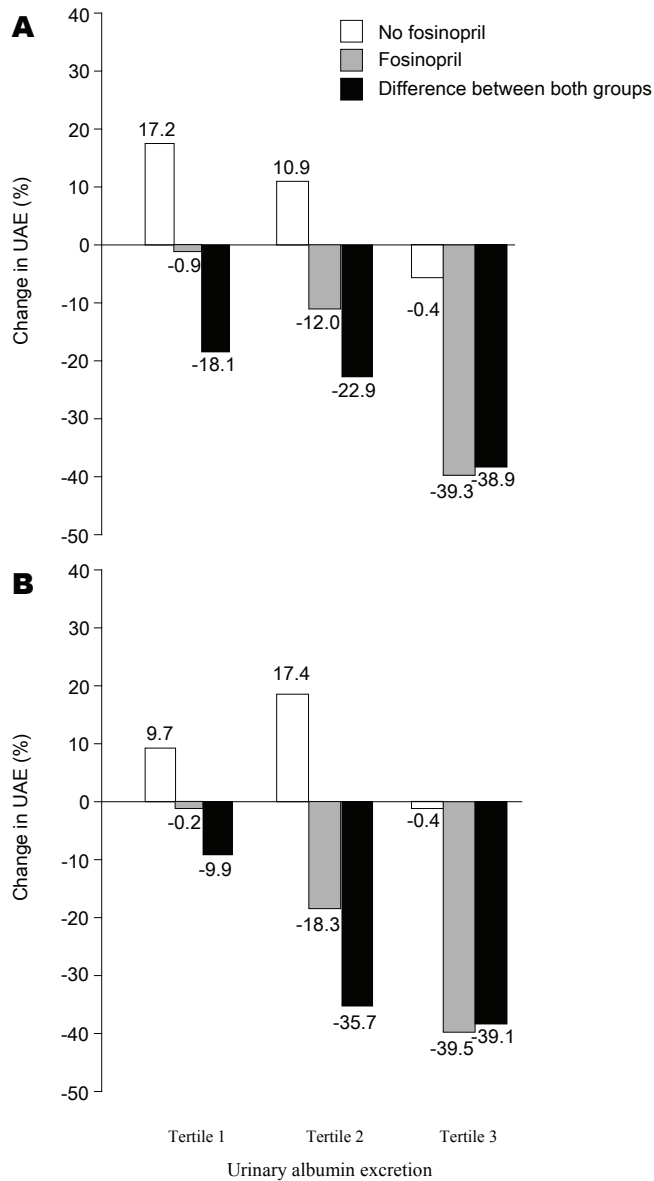


Figure 1. Figure showing the positive association between baseline UAE (divided into tertiles) and the change in UAE after 3 months treatment with placebo (white bars), or fosinopril (grey bars). Furthermore, the effect of fosinopril corrected for placebo is shown (black bars). The association between baseline UAE and change in UAE after three months of treatment was more obvious in fosinopril users than in subjects using placebo (panel A). To test the robustness of this observation we repeated the analysis after exclusion of all subjects in whom urinary creatinine excretion in the 24 hour collections differed by more than 20% (panel B).

This figure shows that in placebo treated subjects at higher levels of UAE a reduction in UAE was observed. The reduction in ACE inhibitor treated subjects was however, more outspoken. Adding the changes induced by placebo and ACE inhibitor in each tertile of baseline UAE, thereby correcting for the placebo effect, indicates that at higher baseline UAE levels ACE inhibition induces more reduction in albuminuria. In addition, a sensitivity analysis was conducted to evaluate the robustness of our findings and to eliminate the possibility of spurious associations due to urine collection errors. Therefore, we repeated the above analysis after exclusion of all subjects in whom 24hr urinary creatinine excretions at baseline and after three months treatment differed by more than 20%. This analysis shows that the association between baseline UAE and ACEi induced changes in UAE remained (Figure 1, panel B). This sensitivity analysis was done based on the assumption that the within-person day-to-day 24hr creatinine excretion is nearly constant. Thus, our findings that subjects with higher baseline UAE will experience more benefit from fosinopril treatment seems quite robust and not spurious.

Interestingly, our data on a baseline UAE-dependent beneficial effect of ACE inhibitors seem in line with studies in different populations and with different outcome parameter. In patients with non-diabetic renal disease it has been suggested that the extra renoprotective effect of ACE inhibitors over other blood pressure lowering agents is dependent on baseline proteinuria.²⁸ A post-hoc analysis of the HOPE Study, which was performed in subjects at high risk for cardiovascular disease, shows that the cardioprotective effect of ACE inhibition was larger in subjects with microalbuminuria compared to subjects without microalbuminuria, both with respect to absolute, as well as to relative risk reduction.^{20;29} In analogy, Asselberg et al noted that in PREVEND IT fosinopril reduced the incidence of cardiovascular events by 29% (from 5.1% to 3.6%) in subjects with an albuminuria level below 50 mg/24hr, but with 60% in subjects with a higher albumin excretions.¹² Our data, in combination with these data from literature, suggest that ACE inhibition may confer a beneficial effect on renal and cardiovascular endpoints beyond the effect that may be expected from blood pressure lowering alone that is dependent on baseline albuminuria levels. Importantly, baseline plasma renin activity was not associated with baseline UAE and did not predict the fosinopril induced reduction in albuminuria independently. Thus, a patient with an activated circulating renin angiotensin system does not necessarily have higher baseline UAE, nor does he experience a greater antialbuminuric reaction when using an ACE inhibitor. An association between plasma renin activity and UAE has previously been suggested in hypertensive patients,³⁰ but our findings do not support the existence of such

an association in a predominantly normotensive to prehypertensive population. Additionally, we specifically questioned whether an interaction between sodium intake and activation of the RAS might play a role in the association between sodium intake and albuminuria. However, this was not the case.

To appreciate the value of the current study a few limitations need to be considered. First, our results should be interpreted with caution as this is a post-hoc analysis of prospective data. The data provided should therefore be regarded as only hypothesis-generating. Second, biological variability in terms of within-subject variability of UAE remains an issue. The variation of this biological marker can be as high as 40%.³¹ Misclassification due to variability may have caused underestimation of some of the observed associations. Third, our results with respect to sodium intake should be seen in the context that the subjects under study remained on their usual diet. Whether the antialbuminuric response to ACEi might be augmented by co-prescription of a salt restricted diet can therefore not be excluded. Fourth, the PREVEND IT Study was performed in a predominantly Caucasian and normotensive to prehypertensive population. Whether our observations hold true for other populations needs therefore corroboration.

Perspectives

We found that mean arterial blood pressure, urinary sodium excretion, and low renal function are independently associated with UAE. Since albuminuria has been shown to be a strong risk marker for future cardiovascular events, we hypothesize that in non-diabetic, microalbuminuric patients without renal disease reduction of sodium intake may be a sensible first approach. However, this hypothesis needs to be evaluated in prospective investigations.

Controversy exist as to whether ACE inhibitors in subjects without renal disease reduce UAE more than might be expected from their blood pressure-lowering action alone. Our analysis demonstrates that fosinopril reduces UAE, partially independent of its antihypertensive effects. The only baseline variable that independently predicts the antialbuminuric effect of fosinopril in this study was found to be baseline UAE. These latter findings have two consequences, when one takes into consideration that recent investigations demonstrated that a short-term change in albuminuria after initiation of blood pressure lowering treatment predicts long-term cardiovascular outcome.^{6;32} On the one hand it is reassuring, as it suggests that ACE inhibition will be cardioprotective in a broad population, irrespective of age, gender, or any other biological characteristic. On the other hand, these data suggest that ACE inhibition may

result in superior cardiovascular protection when compared to other blood pressure lowering agents in subjects with higher baseline levels of albuminuria. This intriguing hypothesis has to be tested though in prospective trials studying the cardioprotective effect of an ACE inhibitor compared to that of other blood pressure lowering agents, in which participating subjects grouped into strata according to baseline albuminuria.

Conclusions

In non-diabetic patients without renal disease, the main predictors of baseline UAE are arterial blood pressure, renal function, and sodium intake. ACE inhibition effectively lowers UAE. This antialbuminuric effect is found to be more than may be expected from blood pressure lowering alone, especially in patients with higher baseline levels of albuminuria.

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CHAPTER 8

General discussion

General discussion

In the present thesis several aspects of dysfunction of the cardiorenal axis are discussed, mainly focussing on the renin angiotensin system and on elevated urinary albumin excretion. In addition, we provide further evidence that even mild renal dysfunction carries a worse long term prognosis. We demonstrate that elevated urinary albumin excretion (UAE) is a common finding in severe chronic heart failure patients, and that spironolactone use activates a RAS feedback mechanism thus leading to high angiotensin II levels in these patients.

Since blood pressure is an important factor in the pathophysiology of UAE, we used an in vitro model, to evaluate some functional aspects of the AT₂ receptor. The vasoactivity of the AT₂ receptor has been the subject of study in human arteries only once before.¹ Most of the textbooks state that stimulation of the AT₂ receptor opposes the effects of the AT₁ receptor. We demonstrated that the AT₂R does not mediate vasodilation in human internal mammary arteries. Given the conflicting results that have been published in literature over the last few years,² it seems safe to conclude that vasoactive competence of the AT₂ receptor is inferior to that of the AT₁ receptor.

Three important general matters considering UAE also need to be discussed here. First, in the present thesis we did not evaluate or discuss the methods we used for measuring UAE. Several methods have been described and they all have their (dis)advantages.³ The golden standard, however, continues to be the procedure of collecting a 24 hour urine, which reduces the influence of circadian rhythm.⁴ We have used several different collection methods in this thesis and we realize that this may have influenced the outcome.

The second issue that needs to be addressed is the current status of increased UAE. Without a doubt the finding of elevated UAE predicts the occurrence of future cardiovascular events. Therefore, elevated UAE seems to link renal to cardiovascular damage. Yet, it is still unclear whether UAE is a risk marker or a risk factor for cardiovascular disease. In diabetics with or without chronic renal disease reduction of UAE by blocking the RAS is accompanied by an improvement of renal prognosis.⁵⁻⁷ A causal link between renal damage and UAE may be suspected and accordingly, elevated UAE is considered to be a risk factor for renal damage. In hypertensive patients and in the general population the pathophysiological role of elevated UAE as a cardiovascular prognostic tool is more controversial. In hypertensive patients UAE is often thought to be a reflection of generalized endothelial and vascular dysfunction.⁸ In accordance with this theory, some authors found increased transcapillary escape rates of

albumin,⁹ others found elevated levels of markers of inflammation (e.g. CRP) in microalbuminuric patients,¹⁰ and some found elevated levels of markers of endothelial dysfunction in diabetic patients with elevated UAE.¹¹ To date, the question whether urinary albumin excretion simply is an epiphenomenon or actually causes cardiovascular disease has not been answered yet.

Third, as we demonstrated in Chapter 7, a large proportion of the UAE reducing potency of ACE inhibitors is the result of the intrinsic blood pressure-lowering effect of these agents. Specific postglomerular vasodilation and concomitant transglomerular pressure reduction, are thought to be responsible for their superior antialbuminuric properties. Yet, other non-blood pressure-related mechanisms such as reduction of Transforming Growth Factor β_1 and Vascular Endothelial Growth Factor, may be involved as well in the renoprotective effects of ACE inhibitors and angiotensin receptor blockers (ARBs).^{12;13} Whether UAE can be used as a marker of endothelial dysfunction remains a question, especially when patients are already using an ACE inhibitor or an ARB, as the specific renal effects of these agents may conceal changes in UAE due to improvement of endothelial function. In the present thesis no clues are found that circulating RAS parameters are involved in this process.

The relationship between elevated UAE and the activity of the RAS merits discussion as well. Even in patients with an activated RAS we did not find a direct association between activity of the systemic RAS and UAE. This leads us to believe that blockade of systemic angiotensin I breakdown into angiotensin II seems to be of minor importance in the beneficial cardiovascular action of ACE inhibitors that has been reported.^{14;15} However, locally generated angiotensin II may be involved in the process that leads to extravasation of plasma albumin and consequent inflammation and atherosclerosis. In parallel to this theory, the RAS may still play a role in the renoprotective properties of these agents as well. In this context, it is important to gain knowledge of the association between circulating and local RAS activity. It is tempting to assume that circulating RAS activity reflects the activity of the local cascade, but assessing local renal and vascular RAS activity in vivo remains a technical challenge.

Importantly, measuring the parameters of the RAS in a venous blood sample is not as easy as it seems. Both physiological and biochemical factors may influence the levels measured, which renders them less useful as markers in a routine clinical setting. These two limitations may partly explain the lack of association between RAS activity and UAE. A third possibility involves the pathophysiological mechanism underlying the effect of ACE inhibitors: the favorable effects of these agents on the RAS may be overestimated, while we should be focusing on the kallikrein kinin system.¹⁶

Future considerations

Measuring UAE is a routine test for nephrologists, and the most recent European Society of Cardiology hypertension guidelines recommend that this test should be performed in all hypertensive patients (and is essential in all diabetic patients).¹⁷ However, many cardiologists still have to discover the value of the test in daily clinical practice. This simple and inexpensive test may be useful in the decision to start therapy, and even to monitor the effect of the prescribed pharmacological intervention. A second advantage of collecting urine is the possibility (or opportunity ?) to evaluate individual sodium excretion, and estimate sodium (and protein) intake (thus assessing dietary compliance), especially in chronic heart failure patients.

Many studies indicate that medications that reduce UAE may provide significant long term cardiovascular benefits for patients with hypertension, diabetes or chronic kidney disease.¹⁸⁻²⁶ Given the beneficial effects of ACE inhibitors and ARBs it will be interesting to see whether the novel RAS inhibitors, such as selective aldosterone receptor blockers and the latest oral renin inhibitors, will also provide end organ protection. Preliminary studies in rats and humans indicate that both eplerenone and aliskiren may protect the kidney,^{27,28} and that eplerenone may have additional cardiovascular protective properties in selected patients.²⁹

Obviously, prevention of (the progression of) end organ damage is an important issue. However, the PREVEND Intervention Trial study demonstrated that in mainly non-diabetic, normotensive, microalbuminuric subjects, fosinopril-induced UAE reduction was also associated with a trend in reducing cardiovascular events.¹⁵ These results suggest that we should screen the general populations for elevated UAE and subsequently turn the subjects found positive into patients and actually treat them with an ACE inhibitor (or an ARB?). Even though pharmaco-economic analysis of the PREVEND study suggested that this strategy may be cost-effective,³⁰ other authors found paradoxical results.³¹ In the present thesis we demonstrate that in normotensive, non-diabetic subjects UAE is determined by sodium excretion, and thus sodium intake. This suggest that modifying the patients diet should be the first sensible step to take, instead of prescribing medication. To reduce the burden on the health care system, future studies should address this issue, and selection criteria for screening patients that qualify for primary prevention should be developed. In secondary prevention, the importance of UAE reduction has been recognized for years.

In chronic heart failure patients the cause of UAE and the value of measuring UAE, need further exploration. In these patients and in diabetics, current guidelines already recommend starting an ACE inhibitor or an ARB,^{32;33} so reduction of UAE in these patients may be a complimentary side effect. However, we do not know whether cardiovascular protection requires the same dosage as renoprotection.

Another issue that still needs to be resolved is the selection of patients that may benefit from combination therapy. Indeed, recent clinical trials suggest superior renoprotective and cardioprotective effects^{34;35} of dual RAS blockade in selected populations, but no selection criteria for the individual patient are currently available. Clearly, further studies are needed to answer this question and to define optimal treatment protocols.

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CHAPTER 9

Summary
Samenvatting
Acknowledgements
Abbreviations
Bibliography

Summary

The kidneys are responsible for regulating the body's fluid volume, mineral composition and acidity. In order to do so, the kidneys depend on an adequate cardiac output. When the cardiac output is reduced, the interplay between kidneys and endocrine tissue will provoke activation of the renin angiotensin system. This neurohormonal cascade forms a strong link between the heart and kidneys. For example, patients with end-stage renal disease are at increased risk of developing cardiovascular disease. Having a less severe renal dysfunction also carries a reduced cardiovascular prognostic value. In Chapter 2 we evaluate the prognostic value of preoperative renal function in over 400 patients undergoing coronary artery bypass grafting (CABG). This post-hoc analysis demonstrates that even a mild renal dysfunction will negatively affect (cardiovascular) prognosis of patients after CABG surgery.

When a chronic heart failure patient develops microalbuminuria, this is usually accompanied by an impaired prognosis as well. Several theories deliberate on the possible origin of microalbuminuria in these patients. One of these theories states that elevated urinary albumin excretion is a reflection of generalized endothelial dysfunction, while another theory suggests hyperfiltration as the underlying mechanism. Possibly, intact nephrones are forced to take over the filtration function of damaged nephrones, which leads to urinary albumin loss. In Chapter 3, a study with 96 severe chronic heart failure patients is described, in which we evaluate the prevalence of microalbuminuria. In 32% of these patients we found elevated urinary albumin concentrations. When this percentage is compared to the prevalence of microalbuminuria in the general population (represented by an age-matched group derived from the PREVEND study) it becomes clear that microalbuminuria is significantly more prevalent in chronic heart failure patients. Other authors demonstrated that antagonists of the renin angiotensin system, such as ACE inhibitors and angiotensin receptor blockers, can effectively reduce elevated urinary albumin excretion. This suggests a relationship between urinary albumin excretion and the renin angiotensin system. In our population, the activity of the circulating renin angiotensin system was increased in the microalbuminuric subjects, but the difference did not reach statistical significance.

When left ventricular function is impaired, which is the case in patients with severe chronic heart failure, the kidneys will respond with increased excretion of the peptide renin. The increased activity of renin will induce breakdown of angiotensin I into angiotensin II. This octapeptide will trigger aldosterone release. As a result, the kidneys will excrete less sodium and less water. The resulting volume overload will eventually induce typical clinical symptoms (e.g.

in chronic heart failure). This process can be delayed by blocking the detrimental effects of the renin angiotensin system. ACE inhibitors have repetitively demonstrated to inhibit the breakdown of angiotensin I into angiotensin II and, by doing so, to improve prognosis in chronic heart failure patients. However, in a considerable percentage of these patients elevated plasma angiotensin II levels can be found, despite the use of an ACE inhibitor (the so-called “ACE-escape”). In Chapter 4 the activation pattern of the renin angiotensin system in 99 severe chronic heart failure patients (New York Heart Association III and IV) is discussed. First, we determined the number of patients in whom angiotensin II levels remained elevated (>16 pmol/L) despite the use of a stable dose of an ACE inhibitor. In a second analysis we identified and evaluated the variables associated with elevated angiotensin II levels. In this study we demonstrated that 45% of our severe chronic heart failure patients have elevated angiotensin II levels despite treatment. Furthermore, the analysis demonstrated that the use of an aldosterone receptor antagonist was the only variable associated with elevated angiotensin II levels, which suggests the presence of a feedback mechanism within the renin angiotensin system. Renal function, dosage and duration of use of the ACE inhibitor seemed to have no predictive value for the occurrence of ACE-escape.

Activation of the renin angiotensin system induces higher angiotensin II and aldosterone levels in the human body. Previous investigators demonstrated that both of these hormones have a range of detrimental effects on the cardiovascular system. The effects of angiotensin II are mediated by stimulation of several receptors, of which the angiotensin II type 1 receptor (AT1R) and the angiotensin II type 2 receptor (AT2R) are the most important. The general opinion is that both these receptors mediate opposing effects. For example, stimulation of the AT1R will induce vasoconstriction, while stimulation of AT2R is thought to induce vasodilation. However, evidence for the latter is contradictory, and in humans also very limited. For this reason we performed the experiments that are reported in Chapter 5. In human internal mammary arteries which were harvested during coronary artery bypass surgery, we evaluated the effect of stimulation of the AT2R on the diameter of the arteries. The experiments demonstrated that both AT1R and AT2R can be found in these blood vessels. Stimulation of AT2R does not induce vasodilation in this experimental model.

Elevated urinary albumin excretion is associated with impaired prognosis. In analogy, lowering of urinary albumin excretion by using ACE inhibitors or angiotensin receptor blockers will improve cardiovascular prognosis. In Chapter 6 we discuss the combined approach: an angiotensin receptor blocker on-top-

of an ACE inhibitor. The theoretical considerations, the indications and the scientific evidence are presented.

The PREVEND intervention trial demonstrated that urinary albumin excretion can be reduced in non-cardiac patients as well. In addition, this study showed that this reduction is accompanied by an (almost significant) improvement of prognosis. In Chapter 7 we identify the variables that are associated with higher baseline levels of urinary albumin in patients from the PREVEND intervention trial. After multivariate regression analysis three variables are associated with a high baseline urinary albumin level: reduced renal function, elevated mean arterial blood pressure, and high sodium intake. This suggests that modification of diet may influence urinary albumin excretion. In addition, we demonstrate that the antialbuminuric effects of ACE inhibitors are partially independent of their blood pressure-lowering effect. Finally, we discuss the variables that determine the antialbuminuric effects of the ACE inhibitor. In this non-diabetic population baseline urinary albumin excretion seems to be the only independent determinant, meaning that the higher baseline the urinary albumin excretion, the more effective is the ACE inhibitor. This is reassuring, because it indicates that ACE inhibitors can be used independent of age, gender, or any other biological characteristic.

Samenvatting

De nieren zijn verantwoordelijk voor het reguleren van de vocht- en elektrolytenbalans in het menselijk lichaam. Om deze taak afdoende te kunnen vervullen zijn de nieren afhankelijk van voldoende bloedaanbod en dus van een adequate cardiac output. Indien de cardiac output daalt, zal middels een samenspel van nieren en endocriene organen het renine-angiotensine-systeem geactiveerd raken. Dit neurohormonale systeem verbindt het hart en de nieren onlosmakelijk met elkaar. Zo hebben dialysepatiënten een sterk verhoogde kans op het krijgen van hart- en vaatziekten. Ook minder ernstige nierfunctiestoornissen blijken al een zekere prognostische waarde te hebben wanneer het gaat om het voorspellen van cardiovasculaire problemen. In hoofdstuk 2 wordt gekeken naar de prognostische waarde van de preoperatieve nierfunctie van meer dan 400 patiënten na coronaire bypass operatie. Deze post-hoc analyse toonde aan dat reeds een milde renale dysfunctie de (cardiovasculaire) prognose van patiënten na deze operatie negatief beïnvloedt.

Wanneer een patiënt met chronisch hartfalen microalbuminurie ontwikkelt, gaat dit gepaard met een verslechtering van de prognose van deze patiënt. Er zijn verschillende theorieën over de pathofysiologie van microalbuminurie in deze patiëntengroep. Zo wordt gedacht dat de verhoogde eiwituitscheiding in de urine het gevolg is van een gegeneraliseerde endotheliale disfunctie. Een andere theorie is die van de glomerulaire hyperfiltratie die veroorzaakt wordt doordat intacte nefronen de functie moeten overnemen van beschadigde nefronen. In de studie zoals beschreven in hoofdstuk 3, worden 96 patiënten met ernstig hartfalen besproken, en wordt bepaald wat de prevalentie is van microalbuminurie. In 32% van de patiënten bleek sprake te zijn van microalbuminurie wanneer gebruik werd gemaakt van een willekeurig urinemonster. Toen dit percentage werd uitgezet tegen de prevalentie van microalbuminurie in een cohort uit de normale populatie (gerepresenteerd door een leeftijdscohort uit de PREVENT-studie) werd duidelijk dat microalbuminurie significant vaker voorkomt bij patiënten met hartfalen. Uit de literatuur blijkt dat patiënten met een verhoogde albumine-uitscheiding kunnen worden behandeld met geneesmiddelen die het renine-angiotensine-systeem afremmen zoals ACE-remmers en angiotensine-receptor-blokkers. Hieruit valt indirect af te leiden dat er een relatie moet zijn tussen albumine-uitscheiding via de nier en het renine-angiotensine-systeem. De activiteit van het renine-angiotensine-systeem was hoger in patiënten met microalbuminurie, maar dit verschil was niet significant.

Wanneer de linkerventrielfunctie afneemt, hetgeen gebeurt bij patiënten met hartfalen, zullen de nieren hierop reageren met een verhoogde uitscheiding van

het peptide renine. De verhoogde activiteit van renine leidt vervolgens tot de aanmaak van angiotensine II en aldosteron, waardoor de nieren minder natrium en water zullen uitscheiden. De volumebelasting die hierdoor ontstaat zal op den duur leiden tot typische klachten bij de patiënt (bv. zoals bij chronisch hartfalen). Door de renine angiotensine cascade te door breken kan dit proces worden afgeremd. In het verleden is dan ook bij herhaling gebleken dat ACE remmers de afbraak van angiotensine I naar angiotensine II kunnen afremmen en hiermee de prognose van patiënten met chronisch hartfalen kunnen verbeteren. Echter, bij een percentage van alle patiënten die worden behandeld kan toch een verhoogde angiotensine II-concentratie worden teruggevonden in het bloed, ondanks de behandeling met een ACE-remmer (de zogenaamde “ACE-escape”). In hoofdstuk 4 wordt ingegaan op het activatiepatroon van het renine angiotensine systeem in 99 patiënten met ernstig chronisch hartfalen (New York Heart Association klasse III en IV) die allen werden behandeld met een ACE-remmer. Ten eerste is bepaald hoeveel patiënten verhoogde angiotensine II spiegels (>16 pmol/L) houden ondanks het gebruik van een ACE-remmer. Ten tweede is in kaart gebracht of deze groep zich misschien onderscheidt van de groep met onderdrukte angiotensine II plasmaconcentraties. Uit dit onderzoek kwam naar voren dat 45% van de patiënten een verhoogde angiotensine II-concentratie houdt ondanks behandeling. De analyse liet verder zien dat deze patiënten vaker een aldosteron receptor antagonist gebruikten, hetgeen zou kunnen duiden op de aanwezigheid van een terugkoppelingsmechanisme binnen het renine angiotensine systeem. De nierfunctie van de patiënt en de gebruiksduur en de dosering van de ACE-remmer leken bij onze patiënten geen voorspellende waarde te hebben voor het optreden van ACE-escape.

Activatie van het renine angiotensine systeem leidt tot verhoogde angiotensine II- en aldosteronconcentraties in het lichaam. In het verleden is aangetoond dat deze hormonen een scala aan ongunstige effecten op het hart en de bloedvaten hebben. Angiotensine II kan zijn werking uitoefenen door stimulatie van onder andere de angiotensine II type 1 receptor (AT1R) en de angiotensine II type 2 receptor (AT2R). Over het algemeen wordt aangenomen dat beide receptoren tegengestelde effecten mediëren. Zo zal stimulatie van de AT1R bijvoorbeeld leiden tot vasoconstrictie, terwijl van de AT2R wordt aangenomen dat deze receptor vasodilatatie geeft. Echter, het bewijs voor dit laatste is tegenstrijdig en zeker in een humaan model flinterdun. Om deze reden werd het in vitro onderzoek uitgevoerd waarvan de resultaten zijn weergegeven in hoofdstuk 5. In humane arteriën die werden verkregen tijdens coronaire bypass operaties werd gekeken naar het effect van stimulatie van de AT2R op de diameter van het bloedvat. Uit de experimenten kwam naar voren dat zowel de AT1R als de AT2R

konden worden aangetoond in de gebruikte humane bloedvaten. Stimulatie van de AT2R leidde echter in het gebruikte model niet tot vasodilatatie.

Verhoogde uitscheiding van albumine middels de urine is prognostisch ongunstig voor de patiënt. Omgekeerd gaat verlaging van de albumine-uitscheiding met behulp van een ACE remmer of een angiotensine receptor blokker gepaard met een verbetering van de cardiovasculaire prognose. In hoofdstuk 6 bespreken we de mogelijkheid van de gecombineerde aanpak: een angiotensine receptor blokker 'on-top-of' een ACE remmer. De achterliggende gedachte, de indicatiegebieden en de gepubliceerde literatuur over dit onderwerp worden besproken.

Dat ACE remmers ook in staat zijn albumine-uitscheiding via de urine te verminderen in niet-cardiale patiënten werd aangetoond in de PREVENT Intervention Trial. Deze studie liet bovendien zien dat dit gepaard ging met een (bijna significante) verbetering van prognose van de patiënt. In hoofdstuk 7 worden de factoren geïdentificeerd die geassocieerd zijn met een hoge baseline albumine-uitscheiding. Uit de multivariate analyse kwamen drie factoren naar voren die samenhangen met een hoge uitscheiding: een verminderde nierfunctie, een hoge gemiddelde arteriële bloeddruk en een hoge natriumintake. Hiermee wordt de suggestie gewekt dat verandering van het dieet mogelijk een invloed kan hebben op de hoogte van de albumine-uitscheiding. Verder laten we in dit hoofdstuk zien dat de albumine-verlagende effecten van ACE-remmers gedeeltelijk onafhankelijk zijn van hun antihypertensive effect. Tenslotte bespreken we de factoren die de albumine-verlagende effecten van de ACE remmer bepalen. In deze groep van niet-diabeten bleek alleen de baseline albumine-uitscheiding van belang te zijn: hoe hoger de baseline albumine-uitscheiding, hoe groter het te behalen effect. Dit is geruststellend, want het betekent dat de ACE remmer in principe gebruikt kan worden ongeachte leeftijd, geslacht en andere biologische karakteristieken.

Abbreviations

ACE	angiotensin converting enzyme
ACh	acetylcholine
AcSDKP	N-Acetyl-Ser-Asp-Lys-Pro
AI	angiotensin I
AIi	angiotensin II
ARB	angiotensin receptor blocker
AT1R	angiotensin II type 1 receptor
AT2R	angiotensin II type 2 receptor
BMI	body mass index
CABG	coronary artery bypass grafting
CHF	chronic heart failure
CRP	C-reactive protein
DM	diabetes mellitus
ESC	European Society of Cardiology
ESRD	end stage renal disease
GFR	glomerular filtration rate
ID	insertion/deletion
JNC	Joint National Committee
KKS	kallikrein kinin system
LDL	low density lipoprotein
LVEF	left ventricular ejection fraction
MAP	mean arterial pressure
MDRD	Modification of Diet in Renal Disease
NO	nitric oxide
NT-proBNP	N-terminal pro B-type natriuretic peptide
NYHA	New York Heart Association
PAI-1	plasminogen activator inhibitor-1
PE	phenylephrine
RAS	renin angiotensin system
TGF- β_1	transforming growth factor beta-1
UAE	urinary albumin excretion
VEGF	vascular endothelial growth factor

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*Here's to beefsteak when you're hungry,
Whiskey when you're dry,
To all the girls you ever wanted,
And heaven when you die.*

Cheers!

Stellingen behorende bij het proefschrift:

**Optimal Blockade of the Renin Angiotensin System
in Cardiorenal Dysfunction**

1. (Micro)albuminurie?
Candesartan, captopril, cilazapril, enalapril, eprosartan, fosinopril, irbesartan, lisinopril, losartan, olmesartan, perindopril, quinapril, ramipril, telmisartan, trandolapril, valsartan, zofenopril ?!
2. Een directe relatie tussen de mate van albuminurie en de activiteit van het circulerend renine-angiotensine systeem is afwezig. (dit proefschrift)
3. Het gebruik van een aldosteron receptor antagonist leidt tot een verhoogde plasma angiotensine II concentratie bij patiënten met ernstig hartfalen. (dit proefschrift)
4. ACE remmers hebben bij gelijke bloeddrukdaling een sterker anti-albuminurisch effect dan andere antihypertensiva, in het bijzonder bij patiënten met een relatief hoge albumineuitscheiding. (dit proefschrift)
5. Dat AT₂ receptor stimulatie vasodilatatie induceert in humane vaten, is nog immer niet bewezen. (Dit proefschrift)
6. Cuando amor no es locura, no es amor.
When love is not madness, it is not love.
(Spaans gezegde)
7. Medicijnen zijn duur, *evidence based medicine* zo mogelijk nog duurder.
8. Uitgerekend het kuddegedrag van de *grijze* massa leidt tot de huidige scheiding van *zwarte* en *witte* scholen.
9. *“Kind words can be short and easy to speak, but their echoes are truly endless.”* (Mother Theresa)
10. Opvallenderwijs zijn *de smurfen* door de jaren heen echte *evergreens* geworden.
11. De huidige adipositas-epidemie is deels te herleiden tot een overdaad aan liefde.
(De liefde van de man gaat door de maag)
12. Generaliseren is altijd gevaarlijk, getuige ook deze stelling.