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ADMA and oxidative stress may relate to the progression of renal disease: rationale and design of the VIVALDI study

Rainer H Böger^a, Edzard Schwedhelm^a, Renke Maas^a, Sabine Quispe-Bravo^b and Cord Skamira^b

Abstract: The renin angiotensin system has been shown to be involved in the pathogenesis of vascular and renal sequelae of diabetes mellitus. In type 2 diabetes mellitus, angiotensin receptor blockers have been shown to exert clinical benefit by reducing the progression of diabetic nephropathy. They also improve endothelium-mediated vascular function. The latter effect is partly due to the reduction of angiotensin II-associated oxidative stress. Moreover, small clinical studies have shown that treatment with angiotensin receptor blockers also reduces the circulating levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide (NO) synthase.

In the VIVALDI trial, the ability of the angiotensin receptor blocker telmisartan to reduce the progression of diabetic nephropathy (associated with proteinuria) in comparison with valsartan in more than 800 patients with type 2 diabetes during 1 year of treatment is being studied. In order to gain more detailed insight into the potential pathomechanisms associated with this effect, further end-points have been defined. Among these are the circulating levels of ADMA and the urinary excretion rate of 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$). The former is an endogenous inhibitor of NO-mediated vascular function(s) and a prospectively determined marker of major cardiovascular events and mortality; the latter is a lipid peroxidation product resulting from the nonenzymatic peroxidation of arachidonic acid, which exerts detrimental vascular effects similar to those of thromboxane A_2 . Urinary 8-iso-PGF $_{2\alpha}$ has been shown in clinical studies to be an independent marker of cardiovascular disease.

Highlighting the effects of telmisartan on ADMA and 8-iso-PGF levels in such a large cohort of diabetic patients will enhance our understanding of the roles of dysfunctional NO metabolism and redox mechanisms in the pathogenesis of end-organ damage and its prevention by pharmacotherapy with angiotensin receptor blockers.

Key words: endothelium; 8-iso-prostaglandin F_2 ; nitric oxide; risk marker; telmisartan

Introduction and background

Despite improved treatment of hypertension, urinary tract obstruction and infection, many forms of renal disease associated with permanent nephron loss still progress inexorably to chronic end-stage renal failure. Although this may be caused by a variety of kidney diseases, in the USA and western Europe this population group is dominated by patients with diabetes or

hypertension. According to the United States Renal Data System registry data for the year 2002, diabetes was the attributed cause of end-stage renal failure in 35.7% of patients who started dialysis. Hypertension was responsible for 24% of cases. Glomerulonephritis accounts for about 15.6% of cases and cystic disease, hereditary causes and urological diseases together constitute another 6.4%. The cause is unknown in 18.3% of these patients.¹

Since the early 1980s the endothelium has been recognized as a major regulator of vascular homeostasis. Endothelial cells, as the inner lining of blood vessels, are strategically located between circulating blood and the vascular smooth muscle. In a person with a body weight of 70 kg, the endothelium covers an area of approximately 700 m² and weighs about 1–1.5 kg.² Functional integrity of the endothelium is crucial for the maintenance of blood flow and antithrombotic capacity because the endothelium releases humoral

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factors that control relaxation and contraction, thrombogenesis and fibrinolysis, and platelet activation and inhibition. Thus, the endothelium contributes to blood pressure control, blood flow and vessel patency. It is now clear that impaired endothelial function contributes substantially to cardiovascular disorders such as atherosclerosis, hypertension and heart failure, which lead to hypoperfusion, vascular occlusion and end-organ damage.

Endothelial dysfunction is also a common feature in patients who are diabetic.³ It is considered a major step in atherosclerosis and acute atherothrombotic events. Furthermore, it predicts cardiovascular mortality.⁴ Endothelial dysfunction has also been documented in a large proportion of patients with type 2 diabetes mellitus.⁵ The most probable explanation for this observation is a disturbed oxidative balance in the vasculature, resulting in endothelial dysfunction.⁶ A major pathophysiological alteration of type 2 diabetes is insulin resistance. Studies carried out in people with type 2 diabetes mellitus show an inverse correlation between measures of oxidative stress and insulin resistance.^{7,8} Both, diabetes mellitus⁹ and renal dysfunction¹⁰ are now considered as major cardiovascular risk factors and are associated with greatly elevated cardiovascular morbidity and mortality.

Endothelial dysfunction: the role of ADMA and oxidative stress

Endothelial dysfunction is mainly characterized by impaired production of the key endogenous vasodilator, nitric oxide (NO). In the endothelium, nitric oxide is formed from L-arginine by the endothelial isoform of nitric oxide synthase (eNOS). In humans NO synthesis may be impaired by asymmetrical dimethylarginine (ADMA), an endogenously formed compound that inhibits NOS activity by displacing L-arginine from the substrate binding site.¹¹ Infusion of ADMA impairs endothelium-mediated vasodilation.^{12,13} Elevated ADMA plasma levels are associated not only with endothelial dysfunction^{14,15} but also with increased oxidative stress,¹⁶ thereby linking endothelial dysfunction and redox mechanisms in vascular disease. Elevated plasma ADMA concentrations have also been found in patients with diabetes.^{17,18} Functional impairment or decreased expression of the ADMA-degrading enzyme dimethylarginine dimethylaminohydrolase (DDAH) has been suggested as one possible mechanism for elevated ADMA concentrations in diabetes.¹⁹ This is brought about at least in part by redox-mediated dysregulation of DDAH activity.^{20,21} Furthermore, several investigators recently demonstrated that an elevated plasma ADMA concentration is an independent risk factor for cardiovascular mortality in patients with cardiovascular disease and in patients with chronic renal failure.^{22,23}

Several groups of drugs have been studied with respect to their ability to lower ADMA plasma concentration and to improve the related impairment in NO-mediated vasodilation. Until now, only metformin, inhibitors of the angiotensin converting enzyme, and AT₁ receptor blockers have been found, in small clinical studies, to lower plasma ADMA concentrations.^{24–26} The strong relationship between elevated ADMA concentration, cardiovascular disease and mortality has made ADMA a goal of primary interest for pharmacotherapeutic intervention in large, controlled clinical trials.

Endothelial dysfunction and oxidative stress: the role of angiotensin II

A growing body of evidence is strengthening the view that angiotensin II is a major stimulus for vascular oxidative stress. Angiotensin II induces a mitogenic response in vascular smooth muscle cells through protein kinase C–extracellular signal-regulated kinase-dependent and -independent pathways,^{27,28} together with increased vascular NAD(P)H oxidase activity²⁹ and modulation of extracellular superoxide dismutase expression.³⁰ The consequence is an aggravation of the oxidative burden associated with atherosclerosis and endothelial dysfunction.³¹ Not surprisingly, AT₁ receptor blockade attenuates low-density lipoprotein oxidation and improves endothelial function in hypercholesterolemia, hypertension and type 2 diabetes.^{32–34}

Isoprostanes: markers and mediators of oxidative injury in the vascular system

Markers of oxidative stress are various and data are often conflicting.³⁵ This may mainly be attributed to the diversity of markers used and the heterogeneity of methods applied. In recent years new markers of lipid peroxidation have shown very promising results: F₂-isoprostanes (reviewed by Morrow³⁶). They are formed endogenously by arachidonic acid oxidation in phospholipids. Subsequently, they are liberated from phospholipids by phospholipases and eliminated via the kidney. F₂-isoprostanes are reliable markers and potentially significant mediators of oxidative stress in disease associated with the cardiovascular system.^{37,38} They have also been validated as reliable markers of oxidative stress in type 2 diabetes.^{39–43} Oxidative stress is involved in the microvascular and macrovascular complications seen in patients with diabetes. These pathophysiological alterations are mainly attributed to atherosclerotic lesions in the vasculature. F₂-isoprostanes have been detected in atherosclerotic lesions and they may be regarded as footprints left by the enhanced oxidative burden. However, it seems reasonable to assume that F₂-isoprostanes are not only an

index of oxidative injury but also contribute to the progression of atherosclerosis.³⁶ They may be quantified by gas chromatography-mass spectrometry (GC-MS), GC-tandem MS, liquid chromatography-tandem mass spectrometry (LC-tandem MS), and immunoassays. Despite this variety of possible methods, reliable quantification of F₂-isoprostanes is hindered by inappropriately performed enzyme immunoassays; this is subject to recent controversy.^{44,45} There does not appear to be a consensus concerning the best method, but chromatographic methods (GC-(tandem) MS, and LC-tandem MS) should be superior to immunoassays.⁴⁶ Moreover, measurement of urinary F₂-isoprostanes seems to be advantageous over plasma F₂-isoprostanes, at least in type 2 diabetes.^{42,43}

Telmisartan: an effective and long-lasting AT₁ receptor blocker

The discovery of a new pharmacological class of antihypertensives, the angiotensin II receptor antagonists (or sartans), has led to the clinical development and marketing of these new therapeutic agents since 1995, with the marketing approval of losartan by the Food and Drug Administration. Telmisartan belongs to the second generation of angiotensin II receptor antagonists. It was developed by Boehringer Ingelheim and was approved for use in hypertension by the Food and Drug Administration for North America in November 1998 and by the European Medicines Agency for Europe in December 1998. Telmisartan is an orally active nonpeptide type I angiotensin II receptor antagonist that lowers blood pressure with once daily dosing over a whole 24 hours dosing interval. The antihypertensive effect of once-daily dosing of telmisartan in patients with mild-to-moderate hypertension results in a significant reduction of sitting, supine and standing systolic and diastolic blood pressure, with usually little or no orthostatic change. The usual starting dose of telmisartan is 40 mg once daily, with the option to increase that dose to 80 mg daily. The 40 mg dosage reduces systolic and diastolic blood pressure by an average of 11.3/7.3 mmHg; with 80 mg this average is 13.7/8.1 mmHg. The antihypertensive activity occurs within 2 hours after single-dose administration and is maintained for the full 24-hour dosing interval. With ambulatory blood pressure monitoring, the 24-hour trough-to-peak ratio for telmisartan was determined to be at least 80% for both systolic and diastolic blood pressure.⁴⁷

AT₁ blockers in the prevention of renal failure in type 2 diabetes mellitus

In September 2001 the *New England Journal of Medicine* published the results of PRIME (Program for Irbesartan Mortality and Morbidity Evaluation).

PRIME was the first clinical program to show beneficial effects in patients with hypertension and type 2 diabetes mellitus across the spectrum of early and late stage kidney disease. It consisted of two studies: IRMA 2 (irbesartan microalbuminuria type 2) and IDNT (irbesartan diabetic nephropathy trial).

IRMA 2, a 2-year multicenter, randomised, double-blind, placebo-controlled study of 590 patients aged 30–70 years, assessed the effects of irbesartan compared with other proven antihypertensive treatments in slowing the progression of diabetic renal disease in hypertensive patients with type 2 diabetes and persistent microalbuminuria (20–200 µg/min).⁴⁸ In this study irbesartan (high dose, 300 mg) led to a 70% reduction in progression to later stage disease, overt diabetic nephropathy. It was therefore shown that irbesartan is renoprotective independently of its blood-pressure-lowering effect in patients with type 2 diabetes mellitus and microalbuminuria.

IDNT, the second study, was conducted in 1715 patients with proteinuria (overt nephropathy), hypertension and type 2 diabetes mellitus.⁴⁹ Irbesartan caused a 20% reduction in progression to doubling of serum creatinine, end-stage renal disease, or all-cause mortality compared with placebo, and a 23% reduction compared with amlodipine. Moreover, there was a 37% reduction in hospitalizations owing to congestive heart failure compared with amlodipine. The conclusion was that irbesartan is effective in protecting against the progression of nephropathy due to type 2 diabetes mellitus independently of the reduction in blood pressure.

In addition, in September 2001 the *New England Journal of Medicine* published the results of a similar trial to IDNT, the RENAAL study (Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan), examining the effects of losartan in patients with type 2 diabetes mellitus and overt nephropathy. The RENAAL study included 1513 patients from 29 countries.⁵⁰ There was a 28% reduction in the risk of developing end-stage renal disease when losartan was included in the antihypertensive treatment regimen, and hospitalization for congestive heart failure decreased by 32%.

In May 2001, data from the MARVAL study (MicroAlbuminuria Reduction with VALsartan) were presented at the 16th Annual Scientific Session of the American Society of Hypertension. MARVAL was a multicenter, double blind, randomized parallel study of 332 type 2 diabetic patients with microalbuminuria and adjusted blood pressure.⁵¹ The patients were randomized to receive either valsartan or amlodipine once daily over 24 weeks. The study was designed to assess the blood-pressure-independent effects of valsartan versus amlodipine on urinary albumin excretion rates, a measure of microalbuminuria. The results showed that more patients returned to a normal albuminuric status after 24 weeks with valsartan (29.9%)

compared with amlodipine (14.5%) ($p = 0.001$). These two groups experienced similar rates of blood pressure reduction. The conclusion was that the angiotensin receptor blocker valsartan is more effective than the calcium channel blocker amlodipine in reducing microalbuminuria in type 2 diabetic patients with adjusted blood pressure.

The effects of angiotensin converting enzyme inhibitors and angiotensin II AT₁-receptor antagonists on mortality and renal outcomes in diabetic nephropathy have recently been reviewed.⁵² Available data suggest that AT₁ receptor antagonists are beneficial for renal outcomes, with about a 22% reduction in risk of end stage renal disease and doubling of the serum creatinine concentration, around a 51% reduction in progression rates from microalbuminuria to macroalbuminuria, and about a 42% increase in regression from microalbuminuria to normoalbuminuria.⁵²

Design of the VIVALDI trial

The VIVALDI trial has been designed as a prospective, multicenter, randomized, double-blind, double-dummy, parallel group trial to investigate the efficacy of telmisartan versus valsartan in hypertensive type 2 Diabetic patients with overt nephropathy. The primary goal is to show non-inferiority of telmisartan 80 mg with valsartan 160 mg in reducing proteinuria after 1 year of treatment. Furthermore, the treatment effects for telmisartan 80 mg versus valsartan 160 mg with regard to several renal function parameters, clinical end-points and parameters of endothelial function and oxidative stress after 1 year of treatment will be evaluated.

Between April 2003 and November 2004, 884 patients aged 30–80 years with type 2 diabetes and hypertension and overt nephropathy (defined by proteinuria in 24 h urine ≥ 900 mg and serum creatinine ≤ 265 $\mu\text{mol/l}$ or ≤ 3.0 mg/dl) were randomized in the study. Study medication is given while other antihypertensive therapy may be maintained, with the exception of angiotensin converting enzyme inhibitors and other angiotensin II receptor antagonists. If the goal blood pressure of 130/80 mmHg cannot be reached with the high dose of study medication, other antihypertensive medication may be given at any time during the study, starting 4 weeks after randomization. The sample size of the per protocol analysis population is set to include a total of 680 patients, 340 administered telmisartan 40 mg daily with mandatory titration after 2 weeks to 80 mg daily, and 340 patients taking valsartan 80 mg daily, with mandatory titration after 2 weeks to 160 mg daily.

The primary efficacy variable is the change from baseline in 24-hour proteinuria, after 1 year of treatment with telmisartan 80 mg versus valsartan 160 mg. Secondary end-points are the following: renal

parameters (24-hour urinary albumin excretion rate, creatinine clearance, glomerular filtration rate, serum creatinine, 24-hour sodium excretion in urine); clinical end-points (composite of doubling of the serum creatinine concentration, end-stage renal disease or all-cause death; composite of morbidity and mortality from cardiovascular causes); parameters characterizing endothelial dysfunction and oxidative stress (change from baseline in C-reactive protein, ADMA and 8-iso-PGF_{2 α} levels); parameters characterizing insulin sensitivity (change from baseline in homeostasis model assessment index in patients not receiving insulin therapy; change from baseline in adiponectin).

Taken together, the VIVALDI trial will evaluate different aspects of the effects of the angiotensin II receptor blockers telmisartan and valsartan on renal function and the processes underlying the pathogenesis and facilitating the prevention of diabetic nephropathy.

ADMA and urinary 8-iso-prostaglandin F_{2 α} as secondary end-points in the VIVALDI trial

As detailed above, serum levels of ADMA and urinary excretion rates of 8-iso-PGF_{2 α} are novel markers of endothelial dysfunction and oxidative stress, respectively. For both, elevated levels have been reported in patients with type 2 diabetes. Moreover, both are associated with cardiovascular disease, although this association was more difficult to assess for 8-iso-PGF_{2 α} because only in a few studies were sufficiently reliable urine collections carried out to allow the assessment of this marker in urine. However, in one study in which we collected urine samples carefully, a significant relationship between urinary excretion of 8-iso-PGF_{2 α} and coronary heart disease was found.³⁷ In the case of ADMA, several prospective as well as numerous cross-sectional clinical studies have confirmed the predictive value of this marker for major cardiovascular adverse events and death. These data have been extensively reviewed elsewhere in this issue.⁵³

The analysis of ADMA in a large cohort of type 2 diabetic patients in the VIVALDI trial will allow us to establish the relationship between ADMA levels and cardiovascular end-points in a prospective manner in the diabetic population. Moreover, it will also allow firm conclusions on the efficacy of the AT₁ receptor blocker telmisartan to lower ADMA levels. Only a few previous studies have been reported in which the effects of AT₁ blockers on ADMA were tested; these included very small numbers of participants (see the overview on therapeutic interventions by Maas in this issue⁵⁴). Moreover, they may have been flawed by the method of assessing ADMA, which has only recently

been improved (see the overview by Schwedhelm, also in this issue⁵⁵).

Although angiotensin II has long been viewed as a major determinant of oxidative stress in the vascular system, the effects of AT₁ receptor blockade on urinary F₂-isoprostane excretion in type 2 diabetes mellitus have not yet been studied.

Thus, the VIVALDI study will give us detailed and high-quality information on the roles of these novel markers of endothelial dysfunction and oxidative stress to allow us to predict outcomes in diabetic patients. It will also allow us to study the inter-relationship between ADMA, lipid peroxidation and angiotensin receptor blockade with telmisartan, and thus to find potential new clues to the mechanisms of action of this class of drugs in cardiovascular disease and diabetes mellitus.

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