Proteinuria and progressive renal disease: towards preventing renal disease

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Introduction

A series of meetings on Proteinuria and Progressive Renal Disease has been conducted over the past few years in Paris (1992), Vienna (1994), Amsterdam (1996) and the fourth meeting in Montreux 1997. Proceedings of the third meeting in Amsterdam were published in Nephrology Dialysis Transplantation 1997; 12 [Suppl 2]: 1–85.

The proceedings of the fourth meeting are organized as extended abstracts, and provide a overview of the different approaches towards progressive renal disease, both in diabetic and non-diabetic nephropathy.

Special attention is given to the concept of microalbuminuria, with special reference to early treatment, within the area of diabetes.

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Progression of renal disease

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Glomerular hypertension has been implicated as a major factor in mediating progressive renal damage after any of a variety of initiating injuries. Amelioration of glomerular hypertension by dietary protein restriction or antihypertensive therapy reduces progressive glomerular damage in experimental models of chronic renal disease. Glomerular hypertension and hyperfiltration also occur in humans with diabetes mellitus, solitary or remnant kidneys, and various forms of acquired renal disease. Therapies shown to limit glomerular hypertension and to ameliorate renal injury in animals have begun to be tested in humans. Dietary protein restriction and antihypertensive drug therapy have been the principal therapeutic interventions tested thus far. A meta-analysis of studies of low-protein diets in renal insufficiency, based only on data from prospective randomized, controlled trials in non-diabetic patients, suggests that dietary protein restriction indeed delays the onset of end-stage renal disease [1]. Evidence also suggests that dietary protein restriction retards the progression of diabetic renal disease as well [2]. The Modification of Diet in Renal Disease Study, a large multicenter clinical trial also demonstrates a benefit of dietary protein restriction and aggressive reduction in blood pressure on the rate of progression of chronic renal disease in humans with significant proteinuria and baseline ongoing deterioration of renal function [3]. Angiotensin converting enzyme (ACE) inhibitors have also been shown to offer protection in patients with diabetic and non-diabetic chronic renal disease [4,5].

Among diabetics, randomized multicenter clinical trials have examined the merits of ACE inhibitors in both type 1 and type 2 patients. Evidence in support of a strong renoprotective effect of this class of antihypertensive therapy was obtained in those with already overt nephropathy as well as in those with incipient nephropathy [4,6]. Based on these findings various professional organizations have issued formal guidelines enthusiastically supporting ACE inhibitors in diabetic subjects. Studies are also in progress to determine whether angiotensin II receptor antagonists are equivalent or possibly more renoprotective than ACE inhibitors. The results of these trials will not be available, however, for at least 3 years.

Abstracts

Population-based studies of microalbuminuria and renal disease
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Microalbuminuria is present when the urinary albumin excretion rate (UAER) in a 24-h urine or a short-time collected urine during day time is in the range of 30–300 mg/24-h (20–200 µg/min equivalent to 0.46–4.6 µmol/24 h). A UAER in the microalbuminuric range is a strong predictor of nephropathy in insulin-dependent diabetes (type 1). Because of this close association between microalbuminuria and progressive renal disease in type 1 diabetic patients this association has been intensely studied. Also the epidemiology of microalbuminuria and renal disease has been investigated. From a number of epidemiological studies it appears that the prevalence of microalbuminuria is 12–22%. There are controversies as to the relation between prevalence of microalbuminuria and diabetes duration. A number of studies observe an almost unchanged prevalence of microalbuminuria independent of diabetes duration. Most of these studies have included only one urine sample for classification of the albuminuria level. This may constitute a problem because of the high day to day variation of UAER. Studies including more than one urine sample describe a three fold increase in prevalence of microalbuminuria after 24 years of diabetes compared to 5–9 years of diabetes.

Despite the close relationship between microalbuminuria and diabetic nephropathy it has been shown that the kidney function in terms of glomerular filtration rate declines only in the patients with a UAER high in the microalbuminuric range. Furthermore, microalbuminuria also has in type 1 diabetic patients shown to be an independent risk factor not only of renal disease but also of cardiovascular disease. The latter association seems to be even more prominent in patients with non-insulin dependent diabetes (type 2) as well as in non diabetic apparently healthy controls. The prevalence of microalbuminuria is as high as 30–40% in type 2 diabetic patients and end-stage renal failure only occurs in 3–8% of these patients. The strong link between microalbuminuria and diabetic nephropathy in type 1, therefore, may also be mediated through mechanisms similar to those associating microalbuminuria to cardiovascular disease in type 1 and type 2 diabetic patients as well as in apparently healthy subjects.


Proteinuria and glomerular heparan sulphate alterations
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Introduction
Heparan sulphate (HS) is the anionic glycosaminoglycan side-chain of heparan sulphate proteoglycan (HSPG) present in basement membranes, in extracellular matrix and on cell surfaces. The impact of HS for the permissive properties of the glomerular basement membrane (GBM) was documented by a number of observations. First, enzymatic digestion of HS by heparinase caused an enhanced permeability of the GBM for native ferritin and albumin [1]. Second, an acute, selective proteinuria could be induced in rats by a single intravenous injection of a monoclonal antibody directed against GBM HS [2]. Third, a reduction in GBM HS-associated anionic sites was demonstrated with cationic probes in several human and experimental proteinuric glomerulopathies [3]. With recently developed antibodies directed against the GBM HSPG core protein and the HS side chain [4], we demonstrated a decrease in HS staining in the GBM in different human proteinuric glomerulopathies, such as systemic lupus erythematosus (SLE), minimal change disease, membranous glomerulonephritis and diabetic nephropathy, whereas the staining of the HSPG core protein remained unaltered [5]. This suggested changes in the HS side-chains of HSPG. To get more insight into the mechanisms responsible for this observation, we studied GBM HS expression in various experimental models of proteinuria. From these investigations four different mechanisms have emerged until now which are discussed below, namely masking of HS by immune complexes, depolymerization of HS by radicals, proteolytic cleavage of HSPG by enzymes released from inflammatory cells and metabolically induced biochemical changes of HS.

Masking of HS by immune complexes
SLE is an autoimmune disease characterized by the occurrence of numerous autoantibodies, primarily directed against nuclear antigens. In both human [5] and murine [6] SLE nephritides, albuminuria is paralleled by a decrease in GBM HS staining and an increase in immune complex depositions in the glomerular capillary wall. This decrease in HS staining was inversely correlated with both albuminuria and with immune complex deposition. Formerly, it was thought that anti-DNA autoantibodies could cross-react with HS in the GBM. Later we could show, however, that antinuclear antibodies are able to bind to HS and to the GBM via nucleosomal antigens [7]. The positively charged histones in these nucleosome-containing immune complexes interact with the negatively charged HS in the GBM. Despite the decrease in HS staining, the HS content in glomeruli of lupus mice is normal, which suggests that HS is masked by immune complexes. This is supported by the finding that the binding of a

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monoclonal anti-HS antibody to HS in ELISA can be inhibited by autoantibodies complexed to nucleosomes but not by purified non-complexed autoantibodies [6]. Therefore, in SLE nephritis and possibly also in other glomerular diseases in which immune complexes are known to play a role in the pathogenesis, deposition of immune complexes in the glomerular capillary may lead to masking of HS, resulting in an enhanced permeability of the GBM for macromolecules. Indeed, inhibition of binding of nucleosome-complexed autoantibodies to the GBM by heparin (oids) prevents both the decrease of HS staining in the GBM and albuminuria in MRL/lpr mice [8].

Depolymerization of HS by radicals

Adriamycin (ADR) nephropathy, an experimental model for the nephrotic syndrome, is characterized by heavy albuminuria and hypalbuminemia. Also in this model, there is a decrease in GBM HS staining which is inversely correlated with albuminuria without changes in the staining of the HSPG core protein and HS attachment sites. Treatment of rats with ADR nephropathy with the hydroxyl radical scavenger dimethylthiourea (DMTU) results in amelioration of albuminuria and partial prevention of HS loss from the GBM, indicating a role for hydroxyl radicals in this model. An explanation for the decrease of HS staining in ADR nephropathy is the in vitro observation that HS is depolymerized by hydroxyl radicals. This depolymerization of HS by reactive oxygen species (ROS) in vitro can be prevented by DMTU. Taken together, these data suggest that in ADR nephropathy HS is depolymerized by hydroxyl radicals leading to loss of GBM integrity and albuminuria [9].

Polymorphonuclear leukocyte (PMN)-derived ROS may have similar effects. Activated PMNs that are attached to extracellular matrix secrete ROS and lysosomal enzymes. In the early phase of passive anti-GBM nephritis, PMN-derived ROS and lysosomal enzymes are responsible for the glomerular damage. In this model, treatment with scavengers of ROS was shown to ameliorate albuminuria, possibly via prevention of HS depolymerization [10,11]. Also nitric oxide (NO) can degrade HS and heparin in vitro via the formation of HNO2 and the cleavage of glycosaminoglycans by cultured endothelial cells can be prevented by inhibition of NO production [12]. Because NO is produced by endothelial cells, PMNs and monocytes after inflammatory stimulation, it may be involved in HS depolymerization and the development of albuminuria in immunologically mediated glomerular inflammation.

Cleavage of HS (PG) by enzymes

Besides the injurious effects of PMN-derived ROS, PMNs also release proteases upon activation. Among the enzymes released are the cationic serine proteases elastase and cathepsin G which are known to digest fibronectin, laminin and collagen type IV. The involvement of these proteases in the development of albuminuria is substantiated by the observation that beige mice, whose PMNs are deficient for elastase and cathepsin G, do not develop albuminuria although they show a comparable influx of PMNs as control mice [13]. Furthermore, in vivo perfusion of elastase in rats results in proteinuria and decrease in GBM HS staining and to a lesser extent of HSPG core protein staining. In vitro experiments showed that elastase, which is highly cationic at physiological pH, can bind to the anionic HS. Next, elastase can cleave HSPG near the HS attachment sites and HS side-chains bound to small peptide fragments are released [14]. Also heparanase, released from leukocytes and phagocytes upon stimulation, can degrade HS in extracellular matrix [15]. Therefore, in forms of glomerulonephritis that are accompanied by inflammatory cell influx, albuminuria may be the result of digestion of HS by released lysosomal enzymes.

Biochemical changes of HS

In human diabetic nephropathy, albuminuria is accompanied by a decrease in GBM HS staining [16]. In streptozotocin (STZ)-induced diabetes in rats, the number of HS-associated anionic sites in the lamina rara externa of the GBM is reduced whereas there is no difference in absolute GBM HS content between control and diabetic rats. This may point to structural alterations in HS, most likely undersulphation [16,17]. HS undersulphation has also been shown for other tissues of STZ-induced diabetic rats. Culture of mesangial cells on mesangial matrix that was non-enzymatically glycated or prolonged exposure of mesangial cells to elevated glucose levels leads to a decreased production of HS which is undersulphated [18]. The undersulphation of HS could be due to a decreased activity of the enzyme glucosaminyl N-deacetylase, the key enzyme in HS sulphation. Indeed, in hepatocytes of STZ-induced diabetic rats, a reduced activity of this enzyme was found [19]. Undersulphation leads to a disturbed interaction of HS with other GBM components and might lead to an increased permeability of the GBM.

Conclusion

From the above-mentioned observations in various experimental glomerulopathies it can be concluded that different mechanisms can lead to an impairment of HS function and thus to the charge-dependent permeability of the GBM. Since HS interacts with other constituents of the GBM, such as collagen type IV, laminin and fibronectin, any change in HS can also lead to a disturbed integrity of the GBM leading to an increased size-dependent permeability. Besides the effects on GBM permeability, changes in HS may have consequences for other processes involved in glomerular pathology, like growth factor activity (bFGF/PDGF), mesangial expansion and proliferation and local coagulation. Therefore, these HS changes may aggravate glomerular inflammation.

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Detecting the Risk of Renal and Cardiovascular Disease in Diabetes

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Elevated albumin excretion rate is the earliest sign of diabetic nephropathy. There is a consistent and independent association of microalbuminuria with higher levels of blood pressure, though often within the normal range, which exceeds by approximately 10–15 percent the blood pressure levels of long-term diabetic patients with normoalbuminuria. This phenomenon has been elegantly documented in studies which have used 24hr blood pressure monitoring. Studies of transition from normo- to microalbuminuria have demonstrated that those diabetic patients who progress already show increases in blood pressure while the albumin excretion rate rises within the normal range. This observation raises the possibility that elevated pressure levels may be one important factor contributing to the development of renal damage. Indeed, diabetic nephropathy clusters in families and diabetic patients who develop albuminuria belong to families with a higher prevalence of arterial hypertension and cardiovascular disease. Of interest diabetic patients with microalbuminuria are more insulin-resistant than patients with normal albumin excretion rate and this may explain why patients with microalbuminuria tend to have a poorer metabolic control and higher arterial pressures. Insulin resistance is an independent risk factor for coronary artery disease in the general population.

Thus diabetic patients with microalbuminuria have a cluster of risk factors for both renal and cardiovascular complications and follow-up studies have shown that microalbuminuria is one of the strongest predictors for cardio-renal disease both in type 1 and type 2 diabetes.


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Antihypertensive treatment in microalbuminuric type 2 diabetic patients

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Both hypertension and type 2 diabetes mellitus are heterogeneous diseases. Blood pressure is determined by cardiac output and systemic vascular resistance, both of which are influenced by multiple interacting hormonal, vasoactive and growth factors. Hypertension in diabetic patients, including those with
type 2 disease, have evidence of salt retention as shown by increased plasma atrial natriuretic peptide and reduced plasma aldosterone and renin concentrations [1]. These patients also have enhanced vascular responsiveness to exogenous administration of catecholamines and angiotensin II (AngII) [2]. Elevated plasma concentrations of these hormones and serum angiotensin converting enzyme (ACE) activity, suggesting activation of these hormonal systems, have also been reported [1]. On the other hand, reduced natriuretic responses, such as reduced urinary dopamine excretion, may also contribute to this salt-retaining tendency [3]. In accord with this pathophysiological heterogeneity, different classes of antihypertensive drugs with different modes of actions have been shown to be effective in reducing blood pressure in diabetic patients, albeit also with marked heterogeneity in treatment responses [4]. The latter is further influenced by potential inter-ethnic pharmacokinetic and pharmacodynamic differences [5,6].

Hypertension and proteinuria frequently coexist in type 2 diabetic patients, especially in non-Caucasian populations [7]. The coexistence of these two conditions markedly increase the risk of cardiovascular death and renal failure in these patients. The pathogenesis of diabetic proteinuria is complex and involves vascular (systemic and intraglomerular hypertension), metabolic (hyperglycaemia and hyperlipidaemia), growth and genetic factors [8]. However, control of glycaemia [9] and blood pressure [10] are two of the most powerful determinants for the progression of proteinuria. In patients with clinical proteinuria and established renal disease, adequate control of blood pressure is of paramount importance [10]. As shown in one of the meta-analyses, a reduction of mean arterial pressure of 10 mmHg is accompanied by an increase of 3.7 ml/min/year of glomerular filtration rate (GFR) [4]. However, since proteinuria and GFR are also determined by metabolic factors such as hyperglycaemia and hyperlipidaemia [11], the metabolic effects of antihypertensive agents may influence their renal effects. In this respect, in view of their relatively neutral effects on carbohydrate and lipid metabolism, ACE inhibitors and calcium channel blocking agents are frequently used as first line antihypertensive agents in diabetic patients [12–14].

There is now a wealth of data confirming the antiproteinuric effects of ACE inhibitors in both type 1 and type 2 diabetic patients, an effect which is independent of blood pressure reduction [4]. The use of this class of drug has also been shown to reduce the rate of decline in renal function in type 1 [15], normotensive type 2 diabetic [16,17] as well as non-diabetic patients with chronic renal failure [18,19]. Although there are sufficient reasons to believe that these beneficial renal effects of ACE inhibitors should also be applicable to hypertensive type 2 diabetic patients, definitive proof in this important therapeutic area is still lacking. To date, there have only been a few long term studies, lasting more than one year, which examined the renal effects of different antihypertensive agents in type 2 diabetic patients with hypertension [20–23]. In all of these studies comparing an ACE inhibitor with a calcium-channel blocking agent, no differences have been found between these 2 classes of drugs. In a 3-year study, hypertensive type 2 diabetic patients exhibited a biphasic pattern in the deterioration in renal function. In the first 12 months, the rate of decline in GFR correlated with the reduction in blood pressure. This was followed by a stabilisation of GFR which correlated inversely with the reduction in blood pressure [23]. These findings further emphasise the importance of conducting long term studies in the evaluation of renal function.

Most studies have shown that the majority of both type 1 [15] and 2 diabetic patients required more than one antihypertensive drug for optimal control of blood pressure [23]. Given the complex nature of diabetes, these findings raise the important question as to which combination therapy will provide the optimal antihypertensive, renal and metabolic effects. Although an ACE inhibitor plus a low dose diuretic is a popular choice of therapy, the combination of an ACE inhibitor and a calcium channel blocking agent has potential additional advantages. Calcium channel blocking agents are potent antihypertensive agents given either alone or in combination with an ACE inhibitor [24,25] especially in patients with salt retention [26], such as diabetic [27] and elderly patients [28]. In some patient groups, such as those with young age or suboptimal metabolic control, a calcium channel blocking agent may be a preferred agent given the potential adverse effects of a diuretic such as those on intermediary metabolism and sexual dysfunction, albeit small and dose-related [13]. Despite the extensive clinical use of calcium-channel blocking agents, the renal effects of this class of agents remain controversial [29]. Although the dihydropyridine calcium-channel blocking agents, such as nifedipine and amlodipine, may increase proteinuria due to their dilating effects on both afferent and efferent glomerular arterioles, their beneficial effects on GFR due to a reduction of systemic blood pressure should be taken into consideration [29].

Despite the potential cardioprotective effects of blocking agents [30] and the beneficial effects of blocking agents on insulin resistance [12], there are no long-term studies examining the use of these agents in type 2 diabetic patients. On the other hand, the new class of antihypertensive agent, the AngII type 1 receptor antagonist, such as losartan, has been shown to reduce proteinuria more effectively than a calcium-channel blocking agent despite similar, and in some patients, less reduction in blood pressure [31]. There is now an ongoing 4-year study, the RENAAL (Reduction in Endpoints in NIDDM with AI Antagonist, Losartan) Study, which examines the renoprotective effects of losartan versus placebo in type 2 diabetic patients with macroalbuminuria and renal impairment. However, more studies are needed to define the optimal antihypertensive regimen in type 2 diabetic patients who make up the majority of patients with renal failure. In this respect, a randomized, parallel study examining the combination of an ACE inhibitor with a low-dose diuretic or a calcium channel blocking or an AngII receptor antagonist on vascular, renal and metabolic indices as well as clinical endpoints will be of particular interest and major therapeutic relevance.

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Glycaemic control, islet function and the development and progression of diabetic nephropathy

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A multicentre study of the efficacy of intensive therapy on the complications of diabetes mellitus, the Diabetes Control and Complications Trial (DCCT) enrolled 1441 patients, nearly all of whom had normal renal function. After an average follow-up of 5–6 years the DCCT Research Group concluded in a decisive manner that intensive diabetic therapy reduced the risk of all diabetic complications (by about 50% for each complication) [1,2]. This conclusion, while of great positive import to the diabetic patient, places the emphasis upon therapeutic application to achieve optimal glycaemic control.

In nearly all studies prospectively evaluating the development and progression of diabetic nephropathy, the diabetic patients who progress to nephropathy have higher haemoglobin A1C values than those diabetic patients who did not advance [3,4]. Thus it is difficult to demonstrate the independence of other factors thought to predict or indicate the likelihood or the actual presence of diabetic nephropathy.

The familial influence in the development of diabetic nephropathy was greatly strengthened by the DCCT DCCT probands and relatives for the development of retinopathy and nephropathy [5]. This relationship was most easily demonstrated in the standard treatment group, with similar trends but a diminished influence in families of patients receiving intensive therapy. Thus these observations re-emphasize glycaemic control as fundamental cause of cardiovascular complications.

Several investigators over the past 2 decades have shown microalbuminuria to be the primary indicator/predictor of diabetic nephropathy [6]. Its relationship to the rise in blood pressure has not always been clear; however, in the DCCT microalbuminuria (verified in at least 2 measurements) occurs 2 years prior to the rise in blood pressure. DCCT data also demonstrated that increasing the level to designate established microalbuminuria enhances its specificity as an indicator or predictor of diabetic nephropathy (e.g. albuminuria of 30 mg/24 h is somewhat less specific than 50 mg/24 h).

The presence of clinical albuminuria (>300 mg/24 h) indicates a clear risk for renal failure. The rate at which the glomerular filtration rate falls in patients with clinical diabetic nephropathy has been reduced with careful implementation of antihypertensive medication. Furthermore observations from pancreas transplantation indicate optimal glycemic control may require several years before efficacy may be demon-
strated [7,8]. However, with careful implementation of therapeutic modalities to improve glycemic control and to control hypertension, kidneys of diabetic patients may experience many more years of function before failing.

In the practice of diabetes care basal insulin secretion or its replacement with long-acting insulin enables efforts to approach near-normal glycaemia. Yet in most type 1 diabetic patients the clear benefit of a sustained level of basal insulin secretion (usually indicated by a rise in c-peptide levels following mixed meal stimulation) cannot be duplicated by exogenous insulin therapy [9]. The DCCT Research Group demonstrated that intensive glycaemic control extends the time of demonstrable islet function [10].

The DCCT Research Group also reported a lower risk for retinopathy and nephropathy for those patients with residual islet function who received intensive therapy compared to intensively treated patients with no residual islet function. Of great interest was the accompanying reduced risk of hypoglycaemia in those DCCT subjects with sustained c-peptide secretion receiving intensive treatment. Thus uniquely in those patients with residual islet function the reduced risk of the microvascular complications was accompanied by a lower risk of hypoglycaemia.

In general those patients who may more successfully reach the goals of intensive therapy have an older age of onset and are more likely to retain higher levels of c-peptide secretion and thus sustained islet function.

### References


### Preservation of normal GFR in type 1 diabetic patients with microalbuminuria under long-term (8 years) ACE inhibition

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The aim was to assess the long term effectiveness of angiotensin converting enzyme inhibition on preservation of glomerular filtration rate (GFR) in type 1 diabetic patients with microalbuminuria. Forty-four normotensive insulin dependent diabetic patients with persistent microalbuminuria (urinary albumin excretion (UAE) 30–300 mg/24 h) were enrolled in an open randomized controlled study of 8 years duration. The treatment group (n=21) was given captopril (50 mg b.i.d.) and low-dose thiazide. The remaining 23 patients were left untreated unless hypertension did develop (n=4). All except 2 patients from each group completed the 8-year follow-up. Sixteen of the 19 patients in the treatment group and 2 patients from the control group were subsequently investigated before and 2 months after cessation of antihypertensive treatment. The percentage of patients progressing to nephropathy was 40% (9/23) in the control group and 10% (2/21) in the intervention group (P<0.02). During the pause in captopril treatment a significant increase in UAE was demonstrated (P<0.001) and 6 of the 16 (38%) patients demonstrated UAE above 300 mg/24 h. During the treatment pause GFR increased 8.6 (SE 3) ml/min (P<0.01) in the captopril group. From baseline to 8 years the fall in GFR was 12.2 (SEDiabetes Control and Complications Trial. Abstract.

Mathiesen ER, Hommel E, Giese J, Parving H-H. Stable glomerular filtration rate in normotensive IDDM patients with microalbuminuria—long lasting and associated with preservation of normal GFR.


Abstracts


**Long-term studies in NIDDM. Effects on nephropathy and retinopathy**

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The last decade of the 20th century has witnessed a marked increase in the incidence of diabetes mellitus, mainly of the non-insulin-dependent variety. This and the rise in life expectancy forecast a major load of end-stage renal disease on the health-care systems of the industrial as well as some developing countries. Effective policies to attenuate the progress of diabetic nephropathy are therefore of increasing importance.

There is a general agreement about the impact of hypertension on the pace of deterioration in kidney function and of the importance of albuminuria both as a prognostic parameter and as a contributing factor to disease progression. There is no consensus however about the desired levels of blood pressure or about the link between decrease in urinary albumin excretion (UAE) and the extend of renoprotection. Also the relative efficacy of the various therapeutic regimes remains unsettled.

ACE inhibitors are advocated by some researchers as the preferred agents for renoprotection. Others, however, show similar effects on the kidney also of calcium channel blocking agents and of beta blocking drugs [1–3]. Meta-analysis of the various therapeutic trials is a frustrating task due to major discrepancies in baseline characteristics of the patients especially in terms of risk factors (e.g control of hyperglycaemia, lipid profile, age, duration of diabetes) and trial design. Some studies include patients with and without nephropathy, with and without hypertension and one or both types of diabetes. Furthermore, in studies of similar design the results are divergent largely due to differences in surrogate end-points, choice and dosage of therapeutic agents and duration of the study [4–6].

Long-term studies in NIDDM have shown a varying degree of decline in GFR in hypertensive and normotensive patients with nephropathy which was partially attenuated by ACE inhibitors and by calcium blockers. The short-term studies, lasting 1 year or less have often failed to demonstrate significant change in kidney function, and therefore also no effect of either class of agents on renal function. The only universally agreed effect is the decline in albuminuria induced by ACE inhibitors although this effect was also observed under calcium blockers.

The studies we have performed are of 5 and 6 years duration. The patient population was uniform and they were placebo controlled [7]. We therefore believe that our results are reliable. They represent, however, highly selected groups of patients low and very low risk. The extrapolation of our results to the diabetic population at large may therefore be problematic. The effect of a given therapeutic intervention is expected to correlate to the degree of risk of the treated patients. The finding of a beneficial effect in a low-risk group may therefore indicate the importance of such treatment in other clinical settings. In the first study normotensive type 2 diabetics with microalbuminuria were randomized to receive enalapril or placebo and were followed for 5 years. A clear renoprotective effect of ACE inhibition was demonstrated.

Analysis of the influence of ACE inhibition on the well known risk factors for the progression of renal disease demonstrates a very modest reduction of mean blood pressure, below statistical significance; a modest decline in plasma total and LDL cholesterol and no change in HDL and HbA1c values. The most important effect of ACE inhibition therefore remains the reduction in albuminuria. These results may therefore be interpreted as supporting the major role of albuminuria in the progression of renal disease. We have examined the protective effect of ACE inhibitors also in the very-low-risk group of normotensive–normoalbuminuric patients with NIDDM. Six-year follow-up on 156 patients, randomized to receive enalapril or placebo showed a modest but a significant effect of enalapril on attenuation of the decline in creatinine clearance and on albuminuria. The mean decrease in creatinine clearance was 2.5 ml/min/year in the placebo group and 1.5 ml/min/year in those who received enalapril ($P = 0.040$). The institution of enalapril treatment was followed by an initial decline in creatinine clearance of about 3 ml/min. Subsequent follow up showed stabilization throughout the trial period.

The difference between the placebo and the treated arms, therefore became significant only after 5 years. There was a significant association between the initial decline in UAE rate by ACE inhibition and the subsequent renoprotective effect. 15/79 placebo treated and 5/77 enalapril treated patients crossed the threshold to microalbuminuria ($P = 0.042$).

The presence of diabetic retinopathy was recorded by an annual ophthalmoscopic examination. In diabetics with microalbuminuria, there were fewer new cases of retinopathy among those treated with enalapril: (7.8 vs 19% among the placebo group patients over a period of 5 years). Among the normoalbuminuric patients fewer new cases of retinopathy were found in those treated with enalapril: (6%—new cases among enalapril-treated vs 17.8% in the placebo group during 6 years). When pulled together there were 2794 patient-years of follow-up. Enalapril treatment resulted in an absolute risk reduction of 11.5 percentage points for the development of retinopathy (95% CI 6–27, $P = 0.004$). There were very few patients with proliferative retinopathy and this development seemed to be independent of therapy. The results of both trials indicate that the introduction of ACE inhibitor therapy may have a long-term protective effect on the microvascular disease of diabetes also in low-risk patients. In the kidney, the main ACE inhibitor induced modification is the decline in albuminuria. The protection of the retinal blood vessels may be partially due to the low blood pressure and possibly also by direct effects on the capillary wall.

ACE inhibitors for protection against microvascular complications in patients with type 1 diabetes

Nishi Chaturvedi and the EUCLID Study Group

Microvascular complications in people with type 1 diabetes continue to pose a major threat to health. ACE inhibitors slow the decline of renal function in advanced renal disease, but their effects at earlier stages are unclear, and the optimal level of albuminuria at which to institute treatment is not known. Further, whilst it is well known that retinopathy shares many of the risk factors for nephropathy, the effects of ACE inhibitors on retinopathy are not clear. We have therefore examined the effects of the ACE inhibitor lisinopril on nephropathy and retinopathy in ‘normotensive’ type 1 diabetic patients. EUCLID is a randomized, double-blind placebo controlled trial of the ACE inhibitor lisinopril in 530 men and women with type 1 diabetes aged 20–59 years with normalalbuminuria or microalbuminuria. Patients were recruited from 18 European centres, and were not on medication for hypertension. Resting blood pressure at entry was between 75 and 90 mmHg diastolic, and 155 mmHg systolic. Urinary albumin excretion rate (AER) was centrally assessed by two overnight urine collections at baseline, 6, 12, 18 and 24 months. Retinal photographs were taken at entry and 24 months, and graded into 5 groups (‘none’ to ‘proliferative’). At 2 years, AER was 18.8% lower in the lisinopril compared to placebo group (95% CI 2.0, 32.7, P = 0.03), adjusted for baseline AER and centre; absolute difference 2.2 g/min.

In people with normalalbuminuria, the treatment difference was 12.7% (95% CI −2.9, 26.0, P = 0.1), absolute difference 1.0 g/min, whilst in those with microalbuminuria, this difference was 49.7% (95% CI −14.5, 77.9, P = 0.1), absolute difference 34.2 μg/min (P = 0.04 for interaction). Retinopathy progressed by at least one stage in 13% of the lisinopril group and in 24% of the placebo group (P = 0.02). Lisinopril also reduced progression to proliferative retinopathy, odds ratio 0.18 (95% CI 0.04, 0.82, P = 0.03. Treatment reduced retinopathy incidence, odds ratio 0.69, 95% CI 0.30, 1.59, P = 0.4. Lisinopril slows the progression of renal disease in normotensive type 1 diabetic patients with little or no albuminuria, but the greatest effect was observed in those with microalbuminuria (AER 20 g/min). Lisinopril also reduces the risk of progression of retinopathy. The use of ACE inhibitors should be considered in the early stages of type 1 diabetes.


Early intervention in microalbuminuria: 24-h BP and exercise changes

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Recent data indicate that substantial pathophysiological changes have taken already in the microalbuminuric stage. Blood pressure is elevated with an attenuated circadian rhythm and vagal function is impaired with an abnormal sympathovagal interaction. Furthermore, new findings indicate abnormals in kidney ultrastructure in type 1 diabetic patients with microalbuminuria. In the light of these abnormalities early intervention in microalbuminuria has acquired increasing interest.

In two randomized placebo controlled double blind studies the effect of 2 years treatment with either lisinopril treatment (20 mg) or placebo was evaluated in normotensive, microalbuminuric type 1 diabetic patients. In collaboration with colleagues in Padova, we here present a post hoc analysis of a subgroup of 60 patients with urinary albumin excretion (UAEx) between 20 and 70 μg/min. Baseline UAEx was almost identical in the two groups (placebo, 36.3 μg/min x = 1.4; lisinopril, 35.5 μg/min x = 1.5 (geometric mean x = tolerance factor)), whereas development in UAEx over the 2 years was significantly different (P < 0.02) in the two groups with final UAEx in the placebo group of 58.8 μg/min x = 3.2 and 29.8 μg/min x = 2.5 in the lisinopril group. In the lisinopril group 22 patients (69%) reverted to normalbuminuria compared to 6 patients (21%) in the placebo group (P < 0.01).

In the subgroup of patients examined in Aarhus (n = 22) we performed 24-h ambulatory blood pressure measurements (AMBp), renal function tests (constant infusion technique) and determinations of exercise-induced albuminuria (bicycle ergometer, 70% of estimated maximal VO2). AMBP showed small increases in 24-h systolic and diastolic AMBP (1.6 ± 2.0 and 0.7 ± 4.9 mmHg over 2 years) in the placebo group, as opposed to significant reductions in the lisinopril group (−6.0 ± 8.2 and −4.1 ± 6.4 mmHg), (P < 0.02 and 0.05 between the values). Clinic BP measurements did not show significant differences. There were no differences in GFR or RPF in the two groups, but development in UAEx and development in filtration fraction (FF) was positively correlated in the intervention group (r = 0.9, P < 0.01), i.e. the patients who showed the greatest fall in UAEx also were the ones with the greatest fall in FF. Exercise testing showed a numerically increased in exercise induced albuminuria in the lisinopril group compared to the placebo group, although the difference was not statistically significant.

In conclusion, ACE-i treatment in patients with low-grade microalbuminuria reduces 24-h AMBP without attenuating diurnal blood pressure variation, reduces UAEx significantly, with changes in UAEx being strongly associated with changes in FF. Furthermore, ACE-i reverses micro- to normoalbuminuria in a significant fraction of patients compared to placebo.


Poulsen PL, Hansen KW, Mogensen CE. Ambulatory blood pressure in the transition from normo- to microalbuminuria; a longitudinal study in IDDM patients. Diabetes 1994; 43: 1248–1253

Mølgaard H, Christensen PD, Hermansen K, Sørensen KE, Christensen CK, Mogensen CE. Early recognition of autonomic

Ace inhibition vs calcium-channel blockade in normotensive type 1 and type 2 diabetic patients with microalbuminuria

G. Jerums on behalf of the Melbourne Diabetic Nephropathy Study Group, Melbourne, Australia

Several studies have examined whether angiotensin converting enzyme inhibitors (ACEI) exert beneficial effects on the course of diabetic nephropathy (DN) which extend beyond blood pressure control. In type 1 diabetes, such an effect has been demonstrated in patients with advanced DN [1] and the antiproteinuric effect of ACEI has been linked to subsequent protection from a decline in glomerular filtration rate (GFR) [2]. However, in normotensive microalbuminuric patients it has been difficult to determine if the initial decline in albumin excretion rate (AER) is linked to protection from the subsequent decline in GFR, even after a follow-up of 8 years [3].

The renal effects of ACEI in overt DN have been compared with those of other antihypertensive agents by meta-analysis. ACEI were shown to decrease proteinuria independently of changes in blood pressure and had an additional favourable effect on GFR that was independent of changes in blood pressure. ACEI and calcium-channel blockers (CCB) other than nifedipine had very similar renal protective effects, expressed as changes in proteinuria or GFR, whereas nifedipine therapy did not reduce proteinuria and was associated with a more rapid decline in GFR [4].

It is not clear to what extent studies in type 1 diabetes can be translated to type 2 diabetic patients. In type 2 diabetes, ACEI has been shown to reduce AER and to present a decline in GFR as estimated on the basis of the reciprocal of plasma creatinine levels, when studied over 7 years in middle-aged Israeli subjects [5]. A similar study performed over 4 years in normotensive Japanese type 2 diabetic patients, showed that ACEI reduced AER but did not change creatinine clearance [6].

Several recent studies have directly compared CCB with ACEI in evolving DN. In normotensive type 1 microalbuminuric patients, a placebo-controlled study compared the effects of lisinopril and nifedipine over 4–10 years [7]. Both drugs were equally effective in delaying the occurrence of overt proteinuria, despite lower systolic blood pressure levels in the lisinopril-treated group. In a 3-year study of 44 hypertensive type 2 diabetic patients with normo- or microalbuminuria, a similar decline in AER and GFR was observed in patients treated with cilazapril or amlodipine [8]. The rate of GFR fall in the study group as a whole was inversely related to baseline ACEI and CCB mediated reductions in proteinuria result in equal slowing of progression of DN, given similar levels of blood pressure control [10]. Type 2 diabetic patients with hypertension and overt DN were treated with either lisinopril (n=18), verapamil or diltiazem (n=18) or atenolol (n=16), with no significant difference in reduction in mean blood pressure over 63 months. Atenolol-treated patients had a greater rate of decline in creatinine clearance and a lesser fall in AER. However, no differences were discerned between the ACEI and CCB groups. In a 42 month study from Denmark, hypertensive type 2 diabetic patients with DN were treated with lisinopril (n=21) or atenolol (n=22), in equihypotensive doses. After 6 months, GFR declined by less than 1 ml/min/month in both groups suggesting that the progressive decline in GFR in DN can be ameliorated equally effectively by both treatments even though AER was reduced more in the lisinopril than the atenolol group [11].

In 1991, the Melbourne Diabetic Nephropathy Study Group reported the results of 12 months of treatment with equihypotensive doses of perindopril or nifedipine (slow release) in type 1 and type 2 diabetic patients with microalbuminuria [12]. Both treatments reduced AER in hypertensive patients and stabilised AER in normotensive patients. A second study is now in progress, with a follow-up of 2–8 years, in normotensive microalbuminuric (AER 20–200 g/min in 2 out of 3 measurements) patients with type 1 or type 2 diabetes. Entry systolic blood pressure (SBP) was <140 mmHg if age <40 years, otherwise <160 mmHg and diastolic blood pressure (DBP) <90 mmHg. Eighty-one patients were randomly assigned to receive placebo (PLAC), perindopril (PER) or nifedipine (NIF) using an open label protocol. Perindopril 2–8 mg/day and nifedipine (slow release) 10–40 mg/day were titrated to achieve a fall in DBP >4 mmHg. In each participant, mean SBP, DBP and MBP during the study, and gradients in AER (% change/year), and calculated GFR (Cockcroft–Gault, ml/min/year), were calculated from 3 monthly measurements. The development of macroalbuminuria was noted. In addition, yearly measurements of GFR using 99mTc-Technetium-DTPA and 24-h ambulatory blood pressure were performed.

In both type 1 and type 2 diabetic patients, HbA1c levels were not significantly different in any treatment group.

In the 47 type 2 diabetic patients, mean clinic arterial pressure during the study was similar in the 3 groups (PER 98, NIF 97, PLAC 100 mmHg). However, mean 24-h ambulatory blood pressure was lower in perindopril treated patients (PER 90, NIF 100, PLAC 97 mmHg, PER > NIF P<0.05). Baseline AER levels were: PER 96, NIF 60, PLAC 86 g/min, n.s. Individual AER gradients showed no tendency to increase in any treatment group (PER—4, NIF —1, PLAC +4%/year, n.s.). Eleven patients developed macroalbuminuria (PER 2, NIF 3, PLAC 6, n.s.). Mean baseline GFR was similar in all three groups (PER 116, NIF 110, PLAC 119 ml/min/1.73 m², n.s.). Individual GFR gradients in the placebo group showed a significant downward trend
to measured or calculated GFR was not evident.
In 34 type 1 diabetic patients, mean clinic blood pressure during the study was similar in each group (PER 92, NIF 96, PLAC 96 mmHg, n.s.), but mean 24-h ambulatory blood pressure was lower in perindopril treated patients (PER 88, NIF 92, PLAC 95 mmHg, PER vs PLAC $P<0.05$). Mean baseline AER was: PER 67, NIF 75, PLAC 78 g/min, n.s. Individual AER gradients showed a significant increase in both the nifedipine and placebo groups, but remained stable in the perindopril group (PER $-$ 4, NIF 49, PLAC 20%/year, NIF vs PER $P=0.02$, NIF vs PLAC $P=0.05$). Eleven patients developed macroalbuminuria (PER 1, NIF 5, PLAC 5, PER vs NIF $P=0.05$, PER vs PLAC $P=0.06$). The mean baseline GFR (PER 118, NIF 132, PLAC 120 ml/min/1.73 m$^2$, n.s.) showed a trend to a higher baseline GFR in the nifedipine group. This was associated with an accelerated decline in GFR in the nifedipine group (PER $-$ 3, NIF $-$ 16, PLAC 0 ml/min/year, NIF vs PLAC $P<0.01$, NIF vs PER $P<0.05$). However, attained GFR at 4 years approximated to 100 ml/min/1.73 m$^2$ in all three treatment groups. Calculated GFR was generally lower than measured GFR and declined by approximately 3 ml/min/year, with no evidence of an accelerated rate of decline in the nifedipine group.

In summary, no evidence of progression of AER or GFR was noted in microalbuminuric type 2 diabetic patients who remained normotensive or whose blood pressure was maintained below 160/90 after a mean follow-up of 4 years. No specific renoprotective effect of perindopril or nifedipine could be demonstrated. By contrast, in type 1 diabetic patients, AER did increase significantly in the placebo and nifedipine groups, and this was prevented by perindopril. Definitive assessment of the effects of nifedipine and perindopril on progression of GFR will require longer-term study as well as further assessments of GFR by: (a) calculation of GFR gradients weighted for duration of therapy to allow for unequal follow up; (b) calculation of GFR gradients with and without baseline values to allow for initial treatment effects on GFR; (c) measurement of GFR after cessation of drug therapy.

If an effect of either drug on GFR progression is found, it will then be necessary to determine if this can be separated from an effect on systemic blood pressure. Current evidence suggests that clinic blood pressures may be an unreliable indicator of 24-h ambulatory blood pressure levels in normotensive patients.

6. Sano T, Hotta N, Kawamura T et al. Effects of long-term enalapril treatment on persistent microalbuminuria in normo-
(<99 ml/min/1.73 m², the mean minus 2 SD of controls). The primary outcome measure was the number of subjects who progressed to glomerular hypertfiltration. The rate of change in the glomerular filtration rate (final minus initial divided by follow-up duration) was also calculated. Urinary albumin excretion and its status (normoalbuminuria <30 mg/24 h, microalbuminuria, macroalbuminuria) and blood pressure were assessed twice a year.

Results. The follow-up was 48 (range 24–72) months. At baseline, 6 subjects on strategy 1 had glomerular hypertfiltration, and 9 normofiltration, whereas 3 subjects on strategy 2 had hyperfiltration, and 7 normofiltration (n.s.). At follow-up, 8 subjects on strategy 1 had hyperfiltration, and 7 normofiltration, whereas one subject on strategy 2 had hyperfiltration, 4 normofiltration, and 5 hypofiltration ($\chi^2 = 10.69; P < 0.01$). On strategy 1 the median rate of change in the glomerular filtration rate was 0.03 vs $-0.86$ ml/min/1.73 m²/month of follow-up on strategy 2 (Mann–Whitney U test $P = 0.0016$). These results were attributable to the enalapril treatment strategy but not to changes in blood pressure, or to the larger number studied.

Conclusions. Treatment with enalapril (20 mg/24 h) prevents the glomerular filtration rate declining in normotensive type 1 diabetic subjects with microalbuminuria.

Marre M, Fabbrt M, Bernt G., Bouhanick B. The concept of structural changes was moderate in all cases. Thus, these results conflict to some extent with those reported by Fioretto et al. [2], who found marked changes in several cases of normo- and low-grade albuminuria. This discrepancy can probably be ascribed to the fact that all of our patients were young with microalbuminuria appearing after 10–15 years of diabetes and none had hypertension. It seems that when microalbuminuria appears after longer duration of diabetes and patients' age thus is higher, a different pattern of the structural changes is seen [3], probably dominated by macroangiopathy.

In a subset of the patients (six pair-biopsies) a comparison of results was made using either three or five glomeruli. The conclusion concerning paired biopsies did not change with the larger number studied.

The baseline biopsies the type 1 diabetic group of patients had significantly increased structural parameters compared with the control group (see Table 1). Each of the parameters was studied. It was found that 5-year mean and AER 33 (15–195) µg/min, i.e. the majority of patients had low-range albuminuria. No patient had elevated blood pressure at entry into the study. Non-diabetic kidney donors (ND) of age <40 years constituted the reference group.

Since the aim was to compare change in structural data over a fairly short period of time (2–3 years) it was mandatory to obtain the parameters with the highest possible precision. Therefore some effort has been made to optimize the methodology. Estimation of mesangial volume fraction is especially demanding since the changes are only borderline at the early stage of diabetic nephropathy. Therefore, three levels were analysed in each of three glomeruli as the standard procedure by electron microscopy (EM) [1]. Further, in one of the series the measurements have been supplemented with quantitation by light-microscopy (LM) of 1-µm-thick sections, using the largest cross-sectional area of seven glomeruli in each case. Light-microscopy as expected led to higher volume fractions but the results in terms of change from first to second biopsy were quite similar by EM and LM.

In the Swedish series the relationship between clinical variables during the years preceding the biopsy and the structural parameters was studied. It was found that 5-year mean HbA1c, diabetes duration and GFR were the variables with an independent influence on the severity of the diabetic glomerulopathy [5].

In this series a comparison was also made with the course of GFR, estimated by determinations during the normalbuminuric stage 2–5 years prior to the renal biopsy and again at the time of the biopsy. Patients showing a decline in GFR >5 ml/min/year had the thickest BM and matrix expansion. In multiple regression analysis of structure vs clinical variables the sole variable independently associated with the fall in GFR was BMT ($P = 0.003$) [6].

Thus, new little bits of information underline the association between diabetes control and the development of structural changes in the kidney and between structure and function. The final analysis of the follow-up biopsies will show if treatment with ACE-inhibitors for 3 years has any

### Table 1. Glomerulopathy data, mean and (CV%)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ND</th>
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<th>$P$</th>
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<tr>
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</table>

Abstracts 1067

Structural changes in microalbuminuria, effect of intervention

Ruth Österby, Hans-Jacob Bangstad, Susanne Rudberg; Electron Microscopy Laboratory, Aarhus Kommunehospital, Aarhus University Hospital, Aarhus, Denmark; Aker Diabetes Research Centre, Aker University Hospital, Oslo, Norway; Pediatric Unit, Karolinska Institutet, Stockholm, Sweden

Two follow-up biopsy series have been obtained in microalbuminuric type 1 diabetic patients with the aim of testing the influence of intervention on renal structural changes. The treatment given during the interval between first and second biopsy was either intensified diabetes control vs conventional control (Norway, n = 20) or ACE inhibitors vs beta-blockers (Sweden, n = 18). The analysis of follow-up biopsies has been completed only in the Norwegian series at the present time.

Both series dealt with young (age, median and (range): 19 (14–29) years) patients with diabetes duration 12 (6–18) years and AER 33 (15–195) µg/min, i.e. the majority of patients had low-range albuminuria. No patient had elevated blood pressure at entry into the study. Non-diabetic kidney donors (ND) of age <40 years constituted the reference group.

Since the aim was to compare change in structural data over a fairly short period of time (2–3 years) it was mandatory to obtain the parameters with the highest possible precision. Therefore some effort has been made to optimize the methodology. Estimation of mesangial volume fraction is especially demanding since the changes are only borderline at the early stage of diabetic nephropathy. Therefore, three levels were analysed in each of three glomeruli as the standard procedure by electron microscopy (EM) [1]. Further, in one of the series the measurements have been supplemented with quantitation by light-microscopy (LM) of 1-µm-thick sections, using the largest cross-sectional area of seven glomeruli in each case. Light-microscopy as expected led to higher volume fractions but the results in terms of change from first to second biopsy were quite similar by EM and LM.

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demonstrable effect on structural changes. Ongoing studies should further clarify if it holds true that morphometric studies are useful in intervention trials over short periods of time.


3. Østerby R, Schmitz A, Nyberg G, Asplund J. Renal structural changes in insulin dependent diabetic patients with albuminuria. Comparison of cases with onset of albuminuria after short or long duration. AMIS (in press)


**Renal interstitial expansion is modified by perindopril in type 2 diabetic glomerulosclerosis (DG) in type 2 (NIDDM) diabetic patients. A 2-year sequential biopsy study**

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Angiotensin-converting enzyme inhibitors (ACE-I) exert a protective effect against the progression of renal insufficiency in type 1 diabetes. Our objective was to study the structural effect of an ACEI, perindopril (PI) in type 2 diabetic patients with diabetic glomerulosclerosis (DG), two biopsies being performed at initiation of the study and 2 years later. 22 patients were randomized, 11 to PI and 11 to placebo (PO), after informed consent. At baseline (MO), 18 were macro and 4 were microalbuminuric; diagnosis of DG (6 ‘early’ and 16 ‘typical’) was assessed by two pathologists; four pts were not included due to predominant ischaemic lesions. 1 patient decided to leave the study at M 21. Compliance was assessed by plasma ACE activity. Tolerance was good. BMI and HbA1c did not change significantly. Blood pressure was slightly but not significantly more elevated in PO group. 24-h albuminuria increased in PO group and diminished in PI group at 2 years (P <0.05). The relative surface of interstitium in the biopsy (stereological analysis/BIOCOM computerized method) increased in PO (31.7±5.3 to 40.5±11.6) and remained stable in PI (33.8±4.8 to 34.6±6.6) (P <0.05). The relative volume of mesangium per glomerulus showed the same trend without reaching significance. Collagen VI, a marker of DG in man, was increased further in interstitium of PO than in those of PI (P <0.01); the same was observed with thrombospondin (P <0.01).

**Conclusion.** Despite limited patient population and observation time, renal biopsies advocate in this study for the role of angiotensin in interstitial expansion, a major factor of renal fibrosis.

**The concept of angiotensin II receptor blockers in hypertension and renal disease**

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Blockade of the renin-angiotensin-aldosterone system with angiotensin-converting enzyme (ACE) inhibitors is now recognized as an effective therapeutic approach to slow the progression of chronic renal failure in diabetic and non-diabetic nephropathies. Recently, non-peptide angiotensin II receptor antagonists have become available which enable to inhibit the renin-angiotensin system by blocking the binding of angiotensin II to its AT1 receptor, this latter being responsible for all the known effects of angiotensin II. The renal effects of these antagonists have now been characterized in normal subjects and hypertensive patients with or without renal failure. The results of these studies suggest that angiotensin II receptor antagonists and ACE inhibitors have rather similar renal effects. Indeed, angiotensin II antagonists have been shown to increase renal plasma flow without affecting glomerular filtration rate; hence filtration fraction decreases. Significant increases in urinary sodium excretion were also observed during angiotensin II receptor blockade. In contrast to other angiotensin II antagonists, losartan has a uricosuric effect which may be useful in patients with hypertension and hyperuricaemia. In hypertensive patients, the uricosuric effect of losartan has been shown to prevent the diuretic-induced increase in plasma uric acid. Recently we have investigated the possible renal interaction between angiotensin II receptor antagonists and non-steroidal anti-inflammatory drugs (NSAID). Interestingly, although angiotensin II antagonists do not affect prostaglandin metabolism, we found that indomethacin blunts both the natriuretic response to the angiotensin II antagonist valsartan and to ACE inhibitor enalapril. Thus NSAID may also attenuate the blood pressure lowering effect of angiotensin II receptor antagonists. Today the important remaining question concerns the renal protective effects of angiotensin II receptor antagonists. Experimentally, angiotensin II blockers appear to have the same effect as ACE inhibitors, but the final clinical response will be provided by the results of the renal study.


**A follow-up study of the course of nephropathy in type 1 diabetes**

Ed Lewis; Section of Nephrology, Rush Presbyterian St, Lukes Medical Center, Chicago, USA

The purpose of the study was to determine the clinical course of type 1 diabetic nephropathy in patients randomized to two
different levels of blood pressure control. 129 patients previ-ously entered into our ACE inhibitor trial were randomized to intensive BP control (MAP 92 mmHg) or standard BP control (MAP 100–107 mmHg).

Baseline characteristics included 47% male; 97% white; age 37 years; age of onset of type 1 DM 11 years; duration of type 1 DM 26 years; duration of proteinuria 7.4 years; duration of retinopathy 9.3 years; mean serum creatinine 1.7 mg/dl (150 pmol/l); mean proteinuria 2.0 g/24h; isothalamate–GFR 66 ml/min/1.73; glycosylated HB 11.6%. Median dose of ramipril in the standard group was 2.5 mg/day and the intensive group 10 mg/day. Median MAP achieved for the standard group was 97 mmHg and the intensive group 90 mmHg. Median 24-h creatinine clearance in the standard group was 70 ml/min at baseline and 65 ml/min at 24 months follow-up. Median creatinine clearance for the intensive group was 55.5 ml/min at baseline and 53.6 ml/min at 24 months follow-up. Median 24-h proteinuria was 1140 at baseline and 1723 mg/24 h at 24 months follow-up for the standard group and for the intensive group it was 1044 at baseline and 1505 mg/24 h at 24 months follow-up.

During the follow-up period the standard group experienced one serum creatinine doubling and 6 ESRD as compared to the intensive group with one doubling and 3 ESRD.

We conclude: (1) ramipril is a renoprotective ACE inhibitor; (2) the combination of ramipril at higher dose level plus between treatments). In this subset of patients the mean reduction in the diastolic blood pressure was 6.4 mmHg in the candesartan group and 3.6 mmHg in the placebo group ($P = NS$).

Conclusions

In this placebo-controlled study, 12 weeks of treatment with the angiotensin II antagonist candesartan reduced microalbuminuria in patients with stable type 2 diabetes and mild hypertension. Thus, candesartan appears to have a nephroprotective potential in this patient category. Further studies are needed to evaluate the long-term beneficial effects of this new class of drugs.


Advanced glycation end products and inhibitors

Mark E. Cooper; Department of Medicine, University of Melbourne, Austin & Repatriation Medical Centre, West Heidelberg 3081, Australia

The process of advanced glycation involves a spontaneous
reaction between glucose and proteins and lipids, particularly on long-lived structural proteins such as the collagens, leading to the formation of advanced glycation end products (AGEs) [1]. This process not only involves glycation but also oxidation steps, and therefore many investigators now consider these products to be as a result of glucoxidation [2]. A range of these products have been detected both in vitro and in vivo, some of these AGEs having a characteristic fluorescence [3].

An inhibitor of this pathway, aminoguanidine, has been shown to decrease AGE formation in rat tissues including the aorta [3] and the kidney [4]. This treatment is associated with attenuation in the rise in albuminuria and prevention of mesangial expansion in the diabetic rat [4]. Similar beneficial effects on other diabetic vascular complications have been reported and recent studies by our group have suggested that the protective actions of aminoguanidine involve prevention of overexpression of prosclerotic cytokines such as transforming growth factor beta (TGF-β1) and matrix proteins such as type IV collagen [5]. Aminoguanidine has now been administered to man and shown to inhibit haemoglobin AGE levels [6]. This drug is at present under trial in patients with end-stage renal disease and in diabetic patients with microalbuminuria and overt nephropathy. The results of these clinical trials are awaited with great interest.

A range of receptors for AGEs have been isolated and shown to be present in the kidney [7–9]. The effects of diabetes and various AGE inhibitors on expression of these receptors is an area of intense investigation. Recently more potent inhibitors of advanced glycation have been developed which are at least five times more potent than aminoguanidine in vitro [10]. Of particular interest is the thiazolidine compound, phenacylthiazolium bromide (PTB), a new compound which reacts with and cleaves covalent, AGE-derived protein cross-links [11]. Such an agent may be particularly useful clinically in the context of established renal disease since it may be able to reverse AGE-mediated tissue damage.

Cytokines and growth factors in diabetic renal involvement: focus on transforming growth factor-β
Kumar Sharma, Fuad N. Ziyadeh; Thomas Jefferson University, University of Pennsylvania, Philadelphia, PA, USA

A variety of growth factors and vasoactive peptides have been implicated in the pathogenesis of diabetic kidney disease. Growth hormone and the related insulin-like growth factors have been shown to play roles in diabetic renal hypertrophy and in mesangial matrix accumulation in experimental animals [1], tumour necrosis factor-α and PDGF-BB have been found to be elevated in glomeruli of diabetic rats [2], and the vasoactive peptides endothelin [3] and angiotensin II [4] are also involved in diabetic renal disease. However, the precise role of these factors in the pathogenesis of diabetic kidney disease is unclear. In the past several years, work from a variety of laboratories has clearly demonstrated an important role for the profibrotic cytokine, transforming growth factor-β in the pathogenesis of diabetic kidney disease. As TGF-β has the unique characteristic of stimulating a wide variety of matrix molecules that are also upregulated in diabetic nephropathy, it is likely that inhibiting TGF-β in the early and late stages of diabetic nephropathy would provide clinical benefit.

The evidence supporting a role for TGF-β has been demonstrated in cell culture, experimental animals and in patients with diabetes [5]. Exposure of mesangial cells to high glucose stimulates TGF-β1 gene expression, protein secretion and its bioactivity. Exposure of mesangial cells to high glucose enhances nuclear transcription of TGF-β1 gene, TGF-β1 promoter activity, and steady-state TGF-β1 mRNA levels. The matrix stimulating effects of high glucose on types I and IV collagen are blocked by neutralizing antibodies to TGF-β1 [6]. In animal models of diabetes, TGF-β1 mRNA and protein are elevated in the kidneys from rodents with spontaneous diabetes (NOD mouse and BB rat) [7] as well as in streptozotocin-induced diabetes in the mouse [8] and rat [9,10]. In addition, there is also upregulation of the TGF-β type II receptor in the STZ-induced diabetic mouse kidney [8].

Neutralization of TGF-β by antibodies against TGF-β1, 2 and 3 inhibits renal hypertrophy, blocks glomerular hypertrophy and attenuates diabetes-induced renal expression of fibronectin and type IV collagen in the early stages of diabetes [8]. In patients with diabetes, glomerular expression of TGF-β is increased in both early [11] and advanced stages of diabetic nephropathy [10]. Assaying TGF-β1 in samples of aortic and renal vein plasma, we recently demonstrated that non-diabetic patients have renal extraction of TGF-β1 from the circulation, whereas the kidneys of diabetic patients uniquely contribute TGF-β1 to the circulation [12]. As angiotensin II has been shown to stimulate TGF-β in cultured
mesangial cells and proximal tubular cells, several studies have demonstrated that ACE inhibition reduces renal TGF-β1 expression in animal models of diabetes (reviewed in [4]).

We recently found that serum TGF-β1 levels were reduced by captopril treatment in a small sample of patients who were enrolled in the Collaborative study [13], and the reduction of serum levels correlated with delayed progression of renal function (JASN [in press]). Apart from the effect of TGF-β1 to stimulate matrix proteins, it may also be involved in the altered vasoreactivity of diabetic renal cells. Pretreatment with TGF-β1 or vascular smooth muscle cells [14] and mesangial cells [15] attenuates all-induced and PDGF-induced intracellular calcium mobilization respectively. The effect of TGF-β1 to inhibit intracellular calcium mobilization may be due to enhanced phosphorylation and decreased expression of the IP3-gated calcium channel, the type I IP3 receptor, in mesangial cells [16]. Interestingly, expression of the type I IP3 receptor is reduced in the kidneys of diabetic rats and diabetic mice, and by immunostaining there appears to be reduced expression of the type I IP3R in glomerular cells and vascular smooth muscle cells (JASN [in press]).

Thus strategies to target and inhibit renal TGF-β production or effect may be beneficial in retarding both the accumulation of mesangial matrix as well as vascular dysfunction in progressive diabetic nephropathy.

In order to propose interventions to block TGF-β1 an understanding of the basic biology of the TGF-system is necessary. The TGF-β system consists of three major isoforms of TGF-β and three receptors [17]. The TGF-β isoform is produced by almost all cell types and is unique in that it also circulates in the blood and may have endocrine type effects. It exists in a latent form bound to the TGF-β latency associated peptide as well as z2-microglobulin. Binding of TGF-β by decorin [18], the latency-associated peptide [19] and the soluble TGF-β type III receptor [17] may prevent its binding to its effector receptors and decrease TGF-β activity. The TGF-β type II receptor primarily acts as the ligand binding receptor at the cell membrane and the complex is then recognized by the signal transducing TGF-β type I receptor [17].

Overexpression of a dominant negative TGF-β type II receptor has the effect of stimulating pancreatic tumour growth in overexpressing cells [20]. Activation of the type I receptor by cross-phosphorylation enhances serine phosphorylation of the intermediary Smad family of molecules [21]. Interaction of phosphorylated Smad 2 or 3 with Smad 4 promotes nuclear translocation and active gene regulation. Recently other Smad isoforms, Smad 6 and 7, have been found to inhibit TGF-β signaling [22].

In conclusion, the evidence implicating TGF-β as an important factor in the early and late stages of diabetic renal involvement is very strong. Inhibiting TGF-β systemically may lead to untoward effects of increasing cellular proliferation and autoimmunity [23]. Greater insight into the signaling pathways mediating TGF-β induced matrix production may allow for selective blockade of these pathways in target tissues thus limiting the potential for generalized undesired effects.

14. Zhu Z, Tepel M, Neusser M, Zidek W. Transforming growth factor beta 1 levels were reduced in diabetic glomeruli and by immunostaining there appears to be reduced expression of the type I IP3R in glomerular cells and vascular smooth muscle cells (JASN [in press]).
Isozyme-specific inhibition of PKCβ. LY333531 new therapeutic approaches to human disease by modulation of protein kinase C (PKC) isozymes
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Protein kinase C (PKC) is a family of closely related serine and threonine kinases. Overactivation of some PKC isozymes has been postulated to occur in several diseases states including diabetic complications. Hyperglycaemia leads to overactivation of PKC and selectively to hyperactivation of PKCβ through elevated diacylglycerol levels. The discovery and development of LY333531 offers an opportunity to test these hypotheses. LY333531 selectively inhibits the PKCβ (IC50 = 4.7 nM) and PKCβII (IC50 = 5.9 nM) isozymes with about 70-fold selectivity for the beta isoforms relative to PKCε [1]. The inhibitor exhibits ATP-dependent competitive inhibition of PKCβ and is selective for PKC in comparison to other ATP dependent kinases (protein kinase A, calcium calmodulin, caein kinase, src tyrosine kinase).

The in vitro selective inhibition of PKCβ by LY333531 has also been demonstrated at the cellular level [2]. When administered orally, LY333531 ameliorated the glomerular filtration rate, albumin excretion rate, and retinal circulation in diabetic rats in a dose-responsive manner, in parallel with its inhibition of PKC activities. The observation that PKCβ regulates vascular endothelial growth factor (VEGF)-induced mitogenesis suggests that a selective PKCβ inhibitor such as LY333531 may be useful in VEGF-mediated disease states [3]. VEGF induced concentration-and time-dependent increases in PKC activation. VEGF stimulation increased the content of Ca(2+) -sensitive PKC isozymes (β and βII) in the membrane fractions, while no changes were observed for PKC isoforms delta and epsilon.

The stimulation of PKC activity by VEGF was preceded by the activation of phospholipase C gamma (PLCγ). VEGF increased phosphatidylinositol 3-kinase activity 2.1-fold, which was inhibited by wortmannin, a phosphatidylinositol 3-kinase inhibitor, without decreasing the VEGF-induced increase in PKC activity or endothelial cell growth. VEGF’s mitogenic effect was inhibited by LY333531, in a concentration-dependent manner. In contrast, antisense PKCζ oligonucleotides enhanced VEGF-stimulated cell growth with a simultaneous decrease of 70% in PKCζ protein content. Thus VEGF appears to mediate its mitogenic effects partly through the activation of the PLCγ and PKC pathway, involving predominately PKCζ isoform activation in endothelial cells.

A spectrum of biochemical and molecular abnormalities associated with chronic changes induced by glucose or diabetes in the cultured mesangial cells and renal glomeruli that can be prevented by LY333531 [4]. Hyperglycaemia increased DAG level in cultured mesangial cells exposed to high concentrations of glucose and activated PKCζ and β isoforms in the renal glomeruli of diabetic rats. LY333531 added to cultured mesangial cells inhibited activated PKC activities by high glucose without lowering DAG levels. When LY333531 was given orally in diabetic rats specifically inhibited the activation of PKCβ isoform without decreasing PKCα isoform activation. Glucose-induced increases in arachidonic acid release, prostaglandin E2 production, and inhibition of Na+ /K+ - ATPases in the cultures were completely prevented by the addition of LY333531. Oral feeding of LY333531 prevented the increased mRNA expression of TGF-β1 and extracellular matrix components such as fibronectin and α1(IV) collagen in the glomeruli of diabetic rats in parallel with inhibition of glomerular PKC activity. These results suggest that the activation of PKC, predominantly the β isoform by hyperglycaemia in the mesangial cells and glomeruli can partly contribute to early renal dysfunctions by alteration of prostaglandin production and Na+ /K+ - ATPase activity as well as the chronic pathological changes by the overexpression of TGF-β1 and extracellular matrix components genes.

These results taken in context suggest selective inhibition of the PKCβ isoforms by LY333531 may offer a new approach to address kidney disease as well as the microvascular complications and cardiomyopathy of diabetes [5].


Progression in inherited renal disease
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The potential mechanisms of renal progression are diverse in the various inherited renal diseases: e.g. cyst progression and compression of adjacent parenchyma in autosomal dominant polycystic kidney disease (ADPKD); persistence and extension of α1/α2 chains of type IV collagen in GBM, in the place of α3/α4/α5 (IV) (IV); focal segmental glomerulosclerosis in type I glycogen storage disease; glycolipid deposition, most particularly in vessels, leading predominantly to ischaemic nephropathy in Fabry’s disease. There is no single mechanism of progression in inherited disorders.

In ADPKD, two main determinants of progression have been identified: (1) PKD1 vs PKD2 disease, the renal progres-
sion being slower in the PKD2 type; interactions between PKD1 and PKD2 genes have been recognized, and these may be crucial to modulate the rate of progression. (2) The sex of the patient, the renal progression being slower in women. Among 126 ADPKD patients in ESRD at Necker, from 1989 to 1996, 42% were females; in the 26 patients in ESRD at 65 years or more, 65% were females, and all 5 in ESRD at 70 years or more were female. Overall, women (n = 53) were in ESRD at 56±11 years and men (n = 73) at 53.9±9 years. Low-protein diet, strict control of blood pressure, and ACE inhibition have been shown to have no significant effect on renal progression. However, the effects of earlier intervention in the course of the disease have not been tested.

Alport syndrome is clinically and genetically heterogeneous. In X-linked disease (due to mutations of the gene encoding for a3 chain of type IV collagen), the rate of progression in males contrasts in juvenile-type (EDRD < 30 years) and adult-type (ESRD > 30 years) families.

The rate of decline of creatinine clearance is also slower in the latter kindreds. No clear-cut relationship has been found so far between phenotype and genotype. The rate of progression is rapid in autosomal recessive disease (defects of a3 or a4 (IV)). So far it has not been tested whether this rate is similar or not in female and male siblings from the same kindreds. In anecdotal cases of Alport syndrome, ACE inhibition has been claimed to be renoprotective, which would not be surprising in a primary glomerular disease. No prospective study is available.

The mechanisms of progression are unknown. Kalluri et al. have suggested that isoform switching (from a1/a2 to a1/a4 (IV)) is developmentally arrested in X-linked Alport disease. This defect would lead to increased susceptibility of GBM to endoproteolysis. Glomerular accumulation of a1/a2 (IV) by itself could be deleterious. The molecular mechanism of progression might be of great interest not only for Alport syndrome, but also for other glomerular diseases.

Similarly, the mechanism of progression of interstitial fibrosis in juvenile nephronophthisis, an autosomal recessive disease, may provide interesting insight into more general mechanisms of initiation and progression of interstitial fibrosis.


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Competing hypothesis in renal scarring: further support for the chronic hypoxia hypothesis

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The hypotheses which have formed the basis for studies of renal pathophysiology have rarely been ‘competing’. Rather, they have invariably emerged as being complementary which is not surprising in that the processes which cause disease are almost always multifactorial in nature. Renal scarring is no exception to this.

The central issue in renal scarring, which is usually secondary to glomerular disease, is: Why does an acute insult not resolve but rather translate itself into a chronic destructive process? To answer this question it is useful to distinguish between (1) factors that per se are responsible for chronicity i.e. the ‘causes’ of the scarring process, (2) factors that affect the rate of scarring, and (3) factors which mediate the scarring process.

Hypotheses that address the ‘cause of chronicity’ must include the following:

1. The initial injurious agent persists and causes chronic damage. This could apply to diabetes but probably cannot be invoked in most primary glomerulopathies.

2. Chronic glomerular scarring is the result of haemodynamic adaptations that are set in train by the initial insult and which, if sufficiently severe, will destroy sufficient filtration surface area to cause the adaptation to be persistent in surviving glomeruli. The ‘adaptation’ thus becomes the injurious agent.

3. Tubulointerstitial injury follows the initial glomerular insult and it is this component that is self-perpetuating. For this hypothesis to be valid, there must be a link between the glomerular injury (or adaptation) and the tubulointerstitial compartment, and injury to the latter must be irreversible and progressive.

What causes tubulointerstitial injury associated with glomerular disease? The two principal ideas are that:

1. Filtration of proteins, lipids and other molecules injure the tubules and this sets up the scarring process. This is supported by the recognition that the magnitude of proteinuria is a major predictor of the rate of progression and reversal of proteinuria slows progression.

2. The ‘chronic hypoxia hypothesis’, which posits that the same alterations in flow and pressure that affect the glomerulus (albeit of lesser magnitude) also cause microvascular disease in the interstitium. This limits oxygen delivery to tubular cells, which respond by producing cytokines and chemokines which initiate and perpetuate fibrosis. Aggravation of this injury occurs in hypertension and where there is a primary vascular component to the disease (amyloid, diabetes) and control of blood pressure limits interstitial injury.

Why does interstitial scarring not resolve?

The fact that some glomerular diseases progress even when proteinuria is completely reversed or when blood pressure is well controlled must mean that these are not ‘causes’ of the progression but rather modulators of its rate.

The chronic hypoxia hypothesis critically requires that interstitial microvessels be surrounded by cells which can sense and respond to hypoxia once the scarring process has been initiated. We have shown that human proximal tubular cells in culture produce cytokines in an oxygen-sensitive manner (endothelin I, VEGF, TGFβ). Others have shown that such cells also release chemokines that attract mononuclear cells which contribute to inflammation and scarring. We have further shown that hypoxia is directly fibrogenic to proximal tubular cells and renal interstitial fibroblasts which produce collagen, decrease their matrix metalloproteinase, and increase their TIMP activities.

Because the fibrogenic process per se obliterates
microvessels, i.e. the ‘healing of a scar’, the hypoxia is per-
petuated and the parenchymal cells which are adjacent to
such ischaemic areas respond by further collagen deposition
and cytokine release. This in turn causes further microvascu-
lar loss and hypoxia and the process is self-perpetuating.

The chronic hypoxia hypothesis therefore requires that a
critical degree of initial hypoxic damage to the tubulointersti-
tium is sufficient to set the vicious cycle in process. If the initial
injury is not sufficiently severe, small or healing scars will
result and the disease will not be progressive.

Studies are in progress which will establish how lowered
oxygen tension triggers collagen production independently of
mediators such as TGF-β. The relevant molecules would be
logical targets for interventional therapy.

**Combination therapy for hypertension and renal disease in
diabetes**

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More than 75% of individuals with diabetes, microalbuminu-
ria and hypertension require two or more antihypertensive
medications to attain the newly recommended level of arterial
pressure control, i.e. <130/85 mmHg [1]. To achieve this
blood-pressure goal, it is common to add a medication whose
antihypertensive action potentiates the initially selected drug
[2]. To further improve compliance and reduce drug side
effect profiles, fixed-dose combinations of antihypertensive
drugs with complementary modes of action have been
recently developed [2]. These medications combine a lower
dose of two different antihypertensive drugs that, in a fixed
dose combination, reduce arterial pressure to a greater extent
than either alone.

While blood-pressure reduction is critical for the preserva-
tion of renal function, it is also important to select antihy-
tensive agents that have unique effects on the kidney. Early
changes observed in the diabetic kidney include an increase in
efferent arteriolar tone and a loss of autoregulation. These
changes lead to an increase in intraglomerular capillary pres-
sure, which results in cell stretch and activation of various
autocrine and paracrine factors associated with tissue
injury [3]. In addition, glomerular membrane permeability is
increased and microalbuminuria ensues, a surrogate marker
for the presence and progression of diabetic nephropathy [3].
The earliest morphologic change is mesangial matrix expan-
sion and in some cases interstitial inflammation, the latter
portends a poor renal prognosis [3,4].

Experimental evidence demonstrates that reductions in
intraglomerular capillary pressure, through either profound
reductions in arterial pressure or dilatation of the efferent art-
eriole, slow progression of diabetic renal disease [3,5]. ACE
inhibitors reduce efferent arteriolar tone and intraglomerular
pressure. In clinical studies, ACE inhibitors demonstrate a
consistent and persistent reduction in proteinuria as well as
an attenuated progression of both type 1 and type 2 diabetes
associated nephropathy [3,6]. Moreover, while blood pres-
sure reduction itself slows progression of early diabetic
nephropathy, the ACE inhibitors appear to preserve renal
function to an even greater extent than other agents [5,6] do.

Antihypertensive agents that do not reduce intraglomerular
pressure may still slow progression of diabetic nephropathy.
This may relate to their specific effects on synthesis or
degradation of mesangial matrix proteins. Notably, non-
dihydropyridine calcium-channel blockers (CCBs), verapamil
and diltiazem, preserve renal morphology in a manner similar
to ACE inhibitors. This subclass of CCBs affects the synthesis
of various matrix proteins such as heparan sulphate and glu-
coaminoglycan as well as fibroblast turnover [7]. Prevention
of mesangial expansion or glomerulosclerosis has not been
shown for either blockers or most dihydropyridine CCBs
[3,6].

Dihydropyridine CCBs do not protect against morpholog-
cal progression of renal disease. Studies using 24-h blood
pressure monitoring in a rat model of renal insufficiency ran-
domized animals to either a dihydropyridine, amlodipine, an
ACE inhibitor, benazepril or a combination of each in a lower
dose to achieve a similar level of blood pressure reduction.
Amlodipine did not reduce development of glomeruloscle-
sis or proteinuria, while the ACE inhibitor and the
combination had similar benefits on these parameters [8].

This lack of protection by dihydropyridine CCBs is also
seen clinically and relates to a lack of effect on glomerular
membrane permeability, in spite of blood-pressure reduction
[9]. While, many factors may contribute to the disparate
effects of the different subclasses of CCBs on both surrogate
end-points as well as progression of renal disease, it is clear
that arterial pressure reduction is the ultimate goal that uni-
formly leads to preservation of renal function. Thus, since the
majority of patients with diabetic nephropathy require more
than one medication to reduce arterial pressure, a combina-
tion of antihypertensive agents, individually shown to reduce
arterial pressure and proteinuria as well as preserve renal
morphology and function would be preferred. Since prelimi-
ary evidence supports the notion that both ACE inhibitors
and non-dihydropyridine CCBs slow progression of diabetic
renal disease to a greater extent than conventional blood
pressure lowering agents, it is predicted that a combination of
these two classes of agents will provide better renal protection
than either alone. Unfortunately, few animal or human stud-
ies have examined this hypothesis.

Of the few animal studies that have evaluated the effects of
an ACE inhibitor/CCB combination on various aspects of
renal disease, only one has actually controlled for blood-
pressure differences [9]. Thus a meaningful comparison
cannot be made between the combination and its individual
components. As previously mentioned this study demon-
strated that combination therapy with a fixed-dose of an ACE
inhibitor, benazepril, and dihydropyridine CCB, amlodipine,
prevented progression to glomerulosclerosis seen with amlodi-
pline alone. It also reduced proteinuria to a greater extent
than amlodipine alone. However, the effects on proteinuria
while greater than benazepril alone were not statistically
different.

In contrast to these findings, two animal studies that com-
bined the effects of a non-dihydropyridine CCB with an ACE
inhibitor demonstrated a potentiation of the ACE inhibitor
associated reduction in proteinuria as well as relatively
greater preservation of renal morphology [5,10]. These effects
were noted at similar levels of blood-pressure reduction.
Moreover, one of the two studies showed greater morpholo-
gical protection of the glomerulus, in the absence of blood-
pressure control, using the fixed-dose combination of verapa-
il with trandolopril in comparison to their individual
components [10]. The potentiating antiproteinuric effects of
a fixed-dose combination of verapamil and trandolopril, vs its
individual components, has also been observed in a recent
randomized clinical study of patients with type 2 diabetes
associated nephropathy and comparable blood-pressure con-

Additionally, a 4-year follow-up study of patients with type
2 diabetes associated nephropathy, randomized to either verapamil or lisinopril, alone or each in a reduced dose combination, demonstrated that combination therapy had a slower rate of decline in renal function, a lower amount of proteinuria and the lowest side-effect profile [12]. No other long term clinical studies have formally evaluated either fixed-dose or reduced dose combination therapy on progression of diabetic renal disease.

It is critically important to remember that effective arterial pressure reduction needs to be achieved in diabetic patients to not only preserve renal function but also reduce cardiovascular mortality, the latter being the primary cause of death in these patients. Evidence supports the concept of arterial pressure reduction to levels of less than 130/85 mmHg maximally protects the kidney and reduces cardiovascular mortality. Diabetic patients have difficulty in controlling blood pressure; thus, combination therapy would be highly recommended. Of the many possible combinations, a non-dihydropyridine CCB/ACE inhibitor combination may be preferred not only because of a low side-effect profile but also the fact that each agent has been individually shown to reduce cardiovascular mortality following myocardial infarctions [3].

In the light of these few studies, however, there are insufficient data to assess whether combination antihypertensive therapy offers a distinct advantage over its individual components to further slow progression of diabetic nephropathy at a given level of blood-pressure reduction.

Rational treatment in diabetic renal disease, what is the evidence?

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Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria (>$300$ mg/24 h), a relentless decline in glomerular filtration rate (GFR), and elevated systemic blood pressure. The prevalence of abnormally elevated albumin excretion rate (>30 mg/24 h) is approximately 40% in patients with both insulin-dependent diabetes mellitus (type 1) and non-insulin-dependent diabetes mellitus (type 2). Diabetes has become the leading cause of end-stage renal failure in the United States, Japan, and in Europe. Identification of patients at high risk of developing diabetic nephropathy is possible by screening for microalbuminuria (30 to 300 mg/24 h) [1,2]. Randomized controlled trials in normotensive type 1 and type 2 diabetic patients with persistent microalbuminuria indicate that angiotensin-converting enzyme (ACE) inhibitors diminish urinary albumin excretion rate and postpone and may even prevent progression to clinical overt diabetic nephropathy [3–5]. These findings suggest that screening and intervention programmes are likely to have life-saving effects and lead to considerable economic savings [6].

Previous studies have demonstrated that the diabetic patients with the most marked proteinuria have the worst prognosis. Recently it has been suggested that proteinuria is not simply a marker of the extent of glomerular damage, but proteinuria per se may contribute to glomerular damage. Furthermore, a decrease in albuminuria shortly after onset of ACE-inhibition predicts an attenuated rate of fall in GFR in diabetic nephropathy. The importance of this finding is highlighted by the demonstration that ACE-inhibition has an antiproteinuric effect independent of the effect on systemic blood pressure.

Impaired nocturnal decline in blood pressure is more prevalent in patients with diabetic nephropathy and autonomic neuropathy. This may contribute to the enhanced cardiovascular morbidity found in that condition. Raised blood pressure accelerates both the development and the progression of nephropathy in both type 1 and type 2 diabetic patients [7]. Arterial blood pressure thus seems to have a complex relationship with diabetic nephropathy—nephropathy raising blood pressure and blood pressure accelerating the course of nephropathy. Effective blood pressure reduction with $\beta$-blockers and/or angiotensin-converting enzyme inhibitors combined with diuretics reduces albuminuria, delays the progression of nephropathy and postpones renal insufficiency in diabetic nephropathy [7–9]. Originally Björck et al. [8] demonstrated that treatment with ACE inhibition combined with diuretics can reduce the rate of decline in kidney function in type 1 diabetic patients with moderately advanced diabetic nephropathy (mean GFR $47$ ml/min/1.73 m$^2$) more than equally effective antihypertensive treatment with metoprolol and diuretics. This finding suggests renoprotection—a beneficial effect on kidney function (and structure) above and beyond that expected from the blood-pressure-reducing effect alone. This suggestion has been confirmed and extended in a recent randomized double blind study comparing the effects of captopril versus placebo (receiving conventional antihypertensive treatment) in type 1 diabetic patients with diabetic nephropathy, whose baseline serum creatinine concentration was above $133$ $\mu$mol/l [9]. The introduction of effective antihypertensive treatment has increased the median survival time to more than 16 years as compared to 5 and 7 years in untreated patients in the past [10]. The potential beneficial effects of ACE inhibitors and calcium antagonists alone or combined on progression of diabetic nephropathy.

Effect of ACE inhibition on the altered intrarenal transforming growth factor-β (TGF-β) system in experimental diabetes

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Several growth factors [1], including the transforming growth factor-β (TGF-β) system [2–7], have been suggested to play a role in the development of diabetic nephropathy. In vitro high glucose concentrations increase TGF-β 1 mRNA levels in both cultured mesangial cells and proximal tubular cells [2,3]. In addition in vivo experiments have been published on changes in the endogenous renal TGF-β axis in various animal models of experimental diabetes [4–7]. It has been suggested that activation of the renal TGF-β system in diabetes may be mediated, beside a direct stimulatory effect of hyperglycaemia per se [2,3], through activation of the renin–angiotensin system. Accordingly, exposure of mesangial cells in vitro to angiotensin II stimulates the expression of TGF-β and extracellular matrix proteins [8]. Furthermore a recent study examined the effect of an ACE inhibitor (captopril) on high-glucose induced changes in the TGF-β system and growth in LLC-PK1 cells, a porcine kidney cell line analogous to the proximal tubule cell [70]. In this cell system high-glucose increased TGF-β 1 mRNA, TGF-β type I and II receptor protein expression and cellular hypertrophy, while cellular mitogenesis was inhibited. Captopril dose-dependently decreased TGF-β type I and II receptor protein expression and cellular hypertrophy, increased cellular hyperplasia, while TGF-β 1 mRNA was unchanged [70]. So far no published data have examined the possible effect of ACE inhibition on the intrarenal changes in the various TGF-β isoforms (TGF-β 1,2,3) and TGF-β type I, type II, type III receptors (TGF-β RI, TGF-β RIII, TGF-β RIIII) in experimental diabetes in vivo. Accordingly, we performed a study in which immunocytohistochemistry was performed on kidney sections from non-diabetic and streptozotocin (STZ)-diabetic rats after 2 and 4 weeks treatment with an ACE-inhibitor (enalapril) or placebo, using specific TGF-β 1,2,3 and TGF-β RI, TGF-β RI, TGF-β RIIII antibodies. Enalapril partially prevented the diabetes associated renal hypertrophy, while no effect was seen on kidney weight in non-diabetic animals. In addition, the diabetes associated increase in 24-h urinary albumin excretion (UAE) was fully prevented by enalapril with no significant effects in non-diabetic animals. Enalapril therapy had no effect on body-weight, metabolic control or food consumption in either groups. Immunoreactivity of the glomerular TGF-β 1 isoform revealed a transient decrease after 14 days with a normalization at 30 days in untreated diabetic animals. In enalapril-treated diabetic animals the immunoreactivity stayed below control level at all time points.

The immunoreactivity of the glomerular TGF-β 1 isoform increased over 30 days in untreated diabetic animals, while this increase was only partially abolished by enalapril-treatment. The glomerular TGF-β 1 immunoreactivity increased over 30 days in untreated diabetic animals with no major effect of enalapril-treatment. The immunoreactivity of glomerular TGF-β 1 RI and TGF-β RIIII isoforms was increased in untreated diabetic animals after 14 and 30 days when compared with control rats, while enalapril treatment decreased the immunoreactivity of both receptors to values below non-diabetic control level at all time points. Similarly, glomerular TGF-β 1 RII immunoreactivity increased over 30 days in untreated animals when compared to non-diabetic controls, while enalapril therapy in diabetic animals was associated with a dramatic decrease over 30 days to almost undetectable levels.

In conclusion, our data suggesting that the TGF-β axis operating through a complex intrarenal system, may be a significant mediator of the renal changes observed in experimental diabetes. Moreover, ACE-inhibition has pronounced inhibitory effects on the elevated levels of the TGF-β receptors required for intracellular signalling through this growth factor system. The present findings suggest a possible new mechanism of action for ACE inhibitors.

Randomized placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy

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Methods

In this prospective double-blind trial, 352 patients were classified according to baseline proteinuria (stratum 1, 1–3 g/24 h; stratum 2, 3 g/24 h), and randomly assigned ramipril or placebo plus conventional antihypertensive therapy targeted at achieving diastolic blood pressure under 90 mmHg. The primary end-point was the rate of GFR decline, and preventing endstage renal disease.

Background

In diabetic nephropathy, angiotensin-converting enzyme (ACE) inhibitors have a greater effect than other antihypertensive drugs on proteinuria and the progressive decline in glomerular filtration rate (GFR). Whether this difference applies to progression of non-diabetic proteinuric nephropathies is not clear. The Ramipril Efficacy In Nephropathy study of chronic non-diabetic nephropathies aimed to address whether glomerular protein traffic influences renal-disease progression, and whether an ACE inhibitor was superior to conventional treatment, with the same blood-pressure control, in reducing proteinuria, limiting GFR decline, and preventing endstage renal disease.

Findings

At the second planned interim analysis, the difference in decline in GFR between the ramipril and placebo groups in stratum 2 was highly significant (P = 0.001). The independent adjudicating panel therefore decided to open the randomization code and do the final analysis in this stratum (stratum 1 continued in the trial). Data (at least three GFR measurements including baseline) were available for 56 ramipril-assigned patients and 51 placebo-assigned patients. The decline in GFR per month was significantly lower in the ramipril group than the placebo group (0.53 (0.08) vs 0.88 (0.13) ml/min, P = 0.03). Among the ramipril-assigned patients, percentage reduction in proteinuria was inversely correlated with decline in GFR (P = 0.035) and predicted the reduction in risk of doubling of baseline creatinine or endstage renal failure (18 ramipril vs 40 placebo, P = 0.04). The risk of progression was still significantly reduced after adjustment for changes in systolic (P = 0.04) and diastolic (P = 0.04) blood pressure, but not after adjustment for changes in proteinuria. Blood-pressure control and the overall number of cardiovascular events were similar in the two treatment groups.

Rational treatment in non-diabetic renal disease: what is the evidence?

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There is controlled information available with respect to halting progression of renal failure on dietary protein restriction and antihypertensive treatment. Although in animal experiments [1] dietary protein restriction interfered with progression, clinical use of dietary protein restriction has been rediscovered only recently [2]. Although in several short-term [3] or well-controlled studies of small sample size [4] a benefit was seen, a large
controlled study, i.e. the MIDRD (Modification of Diet in Renal Disease) study, failed to provide definite statistical evidence for the efficacy of this therapy [5]. The MIDRD study can be criticized on several accounts, i.e. to short duration of observation and failure to consider acute haemodynamically mediated changes of GFR upon institution of a low-protein diet. Any possible beneficial effect of protein restriction, if present [6], must be relatively small compared to that of antihypertensive treatment. In children, a low-protein diet was without any benefit [7]. The evidence is much more convincing for blood-pressure lowering with conventional antihypertensive agents and more recently for ACE inhibitors [8]. Administration of ACE inhibitors retards progression more effectively than can be explained by blood-pressure lowering alone, at least in proteinuric patients. The beneficial effect on progression is predicted by the initial decrease in proteinuria [9]. The selective benefit of ACE inhibitors over alternative antihypertensive agents is particularly pronounced when blood pressure is lowered little or not at all, while the antiproteinuric superiority is progressively lost with increasingly efficacious lowering of blood pressure [10]. A similar phenomenon was also observed with respect to reaching renal end-points (doubling of serum creatinine or renal replacement therapy) in the trial on diabetic nephropathy (E. Lewis, personal communication). The average rate of progression is significantly lower in premenopausal women, while it is similar in men and in postmenopausal women; this raises the issue of whether hormonal replacement therapy should be considered in postmenopausal women with renal disease.

Smoking has a proven deleterious effect on the appearance of proteinuria and progression of nephropathy in patients with diabetes mellitus. The same holds true for patients with non-diabetic renal disease [11].


Concluding remarks
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When we started to organize this meeting over a year ago, I had hoped that I would be giving these remarks at the lowest point on the earth, i.e. at the Dead Sea. Following the ERA-EDTA decision to move their congress to Geneva, we were obliged to move this meeting to Switzerland. I am sure that you will all agree that the choice of the venue has been most conducive to the study of progressive renal disease. I would like to thank Michel Burnier, who agreed, at a very late date, to be responsible for the local arrangements. I must also stress the role of Carl Erik Mogensen, who was the moving force behind the organization of this meeting and without whom we would not have been able to organize this outstanding programme. I also wish to thank the other members of the organizing committee, Ab Donker of the Netherlands, Hans Brunner of Switzerland, and David van Dijk of Israel as well as the members of our advisory committee, Hans-Henrik Parving, Eberhard Ritz, and Mike Steffes for their help and advice.

Before concluding I would like to express a certain concern for the patient, who has progressive renal failure. Over the past day and a half we have heard the latest information on the pathogenesis and treatment of progressive renal failure. Several articles, describing the clinical approach to these patients have been published. In fact their are clear guidelines for the treatment of the patient with diabetic nephropathy [1–4] and the patient with hypertension [5,6]. Unfortunately most patients do not receive optimal treatment. Pommer et al. [7] found that of 66 diabetic patients, referred for nephrological advice, the creatinine clearance was below 30 ml/min in 77% at time of referral. In spite of renal involvement, hypertension was poorly controlled in 97% and 75% were not receiving angiotensin-converting enzyme inhibitors (ACE-I). The time interval from referral to dialysis was less than 6 weeks in 52% of the patients. Thus this group of patients with advanced diabetic nephropathy had not been receiving optimal treatment and were referred to nephrologists late in the course or their disease.

McClellan et al. [8] performed a retrospective analysis of the charts of a random selection of 587 patients admitted to six hospitals, (two urban teaching hospitals), with a primary or secondary diagnosis of diabetes mellitus or hypertension. In less than 4% was any mention made in the history of possible renal disease. Less than 30% of these patients were receiving ACE-I and over 10% were using NSAIDs. Screening for microalbuminuria in patients without a previous history of renal disease was rare. In fact their are clear guidelines for microalbuminuria in patients with diabetes mellitus: the MIDRD as the members of our advisory committee, Hans-Henrik Parving, Eberhard Ritz, and Mike Steffes for their help and advice.
Abstracts


