



# Overt Diabetic Nephropathy and Mineralocorticoid Receptor Antagonism

a report by

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There is accumulating experimental evidence that aldosterone is involved in the progression of nephropathy. In order to translate this evidence to clinical medicine, several studies have been performed with add-on therapy with spironolactone in diabetic patients with nephropathy and persistent proteinuria despite treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor antagonists. In this review, the results of these studies (n=8) are summarized. We conclude that add-on therapy with spironolactone is associated with a pronounced antiproteinuric effect (decrease in proteinuria: 30–54%). Changes in renal hemodynamics or fall in blood pressure can only partly account for this antiproteinuric effect, implicating that other mechanisms play a role. Hard end-point studies with low-dose aldosterone receptor antagonists are required to demonstrate that this mode of therapy is effective in patients with overt (diabetic) nephropathy and proteinuria.

Diabetic nephropathy—characterized by hypertension, macro-albuminuria, progressive loss of renal function, and a high incidence of cardiovascular morbidity and mortality—is the leading cause of end-stage renal failure in the US.<sup>1–5</sup> The predictive power of proteinuria for progressive renal function loss has been demonstrated in patients with and without diabetic nephropathy.<sup>6,7</sup> It has been suggested, therefore, that besides adequate blood pressure and metabolic control, suppression of proteinuria should be a goal of therapy aiming to achieve optimal renal protection in diabetic as well as non-diabetic nephropathy.<sup>6</sup>

According to guidelines, patients with diabetic nephropathy are treated with agents that interfere with the renin–angiotensin system (RAS), as several large intervention studies have demonstrated that these agents provide better renal protection than non-RAS antihypertensive agents.<sup>8–10</sup> Nevertheless, the renal protection provided by these agents is far from complete. For instance, in a study with irbesartan in diabetic nephropathy, end-stage renal failure during follow-up developed in 17.8% of patients on placebo and in 14.2% of patients on irbesartan (absolute reduction of 3.6%).<sup>9</sup> In a study with losartan these values were 25.5% and 19.6%, respectively (absolute reduction of 4.9%).<sup>10</sup> The development of new therapeutic strategies is therefore necessary. In this paper, the effects of aldosterone receptor antagonism on diabetic nephropathy will be discussed. In particular, we will focus on the results of clinical studies evaluating the role of aldosterone receptor antagonism on proteinuria.

## Aldosterone and Nephropathy

Aldosterone is now well recognized as a mediator of the progression of renal disease by causing perivascular inflammation.<sup>12,13</sup> In the rodent remnant kidney model, infusion of aldosterone during losartan administration was associated with hypertension, proteinuria, and glomerulosclerosis.<sup>14</sup> Likewise, in the stroke-prone hypertensive rat on a high sodium intake, aldosterone infusion

during ACE inhibition with captopril was associated with proteinuria and malignant nephrosclerosis,<sup>15</sup> whereas in a hypertensive rat model subjected to angiotensin II and N-nitro-L-arginine methyl ester (L-NAME) infusion, renal and cardiac damage was prevented by aldosterone removal through adrenalectomy or administration of eplerenone.<sup>13</sup> Moreover, in the streptozotocin-induced diabetic rat with increased renal protein excretion, administration of spironolactone markedly attenuated urinary protein excretion and prevented early renal injury, indirectly indicating involvement of aldosterone in this process of renal injury.<sup>16</sup> Similar findings were obtained in the streptozotocin-induced diabetic rat and the db/db (a rodent model of type 1 diabetes) mouse when eplerenone was used as a mineralocorticoid receptor antagonist.<sup>17</sup>

A number of potential mechanisms has been put forward to explain the deleterious effect of aldosterone on the kidney.<sup>12</sup> Recently, it has been demonstrated that the expression of the mineralocorticoid receptor and glucocorticoid-regulated kinase 1 (Sgk1) is significantly increased in renal biopsies obtained from patients with heavy proteinuria.<sup>18</sup> Related to this increased expression, an enhanced expression of inflammatory parameters such as interleukin (IL)-6 and transforming growth factor (TGF)  $\beta$ -1—known to promote renal inflammation—was also observed. Further evidence for the involvement of aldosterone in promoting proteinuria stems from a study in rats showing that aldosterone infusion damages glomerular visceral podocytes.<sup>19</sup> These podocytes are essential for glomerular barrier function. They harbor mineralocorticoid receptors, and damage by aldosterone might be induced by oxidative stress through enhanced Sgk1 expression.<sup>19</sup> Finally, it has been shown that aldosterone has a direct vasoconstrictor influence on afferent and efferent glomerular arterioles, with a higher sensitivity for efferent arterioles. This effect of aldosterone is non-genomic and is caused by activation of phospholipase C, resulting in calcium mobilization through L- or T-type voltage-dependent calcium channels.<sup>20</sup> Therefore, the adverse effects of aldosterone on renal function could be through direct (inflammatory) damage and through alteration of renal hemodynamics.

Although the synthesis and release of aldosterone by the adrenal gland is in part under the control of angiotensin II, escape of aldosterone has been reported to occur in a substantial proportion of patients with diabetic nephropathy treated with ACE inhibitors or angiotensin II receptor antagonists.<sup>21,22</sup> This aldosterone escape is not harmless as it has been shown to be associated with an enhanced excretion of urinary albumin and an enhanced decline of renal function.<sup>22</sup> Because of this aldosterone escape and the knowledge from experimental studies that aldosterone is involved in the development of renal injury, it is not surprising that several clinical studies have been conducted in which the effect of add-on therapy of aldosterone receptor antagonism on proteinuria in (diabetic) nephropathy has been explored.



**Table 1: Overview of Clinical Studies with Aldosterone-receptor Blockade in Type 1 or Type 2 Diabetic Nephropathy**

Reference	Study Design and Duration	Population	RAS Inhibition	Aldosterone-receptor Blocker
Chrysostomou and Becker, 2001	Uncontrolled, 4 weeks	Chronic renal disease, including DNP (n=8)	Enalapril	Spironolactone 25mg
Sato et al., 2003	Uncontrolled, 24 weeks	Type 2 DM with albuminuria and aldosterone escape during ACE inhibition (n=13)	Trandolapril	Spironolactone 25mg
Rachmani et al., 2004	Randomized, uncontrolled, 24 weeks	Type 2 DM, macroalbuminuria (n=46)	24 weeks cilazapril 5mg (n=23), 24 weeks spironolactone 100mg (n=23), 24 weeks cilazapril 5mg+ spironolactone 25mg (n=45)	Spironolactone 25mg
Sato et al., 2005	Non-randomized, uncontrolled, 12 weeks	Chronic renal disease, including DNP (n=32)	Trandolapril	Spironolactone 25mg
Schoedt et al., 2005	Randomized, placebo-controlled, cross-over, 9 weeks	Type 1 DM macroalbuminuria (n=20)	ACE inhibitor or ARB	Spironolactone 25mg
Rossing et al., 2005	Randomized, placebo-controlled, cross-over, 9 weeks	Type 2 DM and macroalbuminuria (n=21)	ACE inhibitor and/or ARB	Spironolactone 25mg
Van den Meiracker et al., 2006	Randomized, placebo-controlled, parallel group, 52 weeks	Type 2 DM and macroalbuminuria (n=29)	ACE inhibitor or ARB	Spironolactone 25–50mg
Schoedt et al., 2006	Randomized, placebo-controlled, cross-over, 8 weeks	Type 1/type 2 DM nephrotic-range proteinuria (n=20)	ACE inhibitor or ARB	Spironolactone 25mg

DNP = deoxyribonucleoprotein; DM = diabetes mellitus; ARB = angiotensin receptor blocker.

## Clinical Studies with Aldosterone Receptor Antagonists

As summarized in *Table 1*, several studies with add-on therapy with spironolactone have been performed in patients with overt diabetic nephropathy.<sup>21,23–29</sup> Patients with both type 1 and type 2 diabetes have been included, and in some studies patients with causes of nephropathy other than diabetes were included as well. Of the eight studies, four were randomized and placebo-controlled with either a cross-over or parallel-group design. The duration of the studies was relatively short, with the longest study lasting one year. The dose of spironolactone ranged from 25 to 100mg once daily. In all the studies but one, spironolactone was added to ACE inhibition and/or angiotensin II receptor antagonism. As shown in *Table 2*, add-on therapy with spironolactone was associated with a substantial decrease in urinary protein excretion, ranging from 30 to 54%. The magnitude of this reduction appears to be unrelated to baseline urinary protein excretion. As also shown in *Table 2*, the effect of spironolactone on serum creatinine concentration or estimates of glomerular filtration rate (GFR) in most studies was small.

In the three randomized cross-over studies reported from the Steno Diabetes Center, changes in ethylenediaminetetraacetic acid (EDTA)-estimated GFR ranged from -3 to -4.3ml/min. In the placebo-controlled, parallel-group study published by our group, GFR estimated from the change in serum creatinine concentration during the one-year follow-up declined on average by 12.9 (9.5–16.5ml/min) in the spironolactone versus 4.9ml/min (0.8–8.9ml/min) in the placebo group (p=0.004). This larger decline in GFR with spironolactone than with placebo was caused by the substantially larger decline in GFR during the first three months of spironolactone administration. In the two studies reported by Sato et al., no values of changes in serum creatinine or GFR are provided. It is stated by the authors that creatinine clearance remained unchanged. In the study reported by Rachmani et al., serum creatinine concentration after addition of spironolactone to cilazapril remained unchanged (125 and 124micromol/l), indicating no significant changes in GFR. As also shown in *Table 2*, addition of spironolactone was associated with modest reductions in blood pressure in most studies, despite the fact that almost all patients had already been treated with at least two antihypertensive agents.

## Mechanism of Antiproteinuric Effect

The antiproteinuric effect of add-on therapy with mineralocorticoid receptor antagonists is theoretically caused by hemodynamic mechanisms, non-hemodynamic mechanisms, or their combination. Experimental studies suggest the involvement of both mechanisms.<sup>12,13,30–32</sup> In a study reported by Dworkin et al., hemodynamic factors were responsible for the glomerular injury in rats with deoxycorticosterone-salt-induced hypertension.<sup>32</sup> Sechi et al. found higher GFR and albuminuria levels in patients with primary aldosteronism (PA) compared with those with essential hypertension. The treatment of PA patients with either adrenalectomy or spironolactone caused a decline in both GFR and albuminuria, suggesting that the effect is, at least in part, hemodynamically mediated.<sup>33</sup>

In more recently published studies, it appears that the antiproteinuric effect is in part dissociated from the induced renal hemodynamic alterations.<sup>30,31</sup> The clinical studies reviewed do not allow firm conclusions to be drawn about the mechanism underlying the antiproteinuric effect of spironolactone. In two of these studies, changes in GFR and proteinuria were correlated.<sup>27,28</sup> However, the magnitude of the decrease in proteinuria by far outweighs the fall in GFR.

It is conceivable that the reduction in blood pressure contributed to the decrease in proteinuria by lowering glomerular filtration pressure, although no relation between blood pressure reduction and antiproteinuric effect could be established. In diabetic nephropathy, renal autoregulation is disturbed.<sup>34</sup> As a consequence, any decrease in blood pressure is likely to have a favorable effect on intra-glomerular pressure, especially when effects of angiotensin II are minimized by ACE inhibition or angiotensin II receptor antagonism.

Mainly in view of the experimental evidence, non-hemodynamic mechanisms may also have contributed to the favorable effect of spironolactone on proteinuria in diabetic nephropathy. There is at this moment only indirect evidence for such a mechanism in the mentioned human studies. In one of the studies reported by Sato and coworkers, administration of spironolactone in

**Table 2: Changes in Proteinuria, Serum Creatinine Concentration, or Estimates of Glomerular Filtration Rate, Blood Pressure, and Serum Potassium in Clinical Studies with Add-on Spironolactone Therapy in Diabetic Nephropathy**

Reference	Decrease in Proteinuria (%)	Change in Serum Creatinine (%)	Change in eGFR (%)	Decrease in Systolic and Diastolic BP (%/%)	Hyperkalemic Patients (%)
Chrysostomou and Becker, 2001	54	n/a	-12	6/14	0
Sato et al., 2003	30*	n/a	No change	n/a	0
Rachmani et al., 2004	38**	3	n/a	1/1	15
Sato et al., 2005	46***	n/a	No change	n/a	0
Schoedt et al., 2005	30	9	-5	4/3	15
Rossing et al., 2005	33	4	-4	7/6	5
Van den Meiracker et al., 2006	44	n/a	-15	5/4	17
Schoedt et al., 2006	32	6	-3	4/5	5

\* Estimated from data on figure. \*\* Combination of spironolactone and cilazapril versus single treatment with these drugs. \*\*\* Subgroup of diabetic patients.

patients with proteinuria persistently greater than 0.5g/l was associated with a decrease in the urinary excretion of type IV collagen.<sup>25</sup> Type IV collagen is the principal component of the glomerular basement membrane and mesangial matrix. Its urinary levels may reflect its rate of turnover. As no decrease in the urinary excretion of type IV collagen is seen with ACE inhibition, it might be that the decrease observed with spironolactone is a specific, non-hemodynamically mediated effect, contributing to the antiproteinuric effect of spironolactone. Finally, administration of spironolactone may also improve glomerular barrier function through an effect on podocyte function via inhibition of the formation of Sgk1 and reduction of oxidative stress.<sup>19</sup>

### Adverse Effects

Although the antiandrogenic effects of spironolactone—such as gynecomastia—are well known and may be troublesome, these side effects were not reported in the referred studies. The relatively low daily dose of spironolactone prescribed and the fact that most studies had a short duration may account for this. Development of hyperkalemia is a feared side effect of aldosterone-receptor antagonism. The risk of hyperkalemia is especially high in patients with impaired kidney function already using an ACE inhibitor or angiotensin II receptor antagonist. Indeed, development of hyperkalemia was reported in five out of the eight studies in 5–17% of participants (see *Table 2*). Baseline renal function is probably the most important determinant for the development of hyperkalemia. For instance, in the study published by our group the median serum creatinine concentration was 162  $\mu\text{mol/l}$  in patients who developed hyperkalemia, defined as a serum potassium concentration  $>5.5\text{mol/l}$ , versus 91  $\mu\text{mol/l}$  in patients in whom serum potassium remained below 5.5  $\mu\text{mol/l}$ . With

progressive renal failure, plasma aldosterone concentration is frequently elevated to counteract the associated hyperkalemia.<sup>35</sup> This defense mechanism is interrupted by the administration of an aldosterone receptor antagonist and hence may increase the risk of hyperkalemia.

### Conclusions and Future Perspectives

From the evidence now available it can be concluded that add-on therapy with aldosterone receptor antagonists in patients with diabetic nephropathy already treated with an ACE inhibitor or angiotensin II receptor antagonist results in a considerably antiproteinuric effect. The mechanism underlying this effect remains speculative, but, based on the rapidly expanding knowledge from experimental studies, hemodynamic and non-hemodynamic mechanisms are likely to be involved.

There is convincing clinical evidence that proteinuria by itself is a determinant for progressive renal function deterioration as well as for cardiovascular morbidity and mortality. It might be inferred, therefore, that reduction of proteinuria should be advantageous both from the perspective of maintenance of kidney function as well as of prevention of cardiovascular disease.

The challenge now is to set up long-term studies with low-dose aldosterone-receptor antagonists in patients with (diabetic) nephropathy and proteinuria in order to confirm the promise that these agents are truly beneficial as evidenced by a decrease in hard end-points, including mortality.

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