CHAPTER 8

ADVERSE RENAL EFFECTS OF THE AGE INHIBITOR PYRIDOXAMINE IN COMBINATION WITH ACEI IN NON-DIABETIC ADRIAMYCIN-INDUCED RENAL DAMAGE IN RATS

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Abstract

Background. Advanced glycation end products (AGEs) are involved in diabetic nephropathy. In non-diabetic proteinuria renal AGEs also accumulate. The AGE inhibitor pyridoxamine (PM) is renoprotective in obese Zucker rats and experimental chronic allograft nephropathy supporting its renoprotective potential in non-diabetic renal damage.

Methods. To investigate whether PM is renoprotective in non-diabetic proteinuria, we studied its effects in adriamycin nephropathy (AN, 1.5 mg/kg iv). Six weeks after disease induction, treatment started with vehicle (VEH), lisinopril (ACEi, 75 mg/L drinking water), PM (2 g/L drinking water) and PM plus lisinopril (PM/ACEi) (n = 12 per group) for 18 weeks. Age-matched healthy rats (n = 6) served as controls (CON).

Results. ACEi reduced proteinuria, blood pressure and renal damage. PM gradually increased blood pressure and did not affect proteinuria. In PM/ACEi the antiproteinuric and blood pressure lowering effects of ACEi were abrogated during long-term treatment. Remarkably, serum creatinine, focal glomerulosclerosis and interstitial fibrosis were considerably increased in PM/ACEi. Pronounced hypercholesterolemia which occurred in both PM treated groups, was accompanied by marked glomerular lipid deposition.

Conclusion. PM was not renoprotective in this model of non-diabetic proteinuria induced renal damage. By contrast, renal damage was aggravated when PM was combined with ACEi. Despite the fact that there is no current evidence that these findings apply to the drug as used in human diabetic nephropathy, we emphasize the importance of close monitoring of blood pressure, lipids and possible direct toxic effects in future studies with PM in renal patients, especially when combining PM with ACEi.
Introduction

Several lines of evidence support a pathophysiological role for advanced glycation end products (AGEs) in diabetic nephropathy. First, accumulation of AGEs occurs in glomerular and tubulointerstitial compartments in proportion to the severity of renal damage. Second, formation of AGEs precedes and correlates with early manifestations of diabetic nephropathy. Moreover, pharmacological intervention in AGE formation with pyridoxamine (PM) and aminoguanidine protects against renal structural lesions, proteinuria and renal function loss in experimental diabetes, supporting the therapeutic potential of AGE inhibition. Interestingly, PM not only provides renoprotection in diabetes, but also in experimental chronic allograft nephropathy and in normoglycemic obese Zucker rats, demonstrating that its beneficial effects are not limited to hyperglycaemia-related models of renal damage.

Involvement of renal AGE accumulation in proteinuria-induced non-diabetic renal damage is supported by several studies. Renal accumulation of AGEs has been shown in focal glomerulosclerosis, hypertensive nephrosclerosis and lupus nephritis. Mesangial accumulation of GA-pyridine, a novel glycolaldehyde-derived AGE, not only occurs in diabetic nephropathy but also in the mesangium of chronic proteinuric renal diseases in man. In experimental renal disease, in adriamycin-induced nephropathy and in subtotally nephrectomized rats, renal AGE accumulation occurs, which can be ameliorated by blockade of the renin-angiotensin aldosterone system (RAAS).

Since AGEs are nephrotoxic and accumulate in adriamycin nephropathy (AN), we hypothesized that renal AGE accumulation, resulting from the primary renal insult, can contribute to further progression of renal damage in non-diabetic proteinuria-induced renal disease. Intervention in AGE formation by PM may thus have renoprotective potential in non-diabetic proteinuria induced renal damage. Currently, standard treatment for proteinuria is RAAS blockade, both in experimental models and in human. In the present study the effect of PM was therefore compared to that of the angiotensin converting enzyme inhibitor (ACEi) lisinopril. In many patients, despite RAAS blockade, a certain residual proteinuria persists, which predicts the rate of subsequent renal function loss. Thus, improvement of antiproteinuric treatment is advocated. Therefore, in the current study we also investigated whether PM allows additional renoprotection in AN when combined with the ACEi lisinopril.
Methods

Animals, experimental groups and treatment
Fifty-four adult male Wistar rats (± 300 g) were studied (Hsd.Cpb. Wu; Harlan Inc., Zeist, the Netherlands). Rats were housed in a temperature-controlled room of 18-20 °C with a 12h-light/dark cycle. The rats had free access to standard chow and drinking water. The local animal ethics committee at the University of Groningen approved all experimental procedures (Committee protocol number: D4091A) and the Principles of Laboratory Animal Care (NIH publication no. 85-23) were followed.

After two weeks of acclimatization and blood pressure measurement training, we induced adriamycin-nephropathy (AN) by injection of 1.5 mg/kg adriamycin in the tail vein under light isoflurane/O2 anaesthesia. Healthy control rats were injected with corresponding volumes of saline (0.9 % NaCl). Rats were sacrificed at week 24.

Rats were randomly divided into five groups, four groups with AN (n=12 per group) and one age-matched healthy control group (CON, n=6). Six weeks after the injection of adriamycin, when proteinuria was stabilized, treatment started. AN rats were treated for 18 weeks, until week 24 with vehicle (VEH), lisinopril (ACEi, 75 mg/L drinking water), pyridoxamine (PM, (PM(HCl)2, 2 g/L drinking water) and with the combination treatment of PM and lisinopril (PM/ACEi).

The dose of PM (2 g/L) was based on previous studies as performed in our lab in Lewis and Fisher rats with allograft nephropathy and isograft controls and by others in non-diabetic Sprague-Dawley rats and Zucker obese rats. In these studies, PM was well tolerated by all strains of rats and did not induce adverse effects on renal function and renal morphology.

Blood pressure and body weight measurements
We trained blood pressure measurements daily for two weeks, prior to the induction of nephrotic syndrome. Body weight was measured weekly and systolic blood pressure (SBP) was measured every other week. An automated multichannel system was used with tail cuffs and photoelectric sensors to detect the tail pulse (Apollo 179; IITC Life Science. Woodland Hills, CA, USA). The rats were placed in a test chamber in restrainers while temperature was maintained at 27 to 29 °C. For each rat, the value was calculated from the mean of two consecutive measurements.

Proteinuria, creatinine, urea, electrolyte and cholesterol measurements
Every other week, we put the rats in metabolic cages (Bioquant™, Merck, Darmstadt,
Germany) to collect 24-hour urine samples. Urinary protein excretion was measured by the pyrogallol red molybdate method. Blood samples were collected at the end of the experiment, immediately prior to sacrifice of the rats. Concentrations of creatinine, urea, sodium, potassium, and cholesterol were all analyzed on a multi-test analyzer system (Merck Mega, Darmstadt, Germany) with Ecoline® MEGA® reagents (Diasys Diagnostic Systems, Holzheim, Germany). Creatinine concentrations in urine and serum were determined with the Jaffé method. Serum values of urea were determined with the urease-GLDH method and concentrations of potassium and sodium were measured with indirect potentiometry. Cholesterol was determined enzymatically.

Sacrifice and assessment of renal morphologic damage
At the end of the studies (week 24) rats were anaesthetized with isoflurane/O₂, kidneys were perfused in situ with saline and rats were sacrificed. One part of kidney tissue was snap frozen in liquid nitrogen. Another part of kidney tissue was fixed in 4% paraformaldehyde and processed for paraffin embedding. Paraffin sections (4μm) were stained with periodic acid-Schiff (PAS) and examined by a qualified pathologist in a blinded fashion by light microscopy to evaluate focal glomerulosclerosis (FGS) and interstitial fibrosis (IF).

To assess the degree of FGS, a blinded pathologist semi-quantitatively scored 50 glomeruli on a scale of 0 to 4. FGS was scored positive when collapse of capillary lumens, mesangial matrix expansion, hyalinosis, and adhesion formation were present in the same quadrant. If 25% of the glomerulus was affected, a score of 1 was given, 50% was scored as 2, 75% as 3 and 100% as 4.

The degree of IF was scored similarly in 30 consecutive visual fields. IF was defined as expansion of the interstitial space, with or without the presence of atrophied and dilated tubules and thickened tubular basement membranes. Medullary tissue, glomeruli, and vessels were excluded from the calculated areas of fibrotic involvement. A score of 0 was given when no interstitial fibrosis was present in a field, 1 for 0-25% with IF, 2 for 25-50%, 3 for 50-75% and 4 for 75-100% of the field showing IF.

To obtain the final score, we multiplied the degree of change by the percentage of glomeruli (for FGS) or visual fields (for IF) with the same degree of injury and added these scores, rendering a theoretical range of 0 to 400.

Immunohistochemistry
Deparaffinized and rehydrated sections (4 μm) were subjected to heat-induced antigen
retrieval by overnight incubation in a 0.1 M Tris/HCl buffer (pH 9.0) at 80°C. Endogeneous peroxidase was blocked with 0.3% H₂O₂ in phosphate-buffered saline (PBS) for 30 minutes. Sections were incubated with an ED1-antibody (Serotec, Oxford, UK) or an anti-pentosidine antibody (16.9 μg/mL) for 60 minutes at room temperature. Binding of the antibody was detected using sequential incubations with peroxidase (PO)-labelled rabbit anti-mouse and PO-labelled goat anti-rabbit antibodies; both for 30 min. Peroxidase activity was developed using 3,3'-diaminobenzidine tetrachloride (DAB) for 10 min. Sections were counterstained with haematoxylin. Macrophages were assessed in fifty glomeruli.

Lipid staining
To assess renal accumulation of neutral fats we used the Oil red O staining on frozen tissue sections (4μm) that were air dried and fixed in 2% paraformaldehyde in PBS at 4°C. For colocalization of intrarenal neutral fats with macrophages, we performed a double staining with ED1 and Oil red O. Frozen tissue sections (4μm), air dried and fixed in 2% paraformaldehyde in PBS at 4°C, were first stained for ED1 and then for Oil red O using the same protocols as described above.

Renal ACE activity
Renal ACE activity was determined using a method previously described in detail. In short, we homogenised tissue in a 50 mM L-1 K₂PO₄ buffer at pH 7.5 and transferred 100 μl of this homogenate to a 0.5 M L-1 K₂PO₄ buffer. Then we added the ACE substrate Hippuryl-His-Leu (12.5 nM L-1, Sigma Zwijndrecht, the Netherlands) and incubated the samples at 37°C for exactly 10 minutes. To stop the conversion of the substrate 1.45 ml of 280 mM L-1 NaOH was added. Thereafter, 100 μl of 1% phthalaldehyde was added to label the free His-Leu product. The amount of labelled His-Leu was determined fluorimetrically at excitation-emission wavelengths of 364/486 nm. Control samples were included in which the conversion of substrate was prevented by adding NaOH before the substrate Hippuryl-His-Leu. Moreover, the substrate was added after the incubation period in these control samples.

Data analysis
Data are expressed as mean ± standard error. Statistical analysis of group differences was performed by a Kruskal-Wallis ANOVA on ranks and Mann-Whitney U tests. Statistical analyses were performed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was assumed at the 5% level (two-tailed).
Results

Body weight and mortality
Throughout the experiments, food and water intake was similar in all groups. The PM and the PM/ACEi treated groups had a significantly lower body weight than the other groups (Table 1). Because of declined condition some rats had to be sacrificed prematurely. At the end of the study data were available in 11/12 rats from the VEH group, 11/12 rats from the ACEi group, 12/12 from the PM group, 10/12 from the PM/ACEi group and 6/6 from the CON group.

<table>
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<tr>
<th></th>
<th>VEH (n=11)</th>
<th>ACEi (n=11)</th>
<th>PM (n=12)</th>
<th>PM/ACEi (n=10)</th>
<th>CON (n=6)</th>
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<tr>
<td>Body weight (g)</td>
<td>491 ± 12</td>
<td>513 ± 13</td>
<td>437 ± 18 **†‡</td>
<td>419 ± 30 **†‡</td>
<td>516 ± 20</td>
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<td>Serum creatinine (μmol/L)</td>
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<td>63 ± 6</td>
<td>81 ± 7</td>
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<td>Creatinine clearance (mL/min)</td>
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<td>0,87 ± 0,14 **†</td>
<td>0,80 ± 0,29 **†</td>
<td>1,85 ± 0,14</td>
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<td>14 ± 2</td>
<td>33 ± 9 *</td>
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<tr>
<td>Serum sodium (mmol/L)</td>
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<td>135 ± 1</td>
<td>134 ± 1</td>
<td>137 ± 1</td>
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<td>Serum potassium (mmol/L)</td>
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<td>5,2 ± 0,2</td>
<td>4,9 ± 0,2</td>
<td>6,9 ± 0,3 *</td>
<td>4,5 ± 0,1</td>
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Table 1. Clinical parameters at the end of the study. Abbreviations are: VEH, vehicle; ACEi, angiotensin converting enzyme inhibition; PM, pyridoxamine; CON, healthy control. *p<0.05 versus all other groups, †p<0.05 versus VEH, ‡p<0.05 versus ACEi.

Blood Pressure and Proteinuria
Systolic blood pressure remained stable after induction of nephrosis until start of treatment. ACEi significantly reduced blood pressure, whereas blood pressure was unaffected by VEH. A gradual, significant rise in blood pressure was observed in the PM group. In the PM/ACEi group blood pressure initially decreased, with a gradual escape afterwards. In the CON group blood pressure remained stable throughout the study (Figure 1A). Proteinuria developed rapidly during the four weeks after adriamycin injection and subsequently levelled off and stabilized. At onset of treatment mean proteinuria in the adriamycin nephrotic rats was 261±14 mg/d. ACEi induced a significant reduction in proteinuria, whereas proteinuria was unaffected by VEH. PM did not affect proteinuria at any time. In the PM/ACEi group, proteinuria initially decreased, but with a gradual escape afterwards, resulting in annihilation of the antiproteinuric response during long term follow-up. The time-course of the antiproteinuric response is shown in Figure 1B. The ab-
solute values of proteinuria at the end of the experiment are given in Figure 3 (right, dark gray bars), showing nephrotic range proteinuria in all AN groups. A significant reduction in proteinuria was only observed in the group treated by ACEi monotherapy.

_Creatinine clearance, Urea and Electrolytes_

Serum creatinine, urea and potassium were significantly higher in the PM/ACEi group compared to all other groups (Table 1). Creatinine clearance was reduced in both the PM

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**Figure 1.** Timecourse of blood pressure development (A) and antiproteinuric response (B) is shown. Abbreviations are: VEH, vehicle; ACEi, angiotensin converting enzyme inhibition; PM, pyridoxamine; CON, healthy control. *p<0.05 versus all other groups, †p<0.05 versus VEH, ACEi and CON.
and the PM/ACEi groups compared to the CON and ACEi groups (Table 1). No significant differences in serum sodium concentration were observed between the groups (Table 1).

Renal structural changes, renal pentosidine accumulation and glomerular macrophages

In all adriamycin nephrotic rats FGS and IF were significantly elevated compared to CON. Compared to VEH, ACEi significantly reduced FGS, but this did not reach statistical significance for IF. In the PM/ACEi group FGS was significantly elevated compared to VEH, ACEi and CON. IF in this group was significantly elevated compared to all other groups (Figure 2A and B, photographs of morphology are presented in the upper 5 panels of Figure 4). Also, in the PM/ACEi group a significant glomerular influx of macrophages was seen (Figure 2C). Pentosidine was found in the brush border and cytoplasm of dilated tubular structures (Figure 5). In PM/ACEi treated rats with AN, total immunoreactive tubular pentosidine was most pronounced, consistent with severe interstitial renal damage.

Figure 2. Quantitative renal parameters of damage, glomerular macrophages and renal ACE activity.

A) Focal glomerulosclerosis, semi-quantitative score (arbitrary units 0 to 400).
B) Interstitial fibrosis, semi-quantitative score (arbitrary units 0 to 400).
C) Mean glomerular macrophage, mean number per glomerulus of 50 counted glomeruli.
D) Renal ACE activity.

Abbreviations are: VEH, vehicle; ACEi, angiotensin converting enzyme inhibition; PM, pyridoxamine; CON, healthy control. *p<0.05 versus all other groups, #p<0.05 versus CON, +p<0.05 versus VEH, ACEi and CON.
Renal ACE activity
To assess the pharmacological efficacy of lisinopril on renal tissue ACE we measured renal ACE activity. In the VEH group renal ACE activity was significantly increased compared to all other groups. ACEi normalized renal ACE activity in both the ACEi group and in the PM/ACEi group. Remarkably, PM also significantly reduced renal ACE activity compared to VEH, albeit not to normal levels (Figure 2D).

Serum and renal lipids
In all AN groups cholesterol was significantly higher than in healthy control rats. Remarkably, in PM and in PM/ACEi treated rats serum cholesterol was significantly higher than in VEH and ACEi treated rats, indicating that in these groups the increase in serum cholesterol was stronger than expected from proteinuria alone (Figure 3).

Oil red O staining of kidney sections showed massive glomerular accumulation of lipid positive cells in PM and even more in PM/ACEi treated rats. In addition, focal accumulation of lipid-positive cells in proximal tubules was observed, but this was not nearly as dramatic as the glomerular accumulation. Less accumulation of Oil red O stainable lipids was observed in glomeruli of VEH treated rats, compared to PM and PM/ACEi treated rats. In ACEi treated rats and CON rats renal lipid deposition was not observed (Figure 4, lower 5 panels). Double staining for macrophages revealed no co-localization of these cells with lipid-positive cells (Figure 6).
Renal morphology & Glomerular lipid deposition

Figure 4. Magnifications are 200x. Upper 5 panels: representative figures of histology. Lower 5 panels: representative figures of Oil red O stained kidney sections. **Renal morphology.** The first of the 5 upper panels is a photograph of a PAS stained section from a kidney of a rat with adriamycin nephrosis treated with vehicle (VEH). Glomerular and interstitial damage was observed, as evidenced by focal glomerulosclerosis and marked tubular basement membrane thickening. The photograph of the section from a kidney of a proteinuric rat treated with lisinopril (ACEi) shows that the overall extent of tubular and glomerular damage is less severe than in vehicle treated rats. In the representative example of a section from a proteinuric rat treated with pyridoxamine (PM) severe glomerulosclerosis and expansion of the interstitial space, with the presence of atrophied and dilated tubules and thickened tubular basement membranes is present. The photograph of a section from a rat with adriamycin nephrosis treated with the combination of pyridoxamine and lisinopril (PM/ACEi) shows that in this group more and severe renal structural damage in glomeruli as well as interstitium was observed compared to all other groups. For comparison of renal morphology, a photograph of a section from a healthy control kidney (CON) is presented as well. **Glomerular lipid deposition.** The first of the five lower panels is a photograph of a section from a healthy control kidney (CON) in which no renal lipid deposition was observed. The photograph of an Oil red O stained section from a kidney of a rat with adriamycin nephrosis treated with vehicle (VEH) shows that some accumulation of Oil red O stainable lipids was observed in glomeruli. In the photograph of section from a kidney of a proteinuric rat treated with lisinopril (ACEi) no renal lipid deposition was observed. The representative photograph of a Oil red O stained kidney section from a rat treated with pyridoxamine (PM) shows that massive glomerular accumulation of lipid positive cells was present. In addition, focal accumulation of lipid-positive cells in proximal tubules was observed. Also, in rats treated with the combination pyridoxamine and lisinopril (PM/ACEi) massive glomerular accumulation of lipid positive cells was observed. This was even more pronounced than in rats treated with pyridoxamine alone as is shown in the lower right panel.
Discussion

In contrast to previous findings in experimental chronic allograft nephropathy from our own laboratory\(^7\) and in rat models of diabetic nephropathy\(^4\) and obese Zucker rats by others,\(^8\) PM in a dose similar to these prior studies was not renoprotective in adriamycin-induced proteinuria, neither as a monotherapy, nor in combination with ACEi. By contrast,
renal damage was markedly aggravated when PM was used in combination with ACEi.

PM is generally believed to be a non-toxic, safe and well tolerated vitamin from the B<sub>6</sub> family, which inhibits the glycoxidative breakdown of Amadori products to AGEs, a key step in the formation of AGEs. Unexpectedly, we found aggravated renal glomerular and interstitial damage along with renal function decline in our adriamycin nephrotic rats when the AGE formation inhibitor PM was given in combination with standard antiproteinuric treatment with ACEi. In contrast, previous studies found renoprotective, and sometimes added effects of combined intervention in the RAAS and in advanced glycation, two different pathways of renal damage. In non-hypertensive diabetic nephropathy in B6 db/db mice, the progression of albuminuria and glomerular lesions was reduced by the combination of PM and the ACEi enalapril. In diabetic spontaneously hypertensive rats the combination of the AGE-inhibitor aminoguanidine and the ACEi perindopril attenuated the development of albuminuria more effectively than either monotherapy. In normotensive streptozocin-diabetic rats no additional renoprotective effect of the addition of the AGE cross-link breaker alagebrum to ACEi with ramipril was observed. However, adverse renal effects of combined intervention as observed in our study have not been described previously.

In our non-diabetic nephrotic rats PM monotherapy did not reduce proteinuria nor ameliorated renal morphological abnormalities. In previous studies, PM prevented the rise in proteinuria or, in line with our current data, was without effect. We observed a gradual increase in blood pressure during PM monotherapy, which is at variance with data in previous studies, in which PM either prevented the rise in blood pressure or was without effect. Renal damage in our PM groups was accompanied by an increase in serum cholesterol which was stronger than expected from proteinuria alone, and by pronounced glomerular lipid deposition. These effects, which were observed in both PM treated groups, have not been described previously. Remarkably, renal ACE activity in PM treated rats was lower than in untreated adriamycin nephrosis. The reduction in ACE activity by PM has been observed previously, but in our study this was apparently not associated with a renoprotective effect. Body weight was significantly lower in both PM treated groups compared to the other groups, in line with adverse effects of PM in these rats. Another explanation of the lower body weight in the PM/ACEi group could be volume depletion, as this group had not only an elevated serum creatinine, but also a disproportionally high urea, consistent with a component of pre-renal failure. However, this does not ex-
plain the lower body weight of the PM-treated group, that did not have elevated serum urea, creatinine or potassium levels. In our previous study in rats with CAN, PM treated Fisher-Fisher isograft controls gained less weight than the vehicle treated controls. Others have observed a decrease in body weight in PM treated diabetic rats, but in most studies, PM had no effect on body weight.

Unexpectedly, combined therapy of PM and ACEi abrogated the renoprotective effects of ACEi. After an initial decrease in proteinuria and blood pressure, these parameters returned to their baseline values during long term therapy. Unexpectedly, the combination of PM/ACEi was associated with a marked increase in glomerular and interstitial renal damage, with a pronounced glomerular influx of macrophages. This was associated with renal function impairment as well. In AN, renal AGEs accumulated in dilated tubular structures, which is in accordance with our prior findings in AN and in CAN. Presumably, this reflects impaired clearance of filtered and reabsorbed AGEs by the dysfunctional tubular cells. We did not observe a clear-cut inhibitory effect of PM on tubular AGE accumulation. In contrast, in PM/ACEi-treated rats, total immunoreactive tubular pentosidine was most pronounced, consistent with the presence of severe interstitial renal damage. To test whether the abrogation of the renoprotective effects of ACEi might be due to a pharmacological interaction, we measured renal ACE activity. This was similarly decreased in the ACEi and the PM/ACEi group, so apparently PM did not interfere with the primary pharmacological effect of the ACEi. Also, model-related factors should be taken into account, as under certain circumstances the adriamycin model is resistant to therapy. However, ACEi monotherapy reduced proteinuria and focal glomerulosclerosis, supporting the responsiveness to ACEi intervention in our experimental animals.

Our study was not designed to unravel the mechanisms underlying the detrimental renal effects of combined therapy of PM with ACEi, but several inferences can be made. It is likely that hypertension, hypercholesterolaemia and intrarenal fat deposition, as observed in both PM groups, contributed to the renal damage. The role of hypertension as a promoter of renal damage is well-established. Lipid deposition could be another mechanism of renal damage observed here, as marked glomerular lipid deposition was observed, in particular in the PM/ACEi rats. Lipid and lipoprotein abnormalities secondary to nephrotic syndrome can contribute to the vicious circle of proteinuria-associated progressive renal damage. Lipid deposition in the kidney occurs in several animal models of renal damage such as experimental diabetes, and obese Zucker rats as well as in man.
In humans, abnormal lipid accumulation has been found in non-fibrotic glomeruli of patients with glomerulopathy. A recent study showed co-localization of renal lipid deposition and increased TGF-β mRNA expression in rats, suggesting that renal lipid deposition can trigger a profibrotic response in the kidney, and thus may play a pathogenetic role in renal damage. It should be noted that prior studies with PM in rat models of renal disease were generally conducted in models where proteinuria was only mild, whereas the AN in our study was characterized by overt nephrotic range proteinuria. Therefore, it cannot be excluded that the severity of the proteinuria in our model was a predisposing factor for the adverse renal effects of the PM/ACEi regimen. Further studies are obviously needed to elucidate the mechanisms underlying the adverse renal effects of the combination of PM and ACEi.

Adverse effects of PM have been suggested by reports showing that pyridoxine, another vitamin from the B6 family, causes dose and duration dependent sensory neuropathy. This vitamin B6 associated neuropathy is enhanced by uremia and a protein-deficient diet. Unfortunately, these pyridoxine toxicity studies did not investigate renal morphology and the uremia study was done in an anephric rat model. Furthermore, recent phase II clinical trials in type 1 and type 2 diabetic patients with overt nephropathy (PYR-206 and PYR-205/207) show that there were slightly more deaths and serious adverse events in the PM compared to the placebo treated group. In these studies PM was given for six months in addition to standard treatment with ACEi and/or angiotensin II type 1 receptor blockade. Although these were randomized trials, the authors ascribed the adverse effects to the existence of medical conditions at baseline predisposing patients in the PM group to the adverse events.

PM is emerging as a promising therapeutic agent, now on the FDA “fast track” to phase III clinical trials for treatment of diabetic nephropathy. However, in a dose that was previously renoprotective in animal models of diabetic nephropathy, Zucker obese rats, and experimental chronic allograft nephropathy, PM was not renoprotective in AN. By contrast, renal damage was aggravated when PM was used in combination with ACEi. These renal effects need further analysis. Despite the fact that there is no current evidence that these findings apply to the drug as used in diabetic nephropathy in humans, we caution that in future studies with PM in renal patients close monitoring of blood pressure, lipids and possible direct toxic effects is needed, especially when adding PM to ACEi.
Acknowledgements

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