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NO-independent activation of soluble guanylate cyclase prevents disease progression in rats with 5/6 nephrectomy

[1,2]Philipp Kalk, [1]Michael Godes, [1,2]Katharina Relle, [3,4]Christiane Rothkegel, [3]Andreas Hucke, [3]Johannes-Peter Stasch & *,[1]Berthold Hocher

[1]Department of Pharmacology and Toxicology, Center for Cardiovascular Research, Charité, Campus Mitte, Hessische Str. 3-4, Berlin D-10115, Germany; [2]Institute of Vegetative Physiology, Charite, Campus Mitte, Berlin, Germany; [3]Cardiovascular Research, Bayer Healthcare, Wuppertal, Germany and [4]Martin-Luther-University, School of Pharmacy, Halle, Germany

1 Chronic renal disease is associated with oxidative stress, reduced nitric oxide (NO) availability and soluble guanylate cyclase (sGC) dysfunction. Recently, we discovered BAY 58-2667, a compound activating heme-deficient or oxidized sGC in a NO-independent manner.

2 We assessed potential of BAY 58-2667 in preventing cardiac and renal target organ damage in rats with 5/6 nephrectomy.

3 Male Wistar rats were allocated to three groups: 5/6 nephrectomy, 5/6 nephrectomy treated with BAY 58-2667 and sham operation. Study period was 18 weeks: blood pressure and creatinine clearance were assessed repeatedly. At study end blood samples were taken and hearts and kidneys harvested for histological studies.

4 BAY 58-2667 markedly lowered blood pressure in animals with 5/6 nephrectomy (untreated *versus* treated animals: 189 ± 14 *versus* 146 ± 11 mmHg, $P < 0.001$). Left ventricular weight, cardiac myocyte diameter as well as cardiac arterial wall thickness significantly decreased in comparison to untreated animals with 5/6 nephrectomy. Natriuretic peptide plasma levels were also improved by BAY 58-2667. Kidney function and morphology as assessed by creatinine clearance, glomerulosclerosis, interstitial and perivascular fibrosis of intrarenal arteries were likewise significantly improved by BAY 58-2667.

5 This is the first study showing that BAY 58-2667 effectively lowers blood pressure, reduces left ventricular hypertrophy and slows renal disease progression in rats with 5/6 nephrectomy by targeting mainly oxidized sGC. Therefore, BAY 58-2667 represents a novel pharmacological principle with potential clinical value in treatment of chronic renal disease.
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Introduction

The prevalence of end stage renal disease in Europe is 700 cases per million inhabitants; the prevalence of chronic kidney disease in earlier stages is estimated to exceed this number by as much as 50 times thus creating a major health care and economic challenge (El Nahas & Bello, 2005). Moreover, cardiovascular disease (CVD) such as coronary disease, heart failure, peripheral vascular disease and cerebrovascular disease has a high prevalence in patients with chronic renal failure (CRF) as a study in the US with patients at the initiation of dialysis showed a prevalence of 52% (Foley *et al.*, 2003; Haffner *et al.*, 2005). Owing to pressure and volume overload in renal disease, left ventricular hypertrophy (LVH) is described as the most frequent cardiac alteration, which has

an important impact on the mortality of these patients (Silberberg *et al.*, 1989; Foley *et al.*, 1995). Those facts create a need for new therapeutic strategies to slow renal disease progression and prevent the detrimental cardiac alterations in CRF patients.

Soluble guanylate cyclase (sGC) is the receptor for the ubiquitous nitric oxide (NO). NO exerts its effects by binding to the prosthetic heme group at the beta-subunit of the sGC and thereby activating the enzyme that catalyzes the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) (Gruetter *et al.*, 1979; 1981; Wedel *et al.*, 1994; Hobbs, 1997). cGMP therefore is a second messenger which affects various physiological processes such as vasodilatation and inhibition of platelet aggregation (Radomski *et al.*, 1990). Those actions make activation of the sGC a promising tool in treating cardiovascular diseases.

*Author for correspondence; E-mail: berthold.hocher@charite.de

Up to now organic nitrates, which mimic the action of NO, are used in the clinic for that purpose, but they require enzymatic bioactivation and suffer from the phenomenon of nitrate tolerance (Feelisch, 1998).

Recently, we identified a novel NO-independent activator of sGC BAY 58-2667 (4-[((4-carboxybutyl){2-[(4-phenethyl-benzyl)oxy]phenethyl}amino)-methyl[benzoic]acid) through an ultra-high-throughput screening (Stasch *et al*., 2002). This compound is characterized by the intriguing feature of activating sGC more potently after oxidation of its heme moiety *via* the sGC inhibitor and hemeoxidant, 1*H*-[1,2,4]ox-adiazolo[4,3-a]quinoxalin-1-one (ODQ). BAY 58-2667 shows remarkable *in vitro* and *in vivo* effects on vasorelaxation and inhibition of platelet aggregation; the vasodilating effect was not altered by pre-existing nitrate tolerance. The oral administration of the compound to spontaneously hypertensive rats led to a long-lasting decrease in blood pressure. Interestingly, BAY 58-2667 exerts its vasorelaxing effect both on arterial and venous vessels (Stasch *et al*., 2002).

Taken those aspects together this compound might be a promising tool in treating hypertension and preventing hypertensive target organ damage such as LVH and ischemic heart disease associated with oxidative stress. As there is currently no data available on the potential of this compound to prevent cardiac target organ damage in hypertension, we conducted this study to investigate the effects of a long-term oral administration of BAY 58-2667 in a rat model of CRF with special attention on hemodynamic and cardiovascular effects.

Methods

Chemicals

The sGC activator BAY 58-2667 (4-[((4-carboxybutyl){2-[(4-phenethyl-benzyl)oxy]phenethyl}amino)-methyl[benzoic]acid) was prepared as recently described (Stasch *et al*., 2002). Unless otherwise stated all other reagents were of analytical grade and were purchased from SIGMA (Seelze, Germany), MERCK (Darmstadt, Germany) and ROTH (Karlsruhe, Germany).

Animal model

In order to perform 5/6 nephrectomy the animals were anesthetized with isoflurane and placed on a heated table to maintain normal body temperature. The right kidney was exposed *via* flank incision and removed. After a 2-week recovery period, the left kidney was exposed accordingly and 2/3 were surgically removed.

Study design

Animal studies were carried out in accordance with local ethical regulations for the use of laboratory animals. Male Wistar rats at the age of 9–10 weeks weighing 320–340 g were randomly allocated to three groups: 5/6 nephrectomy ($n = 15$), 5/6 nephrectomy plus treatment with BAY 58-2667 (3000 parts per million (p.p.m). in the solid feed/about 50 mg day^{-1}; $n = 12$) and sham-operation (OP) ($n = 10$). All animals received a commercial diet (Altromin®; Altromin Co., Lage, Germany) and water *ad libitum* during the study period. After the 5/6 nephrectomy the animals were given 1 week of recovery from surgery (week 0) before the oral administration of the substance was started; furthermore GFR and blood pressure were assessed during this period (see below) in order to exclude differences between uraemic animals before drug treatment. The duration of the study was 18 weeks. During the study period the animals were weighed weekly, blood pressure was assessed *via* the tail-cuff method during week 0, 2, 5, 9, 15. The animals were placed in metabolic cages to obtain 24 h urine samples at week 0, 4, 17; at the same time blood was taken from retro-orbital veins for the single purpose of measuring plasma creatinine levels and calculate creatinine clearance using standard formula. In week 18 animals were killed, blood samples were taken to assess plasma levels of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (AP), glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), creatine kinase (CK), creatinin and as described before (Haffner *et al*., 2005). The hearts and kidneys were harvested for histological studies, organ weights were measured.

Laboratory chemistry

Plasma renin activity (PRA), atrial natriuretic peptide (ANP) and b-type natriuretic peptide (BNP) in plasma were measured at the end of the study period as previously described (Stasch *et al*., 2002; Dumitrascu *et al*., 2006).

Histological studies

Tissue samples were all embedded in paraffin, cut into 3 and 1 μm sections, subjected to hematoxylin–eosin (HE), Sirius red, perjodic acid-Schiff- (PAS) and Elastica-van Gieson staining. Quantitative stereology (i.e. intima/media and lumen area of the arteries) was analyzed using a computer-aided image analysis system as previously described (Hocher *et al*., 1999). Cardiac and renal morphology (interstitial fibrosis, perivascular fibrosis, glomerulosclerosis and media/lumen ratio of blood vessels) were measured as recently described (Hocher *et al*., 2000; Haffner *et al*., 2005). In brief, glomerulosclerosis was defined by the presence of PAS-positive material within the glomeruli. To quantify the amount of glomerulosclerosis a semiquantitative score was used; two investigators, who had no knowledge of the groups to which the rats belonged, judged the results.

The severity of interstitial fibrosis was evaluated after Sirius Red staining using computer-aided histomorphometry devices. In brief, at least 30 microscopic pictures per kidney/heart section were transferred to a PowerMAC *via* Hitachi-CCD-camera. After manually setting a threshold using a randomly chosen subset of the pictures, we measured the relationship of SR-stained area (connective tissue) to total area of the picture using ImageJ, an image processing software (shareware from the NIH).

Accordingly, microscopic pictures of kidney/heart sections after Elastica-van Gieson staining showing arterial blood vessels were generated. We measured the area contents of the media and the lumen of intrarenal/intracardial arteries using the ImageJ program; afterwards media/lumen ratio was calculated serving as marker for arterial wall thickening.

Using 1 μm-sections of the heart in HE-staining pictures were generated as described above and myocyte diameter was measured with ImageJ.

Perivascular fibrosis was judged after Sirius-Red staining using a semiquantitative score by two independent investigators blinded to the groups to which the animals belonged.

Plasma level of BAY 58-2667

Samples were subjected to high-performance liquid chromatography performed on a 2300 HTLC system (Cohesive Technologies, Franklin, U.S.A.) as described (Dumitrascu *et al.*, 2006). Briefly, the mobile phase consisted of 10 mM ammonium acetate (pH 3.0) and acetonitrile. A linear gradient from 20 to 85% acetonitrile (vol vol^{-1}) within 1 min was applied. Tandem mass spectrometry was performed on an API 3000 triple-quadrupole mass spectrometer (PE Sciex, Wellesley, U.S.A.) connected to the 2300 HTLC system through a turbospray interface. The lower limit for quantification of BAY 58-2667 was 0.5 μg l^{-1}.

Statistical analysis

To detect any significant differences between the three groups the Kruskal–Wallis test was applied; the Mann–Whitney test was used to detect significant differences between two groups of interest. Results (given as mean $\pm$ standard deviation (s.d.)) were considered significant when the probability error (P) was less than 0.05.

Results

Mortality

Treatment with BAY 58-2667 was tolerated well without any side effects. During the study, six out of 15 animals (40%) in the 5/6 NX group and three out of 12 animals (25%) of the 5/6 NX + BAY 58-2667 group died, whereas all sham-OP animals survived.

Laboratory results

At the end of the 18 week study period, the animals were killed, organs were harvested for histological studies and blood samples were obtained. The laboratory results from the blood samples are shown in Table 1. There were no significant or clinically relevant differences between the groups regarding AST, ALT, AP, GLDH, LDH and CK. Both groups with 5/6 nephrectomy had higher plasma levels of creatinine and urea compared with the sham-OP group, but in the group treated with BAY 58-2667 this increase was significantly diminished compared with the untreated animals. Plasma levels of protein were lower in both uraemic groups compared with the sham group. Regarding PRA we observed that in both nephrectomized groups the PRA was significantly suppressed compared to sham animals. BNP levels were significantly elevated in both uraemic groups compared with sham controls, but in the group treated with BAY 58-2667 there was a strong trend ($P = 0.05$) towards lower levels compared with untreated uraemic animals. A similar trend was observed with respect to ANP, however, without reaching statistical significance.

Table 1 Plasma laboratory results and body weights at the end of the study

Parameter (unit)	5/6 NX (n = 9)	5/6 NX + BAY 58-2667 (n = 9)	Sham OP (n = 10)
Body weight (g)	415.0 ± 49.3*	391.2 ± 50.7*	502.0 ± 75.4
AST (U/l)	51.0 ± 28.7	51.6 ± 29.5	55.5 ± 11.1
ALT (U/l)	33.5 ± 14.1	46.3 ± 22.0	40.8 ± 9.8
AP (U/l)	77.6 ± 21.4	95.4 ± 28.7	88.7 ± 11.0
GLDH (U/l)	14.4 ± 14.7	10.1 ± 10.4	11.4 ± 11.6
LDH (U/l)	107.3 ± 83.8	104.1 ± 70.1	132.8 ± 45.5
CK (U/l)	96.1 ± 42.0	105.2 ± 53.4	109.5 ± 25.3
Crea (μmol/l)	209.3 ± 110.1**	97.8 ± 29.0**,†	51.7 ± 2.4
Urea (mmol/l)	52.8 ± 46.9**	18.5 ± 6.0**,†	6.0 ± 0.9
Protein (g/l)	55.0 ± 5.0**	54.4 ± 2.7**	65.7 ± 1.7
PRA (ng/ml/h)	0.7 ± 0.5**	1.3 ± 1.0*	2.6 ± 1.2
ANP (pg/ml)	487.3 ± 162.1	346.6 ± 160.8	319.0 ± 162.8
BNP (pg/ml)	47.3 ± 19.3**	33.3 ± 11.0*,(†)	16.7 ± 7.9

Abbreviations: AP = alkaline phosphatase; ALT = alanine amino transferase; ANP = atrial natriuretic peptide; AST = aspartate amino transferase; BNP = B-type natriuretic peptide; CK = creatine kinase; Crea = creatinin; GLDH = glutamate dehydrogenase; LDH = lactate dehydrogenase; PRA = plasma renin activity.
Values are given as mean ± s.d.
*/**$P < 0.05/0.001$ *versus* Sham OP; †$P < 0.05$ *versus* 5/6 NX; (†)$P = 0.05$ *versus* 5/6 NX.
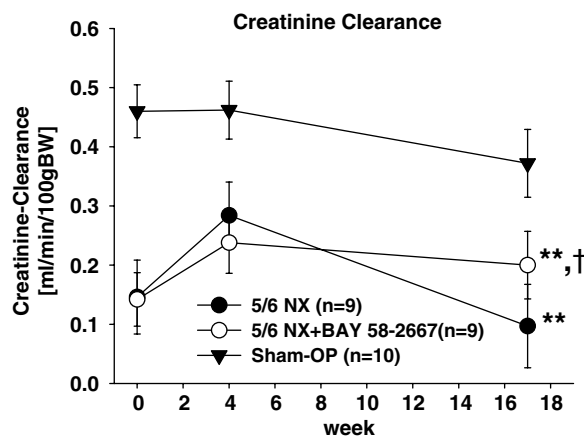

Figure 1 Creatinine clearance. All values are given as mean ± s.d. **$P < 0.001$ *versus* sham OP. †$P < 0.05$ *versus* 5/6 NX.

Creatinine clearance and albuminuria

The creatinine clearance was calculated from blood and urine creatinine at week 0, 4, 17. The results are illustrated in Figure 1. Both groups with 5/6 nephrectomy had markedly lower GFR than the sham group during the time course of the experiment. Both uraemic groups started with the same GFR, but at the end of the experiment the group treated with BAY 58-2667 had a significantly higher GFR than the untreated group.

At the last urine collection (week 17) urinary albumin excretion was measured. The sham-OP animals exhibited a significantly lower urinary albumin excretion (30.7 ± 30.4 mg 24 h^{-1}) compared to both uraemic groups; no difference was detected between uraemic animals treated with BAY 58-2667 (297.9 ± 102.3 mg 24 h^{-1}) and without treatment (274.1 ± 131.2 mg 24 h^{-1}).

Blood pressure

The blood pressure was assessed *via* the tail-cuff method during week 0, 2, 5, 9, 15. As shown in Figure 2 the systolic blood pressure increased markedly in the untreated nephrectomized group compared with the sham controls. Treatment with BAY 58-2667 remarkably diminished this effect during the observation period.

Body and organ weight

Body weight at the time of killing significantly differed between the groups: as shown in Table 1 both uraemic groups weighted less than the sham-OP controls. Left cardiac ventricle weight – expressed as a percentage of body weight – increased significantly in both uraemic groups compared to sham controls, but the effect was significantly diminished by treatment with BAY 58-2667 (Figure 3).

Plasma levels of BAY 58-2667

Food intake and body weight were rising constantly during the study period thus indicating a stable drug administration
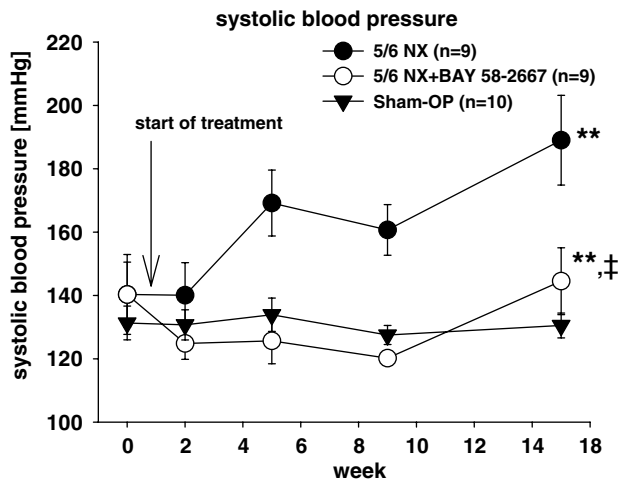

Figure 2 Systolic blood pressure. All values are given as mean $\pm$ s.d.. **$P<0.001$ *versus* sham OP. ‡$P<0.001$ *versus* 5/6 NX.
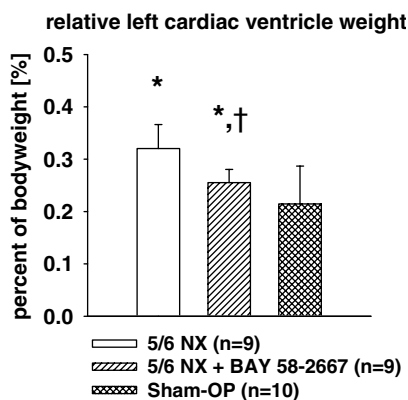

Figure 3 Relative left cardiac ventricle weight. All values are given as mean $\pm$ s.d.. *$P<0.05$ *versus* sham OP. †$P<0.05$ *versus* 5/6 NX.

Table 2 Histology results of the heart

Parameter (unit)	5/6 NX (n = 9)	5/6 NX + BAY 58-2667 (n = 9)	Sham OP (n = 10)
Myocyte diameter (μm)	19.0 ± 4.6**	14.8 ± 2.3*,†	12.0 ± 0.9
Media-lumen-ratio	2.7 ± 1.1*	1.5 ± 0.8†	1.5 ± 0.6
Interstitial fibrosis (% of section)	2.3 ± 1.3	1.9 ± 0.8	2.4 ± 0.7
Perivascular fibrosis (score)	2.1 ± 0.5	1.8 ± 0.4	1.9 ± 0.5

Values are given as mean $\pm$ s.d.*/**$P<0.05/0.001$ *versus* Sham-OP; †$P<0.05$ *versus* 5/6 NX.

throughout the study. Plasma levels of BAY 58-2667 were $233 \pm 29.2 \, \mu g \, l^{-1}$ in the treatment group at the end of the study.

Cardiac histology

At the end of the study period the hearts were harvested for histological study; the results are illustrated in Table 2.

The media-lumen-ratio of cardiac arteries was calculated using computer-aided morphometry devices (for illustration, see Figure 4). The media-lumen-ratio of untreated uraemic animals increased significantly compared to sham controls. Treatment with BAY 58-2667 completely abolished this effect. The diameter of cardiac myocytes was accordingly measured using 1 μm slices in HE staining. We discovered that in both uraemic groups the mean diameter of cardiomyocytes increased significantly *versus* sham controls, but treatment with BAY 58-2667 effectively diminished this increase. Perivascular and interstitial fibrosis of the hearts were judged using 3 μm slices in Sirius-Red-staining; no statistically significant differences between all three study groups could be observed.

Renal histology

At the end of the study period the kidneys were harvested for histological study; the results are shown in Table 3. Regarding the extent of glomerulosclerosis using a semiquantitative scoring system on tissue slices in PAS staining (for illustration, see Figure 4), we found a significant increase in both uraemic groups *versus* sham controls, but this effect was markedly diminished in animals treated with BAY 58-2667 compared with untreated animals. The same pattern was present when we investigated renal perivascular fibrosis in Sirius-Red staining. Using computer-aided histomorphometry devices we also measured the extent of interstitial fibrosis in the kidney (for illustration, see Figure 4). We observed significant increase in renal interstitial fibrosis in untreated uraemic groups *versus* sham controls, but this effect was completely abolished by treatment with BAY 58-2667. No significant differences between all study groups existed regarding the media-lumen-ratio of intrarenal arteries.

Discussion

This study is the first to evaluate and describe the renal and cardiovascular consequences of long-term sGC activation by the novel NO-independent sGC activator BAY 58-2667 in a rat model of CRF (Stasch *et al.*, 2002; Schmidt *et al.*, 2004).
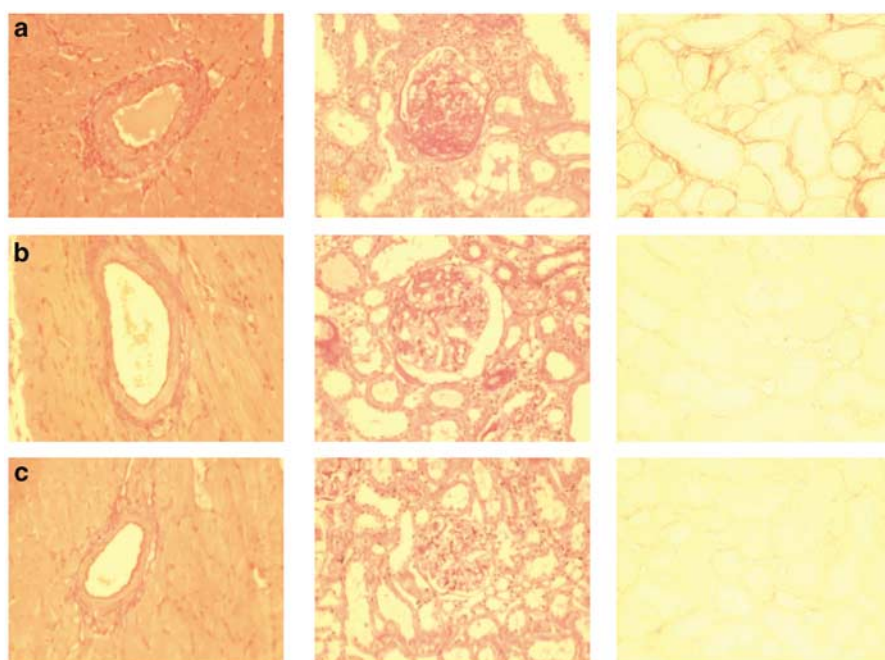
Figure 4 Typical sections of heart and kidney. (a) 5/6 NX; (b) 5/6 NX + BAY 58-2667; (c) sham OP. First column: typical cardiac arteries in Elastica-van Gieson staining, Magnitude × 200. Second column: typical kidney sections in PAS staining, Magnitude × 200. Presence of PAS-positive material (pink color) within glomeruli indicates glomerulosclerosis. Third column: typical kidney sections in Sirius-Red-staining, Magnitude × 200. Red color indicates fibrotic areas.

Table 3 Histology results of the kidney

Parameter (unit)	5/6 NX (n = 9)	5/6 NX + BAY 58-2667 (n = 9)	Sham OP (n = 10)
Glomerulosclerosis (score)	2.4 ± 0.6**	1.4 ± 0.3*,†	1.0 ± 0.04
Media-lumen-ratio	3.5 ± 0.7	3.1 ± 0.8	3.3 ± 0.8
Interstitial fibrosis (% of section)	2.4 ± 1.3**	0.4 ± 0.3†	0.2 ± 0.2
Perivascular fibrosis (score)	2.1 ± 0.3**	1.6 ± 0.3*,†	1.2 ± 0.2

Values are given as mean ± s.d.
*/**$P < 0.05/0.001$ *versus* Sham OP; †$P < 0.05/0.001$ *versus* 5/6 NX.

BAY 58-2667 is characterized by activating sGC in an NO-independent manner and even more potently activating sGC after oxidation of its heme group. We recently showed that the activity of BAY 58-2667 is also potentiated in cells, aortas from different species and *in vivo* under oxidative stress conditions (Rothkegel *et al.*, 2005). CRF is associated with diminished NO availability which is caused by a combination of reduced NO production (Vaziri *et al.*, 1998; Roczniak *et al.*, 1999; Schmidt & Baylis, 2000), depletion/inactivation of NO by reactive oxygen species (Vallance *et al.*, 1992; Vaziri *et al.*, 2002; Vaziri, 2004b) and by a dysfunction of sGC (Sindhu *et al.*, 2004; Vaziri, 2004a). The associated NO deficiency and dysfunctional sGC, in turn, promotes hypertension and accelerates progression of renal disease (Himmelfarb *et al.*, 2002; Vaziri, 2004b). We, therefore, hypothesized that targeting dysfunctional sGC in an NO-independent manner by BAY 58-2667 may attenuate hypertension and retard progression of renal disease by raising the intracellular cGMP.

Regarding plasma parameters provided in Table 1, which were intended to screen for main organ system dysfunctions, we can exclude major side effects of treatment with Bay 58-2667 such as cardio-, nephro- or hepatotoxicity and rhabdo-myolysis. With regard to mortality we did not observe increased mortality in the group treated with BAY 58-2667 when compared to untreated animals. In rats with renal mass ablation PRA was markedly suppressed, whereas BNP, urea and creatinine in plasma were significantly elevated in the nephrectomized, uraemic groups compared to the sham-OR group. These findings are in good agreement with published data of this model of CRF (Strauch & Gretz, 1988; Gretz, 1995).

The NO-independent sGC activator BAY 58-2667 caused lower rise of BNP, urea and creatinine levels within the treated 5/6 nephrectomized group suggesting cardiorenal protective effects of this compound. This is consistent with our data regarding creatinine clearance: both uraemic groups started at the same level, but at the end of the study period the group treated with BAY 58-2667 showed a significantly higher clearance than the untreated group, thus indicating that BAY 58-2667 also slowed the progression of renal disease in our animal model of CRF.

However, this beneficial effect on renal function was not extended on albuminuria; in our study treatment with BAY 58-2667 failed to exert an antiproteinuric effect. This is consistent with literature describing compounds (e.g. certain calcium antagonist subclasses), which are lacking specific antiprotein-uric effects despite potent antihypertensive action (Nathan *et al.*, 2005). Further studies are needed to confirm and elucidate the effects of sGC agonistic compounds on proteinuria.

BNP and ANP are released in conditions related to increased cardiac wall stretch (Angermann & Ertl, 2004;

McCullough, 2004). BNP compared to ANP is recognized as the superior marker for left ventricular dysfunction and has a powerful diagnostic and prognostic value in patients with CRF and is also responsive to medical treatment (Hocher *et al.*, 2004; Silver *et al.*, 2004; Takami *et al.*, 2004). Suggesting that a rise in BNP levels in both uraemic groups reflects a cardiac impairment due to uraemia, lower plasma BNP levels in animals treated with BAY 58-2667 underline the cardiorenal protective effects of this compound.

Regarding cardiac target organ damage we were able to demonstrate a beneficial effect of BAY 58-2667 on morphology, by left ventricular weight and myocyte diameter reduction, as well as on vasculature, by completely abolishing cardiac arterial wall thickening compared with the untreated uraemic group. We suggest that these findings can mainly be attributed to the observed reduction of systemic of blood pressure in the BAY 58-2667 treated group, although there is evidence that arterial wall thickening in CRF also occurs independently from blood pressure (Kakinuma *et al.*, 1992; Amann *et al.*, 1995; Tornig *et al.*, 1996). In addition, it should be noted that animals treated with BAY 58-2667 have a better kidney function as compared to untreated uraemic rats. This might also have a beneficial effect on the cardiac vasculature.

The improved kidney function and morphology in ureamic rats treated with BAY 58-2667 too can most likely be attributed to reduction of systemic blood pressure. Blood pressure is known to be of major impact on renal disease progression in the setting of CRF (Bidani & Griffin, 2004). The antihypertensive potential of BAY 58-2667 in our study is equal to the effect of established antihypertensive agents like enalapril (Okada *et al.*, 2004) or candesartan (Noda *et al.*, 1999) in a similar study design. However, we state that interpretations regarding blood pressure in our study are limited to the observation period: at the last measurement (week 15) blood pressure in the treatment group increased significantly *versus* untreated uraemic animals; as we have no data beyond that point, further studies are needed to elucidate if blood pressure and target organ damage can effectively be controlled by treatment with BAY 58-2667 in long-term studies.

Considering that sGC activating compounds are known to have specific antifibrotic properties in the kidney (Wang *et al.*, 2005), we assume that in our study there might have been additional beneficial effects independent from blood pressure reduction. However, as we observed major differences regarding systemic blood pressure between our groups, our study cannot give evidence for putative blood pressure independent effects of BAY 58-2667. Further studies with low doses of BAY 58-2667 which leave systemic blood pressure unaffected are warranted to clarify this point.

Very recently it has been shown that inhibition of phosphodiesterase 5 by sildenafil (Viagra[R]) treatment prevented hypertension and deterioration of renal function, reduced histologic damage, inflammation and apoptosis, delayed the onset of proteinuria, and preserved renal capillary integrity in 5/6 nephrectomy by increased availability of cGMP (Rodriguez-Iturbe *et al.*, 2005). Moreover, in a second protocol sildenafil was compared with losartan and the combination of both drugs in established renal disease, starting these drugs 4 weeks after 5/6 nephrectomy. Delayed sildenafil treatment failed to improve proteinuria and glomerulosclerosis but ameliorated hypertension and azotemia (Rodriguez-Iturbe *et al.*, 2005).

In conclusion, our study demonstrated for the first time that treatment with the new NO-independent sGC activator BAY 58-2667 in a setting of CRF effectively lowers blood pressure, reduces LVH and preserves renal function and morphology. Therefore, NO-independent activation of sGC as exemplified by BAY 58-2667 is a novel pharmacological principle and has the potential clinical value in the treatment of chronic renal disease.

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