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Comparative effects of nebivolol and atenolol on renal function in rats with chronic renal failure

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ABSTRACT

This study aimed to evaluate the effect of chronic treatment with nebivolol on the renal function of rats with renal failure induced by renal mass reduction and compare it with atenolol. One week after 5/6 nephrectomy, male Wistar rats were divided into three experimental groups: 1) male Wistar rats without treatment (n=14); 2) male Wistar rats treated with nebivolol (n=14); 3) male Wistar rats treated with atenolol (n=14). Treatment was administered in drinking water for six months. Systolic arterial pressure and heart rate were measured in conscious animals using a tail-cuff method once a month following surgery. Creatinine, blood urea nitrogen and electrolytes were measured in serum and/or urine using routine laboratory techniques. Protein concentration in 24-h urine samples was determined using the Bradford method. Histological studies with hematoxylin-eosin and Syrius red staining were performed in the remnant kidney six months after surgery to evaluate renal damage. Changes in plasma levels of creatinine and blood urea nitrogen, creatinine clearance, urinary flow, protein and electrolyte excretion induced by renal mass reduction were similar in the nebivolol and atenolol treated groups. Morphological study revealed that glomerular and tubulointerstitial damage in the nebivolol-treated group was lesser than in the atenolol-treated groups. These results suggest that nebivolol does not have a greater beneficial effect than atenolol on renal function improvement, although it was able to attenuate the structural changes induced by renal mass reduction.

Key-Words:
Atenolol, chronic renal failure, hypertension, nebivolol, nitric oxide, Wistar rats.

INTRODUCTION

Hypertension plays an important role in the progression of chronic renal disease. Chronic renal disease is associated to important structural alterations, such as reduction in renal mass, focal glomerulosclerosis and interstitial fibrosis. These structural changes lead to functional alterations with a progressive diminution in glomerular filtration rate, a decreased urinary concentrating ability and diluting capacity[1,2]. Reduction of renal mass (RMR) is one of the most widely experimental approaches to induce and study the events of chronic renal failure[3-5]. In this model, the animals develop a systemic and glomerular hypertension, proteinuria and a gradual diminution of glomerular filtration rate associated with the presence of glomerulosclerosis and tubulointerstitial fibrosis[6,7].

Nitric oxide (NO) exerts a fundamental role in the regulation of renal function. NO regulates glomerular filtration, renal blood flow and induces natriuresis[8-11].

Alterations in intrarenal NO production may be involved in the pathogenesis of chronic renal failure. In rats with RMR, stimulation of NO production results in normalisation of creatinine clearance, associated with the increase of sodium excretion and the decrease of proteinuria, suggesting that a deficiency in NO production may be partially responsible for the impaired renal function[12]. Therefore, modification of systemic and/or renal NO synthesis may be a logical approach to alter the course of renal failure.

Although effective control of hypertension slows the decline of renal function in progressive renal disease[13,14], some antihypertensive agents offer more effective protection than expected from blood pressure reduction. We have recently found that in rats with RMR the administration of nebivolol, a highly selective beta-1 adrenoceptor blocker possessing additional vasodilator properties, delayed the progression of renal fibrosis and protected against endothelial dysfunction to a greater extent than atenolol despite equivalent blood pressure reduction[15]. As the effects of nebivolol in that study appeared to be mediated in part through their ability to increase NO bioavailability and since increasing evidence suggests that NO deficiency occurs as a result of chronic renal disease and may contribute to injury progression[12,16], we postulate that nebivolol may have the same beneficial effect on the progression of renal failure.

Studies into the effects of nebivolol on renal function are scarce and inconsistent. According to Mangrella *et al.*[17] nebivolol does not modify in a significant way glomerular filtration rate or renal plasma flow. However, Greven and Gabriels[18] showed that nebivolol exerts a positive effect on kidney function in normotensive rats. Thus, in this study, we assessed the effects of nebivolol on renal function in Wistar rats with renal mass reduction. The effect of nebivolol was compared with atenolol, a classic β_1-blocker with no direct vasodilatator properties.

MATERIAL AND METHODS

Animals

Animal care and treatment were performed according to European Community guidelines. The design and experimental procedures of the study were approved by the Animal Care Committee of the University of Salamanca. Male Wistar rats (obtained from Charles River, Barcelona, Spain) with initial body weights of 200 to 250 g were used in this study. All animals were housed under controlled conditions of temperature (20-22ºC), humidity (60--70%), lighting (12-hour dark/12-hour light cycle) and provided with food (standard diet containing 20% protein by weight) and tap water *ad libitum*.

Surgical procedure and experimental design

5/6 nephrectomy (RMR) was carried out under isoflurane anaesthesia and aseptic conditions. The rats' coats were shaved and a medial laparotomy performed. The right kidney was freed and decapsulated with special care not to damage the adrenal glands and removed after the ligation of renal pedicle, to reduce its total mass by 50%. The left kidney was then freed as described and the two poles of the kidney were removed by scissor cutting for a final reduction of approximately 65-70%. The approximate weight of the remaining renal tissue was calculated on the basis of the removed tissue, assuming that the right and left kidneys had equal weights. One week after ablation, surviving animals (n=42) were randomly divided into three experimental groups: RMR: RMR rats without treatment (n=14); RMR+N: RMR rats treated daily with nebivolol (8 mg/kg per day, n=14); and RMR+A: RMR rats treated daily with atenolol (80 mg/kg per day, n=14). Treatment was administered in drinking water for six months and the concentration of nebivolol and atenolol was adjusted weekly by measuring animal weight and water intake. As we did not find significant differences in the fluid intake of rats placed individually in metabolic cages, it was assumed that all rats drank the same volume. At the beginning of the experiment and every month after RMR, systolic blood pressure, heart rate, body weight, haematocrit, plasma creatinine and blood urea nitrogen (BUN), creatinine clearance, proteinuria and urinary excretion of electrolytes were determined. Mortality was also monitored.

Systolic blood pressure and heart rate

Systolic blood pressure (SBP) and heart rate (HR) were measured in conscious animals using a tail-cuff

method (Electro-sphygmomanometer, LETICA LE 5000, Letica Barcelona, Spain) as previously described[19]. Tail-cuff measurements of blood pressure have been shown to be reproducible and correlate well with intra-arterial blood pressures measured in unrestrained, unanaesthetised rats[20]. Rats were trained to get accustomed to the measurement procedure, and SBP and HR were measured every month after surgery. The SBP value for each rat was calculated as the average of six separate measurements that not differ from one another by 10 mmHg.

Renal function

The rats were placed in individual metabolic cages for four days to get accustomed to isolation conditions, and urine was collected during the following two days to measure volume, creatinine concentration, proteinuria and urinary excretion of electrolytes. The day after the metabolic study blood samples (150 µl) were collected from the tail vein into heparinised capillaries. Haematocrit was determined in centrifuged capillaries (10,000 r.p.m. for five minutes) and separated plasma served to measure creatinine concentrations, BUN and potassium concentrations (Hitachi 917 Autoanalyzer). Urinary sodium, potassium and chloride were measured by a flame photometer (Corning 435, Izasa, Barcelona, Spain) and urinary creatinine measurements were performed using the Jaffe method[21]. Protein concentration in 24-h urine samples was determined by the Bradford method[22]. Glomerular filtration rate was estimated from the creatinine clearance, which was calculated by employing a standard formula [UxV/P, where U= urine creatinine (mg/dl), V= urine volume (ml/min./100 g body weight, and P= serum creatinine (mg/dl)].

Histological examination

Surviving animals were euthanised with pentobarbital sodium six months after surgery. Remnant kidney was removed and cut in several pieces, including cortex and medulla, and fixed in 10% formaldehyde for 24 hours. Hematoxylin and eosin (H&E) and Syrius red staining were performed according to standard procedures. Sections were examined by an independent observer blinded to the experimental protocol.

Statistical analysis

The statistical analysis was performed with the SPSS software version 12.0 for Windows (Chicago, IL, USA). Calculations for significant differences between the different groups were made with two-way ANOVA for repeated measures, followed by the Scheffe test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

During this experimental study, seven untreated rats, three nebivolol-treated rats and five atenolol-treated rats died and were not included in the final data analysis. These rats had low body weights, high values of SBP, low values of haematocrit, high levels of plasma creatinine and BUN, and an intense proteinuria (Table I), indicating a severe renal failure that, quite possibly, was the cause of these animals' death. The results presented included only the animals that survived until the end of the study (six months of treatment), meaning a selection, particularly in the

Table I

Parameters of rats that died before the six months of treatment.

Groups (n)	SBP (mmHg)	HR (beats/min)	Ht (%)	UF (ml/min)	PCr (mg/dl)	BUN (mg/dl)	UP (g/day)
RMR (7)	163±23	380±31	35±1	0,024±0,01	2,3±1,1	180±98	0,500±0,19
RMR+N (3)	169±6	341±32	40±2	0,018±0,01	2,1±0,7	144±46	0,705±0,14
RMR+A (5)	154±5	351±51	38±3	0,023±0,01	3,5±1,7	229±111	0,365±0,1

The data correspond to the last measurement made before the death of the animal. Number of rats per group (nº), Systolic blood pressure (SBP), heart rate (HR), haematocrit (Ht), urinary flow (UF), plasma concentration of creatinine (PCr), blood urea nitrogen (BUN) and urinary excretion of proteins (UP). Renal mass reduction (RMR) untreated rats, RMR animals treated with nebivolol (RMR+N) and RMR animals treated with atenolol (RMR+A). Data are expressed as mean ± SD.

group RMR without treatment, as only the animals in better conditions were able to survive such a long experimental period.

Body weight, systolic blood pressure and heart rate

As shown in Table II, at the end of the study there were no statistical differences in body weight between experimental groups. To compare the renal effects of the two drugs used, the doses were selected in order to have a similar effect on the levels of blood pressure. As expected, SBP was significantly increased in the RMR group (Table II). β1-Selective receptor blockade with either nebivolol or atenolol prevented the

Table II

Effect of both atenolol and nebivolol on body weight (BW), systolic blood pressure (SBP) and heart rate (HR) six months after renal mass reduction.

Groups (n)	BW (g)	SBP (mmHg)	HR (beats/min)
RMR (7)	530 ± 26	142 ± 8	376 ± 30
RMR+N (11)	492 ± 23	112 ± 6*	299 ± 9*
RMR+A (9)	538 ± 39	109 ± 6*	292 ± 17*

Renal mass reduction (RMR) untreated rats, RMR animals treated with nebivolol (RMR+N) and RMR animals treated with atenolol (RMR+A). Number of rats per group (nº). Data are expressed as mean ± SD. *P <0.001 *versus* RMR group.

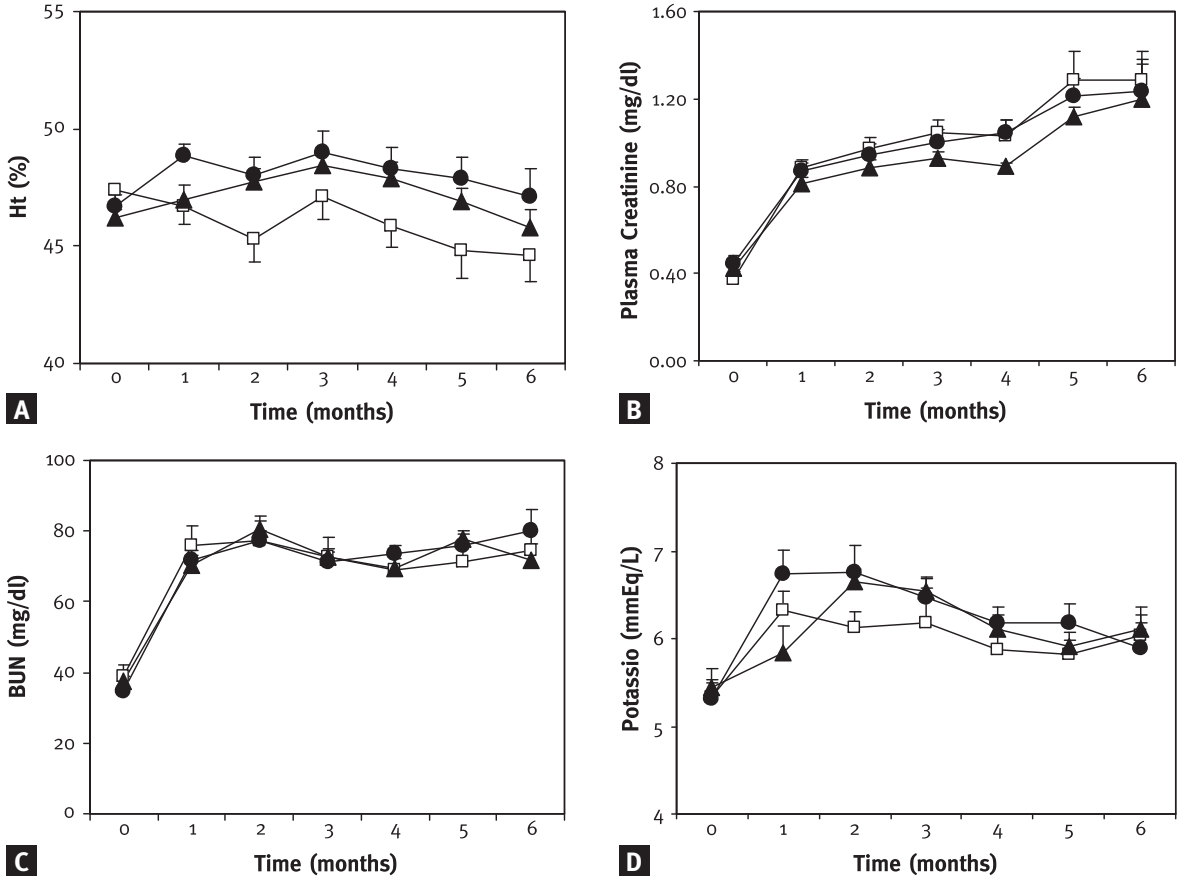

Figure 1

Effect of both atenolol and nebivolol on (A) haematocrit (%), (B) plasma creatinine (mg/dl), (C) BUN (mg/dl) and (D) plasma potassium (mmEq/L) after renal mass reduction. Symbols: RMR untreated animals (open squares), RMR animals treated with nebivolol (closed triangles), and RMR animals treated with atenolol (closed circles). Data are expressed as mean ± SEM.

RMR-induced increase in blood pressure (P<0.001 compared with the RMR group; Table II) and significantly reduced the heart rate to a comparable degree (P<0.001 compared with the RMR group; Table II).

Renal function parameters

The haematocrit values of animals belonging to groups RMR+N and RMR+A were similar during the experimental period, and no statistically significant differences were observed between these groups (Fig. 1A). No significant differences were found between the different groups in any of the parameters of renal function. As we can see in figure 1B, all groups show plasma levels of creatinine above normal range (0.2-0.8 mg/dl)[23] with no significant differences between the groups (Fig. 1B). Plasma levels of BUN and potassium also increased progressively and there were no significant differences among groups (Figures 1C and D). Creatinine clearance progressively decreased after renal ablation and treatment with nebivolol and atenolol did not change these lower values (Fig. 2A). A progressive increase in urinary protein excretion was observed during the study in all groups, with values reaching 0.302±0.15 in the RMR group, 0.256±0.09 in the RMR+N group and 0.214±0.16 in the RMR+A group at study end (Fig.

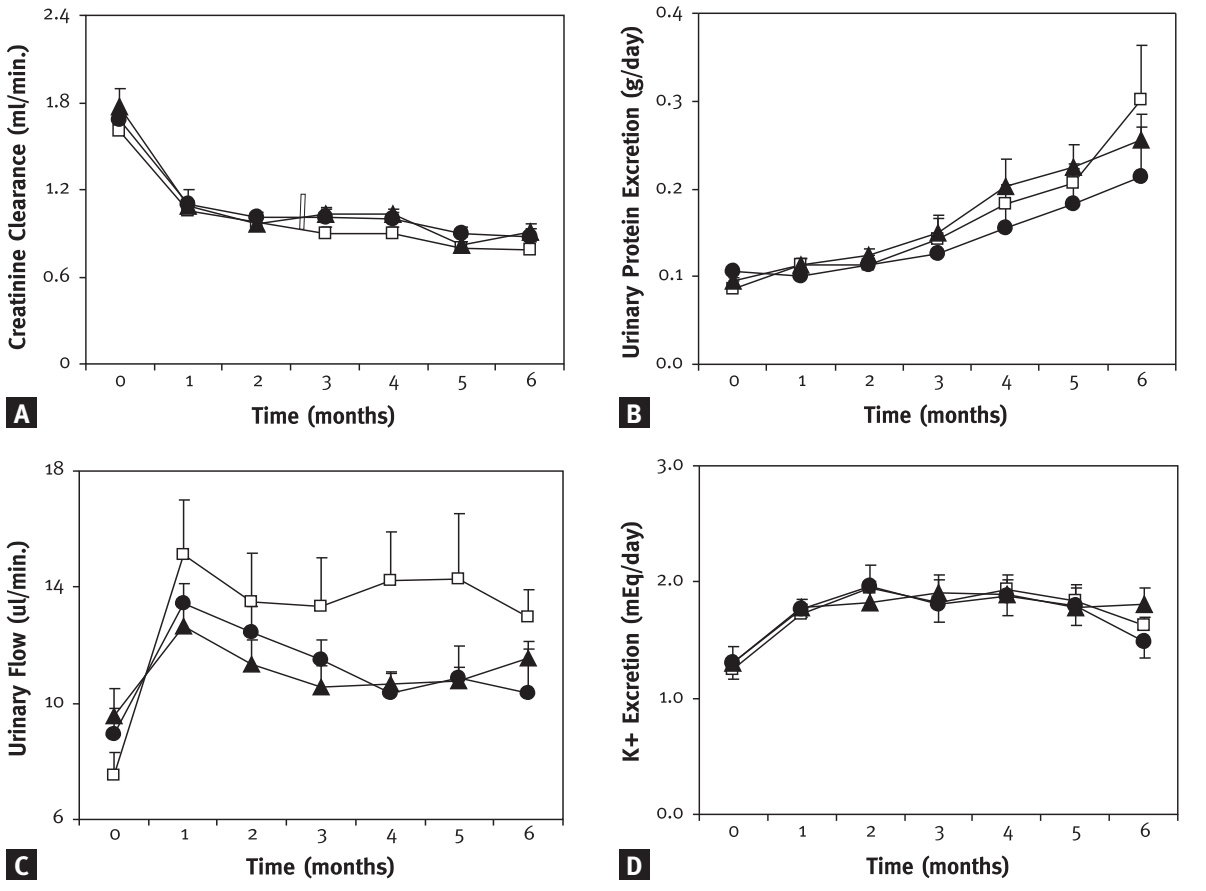

Figure 2

Effect of both atenolol and nebivolol on (A) creatinine clearance (ml/min/100g), (B) urinary protein excretion (g/day), (C) urinary flow (µl/min) and (D) K^+ excretion (mEq/day) after renal mass reduction. Symbols: RMR untreated animals (open squares), RMR animals treated with nebivolol (closed triangles), and RMR animals treated with atenolol (closed circles). Data are expressed as mean ± SEM.

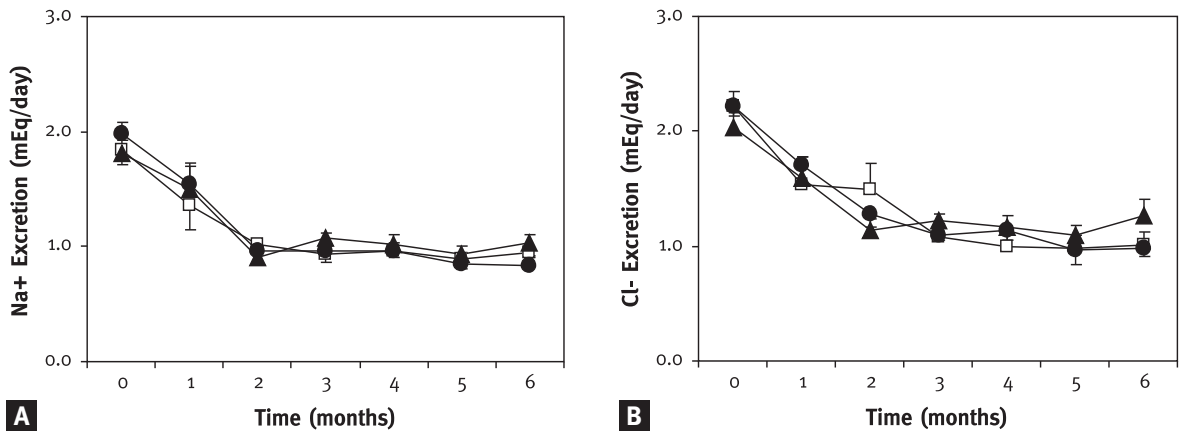

Figure 3

Effect of both atenolol and nebivolol on (A) Na+ excretion (mEq/day) and (B) Cl^- excretion (mEq/day) after renal mass reduction. Symbols: RMR untreated animals (open squares), RMR animals treated with nebivolol (closed triangles), and RMR animals treated with atenolol (closed circles). Data are expressed as mean ± SEM.

2B). Although the animals belonging to the RMR+N group present slightly higher values than those of the RMR+A group, the differences were not statistically significant (Fig. 2B). Urinary flow (Fig. 2C), potassium excretion (Fig. 2D), sodium excretion (Fig. 3A) and chloride excretion (Fig. 3B) were similar in the three experimental groups.

Microscopic findings

Representative images of hematoxylin-eosin and Sirius red staining are shown in figures 4A-F and 5A-F. Six months after renal mass reduction, RMR untreated animals showed sclerotic lesions in more than 70% of their renal glomeruli. The severity of the lesions varied from a marked increase of the mesangial matrix, to a total glomerular sclerosis. In the tubulointerstitial space, a remarkable interstitial fibrosis with proximal tubular dilation, focal tubular atrophy and the presence of numerous hyaline casts in distal and collecting tubules were observed. A perivascular inflammatory infiltrate was also detected. Atenolol-treated rats showed similar glomerular and interstitial lesions to RMR-untreated animals. The percentage of sclerosed glomeruli was reduced in RMR+N group. Tubular dilatation and atrophy, as well as tubulointerstitial infiltration and fibrosis, were substantially reduced in RMR+N group.

DISCUSSION

New beta-blockers open up new perspectives in the treatment of hypertension and its consequences on target organs. In this study, we chose nebivolol, a last-generation beta blocker, a selective β_1-adrenergic receptor antagonist, for the following reasons: (a) it leads to an increase in renal NO excretion and a significant increase in plasma flow and glomerular filtration rate[18], (b) it induces NO release in glomerular endothelial cells[24], isolated renal artery[25] and rat kidney[26], (c) it suppresses the renin-angiotensin aldosterone system and reduces angiotensin II levels[27], (d) it reduces endothelin-1[28] and (e) it has an antioxidant effect[29,30]. All of these effects are related to the pathogenesis of chronic renal failure. No study has evaluated the effects of nebivolol on progression of renal failure during the course of hypertension. We compared the effects of nebivolol and atenolol on renal function loss induced by renal mass reduction.

In this study, to compare the renal effects of the two drugs used, the doses were selected in order to have a similar effect on blood pressure. Treatment with nebivolol (8 mg/ kg/day) and atenolol (80 mg/ kg/day) prevented the increase in systolic blood pressure and there was no significant difference in the anti-hypertensive effects of both treatments. The effectiveness of β blockers to maintain blood pressure

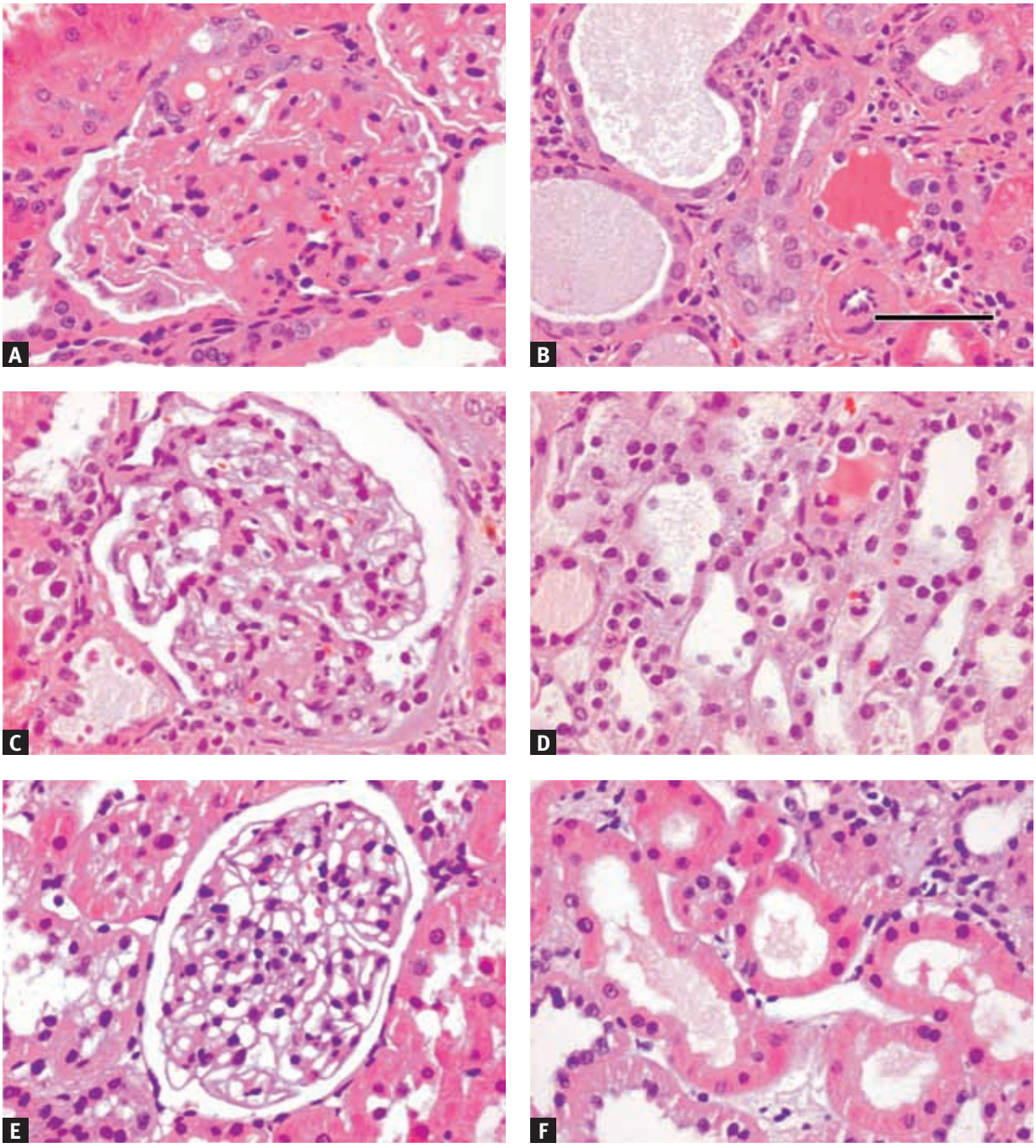

Figure 4

Effect of 6 months' nebivolol and atenolol treatment on renal histological alterations induced by renal mass reduction. Photomicrographs correspond to representative renal sections stained with H&E from RMR-untreated animals (A and B), RMR animals treated with atenolol (C and D) and RMR animals treated with nebivolol (E and F). Black bar indicates 100 µm.

at low levels is widely demonstrated in this and other models of hypertension[31-33].

The fact that body weight was similar in all experimental groups at the end of the study means

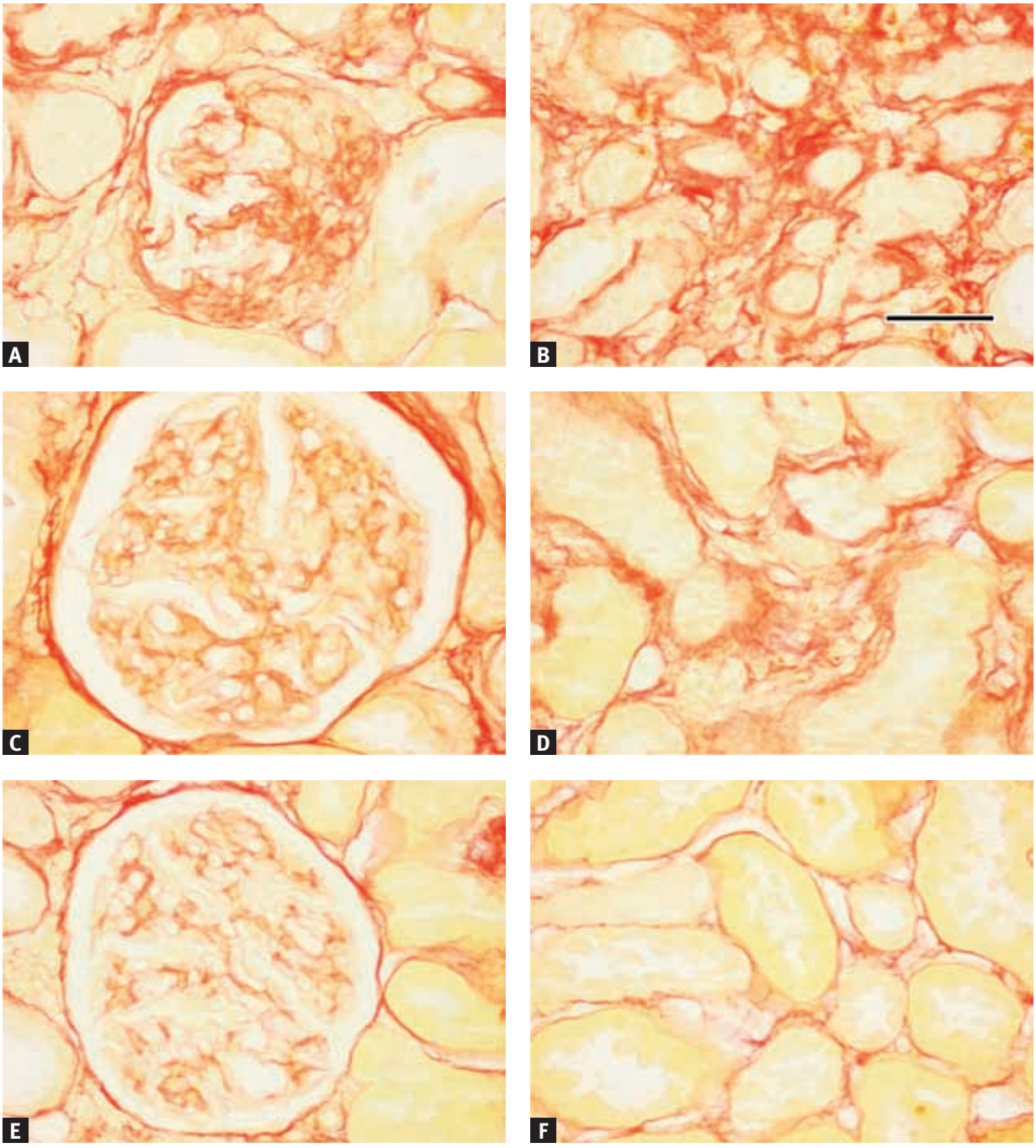

Figure 5

Effect of 6 months' nebivolol and atenolol treatment on renal fibrosis induced by renal mass reduction. Photomicrographs correspond to representative renal sections stained with Syrius red from RMR-untreated animals (A and B), RMR animals treated with atenolol (C and D) and RMR animals treated with nebivolol (E and F). Black bar indicates 100 µm.

that the intake of food was probably similar in these groups, leading us to discard the hypothesis that the differences found in systolic blood pressure between the untreated-group and treated-groups

were due to different sodium consumption. This is important because RMR is a model in which the values of blood pressure vary widely with the consumption of sodium.

Several animal models of renal failure have been developed to investigate various aspects of this syndrome and explore the putative renoprotection potential of innovative molecules. It was been demonstrated that rats with RMR are useful to explore target molecules for renoprotective effect of new molecular entities. The remnant kidney model was established in the rat to create an environment of chronic renal disease. The time course of this model can be divided into three phases (a) the acute phase; (b) a period of stable, but impaired renal function; and (c) a phase in which the animals progress toward terminal renal failure. Immediately after the reduction in renal mass, functional adaptations to increased workload occur, characterised by hypertrophy and hyperplasia of the different compartments in the remaining kidney. Hence, there is a slight recovery in renal function, and a more stable period follows with development of proteinuria[34]. In our study, we observed a consistent increase in plasma creatinine and BUN concentrations, decrease in creatinine clearance and increase in urinary protein excretion which indicates the presence of chronic renal failure in our experimental groups. Some rats progressed to end-stage renal failure at variable time points and died before final analysis, and were thus excluded from statistical analysis.

Chronic renal failure causes secondary anaemia mainly due to a decrease in the erythropoietin production[35]. Haematocrit was used as an indicator of anaemia. In our study, animals of all experimental groups had haematocrit considered in the normal range for the species (36-48%)[36] without significant difference between groups. Yatsu *et al.*[37] conducted a study in male rats with renal mass reduction and observed the presence of anaemia at the end of the study (10 weeks), assessed through red blood cells counts, haemoglobin and haematocrit. In our work none of the rats subjected to RMR and which survived the entire study presented anaemia or a haematocrit lower than 36%. However, haematocrit values lower than 36% were often found in animals that died, mainly in the RMR group, but these animals were not included in the final results.

In rats of all experimental groups, plasma creatinine and BUN concentrations, glomerular filtration rate, determined as creatinine clearance, and urinary protein excretion were not modified by the antihypertensive treatments. At this point it is necessary to consider that only the animals with plasma creatinine and BUN concentrations, glomerular filtration rate and urinary protein excretion within values compatible with life have survived. This emerges from the study of data on renal function of the rats that did not survive. In most of these animals an increase in plasma concentration of creatinine and BUN, and a severe proteinuria is observed, meaning that these parameters being similar between the two antihypertensive treatments may be a consequence of the above problem.

All groups presented proteinuria and the animals treated with nebivolol presented slightly higher values than those treated with atenolol. This is one of the experimental models in which the highest values of urinary protein excretion in rats is observed[38,39]. The rats that presented a severe proteinuria immediately following renal mass reduction died very early, indicating a close relationship between the values of proteinuria and life expectancy of rats with RMR. Proteinuria *per se* may be nephrotoxic. In the past, the degree of proteinuria was merely taken as an indicator of the severity of the underlying renal lesion and as a surrogate marker for renal impairment. More recently, evidence has accumulated suggesting that proteins leaking through a damaged capillary into the nephron may have intrinsic renal toxicity[40,41]. Nebivolol induces NO release in glomerular endothelial cells, isolated renal artery and rat kidney[24-26]. Li *et al.*[42], using isolated rat glomeruli exposed to NO donors, have shown that NO has the ability to impair the glomerular permeability barrier via a mechanism related to tyrosine phosphorylation of glomerular proteins, suggesting that NO may contribute to proteinuria. In addition, NO can increase glomerular capillary pressure by reducing efferent arteriole resistance[43]. These results may explain the slightly higher proteinuria that was observed in animals treated with nebivolol than those treated with atenolol.

The number of glomeruli with focal and total sclerosis was substantially reduced with nebivolol treatment. Moreover, tubulointerstitial changes were improved in the nebivolol-treated group, in contrast

with changes observed in atenolol-treated group. Nebivolol has been shown to possess vasodilatory effects involving the NO dependent pathway[45,46], decrease systemic oxidative stress[29,30], reduce the cell proliferation[47,48] and decrease secretion of endothelin-1[28]. Studies in animals with RMR showed a decrease in NO total production and a reduction in activity of renal nitric oxide synthase[16,49]. Oxidative stress also seems to play an important role in renal injury induced by renal mass reduction and it has been reported that in this model, treatment with antioxidants prevents the progression of renal disease[50-52]. Thus, increasing NO bioavailability, reducing oxidative stress and reducing cell proliferation nebivolol may exert a beneficial effect on structural changes induced by renal mass reduction, as observed in this study.

In this study, the histological findings are not in agreement with the renal function data. Some studies with different experimental models have demonstrated a positive effect of nebivolol on renal function. In normal rats, treatment with nebivolol induced a significant increase in renal plasma flow and glomerular filtration rate[18]. In another study, contrast-induced proteinuria was restored by nebivolol[44]. In our study we were unable to detect a beneficial effect of nebivolol as compared with atenolol on progression of renal function in rats with chronic renal failure induced by RMR. However, the effect of nebivolol in renal function may have been masked by the fact that the animals with lowest renal function, observed mostly in RMR rats without treatment and treated with atenolol, died before the end of the study and were not included in statistical analysis. The number of animals that died before the end of the study was lower in the nebivolol-treated group than in the atenolol-treated or in the untreated group. Thus, there may be a possibility that treatment with nebivolol has not only reduced the progression of renal histological changes, but also slowed the rate of renal function decline.

In conclusion, in this study there was no correlation between the histological changes observed and the renal function data. Nebivolol exhibits renoprotection demonstrated by the reduction of the progression of histological changes induced by renal mass reduction. However, no beneficial effect of nebivolol on renal function is observed in this model.

Conflict of interest statement. None declared.

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