

Effect of unilateral nephrectomy on renal function of diabetic rats

G.S. Lopes, C.C.S. Lemos, C.A. Mandarin-de-Lacerda and R. Bregman

Nephrology Division, Pedro Ernesto Hospital, University of the State of Rio de Janeiro, Rio de Janeiro, Brazil

Summary. Glomerular alterations of experimental diabetes mellitus are observed in animals submitted to a reduction in renal mass, suggesting that some mechanisms responsible for the progression of renal disease are common. The aim of this study was to investigate the effect of nephrectomy on the renal function and morphology of diabetic rats.

Male Wistar rats were divided into 4 groups: control (C), n=8; diabetic (DM), n=8; non-diabetic nephrectomized (Nx), n=8; (DMNx), n=9. DM was induced by streptozotocin (65mg/Kg), and animals were treated with insulin. After 12 weeks, the glomerular filtration rate (GFR), renal plasma flow (RPF) and mean arterial pressure (MAP) were evaluated in unanaesthetized animals. Glomerular volume (GV), glomerular sclerosis index (GSI), mesangial volume density (Vvmes) and glomerular capillary surface density (Svcap) were also evaluated.

Results show that kidney weight increased in Nx groups, being higher in DMNx. GFR was higher in Nx groups as was RPF, being higher in DMNx. RVR was lower in Nx groups, especially in DMNx. MAP was not different among the groups. RPF and GFR showed a high correlation for the DMNx group ($r=0.95$, $p=0.02$). The DMNx group showed a correlation between RVR and GFR ($r=-0.96$, $p=0.005$). The GV increased in Nx groups, and the GSI was higher in DMNx. Vvmes and Svcap increased in DMNx group.

In summary, Nx groups developed similar degrees of glomerular hypertrophy, but only DMNx showed an increased value for GSI. The present data suggest that the acceleration of glomerular lesions in DMNx animals was more closely associated to hemodynamic adaptations than to glomerular hypertrophy.

Key words: Diabetes mellitus, Unilateral nephrectomy, Renal function, Glomeruli, Remnant nephron

Introduction

It was only after 1936 that glomerular injury was associated with diabetes mellitus (DM). Since then, despite hard scientific work, it has not been possible to avoid it becoming the most important cause of end-stage renal failure in the United States of America (Renal Data System USRDS, 2000) and one of the most important causes in other countries including Brazil (Noronha et al., 1997).

Renal alterations in DM within others are characterized by glomerular hypertrophy, thickness of the glomerular capillary membrane and mesangial expansion (O'Donnell et al., 1988; Steffer et al., 1989; Koya et al., 2000). An increase in the glomerular filtration rate (GFR) occurs early in patients with insulin-dependent DM (IDDM) (Christiansen et al., 1981; Mogensen et al., 1983; Raptis and Viberti, 2001) due to alterations of renal hemodynamics with the participation of hormones and growth factors (Hostetter et al., 1981a; Flyvbjerg et al., 1990; Hill et al., 2001). Many alterations observed in the experimental model of DM are also observed in animals submitted to a reduction in renal mass, suggesting that some mechanisms responsible for the progression of renal disease are common (Hostetter et al., 1981a; Flyvbjerg et al., 1988; Haylor et al., 2000; Ward et al., 2001). The objective of this study was to analyze renal function and morphology, in awake diabetic rats with associated reduction in renal mass.

Materials and methods

Male Wistar rats weighing between 150-200 g were divided into 4 groups: control rats (C, n=8), animals with diabetes mellitus (DM, n=8), rats submitted to unilateral nephrectomy (Nx, n=8), and a group with DM and Nx (DMNx, n=9). Rats were made diabetic by a single IV injection of streptozotocin (SPZ) (65 mg/Kg). Only those rats presenting glucose concentration above 300 mg% were used in the study. Rats received daily insulin adjusted to maintain blood glucose concentration between 300-500 mg%.

After 12 weeks animals were submitted to catheterization of the femoral artery and vein as well as the bladder under ether anesthesia. After the surgery animals were maintained in individual cages for 72 hours, afterwards they were placed awake in a restraining cage to evaluate renal function. Saline infusion was initiated in a rate of 1.5 ml/h. After 45 minutes, an infusion of inulin (4 ml, 10%) and para-aminohippuric acid (2.5 ml, 2%) was started. One hour later, three urine samples were collected for 5 to 8 minutes each, in an interval of 10 minutes between each other. In the middle point of each urine collection a blood sample was collected from the arterial catheter. At the end of the experiment mean arterial pressure (MAP) was registered through a Nikon-Kohden polygraph.

Morphology

At the end of the functional studies, kidneys were perfused through the abdominal aorta with 1.25 % glutaraldehyde buffered in phosphate, pH 7.4, at a constant pressure (100 mmHg). Kidney fragments were fixed, and 3-4 μ m sections were stained with periodic acid-Schiff.

Glomerular area (GA) was determined using optic morphometry with a "Mini-Mop". Glomerular volume (GV) was then calculated using the Weibel formula $GV=GA^{3/2} \cdot 1.38/1.1$, the results being expressed as 10^{-3} mm^3 (Weibel, 1979).

The glomerular sclerosis index (GSI) was determined by measuring the frequency of sclerosis in 100 glomeruli from each animal as described before (Raij et al., 1984), and the result is reported as median. A score of 0 to 4+ was assigned to each glomerulus according to the extent of sclerosis: normal glomerulus, 0; up to 25% involvement, 1+; up to 50% involvement, 2+; up to 75% involvement, 3+; and more than 75% involvement, 4+. The GSI score for each animal was the sum of individual glomerular scores multiplied by the percent of glomeruli with the same score.

Stereology

Mesangial volume density (V_{vmes}) and capillary surface density (S_{vcap}) were determined, using a test grid (M42). From each glomerulus 20 electron micrographs with a final magnification of $\times 10,500$ were analyzed. The number of capillary intersections with the test line (I_L) was counted to estimate the capillary surface density ($S_v=2 \cdot I_L$). The reference volume was estimated by point counting using the test points that hit the glomeruli (P_T). The number of points hitting the mesangium (P_p) was counted to estimate the volume density of this structure ($V_v=P_p/P_T$) (Nyengaard, 1999).

Whole kidney function

The glomerular filtration rate (GFR) and renal plasma flow (RPF) were estimated on the basis of inulin

(Fuhr et al., 1955) and para-aminohippuric acid (PAH) clearances (Smith et al., 1945).

Renal vascular resistance (RVR) was calculated as $RVR=(MAP/RPF) \cdot 1332$, the result being expressed as $10^6 \text{ dyne.s.cm}^{-1}$

Statistical analysis

Results are expressed as mean \pm standard error ($X \pm SE$). For comparison of means, one-way analysis of variance was used, and the difference pointed by the Duncan test. The GSI was analyzed by the Kruskal-Wallis test. Correlation coefficients among the GFR, RPF, RVR, GV, glycemia and kidney weight and GSI and GV were calculated. Stereological differences among the groups were tested using the Kruskal-Wallis analysis of variance and when differences were found the Kolmogorov-Smirnov test was used. Statistical significance was considered when $p < 0.05$.

Results

Table 1 shows that the BW was lower in the DMNx group compared to non-diabetic groups. The KW was higher in the Nx groups, and the DMNx group showed a higher value compared to the DM group. Glycemia was higher in the DM groups ($C=278 \pm 16 \text{ mg/dl}$; $DM=412 \pm 34 \text{ mg/dl}$; $Nx=232 \pm 19 \text{ mg/dl}$; $DMNx=453 \pm 47 \text{ mg/dl}$). Mean arterial pressure (MAP) showed similar values among the groups ($C=107 \pm 4 \text{ mmHg}$; $DM=109 \pm 8 \text{ mmHg}$; $Nx=104 \pm 4 \text{ mmHg}$; $DMNx=102 \pm 2 \text{ mmHg}$).

Figure 1 shows that the GFR ($C=1.3 \pm 0.1 \text{ ml/min}$; $DM=1.4 \pm 0.1 \text{ ml/min}$; $Nx=2.0 \pm 0.2 \text{ ml/min}$; $DMNx=2.3 \pm 0.1 \text{ ml/min}$) and RPF ($C=3.9 \pm 0.2 \text{ ml/min}$; $DM=4.7 \pm 0.4 \text{ ml/min}$; $Nx=6.2 \pm 0.4 \text{ ml/min}$; $DMNx=8.4 \pm 0.8 \text{ ml/min}$) were higher in the Nx groups; and the RVR ($C=2.2 \pm 0.1 \times 10^6 \text{ dyne.s.cm}^{-1}$; $DM=1.8 \pm 0.1 \times 10^6 \text{ dyne.s.cm}^{-1}$; $Nx=1.3 \pm 0.1 \times 10^6 \text{ dyne.s.cm}^{-1}$; $DMNx=0.9 \pm 0.1 \times 10^6 \text{ dyne.s.cm}^{-1}$) decreased in all groups when compared to C, and the DMNx showed the lowest value for this parameter. The FF was not different among the studied groups ($C=0.32$, $DM=0.31$; $Nx=0.29$; $DMNx=0.29$). The GV was increased in the Nx groups, and the GSI was higher in the DMNx group when compared to C. No correlations were found between the

Table 1. General data from groups (mean \pm SE).

	BW (g)	KW (g)	KW/BW	GLY(mg%)
C (n=8)	362 \pm 13	1.17 \pm 0.06	0.33 \pm 0.02	278 \pm 16
DM (n=8)	325 \pm 16	1.19 \pm 0.06	0.37 \pm 0.02	412 \pm 34 ^{a,b}
Nx (n=8)	368 \pm 13	1.66 \pm 0.11 ^{a,c}	0.45 \pm 0.03 ^a	232 \pm 19
DMNx (n=9)	291 \pm 15 ^{a,b}	1.97 \pm 0.11 ^d	0.68 \pm 0.04 ^d	453 \pm 47 ^{a,b}

$p < 0.05$: ^a vs C; ^b vs Nx; ^c vs DM; ^d vs all. BW: body weight; KW: kidney weight; GLY: glycemia.

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GV and GFR or GSI for any group. Mesangial volume density (Vvmes) was increased in the DMNx group as was the capillary surface density (Sv cap) when compared to control (Table 2).

There was no correlation between the GFR and glycemia or KW in any studied group. The RPF and GFR showed a high correlation for the DMNx group ($r=0.95$, $p=0.02$); the same was not observed for the Nx group. The DMNx group also showed a strong negative correlation between RVR and GFR ($r=-0.96$, $p=0.005$).

Discussion

One difficulty associated with studying early renal changes in diabetes is the fact that anesthesia and other types of stress influence the GFR (Walker et al., 1983; Vaamonde et al., 1989; Bak et al., 2000, 2001). This problem was overcome in the present study by using conscious rats fully recovered from the operation and trained to participate in the experiment.

Accentuation of the glomerular lesions associated to DM due to renal mass reduction was demonstrated more than a decade ago (Steffes et al., 1978; O'Donnel et al., 1986). The present study had the objective of determining renal function of awake DM rats, submitted to unilateral nephrectomy, as well as the renal morphology of these animals.

Glomerular alterations observed in rats with diabetes mellitus (DM) submitted to unilateral nephrectomy (Nx) are markedly exaggerated. Table 1 shows that BW was significantly decreased in the DMNx group compared to non-diabetic rats. It is known that DM rats maintained moderately hyperglycemic do not gain weight to the same extent as non diabetic rats (Jensen et al., 1986; Bank, 1991). This difference should be pointed out, since BW is correlated to the GFR as well as with renal hypertrophy.

The absolute values for KW were higher in the Nx groups, and showed an exacerbated renal hypertrophy in

DMNx group. Moderate hyperglycemia is an isolated stimulus to renal hypertrophy, its association with the reduction in renal mass resulted in a marked stimulus to this hypertrophy (Steffes et al., 1978; Hostetter et al., 1981b; O'Donnel et al., 1986). Similar values for MAP were observed among the studied groups, excluding the participation of arterial hypertension in the alterations observed in the DMNx group in the present study which was similar to other findings (O'Donnel et al., 1986).

The GFR was elevated in the Nx groups, particularly in the DMNx group, suggesting that a reduction in renal mass associated with DM led to an additional stimulus for hyperfiltration. RPF increased significantly in the Nx groups, associated to a decrease in RVR. These alterations were again more pronounced in the DMNx group. These results suggest that a reduction in renal mass in DM animals exacerbates renal hemodynamic alterations.

Glomerular volume was increased in the Nx groups compared to C. The Nx groups did not show any correlation between the magnitude of variation of the GV or GFR. These results suggest that the elevation of GFR after Nx was not due to expansion of the filtration surface but may be dependent on the hemodynamic adaptations. This is reinforced by the strong negative correlation observed between GFR and RVR, as well as the high positive correlation between RPF and GFR in the DMNx group. These data point to the elevation of RPF secondary to a reduction in RVR as the main determinant of the glomerular hyperfiltration in the DMNx group; these findings are similar to others (Blantz et al., 1982; Woods et al., 1987; O'Donnel et al., 1988; Hirschberg and Kopple, 1989; Raptis et al., 2001).

The glomerular sclerosis index for the DMNx group showed a similar value to that obtained by others (Zatz et al., 1986) in DM rats after 11-14 months of evaluation; suggesting, therefore, that a reduction in renal mass accelerates the appearance of glomerular lesions in DM rats (Steffes et al., 1978; O'Donnel et al., 1986). The mesangial volume density increased in the DMNx group, suggesting mesangial expansion and surface density of glomerular capillaries increased in the experimental groups suggesting hypertrophy.

In summary, the Nx groups developed similar degrees of glomerular hypertrophy, but only the DMNx

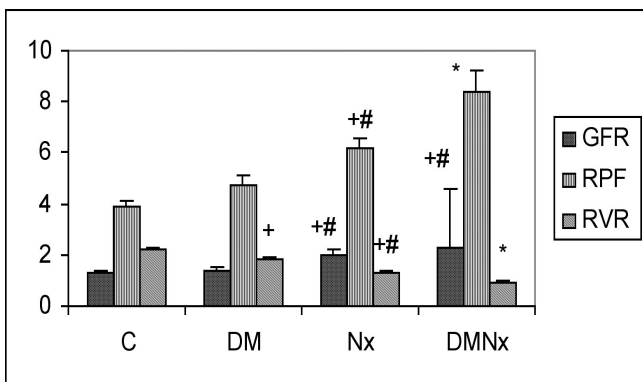


Fig. 1. Renal functions from groups. $p<0.05$: +, vs C; #, vs DM; *, vs all. GFR: glomerular filtration rate (ml/min); RPF: renal plasma flow (ml/min); RVR: renal vascular resistance (10^6 dyne.s.cm⁻¹).

Table 2. Morphological data from groups (mean±SE).

	GV 10^{-3} mm ³	GSI %	Vv mes %	Sv cap mm ² /mm ³
C (n=8)	2.18±0.09 ^a	1.4±0.4	12±1.0	0.31±0.04
DM (n=8)	2.71±0.26	2.1±0.9	13±3.0	0.41±0.08 ^a
Nx (n=8)	2.96±0.17 ^a	1.8±1.1	10±2.0	0.38±0.05 ^a
DMNx (n=9)	3.11±0.23 ^a	5.4±1.9 ^a	15±0.7 ^a	0.40±0.03 ^a

^ax±SEM, $p<0.05$: a, vs C. GV: glomerular volume; GSI: glomerular sclerosis index; Vv mes: mesangial volume density; Sv cap: surface density of the glomerular capillaries.

showed a significantly elevated value for the GSI compared to C. This finding suggests that isolated glomerular hypertrophy is not the main factor for determining glomerulosclerosis. In the same way glycemia was not responsible either, since DM rats did not show an elevation in the GSI. Again, we point out that the main difference between the DMNx group and the others was the hemodynamic alterations leading to different results in this group.

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