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Letter to the Editor

Carvedilol protects the peritubular capillaries and kidney structure in spontaneously hypertensive rats☆

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Carvedilol improves autoregulation of renal blood flow by virtue of its ability to reduce intrarenal vascular resistance [1] and modify the vascular reactivity in non-denervated kidney [2]. Long-term changes in renal blood flow, for example during essential hypertension, may induce progressive modifications in the microvascular (MV) tone, given that the chronic vascular constriction can lead to peritubular capillary loss and increased intrarenal vascular resistance. The severity of these changes may affect the kidney functions, a phenomenon known as vascular rarefaction [3]. Carvedilol is a beta blocker with antioxidant and vasodilating activities, which has proven to provide renal protection in experimental rat models, beyond their antihypertensive activity [4]. However, little is known about the potential preventive role of carvedilol on MV remodeling in spontaneously hypertensive rats (SHR). Eight-week-old male SHR and Wistar-Kyoto (WKY) rats were randomly divided into three groups: SHR (n = 10), SHR-C (n = 10; carvedilol 20 mg/kg/day) and WKY (n = 10). For 16 months, animals were fed on a standard commercial chow (Cooperación, Buenos Aires, Argentina) ad libitum and housed inside an indoor laboratory facility with a 12 h light/dark cycle. At 18 months of age, all the rats were sacrificed and the kidneys excised and processed for quantitative microscopy and immunohistochemistry studies. Animal care was according to the 'Guide for the Care and Use of Laboratory Animals' published by the US National Institutes of Health (NIH publication no. 85-53, revised 1998). At baseline and after every 2 weeks, systolic blood pressure (SBP) was measured by tail-cuff plethysmography. Blood samples were obtained by ventricular puncture and the plasmatic levels of creatinine were assessed with a commercial kit (Sigma Chemical Co., St. Louis, MO). The animals were euthanized under anesthesia and the kidneys were removed, weighed, fixed in 10% buffered formaldehyde and processed for histology. Thin sections from tissue blocks were stained with periodic acid-Schiff (PAS), Masson's trichrome (MT) and Jones methenamine (JM), and the glomerular profile numerical density per area [N_A (glom) per mm^2], glomerular volume (V_G, expressed in μm^3), mesangial matrix (MM, expressed in percentage), tubular metaplasia (TM, expressed in % of glomeruli), interstitial fibrosis (IF, expressed in percentage) and tubular atrophy (TA, expressed in percentage) were evaluated by using an image processing software (Image-Pro Plus version 6; Media Cybernetics, Silver Spring, Maryland, USA). The integrated optical density (IOD) [5] was employed to assess the immunohistochemical labeling that allowed to estimate: peritubular capillary density (PC_D, endothelial immunostaining for CD34), peritubular capillary protection (T_{VEGF}, tubular staining for vascular endothelial growth factor), apoptosis (tubular expression for caspase-3) and oxidative stress status (tubular expression for: thioredoxin-1 (T_{trx1}) and peroxiredoxin-2 (T_{prx2})). The normalized total kidney mass (KM_T) was estimated for comparison purposes.

A significant reduction of total kidney mass was demonstrated in SHR-C (−23.7% vs WKY and −15.83% vs SHR). Also, animals treated with carvedilol showed an elevated N_A (glom) and V_G compared to SHR (+16.64% and +29.21%, respectively), while MM was significantly decreased (−38.94%). In addition, carvedilol induced a significant reduction in TA and IF compared to SHR (−54.66% and −61.08%, respectively), associated to increased peritubular capillaries (+28.91%), that was even better than WKY (+24.12%). Finally, an elevated percentage of tubular metaplasia was demonstrated in SHR-C compared with SHR and WKY (+22.62% and +58.49%, respectively) associated to low expression of caspase-3 and high for VEGF, trx1 and prx2. Moreover, no differences were observed in body weight and plasmatic creatinine between experimental groups (data not shown). Table 1 summarized the analyzed immunohistochemical parameters.

MV remodeling implicates a reduction of peritubular capillary density that may evolve to renal functional loss. The intrarenal blood flow reduction causes oxidative stress, injury of tubular epithelium and release of proinflammatory cytokines [6] favoring leukocyte recruitment,

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Table 1
Normalized total kidney mass, systolic blood pressure, morphometrics and immunohistochemical parameters in the experimental groups.

	Experimental groups		
	WKY	SHR	SHR-C
KM_T (mg/g)	4.6 ± 1.58	4.17 ± 0.65	3.51 ± 0.19*
SBP (mm Hg)	145.5 ± 3.6*	205.3 ± 7.2	183 ± 1**
N_A (glom)/mm^2	7.88 ± 1.63*	6.41 ± 1.61	7.69 ± 1.56*
V_G (10^6 μm^3)	2.74 ± 0.95	2.69 ± 0.83	3.80 ± 0.91**
MM (%)	24.33 ± 7.88	28.53 ± 9.42	17.42 ± 6.3**
TM (%)	20.17 ± 14.04*	37.6 ± 21.38	48.59 ± 7.64**
TA (%)	9.39 ± 3.02*	17.60 ± 12.19	7.98 ± 7.64*
IF (%)	5.26 ± 1.67*	7.58 ± 2.49	4.63 ± 1.78*
PC_D (IOD)	10.80 ± 2.5	9.34 ± 2.99	13.14 ± 1.83**
T_{VEGF} (IOD)	41.77 ± 13.4*	12.90 ± 19.6	55.64 ± 16.14*
T_{trx1} (IOD)	32.70 ± 32.9*	9.86 ± 33.8	88.11 ± 12.5**
T_{prx2} (IOD)	89.1 ± 82.2*	15.81 ± 42.2	157.1 ± 23.65**
$T_{caspase\text{-}3}$ (IOD)	20.52 ± 91.2*	57.31 ± 57.3	23.55 ± 14.9**

Values are means ± SD. Differences were analyzed using the Kruskal–Wallis test and Dunn's Multiple Comparison test. KM_T: total kidney mass; N_A (glom): glomerular profile numerical density; V_G: glomerular volume; MM: mesangial matrix; TM: tubular metaplasia; TA: tubular atrophy; IF: interstitial fibrosis; PC_D: peritubular capillary density; T_{VEGF}: tubular expression for vascular endothelial growth factor; T_{trx1}: tubular expression for thioredoxin 1; T_{prx2}: tubular expression for peroxiredoxin 2; $T_{caspase\text{-}3}$: tubular expression for caspase-3; IOD: integrated optical density units.
* $p < 0.05$ when compared with SHR group.
** $p < 0.05$ when compared with SHR and Wky groups.

myofibroblast mobilization, interstitial fibrosis and contributing to both the reduction of peritubular capillaries and tubular atrophy [7]. In human pathology, the interstitial fibrosis contributes to reduction of peritubular capillaries, constituting a poor prognostic factor. The vasodilatory effect of carvedilol may keep intrarenal blood flow and protect from oxidative stress added to its own antioxidative action, in order to improve the autoregulatory mechanism of intrarenal vascular bed [8]. No differences in PC_D between WKY and SHR groups were observed, in comparison to the significant increase noted in SHR-C, although WKY and SHR-C demonstrated similar reductions of IF compared to SHR. These findings suggest a protective role of carvedilol on microvascular remodeling by tubular increasing of VEGF, trx1 and prx2. The protection of N_A (glom) together with increase of V_G was important structural changes associated to the protective effect of carvedilol on kidney function beyond the blood pressure lowering. In addition, carvedilol prevented the mesangial expansion observed in SHR group, a key factor involved in the evolution toward glomerulosclerosis linked to high immunostaining for trx1 and prx2. Another histological change was the tubular metaplasia in Bowman's capsule, associated for years to renal hypertensive damage. It is now accepted that the parietal epithelium of glomerulus represents a reservoir of renal progenitor cells in adult kidney able to regenerate tubular epithelia [9]. This concept may explain the increased metaplastic glomeruli observed in SHR-C group compared with W and SHR, suggesting that carvedilol helps tubular regeneration, which together with its antioxidant effect ensures protection on glomerulotubular unit. This event may contribute to peritubular capillaries protection via releasing VEGF [10].

In conclusion, carvedilol prevents microvascular remodeling, IF and TA by antioxidant effects, contributing to structural improvement of kidney beyond their functional status. Additionally, carvedilol induced tubular metaplasia that could favor the protection of proximal convoluted tube. Finally, carvedilol prevented mesangial matrix expansion and evolution toward glomerulosclerosis, a protective phenomenon on glomerular population (See Fig. 1).

Conflict of interest

There are no conflicts of interest.

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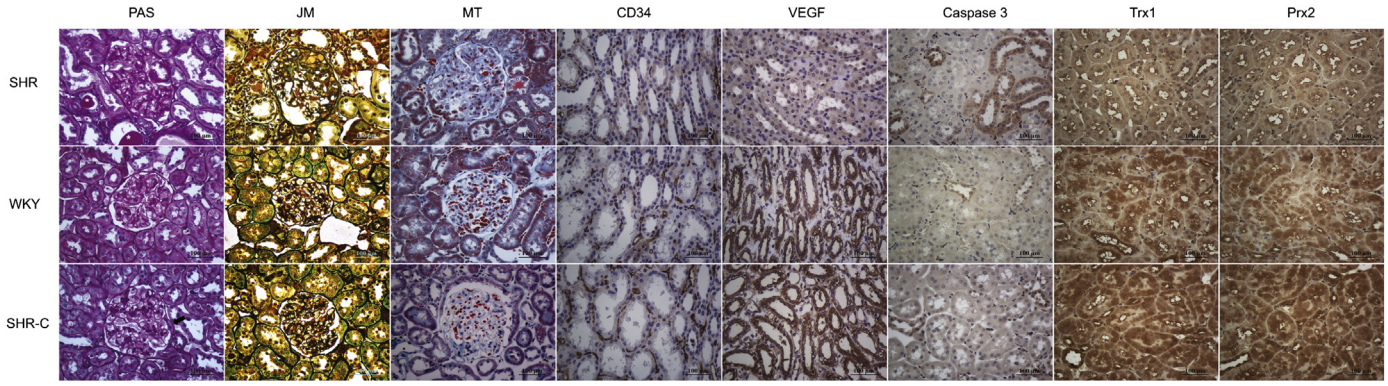

Fig. 1. Representative microphotographs of kidney stained with periodic acid-Schiff (PAS), Jones methenamine (JM) or Masson's trichrome (MT), from experimental groups. SHR rats showed mesangial expantion associated to peripheral capillary collapse, tubular atrophy (asterisks), interstitial fibrosis and chronic inflammation as compared to WKY (normotensive) rats. Carvedilol treatment (SHR-C) improved these morphological parameters, favoring the tubular metaplasia at glomerular level (black arrow). In addition, Carvedilol increased the immunohistochemical expression for CD34 (peritubular capillary density), VEGF, trx1 and prx2 linked to a significant reduction of the immunolabeling for caspase-3 (apoptotic activity). These findings suggest a protective role of Carvedilol on the peritubular capillary bed via the VEGF activity associated to an antioxidant effect. Magnification × 40. Scale bar: 100 μm.