

Original Article

Nephropathy induced by renal microembolism: a characterization of biochemical and histopathological changes in rats

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Abstract: The aim of this study was to investigate some biochemical parameters of renal function and the vascular, glomerular, tubular, and interstitial manifestations in the progression of nephropathy induced by renal microembolism. Renal microembolism was induced by the arterial injection of polymethacrylate microspheres in the remnant kidney of nephrectomized rats. Animals 110-120 days old were randomly divided into three groups: the control group (C; normal), the nephrectomized group (S; nephrectomized that did not undergo renal microembolism), and the model group (M, nephrectomized animals that underwent renal arterial microembolism). The animals were evaluated 30, 60, and 90 days after the induction of a renal microembolism. Blood and urine samples were collected to determine serum creatinine (Cr) and urea (Ur) concentrations and urine total protein (Pt) concentrations. The kidneys were weighed and processed for histopathological analysis using hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Mallory-Azan, and Picro-Sirius staining. The samples were also subjected to immunohistochemistry with a proliferating cell nuclear antigen (PCNA) and a vascular endothelial growth factor receptor (VEGFR). The data demonstrated evidence of the occurrence of vascular, glomerular, tubular, and interstitial abnormalities in the renal tissue, and changes in the biochemical parameters of renal function (serum Cr and Ur and of 24-h urine Pt) in this experimental model of nephropathy induced by renal microembolism, which may indicate the development of chronic kidney disease (CKD). Additionally, the findings indicate that this is a good reproducibility model that may be useful for studying the pathogenesis of CKD that is caused by atheroembolism and possible treatment alternatives.

Keywords: Nephropathy, renal microembolism, chronic kidney disease, animal model

Introduction

Atheroembolism is a multisystem disease that often affects multiple organs, such as the kidneys, the skin, the gastrointestinal tract, and the central nervous system. Although the disease can occur spontaneously in some cases, it is often a consequence of the complications of endovascular procedures or anticoagulant or thrombolytic therapy. In such cases, cholesterol emboli can displace and occlude small arteries and arterioles in different organs, causing ischemia [1, 2]. Renal failure is thus the most common and severe systemic complication, and its progression is one of the major causes of death [3-5].

Atheroembolic renal disease (AERD) is defined as renal failure secondary to the occlusion of renal arteries and arterioles by cholesterol emboli that are deposited as crystals. The exact incidence of AERD is unknown because many cases are undiagnosed. Its clinical presentation varies from subclinical to fatal disease [1, 2, 4].

AERD is a devastating complication of atherosclerotic disease. To date, there are no effective specific treatments [1, 4-8]. Some isolated case reports in the literature have reported improvements in patients' clinical condition with the empirical use of acetylsalicylic acid, corticosteroids, statins, and a prostacyclin ana-

log [7, 9, 10]. However, to our knowledge, no experimental evidence supports such observations.

In this context, the development of animal models is important for identifying signaling pathways that are involved in AERD, elucidating the mechanisms that are involved in the disease, and guiding new treatment strategies [11, 12]. With regard to atheroembolism-induced chronic kidney disease, we found only one study, from 1999, that reported changes in some biochemical parameters and tubular-interstitial damage in an experimental model of AERD [13]. However, the authors did not investigate vascular and glomerular alterations that can manifest in the disease and play an important role in its progression and consequently the development of fibrosis. This was our motivation to study such changes in a model of chronic kidney disease (CKD) induced by renal microembolism.

In this paper, we investigated vascular, glomerular, tubular, and interstitial manifestations, as well as some biochemical parameters of renal function in the progression of nephropathy that is induced by renal microembolism.

Materials and methods

General experimental procedure

The experimental protocol was approved by the Ethics Committee on the Use of Animals in Research of the State University of Maringá (no. 1994250515). All efforts were made to minimize the number of animals used and their suffering. Male Wistar rats, 110-120 days old, were housed in standard animal husbandry conditions (22°C ± 2°C and a 12 h/12 h light/dark cycle) with free access to food and water. After 1 week of adaptation, the animals were randomly divided into three groups (5-6 rats): the control group (C; normal animals), the nephrectomized group (S; nephrectomized animals that did not undergo the induction of renal microembolism), and the model group (M; nephrectomized animals that underwent renal microembolism). The animals from each group were anesthetized and then euthanized 30, 60, and 90 days after the induction of the renal microembolism. Urine was collected for 24 h in a metabolic cage prior to euthanasia. Upon euthanasia, blood samples were collected from

the inferior vena cava and centrifuged at 1000 × g for 15 min at 4°C. The serum was separated and aliquoted using Eppendorf tubes. The samples were kept at 2-8°C until the time of the biochemical analyses (performed on the same day of collection). The kidneys of the different groups of animals were rapidly removed, weighed, and fixed in 10% formaldehyde for 24 h. Afterward, they were dehydrated in an ascending series of ethyl alcohol, diaphanized in xylol, and embedded in paraffin. Serum concentrations of creatinine (Cr) and urea (Ur) and urine concentrations of total protein (Pt) were determined using a chemiluminescence assay with the Immulite-2000 system (Siemens Health Diagnostics, Deerfield, IL, USA) and a Siemens kit. The results are expressed as mg/dl.

Induction of nephropathy by renal microembolism

Nephropathy was induced by an arterial injection of microspheres (poly-methyl methacrylate, Cospheric, Santa Barbara, CA, USA) in the remnant kidney of nephrectomized rats according to the technique of Kimura et al. [13] with modification. The rats were anesthetized with thiopental (45 mg/kg, intraperitoneally) for laparoscopic surgery. The celiac, mesenteric, contralateral renal, and distal regions of the aorta were clamped to guarantee blood flow only in the left renal artery. In the region anterior to the left renal artery ostium, microspheres (20-27 µm diameter) that were suspended in physiological saline (0.8 mg/0.2 ml) were administered. After administration of the solution that contained the microspheres, the arterial clamps were maintained for 15 s, and the aortic puncture site was maintained for 2 min to avoid bleeding. Next, the right kidney was removed. The incisions were sutured, and the animals were kept warm with an incandescent lamp for post-surgical recovery. In the S group, the rats were subjected to the same operative procedure (i.e., nephrectomy) but did not receive administration of the microspheres.

Histopathological analysis of renal tissue

The paraffin-embedded tissue sections were used to obtain 5-µm semi-serial cross-sections. The following staining techniques used were: hematoxylin and eosin (HE), Mallory-Azan, periodic acid-Schiff (PAS), and Picro-Sirius. Hematoxylin- and eosin-stained sections were used

for the microscopic evaluation of the renal corpuscle, the intensity of the inflammatory response in the renal interstitium, and changes in renal tubules. The scores were based on Parlakpınar et al. [14], Kiris et al. [15] and Oruc et al. [16], with modification. The Mallory-Azan trichrome-stained sections were used for the identification of total collagen. The PAS-stained sections were used to measure the area of the basal membrane. For the morphometric analysis of the kidneys, 60 random images were captured in each slide per animal, for a total of 300 images per group, using an Olympus BX41 optical microscope with a 20 × objective (Olympus, Tokyo, Japan). An image analysis system (ImagePro Plus 4.5, Media Cybernetics, Silver Spring, MD, USA) was used for the morphometric analyses of the kidneys. The HE-stained sections were used to measure the Bowman's space area, calculated by subtracting the measurements of the 50 kidney corpuscle areas from the measurements of the 50 glomerulus areas of each animal, for a total of 250 Bowman's space measurements per group. The results are expressed as the mean ± standard error of the mean (in μm^2). The Picro Sirius-stained sections were used to quantify the types of collagen (I and III). The images were captured using a Nikon Eclipse 80i optical microscope coupled with a Nikon DsFiFi camera, using optical polarization, and scanned using Q Capture Pro 5.1 software (Media Cybernetics, Silver Spring, MD, USA). The scanned images were analyzed using ImagePro Plus 4.5 image analysis software (Media Cybernetics, Silver Spring, MD, USA). The results are expressed as a percentage of the amount of each type of collagen per kidney area.

Immunohistochemistry

The kidney samples were dehydrated in an ascending series of ethyl alcohol, diaphanized in xylol, and embedded in paraffin to obtain semi-serial 5- μm cross-sections. The samples were subjected to immunohistochemistry with proliferating cell nuclear antigen (PCNA) antibody (catalog no. PA5-27214, Invitrogen/Thermo Fisher Scientific, Waltham, MA, USA) to immunolabel cell proliferation and vascular endothelial growth factor (VEGF) receptor 2 antibody (catalog no. ab2349, Abcam, Cambridge, MA, USA) to immunolabel angiogenesis. Antigen retrieval was performed with the addi-

tion of sodium citrate and Tween 20 at high temperature for 20 min. After cooling, the sections were incubated in 10% hydrogen peroxide for 20 min to block endogenous peroxidase activity. The slides were then washed three times for 5 min with phosphate-buffered saline (PBS; 0.1 M, pH 7.4) and then incubated with 2% bovine serum albumin in PBS for 10 min to block nonspecific binding sites. Afterward, the sections were incubated with a primary antibody (PCNA or VEGFR) and diluted in PBS (1:200) overnight at room temperature. The sections were then washed twice in PBS for 5 min each and incubated for 90 min at room temperature with mouse anti-rabbit IgG-B secondary antibody (1:200; Santa Cruz Biotechnology, Dallas, TX, USA). Afterward, two washes were performed in PBS for 5 min and incubated with the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature to bind the latter to the secondary antibody. Afterward, two washes were performed in PBS for 5 min, followed by incubation for 30 min at room temperature in the dark with the ImPACT AEC kit (Vector Laboratories, Burlingame, CA, USA) for revelation. The reaction was stopped with distilled water. The sections were counterstained with Meyer's hematoxylin and mounted in the VectaMount AQ aqueous medium (Vector Laboratories, Burlingame, CA, USA) using a 20 × objective. Random images of each slide per animal were captured. ImagePro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA) was used to detect immunoreactivity.

Statistical analysis

The results were expressed as the mean ± standard error of the mean (SEM). Because the data satisfied the assumptions of a normal distribution (D'Agostino and Pearson omnibus normality test), the analysis of variance (ANOVA) followed by Tukey's *post hoc* test was then performed. Values of $P < 0.05$ were considered statistically significant.

Results

Serum creatinine and urea concentrations, 24-h proteinuria, and kidney weight in the control, nephrectomized, and model groups at 30, 60, and 90 days

The mean values of the biochemical parameters at baseline were the following: serum Cr =

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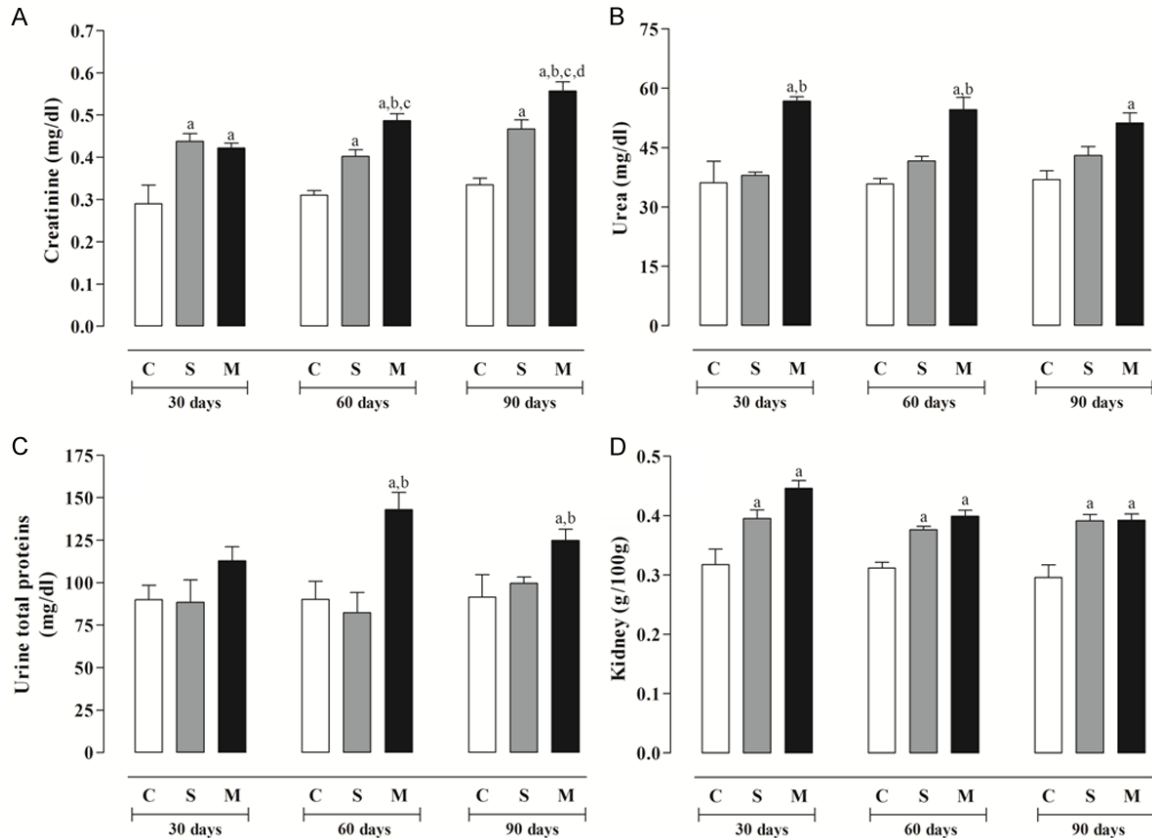


Figure 1. Serum concentrations of creatinine (A) and urea (B), urine protein concentration (C) (expressed as mg/dl), and remnant kidney weight (D) (expressed as a g/100 g body weight of rats) in the control (C), nephrectomized (S), and model (M) groups at different periods of evaluation (30, 60, and 90 days). The data are expressed as the mean ± SEM of six animals per group. ^a $P < 0.05$, compared with C group at a respective period of evaluation; ^b $P < 0.05$, compared with the S group at a respective period of evaluation; ^c $P < 0.05$, compared with the M group at 30 days; ^d $P < 0.05$, compared with the M group at 60 days (one-way ANOVA followed by Tukey's *post hoc* test).

0.30 mg/dl, serum Ur = 38 mg/dl, and 24-h urine Pt = 90.5 mg/dl. Serum Cr and Ur and urine Pt concentrations in the C group remained unchanged at 30, 60, and 90 days (**Figure 1A-C**). However, some of these parameters were significantly altered ($P < 0.05$) in the S and M groups. Serum Cr concentrations in both S and M groups increased over the experimental time period and were significantly higher ($P < 0.05$) compared with the C group at 30, 60 and 90 days. Serum Cr concentrations in the M group progressively increased over the experimental time period and were significantly higher ($P < 0.05$) compared with the S group at 60 and 90 days (**Figure 1A**). Serum Ur concentrations were not significantly altered in the S group compared with the C group at 30, 60, and 90 days. However, in the M group, serum Ur concentrations significantly increased ($P < 0.05$) compared with both the C and S groups

at 30 and 60 days and compared with the C group at 90 days (**Figure 1B**). The 24-h urine Pt concentration in the S group was not significantly altered compared with the C group at 30, 60, and 90 days. In contrast, 24-h urine Pt in the M group significantly increased ($P < 0.05$) at 60 and 90 days compared with both the C and S groups (**Figure 1C**). The weight of the remnant kidneys increased by approximately 33% in both the S and M groups (**Figure 1D**).

Morphological and morphometric evaluation of renal tissue in the control, nephrectomized, and model groups (HE staining)

The histological analysis of the kidneys in the C group, using scoring, showed an intact renal architecture, with a distribution of uniform-sized glomeruli with Bowman's capsule and space preserved at all periods of evaluation

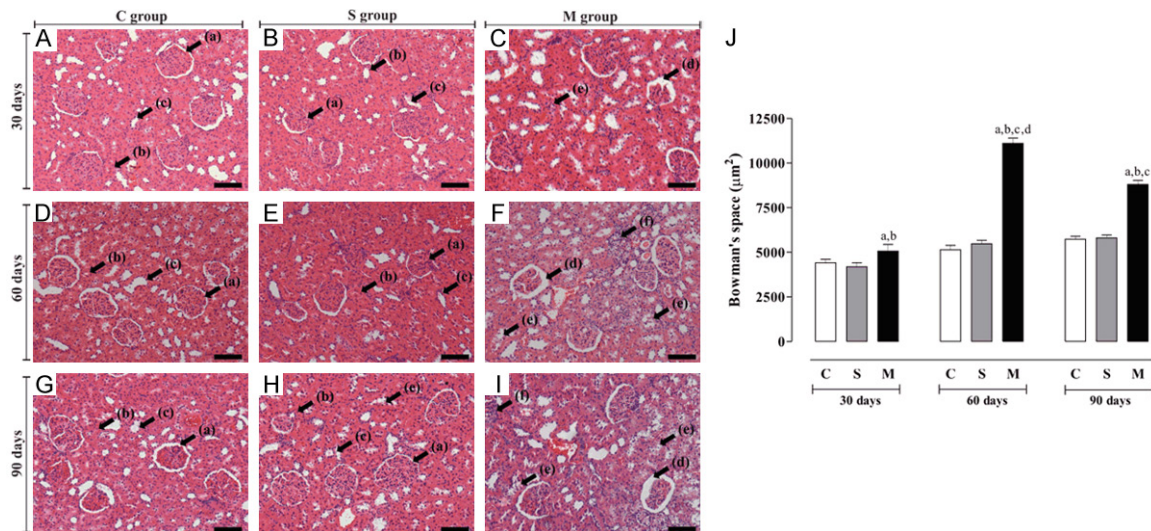


Figure 2. Photomicrographs of histopathological and morphometric changes in Bowman's space in renal tissue in the control (C), nephrectomized (S), and model (M) groups at different periods of evaluation (30, 60, and 90 days). The renal sections were stained with hematoxylin and eosin (HE). A-C. Renal tissue photomicrographs representing the 30-day period in the C, S, and M groups, respectively. D-F. Renal tissue photomicrographs representing the 60-day period in the C, S, and M groups, respectively. G-I. Renal tissue photomicrographs representing the 90-day period in the C, S, and M groups, respectively. J. Morphometric analysis of Bowman's space (in μm^2) in the C, S, and M groups at their respective periods of evaluation. Each value represents the mean $\pm$ SEM of five animals per group. ^a $P < 0.05$, compared with C group at the respective period of evaluation; ^b $P < 0.05$, compared with the S group at respective period of evaluation; ^c $P < 0.05$, compared with the M group at 30 days; ^d $P < 0.05$, compared with the M group at 90 days (one-way ANOVA followed by Tukey's *post hoc* test). Black arrows (a) indicate glomeruli with Bowman's space, preserved capillaries, and cellularity. Black arrows (b) and (c) indicate intact proximal and distal tubules. (d) indicate glomerular changes (i.e., dilatation of Bowman's space, capillary loops, and decrease in cellularity). (e) indicate tubular degeneration. The (f) indicates an inflammatory process. 200 $\times$ magnification.

(30, 60, and 90 days). Additionally, the proximal and distal renal tubules presented a characteristic cellular distribution, and the renal interstitium presented no evident alterations. In the S group, significant glomerular histological changes ($P < 0.05$) were detected, including a decrease in glomerular cellularity and dilation of the glomerular capillaries compared with the C group at all periods of evaluation (30, 60, and 90 days). Significant renal tubular alterations ($P < 0.05$), including cellular degeneration and necrosis, were evident in the S group at 90 days. In the renal interstitium in the S group, an intense inflammatory process was observed at 30 days ($P < 0.05$); this score returned to close to normal at 60 and 90 days (Figure 2 and Table 1). Histological analyses of the kidney in the M group at all periods of evaluation (30, 60, and 90 days) indicated intense morphological alterations compared with the C and S groups. Intense glomerular alterations, such as the dilatation of Bowman's space and capillary loops, and a decrease in cellularity, were observed. Bowman's space dilatation was more pro-

ounced at 60 days. Tubular degeneration and necrosis were evident, progressively occurring at 30 and 60 days and remaining stable at 90 days. An intense inflammatory process was evident in all periods of the evaluation. This change was also progressive at 30 and 60 days and remained stable at 90 days (Figure 2 and Table 1).

Morphometric analysis of the basal membrane in renal tissue in the control, nephrectomized, and model groups (PAS staining)

The morphometric evaluation of the basal membranes of the kidneys in the S group at 30 days presented a significant decrease in the basal membrane area compared with the C group. At 60 and 90 days, the basal membrane area of the kidney in the S group remained similar to the C group. The M group presented a significant and progressive reduction of the basal membrane area at 30 and 60 days, and this decrease was maintained at 90 days, compared with the C and S groups (Figure 3).

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Table 1. Qualitative scoring assessment of the histopathological findings in renal tissue stained using the hematoxylin and eosin (HE) technique in the control (C), nephrectomized (S), and model (M) groups at the different periods of evaluation (30, 60, and 90 days)

Kidney changes		30 days			60 days			90 days		
		C	S	M	C	S	M	C	S	M
Corpuscle	Bowman's space	0.00 ± 0.0	0.00 ± 0.0	2.36 ± 0.1 ^{a,b}	0.12 ± 0.1	0.00 ± 0.0	2.66 ± 0.1 ^{a,b}	0.12 ± 0.1	0.00 ± 0.0	2.26 ± 0.2 ^{a,b}
	Capillary loops	0.66 ± 0.2	2.00 ± 0.3 ^a	1.72 ± 0.3 ^a	0.61 ± 0.2	2.00 ± 0.4 ^a	2.90 ± 0.1 ^{a,b,c}	0.77 ± 0.3	2.13 ± 0.3 ^a	2.66 ± 0.2 ^{a,c}
	Cellularity	0.00 ± 0.0	0.58 ± 0.3 ^a	2.54 ± 0.3 ^{a,b}	0.00 ± 0.0	0.63 ± 0.3 ^a	2.44 ± 0.2 ^{a,b}	0.01 ± 0.1	0.51 ± 0.1 ^a	2.40 ± 0.2 ^{a,b}
Tubules	Degeneration and necrosis	0.33 ± 0.2	0.66 ± 0.2	1.09 ± 0.3 ^{a,b}	0.39 ± 0.1	0.66 ± 0.3	2.63 ± 0.2 ^{a,b,c}	0.44 ± 0.2	1.93 ± 0.1 ^a	3.00 ± 0.1 ^{a,b,c}
Interstitium	Inflammatory cells	0.16 ± 0.2	1.50 ± 0.3 ^{a,d}	1.90 ± 0.2 ^{a,b}	0.16 ± 0.2	0.33 ± 0.2	2.33 ± 0.1 ^{a,b,c}	0.22 ± 0.1	0.33 ± 0.1	2.73 ± 0.1 ^{a,b,c}

Scores were assigned as the following: 0 (integral renal tissue), 1 (mild changes), 2 (moderate changes), and 3 (severe changes). The results are expressed as the mean ± SEM of five animals per group.

^aP < 0.05, compared with S group at the respective period of evaluation; ^bP < 0.05, compared with S group at the respective period of evaluation; ^cP < 0.05, compared with M group at 30 days; ^dP < 0.05, compared with S group at 60 and 90 days (one-way ANOVA followed by Tukey's post hoc test).

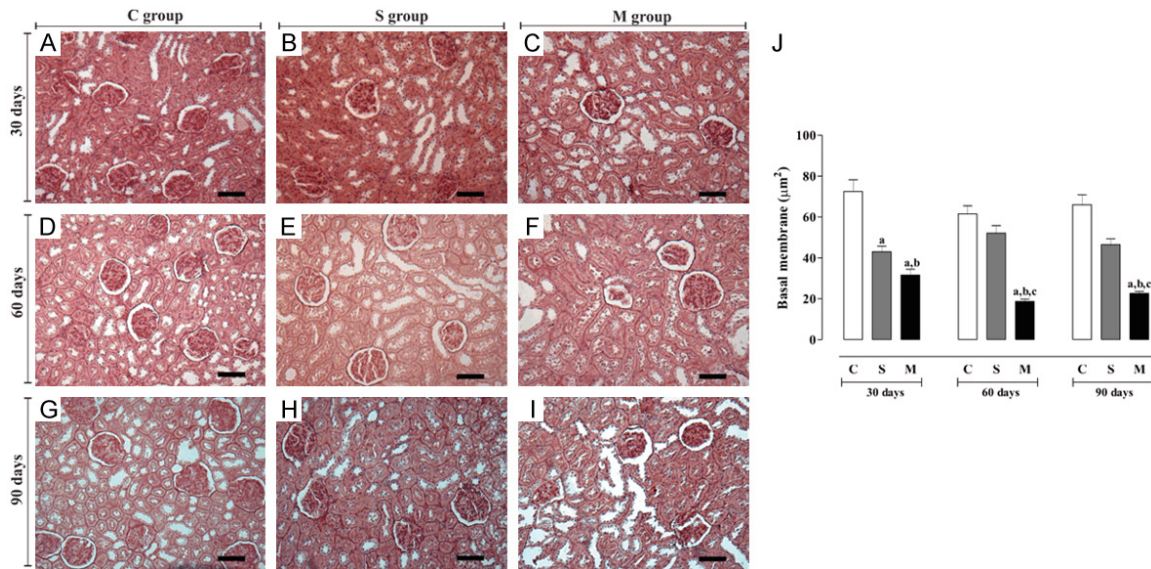


Figure 3. Representative photomicrographs and morphometric analysis of the basal membrane in renal tissue in the control (C), nephrectomized (S), and model (M) groups at different periods of evaluation (30, 60, and 90 days). Renal sections were stained using the periodic acid-Schiff (PAS) technique. A-C. Renal tissue photomicrographs representing the 30-day period in the C, S, and M groups, respectively. D-F. Renal tissue photomicrographs representing the 60-day period in the C, S, and M groups, respectively. G-I. Renal tissue photomicrographs representing the 90-days period in the C, S, and M groups, respectively. J. Morphometric analysis of the kidney basal membrane (in μm^2) in the C, S, and M groups at their respective periods of evaluation. Each value represents the mean $\pm$ SEM of five animals per group. ^a $P < 0.05$, compared with the C group at the respective period of evaluation; ^b $P < 0.05$, compared with the S group at the respective period of evaluation; ^c $P < 0.05$, compared with the M group at 30 days (one-way ANOVA followed by Tukey's *post hoc* test). 200 $\times$ magnification.

Deposition of total collagen in the renal tissue in the control, nephrectomized, and model groups (Mallory-Azan staining)

The deposition of total collagen in the S group was observed at 60 and 90 days (but not at 30 days) compared with the C group. At all periods of evaluation, the presence of intense deposition of collagen was observed in the M group compared with the C and S groups. The development of fibrosis in the M group occurred in a time-dependent manner between 30 and 60 days and remained constant at 60 and 90 days (Figure 4A).

Deposition of type I and III collagen in the kidneys in the control, nephrectomized, and model groups (Picro Sirius Red staining)

Type III collagen deposition was not significantly altered in the S and M groups at 30 days compared with the C group. Type III collagen deposition significantly increased in the S and M groups at 60 and 90 days. The deposition of type I collagen in the C group was similar at 30, 60, and 90 days. In the S group, an increase in

the deposition of type I collagen was observed during progression of the disease, and this increase was significant at 60 and 90 days compared with the C group. In the M group, type I collagen deposition was observed at 30 days, with a greater increase at 60 days, and this parameter remained constant at 90 days. This increase was significant compared with the C group at the respective periods of evaluation (Figure 4B, 4C).

Immunohistochemical evaluation of cell proliferation in the kidney in the control, nephrectomized, and model groups

Cell proliferation was evaluated by PCNA immunohistochemistry, indicating biphasic behavior in the M group compared with the C group at the different periods of evaluation. At 30 days, an increase in cell proliferation was observed. At 60 and 90 days, a decrease in cell proliferation was observed. In the S group, no significant changes in cell proliferation were observed at 30 days compared with the C group. However, a reduction of cell proliferation was observed at 60 and 90 days compared with the C group.

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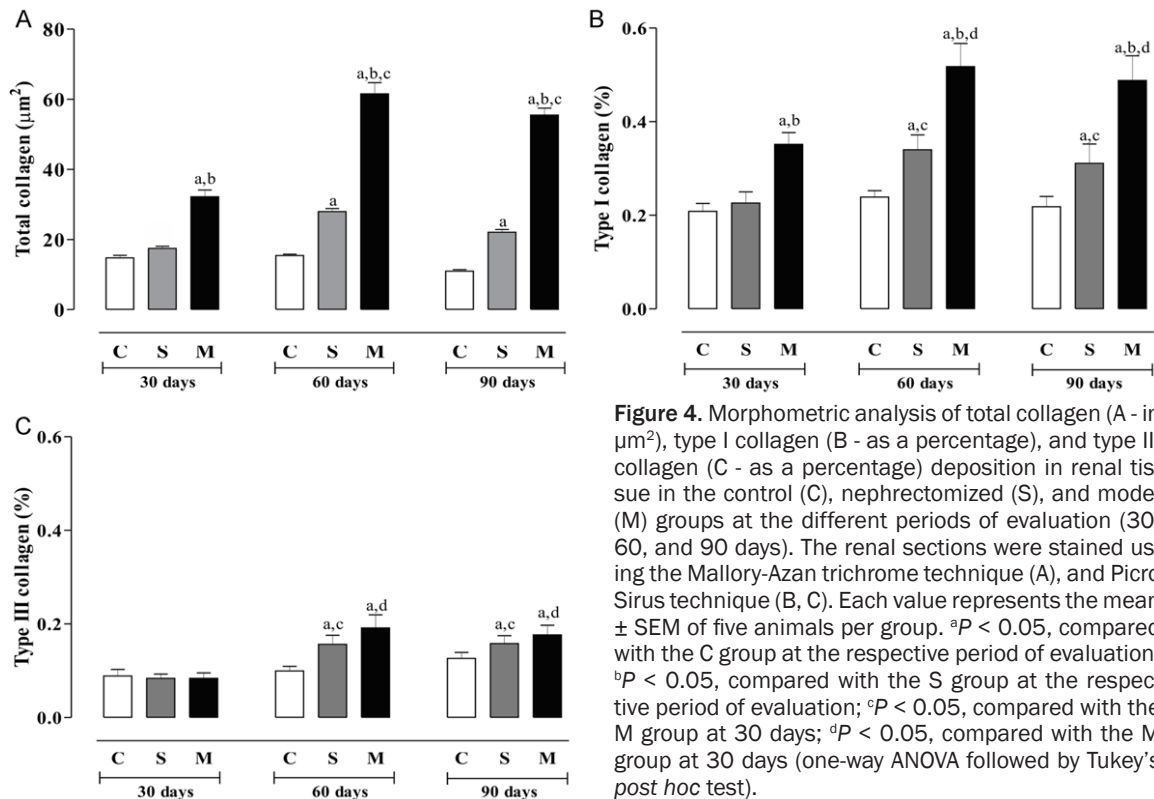


Figure 4. Morphometric analysis of total collagen (A - in μm^2), type I collagen (B - as a percentage), and type III collagen (C - as a percentage) deposition in renal tissue in the control (C), nephrectomized (S), and model (M) groups at the different periods of evaluation (30, 60, and 90 days). The renal sections were stained using the Mallory-Azan trichrome technique (A), and Picro Sirius technique (B, C). Each value represents the mean $\pm$ SEM of five animals per group. ^a $P < 0.05$, compared with the C group at the respective period of evaluation; ^b $P < 0.05$, compared with the S group at the respective period of evaluation; ^c $P < 0.05$, compared with the M group at 30 days; ^d $P < 0.05$, compared with the M group at 30 days (one-way ANOVA followed by Tukey's post hoc test).

In the C group, no significant changes in cell proliferation were observed at 30, 60, or 90 days (Figure 5).

Immunohistochemical evaluation of the angiogenic factor receptor in renal tissue in the control, nephrectomized, and model groups

Immunolabeling for VEGF receptor 2 (VEGFR) was detected in the C group at 30, 60, and 90 days. However, after the induction of renal microembolism, a decrease in VEGFR-positive cells was observed at 60 and 90 days in the M group compared with the C and S groups. The S group presented no significant differences compared with the C group at 30, 60, and 90 days (Figure 6).

Discussion

The present study demonstrated robust evidence of the occurrence of vascular, glomerular, tubular, and interstitial abnormalities in the renal tissue, and changes of biochemical parameters of renal function, in this experimental model of nephropathy induced by renal microembolism, which may indicate the development of chronic kidney disease (CKD). The possible

mechanisms involved in nephropathy induced by renal microembolism are shown in Figure 7. The reproducibility of this model is actually high, and 100% of our animals survived the study.

The present results showed that the injection of microspheres into the renal artery of the remnant kidney in nephrectomized rats (M group) caused significant biochemical, histological, and functional alterations. These changes included increases in serum Cr and Ur levels, intense proteinuria, the dilatation of Bowman's space and of capillary loops, a decrease in glomerular cellularity, tubular degeneration and necrosis, and an intense interstitial inflammatory process. With the exception of Bowman's space dilatation that occurred in a pronounced manner within 60 days after the procedure, the other changes were evident as early as 30 days, progressed at 60 days, and remained stable at 90 days. All of these changes may be associated with impairments in renal function. The process of CKD in both humans and animals is multifactorial [17, 18]. Numerous vascular, glomerular, tubular, and interstitial alterations are involved in the disease process, with

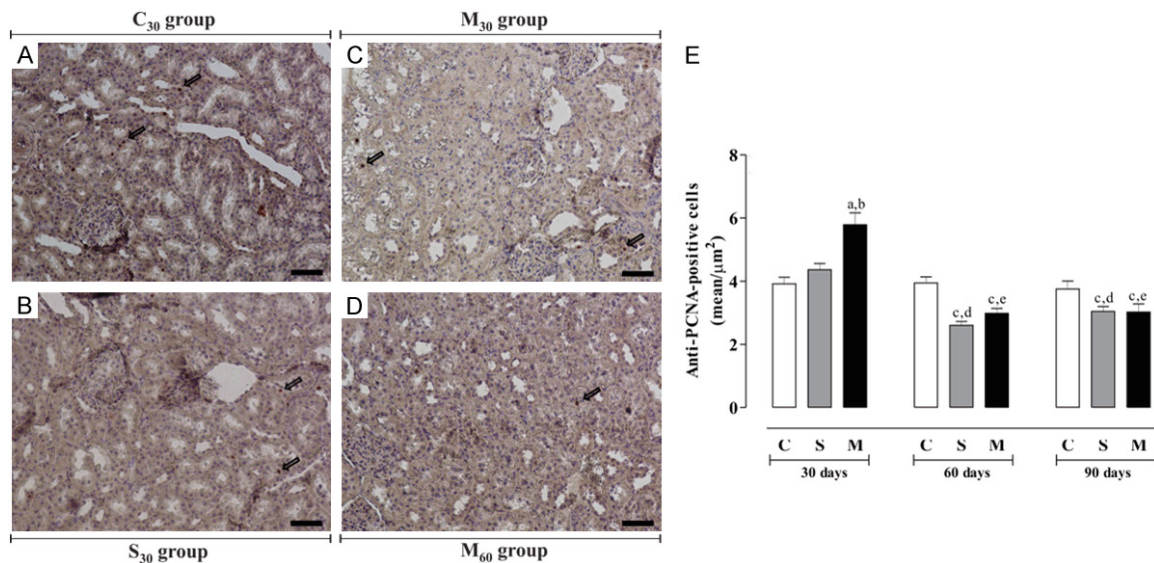


Figure 5. Representative photomicrographs of the control (C), nephrectomized (S) and model (M) groups at 30 and 60 days and quantification of the number of anti-PCNA-positive cells in renal tissue at different periods of evaluation (30, 60, and 90 days). A-C. Renal tissue photomicrographs representing the 30-day period in the C, S, and M groups, respectively. D. Renal tissue photomicrograph representing the 60-day period in the M group. E. Quantitative analysis of anti-PCNA-positive cells (mean/μm²) in the C, S, and M groups. Each value represents the mean ± SEM of five animals per group. ^a*P* < 0.05, compared with the C group at the respective period of evaluation; ^b*P* < 0.05, compared with the S group at the respective period of evaluation; ^c*P* < 0.05, compared with the C group at the respective period of evaluation; ^d*P* < 0.05, compared with the S group at 30 days; ^e*P* < 0.05, compared with the M group at 30 days (one-way ANOVA followed by Tukey's *post hoc* test). 200 × magnification.

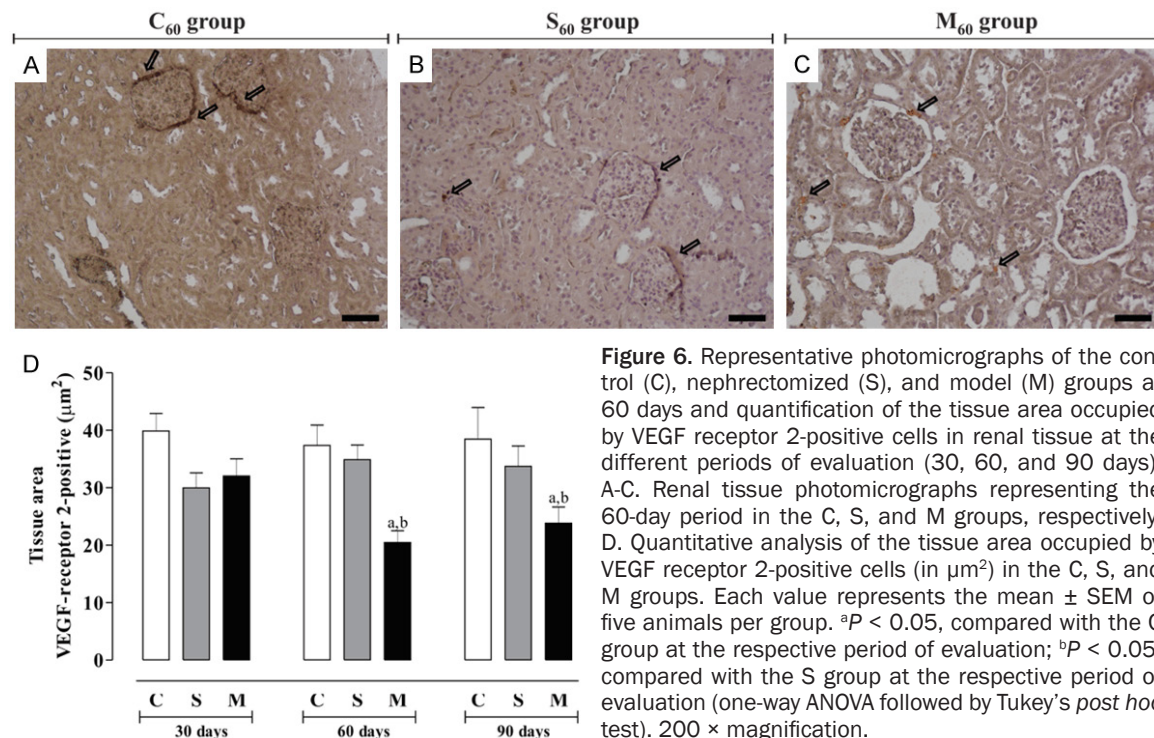


Figure 6. Representative photomicrographs of the control (C), nephrectomized (S), and model (M) groups at 60 days and quantification of the tissue area occupied by VEGF receptor 2-positive cells in renal tissue at the different periods of evaluation (30, 60, and 90 days). A-C. Renal tissue photomicrographs representing the 60-day period in the C, S, and M groups, respectively. D. Quantitative analysis of the tissue area occupied by VEGF receptor 2-positive cells (in μm²) in the C, S, and M groups. Each value represents the mean ± SEM of five animals per group. ^a*P* < 0.05, compared with the C group at the respective period of evaluation; ^b*P* < 0.05, compared with the S group at the respective period of evaluation (one-way ANOVA followed by Tukey's *post hoc* test). 200 × magnification.

the participation of mediators that are activated or released during the period of the noxious stimulus [19-21].

The mechanism by which microsphere injections cause renal injury appears to be related to the occlusion of preglomerular arterioles and

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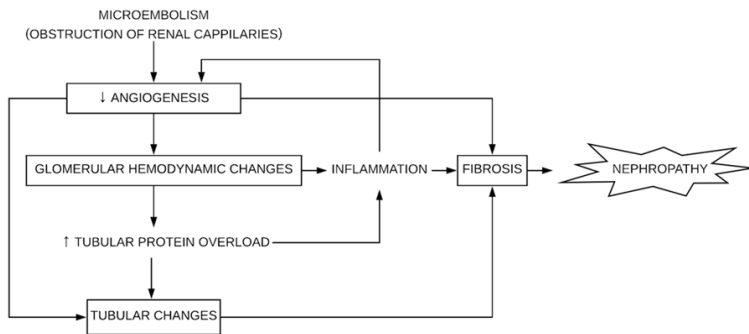


Figure 7. Possible mechanisms involved in nephropathy induced by renal atheroembolism.

intraglomerular capillaries near the vascular pole [13], which induces a sequence of pathophysiological events that may progress to more serious and irreversible kidney damage. Briefly, vessel obstruction may cause hemodynamic changes, resulting in damage to the glomerular and mesangial endothelium. This leads to the accumulation of inflammatory cells and consequently the release of inflammatory mediators and growth factors. Podocytes then become unable to fully cover the basal membrane, resulting in uncovered areas that form synchiae and consequently an elevation of proteinuria [17, 22-24]. In the present study, we considered that the elevation of proteinuria in the M group could also be explained by the decrease in the basal membrane area, detected by PAS staining. Proteinuria and increases in serum creatinine and urea levels are considered markers of renal injury, but when the proteinuria is very high and uncontrolled levels are reached, it may contribute to the progression of lesions, leading to chronic kidney disease (CKD) [17, 25-28].

Histological analyses using the HE technique demonstrated a reduction of intraglomerular cellularity, associated with an involution and/or eventual absence of glomeruli in the M group. These findings corroborate previous studies that reported the apoptosis of glomerular cells in other models of nephropathy [29, 30]. In fact, some studies reported that depending on etiology, glomerular diseases exhibit specific histopathological patterns (e.g., mesangial, membranous, or endocapillary proliferation, membrane alterations, microangiopathy, and vasculitis), leading to changes in the glomerular filtration barrier, which in turn can cause elevations of proteinuria and tubular damage. Additionally,

it can cause destruction of the corpuscular structure, inducing fibrosis [20].

At the same time that intense glomerular damage occurred in the experimental model of microembolism in the present study, tubular and interstitial alterations were observed in the renal tissue in the M group. Such changes may be a consequence of the intense overload of ultrafiltered protein that is attributable to rupture of the

glomerular membrane and that influences the capacity of the tubular reabsorption of nephrons. In the presence of intense protein overload, the tubular cell phenotype is modified by inducing the expression of various inflammatory and fibrogenic mediators. These mediators stimulate fibroblasts, myofibroblasts, and tubular epithelial cells, increasing the production of collagen (interstitial matrix) and resulting in the progression of CKD [23, 31, 32].

In the present study, the deposition of total collagen in renal tissue was evident at 30 days, suggesting a process of fibrogenesis. At 30 days, an increase in type I collagen but not type III collagen was observed. The production of type I collagen that occurs during fibrogenesis can cause a rupture of the basal membrane [33], which appeared to be confirmed in the present study by the decrease in the tubular basal membrane area, revealed by the PAS technique. Additionally, other studies have shown that when epithelial tubular cells lose contact with the basal membrane, they can be converted into mesenchymal cells, which increase collagen synthesis and then subsequently evolve into fibrosis [33]. The present study corroborated these findings. We observed an increase in collagen deposition at 60 and 90 days in the M group, revealed by the Mallory-Azan and Picro-Sirus techniques.

PCNA immunohistochemistry also revealed the intense proliferation of tubular cells at 30 days, which can be a response to phenotypic changes in tubular cells to maintain the population of these cells [34]. Nevertheless, such cell proliferation did not continue to the 60- and 90-day periods of evaluation. Reduction of these cells occurring at 60 and 90 days may indicate the

development of tubular necrosis and the progression of CKD.

The present experimental model resulted in glomerular, tubular, and interstitial changes. These findings led us to speculate on the probable trigger of the development of CKD in this model. One possibility is that vessel obstruction that is caused by microembolism causes hemodynamic changes by reducing the density and/or size of glomerular and peritubular capillaries. This possibility may be clinically important because maintenance of the microvasculature plays a key role in maintaining renal function [35-37]. In the present study, we observed a reduction of the tissue expression of VEGFR, thus inferring a decrease in the number of renal capillaries. Some authors reported an initial vascular proliferation response during the development of renal damage, but such vascular proliferation was not sustained, and a subsequent decrease in proliferation was observed [38-40]. This can be understood as a consequence of the increase in antiangiogenic factors and/or a decrease in angiogenic factors [41, 42]. This possibility was also evidenced in the present study.

The inclusion of a group of nephrectomized rats that did not receive an injection of microspheres (S group) was very important because it demonstrated a difference in the evolution of the disease. In the S group, we observed some biochemical and histological alterations that may be attributable to the physiological compensation process [43, 44]. The fact that collagen deposition has also been evidenced in the nephrectomized group does not reflect loss of function. Notably, the ratio of type I to type III collagen was more pronounced in the M group. Cheng et al. [45] demonstrated that the excessive secretion of type I collagen can result in a disorganized structure of fibers that form hypertrophic wounds on the skin. Another study found that the increase in the type I/type III collagen ratio correlated with hemodynamic changes in the left ventricle and right ventricular hypertrophy in a coronary ligation heart failure model [46]. Importantly, two fundamental factors-proteinuria and the angiogenic factor-in the development of CKD were unaltered in the group of nephrectomized rats (S group), which may reinforce their participation in the development of CKD in the M group.

In conclusion, the present study found robust evidence of the occurrence of vascular, glomerular, tubular, and interstitial abnormalities, and changes in the biochemical parameters of renal function, in the experimental model of renal microembolism. Early vascular impairment induced by renal microembolism may be an important trigger stage for the progression of CKD in this experimental model. Additionally, the findings indicate that this is a good reproducibility model that may be useful for studying the pathogenesis of CKD that is caused by atheroembolism and possible treatment alternatives.

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Disclosure of conflict of interest

None.

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