Impact of gender in early structural changes of contrast induced nephropathy in rats

Impacto do gênero em alterações estruturais precoces da nefropatia induzida por contraste em ratos

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Abstract

Introduction: Contrast-induced nephropathy (CIN) is a major iatrogenic cause of acute kidney injury. Experimental studies have shown that intravascular injection causes intense vacuolization of the contrast agent in the proximal renal tubules cells, preceding the increase in serum creatinine, and that the female may be at a higher risk for CIN. **Objective:** To study the early kidney histomorphometric changes in contrast-induced nephropathy according to the gender. **Methods:** Twenty previously uninephrectomized Wistar rats were divided into 4 groups (n = 5): control males; control females; contrast exposed males; and contrast exposed females. The animals were sacrificed immediately after contrast administration and kidney tissue samples were collected for histomorphometric analysis. The research project was approved by the Research Ethics Committee of the School of Medicine of Universidade Federal Fluminense. **Results:** There was a more intense presence of microvacuoles in proximal tubules in the rats exposed to contrast than in the control groups. Such proximal tubular vacuolation was more intensive in the female rats (p = 0.001). **Conclusion:** Proximal tubular vacuolation is a very early change in CIN and is more intensive in female than in male rats.

Keywords: acute kidney injury; contrast media; risk factors.

Resumo

Introdução: A nefropatia induzida por contraste (NIC) é uma das principais causas iatrogênicas de lesão renal aguda. Estudos experimentais têm demonstrado que a injeção intravascular do agente de contraste provoca vacuolização intensa nas células dos túbulos renais proximais, que precede o aumento da creatinina sérica, e que a fêmea podem estar em maior risco de CIN. **Objetivo:** Estudar as primeiras mudanças histomorfométricas renais na nefropatia induzida por contraste de acordo com o gênero. **Métodos:** Vinte ratos Wistar anteriormente uninefrectomizados foram divididos em 4 grupos (n = 5): machos de controle; fêmeas de controle; machos expostos ao contraste e fêmeas expostas ao contraste. Os animais foram sacrificados imediatamente após a administração de contraste e amostras de tecido de rim foram coletadas para análise histomorfométrica. O projeto de pesquisa foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Medicina da Universidade Federal Fluminense. **Resultados:** Houve presença mais intensa de microvacuolização em túbulos proximais nos ratos expostos ao contraste do que nos grupos de controle. Tal vacuolização tubular proximal foi mais intensa nos ratos do sexo feminino (p = 0,001). **Conclusão:** Vacuolização do tpubulo proximal é uma mudança precoce na CIN e é mais intensa em ratos fêmeas do que em ratos machos.

Palavras-chave: fatores de risco; lesão renal aguda; meios de contraste.

Introduction

Contrasts are substances that have a great capacity to absorb the X-ray and thus enable the visualization of structures that normally cannot be seen in regular X-rays scans.[1] One of the undesirable consequences with the use of contrast media is contrast-induced nephropathy (CIN) with an incidence of less than 5% in unselected populations and > 20% in high-risk populations.[2]

This is an iatrogenic cause of acute renal failure that has been the subject

of numerous clinical and experimental studies with aims to better understand its pathophysiological mechanisms and the search for alternatives that may prevent it.[2]

The histopathological findings in CIN are restricted to the proximal tubule, having as characteristic the intense vacuolization of epithelial cells.[3,4] These changes are reversible after a few days of the use of the contrast agent.[5] The origin of these vacuoles may be due to the contrast-induced diuresis reduction, with a consequent increase in urine concentration.[6]

With high viscosity, which occurs especially with the use of isosmolar dimeric contrast media, there may be slow intratubular flow and longer time of cellular exposure to these molecules.[7] No damage occurs to vital cell organelles, which remain intact.[4]

Several strategies have been proposed to avoid or mitigate the occurrence of CIN. Although hydration with sodium chloride or sodium bicarbonate is the only measure of unquestionable protection, dopamine,[8] adenosine antagonists,[9] endothelin antagonists,[10] prostaglandins,[11] dopamine selective agonists[12,13] and antioxidants such as n-acetyl-cysteine[14] have been tested, with conflicting results.

Wang *et al*.[15] have experimentally demonstrated nephroprotection with Magnolin, the main active component of the Magnolia fargesii herb, which has anti-inflammatory and antioxidant effects. Renalase, a recently discovered monoamine oxidase enzyme in the kidney, has been shown to protect against CIN through anti-inflammatory, anti-apoptosis and anti-inflammatory mechanisms, and appears to be a promising therapeutic intervention to prevent CIN.[16]

Some authors have suggested that females may be at a higher risk for CIN.[17,18] Often, experimental studies with radiological contrasts are performed in male animals, although histochemical, autoradiographic, and ultrastructural studies demonstrate gender-related differences in the kidneys of rats and other rodents.[19]

Schiebler and Danner[20] reported on significant lysosomal differences in the three segments of the proximal tubules, especially in S2 and S3, comparing male and female animals. It is possible that differences in these segments may confer variations in renal sensitivity to contrasts. This study aimed to evaluate the early renal histomorphometric changes of CIN in male and female Wistar rats.

Material and methods

The experimental procedures were carried out according to vivisection standards of animals described by the Brazilian College of Animal Experimentation (COBEA). The research project was approved by the Research Ethics Committee of the School of Medicine of the Fluminence Federal University.

Adult male Wistar rats, albino variant, males and females, weighing between 200 and 250 g, were housed in a refrigerated room with 12 hours of light, constant temperature (22 ± 2 ° C) and relative humidity. The animals were distributed according to the treatment groups, with five animals of the same gender by a plastic bottom cage with a sterilized wood-shavings bed and receiving water and standardized feed (Nuvital®, Nutrientes SA) ad libitum. To make the animals sensitive to nephrotoxicity by contrast, we chose unilateral nephrectomy and water deprivation.[4,21]

After remaining in the adaptation period for two weeks, they were submitted to left nephrectomy under anesthesia with Ketamine 50 mg/kg of body weight and Xylazine 8 mg/kg per body weight intramuscularly. On the fifteenth day after nephrectomy, the animals were submitted to water deprivation for 12 hours and received the drugs according to the following groups, all of them consisting of five animals: males receiving saline (G1); females receiving saline (G2); males receiving diatrizoate (G3); females receiving diatrizoate (G4).

Hyperosmolar ionic contrast, sodium diatrizoate/meglumine (Urografina® Schering, Rio de Janeiro Brazil) was injected into the caudal vein at a dose of 1.9 ml/kg (2.9 g iodine/kg). Immediately afterwards, the animals were slaughtered with the use of intracardiac ketamine injection at the dose of 80 mg/kg and autopsied by opening the abdominal and thoracic cavities. Renal tissue samples were obtained for study under light microscopy.

The samples of renal cortical tissue were fixed in DuBosq Brazil (Bouin alcoholic solution), processed according to the histological routine[22] for light microscopy for preparation of paraffin blocks and obtaining 3-micron thick tissue sections. The staining used for analysis was hematoxylin-eosin (HE).

The histological sections of the kidneys of the experimental groups described, stained by HE, were examined under a Nikon Eclipse E400 light

microscope, using a 20 x 0.40 acroplan objective oblique condenser, with the objective of finding areas of cortical tissue for later morphometric analysis of the CIN proximal tubular lesion, characterized by vacuoles in the lining epithelium. By capturing the areas selected by the Evolution MP 5.0 camera coupled to the computer, images were obtained for this analysis.

The morphometric study was performed using the Image Pro-Plus 4.1 (Media Cybernetic, Silver Spring, USA) image analysis software, selecting "Manual Point Count". From each animal of the groups selected for this evaluation, a total of 100 tubules were counted in cross-sectional tubular sections with a basal tubular membrane outline visible around the structure. Any cross-sections that did not meet these criteria were taken off. Of these 100 tubules with the marking determined in the system, the vacuoles were also marked and counted.

Statistical analysis of the histopathological findings was performed using the Two-way Analysis of Variance (two-way ANOVA) technique: treatment and gender. The Shapiro-Wilk test assessed the normality of the data in the groups formed by the levels of the two factors and the homogeneity of the variances was evaluated by the Levene test.

Multiple comparisons (pairwise comparisons) to identify differences indicated by ANOVA were performed by the Least Significant Difference (LSD) test. Statistical decisions were taken at a significance level of 0.05 (5%).

Results

The animals tolerated well the treatment and all survived until the last stage of the experiment. Renal tissue samples obtained after contrast injection showed a large presence of vacuoles in the proximal tubules (Figures 1 to 4). The two-way ANOVA showed statistically significant differences as a function of treatment: diatrizoate and saline ($p < 0.0001$), gender: higher in females ($p < 0.0001$) and interaction between these two factors ($p = 0.003$).

The animals that received diatrizoate, regardless of gender, had a significantly higher number of vacuoles per tubule (11.32 ± 5.09) than animals receiving saline (3.78 ± 1.42), $p < 0.0001$. The females that received diatrizoate showed a higher mean number of tubule vacuoles (15.54 ± 1.48) than males (7.11 ± 3.41), $p < 0.0001$ (Figure 5). Regarding saline solution, males and females showed no statistically significant difference (2:54 ± 0,40 *versus* 5,03 ± 0,69), $p = 0,055$ (Figure 5).

Figure 1. Optical microscopy showing vacuoles in proximal tubules of male rats after diatrizoate (HE, 20x).

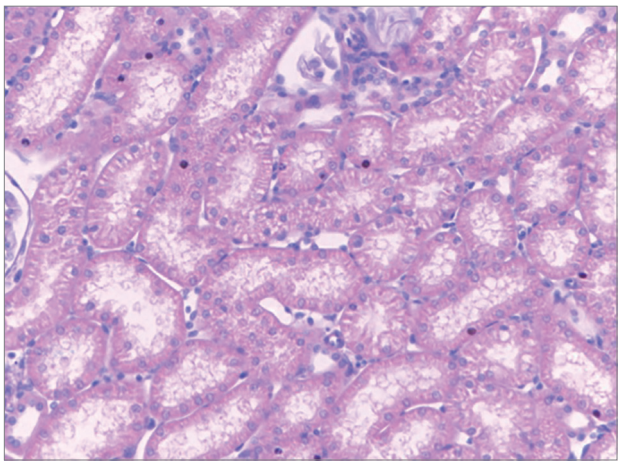


Figure 2. Optical microscopy showing vacuoles in proximal tubules of female rats after diatrizoate (HE, 20x).

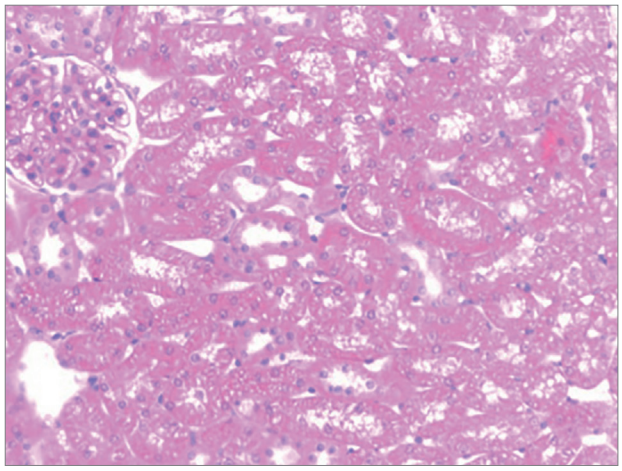


Figure 3. Optical microscopy showing vacuoles in proximal tubules of male rats after saline administration (HE, 20x).

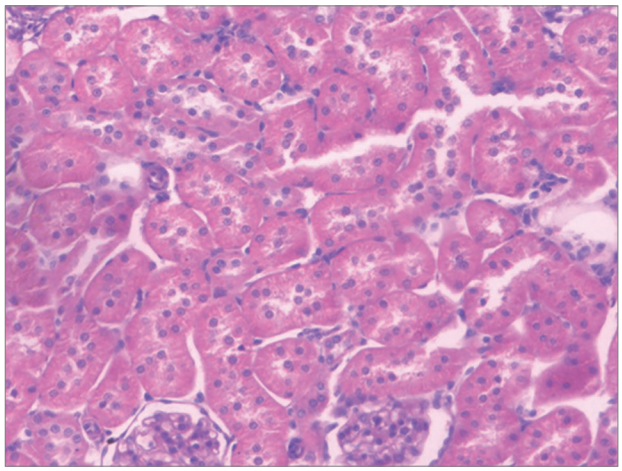


Figure 4. Optical microscopy showing vacuoles in proximal tubules of female rats after saline administration (HE, 20x).

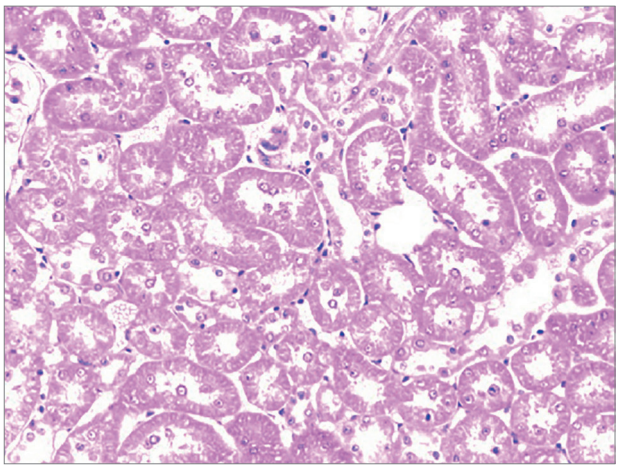
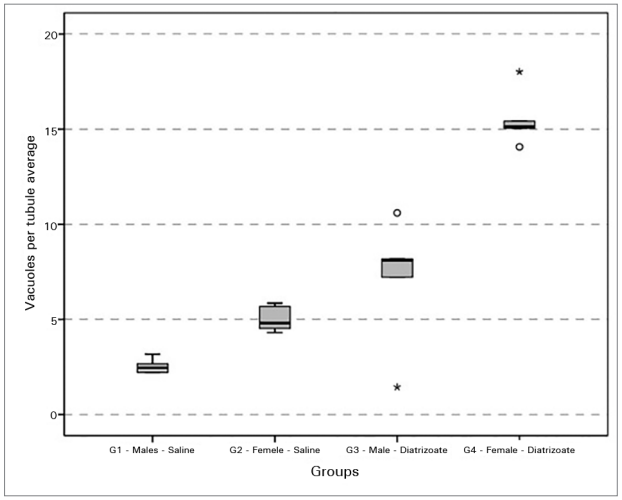


Figure 5. Distribution of the average number of tubule vacuoles, according to the treatment and sex.



Discussion

The clinical indicator of renal impairment due to exposure to contrast media is serum creatinine, which supports the definition of CIN.[2] Experimental studies have demonstrated that intravascular injection of the contrast agent causes intense vacuolization in the proximal tubules preceding creatinine rise.[23]

Under light microscopy, all animals showed intense vacuolization of the proximal tubular cells, in full agreement with the literature findings, which consider proximal tubular vacuolization as a characteristic of contrast exposure.[5,21]

The animals that received diatrizoate showed a mean number of tubule vacuoles three times higher than the mean number found in the saline group. There was a statistically significant difference in the number of vacuoles in the comparison between genders, with females presenting 50% more vacuolization than males.

Normal animals are resistant to the nephrotoxicity caused by contrast media.[23] Exposure to multiple factors of renal aggression, such as single-side nephrectomy, salt depletion, cyclooxygenase inhibitors and induced renal ischemia, sensitizes these animals and enables them to be used as good experimental models of CIN.[23]

In our study, tubular epithelial vacuolization occurred in all animals receiving diatrizoate, confirming that this protocol managed to induce contrast nephrotoxicity. Moreau *et al.*[6] analyzed kidney biopsy material from 211 patients, 10 days after they underwent venous urography or renal arteriography. They found vacuolization of the proximal tubular cells in 47 cases.[6] They reported that there was no necessary correlation between the so-called "osmotic nephrosis" and renal functional decline, which was confirmed in a new study 5 years later.[6,8]

The appearance of the vacuoles can be experimentally demonstrated 5 to 15 minutes after exposure to the contrasts.[21] Tervahartiala *et al.*[24] and Cağlar *et al.*[7] showed tubular vacuolization after two hours of exposure to contrast, in a protocol quite similar to the one presented here. In addition, tubular vacuolation is reported to be dose dependent.[6,25] Batternfeld *et al.*[26] observed that the process of vacuolation induced by high doses of ioxaglate alone is not sufficient to cause renal failure. Other experiments with Wistar rats, not subjected to the various predisposing renal injury procedures, required the use of higher doses of iodinated contrast media, at least twice the amount used in our study, to successfully induce nephropathy.

Although hyperosmolar contrasts are associated with a higher incidence of CIN,[25] Tervahartiala *et al.*[24] reported a lower degree of vacuolization with diatrizoate - a compound of high osmolarity, compared to iotrolan, an isosmolar compound. It is possible that this finding is linked to the other physicochemical properties of the contrasts, especially viscosity, higher in the iso-molecular compounds.[25] Our findings suggest that lysosomal changes are not explicitly dependent on osmolarity of the contrast media.

Iakovou *et al.*,[17] in a prospective study involving 8,628 patients undergoing percutaneous interventions

using contrast, concluded that the female gender is an independent predictor of CIN.[7]

Another study found a higher incidence of CIN in women after percutaneous coronary intervention, but attributed this fact mainly to less favorable baseline characteristics, including lower glomerular filtration rate and a higher incidence of hypertension.[18] Gill *et al*.[27] observed that females may be a risk factor for CIN and that women would have less protection with hydration.

However, a more recent study failed to demonstrate that gender, hypertension or *diabetes mellitus*, cardiac output, and type and amount of contrast medium are significant risk factors for CIN in patients undergoing cardiac catheterization.[28]

In the present experimental study, although we limited ourselves to early evaluation and only histomorphometric aspects, females receiving diatrizoate exhibited significantly more tubule vacuoles than males. The contorted part of the proximal tubules plays an important role in the reabsorption of glomerular filtrate proteins, which are transferred to the lysosomes, where they are degraded and returned to the bloodstream.[29]

Silverblatt and Kuehn[30] demonstrated that lysosomes are the major intracellular organelles for sequestration of gentamycin after uptake by proximal tubular cells. Foreign and nondigestible agents may lead to exhaustion, with consequent changes and enlargements of the lysosome.[26]

Male and female rat kidneys have free binding sites for steroid hormones, and proximal tubule cells have an affinity for estradiol in females and for testosterone in males.[19] The administration of estrogen causes tubular changes, especially lysosomal, in rats[31] and adenocarcinoma of proximal tubules in hamsters.[31]

These observations are, in our view, of particular interest, since all contrast agents induce lysosomal changes in the proximal tubule cells, and could explain our findings of greater vacuolization in females.[24]

The present experimental study confirms the precocity of renal tubular structural changes after administration of a venous contrast medium, and demonstrates a greater intensity of histological renal involvement in females.

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