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Influence of Oxidative Damage on Lithogenesis in Experimental Nephrolithiasis

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Abstract

To assess the impact of oxidative damage to the kidney structure and the severity of lithogenesis, a morphological study of rat kidneys was conducted with experimental oxalate nephrolithiasis. Structural changes were assessed in the kidney medulla, particularly the distribution and size of the calcium compounds. Using immunohistochemistry, the expression of the severity indices of oxidative damage (malondialdehyde) and antioxidant defenses (mitochondrial superoxide dismutase) were determined. On the model of experimental oxalate nephrolithiasis in the rat kidney at the level of light-optical reconstruction, signs of histopathological alterations were revealed in the organ, along with the presence of calcium compounds in the kidney tubular system and interstitial cells. Also, the morphological symptoms of activation of the oxidative damage to the tissues and cells, reducing the antioxidant protective mechanisms of the enzymes was noted. Usage of α-tocopherol revealed a definite reduction in the severity, in terms of the structural adjustment to renal oxidative damage of the tissues and cells, and preservation of the activity of the antioxidant defense system, as well as a reduction in the number and size of the calcium deposits formed. IJBM 2011; 1(4):228-230.

Key words: *experimental oxalate nephrolithiasis, morphology of kidney, free radical oxidation, antioxidants.*

Introduction

Damaging of the cells and tissues plays the important pathogenetic role in the development of oxalate nephrolithiasis. The damage of the urothelium promotes to formation of the initial focus of the lithogenesis in the Randall's plaque in the interstitium adjacent to thin loops of Henle, followed by penetration into the lumen of the collecting tubules, where the final formation of the stones occurs. Damage to the epithelial tubules of the kidney during oxalate nephrolithiasis is directly related to the activation process of free radical oxidation (FRO) in the kidney [1]. Similar results were obtained earlier in our laboratory. During the simulation of experimental nephrolithiasis was found the characteristic signs of oxidative stress in the kidney with the deposition of calcium-positive deposits in the region of the renal papilla [2].

Aim of the study was to assess the effects of oxidative damage in the process of lithogenesis during experimental oxalate nephrolithiasis.

Material and Methods

The experimental model of oxalate nephrolithiasis was performed on 60 certified male Wistar rats weighing 180 to 250 g.

The rats were divided into three groups of 20 each. Rats of the first group were on a general vivarium diet and received a drinking tap water. Urolithiasis was not initiated. This group was used as the control group. The second group of rats was on placed on standard diet with 1% solution of ethylene glycol given as a drink for 21 days, which induces the development of an experimental model of oxalate nephrolithiasis [3]. In the third group of animals, experimental nephrolithiasis was modeled in three weeks. Over the next three weeks the ongoing reception of

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ethylene glycol, the animals received food with α-tocopherol 300 mg/kg. For histological studies the animals were decapitated by cervical vertebrae dislocation, under ether anesthesia, according to the requirements of the European Convention "On Protection of Vertebrate Animals used for experimental or other scientific purposes" (Strasbourg, 1986), and the Federal Law of 01.01.1997, "On protection of animals from cruelty treatment". The research material used was the rat kidney. The organs were fixed in 10% formalin solution, processed by standard methods and embedded in paraffin. Cross sections (through the renal papilla) of 6 mm thickness were stained using hematoxylin and eosin.

To identify the calcium compounds deposited, silver impregnation by von Kossa's method was used. 0.1% hydrochloric acid solution was used to monitor the reaction. [4]. The nature and distribution of the calcium deposits, their average size, and particularly their location in the kidney tissue were evaluated.

To determine the expression of mitochondrial superoxide dismutase (SOD-2) and malondialdehyde (MDA) a two-step indirect streptavidin-biotin method was conducted with control of the specificity reaction. After the standard procedure of de-waxing and rehydration, endogenous peroxidase blocking was performed in accordance with the recommendations of manufacturer's antibody (Santa Cruz, USA).

Restoring antigen specificity was performed by pretreatment of the slices, immersed in citrate buffer (pH 6.0) in a microwave oven at 600 W thrice for 7 minutes [5]. As the primary antibodies used were antibodies to SOD-2 (G-20: sc-18 504, 1:100) and antibodies to MDA (F-25: sc-130 087, 1:30) from Santa Cruz (USA). The reaction product was visualized with the help of Goat ABC Staining system: sc-2023 (Santa Cruz) and diaminobenzidine (DAB).

Morphometric studies were performed using the

$$E\% = 100 - \frac{100 \times D_x}{256}$$

software package ImageJ 1.43 and AxioVision 3.4LE. The intensity of the expression (in points as 1+, 2+, 3+) was assessed by the intensity of the DAB staining. For convenience, interpretation of the results of $\overline{X}$ t h e expected intensity of expression was given by the formula:

where *E%* is the intensity of expression, *256* is the maximum color intensity and D_x is the intensity of DAB staining.

The results are given as values of ±SD. Statistical processing of the data was performed using the R package version 2.12 (License GNU General Public License) for Microsoft Windows®.

Results

The animals in the control group had a normal histological structure of the kidney cortex and medulla. The size of the lumen of the collecting tubules averaged 15.5±0.53 μm. Calcium deposits in the intact rat group were not histochemically verified.

The immunohistochemical study showed moderate (2+) expression of SOD-2 in the cytoplasm of the epithelial cells of the tubules of the nephrons and the epitheliocytes of the collecting tubules. The MDA expression was mild (1+).

Three weeks later of the modeling of oxalate nephrolithiasis in the kidney, the degenerative changes of the epithelium of the tubules and collecting tubules as hydropic degeneration, enlargement of the lumen of the collecting tubules (19.3±0.41μm), desquamated epithelium and protein deposits in the lumen of the collecting tubules were observed. In the epithelium of the tubules and collecting tubules, in the interstitium of the medulla substance, in the lumen of collecting tubules in the protein cylinders numerous deposits of calcium compounds were found (mean 21.4±3.40 in the field of view). The localization of the calcium compounds is characteristically found in the base and the middle third of the renal papilla. The average size of the deposits was observed to be 16.5±0.60 mm. In 10% of the cases, relatively large microlites (up to 30-35 μm) were found with obturation of the lumen of the collecting tubules and their epithelium was encrusted with calcium compounds. In the areas of calcium deposits a proliferation of connective tissue with the formation of peritubular and perivascular fibrosis was detected.

Immunohistochemistry assay showed decreased expression (1+) of SOD - 2 in epitheliocytes of collecting tubules. In the inner zone of the medulla substance, this number was significantly lower (5.3% less) of the values of intact kidneys. In the epitheliocytes of the collecting tubules, obturation by stone, a decreased expression of SOD-2 and reached a maximum that was 7.5% lower than the benchmarks. The weakening expression of the antioxidant enzyme was accompanied by a statistically significant elevation of lipid peroxidation products (LPP), as noted in the tubules of the nephron epitheliocytes, collecting tubules, transitional epithelium pyelocaliceal system and interstitial cells (2+).

With using of α-tocopherol in the experiment, a much smaller intensity of histopathological kidney restructuring was determined. In the epitheliocytes of the collecting tubules of the cortex and medulla, signs of hyaline droplet degeneration were seen. The lumen of the collecting tubules were characterized by relative uniformity in the various fields of view, averaging 16.40±1.56 mm. Single desquamated epithelial cells and protein cylinders were identified In the lumen of some collecting tubules. The moderate amount (up to 17.6±2.39 in the field of view) of calcium compounds, which were located relatively uniformly across the renal papilla area, mainly in the epithelium of the collecting tubules and among desquamated epithelial cells were identify in the renal medulla. Calcium deposits were small, averaging 5.40±0.28 mm in size. Large compounds of calcium, obturation clearance tubules and collecting tubules, or inlays their epithelium were not detected.

Immunohistochemical study of the rat kidney during treatment with α-tocopherol showed moderate (2+) expression of the SOD-2 in epitheliocytes of the collecting tubules, comparable to the intact group and significantly (12.5%) higher than in animals with an experimental model oxalate nephrolithiasis.

Reduction of LPP (1+) was detected in the animals

during the blocking processes of oxidative damage with using α-tocopherol. The intensity of the expression of MDA was similar to that in the intact group and significantly lower than in animals with the experimental oxalate nephrolithiasis.

Discussion

Modern literature indicates that tissue damage in the kidneys is an important factor in the formation of urinary stones. Generally, it is accepted that the deposits of calcium salts are capable of inducing tissue reactions in the epithelium of the distal tubules and collecting tubules, particularly activating the process of free radical oxidation. In the kidney, the initial deposition of calcium occurs in the mitochondria and phagolysosomes, which are highly active phosphatases. In the interstitium, calcium salts get precipitated along membranes of blood vessels and fibrous structures. Multiple data indicate a constant oxidative stress, that significantly contributing to the formation of calcium deposits [1].

A morphological study of rat kidneys was conducted using the ethylene glycol oxalate nephrolithiasis model, which revealed the nature of the distribution of calcium deposits in the kidney medulla and the associated histological features of the restructuring kidney tissue, as well as the severity of oxidative damage to the tissues. Pathomorphological changes in the form of epithelial degeneration, its peeling, or the expansion of the lumen of the tubules and collecting tubules were identified as possibly being due to the damaging effects of the ethylene glycol, and the reaction to the deposition of calcium deposits. Such a restructuring indirectly indicates the presence of local conditions for the development of nephrolithiasis. This was histochemically confirmed by the detection of calcium deposits in the kidney tissue. These facts correspond to published data, according to which the aggregates of calcium crystals get initially fixed at the apical membranes of the epithelial cells and then are transported to the interstitium and are concentrated mainly on the surface of the renal papilla, where it subsequently forms stones [6].

In the field of active lithogenesis a decrease in the intensity of expression of the mitochondrial SOD was recorded, indicating a possible depletion of the antioxidant defense system enzymes. Also, the possibility of reducing the expression of mitochondrial antioxidant enzymes caused by degenerative changes in the epithelial cells near the larger stones cannot be excluded. The observed increase in the expression of MDA in the kidney tissues indicates the activation process of free radical oxidation and the weakening of antioxidant defenses in the early stages of modeling the experimental oxalate nephrolithiasis. These impairments have a deleterious effecton kidney tissue, stimulating the process of lithogenesis.

In blocking the process of oxidative damage by using α-tocopherol expression of the mitochondrial SOD and MDA matches to performance of intact groups in the whole. Also, a lesser degree of severity of renal structural adjustment was observed compared with the group of animals with nephrolithiasis. Although the tubular system of the kidney showed signs of hyaline droplet degeneration, the lumen of the collecting tubules was only slightly enlarged and the renal papilla within were characterized by relative uniformity. Against this background, a significant slowdown in lithogenesis was noted. Although the amount of calcium deposits in the field of view were not as reduced as compared with the experimental group of animals, their size during treatment with antioxidants decreased by more than three times. In the animals, in the light of the blocking processes of oxidative damage by using tocopherol expression of the mitochondrial SOD and MDA matches to performance of intact groups in the whole. The data obtained are consistent with the results of biochemical studies conducted earlier, in which a three-week consumption of a 1% solution of ethylene glycol by rats resulted in an increase in the concentration of LPP products in the 1.8 times [2].

Conclusion

During the simulation of the experimental oxalate nephrolithiasis in the rat kidney, marked morphological signs of activation of oxidative damage to the tissues and cells and a weakening of the enzymatic antioxidant defense system, accompanied by an acceleration lithogenesis were noted. The usage of antioxidants has beneficial effects on the renal morphologic reorganization in animals with induced nephrolithiasis, as it reduces the degree of oxidative damage to the cells and tissues, while it helps to reduce the number and size of the calcium deposits formed.

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