Role of Individual Structural Features of Rabbit Kidneys in the Predisposition to Gentamicin Nephrotoxicity

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A relationship between congenital features in the structure of rabbit kidneys and sensitivity to gentamicin nephrotoxicity was revealed. Gentamicin nephrotoxicity is more pronounced in rabbits with lower diameter of nephron convoluted tubules and with smaller cells lining their lumen.

Key Words: *rabbits; gentamicin; nephrotoxicity; predisposition*

Different individual sensitivity of animal and human kidneys to gentamicin toxicity is well known [5,6]. However, the mechanisms determining predisposition of humans and animals to gentamicin nephrotoxicity remain unknown.

We propose a model for detecting the morphological markers of animal hypersensitivity to nephrotoxic effect of gentamicin.

MATERIALS AND METHODS

Experiments were carried out on 18 female rabbits with the initial weight of 2.5-3.5 kg. Right-side nephrectomy was carried out under sterile conditions and general anesthesia (diethyl ether). The structure of the cortical (CN) and juxtamedullary (JN) nephrons of the removed kidney was examined: tubule diameter and height of cells lining the tubular lumen. A "Morphological Passport" was charted for the kidney of each rabbit on the basis of these results.

Gentamicin (60 mg/kg daily, intramuscularly) was started 5 months after surgery. The severity of nephrotoxic effect of gentamicin was evaluated using morphological (kidney: hematoxylin and eosin staining), biochemical, and clinical (urine, blood) methods.

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Proteins, leukocyte, erythrocytes, and epitheliocytes were measured in the urine collected under sterile conditions from the bladder of sacrificed rabbits. Blood urea was measured by the urease test with Abbott reagents, creatinine by the Jaffe kinetic method with Hospitex Diagnostics reagents, and the content of medium-weight molecules was estimated [3].

The relationships between specific features in the nephron structure before gentamicin treatment and the type and severity of damage to the kidney produced by antibiotic were evaluated by analysis of correlations, staged multifactorial regression, dispersion, and canonical analyses.

RESULTS

Morphological studies showed variability in the structure of CN and JN in intact rabbits (Table 1).

By the end of gentamicin intoxication urinary protein content was 0.11-2.60 g/liter, erythrocyte count was 2-50 in visual field, epitheliocyte count 1-6 in visual field. Blood values also varied within a wide range: urea content was 3.83-23.80 mmol/liter, creatinine 0.08-0.71 mmol/liter, medium-molecular-weight molecules 0.18-0.52 mmol/liter. These data indicate gentamicin damage to the rabbit kidneys and pronounced individual variability of the nephrotoxic effect of this drug.

Analysis of correlations showed no relationship between urinary levels of proteins, leukocytes, ery-

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throcytes, and epithelial cells (after gentamicin intoxication) and structural features of the rabbit nephrons (before intoxication) (Table 1).

Pronounced hyperammoniemia after gentamicin intoxication was recorded in the population of rabbits with little diameters of CN and JN proximal convoluted tubules (PCT), JN distal convoluted tubules (DCT), and the minimum size of epithelial cells lining the CN and JN PCT (Table 1). The highest blood content of creatinine after antibiotic intoxication was detected in animals with small epithelial cells lining the CN and JN PCT, JN distal straight tubule, and with little diameter of JN DCT (Table 1).

The content of medium-molecular-weight molecules in the blood after antibiotic intoxication was maximum in rabbits with initially small epithelial cells lining CN PCT and with small DCT diameter in CN and JN (Table 1).

The content of urea in the blood after gentamicin intoxication was maximum in animals with the following structural features of the nephrons: little diameter of CN PCT in combination with small height of cells lining its lumen; little diameter of JN PCT; little height of cells lining JN PCT and little diameter of JN DCT. These relationships are described by equations of linear and multiple linear regression (Table 2).

Gentamicin-induced hyperammoniemia is associated with small height of cells lining the CN PCT lumen in combination with little diameter of JN PCT, small height of epithelial cells lining its lumen, and little diameter of JN DCT. These relationships are described by the multiple linear and linear regression equations (Table 2). The maximum content of urea in the blood after gentamicin intoxication was recorded in rabbits with initially little diameter of JN PCT in combination with small height of cells lining its lumen and little diameter of JN DCT (multiple linear regression equations).

High level of blood urea after gentamicin intoxication is also observed in animals with initially small cells lining the JN PCT in combination with little diameter of JN DCT (multiple linear regression equation; Table 2).

High blood level of creatinine by the end of antibiotic intoxication was observed in rabbits with initially small epithelial cells lining the CN PCT lumen, in combination with small JN PCT cells, little diameter of JN DCT or small size of JN distal straight tubules (multiple linear and linear regression equations; Table 2). High level of blood creatinine by the end of gentamicin intoxication was associated with initially small size of JN PCT cells in combination with little diameter of these DCT and small size of JN distal straight tubule cells (multiple linear regression equations).

High creatinine level in the blood after gentamicin treatment was observed in animals with initially little

TABLE 1. Coefficients of Correlation between Renal Morphology before Gentamicin Intoxication and Its Nephrotoxic Effect

Object of analysis			Range of values before gentamicin intoxication	Gentamicin nephrotoxicity						
				urine				blood		
				protein	leukocytes	erythrocytes	epithelium	urea	creatinine	medium-weight molecules
CN	PCT	diameter	28.90-36.29	-0.41	+0.37	-0.07	-0.30	-0.65*	-0.51	-0.33
		cell height	10.28-14.20	-0.18	+0.30	+0.03	-0.16	-0.81*	-0.72*	-0.56*
	DCT	diameter	21.16-29.67	-0.24	+0.55	+0.13	+0.04	-0.25	-0.41	-0.56*
		cell height	7.50-11.43	+0.13	+0.44	+0.18	+0.29	+0.06	-0.12	-0.24
JN	PCT	diameter	30.14-36.31	-0.57	+0.21	+0.16	-0.30	-0.65*	-0.50	-0.33
		cell height	10.07-14.87	-0.11	+0.44	-0.05	-0.26	-0.62*	-0.67*	-0.35
	DCT	diameter	20.98-30.38	-0.22	+0.25	+0.36	+0.07	-0.64*	-0.60*	-0.68*
		cell height	7.53-12.71	-0.38	+0.53	+0.12	+0.06	+0.10	+0.00	-0.07
	Distal straight tubule	diameter	21.18-29.26	+0.00	+0.34	+0.43	+0.25	-0.25	-0.47	-0.15
		cell height	6.72-11.57	-0.53	+0.41	-0.04	-0.36	-0.22	-0.58*	-0.21

Note. *p<0.05 compared to the corresponding parameter before intoxication.

TABLE 2. Multiple Regression Equations Describing the Relationship between Gentamicin Nephrotoxicity and Congenital Structure of the Rabbit Nephrons

Nephrotoxicity index (z)	Before intoxication		Regression equations; R; F values	p	Regression type
	x	y			
Blood urea	CN PCT diameter	CN PCT cell height	z=67.92-4.27y; R=0.81; F=21.48	<0.00072	Linear
		JN PCT diameter	z=81.27-2.07y; R=0.65; F=8.08	<0.02	Linear
		JN PCT cell height	z=67.47-1.11x-1.47y; R=0.69; F=4.66	<0.03	Multiple linear
		JN DCT diameter	z=76.52-1.15x-0.99y; R=0.75; F=6.21	<0.02	Multiple linear
	CN PCT cell height	JN PCT diameter	z=82.24-3.51x-0.73y; R=0.83; F=11.29	<0.003	Multiple linear
		JN PCT cell height	z=67.92-4.27x; R=0.81; F=21.48	<0.0007	Linear
		JN DCT diameter	z=67.92-4.27x; R=0.81; F=21.48	<0.0007	Linear
	JN PCT diameter	JN PCT cell height	z=78.49-1.39x-1.53y; R=0.70; F=4.90	<0.03	Multiple linear
		JN DCT diameter	z=88.05-1.45x-1.02y; R=0.75; F=6.52	<0.03	Multiple linear
	JN PCT cell height	JN DCT diameter	z=67.46-1.12x-1.94y; R=0.75; F=6.44	<0.02	Multiple linear
Blood creatinine	CN PCT cell height	JN PCT cell height	z=1.82-0.08x-0.04y; R=0.75; F=6.27	<0.02	Multiple linear
		JN DCT diameter	z=1.75-0.12x; R=0.72; F=11.54	<0.006	Linear
		Cell height of JN distal straight tubules	z=2.11-0.11x-0.07; R=0.83; F=11.27	<0.003	Multiple linear
	JN PCT cell height	JN DCT diameter	z=1.95-0.07x-0.03y; R=0.76; F=6.96	<0.012	Multiple linear
		Cell height of JN distal straight tubules	z=1.75-0.08x-0.06y; R=0.79; F=7.67	<0.009	Multiple linear
	JN DCT diameter	Cell height of JN distal straight tubules	z=1.67-0.03x-0.06y; R=0.71; F=5.13	<0.03	Multiple linear
Blood medium-weight molecules	CN PCT cell height	CN DCT diameter	z=1.04-0.03x-0.01y; R=0.66; F=3.86	<0.05	Multiple linear
		JN DCT diameter	z=1.03-0.02x-0.02y; R=0.66; F=4.76	<0.03	Multiple linear
	CN DCT diameter	JN DCT diameter	z=1.08-0.01x-0.02y; R=0.73; F=5.74	<0.02	Multiple linear

diameter of JN DCT in combination with small cells of the distal straight tubule of these nephrons (multiple linear regression equation; Table 2).

The content of medium-weight molecules in the blood of rabbits by the end of gentamicin intoxication also depended on the nephron structure before intoxication. In animals with initially small height of CN PCT cells in combination with little diameter of CN or JN DCT gentamicin intoxication led to a more pronounced increase in the blood level of medium-weight molecules. A similar regularity was detected in animals with little diameter of DCT in both nephron types (multiple linear regression equations; Table 2).

Figure 1 presents a graphic image of multiple linear regression.

The results of dispersion analysis indicate high informative value of the developed models ($p<0.05$).

The results of canonical analysis confirmed a strong statistically significant correlation ($R=1$; $p=0$) between the pre-intoxication values and the values after gentamicin intoxication of the kidneys. Judging by clinical and biochemical findings (analysis of the urine and blood), predisposition to gentamicin nephrotoxicity in 100% cases was explained by the initial morphological features of the studied nephrons.

A strong direct relationship between the first canonical (latent) variables is early seen on the scheme by the compact position of the dotted field stretched upward to the right (Fig. 2).

Hence, the decrease in the nephron tubule diameter and their cell size can reflect predisposition of the renal parenchyma to the nephrotoxic effect of gentamicin. In our interpretation of this relationship we proceeded from the fact that the most important sign for any tissue, including the renal one, is the relationship between its constitutive elements [2]. Decreased diameter of the tubules and height of epitheliocytes is as a rule paralleled by a justified decrease of the bulk (number and size) of intracellular functioning structures, such as mitochondria, microvilli plication of the basal cytolemma, cytosomes, *etc*. Changes in the quantitative parameter of epitheliocyte cytoplasm (in comparison with wider tubules and larger epitheliocytes) are paralleled by appropriate decrease of cell metabolism. This, in turn, causes a decrease in the main function of the nephron tubular system, the intricate process of urine production, starting from reabsorption of ultrafiltrate from primary urine until the formation of the final urine [1,4]. Overloading of these tubules and epitheliocytes under conditions of severe acute or chronic experiment can lead to failure of oxidative phosphorylation and suppression of the activity of respiratory enzymes in the tubular epitheliocytes. This can result in renal failure. In this case the maintenance of structural homeostasis by compensatory

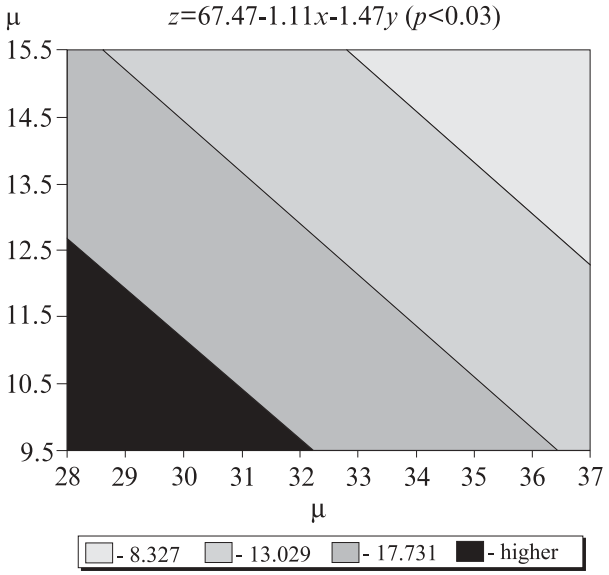

Fig. 1. Relationship between blood plasma urea content in rabbits (*z*; after gentamicin intoxication), diameter of proximal convoluted tubules of the cortical nephrons (*x*), and cells height in the proximal convoluted tubules of juxtamedullary nephrons (*y*). The degree of hyperammoniemia is shown by the intensity of coloring and figures presented on the right scale.

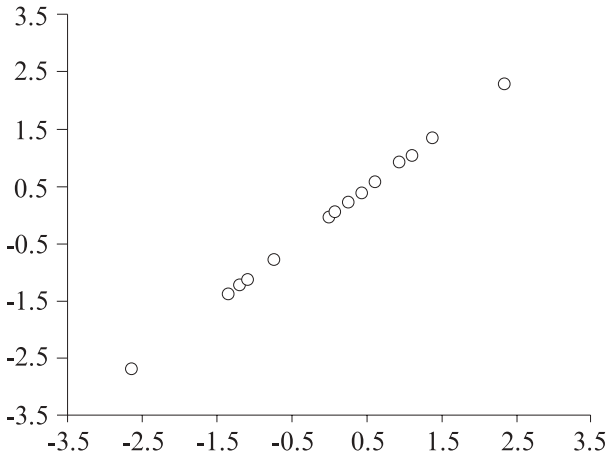

Fig. 2. All rabbits examined. Abscissa: before gentamicin intoxication; ordinate: after intoxication.

mechanisms will be realized in the presence of obvious disagreement between the level of functioning and its plastic support. On the other hand, it is well known that the reliability of any biological system is based on its structure, namely, on regulation of the number of actively functioning reserve systems [2].

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