Role of Nitric Oxide in the Mechanisms of Verografin Nephrotoxicity

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 10, pp. 396-399, October, 1997
Original article submitted March 6, 1996

Nephrotoxicity of radiopaque agents and the role of nitric oxide in its realization are studied in Wistar rats with Verografin-induced acute renal insufficiency. Experiments demonstrate a significant decrease in nitric oxide production in the kidneys due to inhibition of constitutive NO-synthetase and disturbances of renal function, hemodynamics, and electrolyte balance. These changes are prevented by simultaneous injection of verapamil.

Key Words: *nitric oxide; nephrotoxicity; radiopaque agents; verapamil*

It has been established that nitric oxide (NO) is formed from L-arginine after enzymatic oxidation of immune group in its guanidine residue. This process is catalyzed by NO-synthase (NOS). In some tissues this enzyme is constantly expressed (constitutive NOS), while in others it can be induced by some agents (inducible NOS) [12]. NO is an active component of endothelium-derived relaxation factor [9]. In light of this of particular interest is its participation in pathological processes characterized by disturbances of vascular homeostasis and hemodynamics. The key role in the development of acute renal insufficiency (ARI) caused by radiopaque agents is played by disturbances of renal circulation, resulting in a reduced rate of glomerular filtration [2,6].

In the kidneys, NO is a product of L-arginine oxidation catalyzed by constitutive NOS of endothelial and mesangial cells and regulated by a number of activators and inhibitors [5]. It has been found that NO in the kidneys regulates capillary pressure and renal circulation through relaxation of afferent arteriole and regulation of glomerular mesangial cell contractility [6,11]. In the present study we evaluated changes in NO synthesis and their role in the development of ARI induced by the radiopaque agents Verografin.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 280-300 g and random-bred albino male mice weighing 20 g. Acute renal insufficiency was modeled by a single intravenous infusion of 76% Verografin, a high-osmolar ionogenic radiopaque agent, in a dose of 10 ml/kg body weight at a rate of 1 ml/min (group 2) [14]. Some animals (group 3) received intravenous infusion of verapamil, a calcium channel blocker, in a dose of 1.3 mg/kg and at a rate of 10 μg/min immediately before Verografin. Control animals (group 1) were given an equivalent volume of physiological saline.

In all animals serum creatinine and the rate of glomerular filtration were determined, and the content of sodium and calcium in the plasma and their urinary excretion were measured by atomic adsorption spectrophotometry. Renal hemodynamics was assessed by the volume of circulating blood using ^{131}I-albumin. Plasma renin activity and aldosterone concentration were measured by radioimmunoassay using Sorin kits.

In morphological study, paraffin section of the kidneys stained with hematoxylin and eosin and

0007-4888/97/0010-0968$18.00

using PAS reaction were examined under a light microscope.

NO synthesized in the kidneys *in vivo* was detected by electron paramagnetic resonance using Fe^{2+}-diethyldithiocarbamate (DETC) complexes as an NO trap [1]. The binding of NO to this trap yields paramagnetic mononitrozyl iron complexes with DETC (MNIC—DETC), characterized by an electron paramagnetic resonance signal with maxima at $g_{\perp}$= 2.035 and $g_{||}$=2.02 and superfine triplet structure at $g_{\perp}$=2.035 [7]. This approach was successfully used for detection of *in vivo* formed NO in animal tissue [1].

For quantitative evaluation of NO in the kidneys the animals were intraperitoneally injected with 500 mg/kg DETC-Na (Sigma) in 0.2 ml physiological solution 30 min before sacrifice. The competitive NOS inhibitor N-nitro-L-arginine (Sigma) was injected in a dose of 300 mg/kg in 0.5 ml physiological solution 1 h before sacrifice. The animals were decapitated, kidneys were removed, placed into glass tubes, and frozen in liquid nitrogen. Electron paramagnetic resonance spectra were recorded at -196°C in a Rubin and a Radiopan spectrometers (Poland) in the X range (5 mW microwave power and 0.5 mT amplitude of magnetic field modulation). The concentration of MNIC—DETC complexes in the kidneys was determined by comparing the signal from the sample with a standard signal from MNIC—DETC of a known concentration) synthesized in dimethyl sulfoxide solution as described elsewhere [13].

RESULTS

Injection of Verographin to group 2 animals significantly decreased the parameters of renal hemodynamics (reduced circulation volume) and impaired renal function: serum creatinine level increased 2.7-fold, while the rate of glomerular filtration decreased 2-fold in comparison with the control; there were also electrolyte disturbances, in particular, enhanced sodium excretion and hypercalcemia (Table 1). NO production in the kidneys was reduced significantly in this group. Morphological study revealed marked narrowing of Henle's loops. Moreover, hypercellularity in the mesangial zone of the glomerulus was seen. Destruction of distal tubular epithelium was accompanied by infiltration of injured zone with mononuclear cells.

In group 3 animals, the calcium channel blocker verapamil preserved renal function: renal circulation, plasma creatinine, and the rate of glomerular filtration did not differ from the control. Fraction excretion of serum sodium and calcium remained normal. The content of NO surpassed the control values, which can be attributed to the effect of verapamil.

The increased concentration of MNIC—DETC can be explained on the basis of two assumptions. First, additional NO is delivered by the blood in the form of nitrozyl complexes; second, additional NO is synthesized by inducible NOS stimulated by verapamil. Experiments with the competitive NOS inhibitor N-nitro-L-arginine in group 3 animals confirmed activation of inducible NOS.

Thus, Verographin significantly decreases the production of NO in the kidneys and impairs their function, renal hemodynamics, and electrolyte balance. The Ca blocker verapamil injected simultaneously with Verographin prevents the development of ARI.

Hypercalcemia observed in our experiments as well as concomitant hyponatremia and hypovolemia play an important role in the pathogenesis of Verographin-induced ARI [3]. Hyponatremia and hypovolemia manifest themselves as a reduced volume of circulating blood and enhanced Na excretion. Hypercalcemia, on the one hand, is responsible for a pronounced vasoconstrictory effect of radiopaque agents (constriction of the afferent arteriole) leading to hemodynamic disturbances and renal insufficiency. In our study this was confirmed by a decrease in the circulating blood volume and glomerular filtration rate and a rise of serum creatinine. On the other hand, hypercalcemia is responsible for the direct cytotoxic effect of radiopaque agents [3], which was observed under our experimental conditions both at the morphological (destruction of the distal tubular epithelium) and functional (enhanced Na excretion) levels.

Our experiments demonstrated a considerable decrease in the content of NO in renal tissue, probably, due to inhibition of constitutive NOS in the kidneys in Verographin-induced ARI. This promotes the development of hemodynamic disturbances due to both a spasm of the afferent arteriole and decreased ultrafiltration coefficient, which is an additional component of the pathogenesis of the studied ARI.

Published data suggest that activation of different NOS isoforms functionally depends on intracellular calcium homeostasis [8,10]. Endothelial constitutive membrane-bound NOS is activated by calcium-dependent mechanisms, in particular, activation of various NO receptor agonists such as acetylcholine, bradykinin, and atrial natriuretic factor [5,8,10]. First, constitutive and inducible NOS isoforms were distinguished by the dependence on and independence of calcium, respectively. However, recent studies suggest that some Ca-dependent NOS isoforms are inducible [4,8,10]. There is evidence that NO mediates relaxation of vascular smooth muscles via direct activation of calcium-dependent potassium channels [4]. Thus, disturbances of calcium metabolism are

TABLE 1. Parameters of Renal Functions and Regulatory Factors after Injection of Test Preparation (*M*±*m*)

Parameter	Group		
	1st (*n*=14)	2nd (*n*=28)	3rd (*n*=27)
Serum creatinine, mg/dl	1.04±0.12	2.9±0.6***	1.2±0.04++
Rate of glomerular filtration, ml/min/100 g	0.94±0.03	0.46±0.01***	0.79±0.06++
Serum calcium, mmol/liter	2.60±0.03	3.01±0.1*	2.56±0.1+
Sodium excretion fraction, %	0.26±0.02	1.60±0.4*	0.35±0.1+++
Plasma aldosterone concentration, ng/ml	260±18.4	96.4±10**	101.3±12
Plasma renin activity, ng/ml/h	7.84±0.8	9.32±0.2*	8.92±0.1
Circulating blood volume in the kidneys, ml/g	0.292±0.4	0.134±0.01****	0.264±0.02+
NO concentration, ng/g wet tissue	5.7±1.7	3.0±1.0**	7.3±3.0+++
NO concentration after NOS inhibition, ng/g	0	0	0.35±0.04

Note. *p<0.05, **p<0.01, ***p<0.001, ****p<0.005 compared with the control (group 1); +p<0.05, ++p<0.01, +++p<0.001 compared with group 2.

evidently related to the inhibition of different NOS isoforms.

Thus, on the basis of our experimental findings and published data we propose the following role for NO in the pathogenesis of ARI caused by radiopaque agents, which, being high-osmolar ionogenic substances (Verografin), disturb calcium metabolism (induce hypercalcemia). The hypercalcemia-related direct cytotoxicity leads to inhibition of NO synthesis in glomerular endotheliocytes and, consequently, increases the tone of afferent arteriole and contraction of mesangial cells. On the other hand, constitutive NOS is activated by the rise of cell calcium concentration [8,10]. However, the observed decrease in the NO content instead of its increase in hypercalcemia attests to inactivation of this enzyme caused by the cytotoxic action of calcium ions. The decrease of NO content leads to a drop in hydrostatic pressure in glomerular capillaries, and reduces ultrafiltration coefficient and, consequently, the rate of glomerular filtration. Taking into account the antiaggregation activity of NO [8], it can be assumed that NO deficiency impairs glomerular microcirculation due to enhanced platelet aggregation.

Normalization of Ca metabolism by the calcium channel blocker verapamil injected immediately before the radiopaque agent maintained the parameters of electrolyte metabolism, circulating volume, serum creatinine and rate of glomerular filtration within normal, i.e., prevented the development of ARI.

It can be hypothesized that the protective effect of verapamil is, on the one hand, due to compensatory dilation of the afferent arteriole and, on the other hand, is associated with reduction of direct cytotoxic effect of hypercalcemia and normalization of Ca-mediated regulation of NOS.

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