ULTRA STRUCTURE OF RENAL TUBULAR EPITHELIAL CELLS OF RAT’S KIDNEYS AFTER ADMINISTRATION OF L-ARGININE

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Abstract

Sixteen white Wistar female rats were divided into two equal groups. Experimental group received per os 40 mg/kg b.w. of L-arginine, every other day for 2 weeks and were decapitated after 3 weeks of the experiment. Control rats received in the same manner 2 ml of distilled water and were decapitated after 3 weeks of the experiment. The renal lesions observed under electron microscope were of focal character and concerned only the experimental group. The tubules with necrotic cells were observed among normal tubules or single normal epithelial cells of the tubular wall. The boundaries between epithelial cells of the tubule wall were blurred. The mitochondria indicated abnormal structure. Numerous lysosomes and peroxysomes with dark, homogenous content were observed. The rough endoplasmic reticulum had widened channels and was focally completely destroyed. The nucleus of damaged cells was most commonly located in one of the cell poles; its shape was changed and visibly smaller than the nuclei of normal cells. Condensation and peripherally located chromat in were noticed. The lesions observed were characteristic for apoptotic cells.

Key words: rats, L-arginine, kidneys, ultrastructure.

In 1998, Robert F. Furchgott, Luis Ignarro and Ferid Murad (5) received the Nobel Prize in medicine for their research on the sequence of metabolic changes of L-arginine to nitric oxide (NO). In the organism, NO is produced from L-arginine with help of nitric oxide synthase (NOS), also called digoxigenase (9).

Five isoforms of NOS were discovered: brain (bNOS), endothelial (eNOS), macrophage (macNOS), hepatocyte (hepNOS), and mitochondrial (mtNOS). The studies on their structure and regulation are being conducted.

The organism produces NO by endothelial cells, macrophages, hepatocytes, nerve endings, some neurons (10), neutrophils, monocytes, mastocytes, and blood platelets (23). Nitric oxide induces cell apoptosis via free radicals and oxidative stress. NO is also known to have anti-apoptotic effects. The capability to induce and suppress apoptosis is currently extremely desirable in medicine and its importance in the treatment of neoplasms and other difficult-to-treat diseases cannot be overestimated.

During the recent years, an increasing number of reports have been published concerning exogenous and endogenous NO as well as its favourable action in many diseases. On the other hand, there are opinions that NO exerts numerous adverse effects. Exogenous NO is administered to patients with venous atherosclerosis of the lower limbs, coronary disease, and pregnancy-induced hypertension. Exogenous NO is an easily available medicine. However, its influence on cells, including renal cells, has not been fully explained.

It was demonstrated that the proapoptotic influence of NO was caused by oxidative stress induced in the cell. The so-called “nitrosative stress” was described in the rat’s macrophages, where NO-induced apoptosis was observed (6).

In the study, the effects of L-arginine - exogenous nitric oxide, on renal tubular epithelial cells were investigated.
Material and Methods

The study material consisted of 16 white Wistar female rats of 200-250 g of the baseline body weight and 2.5-3 months of age. The animals received standard feed and water ad libitum. They were kept in the Animal Quarters of the Department of Histology and Embryology, Medical University of Lublin, at the temperature of 20±2 ºC and humidity about 60%. The air temperature, lightening, and noise slightly changed during the day. The rats were kept in 0.2 m² metal cages, four individuals in each cage. The cage lining was suitably changed. The study was approved by the Local Ethics Committee.

The animals were divided into two equal groups:

- group I – rats, which received through the stomach tube (Argininum, Curtis Healthcare, Poland) 40 mg/kg b.w. of L-arginine (5 mg of L-arginine in 1 ml of distilled water), every other day for 2 weeks and were decapitated after 3 weeks of the experiment;
- group II – control rats, which received in the same manner 2 ml of distilled water and were decapitated after 3 weeks of the experiment.

After decapitation, the kidneys were examined macroscopically and the specimens from the left kidney were collected, fixed in the solution consisting of 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1M phosphate buffer according to Sorensen (4) and in osmium tetroxide (OsO₄), contrasted in uranyl acetate, dehydrated in the alcohol-acetone series, and embedded in the Araldit AMC Fluka resin. Then, ultrathin (60 nm) sections were prepared and stained with 8% solution of uranyl acetate in 0.5% acetic acid and lead citrate. The material was evaluated under the electron microscope Tesla BS-500.

Results

The rats of experimental and control groups behaved in a similar way throughout the experiment and showed similar appetite, thirst, and physical activity.

In both groups, the kidneys did not macroscopically differ. They were bean-shaped, surrounded by an easy-to-remove capsule. Their surface was smooth and red-brown in colour. The cross-sections showed the kidney cortex and medulla, which were different in shade. The blue-red medulla was indented towards the yellowish-red cortex dividing it into renal columns. In the cortex inspected against the light, the punctuates corresponding to renal glomerules were visible.

The renal tubules of animals from control group had normal structure. Their wall was built of cubic epithelial cells located on the basal membrane of normal thickness (Fig. 1). The boundaries between cells were clearly visible. The cells forming the wall of primary convoluted tubule at the pole directed towards the lumen had the brush border composed of microvillae (Fig. 1). The cellular membrane of the basis of epithelial cells of proximal and distal tubules was indented into the cytoplasm forming the peribasal labyrinth (striaion) with parallel rows of mitochondria inside (Fig. 1). The mitochondria had normal shape, membranes, and mitochondrial crests (Fig. 1). The nucleus in the epithelial cells of the tubules was located above the striaion, medially, and eccentrically (Fig. 1). It was round or oval, large, surrounded by the nuclear membrane and had the nucleolus (Fig. 1). Above the nucleus, there was a well developed endoplasmic reticulum – rough and smooth, and lysosomes, as well as dark peroxysomes with crystalline medulla (Fig. 1).

Fig. 1. Control group. The epithelial cell of the wall of the convoluted tubule of the nephron. The photomicrograph reveals a round, large nucleus (N) surrounded by perinucleolar heterochromatin under the internal lamina of the nuclear capsule, normal mitochondria within peribasal striaion in the cytoplasm. TEM 4,000x.

In the kidney of the experimental rats, focal lesions, in the form of necrotic tubular cells, were observed (Fig. 2). The boundaries between epithelial cells of the tubule wall were blurred. Some cells were

Fig. 2. Experimental group. The renal tubule section of the rat treated with L-arginine. Numerous peroxysomes (P), lysosomes, and vacuoles (V) as well as slightly oedematous mitochondria (orthodox condition) (M) are visible. TEM. 3,000x.

In the kidney of the experimental rats, focal lesions, in the form of necrotic tubular cells, were observed (Fig. 2). The boundaries between epithelial cells of the tubule wall were blurred. Some cells were
flattened, with reduced volume, partially, or completely destroyed. The tubular lumen was widened with homogenous deposits inside, as well as nuclei, mitochondria, and single epithelial cells. The brush border in the proximal tubules was focally destroyed.

The partially destroyed cells had numerous vacuoles surrounded by the smooth or rough membrane in the cytoplasm (Fig. 2). The rough endoplasmic reticulum had widened channels and was focally completely destroyed. The mitochondria showed abnormal structure. They were oedematous with brightened matrix and destroyed crests (Fig. 2). Moreover, the architectonics of peribasal striation was damaged. Numerous lysosomes and peroxysomes with dark, homogenous content were observed (Fig. 2). Some cells had only a nucleus and only few organelles, and brightened structure of the cytoplasm. The nucleus of damaged cells was most commonly located in one of the cell poles; its shape was changed and markedly smaller than the nuclei of normal cells. Condensation and peripherally located chromatin were observed.

Moreover, a prominent cell membrane (best visible in the lumen) and formation of apoptotic alveoli (“cell boiling”) were observed. The alveoli contained complete or fragmented nucleus and other organelles. The lesions observed were characteristic for apoptotic cells.

**Discussion**

Nitric oxide as a “free radical scavenger” is about 10,000-100,000 times more effective than vitamin E. However, too high concentrations make it harmful. It inhibits glutathione peroxidise, therefore it cannot neutralise peroxides converting them into alcohols. Such a relation was noted by Kronon et al. (18) who described the so-called nitric oxide paradox, i.e. small doses of L-arginine, a precursor of NO (L-arginine solution in the concentration of 4 mmol/L) exerted protective effects on the myocardium of newborns by reducing oxygen free radicals, while its high doses (10 mmol/L) caused an increase in the number of oxygen free radicals, which resulted in the damage to vessels and myocardium.

Nitrosative stress can lead to reactions that alter protein structure, thus interfering with normal body functions.

Free radicals damage cellular elements by reacting with them in a non-specific way. Due to these reactions the hydrogen atom is detached from organic particles and transferred onto reactive forms of oxygen. Such particles become free radicals – a vicious circle arises.

Such way of oxidation by free radicals mainly involves: proteins (loss of activity of enzymes, membranous transporters, regulatory proteins) (11); nitrogen basis in DNA (neoplasia formation); lipids (peroxidation of membrane lipids - damage to biological membranes (21, 27, 28)); sugars.

Moreover, free radicals damage cell organelles such as mitochondria (17) and biological membranes (increased permeability and depolarisation of the cell membrane). They also decrease the ATP level, impair intracellular homeostasis of Ca\(^{2+}\), and change the antigenic properties of cells.

Evans et al. (7) administered L-arginine to healthy people in doses of 3, 9, 21, and 30 g/d and noticed that the 3 g/d dose caused only a few side effects even if administered for a week (similarly to the dose used in the study). Such a dose is sufficient to decrease pregnancy-induced hypertension. This was observed by Rytlewski et al. (23) who administered L-arginine in the dose of 3 g/d for 3 weeks to pregnant women. Other authors (26) who administered L-arginine to rats in lower doses – from 0.2% to 2% in drinking water (in the study 0.5%) also noted its clinical effectiveness in reducing blood pressure in pregnancy (1). Pregnant women with hypertension also received much higher amounts of L-arginine, e.g. Facchinetti et al. (8) administered 30 g in the intravenous drip infusion and observed increased nitric oxide (NO) production and hypotension.

In the study, the dose of L-arginine was similar to that used in pregnant women treated for gestosis. This dose should be safe for a mother and fœtus (the so-called dose scavenging free radicals) (23). The study also reveals, that L-arginine as a donor of exogenous nitric oxide induced the apoptotic signal in normal renal tubular cells of the rats. The focal lesions in the renal tubules observed under electron microscope revealed qualitatively and quantitatively increased apoptosis. The ultrastructural examination showed an extremely large number of lesions in the mitochondria (mitochondrial pathway of apoptosis). The mitochondria were oedematous, their matrix brightened, and crests destroyed. It was found that low-amplitude swelling of mitochondria was reversible, which indicated the adaptation of the mitochondria to higher energy demand. If the mitochondria are swollen by more than 20% of their physiological volume, the change is irreversible and denotes the cell death. In the study, the oedema was very highly intensified, which irreversibly destroyed mitochondria.

Another characteristic lesion seen under electron microscope was L-arginine-increased number of peroxysomes in the cytoplasm of dying cells, which confirms their significant involvement in the detoxicating processes. The peroxysomes play a significant role in detoxication of many metabolites and xenobiotics by their oxidation. They are formed by blastogenesis from the smooth endoplasmic reticulum and their enzymes are synthesised in the rough endoplasmic reticulum. An increased number of peroxysomes in the myocardium following the administration of adriamycin (200%-400%) was observed by Zipper (29). He described these changes as a cell response to oxidative stress.

In the study the effects of exogenous NO on the renal tubular epithelial cells were examined by administering L-arginine as a substrate of NO (3). Gryglewski (12) demonstrated, however, that L-arginine administered exogenously was converted into NO. The synthesis of NO from exogenously administered L-arginine is visible in an increase in plasma cGMP after
L-arginine administration. L-arginine is a substrate for NO synthase (NOS) (16).

Various exogenous precursors of nitric oxide were used in the studies on the effects of NO on various tissues and organs in experimental animals, as well as humans (15), including non-steroidal anti-inflammatory drugs (NSAID), (22) so-called donors of nitric oxide (NO-NSAID) (15): NO-inbuprofen (NCX 2111), NO-aspirin (NCX 4060) (22), and nitrosulindac (NCX 1102) (15). Other precursors of exogenous nitric oxide also included: sodium nitroprusside (SNP) (2), and S-nitroso-N-acetyl-penicillamine (SNAP) (19). L-arginine used in the study is also commonly applied precursor of NO (20).

This drug is administered in many different ways. Holm et al. (13) administered L-arginine to rats orally in the amount of 2.25% in the drinking water; Raff et al. (20) gave the compound intraperitoneally.

Tong et al. (25) demonstrated that exogenous NO protected the neoplastically modified prostate cells against apoptosis. Other authors observed that NO inhibited proliferation and had proapoptotic effects on the epithelial cells of the prostate (15, 22).

Hortelano et al. (14) studied macrophages and Jurkat cells (lymphoid leukaemic cells), which underwent apoptosis induced via the intrinsic-mitochondrial pathway. NO in the macrophages elevated the potential of the internal mitochondrial membrane (ΔΨ(m)) and decreased it in the Jurkat cells. The authors concluded that the changes in the potential of the internal mitochondrial membrane caused by exogenous NO were dependent on the type of cells.

The lesions observed in the study in renal tubular epithelial cells were characteristic for apoptotic cells. This indicates that L-arginine induces proapoptotic effect in kidney’s cells.

References

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