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Natural Antioxidant L-Carnosine Inhibits LPO Intensification in Structures of the Auditory Analyzer under Conditions of Chronic Exposure to Aminoglycoside Antibiotics

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Intragastric administration of L-carnosine suspension to Wistar—Kyoto rats 3 days before and after 7-day course of intraperitoneal injections of ototoxic aminoglycoside antibiotic kanamycin compensated expenditures of tissue antioxidant systems and significantly eliminated kanamycin-induced intensification of MDA production in tissues of the membrane part of the cochlea and in the auditory cortex of the temporal lobe. L-NAME (competitive NO synthase inhibitor) also inhibited LPO, increased total antioxidant activity, and decreased ototoxicity of kanamycin, which confirms the contribution of NO into LPO intensification under conditions of aminoglycoside treatment. Inhibition of pathological intensification of LPO processes and increase in total antioxidant activity under conditions of induced acute aminoglycoside ototoxicity characterizes L-carnosine as a highly effective otoprotector.

Key Words: *auditory cortex; L-carnosine; aminoglycoside ototoxicity; nephrotoxicity; otoprotection*

Neuroepithelium of the cochlea (the receptor component of the auditory analyzer) is a non-dividing cell population during the entire human life-span starting from month 4 of intrauterine development [6]. This characteristic of hair cells (considering their mechanoelectrical transduction function creating permanent oxidative stress because of permanent auditory stimulation) suggests that their natural defense antioxidant potential is very high. However, some pathological situations can cause excessive generation of reactive oxygen species (ROS) surpassing the compensatory potential of natural antioxidant defense of the auditory analyzer [4,9,12,14,15].

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The process of ROS production in stimulated structures of the auditory analyzer is unspecific and underlies the mechanisms of adequate (sound) and pathological stimulation. The etiological factors of pathological oxidative stress developing primarily in the cochlear tissues are exposure to ototoxic drugs [9,14], chronic and acute auditory traumas [4,15], aging of the auditory analyzer [12], *etc.* Excessive LPO production causes death of the neuroepithelium by the mechanism of apoptosis [7,10] with subsequent retrograde degeneration of the corresponding nerve structures.

We studied the severity and type of oxidative damage in the auditory analyzer induced by injections of aminoglycoside antibiotic (AA) kanamycin, involvement of NO in this process, and the effects of well-known physiological antioxidant L-carnosine on LPO

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intensity in the membrane part of the cochlea and auditory cortex.

MATERIALS AND METHODS

Chronic experiment was carried out on 14 male Wistar—Kyoto rats (300-400 g) without signs of somatic or otological diseases (according to otoscopy data and Prayer reflex evaluation). Group 1 animals (n=3) were daily injected (intraperitoneally) with kanamycin sulfate (60 mg/kg) for 7 days in order to induce an ototoxic sensorineural injury. Group 2 rats (n=3) daily received L-carnosine suspension (200 mg/kg) through a gastric tube 3 days before and during kanamycin treatment. Group 3 animals (n=3) were subcutaneously injected with L-NAME (competitive NO synthase inhibitor, ICN) in a daily dose of 50 mg/kg in normal saline 4 h before kanamycin injection. Controls (n=5) were injected with normal saline.

Homogenates of the membrane part of the cochlea and auditory cortex were studied. Samples of the cerebellum, which is not associated with auditory sensitivity, served as the control for the auditory cortex tissue. In parallel, blood serum and liver homogenates (one more target organ for the toxic side effects of AA) were studied.

LPO processes and the status of antioxidant system (AOS) were evaluated in the studied biological samples. LPO intensity was evaluated by changes in MDA level (LPO end product) [1]. AOS status was evaluated by the total SOD activity [1] and total antioxidant activity, which was assessed by the degree of LPO activation with iron sulfate in brain and inner ear homogenates [2].

The animals were decapitated 4 h after the last kanamycin injection, temporal bones and membrane part of the cochlea were isolated [4]. The auditory cortex was isolated at the level of the temporal lobes between two frontal sections, in accordance with the cytoarchitectonic map of rodent neocortex [5]. Tissue homogenates were prepared using glass homogenizers in 50 μM Na phosphate buffer (pH 7.4). Supernatants after 20-min centrifugation at 1000g were analyzed. The measurements were carried out on an SF-2000 spectrophotometer.

The animals were kept and subjected to all manipulations in accordance with the requirements of Ethic Committee of I. P. Pavlov University developed on the basis of Helsinki Declaration.

RESULTS

Control animals were characterized by unchanged (active) Prayer reflex. In group 1 Prayer reflex was drastically inhibited or absent. In group 2 Prayer reflex was retained, though inhibited, by the end of the experiment. A similar picture was observed in group 3 animals.

In vivo 7-day treatment with kanamycin in subtoxic doses intensified LPO processes in tissues of the auditory analyzer (both peripheral and central), which manifested in MDA hyperproduction. Serum MDA concentration remained unchanged, and just a trend to its increase was detected in the renal tissue (Table 1).

This reaction of the auditory analyzer tissue structures is unspecific and is observed during exposure to ototropic factors of different nature [4,9,12,14,15]. The effects of these factors at the morphological and ultrastructural levels manifest by identical degenerative changes in the spiral organ [8]. This gives us grounds to consider kanamycin (judging from the toxic type changes in the auditory analyzer) as a pharmacological equivalent of adequate (auditory) stimulus. Nonspecific response of the sensitive cochlear structures to otopathogenic factors of different nature can be due to common components in their pathogenesis, one of which can be excitotoxicity. The probability of this toxicity is determined by the presence of glutamate (NMDA) synaptic receptors on hair cells of the spiral organ and on spiral ganglion neurons [11].

Activation of LPO in the central part of the auditory analyzer under conditions of AA treatment was detected for the first time. Aminoglycoside-induced activation of LPO in the auditory cortex was much more intensive than in the cerebellum. The mechanism of MDA hyperproduction in the auditory cortex can also be explained by excitotoxic excitation developing after afferent impulsation induced by inadequate (ototoxic) stimulation in the peripheral part of the analyzer at the level of inner hair cell—nerve fiber.

Presumably, the absence of changes in serum LPO can be due to the absence of the substratum for realization of AA cytotoxic effects in the peripheral blood (aminoglycoside ototoxicity is a specific injury).

The trend to an increase in MDA production detected in the renal tissue *in vivo* is in line with a slight increase of ROS production in the renal cortex tissue culture after incubation with AA [13]. This can indicate a lesser significance of the mechanism of free radical damage in the pathogenesis of nephrotoxic complications in comparison with ototoxic damage. One more important factor for the mechanism of the formation of aminoglycoside nephrotoxicity seems to be the absence of the excitotoxic factor drastically augmenting the depth of injury in oxidative stress of the nervous tissue.

NO forming in the reaction with participation of NO synthase is involved in the pathogenesis of aminoglycoside cytotoxicity of hair cells [10]. Peroxynitrite possessing high reaction potential typical of free radi-

TABLE 1. LPO Intensity and Total Antioxidant Activity in Studied Tissues and Blood (*M*±*m*)

Object, group		MDA, nmol/mg lipids	Degree of LPO activation, Fe^{2+}, rel. units	SOD, arb. units
Cochlea	Control	0.570±0.023	1.810±0.068	11.10±0.67
	1	1.060±0.125**	2.85±0.38*	18.8±2.19*
	2	0.65±0.03^{+}	2.05±0.25	13.6±1.4
	3	0.670±0.025^{+}	2.190±0.315	15.7±1.6*
Temporal cortex	Control	6.800±0.648	1.290±0.038	9.90±1.33
	1	11.40±0.72**	2.62±0.12**	7.310±0.978
	2	4.0±0.3*$^{++}$	1.490±0.123^{++}	16.00±2.58
	3	5.40±0.42^{++}	1.79±0.03*$^{+}$	15.0±1.8^{+}
Cerebellum	Control	5.72±0.34	2.67±0.10	11.72±1.35
	1	7.86±0.48**	3.44±0.69	8.50±1.11
	2	5.83±0.33^{++}	2.47±0.18	13.80±2.67
	3	6.77±0.87	2.63±0.336	14.80±2.16^{+}
Kidneys	Control	16.7±2.5	—	31.40±0.57
	1	18.80±1.35	—	34.70±0.33
	2	14.50±1.89	—	36.20±0.78
	3	18.4±3.63	—	34.90±1.17
Blood	Control	13.20±0.49	—	15.50±1.98
	1	12.70±0.98	—	11.60±2.52
	2	11.500±0.531	—	15.8±3.0
	3	12.10±0.52	—	25.4±5.1

Note. *p<0.05, **p<0.001 compared to the control; ^{+}p<0.05, ^{++}P<0.001 compared to group 1.

cal forms under conditions of intracellular hypoxia as a result of NO reaction with $O_2^{\bullet-}$. It initiates peroxidation of various cell substrates, *e.g.* membrane lipids. Prevention of kanamycin-induced increase in MDA concentration in the cochlea and in the temporal lobe and cerebellar tissue during treatment with L-NAME (competitive inhibitor of NO synthase) suggests that ROS inducing pathological LPO under conditions of aminoglycoside treatment are mainly generated through the formation of peroxynitrites.

Kanamycin treatment provoked a decrease of AOS activity in the membrane part of the cochlea and temporal lobe of the brain, which was seen from more pronounced activation of LPO processes with Fe^{2+}. Changes in SOD activity in the cochlea and cerebral tissues were oppositely directed. In the cochlea the increase in SOD activity seemed to be an adaptation reaction, while the trend to SOD inhibition in the cerebral tissues reflected adaptation insufficiency of AOS.

Hyperproduction of MDA, observed during kanamycin treatment and associated with LPO intensification at all levels of the auditory analyzer, was fully compensated by simultaneous treatment with natural antioxidant L-carnosine. This was due to certain nonspecific polyvalent potentialities of the dipeptide (neutralization of free radicals, pH buffer properties, chelating of metals with alternating valency, protection of enzyme complexes) [3]. Decrease of LPO activation after treatment with L-carnosine was paralleled by significant normalization of AOS parameters only in the temporal lobe nervous tissue, but not in the cochlear and cerebellar tissues, where just an insignificant antioxidant effect of the dipeptide was observed. The increase in SOD activity in the cochlea and temporal cortex in rats treated with carnosine confirms the antioxidant effects of this dipeptide.

Treatment with L-NAME promoted a decrease of LPO activation by Fe^{2+} and an increase of SOD activity in the temporal tissues, but it was not paralleled by normalization of AOS. The increase in SOD activity in the ear and temporal cortex in rats attests to a preventive antioxidant effect of L-NAME.

The results of this study and the data on similar effects of L-carnosine under conditions of acute auditory injury against the background of reduced severity of degenerative changes in the cochlear neuroepithelium [4] indicate a positive otoprotective potential of exogenous L-carnosine. A pronounced protective effect manifesting on different models of sensorineural injury to the auditory analyzer prompts further investigation of L-carnosine capacity to penetrate through the blood-labyrinth barrier.

Hence, our study confirmed that the protective effect of L-carnosine [3], manifesting, among other things, in inhibition of LPO induced by pathogenic factors, is particularly intense in sensitive tissue structures and in the presence of pathological uncompensated hyperproduction of ROS paralleled by decrease in the antioxidant defense system reserve.

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