Correction of L-NAME-Induced Disturbances in DNA Synthesis and Free Radical Oxidation in Respiratory Organs of Newborn Albino Rats with Dalargin

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 5, pp. 501-503, May, 2002
Original article submitted April 18, 2002

Intraperitoneal injections of L-NAME (5 injections, 9.3×10^{-5} mol/kg each) to albino rats from the 2nd to 6th day of life inhibited DNA synthesis in epithelial cells, stimulated this process in bronchial smooth muscle cells, and intensified free radical oxidation in the lungs. Dalargin administered in a dose of 1.4×10^{-7} mol/kg 30 min after treatment with N^G-nitro-L-arginine methyl ester abolished its effect on epithelial cells and attenuated changes in smooth muscle cells. Correcting activity of dalargin is associated with its antiradical and antioxidant properties.

Key Words: *DNA synthesis; nitric oxide; free radical oxidation*

Our previous studies showed that the disturbances in constitutive nitric oxide (NO) synthesis promoted changes in structural homeostasis in epithelial and smooth muscle cells of the respiratory tract in newborn albino rats [4]. The synthetic analogue of leu-enkephalin dalargin possesses pronounced antioxidant and cytoprotective properties [1,5]. Here we studied the effects of dalargin on DNA synthesis in epithelial and smooth muscle cells and free radical oxidation (FRO) in the lungs of newborn albino rats treated with constitutive NO-synthase inhibitor L-NAME.

MATERIALS AND METHODS

Experiments were performed on 105 newborn albino rats. The animals received intraperitoneal injections of substances from the 2nd to 6th day of life. Group 1 and 2 rats were injected with 1.4×10^{-7} mol/kg dalargin (D-Ala^2-Leu^5-Arg^6-enkephalin) and 9.3×10^{-5} mol/kg L-NAME (N^G-nitro-L-arginine methyl ester, ICN Biomedicals Inc.), respectively. Group 3 rats received dalargin 30 min after administration of L-NAME. Control animals were injected with isotonic NaCl. The rats were decapitated 24 h after the last treatment.

DNA synthesis was studied by autoradiography. The rats were intraperitoneally injected with ^{3}H-thymidine in a dose of 1 μCi/g (1570 TBq/mol) 1 h before decapitation. Autoradiographs were prepared routinely. The number of S-phase cells (index of labeled nuclei, ILN) was estimated in epithelial and smooth muscle cells of cartilaginous bronchi. The intensity of FRO in lung homogenates was estimated by chemiluminescence (CL). CL was recorded on a LS 50B luminescence spectrometer (Perkin Elmer). Signals were standardized using Finlab software. The intensity of spontaneous and Fe^{2+}-induced CL was measured as described elsewhere [3]. Total CL estimated over 1 min of spontaneous CL (S_{SP}) correlated with the intensity of free radical processes. The maximum flash amplitude (h) of Fe^{2+}-induced CL reflected the content of lipid hydroperoxides. Total CL measured over 4 min of the post-flash period (S_{IND}) reflected the rate of free radical formation. Kinetic parameters of H_2O_2-induced luminol-dependent CL [2,9,12] were analyzed by the maximum amplitude of flash 1 (H_1) reflecting

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0007-4888/02/1335-0433$27.00

the intensity of radical generation in Fenton-like reactions, maximum amplitude of flash 2 (H_2) correlating with the activity of antiradical systems, and interval (t) between H_1 and H_2 that depended on the state of antioxidant systems. Parameters of CL were calculated per 1 mg lipids and expressed in relative units. The total lipid content was estimated by the phosphovanillin method using Lachema kits.

The results were analyzed by Student's t test.

RESULTS

Treatment with L-NAME produced various changes in DNA synthesis in epithelial and smooth muscle cells of bronchi (Table 1). ILN decreased by 1.3 times in epithelial cells, but increased by 1.6 times in smooth muscle cells. S_{SP} increased by 2.4 times, hence L-NAME intensified FRO in the lungs (Table 2). These changes were primarily associated with accumulation of lipid hydroperoxides, acceleration of free radical generation, and accumulation of hydroxyl radicals (h, S_{IND}, and H_1 increased by 1.7, 1.9, and 5.3 times, respectively). Disturbances in the free radical state were related to inhibition of antioxidant and antiradical systems: t decreased by 1.6 times, while H_2 increased by 1.7 times. Our results are consistent with published data that repeated treatment with L-NAME is accompanied by the development of oxidative stress [10,13, 15]. The inhibition of DNA synthesis in epithelial cells probably results from tissue-specific oxidative damages, because extremely toxic FRO product peroxynitrite [7] formed in the respiratory system is localized primarily in the epithelium [6,8]. Activation of DNA synthesis in smooth muscle cells of the respiratory tract reflects their reaction to oxidative stress [11,14].

Dalargin had no effect on the count of DNA-synthesizing cells in the epithelium and smooth muscle tissue. ILN remained unchanged (Table 1). We observed inhibition of FRO in the lungs, which was confirmed by a 1.2-fold decrease in S_{SP} (Table 1). S_{IND} decreased by 1.3 times, which indicated deceleration of free radical generation. These changes were accompanied by an increase in the buffer capacity of antioxidant and antiradical systems. H_2 decreased by 1.4 times, while t increased by 1.2 times.

Treatment with dalargin after L-NAME administration prevented the decrease in the count of DNA-synthesizing epithelial cells in the bronchi. Under these conditions ILN did not differ from the control. The content of S-phase smooth muscle cells surpassed the baseline level. ILN 1.3 times surpassed the control. However, in rats receiving L-NAME these changes in ILN were less pronounced (Table 1). Dalargin markedly inhibited FRO and stimulated the antioxidant and antiradical systems in animals receiving L-NAME (Table 2). Parameters of CL in the lungs approached the control level and significantly differed from those in animals receiving L-NAME alone. The intensity of FRO in the lungs decreased, which was confirmed by a 1.6-fold decrease in S_{SP}. These changes were associated with a decrease in lipid hydroperoxide content

TABLE 1. Effects of Dalargin and L-NAME on DNA Synthesis (ILN, %) in Epithelial and Smooth Muscle Cells of Bronchi in Newborn Albino Rats ($M\pm m$)

Cells	Control	Dalargin	L-NAME	L-NAME+dalargin
Epithelial cells	1.86±0.12	1.88±0.14	1.45±0.11*	1.92±0.12
Smooth muscle cells	0.526±0.034	0.550±0.048	0.854±0.071*	0.665±0.039*+

Note. Here and in Table 2: $p<0.05$: *compared to the control, +compared to L-NAME.

TABLE 2. Effects of Dalargin and L-NAME on Chemiluminescence in Lung Homogenates from Newborn Albino Rats ($M\pm m$)

Parameter	Control	Dalargin	L-NAME	L-NAME+dalargin
S_{SP}, rel. units	6.27±0.20	5.13±0.12*	15.14±0.63*	9.48±0.35*+
Fe^{2+}-induced CL				
h, rel. units	0.086±0.002	0.082±0.004	0.145±0.005*	0.109±0.003*+
S_{IND}, rel. units	44.48±1.75	35.25±1.16*	85.49±2.37*	56.62±1.53*+
H_2O_2-induced luminol-dependent CL				
H_1, rel. units	0.138±0.007	0.129±0.004	0.742±0.005*	0.178±0.005*+
t, min	0.84±0.04	0.99±0.03*	0.52±0.02*	0.69±0.04*+
H_2, rel. units	0.473±0.023	0.338±0.028*	0.794±0.048*	0.636±0.012*+

and deceleration of free radical generation (including hydroxyl radicals). S_{IND}, h, and H_1 decreased by 1.5, 1.3, and 4.3 times, respectively. Activation of antioxidant and antiradical systems promoted changes in the oxidative state; t increased by 1.3 times, while H_2 decreased by 1.3 times.

Our findings suggest that in the early postnatal period dalargin shifts the balance between oxidizing and reducing components of the free radical system in the lungs toward the prevalence of reducing components. This is observed not only under normal conditions, but also during oxidative stress caused by L-NAME treatment. These data indicate that dalargin corrects DNA synthesis in bronchial epithelial and smooth muscle cells during inhibition of constitutive DNA synthesis, which is associated with antioxidant and antiradical properties of the preparation.

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