Effect of Dalargin on DNA Synthesis in the Gastric Mucosa of Albino Rats

E. U. Zivotova, M. U. Fleishman, O. A. Lebedko*, E. N. Sazonova, and S. S. Timoshin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 9, pp. 288-290, September, 2007
Original article submitted June 29, 2007

^{3}H-thymidine autoradiography and chemiluminescence study demonstrated pronounced effect of dalargin on the state of the gastric mucosa in albino rats. Dalargin stimulated DNA synthesis in the epithelium of the gastric mucosa and increased buffer capacity of its antiradical and antioxidant systems. Dalargin analogue not containing arginine ([D-Ala2]-leu-enkephalin) had little effect on these parameters. NO synthase inhibitor L-NAME abolished the effects of dalargin on DNA synthesis in the gastric mucosa. Our results suggest that the NO system plays an important role in the effect of dalargin on the gastric mucosa.

Key Words: *opioid peptides; stomach; proliferation; nitric oxide*

Dalargin is a synthetic analogue of leu-enkephalin. This pharmacological agent is extensively used for the therapy of ulcer disease of the stomach and duodenum [2]. Previous studies showed that dalargin improves the course of gastric erosions and ulcers induced by nonsteroid antiinflammatory drugs [4]. The mechanism of the gastroprotective effects of dalargin is poorly understood. The influence of dalargin on the gastric mucosa (GM) can be related to the presence of amino acid L-arginine in its molecule. Arginine is an important component of the NO and NO synthase (NOS) system [10]. Published data show that NO plays a key regulatory role in the maintenance of GM integrity and function [7,12].

We compared the effects of two peptide compounds, dalargin and analogue not containing arginine [D-Ala2]-leu-enkephalin, on proliferative processes and free radical oxidation in GM of experimental animals.

Central Research Laboratory, Far-Eastern State Medical University, Khabarovsk; *Institute of Maternity and Child Welfare, Khabarovsk Branch, Far-Eastern Research Center for Physiology and Pathology of Respiration, Siberian Division of the Russian Academy of Medical Sciences. ***Address for correspondence:*** timoshinss@yandex.ru. S. S. Timoshin

This work was designed to evaluate the role of arginine in the effect of dalargin on GM.

MATERIALS AND METHODS

Experiments were performed on 106 male outbred albino rats weighing 180-250 g. The test peptides, dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg) and non-arginine-containing analogue [D-Ala2]-leu-enkephalin (Tyr-D-Ala-Gly-Phe-Leu, Laboratory of Peptide Synthesis), were injected intraperitoneally in a single dose of 100 μg/kg for 5 days. Control animals received an equivalent volume of sterile isotonic NaCl.

Nonselective NOS inhibitor (L-NAME, ICN Biomedicals Inc.) in a dose of 9.3×10^{-5} mol/kg was administered 30 min before dalargin.

Proliferative activity of the GM epithelium was studied by ^{3}H-thymidine autoradiography. ^{3}H-thymidine in a dose of 0.6 μCi/g (specific activity 1570 TBq/mol) was administered to experimental animals 1 h before euthanasia. Autoradiographs were prepared by the standard method with Kodak photoemulsions for autoradiography [6].

The index of labeled nuclei (ILN) was estimated by counting of 2500-3000 epitheliocytes in GM

0007-4888/07/1443-0314

(%). Longitudinal full-thickness sections of gastric glands were used to evaluate ILN. The labeling intensity was calculated as the mean number of tracks per 50 epitheliocyte nuclei.

The method of chemiluminescence (CL) was used to study free radical oxidation. CL was recorded on a LS-50B luminescence spectrometer (Perkin Elmer). The signal was standardized with Finlab software. Spontaneous and Fe^{2+}-induced CL was estimated as described elsewhere [5]. The total yield of spontaneous CL (S_{SP}) measured over 1 min reflected the intensity of free radical processes. The maximum of fast flash (H1) of induced CL reflected the content of lipid hydroperoxides. The total yield of CL ($S1_{IND}$) over 4 min after fast flash reflected the rate of peroxide radical formation.

Kinetic parameters of H_2O_2-induced luminol-dependent CL were estimated as described elsewhere [3,9,11]. The maximum of fast flash (H2) reflected the intensity of radical generation in a Fenton-like reaction. Total CL yield ($S2_{IND}$) was recorded over 4 min and depended on activity of the antioxidant and antiradical defense system. Parameters of CL were studied in homogenates of the stomach from albino rats.

Intergroup differences were significant at $p<0.05$ (Student's t test).

RESULTS

Fivefold administration of dalargin to male albino rats was followed by a significant increase in DNA synthesis in the GM epithelium. ILN increased by 40.3%, while the labeling intensity remained unchanged (Fig. 1). Hence, dalargin increased the number of proliferating epitheliocytes in GM. The stimulatory effect of dalargin on proliferative activity of the GM epithelium was reported previously [8].

Fivefold administration of [D-Ala2]-leu-enkephalin had little effect on proliferative activity of GM (Fig. 1). ILN and labeling intensity in treated animals did not differ from the control. Previous studies showed that leu-enkephalin does not modulate DNA synthesis in the GM epithelium of albino rats [1].

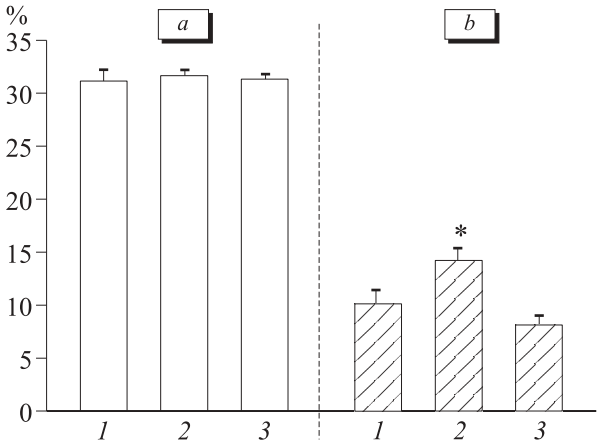

Fig. 1. Effects of dalargin and [D-Ala2]-leu-enkephalin on DNA synthesis in the GM epithelium of albino rats. Labeling intensity (*a*) and ILN (*b*). Control (*1*); dalargin (*2*); and [D-Ala2]-leu-enkephalin (*3*). *$p<0.05$ compared to the control.

Dalargin increased buffer capacity of antiradical and antioxidant systems in stomach homogenates, which was seen from significant changes in all CL parameters. As differentiated from dalargin, its analogue containing no arginine had no effect on free radical processes in GM (Table 1).

The absence of arginine in the peptide molecule abolished its effect on DNA synthesis and free radical oxidation in the GM epithelium. The key role of arginine in the influence of dalargin is probably associated with the fact that this amino acid serves as a substrate for NO.

Fivefold administration of L-NAME had little effect on DNA synthesis in the GM epithelium of experimental animals (Table 2). It should be emphasized that dalargin did not modulate DNA synthesis in GM after inhibition of NOS activity with L-NAME. No significant changes in ILN and labeling intensity were found in animals receiving dalargin and L-NAME. Therefore, NOS inhibitor abolished the stimulatory effect of dalargin on proli-

TABLE 1. Effects of Dalargin and [D-Ala2]-Leu-Enkephalin on CL Parameters in Homogenates of the Stomach from Albino Rats (rel. units, $M\pm m$)

Parameter	Control	Dalargin	[D-Ala2]-leu-enkephalin
S_{SP}	1.330±0.093	0.531±0.050*	1.405±0.078
H1	2.280±0.077	1.210±0.075*	2.150±0.079
$S1_{IND}$	2.882±0.141	1.638±0.076*	3.030±1.183
H2	2.150±0.106	1.390±0.130*	2.260±0.154
$S2_{IND}$	1.090±0.099	0.440±0.030*	1.290±0.084

Note. *$p<0.05$ compared to the control.

TABLE 2. DNA Synthesis in the GM Epithelium of Rats after 5-Fold Administration of Dalargin and L-NAME (*M*±*m*)

Group	ILN, %	Intensity of label
Control	7.19±1.13	11.92±0.52
L-NAME	8.32±0.80	12.90±0.46
L-NAME+dalargin	5.60±0.80	9.72±0.34

ferative processes in the GM epithelium. Our results support the data that the presence of arginine in the dalargin molecule contributes to the effect of this peptide on DNA synthesis.

We conclude that amino acid arginine and NO-NOS system play an important role in the gastroprotective effect of dalargin.

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