Gastroprotective Effect of Dalargin in Gastropathy due to Treatment with Nonsteroid Antiinflammatory Drugs

E. Yu. Zhivotova, M. Yu. Fleishman, E. N. Sazonova, O. A. Lebed'ko*, and S. S. Timoshin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 4, pp. 422-425, April, 2009
Original article submitted October 21, 2008

We studied the effect of dalargin and its analogue [Dala]^2-leu-enkephalin on the gastric mucosa in indomethacin-receiving animals. Indomethacin treatment was followed by severe injury to the gastric mucosa, decrease in proliferative activity of the epithelium, and stimulation of free-radical processes in gastric tissues. Dalargin significantly decreased the area of erosive and ulcerative lesions, had a normalizing effect on proliferation of epithelial cells, and reduced the degree of oxidative stress. Administration of [Dala]^2-leu-enkephalin did not improve the state of the gastric mucosa.

Key Words: *dalargin; nonsteroid antiinflammatory drug-induced gastropathy; DNA synthesis; free radical oxidation*

Nonsteroid antiinflammatory drugs (NSAID) are extensively used in clinical practice. More than 300 million people annually receive NSAID. It should be emphasized that more than 50% patients have NSAID-induced gastropathy with a variety of endoscopic and clinical signs, including subepithelial hemorrhages, erosions, and ulcers [7].

Dalargin is a synthetic analogue of leu-enkephalin. This pharmaceutical product is extensively used for the therapy of ulcer disease of the stomach and duodenum [1]. Our previous studies showed that dalargin improves the course of gastric erosions and ulcers induced by NSAID [3]. The mitogenic effect and antioxidant properties of dalargin are manifested only in the presence of amino acid arginine [5]. It is interesting to evaluate the gastroprotective effect of dalargin under conditions of impaired of tissue homeostasis in the gastric mucosa (GM) caused by indomethacin treatment.

Here we studied the mechanisms for gastroprotective action of dalargin during NSAID-induced gastropathy.

MATERIALS AND METHODS

Experiments were performed on 142 male random-bred albino rats (180-250 g) and male albino mice (25-30 g). Indomethacin in a dose of 250 mg/kg was administered intragastrically through a probe to produce NSAID-induced gastropathy.

The test peptides, dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg) and non-arginine-containing analogue [Dala]^2-leu-enkephalin (Tyr-D-Ala-Gly-Phe-Leu, Laboratory of Peptide Synthesis, Russian Cardiology Center), were injected intraperitoneally in a single daily dose of 100 μg/kg for 5 days. On day 5, the animals were treated intragastrically with indomethacin. The animals were killed after 48 h.

A special series was performed with a non-selective NOS inhibitor NG-nitro-L-arginine methyl

Central Research Laboratory, Far-Eastern State Medical University; *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, Khabarovsk, Russia. ***Address for correspondence:*** timoshinss@yandex.ru. S. S. Timoshin

0007-4888/09/1474-0441

ester (L-NAME, ICN Biomedicals Inc.). L-NAME in a dose of 9.3×10^{-5} mol/kg was injected intraperitoneally 30 min before dalargin treatment.

The area of erosive and ulcerative lesions in GM was measured by means of computerized morphometric analysis on a MEKOS-Ts image analyzer. The study was performed after video capture under a binocular magnifier (×6).

Proliferative activity of 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 animals 1 h before euthanasia. Autoradiographs were prepared by the standard method with Kodak Autoradiography photoemulsions (Emulsion NTB Product code 8895666).

The index of labeled nuclei was estimated by counting of 2500-3000 GM epitheliocytes in longitudinal full-thickness sections of the gastric glands. The intensity of labeling was calculated as the mean number of tracks per 50 epitheliocyte nuclei.

Chemiluminescence assay was used to study free radical oxidation (FRO). Chemiluminescence was recorded on a LS-50B luminescence spectrometer (Perkin Elmer). The signal was standardized with Finlab software. Spontaneous and Fe^{2+}-induced chemiluminescence was determined as described elsewhere [4]. Total spontaneous chemiluminescence is measured over 1 min and correlates with the intensity of FRO. The first flash maximum of induced chemiluminescence reflects the content of lipid hydroperoxides. Total chemiluminescence is recorded over 2 min of the post-flash period and reflects the rate of peroxide radical formation.

Kinetic parameters of H_2O_2-induced luminol-dependent chemiluminescence were estimated as described elsewhere [2,8,11]. The first flash maximum reflects the intensity of radical generation in a Fenton-like reaction. Total chemiluminescence is recorded over 2 min and depends on activity of the antioxidant and antiradical defense system.

The results were analyzed by Student's *t* test. Intergroup differences were significant at $p<0.05$.

RESULTS

Intragastric administration of indomethacin induced severe erosive and ulcerative lesion of GM. Morphometry showed that the average area of lesions was 10.92±2.05 mm^2 (no abnormalities in the control). DNA synthesis was suppressed in the GM epithelium of the gastric fundus. The index of labeled nuclei and labeling intensity were reduced by 36 and 22.4%, respectively (Table 1). Indomethacin-induced decrease in proliferative activity of GM was accompanied by significant activation of FRO in gastric tissues (Table 2). All parameters of chemiluminescence in stomach homogenates were increased by 2.84-5.17 times.

Fivefold pretreatment with dalargin before administration of indomethacin had a strong effect on the response of GM to NSAID. The area of erosive and ulcerative lesions in GM decreased to 1.79±0.54 mm^2. Dalargin completely abolished the adverse effect of indomethacin on proliferative activity of the gastric epithelium. DNA synthesis in animals receiving dalargin and indomethacin did not differ from the control (Table 1). Administration of NSAID after pretreatment with dalargin had a less pronounced effect on FRO (Table 2). The intensity of FRO in stomach homogenates from treated animals was shown to increase by 1.22-1.68 times compared to the intact control. Therefore, dalargin significantly reduces the degree of oxidative stress in experimental animals.

Other results were obtained after injection of a non-arginine-containing dalargin analogue [Dala]^2-leu-enkephalin. The area of erosive and ulcerative lesions in GM of animals receiving NSAID after pretreatment with [Dala]^2-leu-enkephalin (6.92 mm^2) practically did not differ from that in specimens of the indomethacin group. DNA synthesis in GM was significantly suppressed in animals of this group (Table 1). The intensity of FRO in stomach homogenates from animals receiving [Dala]^2-leu-enkephalin and indomethacin was much greater than in control specimens (by 2.24-2.41 times). The degree of changes in FRO in these animals was lower than in indomethacin-receiving specimens, but higher than in the "dalargin+indomethacin" group (Table 2).

Therefore, the gastroprotective effect of dalargin during NSAID-induced gastropathy is not observed in the absence of arginine. Arginine plays a key role in the protective effect of dalargin on GM, which is probably related to the fact that this amino acid serves as a NO substrate. To test this hypothesis, we evaluated the ability of dalargin to normalize proliferative activity of the gastric epithe-

TABLE 1. DNA Synthesis in GM Epithelium in Animals of Various Groups (*M*±*m*)

Group	Index of labeled nuclei, %	Labeling intensity
Control	9.19±0.97	18.11±0.50
Indomethacin	5.88±1.11*	14.05±0.98*
Dalargin+indomethacin	9.07±1.36	16.21±0.97
[Dala]^2-leu-enkephalin+indomethacin	5.39±0.90*	13.60±0.91*

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

TABLE 2. Effects of Dalargin and [Dala]^2-Leu-Enkephalin on Indomethacin-Induced Changes in Chemiluminescence of Stomach Homogenates from Albino Mice (*M*±*m*)

Parameter	Control	Indomethacin	Dalargin+ indomethacin	[Dala]^2-leu-enkephalin+ indomethacin
Total chemiluminescence over 1 min of spontaneous chemiluminescence, rel. units	1.04±0.06	3.52±0.21* (338)	1.75±0.09* (168)	2.44±0.14*+ (235)
Fe^{2+}-induced chemiluminescence				
first flash maximum, rel. units	1.52±0.08	4.31±0.27* (284)	2.44±0.10* (161)	3.40±0.15*+ (224)
total chemiluminescence over 2 min of the post-flash period	2.43±0.09	9.24±0.51* (380)	3.98±0.19* (164)	5.53±0.34*+ (228)
H_2O_2-induced luminol-dependent chemiluminescence				
first flash maximum, rel. units	1.58±0.07	6.77±0.32* (428)	2.11±0.13* (136)	3.78±0.22*+ (239)
total chemiluminescence over 2 min of the post-flash period	0.86±0.05	4.45±0.21* (517)	1.05±0.08* (122)	2.07±0.11*+ (241)

Note. Percent of the control (100%) is shown in brackets. +$p<0.05$ compared to the "dalargin+indomethacin" group.

TABLE 3. DNA Synthesis in GM Epithelium after Bloc-kade of the NO System (*M*±*m*)

Group	Index of labeled nuclei, %	Labeling intensity
Control	4.80±0.74	18.68±0.73
L-NAME+indomethacin	2.09±0.89*	16.35±0.45*
Dalargin+L-NAME+ indomethacin	2.63±0.60*	17.05±0.58

lium under the influence of indomethacin after pharmacological blockade of the NO system (Table 3).

Injection of nonselective NOS inhibitor L-NAME did not modulate the effect of indomethacin on DNA synthesis in the GM epithelium. Administration of indomethacin after 5-fold treatment with L-NAME induced a significant decrease in the index of labeled nuclei (by 57.5%) and labeling intensity (by 13.5%) in the epithelium of albino mice. L-NAME completely abolished the corrective effect of dalargin on DNA synthesis in GM of animals with NSAID-induced gastropathy.

NO is one of the major factors regulating blood flow in GM and, therefore, maintaining the integrity of GM [9]. Previous studies showed that a NO donor Sydnopharm has antiulcer activity [6]. Combined treatment with L-arginine and ibuprofen significantly reduces the adverse effect of NSAID on GM [10].

We conclude that the gastroprotective effect of dalargin during NSAID-induced gastropathy is observed only in the presence of amino acid arginine in an opioid peptide molecule. This effect is mediated by activation of the NO system.

REFERENCES

1. S. A. Alekseenko and S. S. Timoshin, *Klin. Med.*, No. 9, 52-64 (1996).
2. A. V. Arutyunyan, E. E. Dubinina, and N. N. Zybina, *Methods to Study Free Radical Oxidation and Antioxidant System in the Organism. Methodical Recommendations* [in Russian], St. Petersburg (2000).
3. N. A. Bolonyaeva, E. Yu. Zhivotova, M. Yu. Fleishman, *et al.*, *Dal'nevost. Med. Zh.*, No. 2, 28-30 (2005).
4. Yu. A. Vladimirov, O. A. Azizova, A. I. Deev, *et al.*, *Itogi Nauki Tekhniki. Ser. Biofizika*, **29** (1991).
5. E. Yu. Zhivotova, M. Yu. Fleishman, E. N. Sazonova, *et al.*, *Byull. Eksp. Biol. Med.*, **144**, No. 9, 241-244 (2007).
6. S. E. Zhuikova and G. E. Samonina, *Uspekhi Fiziol. Nauk*, **33**, No. 1, 77-87 (2002).
7. A. E. Karateev and V. A. Nasonova, *Ter. Arkhiv*, No. 2, 79-82 (2004).
8. N. Gulyaeva, M. Onufriev, and M. Stepanichev, *Neuroreport*, **6**, No. 1, 94-96 (1994).
9. P. Holzer, *Adv. Exp. Med. Biol.*, **298**, 3-16 (1991).
10. M. D. Jimenez, M. J. Martin, C. Alarcon de la Lastra, *et al.*, *Free Radic. Res.*, **38**, No. 9, 903-911 (2004).
11. A. I. Kuz'menko, *Ukr. Biokhim. Zh.*, **71**, No. 4, 63-66 (1999).