Protective Effect of Dermorphin Analogue Sedatin on Indomethacin-Induced Injury to the Gastric Mucosa

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Administration of indomethacin (250 mg/kg) to mice was followed by the formation of severe ulcerative and erosive injury to the gastric mucosa, inhibition of DNA synthesis, and development of oxidative stress. Fivefold pretreatment with sedatin (100 μg/kg) decreased the area of indomethacin-induced ulcers and erosions, stimulated DNA synthesis, and reduced the severity of oxidative stress. Non-arginine dermorphin analogue did not stimulate DNA synthesis and had no effect on the degree of oxidative stress. After pretreatment with L-NAME, sedatin did not modulate the synthesis of DNA under conditions of indomethacin-induced injury to the gastric mucosa.

Key Words: *sedatin; NSAID gastropathy; DNA synthesis; free radical oxidation*

Synthetic arginine-containing analogue of dermorphin, sedatin (H-Arg-Tyr-D-Ala-Phe-Gly-OH), has a wide range of biological properties. The peptide in various doses can stimulate proliferation [5], *e.g.*, in the epithelium of the gastric mucosa (GM). Sedatin possesses antioxidant and antiradical properties. The presence of arginine is required for a variety of biological effects of sedatin [6]. It is important to evaluate the effect of sedatin on proliferation during the impairment of tissue homeostasis induced by nonsteroid antiinflammatory drug (NSAID) indomethacin. This problem is of particular significance for medical practice. The number of patients with NSAID gastropathy tended to increase in recent years [4]. Despite advances in the prevention and therapy of NSAID gastropathy, the mechanisms for adaptation of GM remain unknown. It is necessary to develop new methods for the therapy of this disorder [13].

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Here we studied the effect of sedatin and its non-arginine analogue on DNA synthesis and antioxidant status during indomethacin-induced injury to GM.

MATERIALS AND METHODS

Experiments were performed on male mice weighing 25-30 g. The study was conducted in the following two series: series I, 4 groups of animals (Tables 1 and 2); and series II, 3 groups of animals (Fig. 1). The control group consisted of intact mice. NSAID gastropathy was induced by intragastric administration of indomethacin in a dose of 250 mg/kg. Sedatin was synthesized at the Peptos Research-and-Production Company. To study the protective effects, sedatin and its non-arginine analogue in a dose of 100 μg/kg were injected intraperitoneally for 5 days. Indomethacin was administered intragastrically (through a probe) on day 5 after peptide treatment. Destructive changes were most pronounced 48 h after the last injection of sedatin and administration of indomethacin. The animals were decapitated. ^{3}H-thymidine in a dose of 0.6 μCi/g (specific activity 1570 TBq/mol) was injected intraperitoneally to animals 1 h before decapitation. The stomach was removed and opened along

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the greater curvature. Images of the damaged mucosa were video captured under a binocular lens (×6) and analyzed using a computerized morphometric attachment (MEKOS-Ts). The protective effect of the test peptide was evaluated morphometrically by calculating the average area of ulcerative and erosive lesions in GM (mm^2).

Samples of the gastric fundus were fixed in a mixture of 96% ethanol and acetic acid in the 3:1 ratio. The preparation of autoradiographs and evaluation of the index of labeled nuclei and intensity of the label (mean number of tracks above 50 nuclei) were performed by the standard method with Kodak Autoradiography Emulsion (Type NTB). The index of labeled nuclei was estimated in the generative zone by counting of 1700-2100 cells. It was expressed in percent.

Free radical oxidation in gastric tissue homogenates from some animals was studied by the method of chemiluminescence (CL). CL was recorded on a LS 50B luminescence spectrometer (Perkin Elmer). Spontaneous and Fe^{2+}-induced CL was evaluated as described elsewhere [2]. The total yield of spontaneous CL (S_{SP}) over 1 min was measured. This parameter correlates with the intensity of free radical generation. The first flash maximum (H1) of induced CL reflects the content of lipid hydroperoxides. Total yield of CL was recorded over 2 min of the post-flash period ($S1_{IND}$); this parameter reflects the rate of lipoperoxide radical accumulation. Kinetic parameters of H_2O_2-induced luminol-dependent CL were estimated as described previously [7,11]. The maximum luminescence (H2) reflects the ability of a biological object to undergo peroxidation. Total CL is recorded over 2 min ($S2_{IND}$) and depends on activity of the antioxidant and antiradical defense system. The intensity of CL (in mV) was calculated per 1 g wet tissue and expressed in relative units.

NOS activity was suppressed by nonspecific inhibitor L-NAME (N^G-nitro-L-arginine methyl ester; ICN Biomedicals Inc.). L-NAME in a dose of 9.3×10^{-5} mol/kg was injected intraperitoneally 30 min before sedatin treatment. One animal received injection of L-NAME in the same dose without sedatin treatment.

The results were analyzed by Student's *t* test (Statistica 5.0 software). Inter-group differences were significant at $p<0.05$.

RESULTS

Administration of indomethacin to animals was followed by destructive changes in GM. This conclusion was derived from the appearance of numerous hemorrhages, erosions, and ulcers. Morphometry showed that the mean area of ulcerative and erosive lesions was 7.43±1.00 mm^2 (Table 1). Dysfunction of GM was not revealed in intact animals.

Activation of free radical oxidation was observed in the gastric tissue of indomethacin-treated mice (3.1-fold increase in S_{SP}; Table 2). The content of lipid hydroperoxides and formation of peroxide radicals were shown to increase under these conditions. Our conclusion was confirmed by the corresponding changes in CL. The amplitude H1 and $S1_{IND}$ increased by 2.7 and 3.8 times, respectively. These changes in the free radical state are related to a decrease in the antioxidant and antiradical protection (4.8-fold increase in $S2_{IND}$) and reduction of peroxide resistance (3.8-fold increase in the amplitude H2). These changes in the free radical state (*i.e.*, variations in CL) reflect the development of oxidative stress. Our results are consistent with published data on the free radical mechanisms for gastric toxicity of NSAID [3].

The inhibition of DNA synthesis is a cause of NSAID-induced ulceration of GM. We revealed a significant decrease in the index of labeled nuclei (by 1.8 times compared to the intact control; Table 1). Significant decrease in the intensity of the label serves as indirect evidence for the delay of DNA synthesis. Pretreatment with sedatin significantly decreased the area of erosive and ulcerative lesion in GM (by 1.75 times). The index of labeled nuclei in these mice was 1.5-fold higher than in indomethacin-treated animals. The intensity of the label in mice of the treatment group did not differ from that in the intact control.

Studying the kinetics of CL showed that pretreatment with sedatin has a normalizing effect on the pro-

TABLE 1. DNA Synthesis and Area of Erosive and Ulcerative Injury to GM in Mice after Administration of Indomethacin and Dermorphin Analogues (*M*±*m*)

Group	Area of GM injury, mm^2	ILN, %	IL
Control	—	9.60±0.34	19.16±0.70
Indomethacin administration	7.43±1.0	5.28±0.20*	11.16±0.90*
Fivefold administration of sedatin and indomethacin	4.24±1.12*	7.92±0.44*	19.20±1.17
Fivefold administration of non-arginine sedatin and indomethacin	6.60±1.02	5.38±0.51*	14.95±1.69*

Note. Here and in Table 2: *$p<0.05$ compared to the control. ILN: index of labeled nuclei; IL: intensity of label.

oxidant-antioxidant state in gastric tissues from indomethacin-treated animals (Table 2). All parameters of CL approached the control level. Parameters of CL in mice of the sedatin group differed significantly from those in indomethacin-treated animals (no correction). Peroxide resistance and activity of the antioxidant and antiradical defense system increased by 2.0 and 2.2 times, respectively. This conclusion was derived from variations in H2 and $S2_{IND}$. Changes in the antioxidant status was accompanied by inhibition of free radical oxidation (1.5-fold decrease in S_{SP}). It was related to inhibition of the initial stage of lipid peroxidation and delayed formation of peroxide radicals (decrease in the amplitude H1 and $S1_{IND}$ by 1.3 and 1.9 times, respectively). Partial correction of the free radical state with sedatin contributes to optimization of reparative processes. Moreover, excessive amounts of free radicals trigger the system of apoptosis that plays an important role in impairment of tissue homeostasis after treatment with NSAID.

Administration of non-arginine analogue did not decrease the area of GM ulceration. This compound had no stimulatory effect on DNA synthesis (Table 1). The intensity of free radical oxidation in mice of the non-arginine analogue group was similar to that in indomethacin-treated animals (Table 2). The absence of the arginine residue in this peptide is accompanied by the loss of antiradical activity. The protective effect of sedatin during indomethacin-induced injury to GM is probably associated with selective correction of abnormalities in the L-arginine—NOS—NO system, which plays an important role in the prooxidant-antioxidant balance [9]. This assumption is confirmed by the results of our experiments. The test parameters did not improve in animals receiving indomethacin and non-arginine analogue of sedatin (as compared to mice of the indomethacin group).

The results of experiments with NOS inhibitor L-NAME also confirm the important role of the L-arginine—NOS—NO system in GM adaptation. Administration of L-NAME to animals receiving indomethacin and sedatin was shown to abolish the cytoprotective effect of sedatin. The index of labeled nuclei in GM of mice receiving L-NAME, sedatin, and indomethacin did not differ from that in animals of the L-NAME+indomethacin group. These parameters in animals of both groups were lower compared to the intact control (Fig. 1).

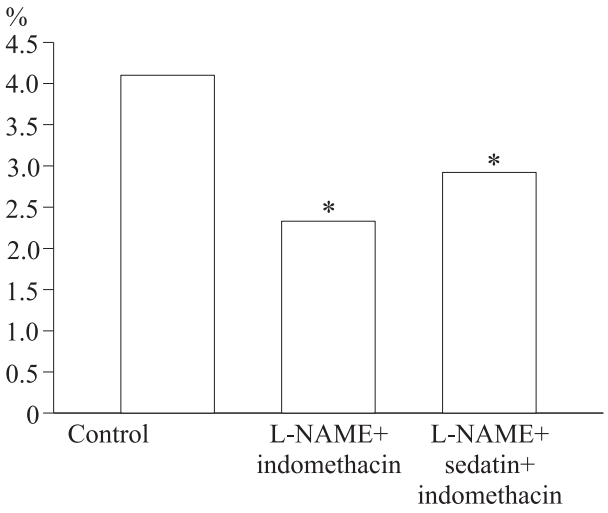

Fig. 1. Index of labeled nuclei in the epithelium of GM from mice after administration of L-NAME. *$p<0.05$ compared to the control.

These data confirm the results of our previous experiments. We conclude that arginine molecule play an important role in the realization of morphogenetic and antioxidant properties of sedatin [6]. Our findings are consistent with published data that the nitroxidergic component is important for GM adaptation to a variety of adverse factors, including NSAID [8,10,12].

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TABLE 2. Effect of Bioactive Peptides on CL of Gastric Homogenates from Albino Mice (*M±m*, rel. units)

Group	S_{SP}	Fe^{2+}-induced CL		H_2O_2-induced luminol-dependent CL	
		H1	$S1_{IND}$	H2	$S2_{IND}$
Control	1.04±0.06	1.52±0.08	2.43±0.09	1.58±0.07	0.86±0.05
Indomethacin	3.26±0.24*	4.17±0.22*	9.32±0.60*	5.94±0.37*	4.12±0.20*
Sedatin and indomethacin	2.17±0.11*	3.10±0.19*	4.87±0.25*	2.95±0.19*	1.89±0.14*
Non-arginine sedatin and indomethacin	3.12±0.21*	4.05±0.26*	8.85±0.57*	6.15±0.30*	4.61±0.25*

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