Effect of HMG-CoA-Reductase Inhibitor on DNA Synthesis and Free Radical Oxidation in the Gastric Mucosa under Normal Conditions and during Indometacin-Induced Ulcerative Process in the Stomach of Albino Mice

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We studied the effect of simvastatin (24 mg/kg *per os* for 30 days) on DNA synthesis (^{3}H-thymidine autoradiography) and free radical oxidation (chemiluminescent method) in the gastric mucosa of albino mice under normal conditions and in ulcerative process induced by single indometacin administration. Simvastatin treatment activated free radical oxidation, which was seen from enhanced chemiluminescence in the mucosa homogenate (by 1.7-4.6 times). Administration of indometacin against the background of simvastatin treatment potentiated local oxidative stress and inhibited DNA synthesis. Under these conditions, the area of ulcerative lesion in the gastric mucosa increased by 3.0 times.

Key Words: *aimvastatin; gastric mucosa; free radical oxidation; DNA synthesis*

HMG-CoA-reductase inhibitors, statins, are obligate preparation for atherosclerosis prevention and treatment. Apart from basic hypolipidemic effect, statins induce a wide spectrum of pleiotropic effects [2]. Published data on the effects of statins on tissue homeostasis are scanty and are primarily focused concentrated essentially on their inhibitory effects on the growth of tumor cells [10] and cell populations of the cardiovascular system [9,14]. The effects of statins on proliferative processes in endodermal epithelia, *e. g.* in the gastric mucosa (GM) are poorly studied.

Non-steroidal antiinflammatory drugs (NSAID) are the most popular type of medicines. A possible complication of NSAID treatment is the development of ulcers and erosions accompanied by bleeding. In clinical practice, statins and NSAID are often used in combinations.

According to experimental data, statins exhibit protective properties in case of NSAID-induced gastrointestinal ulcers [12,13], though there are no data on activity of proliferative processes under these conditions.

Free radicals take part in various pathological processes, including NSAID-gastropathies, and play a role in the regulation of cell division [8]. The data on the influence of statins on free radical oxidation are contradictory [4,5].

Here we evaluate the effect of simvastatin on proliferative processes and local free radical status of GM in intact mice and in mice with NSAID-induced ulceroative gastropathy.

MATERIALS AND METHODS

Experiments were carried out on male albino mice (n=150) weighting 25-30 g. The mice were divided into 4 groups. Group 1 mice received simvastatin (Zo-

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cor, Merck Sharp & Dohme, 24 mg/kg for 30 days) *per os* through a probe in 0.3 ml water suspension [6]. In group 2 (indometacin), ulcers and erosions were induced by single intragastric administration of indometacin in a dose of 250 mg/kg. In group 3 (simvastatin+indometacin), indometacin in dose of 250 mg/kg was administered after 30-day simvastatin treatment and the mice were decapitated 48 h after indometacin injection when the most pronounced destructive changes were noted. Group 4 (control) comprised intact mice.

One hour before decapitation, ^{3}H-thymidine (0.6 μCi/g, specific activity 1570 TBq/mol) was injected intraperitoneally. After decapitation, the stomachs were dissected by the greater curvature. Images of destructed regions were grabbed under a binocular lens (×6). The area of lesions was measured by computer morphometry using Mecos-Ts apparatus. The mean area of ulcers in GM was expressed in mm^2.

Fragments of gastric fundus were fixed in 96% ethanol:acetic acid mixture (3:1). Radioautographs were prepared and index of labeled nuclei (ILN) were determined routinely using KODAK Autoradiography Emulsion (Type NTB). ILN was evaluated in the generative zone after examination of 1700-2100 cells and was expressed in percents.

Integral estimation of free radical oxidation processes in stomach tissue homogenates was made using the chemiluminescence method (CL). CL were registered on chemiluminescent spectrometer LS 50 V PERKIN ELMER. Spontaneous and Fe^{2+}-induced CL were studied as described previously [3]. We determined the yield of spontaneous CL per minute (Ssp) reflecting the intensity of free radical generation, amplitude of fast flash of induced CL (H1) reflecting the contents of lipid hydroperoxide contents, and the yield of Ch during the second minute after the fast flash (S1ind) reflecting the rate of lipoperoxide radical accumulation. CL kinetics initiated by H_2O_2 in the presence of luminol [11] was analyzed by 2 parameters [1]: CL maximum (H2) reflecting the potential of biological object to peroxidation, and CL yield over 2 minutes (S2ind) reflecting activity of antioxidant defense. CL intensity (in mV) per 1 g wet tissue was calculated and expressed in relative units.

The data were processed by Student's *t* test using Statistica 6.0 software. The differences were significant at $p<0.05$.

RESULTS

Chronic administration of simvastatin induced oxidative stress in homogenates of the stomach, which was seen from the increase in the parameters of spontaneous and induced CL by 1.7-4.6 times (Table 1). Our results agree with the data on prooxidant effect of simvastatin [5].

Similarly to our previous experiments, the dynamics of CL parameters in GM in animals receiving indometacin attests to local oxidative stress [7]. Under these conditions ILN decreased by 1.25 times, but these changes were insignificant. Indometacin treatment induced intensive destructive processes in GM: mean area of ulcerative lesions was 2.26±0.78 mm^2 (Table 2).

Combined treatment with simvastatin and indometacin significantly increased the area of ulcerative lesions in GM by 3 times (to 6.93±1.40 mm^2) in comparison to the corresponding parameter in animals receiving indometacin alone.

The increase in lesion area was accompanied by aggravation of oxidative stress; all parameters of free radical status in GM exceeded the corresponding values in "indometacin" group by 1.17-1.25 times (Table 1). Under these conditions ILN in GM of mice receiving simvastatin+indometacin decreased by more then 2 times in comparison with the control level (Table 2).

Thus, combined injection of simvastatin and indometacin aggravates ulcerative process increasing the lesion area and manifestations of local oxidative stress in GM. ILN remained at the level of the cor-

TABLE 1. Effects of Simvastatin and Indometacin on CL Parameters in Homogenates of the Stomach of Male Albino Mice (*M*±*m*; rel. units)

Group	Ssp	Induced CL (Fe^{2+})		Induced CL (luminol-H_2O_2)	
		H1	S1ind	S2ind	H2
Control	1.07±0.07	1.74±0.09	2.54±0.17	1.70±0.09	1.08±0.06
Indometacin	3.70±0.12*	5.13±0.24*	10.88±0.38*	7.16±0.20*	6.12±0.27*
Symvastatin	2.82±0.13*+	3.05±0.12*+	6.36±0.25*+	5.81±0.20*+	4.97±0.25*+
Symvastatin+indometacin	4.63±0.21*+	6.43±0.29*+	13.20±0.55*+	8.40±0.29*+	7.59±0.36*+

Note. Here and in Table 2: $p<0.05$ compared to *control, +indometacin treatment.

responding parameter in mice received indometacin alone (Table 2).

Our previous studies showed that the protective effect of opioid peptides (dalargin and sedatin) in NSAID-induced gastropathies is determined by combination of their antioxidant activity and stimulation of DNA synthesis [7]. Our results confirm of importance of combination of increasing proliferative potential and antioxidant activity in tissue homeostasis maintenance in NSAID-induced gastropathies. The discrepancy between our results and published data on antiulcer activity of statins can be explained by dose-dependent and tissue-specific character of effects of simvastatin on pleiotropic processes, *e.g.* angiogenesis, and their dependence on activity of free radical oxidation [15].

TABLE 2. Parameters of DNA Synthesis and Areas of Ulcerative Lesions in GM of Albino Mice (*M*±*m*)

Group	Area of lesions, mm^2	ILN, %
Control	—	9.48±1.33
Indometacin	2.26±0.78*	4.880±0.764*
Simvastatin	—	7.58±0.81
Simvastatin+ indometacin	6.93±1.40*+	4.250±1.502*

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