Acetylsalicylic Acid as a Modulator of Neutrophil Peroxidase System

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Effect of acetylsalycilic acid (aspirin; Ron-Pulenk) on activity of mouse neutrophil peroxydase system was investigated. Using luminol-dependent chemiluminscence and cytochemical methods we demonstrated that neutrophil peroxydase system in mice receiving aspirin for 14 days is probably determined by stimulation of myeloperoxydase synthesis.

Key Words: *acetylsalycilic acid; leukocyte*

Leukocyte peroxidase system is the molecular basis of nonspecific immunity. It is localized in azurophilic peroxidase-containing granules and consists of myeloperoxidase enzyme (MPO), hydrogen peroxide, and oxidizable cofactor (chloride, bromide) [2]. Peroxidase-containing granules differ from both lysosomes and peroxisomes. Oxygen-dependent activity of the peroxidase system determines the capacity of polymorphonuclear leukocytes to kill bacteria, viruses, and other pathogens [6]. Strengthening of the peroxidase-dependent nonspecific immunity by means of known and widely applied drugs substances is a popular approach. We previously demonstrated potentiation of mouse peroxidase system under the effect of hypolipidemic agent clofibrate (due to stimulation of MPO synthesis in maturating neutrophils) and synthetic peptide dalargin (activates hydrogen peroxide production) [3].

Aspirin is one the most widely used drug around the world. Its anti-inflammatory, antipyretic, analgesic, and antiplatelet effects are well known. Aspirin can reduce the incidence of heart attacks due to its effect on prostacyclin/tromboxane ratio at the stage of platelet hemostasis; the efficiency of aspirin in the treatment of cardiovascular diseases was confirmed by numerous studies in nearly all patient groups [1]. Aspirin is often taken for a long period of time. Bone marrow leukocytes maturate for about 2 weeks, therefore the influence of aspirin on myeloperoxidase synthesis in maturing neutrophils cannot be excluded.

Here we studied the effect of aspirin (Ron-Pulenk) on activity of peroxidase—hydrogen peroxide system in blood neutrophils of outbred albino mice using the method of luminol-dependent chemiluminscence (CL) and cytochemically.

MATERIALS AND METHODS

Outbred albino male mice weighing 18-20 g were used in the study. Experimental mice (n=25) received 20 mg aspirin for 14 days (*per os* 1 time a day, milk suspension). Control group (n=25) received only milk. The control and experimental mice were decapitated under ether anesthesia and the blood was collected into heparinized tubes. For CL analysis, the blood samples were diluted 1:9 with sterile physiological saline. The level of luminol-enhanced CL was detected after phagocytosis induction with polystyrene latex particles (0.8 μ, Serva, 4×10^7 per sample). Luminol (5-amino-2,3-dihydro-1,4-phtalazinedione, Serva) was dissolved in DMSO (Sigma) to a concentration of 10^{-2} M. The final luminal concentration in the cuvette was 10^{-6} M. CL was measured on a Model 1251 luminometer (LKB-Wallac) at 37°C. The results were analyzed by maximum integrated signal in sample triplicates.

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Dried blood smears from the control and experimental animals were fixed for 1 h in alcohol-formalin mixture (9:1). After the fixative was washed out with water, blood smears were incubated at 37°C for 15 min in a medium containing 3,3'-diaminobenzidinee hydrochloride (DAB, Sigma) dissolved in 0.2 M tris buffer (8 mg DAB/10 ml of buffer, pH 7.6) and 3×10^{-3} M H_2O_2 in order to reveal neutophil MPO. For detection of peroxidase—endogenous hydrogen peroxide system in neutrophils, blood smears were incubated for 1 h 15 min at 37°C and H_2O_2 was not added to the incubation medium. After incubation, cell nuclei were stained with 1% methylene green for 30 sec. Activity of peroxidase—endogenous hydrogen peroxide system was estimated by the number of azurophilic peroxidase-containing granules stained with oxidized DAB per 100 neutrophils, and cytochemical index was calculated. To this end, neutrophils were divided into 3 groups: with solitary granules (group 1), with numerous not fusing granules (group 2), and with fused granules (group 3). The number of neutrophils in each group was multiplied by corresponding coefficient (1, 2, or 3) and the results were summed up. Thus, each blood smear was described by a number, cytochemical index. Experiment was repeated 5 times.

Statistical significance of difference between the control and experimental groups was estimated using Student *t* test for related samples.

RESULTS

In our experiments, activity of peripheral blood neutrophil peroxidase system in mice receiving aspirin for 2 weeks increased 2-fold (Fig. 1). The applied method of luminol-dependent CL reflects the level of oxidative metabolism in neutrophils. The cytochemical method demonstrated activation of the peroxidase system in neutrophils only in the incubation medium with hydrogen peroxide (Fig. 2), *i.e.* in the presence of hydrogen peroxide excess when activity of the peroxidase system depends only on peroxidase enzyme (t=-3.16; n=15; p<0.01). The effect of aspirin on peroxidase—endogenous hydrogen peroxide system was statistically insignificant (Fig. 2; t=-0.267; n=15; p<0.05).

These results suggest that the effect of aspirin manifests in stimulation of MPO synthesis in maturating neutrophils or through other way of activation of this enzyme, the major component of non-specific immunity.

There is no intelligible explanation of the mechanism of aspirin action on inflammation, pain, and immune system activation. The effects of antipyretics (*e.g.* aspirin) on the metabolism of immunocompetent cells are mainly studied on isolated cells *in vitro*. It was shown that aspirin suppresses tryptophan degradation and neopterin production by mitogen-stimulated human monocytes [10], inhibits intracellular signal transduction systems, so called NF-κB [9], modulating cytokine production [8], and reduces transport and release of antimicrobial agents azithromycin and moxifloxacin by leukocytes [7]. Effect of pretreatment with aspirin (up to 30 min) in concentrations of 25, 250, 2500 μg/ml on respiratory burst in polymorphonuclear leukocytes was studied *in vitro* [4]. The suppression of the production of superoxide (measured by cytochrome C reduction) and hydrogen peroxide (detected by phenol red microtechnique) depended on aspirin concentration and pretreatment time. Antioxidant and radical-trapping activities of aspirin were revealed [6]. However, these data do not contradict the results ob-

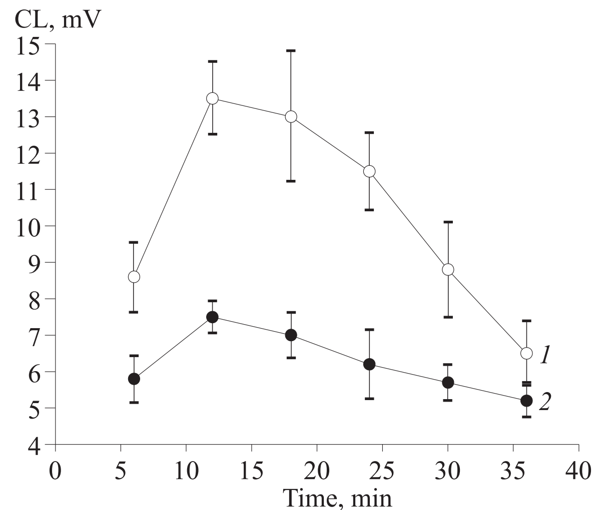

Fig. 1. Luminol-dependent CL of whole blood of mice receiving 20 mg aspirin daily for 2 weeks (*1*) and mice not receiving aspirin (*2*). Each point represents the mean of three measurements±standard deviation. Here and on Fig. 2: results of one experiment are shown.

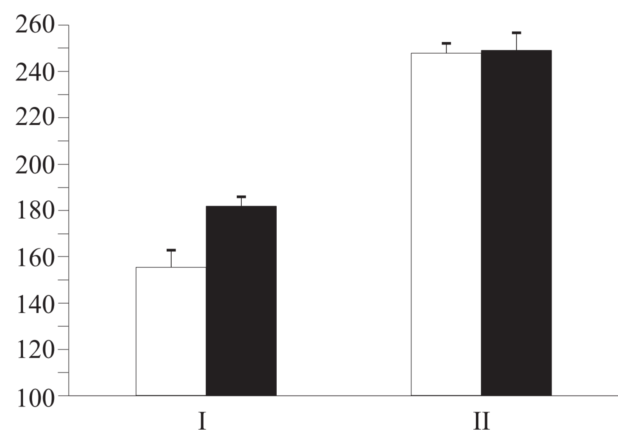

Fig. 2. Cytochemical index calculated for estimation of peroxidase system activity in mouse peripheral blood neutrophils. Light bars: mice not treated with aspirin, dark bars: mice receiving 20 mg aspirin daily for 2 weeks. I: incubation medium with 3×10^{-3} M H_2O_2; II: incubation medium without H_2O_2.

tained in our experiment. Even if aspirin reduces hydrogen peroxide production in leukocytes, activation of MPO or stimulation of its synthesis by aspirin can lead to potentiation of the peroxidase system.

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