Clofibrate and Dalargin Increase Luminol-Dependent Chemiluminescence of Mouse Blood

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The effects of hypolipidemic drug clofibrate and polypeptide dalargin on activity of the neutrophil peroxidase system in mice were studied using the method of luminol-enhanced chemiluminescence. Clofibrate and dalargin increased the chemiluminescence of mouse whole blood. Their combined use several-fold potentiated this effect. It is expected that combined use of hypolipidemics and polypeptides will open a new trend in the search for stimulators of oxygen-dependent nonspecific immunity.

Key Words: *clofibrate; dalargin; neutrophil*

CO-dependent capacity of polymorphonuclear leukocytes to kill bacteria, viruses, and neutralize other pathogens is the key point in defense from infection. The molecular basis of this phenomenon is the myeloperoxidase system located in special organelles (peroxisomes) and consisting of myeloperoxidase, H_2O_2, and oxidizable cofactor (chloride, bromide, iodide, and thiocyanate) [2]. Activity of the peroxidase system can be increased by stimulation of H_2O_2 production via additional activation of superoxide-generating oxidases or the synthesis of myeloperoxidase in maturing neutrophils.

We studied the effects of polypeptide dalargin, a known stimulator of oxidative activity of mature phagocytic neutrophils [12], and hypolipidemic clofibrate, a stimulator of oxidase synthesis and peroxisome formation in animal and plant cells [5,10], on mouse neutrophilic leukocytes.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino mice (18-20 g). Twenty experimental animals were treated with clofibrate (ethyl ester of α-(p-chlorophenoxy)isobutyric acid; Chinoin) in a daily dose of 375 mg/kg (0.05 ml water suspension orally) for 14 days. Twenty controls received 0.05 ml water orally. Dalargin (D-Ala-Gly-Phe-Leu-Arg), a gift from Laboratory of Peptide Synthesis (Russian Cardiology Research-and-Production Complex, Federal Agency of Health Care and Social Development) was added to cuvettes in a concentration of 10^{-4} M before chemiluminescence (CL) measurements. The animals were decapitated under ether narcosis, the blood from control and experimental animals was collected into tubes with heparin. Blood samples for CL measurements were diluted with sterile saline (1:9). Luminol-enhanced CL of leukocytes was measured after induction of phagocytosis. To this end, 0.8-μ polystyrene latex beards (Serva) were added (4×10^7 per cuvette). Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Serva) was dissolved in DMSO (Sigma) to a concentration of 10^{-2} M. The final concentration of luminol in the cuvette was 10^{-6} M. Chemiluminescence was measured on a Model 1251 luminometer (LKB-Wallac) at 37°C. The results of CL measurements were analyzed by maximum values of integral signal in 3 repetitions.

RESULTS

The effect of hypolipidemic clofibrate on animal and human liver cells is well studied. Clofibrate

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causes hepatomegalia, proliferation of endoplasmic reticulum, peroxisomes, and less markedly increases the number of mitochondria [11]. Clofibrate induces changes of this kind in plants as well. The number of peroxisomes increased 5-fold and of mitochondria 2-fold in pea leaves treated with 1 mM clofibrate [10]. Ultrastructural changes are paralleled by induction of some peroxisomal oxidative enzymes, which leads to an increase in the concentrations of active oxygen forms (H_2O_2, O_2^-, $OH^•$) in animal and plant cells and cytochrome P-450 activity (peroxidase compound III) [4,5,8]. On the other hand, activities of catalase and SOD protecting cells from the destructive effects of oxygen radicals decreased under the effect of clofibrate [9,10]. Since H_2O_2 serves as the substrate for neutrophilic leukocyte myeloperoxidase, the increase in its concentration in cells can lead to activation of the peroxidase system and, hence, to stimulation of the neutrophil defense function. In our experiments, luminol-dependent CL in mouse whole blood reflecting activity of neutrophilic peroxidase system increased 2-fold under the effect of clofibrate (Fig. 1). Additional treatment with polypeptide dalargin, an activator of the phagocytic neutrophil oxidase system [12], resulted in a 6-fold increase of CL. Luminol-enhanced CL of the whole blood is a result of total oxidative activity of neutrophilic leukocytes. The CL level can increase not only because of increased production of superoxide radical and then H_2O_2, but also at the expense of myeloperoxidase overproduction under the effect of clofibrate during neutrophils maturation, because in our experiment the animals were treated with clofibrate for 14 days. We previously demonstrated a 2-fold increase in peroxisome volume in neutrophils of mice treated with clofibrate for 2 weeks [3].

It was hypothesized that oxidative stress associated with long proliferation of peroxisomes can serve as initiator of carcinogenesis with participation of active oxygen forms [5,7]. Hypolipidemics are used in combined therapy of atherosclerosis$ they reduce triglyceride level and increase HDL level [1,6]. Our data on clofibrate-induced increase in activity of the neutrophilic peroxidase system characterized by potent antibacterial, antiviral, and antitumor effects indicate that the balance between increased defense potential of the organism and the carcinogenic effect of clofibrate can be attained at certain doses and duration of treatment. Combined use of hypolipidemics and polypeptides opens a new trend in the search for stimulators of oxygen-dependent nonspecific immunity.

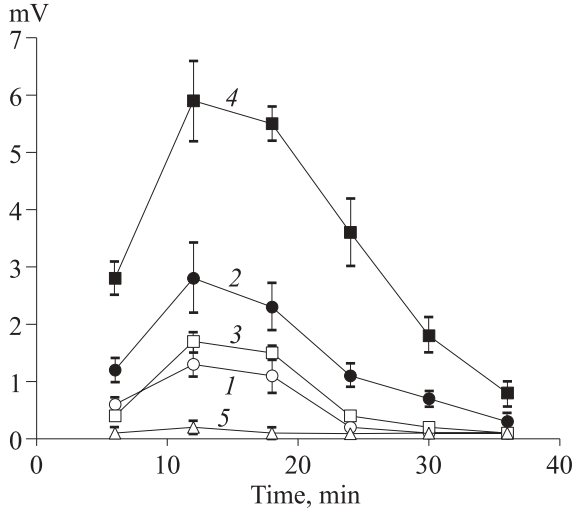

Fig. 1. Effects of clofibrate and dalargin on luminol-enhanced CL of mouse whole blood. *1*) control; *2*) clofibrate; *3*) 10^{-4} M dalargin; *4*) clofibrate+10^{-4} M dalargin; *5*) clofibrate+10^{-4} M dalargin+0.1 M NaN_3.

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