MECHANISMS OF CINNAMATES EFFECT ON REACTIVE OXYGEN AND CHLORINE SPECIES GENERATION IN NEUTROPHILS

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Cinnamates are a wide class of phenolic compounds, the most important of which are cinnamic acid and its hydroxy derivatives. These compounds are found in large quantities in cereals, legumes, oilseeds, vegetables, fruits. It has been shown that various cinnamates representatives have anti-inflammatory, antibacterial, antiproliferative, antitumor, and antioxidant effects [1]. It is known that the level of reactive oxygen and chlorine species (ROCS) produced in blood cells is an important characteristic of the body's metabolic state. Its change serves as a signaling mechanism for triggering various cellular processes, such as differentiation, proliferation, and apoptosis [2]. The molecular-cellular basis of cinnamates' effect on ROCS production by cells is poorly studied.

The purpose of the work is to establish the effect of cinnamic acid and its hydroxy derivatives (caffeic, ferulic, and sinapic acids; concentration range 0.001-10 μM) on the mechanisms of the "respiratory burst" formation in human blood neutrophils.

Neutrophils were isolated from the peripheral blood of healthy people according to the standard method. Myeloperoxidase (MPO) was isolated from the resulting suspension of neutrophils by three freezing/thawing cycles, followed by centrifugation for 20 min at 3000 rpm. The MPO containing supernatant was then analyzed. ROCS production was assessed using the luminol-dependent chemiluminescence (LumCL) method on a BCL-1 chemiluminometer (Minsk, Belarus). Cinnamates' effect on intracellular signaling processes was studied using specific inhibitors of signaling pathways components. MPO activity was assessed by the LumCL in the system "neutrophil lysate-luminol-H_2O_2". The anti- and prooxidant effect of cinnamates was studied in the model systems "luminol-HOCl" and "luminol-H_2O_2".

It has been found that studied cinnamates, depending on the concentration, are capable of exerting anti- or prooxidant effects on neutrophils stimulated by fMLP. Cinnamic, caffeic, and sinapic acids have a multidirectional effect on HOCl formation in the halogenating cycle of MPO localized inside cells or isolated from them, which may be associated with the modification of intracellular signaling pathways in activated neutrophils. Cinnamic acid and its hydroxy derivatives alter the output of ROCS by selective multidirectional correction of the signaling pathway components contribution such as PKC, PI3 kinase, ERK1/2 kinase, and p38-MAP kinase in fMLP-stimulated neutrophils. In model systems caffeic, ferulic, and sinapic acids inhibit ROS release during the luminol oxidation by H_2O_2. According to the inhibition effectiveness the analyzed cinnamates form the following series: sinapic acid > caffeic acid > ferulic acid.

Thus, cinnamic, caffeic, ferulic, and sinapic acids are regulators of neutrophils' functional activity, which is due, firstly, to their pro- or antioxidant action associated with direct interaction with ROS, and secondly, to the cinnamates' effect on signal transduction pathways activation in these cells.

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Bibliographic references

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