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CYTOKININ FRACTION OF THE *Hericium coralloides* INCREASES OXIDATIVE METABOLISM OF MURINE PERITONEAL MACROPHAGIES

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This study was *aimed* to examine influence of cytokinin fraction of basidiomycete *Hericium coralloides* on the spontaneous and induced phagocytic activity of murine peritoneal macrophages

Materials and methods. Mononuclear phagocyte (macrophage) fraction of peritoneal exudate of C57BL/6 mice was used. Macrophages were incubated under standard conditions at 37 °C, 100% humidity and 5% CO_2 for 4 hours. Phorbol 12-myristate 13-acetate (PMA) was added to part of the samples to activate oxidative metabolism. *Hericium coralloides* was added in two concentrations that were 5 and 10 times lower than the IC50, defined as an antiproliferative effect on colon cancer cells. Incubation with samples was carried out for 2 hours.

Result. When adding PMA, *Hericium coralloides* (0.017 and 0.035 μg/ml) and in the combination of PMA with *Hericium coralloides*, activation of reactive oxygen species (ROS) in peritoneal macrophages was revealed by 1.37–1.7 times, compared to the spontaneous activity of phagocytes.

Conclusions. Thus, the effect of the cytokinin extract of the basidial fungus *Hericium coralloides* was manifested by an increase in the phagocytic activity of peritoneal macrophages as one of the possible mechanisms of immunomodulatory action.

Key words: *Hericium coralloides*, NBT test, oxidative metabolism, peritoneal macrophages.

Fruiting bodies and cultivated mycelium of many basidial fungi contain biologically active substances that enhance innate and acquired immune responses and demonstrate antitumor activity in animal and human cell culture [1]. Growing mushrooms in culture is a convenient way to obtain a significant amount of mushroom mycelial biomass, which has all the valuable properties inherent in mushroom fruiting bodies. Previously, we found an antiproliferative, proapoptotic effect and modification of the metabolism of tumor cells *in vitro* for a number of medicinal basidiomycetes (crude extracts and cytokinin fractions) [2, 3].

The most active mushroom was *Hericium coralloides* (Scop.) Pers., strain 2332. Since the enhancement of the antiproliferative effect may be associated with immunomodulatory activity, it was important to determine the effectiveness of the cytokinin fraction of this mushroom on the primary link of innate immunity.

To examine the effect of cytokinin fraction of *Hericium coralloides* on the spontaneous and induced phagocytic activity of murine peritoneal macrophages.

Materials and Methods

Peritoneal macrophages were isolated from C57BL/6 mice by standard procedure according to Pietrangeli [4]. Spontaneous and induced oxidative metabolism was examined in the nitroblue tetrasolium (NBT) test. Briefly, 0.1 ml of NBT in a phosphate-buffered solution was added to the test samples to determine spontaneous activity.

To determine the stimulating activity, 0.1 ml of NBT and 10 ng/ml of phorbol 12-meristat-13-acetate (PMA) were added as an additional stimulus under standard conditions. Cytokinin fraction of *Hericium coralloides* was used at concentrations that were lower than IC_{50} (0.17 μg/ml) by 5 and 10 times (0.035 μg/ml and 0.017 μg/ml, respectively). Only 0.1 ml of phosphate buffer was added to the control wells. The cells were incubated for 1 hours at a temperature of 37 °C in a CO_2 incubator. After incubation, the 96-well plate was centrifuged for 10 min at 400 g. The supernatant was removed, and 0.2 ml of ethanol was added to the sediment. Centrifugation was repeated under the same conditions. After removing the supernatant, 0.1 ml of 0.1M KOH and 0.1 ml of DMSO were added to all wells, and the contents were carefully resuspended. The results were calculated using the spectrophotometric method at a wavelength of 540 nm. Spontaneous activity of peritoneal macrophages was expressed in conventional units. The percentage of stimulation of the activity of peritoneal macrophages was calculated according to the formula:

$$(St - Sp) / 0\ Sp \times 100\%,$$

where Sp — value of the optical density of a spontaneous sample; St — value of the optical density of the sample stimulated with PMA.

Results and Discussion

Spontaneous value of ROS generation in peritoneal macrophages was 0.43±0.035 a.u. (Fig. *A*). Incubation of macrophages with PMA led to an increase in this indicator to 0.63±0.021, which was almost 1.5 times more than spontaneous activity ($P < 0.05$). The addition of *Hericium coralloides* at a concentration of 0.017 μg/ml slightly increased the activity of NBT absorption by macrophages, which was 0.51±0.03 and was not significantly different from the control, while the concentration of *Hericium coralloides* of 0.035 μg/ml led to an increase in phagocytic activity by 37%, compared to the control and was 0.58±0.029 (Fig. *D*). Cell treatment with cytokinin fraction (concentration 0.035) μg/ml along with PMA resulted in the increase of ROS generation by 1.7 times.

It is known that cytokinins exhibit cytotoxic and immunomodulatory effects, they are considered as promising modern biologically active compounds with high therapeutic potential [5].

The content of the following cytokinins trans-zeatin, zeatin riboside and isopentenyl-adenine in *Hericium coralloides* most likely determines their ability individually and together with PMA to stimulate phagocytic activity. Therefore, the issue of

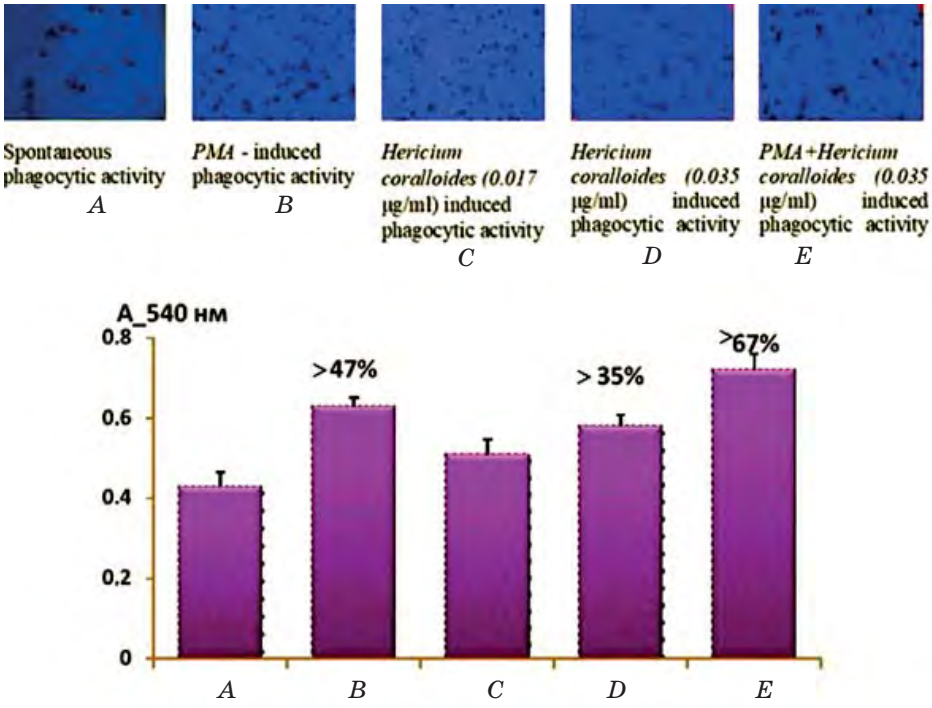

Fig. **Levels of the spontaneous and induced (PMA, *Hericium coralloides* and *Hericium coralloides*+PMA) oxidative metabolism of the peritoneal macrophages showed by the nitroblue tetrasolium (NBT) test**

pharmacological properties of phytohormones produced by mushrooms is extremely relevant and requires further research.

Conclusions

Thus, the effect of the cytokinin extract of the basidial fungus *Hericium coralloides* was manifested by an increase in the phagocytic activity of peritoneal macrophages as one of the possible mechanisms of immunomodulatory action.

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