Antiradical activity of compositions with antimicrobial and wound-healing properties based on humic substances in the cell culture *in vitro*

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Oxidative stress is one of the main reasons of disruption in the process of wound healing, especially if a high level of reactive oxygen species (ROS) persists for a long time. Therefore, investigation of antiradical activity (ARA) for new wound healing substances is a relevant task. The synthesis of nanoparticles containing biologically active silver ultradispersed in the matrices of humic substances and their derivatives (HS-AgNPs) was carried out in the laboratory of natural humic systems (Medicinal Chemistry and Fine Organic Synthesis Division, Lomonosov Moscow State University).

The aim of the current study was to investigate the effect of the initial humic matrices (HS), as well as HS-AgNPs, on the basal and prooxidant-stimulated production of ROS in 3T3-L1 and HepG2 cells *in vitro*.

Intracellular ROS production was assessed using a 2,7-dichlorofluorescein diacetate (DCFDA) fluorescent probe (final concentration 5 μ M) as described [1]. Briefly, hydrogen peroxide at a final concentration 100 μ M was used to stimulate the production of ROS. After 60 minutes of incubation fluorescence was determined in the wells of the culture plate (λ_{ex} =485 nm, λ_{em} =530 nm) using a Tecan® Infinite 200 PRO multifunctional microplate reader (Tecan, Switzerland).

To identify the significance of differences, one-way ANOVA was used. The differences were considered significant at $p < 0.05$.

As a result, it was found that studied HS and HS-AgNPs have an ability to reduce the ROS level stimulated by hydrogen peroxide *in vitro*. Prooxidant properties were not shown for any sample of HS and HS-AgNPs. In general, all samples demonstrated a significantly greater effect on the stimulated ROS level compared with the basal one. The studied samples of HS-AgNPs have a more pronounced ARA in comparison with the initial HS.

Depending on the decrease in their ARA (basal and stimulated ROS levels), all studied HS samples can be arranged in the following row: FA $\rightarrow$ CHP-K $\rightarrow$ CHE-K $\rightarrow$ CHS-K $\rightarrow$ PHF-T3 $\rightarrow$ Peat1 $\rightarrow$ Peat2. When constructing a similar row for the studied HS-AgNPs, the following sequence was obtained: FA-AgNPs $\rightarrow$ CHP-AgNPs $\rightarrow$ CHS-AgNPs $\rightarrow$ CHE-AgNPs $\rightarrow$ PHF-T3-AgNPs $\rightarrow$ Peat2-AgNPs $\rightarrow$ Peat1-AgNPs.

FA sample has the most pronounced ARA (stimulated level of ROS was decreased in 3T3-L1 by 27.9% ($p < 0.05$), in HepG2 - by 32.1% ($p < 0.05$)). FA-AgNPs reduces the basal ROS level in the 3T3-L1 by 20.5% ($p < 0.05$), in HepG2 - by 43.3% ($p < 0.05$), the stimulated level of ROS in 3T3-L1 - by 48.7% ($p < 0.05$), in HepG2 - by 55.9% ($p < 0.05$)).

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References

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