CYCLE BUT THEY OXIDISE ASCORBATE BOTH *IN VITRO* AND *IN VIVO*

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Silver nanoparticles (Ag NPs) have gained particular attention from industrialists due to their relatively low cost of production and tremendously enhanced physical/chemical characteristics. Since the 19th century, the unique antimicrobial and fungicidal properties have been encouraging very wide utilization of Ag NPs in medical products, fabrics, antiseptics, food containers, cosmetics, paints, and even plush toys. As a result, Ag NPs have been the most widely used nanotechnology products in the world over the last century. Nowadays, nearly 25% of all nanotechnology consumer products include Ag NPs (according to Inventory of Nanotechnology Consumer Products). The dramatic increase in industrial use of Ag NPs has raised considerable concern about their potential release and effects on flora and ecosystems, as well as the possibility of it entering human food chain through plants. Silver does not have any established function in plants or animals and has been acknowledged as a trace element. Only few reports have dealt with investigation of the effects of Ag^+ and engineered Ag NPs on living systems at the molecular and cellular level. Ag NPs were shown to promote long-term redox imbalance and oxidative stress in a number of organisms however the mechanism of this toxic effect remains largely unclear. It was recently shown that, under very alkaline pHs, Ag NPs react with H_2O_2, forming Ag^+ and $^{\bullet}O_2^-$, and further with $^{\bullet}O_2^-$ to give "negatively charged Ag NPs" and O_2. Negatively charged Ag NPs can interact with ionic silver (Ag^+) by reducing it to metallic Ag^0, promoting formation of new Ag NPs. Thus Ag NPs can potentially behave as a transition metal ions (such as copper or iron). This has raised a question about possible production of hydroxyl radicals through transition metal catalysed Haber-Weiss cycle involving H_2O_2 and ascorbate, which are both are present in living cells. Here, this hypothesis was tested using Electron Paramagnetic Resonance (EPR) spectroscopy. Another possibility is that Ag NPs affect cell antioxidants, such as L-ascorbic acid, thus decreasing the reducing power and causing accumulation of oxidizing and oxidized species. This question was also addressed in the present study. To examine the capacity of inducing hydroxyl radical generation by Ag NPs, 5,5-dimethyl-pyrroline N-oxide (DMPO) was applied with Fenton-like mixture with Ag NPs or bulk used in-

stead of the transition metal (30-15000 mg L^{-1} Ag NPs or bulk were tested). In contrast to the standard mixture (containing 1 mM $CuCl_2$ instead Ag NPs), the NP-containing mixture was ineffective in the producing characteristic four-peak spectrum of hydroxyl radical DMPO adducts. However, Ag NPs caused the appearance of an ascorbyl radical-specific signal which showed the classical two-peak shape spectrum. Ascorbyl radicals are the products of ascorbate oxidation, relatively long-living and having distinct characteristic spectrum. An addition of natural plant radical scavenger mannitol (1 mM) or key antioxidant glutathione (1 mM) resulted in the disappearance of the ascorbyl radical signal, suggesting that these substances are capable of protecting ascorbate from Ag-NP mediated oxidation. Interestingly, Ag NPs did not induce generation of mannitol or glutathione radicals. Thiourea, caused an effect, which was similar to that of glutathione while bulk or supernatant did not induce ascorbyl radical signal. Application of Ag NPs (3000 and 15000 mg L^{-1}) to intact roots (sterile seedlings of *Arabidopsis thaliana* L. plants) resulted in characteristic ascorbyl radical peaks, which was sensitive to a number of free radical scavengers. High concentrations of H_2O_2, which are known to cause the oxidation of L-ascorbic acid, also induced the formation of the ascorbyl radicals (used as a positive control). Addition of bulk particles or supernatant did not result in the formation of ascorbyl radicals in intact roots. Overall, these results showed that Ag NPs can promote redox imbalance and oxidative stress through affecting cell ascorbate pool but not via production of hydroxyl radicals.

REACTION OF CHOOSEN COMPOUNDS FOUNDED IN EEP WITH FREE RADICALS

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Antioxidant activity of ethanolic extract of Polish propolis (EEP) depends on its chemical composition. Our previous study allowed to identify main phenolic compounds, flavonoids and their derivatives in our EEP. In this study we tested selected compounds (the highest concentration in sample of EEP): caffeic acid, *p*-coumaric acid, ferulic acid and chrisin using three below methods:

1. DPPH radical scavenging activity.

To 0.04 ml test sample was added 0.120 ml methanol. Then the sample was mixed with 0.04 ml DPPH in methanol about (A_{524nm} = 0.900 ± 0.020).