Metabolic effects of silver nanoparticles assessed by NMR metabolomics of mice liver and serum

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Abstract

Silver nanoparticles (Ag-NPs) are among the nanomaterials with highest propensity for human exposure, arising from their established use in wound dressings and increasing incorporation into consumer products (e.g. clothing, food packaging), mainly due to their remarkable antimicrobial properties. However, there is a narrow window between the bactericidal activity of Ag-NPs and their toxicity to human cells[1], making the further understanding of their biological effects a relevant up-to-date subject. Development of metabolic profiling (metabolomics) strategies for assessing the cellular and systemic effects of these nanoparticles may provide a unique and important tool that can be broadly applied in the areas of nanotoxicology and nanomedicine[2].

In this work, male mice were randomly divided into three groups, a control group (n 10) and two experimental groups (n 5 each) i.v. administered with Ag-NPs suspensions (1 mg/mL) and sacrificed at 24 and 48 hours post-injection. A complete necropsy was conducted on all mice. The necropsies included, but were not limited to, examination of the external surface, the cranial, thoracic, abdominal and pelvic compartments, including viscera. Liver, spleen, heart and kidneys were collected, rinsed with physiological serum and weighted. Tissue histopathology parameters and complete haemogram were also assessed. Based on a preliminary biodistribution study, liver tissues and blood serum were collected for metabolic profiling analysis. In particular, the samples were analysed by ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy, using High Resolution Magic Angle Spinning (HRMAS) for direct tissue analysis, and the spectral data subjected to multivariate analysis, namely Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), to highlight the metabolic differences between the groups.

The livers of control and Ag-NPs-exposed mice showed several significant differences in their metabolic composition, already apparent by simple visual inspection of ^{1}H HRMAS spectra (Figure 1A). Indeed, control and exposed groups showed a trend for separation in the PCA scores scatter plot and were clearly discriminated by PLS-DA (Figure 1B). The main metabolic alterations explaining this separation were in the levels of glucose, glycogen and reduced glutathione (decreased in exposed animals compared to controls) and in the levels of choline compounds and taurine (increased in mice exposed to nanoparticles for 24 and 48h, respectively). In regard to serum NMR profiles, while the most apparent alterations were in the levels of lipoprotein subclasses (Figure 2A), several other differences could be found in small metabolites, including increased levels of amino acids (alanine, valine, lysine, histidine, tyrosine, phenylalanine), creatine, choline and glycerol, together with decreased levels of glucose, acetate and fumarate. Interestingly, most of these changes showed a stronger magnitude at 24h than at 48h of Ag-NPs exposure, which explains the time-dependent group separation observed in the PCA and PLS-DA scores plots (Figure 2B). Overall, the results show that Ag-NPs, at a sublethal dose, disturb cellular and systemic metabolism, mainly affecting pathways involved in energy production and antioxidant protection.

References

[1] Kim S and Ryu DY, J. Appl. Toxicol., **33** (2013) 78.
[2] Duarte IF, J. Control. Release **153** (2011) 34.

Figures

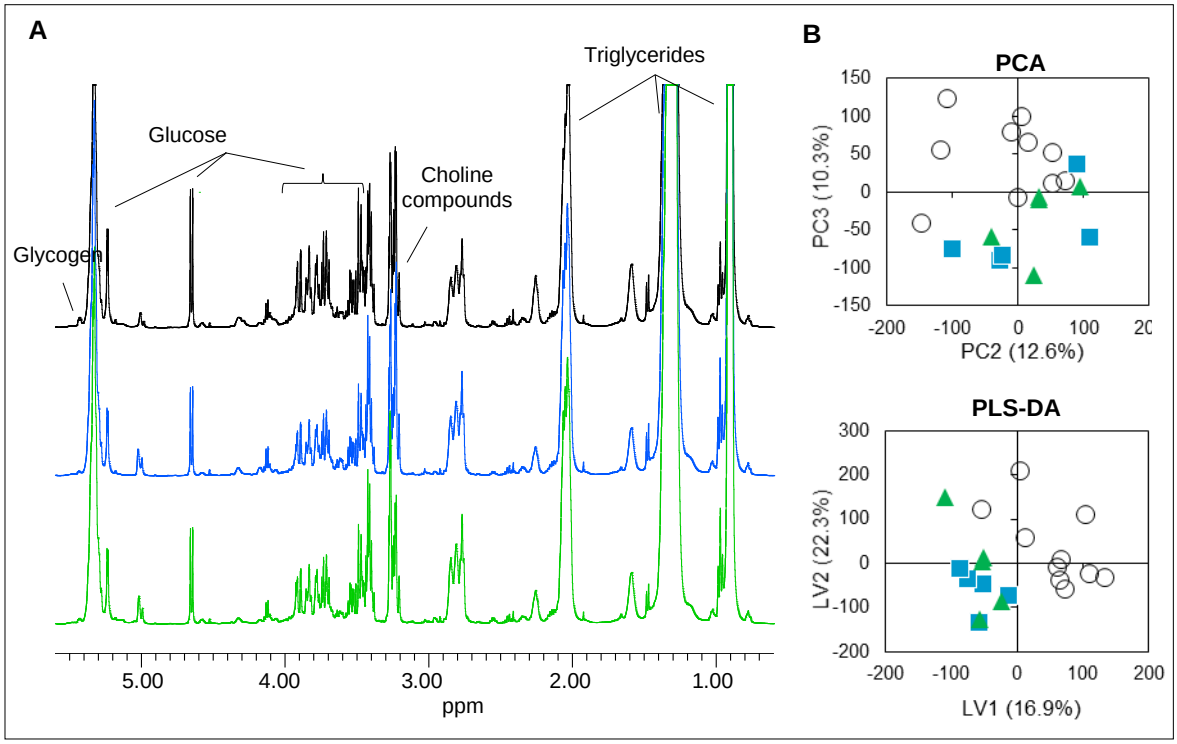

Figure 1. A) Average ^{1}H HRMAS NMR spectra of liver tissue from control mice (top), and mice exposed to Ag-NPs for 24h (middle) and 48h (bottom). **B)** Scores scatter plots obtained by PCA and PLS-DA of NMR liver spectra (○ controls; ■ exposed 24h; ▲ exposed 48h).

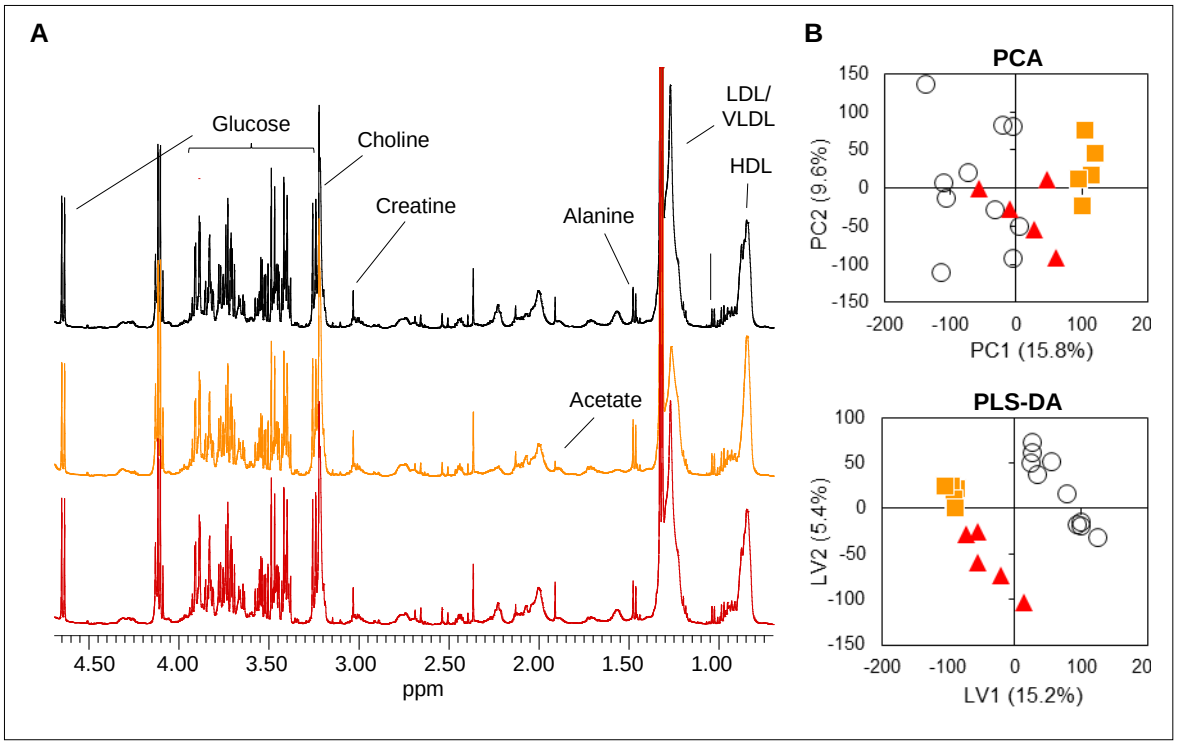

Figure 2. A) Average ^{1}H NMR spectra of blood serum from control mice (top), and mice exposed to Ag-NPs for 24h (middle) and 48h (bottom). **B)** Scores scatter plots obtained by PCA and PLS-DA of NMR serum spectra (○ controls; ■ exposed 24h; ▲ exposed 48h).