Polyvinylpyrrolidone-coated silver nanoparticles induce the expression inducible nitric oxide synthase in intestinal C2BBe1 cells

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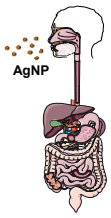
BACKGROUND

Silver nanoparticles (AgNP) present peculiar physical, chemical and biological properties, which makes them even more interesting in their practical application in several sectors, from medicine to food industry. Therefore, AgNP have become part of our daily life, leading to an inevitably increased human exposure, especially by the oral route.

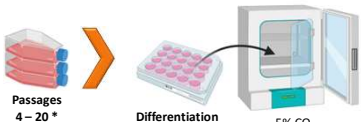
Considering that the intestine contains the first tissues exposed to dietary AgNP, it can be assumed that it may be particularly susceptible to their toxicity and possible pro-inflammatory effects. Despite this, the adverse effects of AgNP on intestinal cells are still unexplored.



***Aim**: Investigate the potentially toxic and pro-inflammatory effects of polyvinylpyrrolidone (PVP)-coated AgNP (5 and 50 nm), in intestinal epithelial C2BBe1 cells*

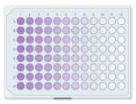
METHODS

C2BBe1 cell culture and differentiation



*Subculturing according to ATCC, with Dulbecco's Modified Eagle's Medium (4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate.)

I. Metabolic activity



MTT reduction assay
(Absorbance reading at 570 nm)

Postive control: Triton-X (1%)

II. Cellular viability

Annexin V/ Propidium Iodide
(Flow Cytometry)

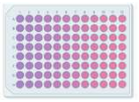
Postive control: DMSO 10%

III. iNOS ; iκBα

Western Blot

Postive control: CytoMix (20 ng/mL IFN-γ; 10 ng/mL IL-1β; 10 ng/mL TNF-α)

IV. Production of NO•



Griess reagent
(Absorbance reading at 550 nm)

Postive control: CytoMix (20 ng/mL IFN-γ; 10 ng/mL IL-1β; 10 ng/mL TNF-α)

RESULTS

I.Metabolic activity

For 50 nm PVP-AgNP (12.5 and 25 µg/mL), it was observed a reduction of metabolic activity of C2BBe1 cells.

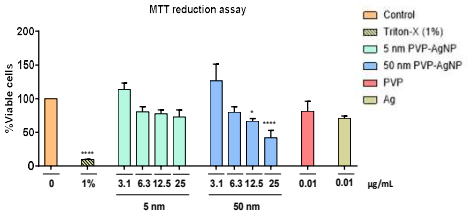
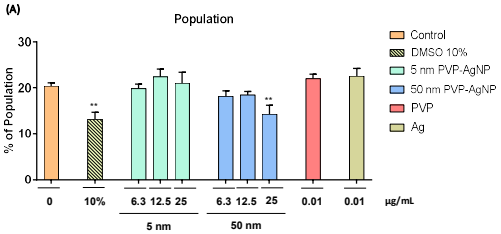


Fig. 1 - Effect of 5 and 50 nm PVP-AgNP on the metabolic activity of C2BBe1 cells, after 24 hours of exposure, measured by MTT reduction assay. *p<0.05, ****p<0.0001, when compared with the control (untreated cells). Values are presented as the means ± SEM (n≥3).

II.Cellular viability

It was also observed a tendency, for the occurrence of early and late apoptotic events, for the 50 nm PVP-AgNP (12.5 and 25 µg/mL).



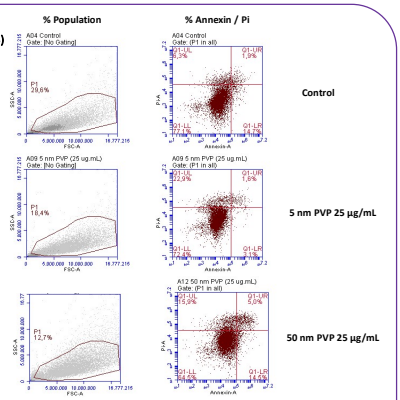
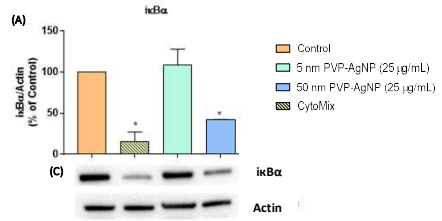


Fig. 2 – (A) Effects of 5 and 50 nm PVP AgNP on the viability C2BBe1 cells, after 24 hours of exposure, measured by annexin V/propidium iodide assay. **p<0.01, when compared with the control (untreated cells). Values are presented as the means ± SEM (n≥3). **(B)** Representative flow cytometry plots of annexin V/propidium iodide assay for control and for 5 and 50 nm PVP-AgNP, at the maximum tested concentration (25 µg/mL).

III. iNOS ; iκBα



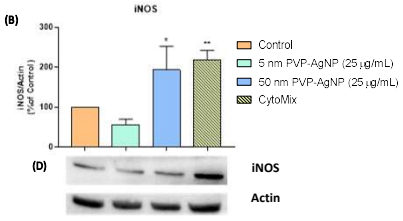


Fig. 3 – (A)(B) Assessment of iκBα and iNOS transcriptional activation, respectively, in C2BBe1 cells, after 24 hours of exposure. Values are presented as the means ± SEM (n≥3). *p < 0.05, **p<0.01, when compared to control (untreated cells). **(C)(D)** Representative western blotting bands showing the expression levels of IκBα and phosphoIκBα. β-actin was used for equal loading of protein.

A pro-inflammatory response was observed after the exposure of C2BBe1 cells with 50 nm PVP-AgNP, reflected by the decrease of iκBα levels and the increase of iNOS levels.

IV.Production of NO•

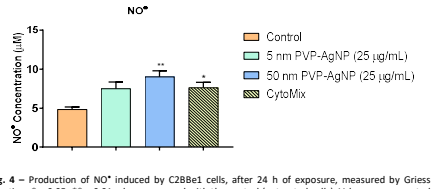


Fig. 4 – Production of NO• induced by C2BBe1 cells, after 24 h of exposure, measured by Griess reaction. *p<0.05, **p<0.01 when compared with the control (untreated cells). Values are presented as the means ± SEM (n≥3).

The increased expression of iNOS levels was followed by increased production of NO•, after exposure to 50 nm PVP-AgNP.

CONCLUSIONS

- It was observed a **decrease of cellular metabolic activity**, which was accompanied by a significant **decrease in the cellular viability**. These events were probably associated with the occurrence of early and late apoptotic events, for the **50 nm PVP-AgNP.**
- The expression levels of **iκBα** decreased after exposure to **50 nm PVP-AgNP.**
- The expression of **inducible nitric oxide synthase (iNOS)** levels **increased**, after exposure to **50 nm PVP-AgNP**. This increase was followed by an **increase in NO• levels**.

Therefore, it can be concluded that larger PVP-AgNP induce prominent activation of a putative inflammatory response by intestinal cells. However, further studies are needed to disclose the mechanistic pathways involved in intestinal pro-inflammatory effects of AgNP.

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