The influence of silver nanoparticles on active caspases levels in Glioblastoma multiforme cells cultured on *in ovo* model

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Objective

The prime feature of tumor development is the escape cells from programmed cell death due to a metabolic abnormalities or genetic mutations. Activation of apoptosis pathways is a key mechanism by which cytotoxic drugs kill tumor cells. However in anticancer therapy of central nervous system tumors drugs concentration in brain tissue is limited because of blood-brain barrier. Due to ability to cross the blood-brain barrier and affinity for acidic environment, silver nanoparticles (AgNPs) can be a useful tool in anticancer therapy of central nervous system tumors, especially those of neuropeithelial origin. The most promising AgNPs property is the induction of tumor cell death by activation of the cascade of caspases which is a critical component of the execution phase of cell death in most forms of apoptosis.

Aim

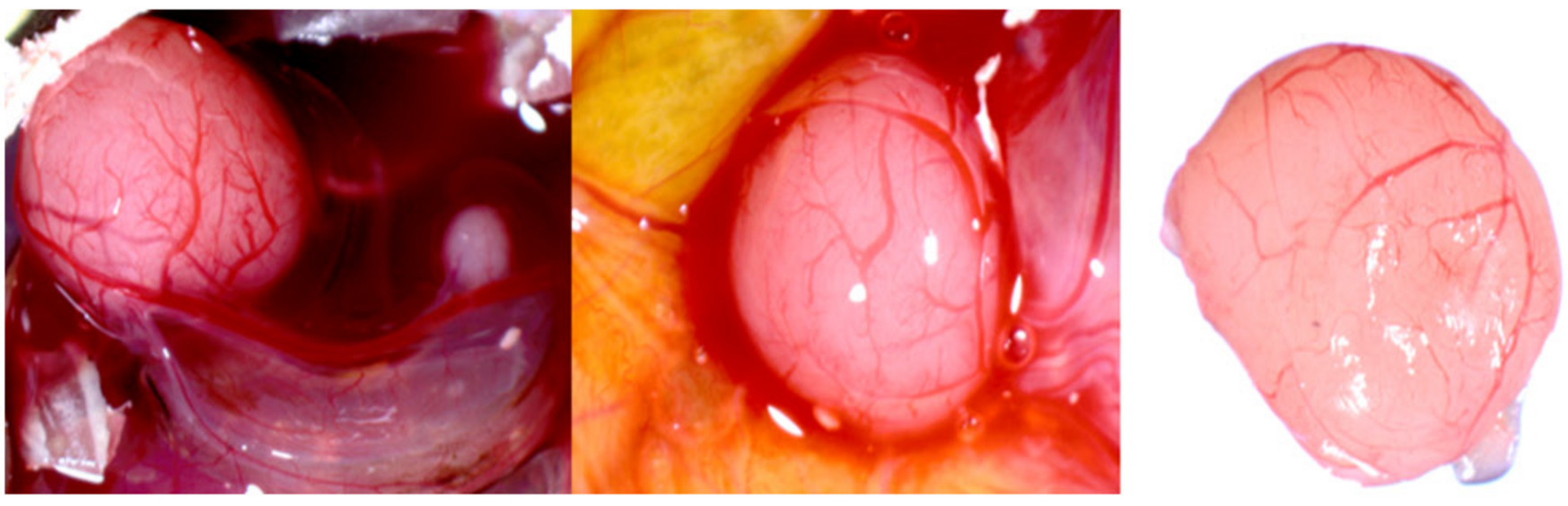
The aim of this study was to evaluate the influence of AgNPs on activation of the intrinsic apoptotic pathway of Glioblastoma multiforme (GBM) cultured on *in ovo* model.

Methods

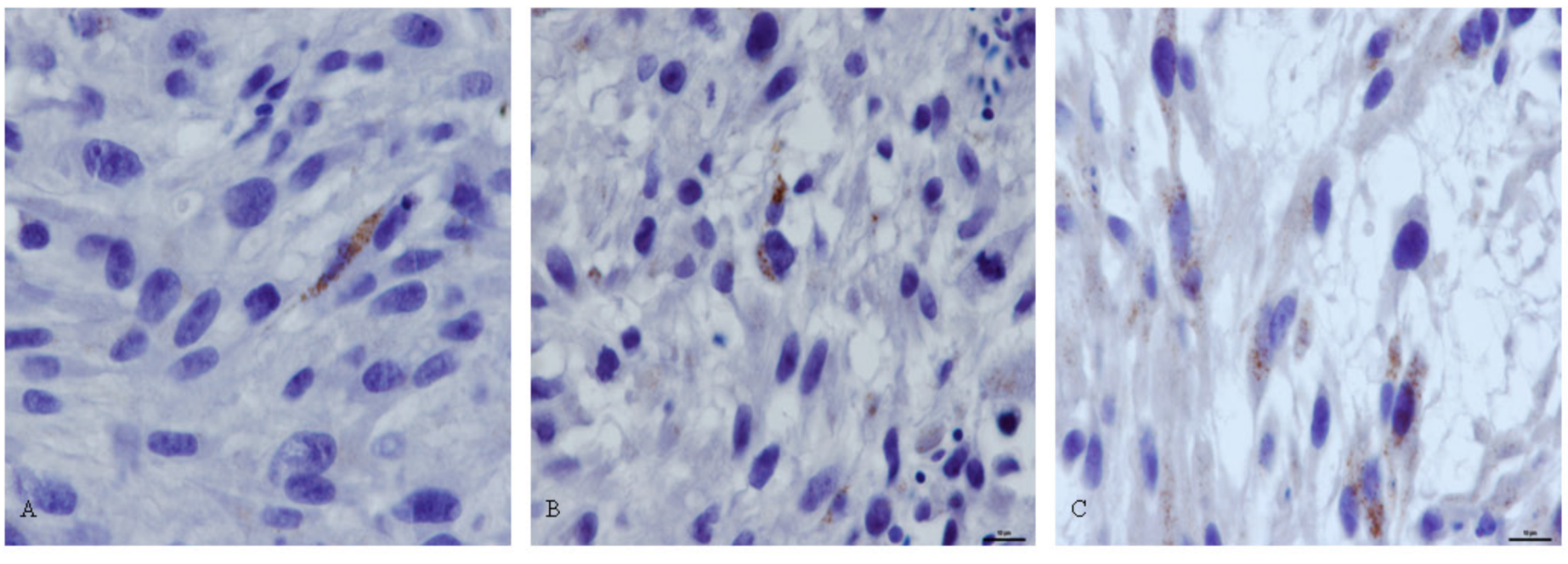
Human GBM cells line U-87 were placed on the chorioallantoic membrane of the chicken embryos on 7^{th} day. At day 14^{th} the tumors were divided into three groups: control (n=20), AgNPs (n=20) – tumors treated with colloidal AgNPs (40 ppm) and placebo (n=15) – tumors supplemented with aqua pro injectione. Four days later tumors were isolated, fixed in 10% neutral buffered formalin and processed by common paraffin technique. Slides were stained immunohistochemically with antibodies against cleaved caspase-9 and cleaved caspase-3. The cleaved caspase-3 and cleaved caspase-9 indices were determined at a magnification of 400x, by counting the number of positive cells/1000 cells.

Results

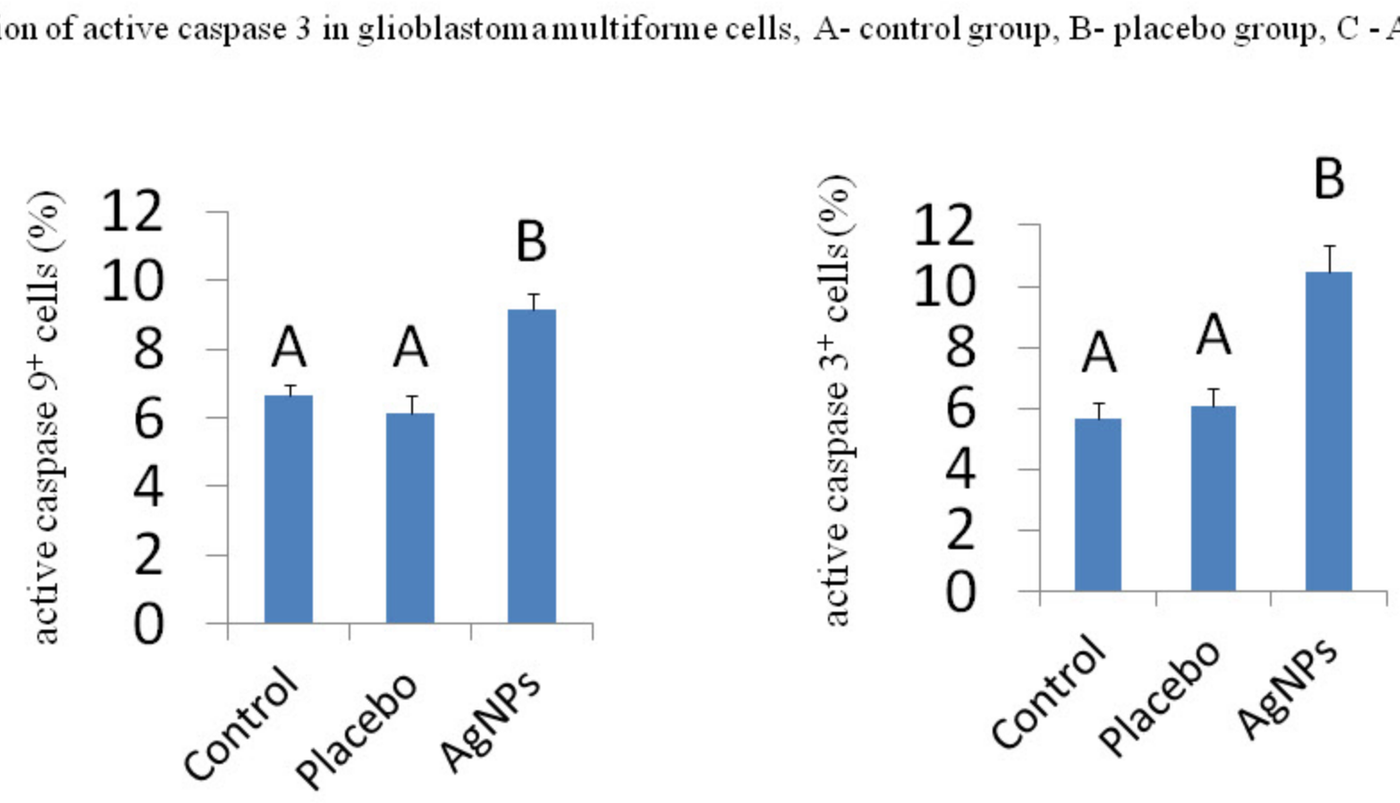
In the control group the number of cleaved caspase-9^+ cells was ranging 4,0%-9,8% (6,71%), while the number of cleaved caspase-3^+cells for GBM control group was 3,2%-12,6% (5,92%). The same examined parameters for AgNPs and placebo group were as follows: 5,3%-12,3% (9,01%), 5,5%-19,5% (10,54%), and 2,8%-10,8% (6,14) , 3,4%-11,3% (6,07), respectively. The mean values for all examined parameters differ significantly between AgNPs and other groups.



Glioblastoma multiforme tumors.



Expression of active caspase 3 in glioblastoma multiforme cells, A- control group, B- placebo group, C - AgNPs group.



Analysis of active caspase 9 and active caspase 3 expression in glioblastoma multifrome cells in groups. Different capital letters denote statistically significant $P \leq 0,01$.

Conclusion

The results of this study have shown that the AgNPs can influence on tumor's growth inhibition, however it required more studies.