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The Effects of Silver Nanoparticles on the Sex Hormones and Fetal Development in Pregnant Wistar Rats

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Abstract

Different nanoparticles have their own unique properties and are used in various fields, but their toxicities to living organisms are less known. The purpose of this study was to evaluate the effects of silver nanoparticles on the sex hormone blood levels and fetal development in pregnant Wistar rats. Five experimental groups were divided, including three treatment groups, one control group, and one injection control group. The treatment groups were administrated silver nanoparticles (250, 500, and 1000 ppm, respectively) intraperitoneally every other day from the 7th day to the 18th day of pregnancy. The injection control and the control groups received normal food and water with and without intraperitoneal injection of 0.5 ml of distilled water, respectively. On the 18th day, rats were investigated for the progesterone and estrogen hormone levels and fetal development. The data showed that silver nanoparticles could lead to hormone level changes and fetal abortion at the used concentrations. Treatment with silver nanoparticles at the concentration of 250 ppm resulted in the highest increase of the progesterone level, greatest reduction of the estrogen level, and abortion of fetuses ($P < 0.05$). Further studies are required to understand the mechanisms underlying these changes.

Keywords: Silver nanoparticles, toxicity, estrogen, progesterone, abortion, and pregnancy

Introduction

Nanotechnology leads to a technological revolution in the new millennium and its implications have a huge potential influencing the world. Nanotechnology is also affecting almost all aspects of human life (1-6). Despite the bright prospects of nanotechnology, the efforts may intentionally or unintentionally endanger human health and environment (7). The people who are susceptible to diseases may be at a great risk. Nanoparticles are attractive from the fundamental science and technological reasons, and human exposure to nanoparticles is progressively being increased (8). Because nanoparticles have distinctive characteristics, evaluation of their toxicities is essential with developed and precise prevention methods (9, 10). At the present time, research in this area is rapidly growing. Modern studies are mostly concentrated on the effects of nanoparticles on human life and environment (11-14).

Silver nanoparticles, one type of the most popular nanoparticles, are used in various industries and medicine because of their antibacterial activities. Despite the widespread use of silver nanoparticles, there is still less information about their biological effects on human cells and environment (15-17). Because of the differences in the application of silver materials, tests, and original tissues (human or animal), the results are inconsistent among different studies. Given that embryonic development in mammals is influenced by the environmental factors, exploring the effects of these nanoparticles on the fetal development is necessary. There are many *in vitro* studies on the toxicity of silver nanoparticles (18-20), but few in *vivo*. The toxic effects of silver nanoparticles on sex hormones have not been investigated yet. This study was attempted to investigate the effects of silver nanoparticles on the progesterone and estrogen hormones and fetal development in pregnant Wistar rats.

Materials and Methods

Thirty female Wistar rats weighing 200 to 250 grams were used for mating in the current study. All rats were randomly divided into five groups (six rats per group) including three treatment groups, one control group, and one injection control group. Considering the formation of vaginal plug (G0), the pregnant rats were maintained for seven days.

Silver nanoparticles at various concentrations were then injected intraperitoneally to the rats every other day from the 7th to the 18th day of pregnancy. The silver nanoparticles used in this study were purchased from Sigma (Ontario, Canada). The nanoparticles have a spherical shape with an average diameter of 10 nm. The detailed information for each group was:

Group 1: Control group: normal food and water

Group 2: Injection control group: injection of 0.5 ml of distilled water to monitor the potential shock induced by injection.

Group 3: Treatment group 1: 0.5 ml of silver nanoparticles at the concentration of 250 ppm.

Group 4: Treatment group 2: 0.5 ml of silver nanoparticles at the concentration of 500 ppm.

Group 5: Treatment group 3: 0.5 ml of silver nanoparticles at the concentration of 1000 ppm.

After the last injection on the 18th day of pregnancy, blood was taken from the rats' heart. The levels of estrogen and progesterone were assessed with the LIAISON kit (DiaSorin, Italy). The numbers of healthy embryos and aborted fetuses were examined.

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Data analysis

ANOVA and Tukey tests were conducted for data analysis using the SPSS software (version 20). All data were presented as mean ± standard deviation (SD). $P < 0.05$ was considered significant.

Results

Increase of the progesterone level

Among the three treatment groups, the highest blood level of progesterone was observed in the rats treated with the silver nanoparticles at the concentration of 250 ppm (26,290 pg/ml ± 3,950.24) and the lowest level (7,836.7 pg/ml ± 1,041.64) at the concentration of 1000 ppm. The blood level of progesterone was the lowest in the control group among all groups including the injection control group (7,775.72 pg/ml ± 912) (Figure 1). Tukey test showed a significant difference in the progesterone level between the treatment groups and the injection control group as well as the control group (both $P < 0.05$). It was also significantly different between the 250 ppm treatment group and the other two treatment groups ($P < 0.05$).

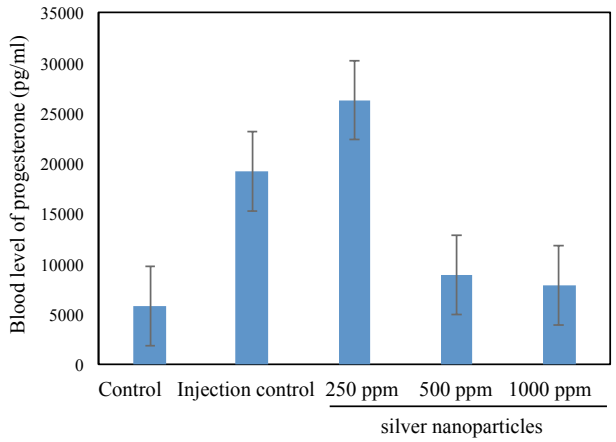

Fig. 1. Progesterone blood levels following treatment with silver nanoparticles.

Decrease of the estrogen level

Evaluation of the estrogen levels showed that, among the treatment groups, the group treated at the concentration of 500 ppm had the highest level of estrogen hormone (70,759 pg/ml ± 880.75) and the group treated at the concentration of 1000 ppm had the lowest level (45,825 pg/ml ± 3,256.23). Among the all five groups, the injection control group presented the highest estrogen level (76,765 pg/ml ± 4,813.23) (Figure 2).

Tukey test revealed significant differences for the estrogen hormone level between the following any two groups: control group *vs*. injection control group, 250 *vs*. 500 ppm groups, 1000 ppm group *vs*. injection control group, and 250 or 500 ppm groups *vs*. control group ($P < 0.001$).

No change in the number of healthy embryos

The results showed that the number of healthy embryos was higher with the 1000 ppm concentration ($n = 9$) than with the 250 and 500 ppm concentrations (both, $n = 8$). The number of healthy embryos was 10 in both control and injection control groups (Figure 3). There was no significant difference among groups regarding the number of healthy embryos.

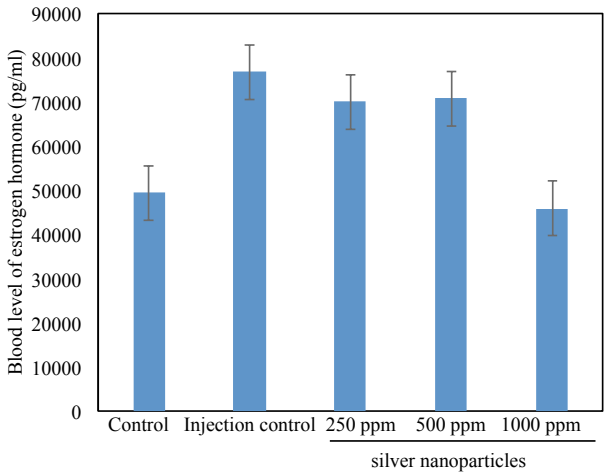

Fig. 2. Estrogen blood levels following treatment with silver nanoparticles.

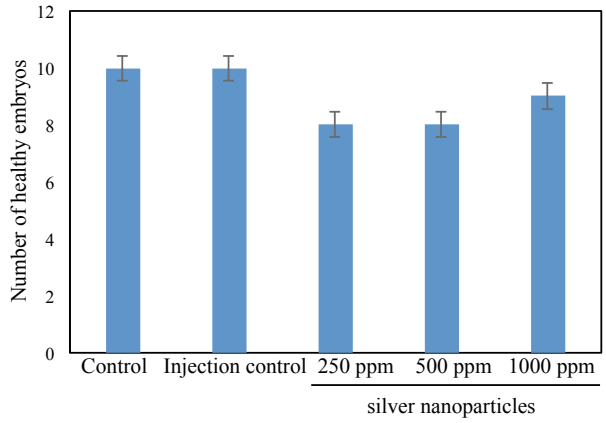

Fig. 3. Number of healthy embryos following treatment with silver nanoparticles.

Silver nanoparticles led to fetal abortion

As shown in the Figures 4-6, the numbers of aborted fetuses were different among the groups. The highest number of aborted fetuses was observed in the group treated at the 250 ppm concentration ($n = 9$). No aborted fetuses were observed in the 1000 ppm group and the control group. The number of aborted fetuses in the injection control group was 8 (Figure 4).

The differences reached significance for the number of aborted fetuses ($P < 0.001$) between the following any two groups: control group *vs.* injection control group, 250 *vs*. 500 ppm groups, and 1,000 ppm group *vs*. the injection control group.

Discussion

In the present study, the blood levels of estrogen and progesterone hormones as well as the development of embryos were analyzed following exposure of the pregnant rats to different concentrations of silver nanoparticles (250, 500, and 1000 ppm, respectively).

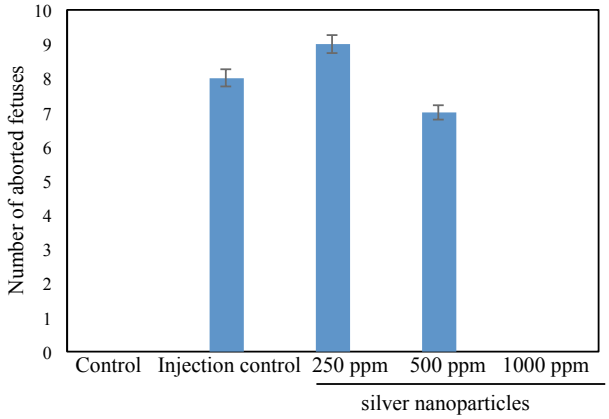

Fig. 4. Number of aborted fetuses following treatment with silver nanoparticles.

Changes in the progesterone and estrogen blood levels

The assessment on the effect of silver nanoparticles on the progesterone showed increase in the progesterone level compared to the control group in a way that treatment at the concentration of 250 ppm led to the highest level, followed by the treatment at the other two concentrations. The lowest level of progesterone hormone was observed in the control group. The mechanisms underlying the change of progesterone level need to be studied. Given the observed changes, it could be concluded that silver nanoparticles at these three concentrations could result in increase of progesterone level and the increase was more prominent at the low concentration (250 ppm). The results also suggested that higher concentrations of silver nanoparticles had limited effect on the progesterone hormone level.

Regarding the effect on estrogen, it was shown that silver nanoparticles could change the estrogen hormone level in a way that lower concentrations (i.e. 250 and 500 ppm) increased the estrogen level and higher concentration (1000 ppm) decreased the estrogen level in comparison with that of control group. Change of the estrogen level induced by the silver nanoparticles was associated with the concentrations of the silver nanoparticles. Recent studies have proven that silver nanoparticles can have different effects on different body parts of living organisms, although the mechanisms are unknown. For both of the progesterone and estrogen hormones, high concentration of silver nanoparticles induced less change of their blood levels. Studies have shown that aggregation may occur at high concentrations and this phenomenon may lead to weakening of the biological activities of silver nanoparticles. Therefore, less change of the hormone levels at high concentration of silver nanoparticles might be due to their aggregation in the present study (21-24)

Effects on the development of healthy embryos

Regarding the development of healthy embryos, changes in the number of embryos were observed following treatment with silver nanoparticles. Silver nanoparticles reduced the number of embryos in a way that the number of embryos was equal at the concentrations of 250 and 500 ppm, and this change was less in the group treated with 1000 ppm concentration, although the difference among the three treatment groups was not significant ($P > 0.05$) (Figure 3). Overall, we could conclude that the concentrations of silver nanoparticles used in this study could not result in a significant effect on the development of embryos.

To compare the effects of silver nano ions and silver nanoparticles coated with silver nano ions on the survival of Zebrafish embryos, they were treated with these two types of nano-silver ions at the concentrations of 10 and 20 ppm, respectively, and the induced acute reaction during embryogenesis was investigated (25, 26). The results of this study have shown that the groups treated with silver ions had lower survival rate, compared to the groups treated with silver nanoparticles. Phenotype changes in Zebrafish larvae treated with the silver nanoparticles and silver ions were created due to changes in gene expression in Zebrafish embryos showing increase of apoptosis and incomplete formation of an axis (25, 26). Silver nanoparticles cause widespread anatomical and histological changes in the structure of the placenta, but have no effect on survival of neonatal rats (27).

Effects on the abortion of fetuses

The results regarding changes in the number of aborted fetuses revealed that silver nanoparticles at the concentrations of 250 and 500 ppm led to increased rate of abortion. Comparing with the control group, the highest rate of abortion was observed at the 250 ppm concentration ($P < 0.001$). No aborted fetus was observed in the group treated with the concentration of 1000 ppm and this was significant compared to other groups ($P < 0.001$) (Figure 4). The results indicated that silver nanoparticles had the ability to reduce survival rate of rat fetuses, leading to fetal abortion. It was noticed that the abortion rate was higher at lower concentrations, the underlying mechanisms were unknown.

In a study conducted by Aerle *et al*. in 2013 for the mortality in Zebrafish embryos, it was found that the abortion rate of fetuses treated with silver nanoparticles was 4.6 ± 2.5 (28). No significant difference was observed in the mortality rate in fetus treated with silver nanoparticles at the concentration of 5 mg/L. The data obtained in the present study was in line with the hypothesis that the availability of silver ions in the embryos exposed to silver nanoparticles is important (28).

In another study for the effect of drinking silver nanoparticles (10 and 1 ppm, respectively) on the fetal mortality of rats, the results have shown that high concentration of silver nanoparticles can have toxic effects on the fetus, leading to death of them (29). The studies on the effects of silver nanoparticles on the mortality of fetuses by Yu *et al*. have demonstrated that similar nanoparticles have no effects on fetus mortality at the dosage of 100, 300, and 1000 mg/kg/day. Therefore, the dosage may play a key role in the severity of damages in pregnancy and embryonic development (30).

Conflicts of Interest: None

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