IN VIVO AND IN VITRO TOXICITY OF Ag-NPs-CONTAINING-HYDROGEL IN REPRODUCTIVE ORGANS AND IN HELA CELLS

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Introduction:

Following the nanotechnology rapidly advancing, a various kinds of nanomaterials have been commercially used in a wide range of areas, such as medical, food, cosmetic, and so on. Silver nanoparticles (Ag-NPs) are among the most commercialised nanoparticles due to its anti-bacterial activity. However increasing data have demonstrated that silver nanoparticle could induce cytotoxicity in several cells in vitro [1], and cause damage in the some organs in vivo [2]. Silver nanoparticle-containing hydrogel (Ag-NPs-Gel) is one of commercially used nanoparticle-containing products, for treating the cervicitis or the cervical erosion. However, little is know its uptake, distribution, and whether Ag-NPs-Gel accumulate in the reproductive organs, has some reproductive toxicity risks. This study focused on investigating in vivo toxicity and in vitro cytotoxicity of Ag-NPs-Gel in reproductive organs and in HeLa cells.

Materials and Methods:

Test materials. New Zealand white rabbits, body-weight was 2.1-2.4 Kg, adult female, were used. The HeLa cells were used as target cells because the Ag-NPs-Gel was used for treating cervicitis. Ag-NPs-Gel was provided from co-laboratory company. The content of Ag-NPs in Ag-NPs-Gel was 0.38μg/mg. The size of Ag particles was less than 30nm.

Exposure of Ag-NPs-Gel in vivo: The dose was selected according to the human/rabbit drug dosing ratio, the dose in rabbit = g/kg (human) x 2.3, and based this, the dose was up to 5 times, continually exposure for 6 days, wishing to mimic a high-dose exposure in the human use.

Cell culture and treatment: HeLa cells were cultured in DMEM, with 10% FBS and 100u/ml penicillin, 100μg/ml streptomycin, at 5% CO2, 37℃.

Pathological anatomy: The experimental rabbits were set as control group and Ag-NPs-Gel group. After 6 days Ag-NPs-Gel exposure, the animal were killed, and the blood, reproductive organs were dissected. After rinsed by PBS, tissues were seperated for HE stain, TEM, and silver content detection, respectively.

ICP-MS: The Ag contents in the tissues or cells were detected by inductively coupled plasma – mass Spectrometer (ICP-MS).

Results and Discussion

Morphological changes after 6 days exposure: as shown in Fig. 1, vagina mucus-membrane shrinkage, vascular expansion in the cervix of the uterus under-mucus membrane, and mucus-membrane shrinkage of uterine tuber were observed in the 6-days exposure.

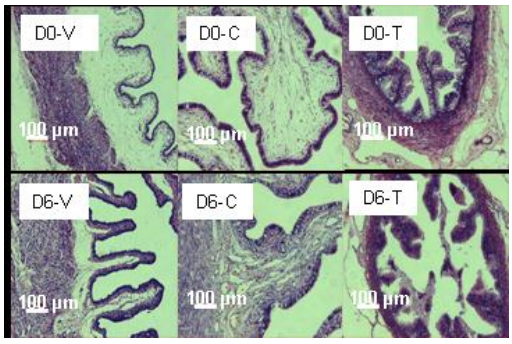

Fig. 1 Tissue morphological changes (HE stain) after 6 days exposure of Ag-NPs-Gel. D0: non-treatment control; D6: Ag-NPs-Gel exposure for 6 days; V: Vagina; C: Cervical tissue; T: Uterine tube.

The results above suggested that high-dose exposure of Ag-NPs-Gel was able to cause damage in some reproductive organs.

Ag contents in blood and reproductive organs: To further know that whether the damage is due to Ag-directly induced, the Ag contents were measured by ICP-MS. The results showed that the Ag contents were higher in all reproductive organs and blood as 5- to 25-fold compared to that in the control group (Fig.2). These results suggested that the reproductive organ damage is primarily due to Ag nanoparticles and/or Ag-ion release from Ag-NPs directly induced. It is considered that Ag nanoparticles and/or Ag-ion are uptaken by the vagina mucus-membrane, and following blood circulation disposed in the reproductive organs.

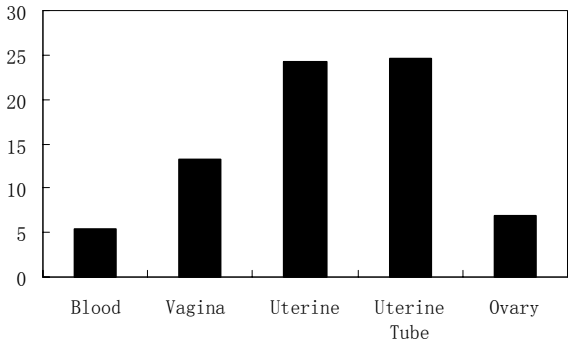

Fig. 2 Ag contents in the reproductive organ tissues after 6 days exposure of Ag-NPs-Gel. The values showed as fold-changes compared to that in the un-treatment control animals.

Ultra-micro-structural observation: The ultra-microstructural changes were observed by transmission electron microscopy (TEM). The nucleus showed un-normal changes such as, micronuclei formation (Fi.3 A), nuclei disruption (Fig.3 D), and chromosome accumulation on the side, stronger stain (Fig.3 A, C and D). The Ag-like round and high-density particles were observed in cells of reproductive organs, such as showed in Fig.3 B. These observations suggested that the Ag-NPs and ions released by Ag-NPs remained in the tissue could cause ultra-microstructural changes.

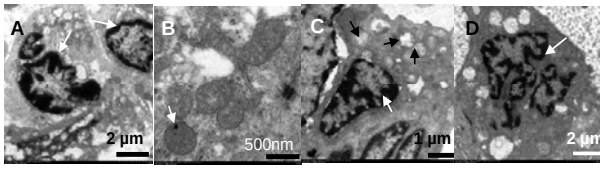

Fig. 3 Ultra- microstructural changes of the reproductive organ tissues were observed by TEM after 6 days exposure of Ag-NPs-Gel. A, vagina; B, cervical tissue; C, uterus; D, ovary.

Cytotoxicity in HeLa cells: To know whether the in vitro cytotoxicity linked to the in vivo toxicity, the morphological and ultra-microstructural changes of the cells exposed to Ag-NPs-Gel were observed by normal microscopy and TEM. As showed in Fig.4, the HeLa cells exposed to the Ag-NPs-Gel (45mg/ml, contained 17.1μg/ml of Ag-NPs) for 48h, the cells lost its epithelial cell morphology, become to longer, swelling, proliferation inhibition and died. In contrast, hydrogel (without Ag-NPs) treatment did not showed significant difference compared to the control (Fig.4 up-panel). Ag-NPs like particles were observed within and around a lysosome and between the endosomes (Fig.4 lower panel, A and B, respectively). The expansion of endoplasmic reticulum and vacuolar formation were observed (Fig.4 C). These changes in the cell morphology and ultra-microstructure suggested that Ag-NPs-Gel damage the cells, as well as in vivo tissues, represented at the macro-levels and ultra- microstructure-levels.

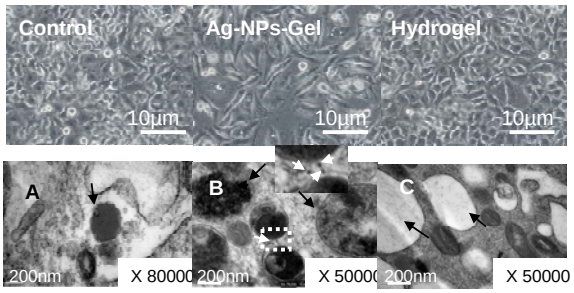

Fig.4 Cell morphological and ultra-microstructure changes post-48h exposure of Ag-NPs-Gel. A, Ag-NPs like high density particles translocated within or around lysosome (A, arrow) and translocated between endosomes (B, white arrow); Endoplasmic reticulum expansion and de-particles (C, black arrow) .

Conclusion:

The Ag-NPs-Gel exposure both in vivo and in vitro could cause ultra-microstructural changes, as well as damage in tissue or cell level in reproductive organs and HeLa cells. The damage may directly was induced by Ag-NPs, because the hydrogel only (without Ag-NPs) did not cause significant changes. The mechanisms of toxic effects may be due to Ag particles and Ag-ions, and the Ag-ions released from Ag particles may play a key role than Ag particles themselves as demonstrated in our previous study [1].

References:

[1] Liming Xu, et al. Material Express, 2010 in press.
[2] Jinglong Tang, et al. J Nanosci Nanotechnol. 2009 Aug;9(8):4924-32.